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CONTENT

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RESPONSES OF ANTIOXIDANT SYSTEM OF VARIOUS COTTON GENOTYPES TO HEAT STRESS AT THE JUVENILE STAGE OF ONTOGENESIS

SUMMARY

Extremely high temperatures affecting cotton plants at various stages of ontogenesis. Oxidative damage of cell structures inflicted by moderately high temperatures is a main cause for the deleterious effect of heat stress. However, the status of the protective antioxidant system (AOS) in cotton plants has been studied mainly at the generative stages of development, while peculiarities of its functioning in the juvenile plants of various genotypes remains underexplored. The work was done to study the reaction of growth and the AOS response to the heat stress in the 7-8-day seedlings of various cotton cultivars obtained by various selection methods differing in economically valuable features. Surkhan-103 and Bukhara-102, the cultivars generated by classical selection, as well as those generated by biotechnological methods with higher fiber quality, to cultivars Porlok-1, Porlok-2, Ravnak-1 and Ravnak-2, were the objects of experiments. The 7-day seedlings were exposed to the 6-hour effect of high temperature (45°C) to subsequently assess the increment of control and stress-inflicted plants and to determine parameters of the AOS state and development of oxidative damages. Surkhan-103, Porlok-4 and Ravnak-2 cultivars were found to distinguish by significantly lower post-stress inhibition of growth of both seedlings and roots than those of Bukhara-102, Ravnak-1 and Porlok-1. The sensitive cultivars affected by heat stress demonstrated higher post-stress contents of hydrogen peroxide and malondialdehyde (MDA), a product of lipid peroxidation, in the leaves. At the same time, the resistant cultivars demonstrated higher activity of catalase and superoxide dismutase. The higher post-stress

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content of proline were typical of the resistant cultivars. A close direct correlation between inhibition of growth of seedlings and higher contents of hydrogen peroxide and MDA was demonstrated. In addition, high-level inverse correlation was demonstrated between the growth inhibition and higher catalase activity and proline content. The juvenile cotton plants were concluded to be promising in assessment of heat resistance and its dependence on the AOS state.

Keywords: *Gossypium*, heat resistance, reactive oxygen species, antioxidant enzymes, proline

INTRODUCTION

Cotton (*Gossypium*) is relatively well adapted to growing in moderately arid regions. However, the plants are vulnerable to high temperatures both at the early stages of development (James *et al.*, 2005) and during flowering and development of bolls (Saini *et al.*, 2023). Due to recent changes in the climate, waves of heat in the regions of cotton growing are being more intensive and less predictable (Saleem *et al.*, 2021). That is the reason why the screening of resistibility of the basic material intended for generation of improved-yield heat resistant cotton cultivars with plastic phenotypes is the main task for up-to-date program of selection of the culture (Zafar *et al.*, 2021). Resistance assessment of novel cultivars including those generated using biotechnological methods is not less significant.

Effects of temperatures significantly exceeding physiological optimum, as a rule, result in development of oxidative stress. Fluidization of lipid basis of cell membranes, including membranes of chloroplasts and mitochondria, is a main cause for the damaging effect of high temperatures (Yoshioka, 2016; Choudhury *et al.*, 2017). The temperature rises due to increase in fluidity of thylokoid membranes, electron-transport chain of chloroplasts may disunite; that is believed to be a main cause for increase of generation of reactive oxygen species (ROS) under heat stress (Asthir, 2015). Photosystem II and its Mn-containing oxygen-releasing complex is the most sensitive to effects of high temperatures (Kreslavski *et al.*, 2012; Bernfur *et al.*, 2017).

However, generation of ROS under heat stress increases not only in chloroplasts. Stochastic generation of ROS increases in the membranes of mitochondria, as well, possibly associated both with changes in the status of membrane lipid phase and with the overrecovery of electron transport chain due to insufficient consumption of pool of reductants (Choudhury *et al.*, 2017). This state of mitochondrial complexes leads to the formation of radical ROS and, in particular, superoxide anion-radical in them.

Excessive accumulation of ROS is known to result in pro-/antioxidant imbalance and damage of redox-regulation. That may be an underlying cause of cell damage and death (Kumar, Rai, 2014; Kolupaev *et al.*, 2023a).

To prevent damages of redox-regulation processes and development of stress-induced oxidative damages, plants in evolution generate powerful multicomponent antioxidant system (AOS). The system is represented by enzymatic and low molecular antioxidants (Kolupaev *et al.,* 2019; Hasanuzzaman *et al.,* 2020). Among the enzymes, there are ROS - neutralizing catalyzers, to name superoxide dismutase (SOD), catalase (CAT) and various peroxidases (POX), enzymes of lipid detoxication including glutathione S-transferase, glutathione reductase, and regenerators of active forms of antioxidants, such as monodehydroascorbate reductase, dehydroascorbate reductase and glutathione reductase. In addition, plants have complex system of thioredoxins and peroxiredoxins which in combination with other redox-active proteins participate in the regulation of thiol groups state (Hasanuzzaman *et al.,* 2020).

The complex of low-molecular antioxidants is represented by many chemically heterogeneous compounds (Das, Roychoudhury, 2014). Recently, some compounds with antioxidant function as non-basic one, but having marked antioxidant properties and accumulating as the response to stress-factors, particularly, proline, are believed to belong to antioxidants (Liang *et al.,* 2013).

Attempts to find associations between the AOS function and resistance of specific genotypes of plants to one or other stress-factors have being made for many years. At the same time, the strategies for plant adaptation to oxidative stress depend on their taxonomic belonging (Kolupaev *et al.,* 2016; 2022; Pržulj *et al.,* 2020). For example, peculiarities of functioning of the AOS of wheat and other cereals under stress conditions were studied in enough details. The correlation between some AOS parameters and heat- and drought resistance in the wheat cultivars was demonstrated (Kirova *et al.,* 2021; Kolupaev *et al.,* 2023b).

At the same time, peculiarities of responses of various cotton genotypes to high temperatures are underexplored. Herewith, in several works, the AOS status under heat stress at the generative stages of plant development was focused on. Thus, activity of SOD, CAT and POX, as well as content of proline was found higher in the heat resistant cultivars on the stage of flowering in the field conditions by means of a tunnel constructed using bamboo sticks and plastic sheets (Zafar *et al.,* 2023). In contrast, the resistant cultivars under the effect of excessive temperatures were found to retain values of oxidative stress markers, including hydrogen peroxide and MDA, close to the control ones. In another work with a large set of cultivars, high correlation of cotton yield with activities of peroxidase and SOD under heat stress generated in the field conditions by sowing later (Yousaf *et al.,* 2022).

In experiments with cotton plant anthers, significant inverse correlation between the pollen viability and concentrations of superoxide anion-radical and hydrogen peroxide was demonstrated (Zhang *et al.,* 2023). However, Mahan and Mauget (2005) failed to register significant correlation between MDA concentrations and temperature increase in field experiments on studying responses of cotton cultivars with different genotypes. As compared to the stress-sensitive cotton cultivars, Sarwar *et al.* (2023) demonstrated higher activities of antioxidative enzymes, including SOD, catalase and peroxidase, in stress-resistant cotton cultivars in the greenhouse. The stress-resistant cultivars had lower MDA contents, as well.

Still, there are no studies on effects of high temperatures on the wide spectrum of cotton cultivars under controlled conditions at early stages of development.

The purpose of the work is to study the possible connection between the heat resistance of cotton cultivars in the early stages of development and the state of their antioxidant system. For the work, we used varieties created by classical breeding and biotechnological methods (marker-associated selection and gene knockout).

MATERIAL AND METHODS

Plant material

Cotton plants of *G. hirsutum* (cultivar Bukhara-102) and *G. barbadense* (cultivar Surkhan-103) species generated using classical cotton breeding were used in experiments. Plants of gene-knockout cultivars G. hirsutum Porlok-1 and Porlok-4 with suppression of the PHYA1 gene were also used, which shortens the growing season of plants (Pat. NAP 20130014, 15.11.2013 and Pat. NAP 20130017, 15.11.2013). In addition, two cultivars of G. hirsutum (Ravnak-1 and Ravnak-2), created by the method of marker-associated selection aimed at increasing the strength of cotton fiber, were used for experiments (Pat. NAP 201600228, 30.08.2019 and Pat. NAP 201600229, 30.08.2019).

Seeds of the gene-knockout cotton cultivars, such as Porlok-1 and Porlok-4, as well as of Ravnak-1 and Ravnak-2 generated by marker-associated selection were provided by the Center of Genomics and Bioinformatics, Uzbekistan Academy of Sciences. Seeds of the Surkhan-103 and Bukhara-102 cultivars were provided by the Cotton Breeding, Seed Production and Agrotechnologies Research Institute, Uzbekistan Ministry of Agriculture and Water Resources.

Production of plants and exposure to heat stress

Seeds of the cotton cultivars under study were denuded in concentrated sulfuric acid, washed under cold running water for 15 minutes and kept in the distilled water for 12 hours (Babaeva *et al.* 2020). The swollen seeds wrapped in the paper rolls were germinated in a dark wet chamber for 7 days at 30°C. After the 7th day of germination, 50% of the seedlings were placed in the climatic chamber at 30°C as the controls, another half of the seedlings were exposed to heat stress. To simulate heat stress, the temperature gradually increased from 30°C to 45°C by 1°C every 10 minutes. When the desired temperature was reached, the plants were exposed to 45°C for 6 hours. The conditions were chosen based on the findings from the preliminary experiments with variations of temperature and duration of thermal effects on the plants (the findings are not presented).

After exposure to heat stress, the samples of leaves were collected to be frozen in liquid nitrogen and subsequently analyzed. Part of control and heat-exposed seedlings were used to assess either the growth response by determining biomass of shoots and roots before exposure to heat stress and in 24 hours after stress or growth of plants under optimum conditions. In addition, biochemical parameters were measured in 24 hours after exposure to the stress. The cotyledon leaves were used for the analysis.

The inhibition of seedlings and root growth of the seedlings was determined according to the formula (Kolupaev *et al.,* 2023a):

$$I = \frac{(C_2 - C_1) - (E_2 - E_1)}{C_2 - C_1} \cdot 100\%,$$

where I is growth inhibition (%); C1 and C2, E1 and E2 are, respectively, the initial and final values of seedling organ fresh weights in the control and experimental (heat stress) variants.

Contents of hydrogen peroxide

The method based upon potassium iodide (PI) oxidation by hydrogen peroxide in the acidic medium was used (Junglee *et al.,* 2014). The absorption was measured spectrophotometrically at 390 nm using Shimadzu UV-1800 (Shimadzu, Japan).

Measurement of MDA contents

The MDA content were measured by the reaction with 2-thiobarbituric acid (2-TBA), as described by Gür *et al*. (2010). Frozen leaf samples were homogenized in a solution containing 0.25% 2-TBA in 10% trichloroacetic acid (TCA), while the controls were homogenized in 10% TCA without 2-TBA. The samples were covered with foil caps and boiled for 30 minutes. The samples were cooled and centrifuged at 10.000 g for 10 minutes using DLAB D1524R microcentrifuge (Shandong, China). The absorbance of supernatant was measured at 532 nm. The value for absorption at 600 nm adjusted for the non-specific absorption subtracted from the basic result was measured as well.

Analysis of activities of antioxidant enzymes

To get the enzymatic extract a sample of tissue (500 mg) previously frozen in the liquid nitrogen was ground in a cold porcelain mortar with addition of appropriate extraction buffer (0.1 M Na-phosphate buffer pH 7.0 containing 20 mM EDTA, 2 mM PMSF, 1% triton X, 150 mM PVP) in 1:10 ratio. The homogenate was centrifuged at 8000 g for 15 min at temperature not higher than 4°C.

Total superoxide dismutase (SOD) activity was determined by measuring its capacity to inhibit the photochemical reduction of nitroblue tetrazolium chloride in accordance with a method by Giannopolitis and Ries (1977) with some modifications by Nikerova *et al*. (2019). To eliminate the effects of methionine- and riboflavin-like substances, two additional series of measurements were made without alternative addition of methionine and riboflavin into the incubation medium.

The peroxidase activity was determined using hydrogen peroxide as a substrate and *o*-dianisidine as a reducing agent using method described by

Christensen *et al.* (1998). The deviations in absorbance during the reaction was determined at 460 nm.

The catalase activity was measured using method described by Sinha (1972) in which dichromate in acetic acid is reduced to chromic acetate in a molar ratio of 1: 3. Lowry's method was used for protein measurement (Lowry *et al*., 1951).

Proline contents

Proline concentrations were measured using ninhydrin reagent in accordance with the method by Bates *et al*. (1973) with some modifications by Shihalyeyeva *et al*. (2014).

Reproducibility of experiments and statistical processing of results

The experiments were repeated thrice, each of which was independently reproduced thrice. The data were statistically processed using Atte Stat V.10.9.6 program as a computer application to "Microsoft Excel-2007". Student's *t*-test was used to determine the significance of inter-variant differences. Different letters denote values with differences significant at $p \le 0.05$.

Correlation coefficients were estimated using the R programming language version 4.1.1 (R Core Team).

RESULTS AND DISCUSSION

Growth of cotton seedlings after heat stress

Exposure to stress temperature caused inhibition of growth in cotton seedlings of all cultivars under study (Table 1). Growth of roots turned out to be more sensitive to heat stress, as compared to the growth of shoots. Thus, in Bukhara-102 and Porlok-1, complete termination of root growth within 24 hours after stress exposure; more than 90% root growth inhibition was seen in Ravnak-1. In other cultivars, the root growth was inhibited more than by 50-70%.

Table 1. Growth inhibition (%) in cotton shoots and roots in 24 hours after 6-hour exposure to 45°C

Cultivar	Growth inhibition, %	
	Shoots	Roots
Surkhan-103	63.7±2.4 b*	78.6±2.9 bc
Bukhara-102	68.9±2.7 b	100.0±0.0 a
Porlok-1	92.9±3.3 a	100.0±0.0 a
Porlok-4	63.5±2.5 b	53.8±2.6 d
Ravnak-1	95.5±3.2 a	90.5±2.8 b
Ravnak-2	68.0±2.8 b	71.4±3.1 c

* The table shows: M ± m (n = 3), different letters denote values with differences significant at $p \le 0.05$ (Student's t-test).

Potent inhibition of shoots growth (more than by 90%) after exposure to high temperature was seen in Ravnak-1 and Porlok-1; in other cultivars the inhibition of shoots growth turned out ranging from 60 to 70%.

Generally, the highest resistance to growth after exposure to high temperature could be seen in Porlok-4; growth of both shoots and roots remained rather intensive (Table 1). Relatively high growth potential of plant organs was retained in Ravnak-2 and Surkhan-103. Roughly, the three cultivars can be considered as the relatively resistant among 6 cultivars under study. At the same time, in the cultivars Porlok-1, Ravnak-1 and Bukhara-102, the values of inhibition of organ growth were significantly greater. (Table 1).

Parameters of oxidative stress in cotton seedlings after exposure to high temperature

Constitutive concentrations of hydrogen peroxide in various cultivars were different; thus, it was high in Porlok-1, Porlok-4 and Ravnak-1, being slightly lower in Bukhara-102 and Ravnak-2 and even lower in Surkhan-103 (Table 2). The associations between the basic concentrations of H_2O_2 and heat resistance of seedlings could not be seen. Constitutive MDA concentrations in the seedlings were different by the cultivar. Thus, it was high in Porlok-1, Porlok-4 and Ravnak-1; while lower values were registered in other three cultivars (Table 2).

Table 2. Effect of heat stress on hydrogen peroxide and MDA contents in the cotton seedlings leaves

Cultivar	H_2O_2, nmol/g of fresh weight			MDA, nmol/g of fresh weight		
	Control	Heat stress		Control	Heat stress	
		After 6-hour exposure to 45°C	After 6-hour exposure to 45°C and in 24 hours of exposure to 30°C		After 6-hour exposure to 45°C	After 6-hour exposure to 45°C and in 24 hours of exposure to 30°C
Surkhan-103	117±4 e*	139±5 d	118±4 e	12.5±0.4 d	14.3±0.5 c	13.7±0.5 cd
Bukhara-102	159±6 c	195±7 bc	183±5 c	14.8±0.3 c	17.5±0.4 b	16.5±0.3 b
Porlok-1	208±8 b	285±4 a	291±5 a	17.5±0.5 b	24.0±0.8 a	26.4±0.9 a
Porlok-4	196±6 b	220±5 b	208±3 b	16.5±0.5 b	16.7±0.4 b	15.0±0.3 c
Ravnak-1	201±7 b	284±6 a	294±5 a	17.8±0.5 b	23.5±0.8 a	26.7±0.9 a
Ravnak-2	140±3 d	199±5 bc	143±5 d	13.4±0.4 cd	18.0±0.6 b	15.6±0.6 b

* The table shows: M ± m (n = 3), different letters denote values with differences significant at $p \leq 0.05$ (Student's t-test).

The 6-hour exposure to high temperature caused increase in the concentrations of hydrogen peroxide and MDA in the leaves of all plants under study, excluding those of Porlok-4 cultivar. Ravnak-1, Ravnak-2 and Porlok-1 cultivars demonstrated the most significant increase of the heat stress parameters

(Table 2). The less significant effect of increase in concentrations of H_2O_2 and MDA under heat stress was observed in Bukhara-102 and Surkhan-103.

Within 24 hours after stress, the concentrations of H_2O_2 and MDA tended to decrease in Surkhan-102, Ravnak-2 and Bukhara-102 cultivars; that is, the values reached the control ones (Table 2). At the same time, the parameters in Porlok-1 and Ravnak-1 remained increased, as compared to the control values. In Porlok-4 cultivar, as it was earlier pointed out, concentrations of H_2O_2 and MDA remained unchanged in 6 hours of stress exposure; no changes in the parameters could be seen in 24 hours of incubation at optimum temperature, as well.

Generally, no signs of oxidative stress after exposure to 45°C could be seen in Porlok-4 cultivar only. On the contrary, significant increase of hydrogen peroxide and MDA in the leaves after heat stress could be seen in Porlok-1 and Ravnak-1 cultivars; the rest cultivars kept intermediate position by the parameters in question.

Activities of antioxidant enzymes in leaves of cotton seedlings after heat stress

Basic SOD activity in the leaves of different cultivars varied a lot (Table 3). The highest values were registered in Surkhan-103, the only representative of *G. barbadense*. Alterations in activity of the enzyme under heat stress and in post-stress period manifested differently depending on peculiarities of the cultivars. A significant increase in the SOD activity after 6-hour exposure to stress temperature was found in Surkhan-103 cultivar only. In Porlok-1, Ravnak-1 and Ravnak-2 cultivars, activity of the enzyme under the stress of the kind did not significantly change; in Bukhara-102 and Porlok-4 cultivars the SOD activity was found to be reducing (Table 3). In post-stress period, a significant increase in the SOD activity could be seen in Bukhara-102 and Porlok-4. In other cultivars, no alterations in activity of the enzyme significant at $P \leq 0.05$ could be seen.

Basic activity of catalase in various cotton cultivars under study differed significantly (Table 3). After 6-hour exposure to 45°C, activity of the enzyme decreased in all cultivars excluding Surkhan-103 cultivar. In post-stress period activity of the enzyme remained steadily high in Surkahn-103 cultivar (*G. barbadense*). A significant increase in the activity up to the values, exceeding the control ones, was registered in Bukhara-102, Porlok-4 and Ravnak-2 cultivars. At the same time activity of CAT in Porlok-1 cultivar remained significantly decreased, while in Ravnak-1 it slightly increased not exceeding the control values (Table 3).

As compared to the activities of the other two enzymes, activity of POX in various cultivars in the control varied less significantly. In response to a 6-hour stress exposure, most cultivars experienced an increase in peroxidase activity. However, in Ravnak-1 and Ravnak-2 cultivars, activity of the enzyme decreased. In 24 hours after stress exposure, the activity in most cultivars remained on the level registered in 6 hours after stress exposure, respectively did not change significantly. Ravnak-1 was the exclusion with significant increase in activity of the enzyme.

Table 3. Effect of heat stress on activity of antioxidant enzymes in the cotton seedlings leaves

Cultivar	Control	Heat stress	
		After 6-hour exposure to 45°C	After 6-hour exposure to 45°C and in 24 hours of exposure to 30°C
SOD activity (U/mg protein)			
Surkhan-103	20.1±0.7 c*	25.8±0.5 ab	28.0±0.9 a
Bukhara-102	15.8±0.3 d	13.1±0.4 e	20.2±0.6 c
Porlok-1	15.0±0.6 de	15.9±0.5 d	17.0±0.6 cd
Porlok-4	17.4±0.5 cd	11.5±0.3 f	24.0±0.5 b
Ravnak-1	12.8±0.4 e	12.5±0.4 e	14.0±0.5 de
Ravnak-2	14.4±0.6 de	15.6±0.6 de	15.0±0.6 de
CAT activity (U/mg protein)			
Surkhan-103	150.0±4.8 b	160.0±2.5 b	170.0±6.5 ab
Bukhara-102	130.0±5.9 bc	90.0±4.5 d	190.3±4.5 a
Porlok-1	92.5±3.6 d	54.4±1.5 ef	52.5±1.4 ef
Porlok-4	110.0±3.2 c	78.0±2.7 d	175.0±6.5 ab
Ravnak-1	55.8±1.4 ef	30.3±0.9 h	50.0±1.2 f
Ravnak-2	60.4±1.8 e	42.0±1.0 g	98.0±3.7 cd
POX activity (U/mg protein)			
Surkhan-103	18.0±0.6 cd	23.0±0.9 b	22.0±0.8 b
Bukhara-102	15.8±0.5 d	28.0±0.7 a	25.0±0.8 ab
Porlok-1	17.4±0.3 cd	19.3±0.8 b	25.0±0.7 ab
Porlok-4	18.0±0.5 cd	25.0±0.9 ab	25.4±0.6 ab
Ravnak-1	18.8±0.5 c	11.2±0.3 e	28.0±05 a
Ravnak-2	14.4±0.4 de	10.5±0.5 e	12.6±05 e

* The table shows: M ± m (n = 3), different letters denote values with differences significant at $p \leq 0.05$ (Student's t-test).

Contents of proline in the cotton seed leaves under heat stress

Constitutive contents of proline in the cotton leaves were found to vary from 0.28 μmol/g in Porlok-4 to 0.73 μmol/g in Ravnak-2 cultivar (Table 4). The 6-hour exposure of seedlings at 45°C caused various effects depending on the cultivar. A significant increase of the proline contents could be seen in Ravnak-2 cultivar only. In Porlok-1 and Porlok-4 cultivars, after the 6-hour stress exposure proline did not change significantly. Subsequent 24-hour exposure of seedlings exposed to optimum temperature (30°C) caused significant increase in concentrations of proline in Surkhan-103, Bukhara-102 and Porlok-4 cultivars.

In other cultivars, after transfer of seedlings to the optimum conditions proline either unchanged significantly, or tended to slight decrease (Porlok-1, Ravnak-1 and Ravnak-2 cultivars).

Table 4. Effect of heat stress on concentrations of proline (μmol/g of fresh weight) in the cotton seedlings leaves

Cultivar	Control	Heat stress	
		After 6-hour exposure to 45°C	After 6-hour exposure to 45°C and in 24 hours of exposure to 30°C
Surkhan-103	0.34±0.01 d	0.24±0.01 ef	0.45±0.01 c
Bukhara-102	0.67±0.02 b	0.40±0.01 c	0.83±0.03 ab
Porlok-1	0.66±0.02 b	0.59±0.03 bc	0.44±0.01 c
Porlok-4	0.28±0.01 e	0.25±0.01 ef	0.48±0.02 c
Ravnak-1	0.34±0.01 d	0.26±0.01 ef	0.22±0.01 f
Ravnak-2	0.73±0.03 b	1.00±0.05 a	0.92±0.01 a

* The table shows: M ± m (n = 3), different letters denote values with differences significant at $p \leq 0.05$ (Student's t-test).

Correlations between growth response of plants to heat stress and biochemical parameters

Absolute values of contents of hydrogen peroxide and MDA in leaves at the moment of elimination of the 6-hour exposure to 45°C were found to closely correlate with the growth inhibition (Figure 1). Activities of antioxidant enzymes were in inverse correlation with the growth inhibition of seedlings, though their values were not significant at $P \leq 0.05$. Attention should be paid to high coefficient of inverse correlation between catalase activity and concentrations of hydrogen peroxide, being the evidence for the contribution of the enzyme in the control of H_2O_2 contents in the cotton leaves.

Correlation between growth inhibition of seedlings and the AOS parameters was found closer not immediately after stress, but after subsequent 24- hour very high correlations between growth inhibition and oxidative stress parameters (contents of H_2O_2 and MDA) could be seen. Inverse correlation between growth inhibition and catalase activity was registered, as well (Figure 2).

Thus, absolute values of some parameters characterizing the status of pro-/antioxidant balance, including of hydrogen peroxide and MDA contents and CAT activity, significantly correlated with the seedlings growth potential in post-stress period. Still, the data obtained for other plant species demonstrate that in some cases not absolute values of biochemical parameters but the relative ones (in % to the controls) reflecting the character of changes in these parameters under stress and in the post-stress period (Kolupaev *et al.*, 2023a).

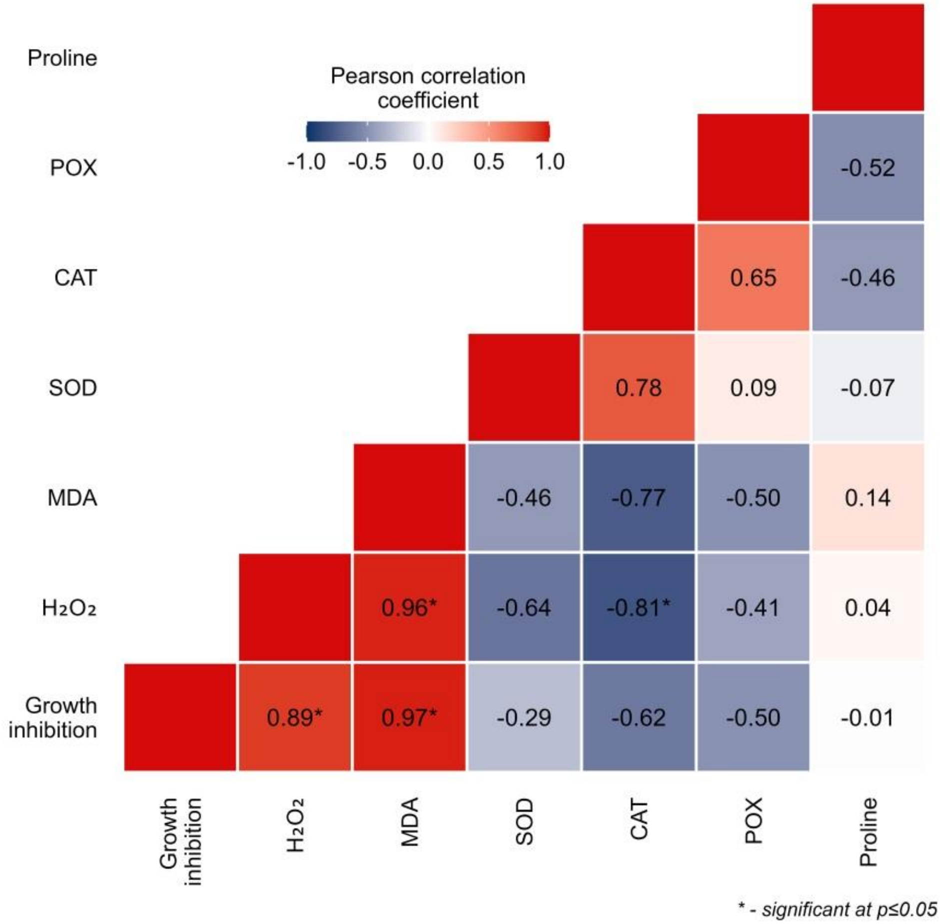

Figure 1. Correlation between growth inhibition and absolute values of biochemical parameters in cotton cultivars after 6-hour exposure to 45°C, CAT – catalase, MDA -malondialdehyde, POX – peroxidase, SOD – superoxide dismutase.

That was the reason for us to present the results as the heat map of values calculated in % to respective control values (Figure 3). This helped see some patterns less noticeable in analysis of absolute values. Thus, in Surkhan-103 and Porlok-4 cultivars with the lowest growth inhibition of the above ground part of plant (Table 1) only insignificant increase in H_2O_2 and MDA contents could be seen after the 6-hour stress exposure; while subsequently after transfer of plants to optimum conditions the parameters decreased to the control levels.

It is of note, that in the stress resistant Surkhan-103 and Porlok-4, the SOD activity was increased more significantly than the one in other cultivars in 24 hours after elimination of stress exposure (Figure 3). Significant increase in catalase activity in post-stress period took place in the stress resistant cultivars – Porlok-4 and Ravnak-2. At the same time, dynamics of changes in the peroxidase activity was found to slightly depend on the resistance of cultivars.

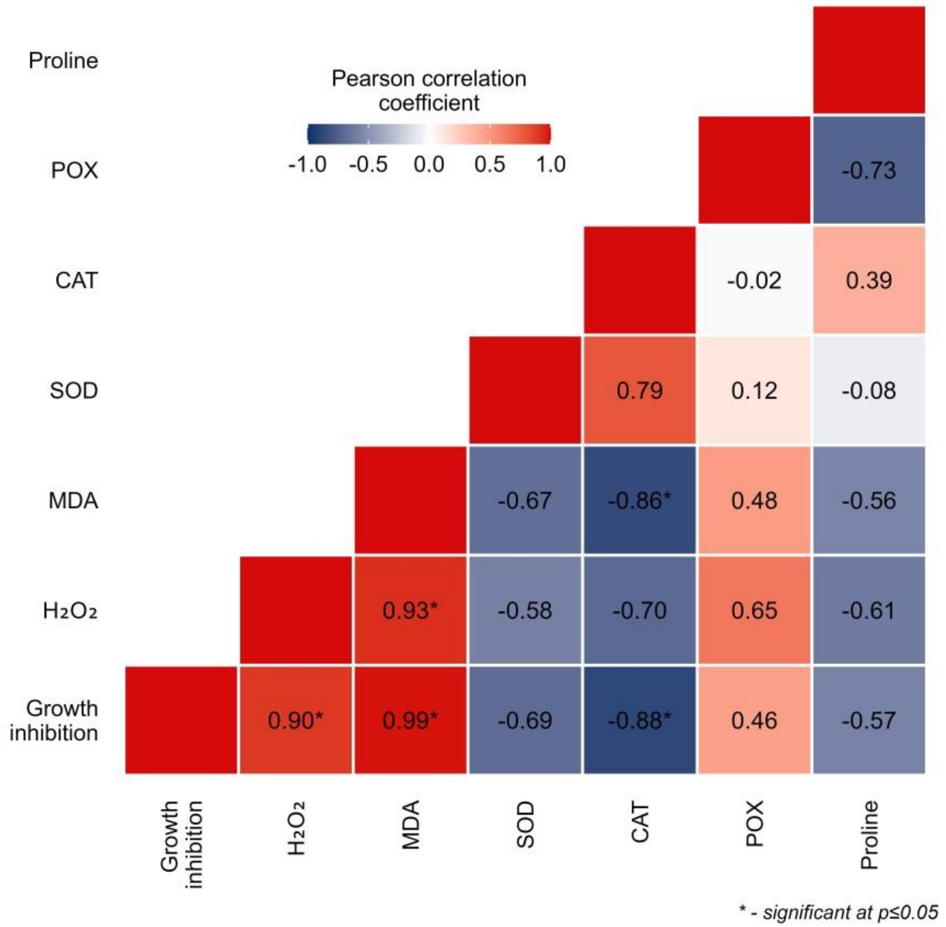

Figure 2. Correlation between growth inhibition and absolute values of biochemical parameters in cotton cultivars after 6-hour exposure to 45°C and subsequent 24-hour exposure to 30°C, CAT – catalase, MDA -malondialdehyde, POX – peroxidase, SOD – superoxide dismutase

Contents of proline immediately after stress exposure slightly depended on the growth parameters. However, the contents in post-stress period significantly increased in two cultivars maintaining high growth potential, to name Porlok-4 and Surkhan-103.

Based on the values expressed in percent to the control, we calculated coefficients of correlation between growth inhibition and parameters characterizing the AOS functioning (Figure 4). It turned out that immediately after stress exposure rather high direct correlation was registered between the values of growth inhibition of seedlings and increase in contents of oxidative stress markers – hydrogen peroxide and MDA, as well as the inverse correlation between growth inhibition and increase in catalase activity. However, the correlations were not significant at $P \leq 0.05$. Correlations of growth inhibition with other biochemical parameters turned out relatively low.

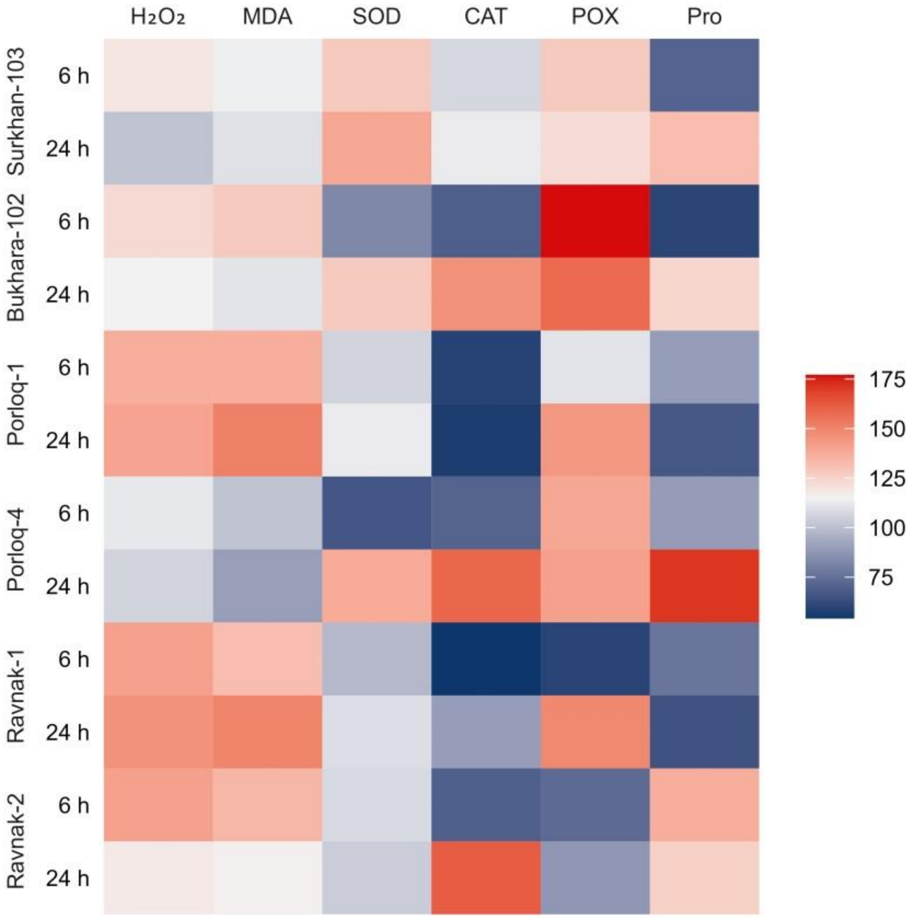

Figure 3. The heat map of alterations in biochemical parameters in cotton cultivars after 6-hour exposure to 45°C and after subsequent 24-hour exposure to 30°C. All values are presented in % to values in the controls, CAT – catalase, MDA -malondialdehyde, POX – peroxidase, SOD – superoxide dismutase

More explicit picture of correlations between growth and relative biochemical parameters could be seen in 24 hours after of stress effect. In this case, the correlation between growth inhibition and alterations in contents of H_2O_2 and MDA (0.98 and 0.95, respectively) turned out the highest one (Figure 5). Significant ($P \leq 0.05$) inverse correlations between growth inhibition and alterations in activity of catalase and contents of proline were found, as well. In addition, attention should be paid to the high inverse correlation between alterations in the contents of MDA and proline, as well as to the one between alteration in activity of catalase and MDA contents. Alterations in contents of proline were found closely inversely correlated with alterations in contents of hydrogen peroxide in leaves. It is reasonable to think that the activity of catalase and contents of proline might be essential elements in the strategy of prevention for irreversible progress of oxidative stress and excessive inhibition of growth. It is noteworthy that a high direct correlation between alterations in proline contents and catalase activity could be seen as well. Rather high but insignificant ($P \leq 0.05$) inverse correlation between growth inhibition and alteration in the SOD activity was registered to be indicative of considerable contribution of the enzyme to the maintenance of post-stress pro-/antioxidant balance.

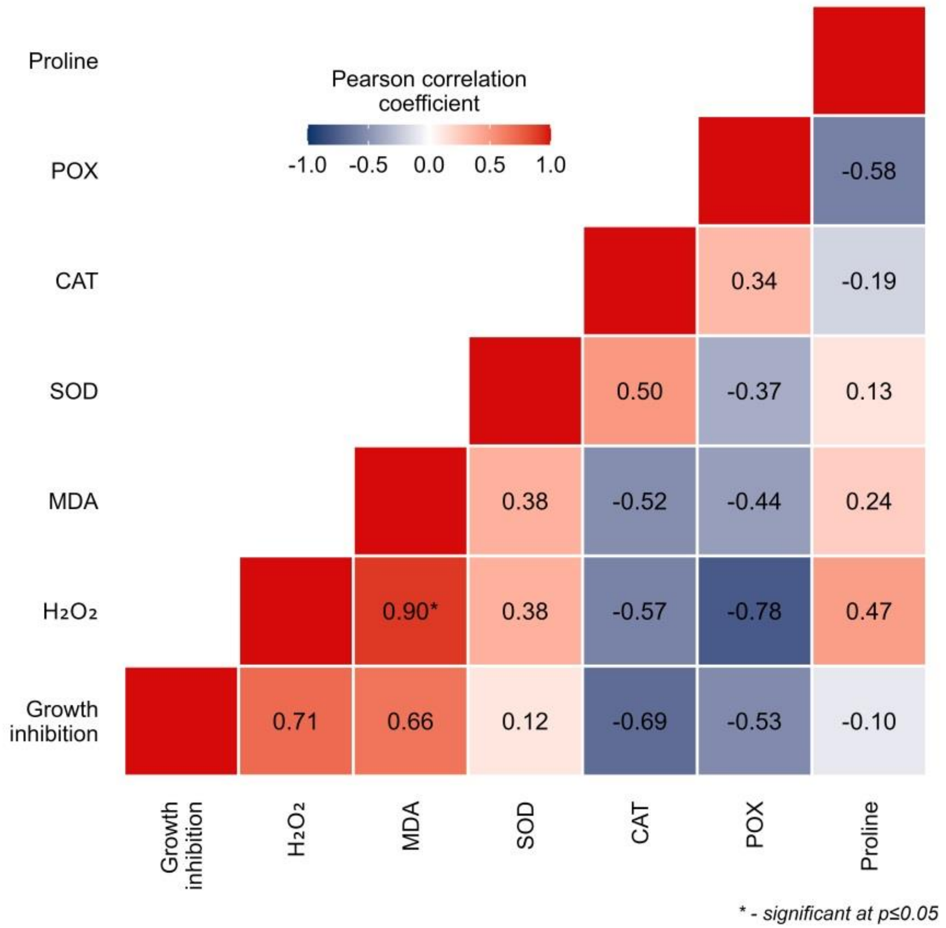

Figure 4. Correlation between growth inhibition and alterations in biochemical parameters (in % to control) in cotton cultivars after 6-hour exposure to 45°C, CAT – catalase, MDA -malondialdehyde, POX – peroxidase, SOD – superoxide dismutase

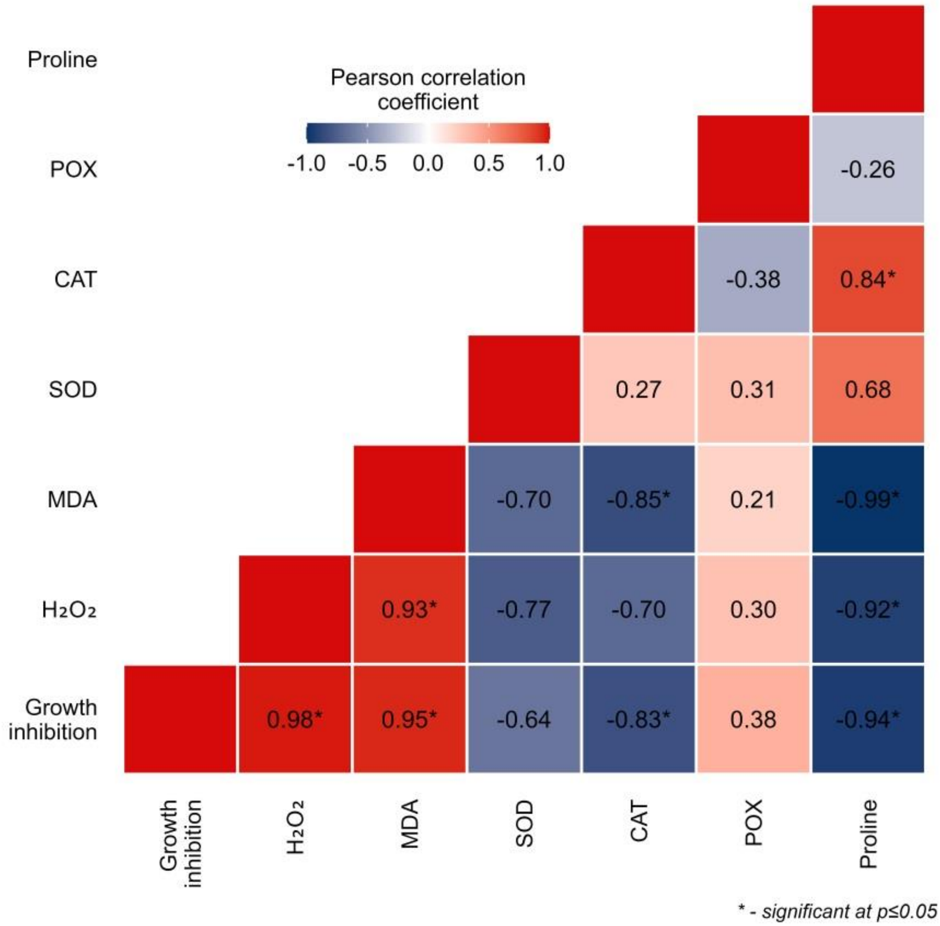

Figure 5. Correlation between growth inhibition and alterations in biochemical parameters (in % to control) in cotton cultivars after 6-hour exposure to 45°C and subsequent 24-hour exposure to 30°C, CAT – catalase, MDA - malondialdehyde, POX – peroxidase, SOD – superoxide dismutase

Generally, our findings are consistent with those of Hasan et al. (2018). This study compares the AOC status of three cotton cultivars having different drought tolerance and belonging to three different species including *Gossypium hirsutum*, *Gossypium arboreum* and *Gossypium barbadense* under drought conditions. According to the authors, the most frequently used species in breeding, *Gossypium hirsutum*, exceeds the other two in terms of resistance (Hasan *et al.*, 2018). The experimental data obtained by the authors indicated the presence of correlations between resistance and concentrations of proline, soluble proteins and carbohydrates, as well as the activities of SOD, POX, ascorbate peroxidase and oxidative stress parameters. In study on correlations between antioxidant activity and drought resistance of the cotton species belonging to

Gossypium hirsutum, the reduction in the activities of catalase and peroxidase was demonstrated in non-resistant genotypes and the increase in the parameters of the resistant ones (Singh *et al.,* 2021). Upon comparison responses of tolerant and resistant cotton cultivars to the combined effect of drought and high temperature, Sekmen et al. (2014) established that the resistance to the stresses was associated with the capability of the cultivars to preserve constitutive activity of SOD and ascorbate, as well as to increase activity of catalase and non-specific peroxidase. The cultivar most resistant to the combined effects of stressors had the capacity of accumulating higher concentrations of proline.

As it was pointed out, in the field experiments on the stage of cotton flowering activities of catalase, SOD, POX as well as contents of proline under heat stress were found increased in the resistant cultivars, while contents of MDA and hydrogen peroxide were typically lower in them (Zafar *et al.,* 2023).

Yousaf et al. (2022) demonstrated significant positive correlation of seed cotton yield with activity of POX (r = 0.974) and SOD (r = 0.868) under heat stress field conditions by shift in sowing period. In several works, attempts to assess the association between heat resistance of cotton cultivars to high temperatures and accumulation of MDA as a main marker of oxidative stress have been made. Sarwar et al. (2023) demonstrated close correlation of MDA contents with the cell damage in plants grown in the hothouse under local terminal heat stress. At the same time, no inverse correlation between heat resistance and MDA accumulation could be seen in the control plants and those exposed to heat stress due to late sowing in the field (James *et al.,* 2005).

In our findings, cotton cultivars in the post-stress period showed high correlation between growth inhibition and accumulation of markers of oxidative damages, to name hydrogen peroxide and MDA. High negative correlation has been found between growth inhibition and increased catalase activity and proline contents in post-stress period (Figure 5), as well. Close inverse correlation between alterations in MDA concentrations and those in proline contents was registered. This indicates considerable contribution of the AOS components above to the protection of cotton seedlings from oxidative stress. It is of note, that the inverse correlation between contents of MDA and proline under stress can be seen not in all plant species. Thus, the wheat cultivars under heat stress demonstrated high direct correlation between the increased contents of MDA and proline (Kolupaev *et al.*, 2023b). Close direct correlation was demonstrated in the rapeseed plants exposed to toxic effect of copper (Kholodova *et al.,* 2018). The accumulation of proline in these plant species is possible to take place when there are the oxidative damage occurrences; this can be the basis for argument about contribution of proline to the antioxidant protection. Participation of proline in response to heat stress and, possibly, to other ones can be suggested to depend on the plant species peculiarities. This seems to increase the relevance of special study on the AOS behavior in plants of definite species under definite types of stress. Generally, status of the AOS components in plants of different genotypes can be a significant marker for their resistance to stresses of various natures. In particular, the association found between resistance of the wheat cultivars to the drought and heat stress and their capability to withstand damages under direct effect of oxidative stress agents, including hydrogen peroxide and iron sulfate (III) (Yastreb *et al.*, 2023). The association between the drought resistance of the rice cultivars and their resistance to methyl viologen, an active ingredient of herbicides causing oxidative damages was demonstrated, as well (Iseki *et al.*, 2014).

Our work presents the comparative estimation of the thermal stability in cotton seedlings of two botanical species and 6 cultivars obtained by various methods. Of note, under extreme conditions, Surkhan-103 cultivar (*G. barbadense* species) demonstrated rather high resistance to high temperature; low parameters of growth inhibition and accumulation of oxidative damage markers being the evidence for that (Tables 1, 2). As compared to others, Porlok-4, a cultivar with a phytochrome knocked out gene, demonstrated high resistance. Ravnak-2, a cultivar generated using marker-associated selection, was found to be rather resistant to high temperatures. It should be noted, two other cultivars generated using biotechnological methods, to name Porlok-1 and Ravnak-1 turned out less resistant to high temperatures. By this parameter, they conceded to the moderately resistant Bukhara-102 generated using classical selection.

CONCLUSIONS

The cotton seedlings can serve as a model for estimation of heat resistance of cultivars and study on contribution of components of stress-protective systems in manifestation of heat resistance as a feature. A direct correlation has been established between the accumulation of hydrogen peroxide, the LPO product of MDA, and the amount of growth inhibition of cotton cultivars after heat stress. An inverse relationship has also been established between the inhibition of plant growth caused by heat stress and the accumulation of proline, an increase in the activity of CAT and, partly, SOD in the period after stress. These parameters can be used in assessment of the selection cotton material by heat resistance.

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CHEMICAL COMPOSITION AND FIBROUS STRUCTURAL COMPONENTS OF CELL WALLS OF DRY LEAF MASS OF SUGAR BEET, FODDER BEET AND TABLE BEET

ABSTRACT

In 2018, field experiments were conducted at the Agricultural Institute - Shumen, Bulgaria to assess the feed value of leaf mass of standard cultivars of beetroot (the institute's gene pool). For this purpose, the chemical composition of dry leaf mass of Bulgarian cultivars of sugar beet, fodder beet and table beet and their pollinators were analyzed.

Data indicated that the cultivars with the highest content of crude protein in dry matter were: Vesi (272.2 g kg^{-1}) - from fodder beet, Diex (271.0 g kg^{-1}) - from sugar beet and Radost 1 (247.5 g kg^{-1}) – from table beet.

In the sugar beet cultivars, the leaf mass has the lowest content of acid-detergent fibers (from 25.7 to 50.8%), acid-detergent lignin (from 25.7 to 50.8%) and cellulose (from 10.9 to 14.3%), but with a higher concentration of neutral-detergent fibers (with from 15.1 to 15.4%) and hemicellulose (with from 54.0 to 74.0%) compared to that of fodder and table beets.

Regression models have been developed through significant dependence between some quality indicators of the plant cell, which can be used for indicative prediction of the traits.

Keywords: sugar beet, fodder beet, table beet, leaves, chemical composition

INTRODUCTION

One of the ways to solve the problem of balanced feeding of animals is the inclusion of fodder, sugar and semi-sugar beets in the daily ration (Tanosiunier and Noskaitit, 2000). High yields and concentrations of nutrients and vitamins, and their good palatability determine the use of fodder and semi-sugar beets for fodder and their inclusion in ruminant rations in the autumn-winter period (Badawi et al., 2002; Kikindonov, 2011; Enchev and Bozhanska, 2022).

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Sugar beet (*Beta vulgaris* L.) is a technical crop that is grown for the production of granulated sugar and sweet syrups. The high content of amino acids in its biomass is a prerequisite for its use as a raw material for animal husbandry (Vladimirov, 1973). Of the root crops, sugar beet has the highest content of feed units, with digestibility of 75%. In the processing of root crops, valuable fodder for animal husbandry are the leaves, beetroot and beet slices, and molasses. For industrial purposes, alcohol, citric, lactic and glycolic acid, glycerin, betaine, baker's yeast, yeast, etc. are obtained from molasses. (Uchkunov, 2008; Kikindonov and Kikindonov, 2012).

Fodder beet (*Beta vulgaris* var. crassa) is grown for the production of succulent fodder. Its inclusion in the ration of animals improves their balanced nutrition. Root crops are an easily digestible forage and are readily accepted by cattle, pigs, sheep and horses (Todorov et al., 1995). The dry matter in the roots of fodder beet is composed of nitrogen-free extractive substances, 1.3% protein, 0.1% oils, 0.9% cellulose and 0.9% ash substances, crude protein, crude fats and fibers, methionine, cystine, tryptophan, calcium, phosphorus, etc. (DAF, 1998).

Table beet is a traditional and popular vegetable in many parts of the world. It is consumed regularly as part of the normal diet, fresh, after heat treatment or fermentation. In production, it is used as a food coloring - E162 (Clifford et al., 2015; Sawicki and Wiczkowski 2018). It contains large amounts of biologically active substances, including betalains, carotenoids, phenols, B-vitamins (B1, B2, B3, B6 and B12), folate minerals, fiber, as well as low-energy sugars (Kale et al., 2018) and inorganic nitrate (Clifford et al., 2015). All parts of this plant have various medicinal uses, such as antioxidant, antidepressant, antimicrobial, antifungal, anti-inflammatory, diuretic, expectorant and carminative (Jasmitha et al., 2018), and hepatoprotective agents (Olumese and Oboh, 2018).

Beetroot leaves are underutilized due to a lack of sufficient knowledge, especially of their nutritional value, both for animal husbandry and as human food. Beet leaves are rich in total phenolic compounds, vitamins and iron (Kaushik and Kavita, 2020, Lorizola et al., 2018). They are defined as secondary products (waste) in the harvest period (Fernandez et al., 2017). The byproduct in sugar beet constitutes almost half of the whole plant (Bengardino et al., 2019; Pellegrini and Ponce 2020; Ebrahimi et al., 2022). Beetroot leaves are a rich source of bioactive compounds such as fatty acids, minerals (Biondo et al. 2014), proteins (Akyüz and Ersus 2021) and polyphenols (Nutter et al., 2020). Among these compounds, polyphenols are powerful substances that improve human health through their antibacterial, antifungal, anti-inflammatory and anti-tumor properties. These compounds are a group of secondary metabolites synthesized in plants that possess one or more phenolic rings with attached hydroxyl groups. They are considered natural antioxidants, improving food quality by delaying lipid oxidation (Ebrahimi and Lante 2021; Kolev, 2022).

Since these food by-products can be major sources for the recovery of bioactive compounds, many studies have proposed a strategy to valorize them using extraction techniques (Tinello and Lante 2019; Cisneros-Yupanqui et al., 2021; Lante et al., 2020).

The aim of the present study was to determine the chemical composition and the content of the structural fibrous components of the cell walls in the dry matter of the leaf mass of sugar, fodder and table beet cultivars.

MATERIAL AND METHODS

In 2018, in the experimental field of the Agricultural Institute - Shumen, Bulgaria (43.315152/N43°18'54.547 27.029729/E27°1'47.022). field experiments were carried out to assess the feed value of leaves from standard cultivars and their pollinators sugar beet, fodder beet and table beet. Within the framework of the research, the developed technologies for beet cultivation are being optimized with an emphasis on the leaf mass as raw material for fodder. Pollinators and their hybrids from the comparative trials were studied to establish the genotypic response.

The experimental set-up was by the method of long plots in 4 replications, with a harvest plot size of 8.4 m^2. The soil type is medium-strength, heavy-sandy, carbonate chernozem with a slightly alkaline reaction of the soil solution. Sowing was done manually at 70 cm row spacing (10.000 plants/da). The leaf samples were realized together with the harvesting of the root crops on 23.10.2018.

The experiment included determination of the chemical composition and energy value of the leaf mass of sugar, fodder and table beet cultivars and their pollinators (Table 1).

Table 1. Standard cultivars sugar beet, fodder beet, table beet and pollinators

Type	Standard cultivars	Pollinators
Sugar beet	*Diex - diploid hybrid on monocot basis *KOM - triploid hybrid	*M 985 - tetraploid pollinator of Kom variety *MS 142 - maternal component of Hybrid 56 and KOM *RPK-multiseeded diploid pollinator of Diex variety
Fodder beet	*Sasha - multi-seeded tetrafruit variety *Preslav - diploid multiseed synthetic population *Hybrid 56 – triploid semi-sugar hybrid *Vesi – semi-sweet, diploid hybrid	Sg x 142 – Tetragold Semi-Sugar Yellow Pollinator
Table beet	Radost 1-diploid fertile multi-seeded variety *Radost 3- diploid fertile multifertile population	

The main chemical composition of the dried leaf mass was analyzed, including the following indicators:

• Crude fiber (CFr, g kg^{-1} DM) according to the Weende analysis - the sample was treated sequentially with solutions of 1.25% (w/v) H_2SO_4 and 1.25% (w/v) NaOH under special conditions. The residue was dried and incinerated;

• Crude protein (CP, g kg^{-1} DM) according to Kjeldahl (according to BDS - ISO-5983)

• Crude fats (CF, g kg^{-1} DM) by extraction in an extractor of Soxhlet type (according to BDS - ISO-6492). After extraction, the sample was dried at 95°C;

• Ash (g kg^{-1} DM) - decomposition of organic matter by gradual combustion of the sample in a muffle furnace at 550°C (according to BDS - ISO-5984);

• Dry matter (DM, g kg^{-1}) - empirically calculated from % of moisture;

• Calcium (Ca, g kg^{-1} DM) - according to Schottz (complexometric);

• Phosphorus (P, g kg^{-1} DM) - with vanadate-molybdate reagent by the method of Guericke and Curmis and spectophotometer (Agilent 8453 UV - visible Spectroscopy System), measuring in the range of 425 ηm.

• NFE (%) = 100 - (CP, % + CFr, % + CF, % + Ash, % + Moisture, %) in g kg^{-1} DM.

The fibrous structural elements in the plant cell are analyzed in laboratory: Neutral Detergent Fibers (NDF, g kg^{-1} DM); Acid detergent fiber (ADF, g kg^{-1} DM) and Acid detergent lignin (ADL, g kg^{-1} DM) by the Van Soest and Robertson (1979) detergent assay and in vitro dry matter digestibility (IVDMD, g kg^{-1}) according to a two-way pepsin-cellulase method of Aufrere (1982). The polyosides are empirically calculated: Hemicellulose (g kg^{-1} DM) = NDF-ADF and Cellulose (g kg^{-1} DM) = ADF-ADL. The lignification degree is expressed as the percentage of ADL and NDF.

The experimental data were statistically processed by analysis of variance (ANOVA) and the program Statistica for Windows 10.

RESULTS AND DISCUSSION

Main chemical composition of dry matter of sugar beet, fodder beet and table beet cultivars

The nutritive value of *Beta vulgaris* L. result mainly from its high content of protein gathered both in leaf blades and in petioles, and from high content of mineral salts, mainly iron and calcium, as well as vitamins C, A, B1, B2 (Dzida et al., 2011). Dehydrated beet leaves contain high crude protein (26.4–31.0%) and carbohydrate (30.7–41.0%) (Biondo et al., 2014; Glencross, 2009). The chemical analysis of the dry leaf mass of fodder, sugar and table beets indicates that the cultivars with the highest crude protein content are: Vesi (272.2 g kg^{-1}) – from fodder beet, Diex (271.0 g kg^{-1}) – from sugar beet and Radost 1 (247.5 g kg^{-1}) - from table beet - Table 2.

Table 2. Main chemical composition of dry matter of sugar beet, fodder beet and table beet cultivars (g kg-1 DM)

Cultivars	CP	CFr	CF	Ash	БЕВ	Ca	P	N
Sugar beet								
M 985	251.9	110.8	24.8	178.0	324.2	21.6	5.9	35.9
РПК	250.1	118.4	28.1	194.6	306.2	19.2	5.6	35.9
КОМ	265.1	138.1	25.5	174.1	298.3	19.1	5.6	38.2
Diex	271.0	115.9	25.2	185.0	302.3	32.5	6.1	39.0
MC 142	226.0	102.5	29.2	185.7	351.6	23.7	6.3	32.4
Average	***252.8***	***117.1***	***26.6***	***183.5***	***316.5***	***23.2***	***5.9***	***36.3***
CV	***6.9***	***11.3***	***7.4***	***4.3***	***6.9***	***23.8***	***5.2***	***7.1***
SD	***17.4***	***13.2***	***2.0***	***7.9***	***22.0***	***5.5***	***0.3***	***2.6***
Fodder beet								
Preslav	271.3	106.2	25.2	181.8	313.6	23.6	6.0	39.0
Vesi	272.2	95.3	30.5	194.8	302.7	21.4	5.9	39.0
Tetragold	260.0	124.3	24.2	185.7	287.5	21.8	10.0	36.7
Sasha	239.3	123.0	23.2	222.6	283.1	21.5	6.6	34.1
Sg x 142	271.4	109.9	25.1	178.7	307.9	10.3	8.7	38.8
Hybrid 56	233.9	120.9	27.4	220.7	296.6	10.2	7.6	33.7
Average	***258.0***	***113.3***	***25.9***	***197.4***	***298.6***	***18.1***	***7.5***	***31.4***
CV	***6.7***	***10.1***	***10.2***	***9.9***	***4.0***	***34.0***	***21.9***	***44.0***
SD	***17.3***	***11.5***	***2.6***	***19.6***	***11.8***	***6.2***	***1.6***	***13.8***
Table beet								
Radost 1	247.5	128.1	25.6	223.5	283.3	18.9	8.4	36.0
Radost 3	216.5	132.7	22.2	209.7	323.5	14.6	7.5	31.3
Average	***232.0***	***130.4***	***23.9***	***216.6***	***303.4***	***16.8***	***8.0***	***33.7***
CV	***9.4***	***2.5***	***10.1***	***4.5***	***9.4***	***18.2***	***8.0***	***9.9***
SD	***21.9***	***3.3***	***2.4***	***9.8***	***28.4***	***3.0***	***0.6***	***3.3***

For the sugar beet cultivars, the difference in crude protein values was from 5.9 g kg^{-1} (KOM) to 45.0 g kg^{-1} (MC 142) relative to the maximum. Only the cultivars KOM and Diex registered a higher concentration of the indicator by 4.9% and 7.2%, respectively, compared to the average (252.8 g kg^{-1}). Cultivar KOM has the highest crude fiber content (138.1 g kg^{-1}) and the least amount of nitrogen-free extractives (298.3 g kg^{-1}). The values of these indicators are completely opposite for variety MC 142, where the fiber content (102.5 g kg^{-1}) in the composition of the dry leaf mass is the lowest, and the amount of BEV (351.6 g kg^{-1}) is the highest. In the sugar beet cultivars, the content of mineral substances and crude fat varied from 174.1 g kg^{-1} (KOM) to 194.6 g kg^{-1} (RPK) and from 24.8 g kg^{-1} (M 985) to 28.1 g kg^{-1} (RPK). The dry leaf mass of sugar beet cultivars is rich in the macroelement calcium. With the highest values of the indicator is the Diex cultivar (32.5 g kg^{-1}), and with the lowest - the KOM cultivar (19.1 g kg^{-1}). The amount of phosphorus and nitrogen varies from 5.6 g kg^{-1} (RPK and KOM) to 6.3 g kg^{-1} (MC 142) and from 32.4 g kg^{-1} (MC 142) to 39.0 g kg^{-1} (Diex).

The beetroot leaves showed significant levels of protein and lipids in all developmental stages (Biondo et al., 2014). In fodder beet cultivars, the difference in crude protein values was from 0.8 g kg^{-1} (Sg x 142) to 38.3 g kg^{-1} (Hybrid 56) compared to the maximum. The cultivars Vesi (by 5.5%), Sg x 142 (by 5.2%), Preslav (5.1%) and Tetragold (by 0.8%) have a higher content than the average value of the indicator (258.0 g kg^{-1}).

Carbohydrate is an important non-protein and energy source (Bansemer et al., 2016; Lee et al., 2017). The leaf mass of the cultivar with the highest protein content (Vesi) has the lowest crude fiber values (95.3 g kg^{-1}). For fodder beet cultivars, the content of non-nitrogen extractive and mineral substances in dry matter varies from 283.1 g kg^{-1} (Sasha) to 313.6 g kg^{-1} (Preslav) and from 178.7 g kg^{-1} (Sg x 142) to 222.6 g kg^{-1}(Sasha), respectively.

Mineral substances are of great importance for the nutrition of farm animals. The leaf mass of the Preslav cultivar has the highest concentration of calcium (23.6 g kg^{-1}). Nitrogen is a very important structural element in plants, which contain between 1 and 6% of nitrogen in dry mass (Dzida et al., 2012). In the Vesi and Preslav cultivars, the amount of nitrogen prevails by 24.2% compared to the average value of the indicator (31.4 g kg^{-1}). The dry matter of the cultivars: Tetragold (33.3%), Sg x 142 (16.0%) and Hybrid 56 (1.3%) has a higher phosphorus content than the average value (7.5 g kg^{-1}).

Beetroot leaves are underused due to lack of proper knowledge, specially of their nutritive value (Vilhena and Silva, 2007).

In table beets, the amount of the protein fraction in the composition of Radost 1 is higher by 6.7% compared to the average value of the indicator (323.0 g kg^{-1}) and by 14.3% compared to that of Radost 3 (216.5 g kg^{-1}). The leaf mass of the Radost has a higher crude fat content (by 15.3% compared to the Radost 3 cultivar and by 7.1% compared to the average value of the indicator), ash (by 6.6% compared to the Radost 3 cultivar and by 3.2% compared to the average value of indicator), calcium (by 29.5% compared to the Radost 3 cultivar and by 12.5% compared to the average value of the indicator), phosphorus (by 12.0% compared to the Radost 3 cultivar and by 5.0% compared to the average value of the indicator) and nitrogen (by 15.0% compared to cultivar Radost 3) and by 6.8% compared to the average value of the indicator), but with a lower content of raw fibers (by 3.6% compared to cultivar Radost 3 and by 1.8% compared to the average value of the indicator) and nitrogen-free extractive substances (by 14.2 % compared to cultivar Radost 3 and by 7.1% compared to the average value of the indicator).

Fibrous structural components of cell walls and in vitro digestibility of dry matter in sugar, fodder and table beet cultivars Beet leaves containing high fiber content (Fernandez et al., 2020). Research indicates that sugar beet leaf mass has the lowest content of acid-detergent fiber, acid-detergent lignin and cellulose, which positively affects dry matter digestibility (Table 3). The average values of these indicators are lower than those of fodder and table beets by 26.0-38.7% (for

ADF), 25.7-50.8% (for ADL) and 10.9-14.3% (for Cellulose), respectively and are similar to those found by Alhan and Can (2017).

Table 3. Fibrous structural components of cell walls and in vitro dry matter digestibility of sugar, fodder and table beet cultivars (g kg^{-1} DM)

Cultivars	NDF	ADF	ADL	Hemicellulose	Cellulose	IVDMD of Aufrere
Sugar beet						
M 985	406.0	142.2	97.5	263.9	44.6	783.8
РПК	348.4	111.5	80.7	236.9	30.8	810.6
КОМ	414.4	157.4	102.3	257.0	55.1	774.6
Diex	412.9	173.2	121.4	239.8	51.8	761.4
MC 142	400.4	126.9	72.3	273.6	54.5	799.8
Average	***396.4***	***142.2***	***94.8***	***254.2***	***47.4***	***786.0***
CV	***6.9***	***17.1***	***20.3***	***6.2***	***21.4***	***2.5***
SD	***27.4***	***24.3***	***19.2***	***15.7***	***10.2***	***19.6***
Fodder beet						
Preslav	337.2	204.1	95.2	133.1	108.8	741.4
Vesi	318.6	193.3	138.8	125.3	54.5	744.8
Tetragold	360.9	201.0	155.2	159.9	45.8	745.2
Sasha	335.7	171.4	136.4	164.3	35.0	764.5
Sg x 142	332.2	204.2	151.1	128.1	53.0	731.9
Hybrid 56	375.5	209.4	181.3	166.1	28.0	730.7
Average	***343.4***	***197.2***	***143.0***	***146.1***	***54.2***	***743.1***
CV	***6.1***	***7.0***	***19.8***	***13.2***	***52.9***	***1.6***
SD	***20.9***	***13.7***	***28.4***	***19.2***	***28.7***	***12.2***
Table beet						
Radost 1	385.4	148.6	81.5	236.8	67.1	782.3
Radost 3	303.1	209.8	156.9	93.3	53.0	731.6
Average	***344.3***	***179.2***	***119.2***	***165.1***	***60.1***	***757.0***
CV	***16.9***	***24.1***	***44.7***	***61.5***	***16.6***	***4.7***
SD	***58.2***	***43.3***	***53.3***	***101.5***	***10.0***	***35.9***

The results obtained regarding the average amount of neutral detergent fibers and hemicellulose are completely opposite. The values of these indicators are higher (by 15.1-15.4% for NDF and by 54.0-74.0% for Hemicellulose) in the dry mass composition of sugar beet cultivars compared to fodder and table beet cultivars.

In the sugar beet cultivars, the excess in the values of neutral-detergent fibers compared to the average for the species (396.4 g kg^{-1}) is from 1.0% (MS 142 - line) to 4.5% (KOM).

The sugar beet leaves which are rich in WSC can lead to a reduction in cell wall constituents, particularly cellulose and hemicellulose and to a lower degree lignin (Larsena et al., 2017). The amount of ligno-cellulose complex (ADF) is a determining factor for dry matter digestibility. This amount is lower compared to the average value of the indicator (142.2 g kg^{-1}) for the cultivars MS 142 - line and RPK (by 12.1-27.5%) and higher for the cultivars KOM and Diex (by 10.7-21.8%). Similar are the results regarding the concentration of acid-detergent lignin. In the composition of the sugar beet cultivars, the values of hemicellulose and cellulose polyosides range from 236.9 g kg^{-1} (RPK) to 273.6 g kg^{-1} (MS 142) and from 30.8 g kg^{-1} (RPK) to 55.1 g kg^{-1} (KOM), respectively. With the highest in vitro digestibility of dry matter is the RPK cultivar (810.6 g kg^{-1}) followed by the MC 142 cultivar (799.8 g kg^{-1}). The values of the indicator exceed the average for the species by 3.1% and 1.4%, respectively.

In fodder beet, the leaf mass of the cultivars Tetragold and Hybrid 56 has a higher content of neutral-detergent fibers by 5.1% and 9.3%, respectively, compared to the average for the species (343.4 g kg^{-1}). The values are close to those established by Özkan et al., (2017). Acid-detergent fibers prevail in the dry matter of the cultivars Tetragold, Preslav, Sg x 142 and Hybrid 56 with from 1.9% to 6.2% compared to the average value (197.2 g kg^{-1}) of the indicator. The Preslav cultivar has the lowest concentration of acid-detergent lignin (95.2 g kg^{-1} with an average value of 143.0 g kg^{-1}) and the highest (almost twice compared to the other cultivars of the species) of the cellulose that is not completely digestible by animals (108.8 g kg^{-1}). The differences in ADF end NDF contents of leaves are likely associated with harvesting stage of plant. The ADF content increased with advancing maturity (Kaplan et al., 2014). The hemicellulose values of fodder beet cultivars ranged from 125.3 g kg^{-1} (Vesi) to 166.1 g kg^{-1} (Hybrid 56) with an average of 146.1 g kg^{-1}. With the highest in vitro digestibility of dry matter is the cultivar Sasha (764.5 g kg^{-1}) followed by the cultivar Tetragold (745.2 g kg^{-1}) and the cultivar Vesi (744.8 g kg^{-1}).

The neutral-detergent fiber content of the table beet cultivars varied from 303.1 to 385.4 g kg^{-1}. A significant difference in the values of acid-detergent fiber, acid-detergent lignin and hemicellulose was observed in the species. In the dry matter of the cultivar Radost 1, the concentration of KDF and KDL is respectively 41.1% and 92.5% lower compared to Radost 3, and the content of hemicellulose (a polyoside fully digestible by ruminants) is 153.8% higher. The digestibility of the leaf mass in the cultivar Radost 1 is higher by 3.3% and 6.9%, respectively, compared to the average value of the species and compared to the digestibility of Radost 3.

The leaf mass of fodder beet has the highest degree of lignification (average value of the indicator – 41.54) compared to that of sugar (average value of the indicator – 23.87) and table (average value of the indicator – 36.46) beet – Fig. 1.

Only the leaf mass of the cultivars Preslav (coefficient – 28.25) and Sasha (coefficient – 40.63) registered a lower degree of lignification compared to the average for the species. In sugar beet cultivars, the degree of lignification is characterized by coefficients from 18.07 (MS 142 - line) to 29.39 (Diex), and in table beet with a coefficient of 21.16 - for the Radost 1 cultivar and a coefficient of 51.75 - Radost 3.

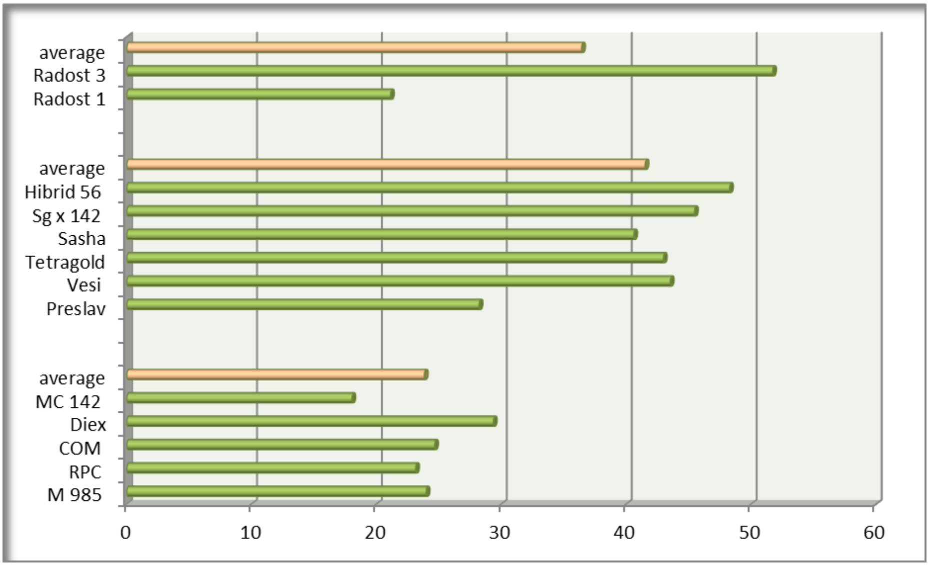

Figure 1. Degree of lignification of dry leaf mass of sugar, fodder and table beet cultivars (coefficient)

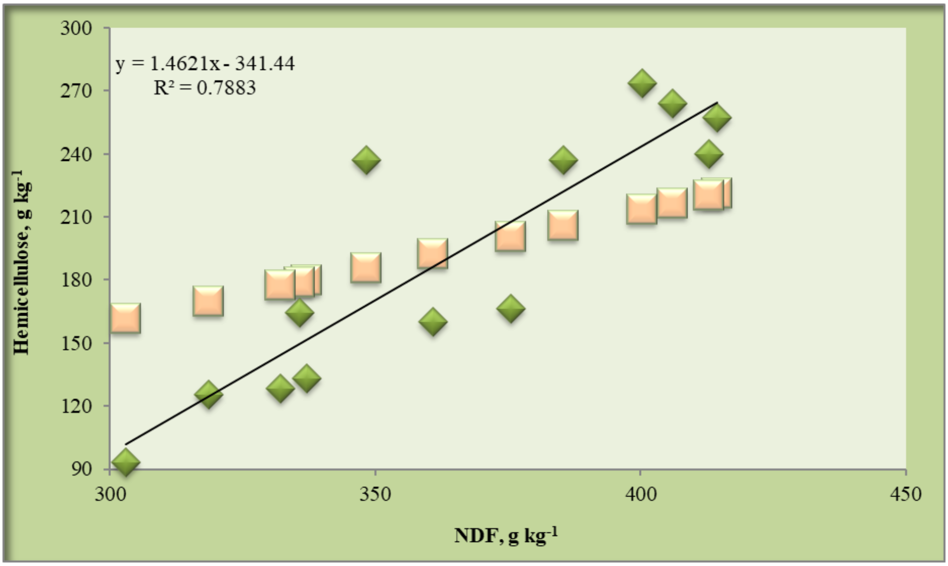

Figure 2. Regression relationship between the content of neutral detergent fibers and the amount of hemicellulose in the dry matter of sugar, fodder and table beet cultivars

Given the chemical and biochemical analyses, and the graphic regression models developed on this basis, it is possible to determine with relatively high accuracy, the variation of some indicators determining the quality and nutritional value of the dry leaf mass of the studied beet types.

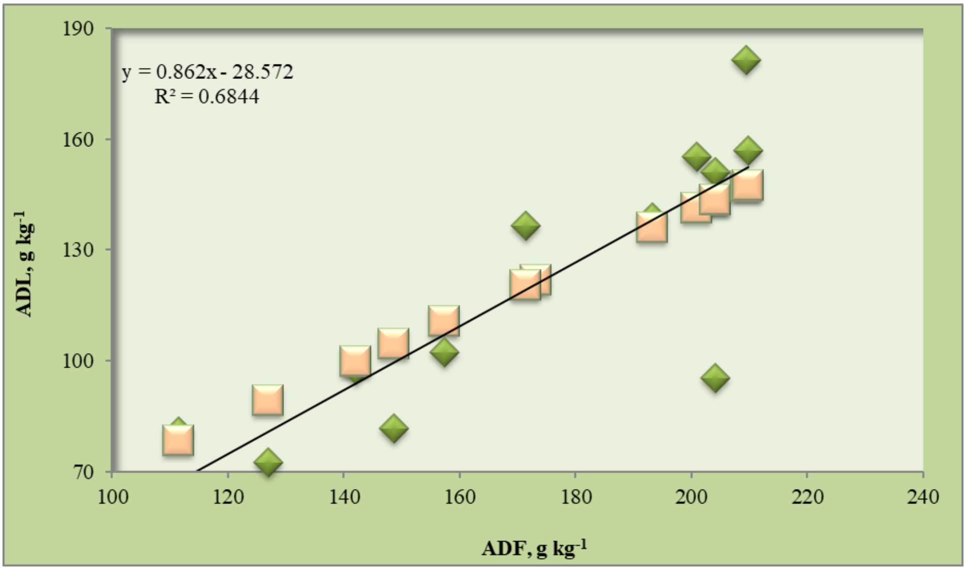

Figure 3. Regression relationship between the content of acid-detergent fibers and acid-detergent lignin in the dry matter of sugar, fodder and table beet cultivars

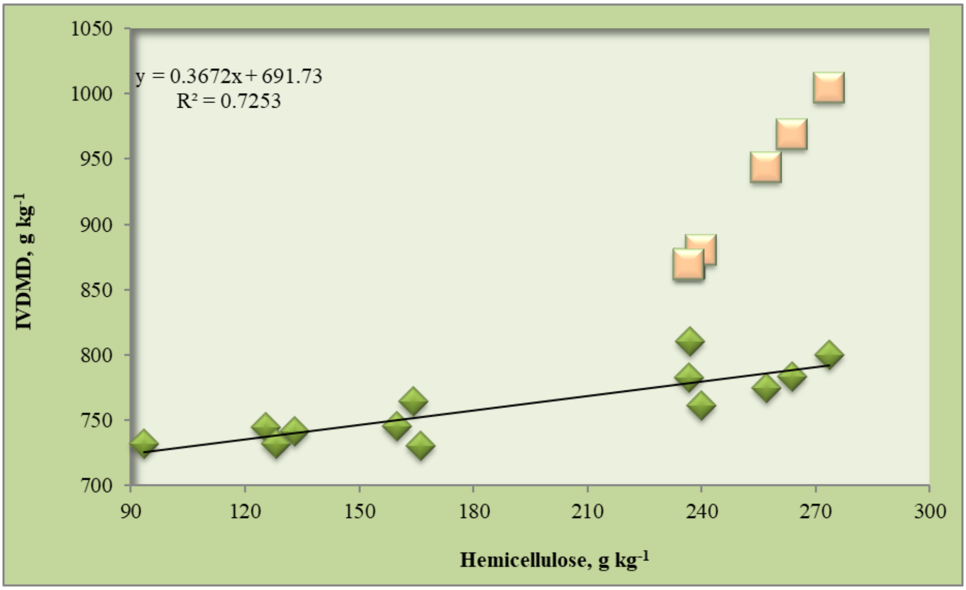

Figure 4. Regression relationship between hemicellulose content in dry matter and in vitro dry leaf mass in sugar, fodder and table beet cultivars

A regression relationship was established between the content of neutral-detergent fibers and the amount of hemicellulose in the sugar, fodder and table beet cultivars. The resulting equation has a high coefficient of determination $R^2 = 0.7883$ proven statistically at $P < 0.05$ – Figure 2.

The equation by which it is possible to predict the amount of acid-detergent lignin by acid-detergent fibers is: y = 0.862x – 28.572 with a coefficient of determination – R^2 = 0.6844 (with significance level P <0.05) - Figure 3.

Hemicellulose as a structural component of the plant cell is found in a significant relationship with the in vitro digestibility of the dry leaf mass (Figure 4). The established regression equation (y = 0.3672x + 691.73 with a high coefficient of determination R^2 = 0.7253) can be used to tentatively predict the digestibility of the investigated sugar, fodder and table beet cultivars.

CONCLUSIONS

- It was found that the cultivars with the highest content of crude protein in the dry matter are: Vesi (272.2 g kg^{-1}) - from fodder beet, Diex (271.0 g kg^{-1}) - from sugar beet and Radost 1 (247.5 g kg^{-1}) – from table beets.
- The leaf mass of the sugar beet cultivars has the lowest content of acid-detergent fibers (from 25.7 to 50.8%), acid-detergent lignin (from 25.7 to 50.8%) and cellulose (from 10.9 to 14.3%), but with a higher concentration of neutral-detergent fibers (with from 15.1 to 15.4%) and hemicellulose (with from 54.0 to 74.0%) compared to that of fodder and table beets.
- Regression equations have been developed as an expression of the regression dependence between the fibrous structural components in the plant cell and can be used for indicative prediction of the traits.

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ASSESSMENT OF LAND USE CHANGE IMPACTS ON LAND CAPABILITY IN NGADIROJO, INDONESIA

SUMMARY

Since the pro-investment program was implemented in 2011, Wonogiri District including Ngadirojo Sub-district has experienced a shift in the economic sector from agriculture to industry, and land conversion has occurred. This research aims to determine the rate of land conversion in the Ngadirojo Sub-district and its effect on land capability class. The rate of conversion is determined based on the shrinkage of land use area from 2009 to 2020. The land unit for observing the land capability class is determined by overlaying the land use change map with the soil type map. Observation of land capability class is carried out on agricultural land that is experiencing conversion. As comparison data, land capability classes were observed at points that did not experience conversion. Land use change in the Ngadirojo Sub-district in 2009-2020 covering an area of 1,308.53 ha (14.03%) with a rate of conversion of agricultural land to non-agricultural areas of 264 ha (24 ha year^{-1}). Changes in land use have a very significant effect on the land capability class. Land use causes soil permeability to be slower and degrades land capability. The recommended land management efforts in the research area are the addition of organic matter to soil properties and agroforestry system implementation.

Keywords: Agricultural land, Land capability, Land conversion, Land management, Limiting factor

INTRODUCTION

Population growth and increasing economic development have led to an increase in land demand. The availability of land that is fixed but needed for all sectors causes land conversion, especially on agricultural land (Aini *et al.* 2019; Widhiyastuti *et al.* 2023). Developmental factors, either economic or demographic, have an impact on the conversion of agricultural land (Egidi *et al.*

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2020; Kumar and Agrawal 2019; Kocur-bera and Pszenny 2020). Development factors and agricultural land tend to work in opposite directions (Palšov, 2019).

Wonogiri District is one of the areas facing the problem of land conversion. The pro-investment policy since 2011 has made it easier for investors to build a business (Nugroho *et al.* 2020). Since then, there has been a shift in the economic sector, which was originally based on agriculture to become industrial. The influx of investors recruits a lot of labor but also causes land use conversion (Palšov, 2019). The construction of industrial factories causes the conversion of productive agricultural land. This will result in a reallocation of land use from less profitable activities to more profitable activities. Activities that are always threatened are agricultural activities that are considered less profitable than other economic activities (Catur and Joko 2010).

According to Dellamitha *et al.* (2018), Ngadirojo Sub-district is predicted to have the largest regional growth rate in Wonogiri District. Regional growth of the area means an increase in built-up land for residential and industrial purposes. This causes the conversion of agricultural land into settlements is also getting higher. The conversion of agricultural land to non-agriculture reduces the amount of productive land (Wardhana *et al.* 2018). The conversion of agricultural land is of particular concern because it threatens farmers to lose their land (Stanny *et al.* 2021). Changes in land use, especially in paddy fieldss that have high productivity, will have a negative impact on food availability and environmental quality (Nurliani and Rosada 2016; Wahyuti *et al*. 2023).

Land use change also includes changes in the use of agricultural land into other agricultural lands, for example, changes in paddy fields into moor or vice versa. Land use change does not only affect the decrease in land area but also changes the characteristics of land and soil (Rendana *et al.* 2022; Wardhana *et al.* 2018). (Mujiyo *et al.* 2018) mentions changes in land use causing changes in soil physical properties. Fields that turn into paddy fields experience changes in the permeability aspect, which is slower. Changes in the characteristics of land and soil will change the land carrying capacity. Land carrying capacity to be used for certain uses without causing permanent damage is the definition of land capability (FAO, 1983; Wells, 2001).

Considering that land use change affects several aspects, it is necessary to evaluate, observe, and resolve the problem of conversion that occurs (Riao *et al.* 2020). Efforts that can be made to suppress the conversion of agricultural land are by periodic identification and mapping (Mujiyo *et al.* 2008). Until now, data and information regarding land use change in Ngadirojo Sub-district are not yet available, so there is a need for an inventory of land use change data. The purpose of the study was to determine the land conversion rate and its effect on land capability, and to determine the factors causing a land conversion.

MATERIAL AND METHODS

This research was conducted from November 2020 - February 2021. Sampling and field observations were carried out in Ngadirojo Sub-district, Wonogiri District. The area of Ngadirojo Sub-district is 9,325.56 ha with dense hilly and mountainous areas, as well as inundation areas of the Waduk Serba Guna Gajah Mungkur. The largest land use is dry land which covers 50% of the total land area followed by paddy fields (26%), buildings and yards (20%), and other uses (3%) (BPS Kabupaten Wonogiri, 2018). The soil types are Alfisols which dominate on the north side and Inceptisols are on the south side.

Land use change is determined by overlaying land use maps in 2009 and 2020. The land use maps are the result of the interpretation of Google Earth images. The resulting land use change map is then inventoried for its area, type, location, and rate of land use change. The rate of land conversion is determined by calculating the rate of shrinkage of agricultural land into non-agricultural land. The total area in 2020 (L_t) is subtracted by the land area in 2009 (L_{t0}) then divided by the period 2009 (T_0) to 2020 (T). This equation produces the average rate of land conversion per year.

$$V = \frac{(Lt - L_{t0})}{(T - T_0)}$$

Remarks: V = land conversion rate (ha year^{-1}), L_t = land area in 2020 (ha), L_{t0} = land area in 2009 (ha), T = Year 2020, T_0 = Year 2009

Land capability class is observed on agricultural land converted into other agricultural lands. An observation made using the descriptive exploratory survey method. This method is separated into three stages: (1) pre-survey, (2) surveys, and (3) post-survey. The first stage is pre-survey, which consists of mapping the research region by constructing a working map from an overlay of thematic maps (area administration map, soil type map, and land use map) and defining the distribution of sampling locations. The survey stage is then the most important stage in the investigation. Land verification and soil samples are taken at this step. The land verification stage is required to determine whether the condition of the land in the field (actual conditions) matches what is on the map and whether the condition of the land allows it to be used as a sample point in terms of land characteristics. Farmers and local stakeholders provide detailed verification, such as evaluating soil type and land use information. Soil samples are collected after confirming the compatibility of the conditions on the map and in the field. Soil samples were gathered at various depths inside the tillage layer (1-20 cm). The final stage is post-survey, in which sample preparation and parameter analysis are performed in the laboratory.

The land unit (Figure 3) for observing the land capability class is determined by overlaying the land use change map (Figure 2) with the soil type map. Determination of sampling points was carried out purposively on agricultural land that experienced conversion during 2009-2020. As a comparison data, observations and soil samples were taken at the nearest point that did not experience any transfer of function. Land capability class classification is carried out by matching land characteristic data with land capability criteria according to (Arsyad, 2010), (Table 1). T-test analysis was conducted to determine the effect of land conversion and soil type on land capability class.

Table 1. Table of Land Capability Classification Criteria

Inhibiting Factors / Limiting	Land Capability Class							
	I	II	III	IV	V	VI	VII	VIII
Surface Slope	I_0	I_1	I_2	I_3	I_0	I_4	I_5	I_6
Erosion Sensitivity	KE_1 KE_2	KE_3	KE_4 KE_5	KE_6	(*)	(*)	(*)	(*)
Erosion Rate	e_0	e_1	e_2	e_3	(**)	e_4	e_5	(*)
Soil Depth	k_0	k_1	k_2	k_3	(*)	(*)	(*)	(*)
Upper Layer Texture	t_1,t_2,t_3	t_1,t_2,t_3	t_1,t_2,t_3,t_4	t_1,t_2,t_3,t_4	(*)	t_1,t_2,t_3,t_4	t_1,t_2,t_3,t_4	t_5
Lower Layer Texture	t_1,t_2,t_3	t_1,t_2,t_3	t_1,t_2,t_3,t_4	t_1,t_2,t_3,t_4	(*)	t_1,t_2,t_3,t_4	t_1,t_2,t_3,t_4	t_5
Permeability	P_2,P_3	P_2,P_3	P_2,P_3	P_2,P_3	P_1	(**)	(**)	P_5
Drainage	d_1	d_2	d_3	d4	d_5	(**)	(**)	d_0
Gravel/Rock	b_0	b_0	b_1	b_2	b_3	(*)	(**)	b_4
Flood Threat	O_0	O_1	O_2	O_3	O_4	(* *)	(**)	(*)

Remark: (*)= can have any properties, (**)= not applicable, (***)= generally found in dry climates

RESULTS AND DISCUSSION

Land Use Change

Land use change in Ngadirojo Sub-district in 2009-2020 covers an area of 1,308.53 ha (14.03%) and the fixed land area is 8,017.03 ha (85.97%).

Table 2. Changes in Land Use Areas in Ngadirojo Sub-district in 2009-2020

Types of Land Use	Total Area (ha)		Changes (ha)
	2009	2020	
River	33.20	32.93	-0.27
Buildings	2,155.55	2,335, 20	179.65
Dry Land	4563.24	4822.17	258.93
Forest	193.92	201.99	8.07
Open Land and Shrubs	21.75	53.50	31.75
Paddy fields	2357.90	1879.77	-478.13

The results showed that paddy fields were reduced the most (478.13 ha) among other land uses (Table 2). Many paddy fields are converted into moor. Based on the survey, the relatively easier management of dry land is one of the factors in the conversion of paddy fields to moor. Shortage of water is also one of the factors causing land conversion.

Wonogiri District's spatial planning section public housing service revealed that, generally, agricultural land conversion occurs because the certificate owner feels he has full rights to the land that will be converted for personal use without considering the regional spatial planning plan. On average, landowners have a low level of formal education. They are easily tempted by offers of high land purchase prices so that much of the land is ultimately sold to other parties to build housing, factories, and other infrastructure. Weak government policies regulating land conversion without sanctions and a lack of strict assistance to agricultural land owners are other factors in many agricultural land conversions. Local people who sold their land expressed regret because they realized that land is a non-renewable resource that can be an investment for the next generation of children and grandchildren. Dewi and Rudiarto (2013), revealed that people who were previously farmers will experience changes in socio-economic conditions because loss of agricultural land causes a decrease in income. The preventive step taken by the government as a policy maker is to provide outreach and education to the community regarding the risks and negative impacts of changing the function of agricultural land.

The cost of providing water for paddy fields becomes more expensive, so farmers choose to convert their land into dry land (Al Viandari *et al.* 2022). Landowners convert their land to expect more profits (Sianipar, 2016). Aini *et al.* (2019) stated that three factors influence the conversion of agricultural land, which is more common in paddy fields. First, the development of housing, shops, offices, and industrial areas is easier to do on flatter paddy fields. Second, past developments were more focused on efforts to increase rice production, so that more economic infrastructure was available in paddy fields. Third, paddy fields are generally closer to consumer areas or urban areas which are relatively densely populated. However, practical implementation of the Wonogiri Regency Local Regulation No. 2 of 2020 concerning the spatial planning of Wonogiri Regency from 2020 to 2040 lacks actions towards those shifting land from agriculture to non-agricultural purposes, insufficient support to encourage landowners to maintain their agricultural plots, and a lack of surveillance in ensuring the preservation of sustainable farming lands, especially for food crops. These factors make landowners susceptible to high offers for their land, leading to extensive sales for housing, industrial, and infrastructure development. The certificate owners often feel entitled to convert land for personal use without considering the regional spatial planning. Another factors besides economy is social factor. In terms of social factors and infrastructure, the most common occurrence in society is the need for residential and business spaces, leading to the conversion of agricultural land to non-agricultural use. The proximity of agricultural land to public access and transportation also contributes to its conversion. According to Hendrawan and Dewi (2016), there are three fundamental constraints in implementing land use conversion: policy coordination, policy implementation, and planning consistency.

The area of the dry land has increased by 258.93 ha. The dry land is used to cultivate corn, cassava, and peanuts. Rivers and water bodies experienced a decrease in the area of 0.27 ha. Although the area of the river is reduced, its use is still intended as a river and is not cultivated as agricultural land. In 2020 the river on the southeast side becomes less clear and sedimentation is visible. Sediment is part of the dynamics of the natural balance of the river if its presence is within a certain limit. However, if its presence is excessive, it can impact the characteristics and cause various problems in the environment and human life (Hambali and Apriyanti 2016).

Forest, open land and shrubs, buildings and infrastructure have increased in area from 2009. Forests increased by 8.07 ha. Open land and shrubs increased by 31.75 ha. Unproductive and unutilized dry land, paddy fields, and forests have turned into open land and shrubs. Open land and shrubs can be used to expand agricultural land to compensate for the conversion of functions provided that the land has potential and meets its biophysical criteria (Hidayat, 2009).

The use of building land and infrastructure which includes residential areas, business premises such as factories, offices, and buildings increased by 179.65 ha. The increase is due to the increasing need for residents both for housing and business places. In line with (Janah *et al.* 2017) that in general, the rate of land use change is associated with the rate of population growth which then increases the need for land use such as settlements and other public facilities. The construction of a factory in Ngadirojo Sub-district which has been rampant since 2014 has also caused an increase in the area of conversion of agricultural land into buildings.

Various agricultural land uses changed in the period 2009-2020 (Figure 1). Agricultural land that experienced the most conversion converted to non-agricultural land is dry land into buildings and infrastructure (15.83 ha year^{-1}) (Table 3). The characteristics of dry land that are never flooded are ideal for use as buildings (Romadhon and Aziz 2022). At a high rate of population growth, development level is one of the factors causing land use change (Iskandar *et al.* 2016).

(A) Paddy field-Moor, Alfisols (2009) (B) Paddy field-Moor, Alfisols (2020)

(C) Moor-Paddy field, Alfisols (2009) (D) Moor-Paddy field, Alfisols (2020)


(E) Moor-Forest, Inceptisols (2009)


(F) Moor-Forest, Inceptisols (2020)


(G) Forest-Open land and shrubs, Inceptisols (2009)


(H) Forest-Open land and shrubs, Inceptisols (2020)


(I) Paddy fields-Moor, Inceptisols (2009)


(J) Paddy fields-Moor, Inceptisols (2020)


(K) Moor-Open land and shrubs, Inceptisols (2009)


(L) Moor-Open land and shrubs, Inceptisols (2020)

Figure 1. Land Use Change Areas in Ngadirojo District.

Table 3. Agricultural Land Use Change Rate

Land Use Change	Area (ha)	Rate (ha year^{-1})	Percentage (%)
Paddy fields-Open and Shrub	2.21	0.20	0, 02
Dry land-Open Land and Shrub	12.18	1.11	0.13
Forest-Open Land and Shrub	21.55	1.96	0.24
Paddy fields-Building and Infrastructure	53.95	4.90	0.59
Dry land-Building and Infrastructure	174.11	15.83	1.90
Total	264.00	24.00	2.88

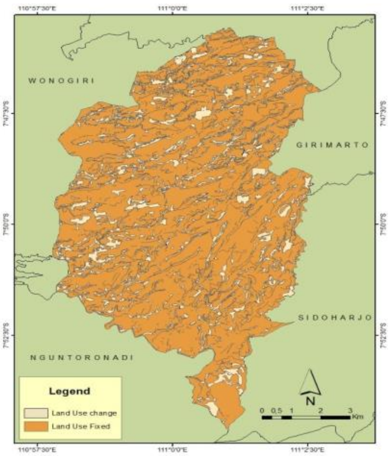

Figure 2. Map of Agricultural Land Use Change in Ngadirojo Sub-district

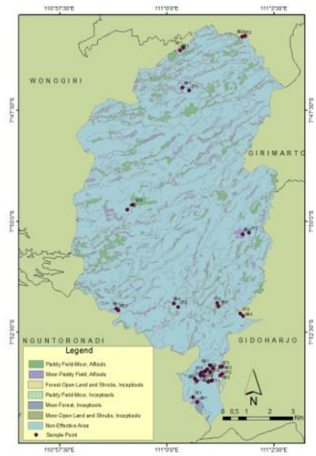

Figure 3. Land Unit Observation of land capability

The rate of conversion of paddy fields into buildings is 4.90 ha year^{-1} with a total conversion area of 53.95 ha. Forests into open land and shrubs is 1.96 ha year^{-1} with a converted area of 21.55 ha, moor into open land and shrubs of 1.11 ha year^{-1}, and paddy fields into open land and shrubs of 0.20 ha year^{-1}. The rate of conversion of agricultural to non-agricultural functions is mostly buildings and infrastructure such as industrial areas, buildings, and offices. According to (Kusumastuti *et al.* 2018), people prefer to convert the land into shops, housing, or other service sectors that generate greater profits when compared to agriculture.

The implementation of pro-investment in Wonogiri District, one of which is in Ngadirojo Sub-district, makes land demand increase and makes the conversion of agricultural land to non-agricultural land unavoidable. Land use change will increase every year because it is generally contagious. Land use change that occurs in one location will affect the surrounding locations (Nofita *et* *al.* 2016). A holistic and comprehensive strategy is needed to control the rate of land conversion (Kaputra, 2013).

Land Capability

Land capability classification is the determination of land potential to be used both as agricultural and non-agricultural land (Amelia *et al.* 2021). Land capability classification is based on the chemical and physical properties of the soil (Scopesi *et al.* 2020). Observation of land capability was carried out on six land units as a result of overlaying land use conversion maps.

Table 4. Land Capability in Land Use Change Areas

LU	Land Use	Capability of	
		Fixed Land	Changes Land
1	Paddy field-Moor, Alfisols	IV-d_4	IV-d_4
2	Moor-Paddy field, Alfisols	IV-d_4	VP_1
3	Moor-Forest, Inceptisols	IV-I_3,d_4,b_2	VP_1
4	Forest-Open land and shrubs, Inceptisols	IV-I_3,d_4,b_2	VP_1
5	Paddy fields-Moor, Inceptisols	IV-I_3,d_4	VP_1
6	Moor-Open land and shrubs, Inceptisols	VP_1	IV-I_3,d_4

Land capability at all points includes class IV-V. According to Feudis *et al.* (2021) land classes I-IV are suitable for agricultural land development, while classes V-VIII are not suitable for agricultural land but for forest areas, protected areas, and grasslands. Overall, changes in land use in Ngadirojo Sub-district cause changes in land capability class. The results of the T-test prove that on fixed land and changed land there are significant differences in land capability class ($t = 5.464$, $P = 0.025$). Land characteristics and ability class in LU 1 remain the same. Changes in land capability class occur at LU 2, 3, 4, 5, and 6.

Changes in land capability class are caused by changes in land characteristics. At LU 2, 3, 4, and 5, changes in land use cause soil permeability to be slower. Permeability value for each land use is highly correlated with land capability ($P = <0.05$). The change of dry land to paddy fields at LU 2 causes the permeability to be slower. The puddling of paddy fields destroys soil structure, resulting in a dense plow pan (Keen *et al.* 2013), increasing bulk density and ultimately causing slow permeability (Verma and Dewangan 2006). The change of forest into open land/shrub in LU 4 also causes slower permeability. Forest change causes a decrease in organic matter input, thereby increasing bulk density (Li *et al.* 2021) and slowing permeability.

Due to the conversion of the land function, LU 6 which is dry land that becomes open land and shrubs, has a better ability from class V to class IV. Soil characteristics that increase at LU 6 include erosion sensitivity and permeability, and both have a very significant correlation ($P = <0.05$) to the land capability class.

Table 5. Land Characteristics at Land Unit Observation

Sampling Point	Slope (%)	Erosion Sensitivity	Erosion Rate	Depth (cm)	Upper Layer Texture	Lower Layer Texture	Permeability (cm $hour^{-1}$)	Drainage	Rock/ Gravel	Flood	Soil Capability
1T	9.17	0.15	Low	77.33	Silty Clay	Silty Clay	1.36	Bad	Slightly	Sometimes	IV-d_4
1B	8.33	0.17	Low	67.67	Silty Clay	Clay	0.72	Bad	Slightly	Sometimes	IV-d_4
2T	6.67	0.17	Low	79.33	Silty Clay	Silty Clay	0.54	Bad	None	Sometimes	IV-d_4
2B	4.73	0.24	Low	74.67	Silty Clay	Clay	0.42	Rather Bad	None	Sometimes	VP_1
3T	19.27	0.24	Low	42.33	Silty Clay	Silty Clay	0.87	Bad	Medium	Never	IV-I_3,d4,b_2
3B	20.67	0.16	Low	35,00	Clay	Clay	0.27	Bad	Medium	Never	VP_1
4T	30.00	0.20	Low	57.67	Clay	Clay	0.63	Bad	Medium	Never	IV-I_3,d4, b_2
4B	30.00	0.18	Low	55.33	Clay	Clay	0.41	Bad	Medium	Never	VP_1
5T	15.33	0.27	Low	70.33	Clay	Clay	0.73	Bad	Slightly	Sometimes	IV- I_3,d_4
5B	24.33	0.35	Low	76.00	Silty Clay	Clay	0.26	Bad	Medium	Sometimes	VP_1
6T	28.33	0,18	Low	65.00	Silty Clay	Clay	0.28	Bad	None	Never	VP_1
6B	25.00	0.15	Low	49.67	Silty Clay	Silty Clay	1.53	Bad	None	Never	IV-I_3,d_4

Soil physical characteristics influence each other, especially soil permeability is directly influenced by soil pore size, porosity percentage, and soil texture composition, where these properties contribute to the water flow process (Abdullah *et al.* 2021). Some of the physical properties of the soil, including the structure, density, and porosity of the soil are formed from the accumulation of organic matter. Soils with low SOC content tend to be dense and have low water absorption capabilities. The results of the study (Sánchez–González *et al.* 2017) proved that dry land has a lower SOC content of up to 50% compared to the SOC content in soil under natural stands (natural forests and shrubs). This is caused by the content of SOC which in intensive agricultural land is also widely used for crops and is limited to returns/inputs to the soil so that it decreases continuously. Land conversion clearly has an impact on the physical condition of the soil.

In addition to changes in land use, the basic nature of each soil type will also affect the physical, chemical, and biological characteristics of the soil. Changes in land management have different impacts on changes in soil physical properties based on soil characteristics for each type of land use (Wardhana *et al.* 2018). The results showed that the soil type on the research land that was fixed and underwent a change of a function with different types had a very significant effect on land capability (F = 7.556, P = 0.010). From Table 5, it can be seen that the conversion of paddy fields to moor on the Alfisols soil type (LU 1) did not result in a difference in land capability, while the land use change while the land with the same conversion function on the Inceptisols soil type (LU 5) changed the land capability, namely from class IV to class V where for the ability of land V is not suitable to be used as agricultural production land including moor. Focusing solely on soil characteristics in assessments is preferred for their direct impact and specificity, simplifying analysis and enabling targeted evaluations. Soil types crucially influence fertility, water retention, and nutrient content, vital for determining land suitability in agriculture. Soil heterogeneity within an area affects its capability for diverse land uses, making consideration of soil types vital for sustainable agricultural production assessments (Ljuša et al, 2016; Zahra et al, 2023; Dingil et al, 2010). Alfisols, common in moderate to high rainfall areas, vary in land capability, impacting soil-land interactions and crop suitability. With clay-rich subsurface and high organic matter, they are suitable for a variety of crops but require specific management for optimal yields (Salari, M., & Baghernejad, 2014). Inceptisols present in similar rainfall regions have sandy subsurface that inhibits root growth and nutrient transport. This leads to lower fertility and limited crop suitability compared to other soils (Supriya et al, 2018).

Land Management Strategies

Results of land capability can be used to allocate areas of agricultural land sustainably as a basis for consideration of land use planning and policies (Bhermana *et al.* 2021). Land use capability classification is a fundamental argument that shows the suitability of agricultural production from the land. Improperly managed land will cause land degradation. Land degradation is a major threat to natural resources (Özcan, 2021). Thus, it is necessary to pay attention to the management of land that is undergoing conversion to avoid a decrease in land capability and land productivity for agriculture.

In LU 3, and LU 4 the land has been converted and has decreased land capability class, even though the type of land use with land capability class is appropriate, so it is hoped that land use can be maintained as land with natural vegetation. Land with capability classes II, III, and IV can be designated as arable land for agriculture (Amelia *et al.* 2021). Meanwhile, on land resulting from conversion (land change) LU 1, LU 2, and LU 5, the land use is not in accordance with the ability of the land, and the future impact is the emergence of serious problems, both in terms of productivity of agricultural products on the land, as well as damage to the land that has been damaged. make land critical. Inappropriate use is that LU 1, LU 2, and LU 5 are currently used as agricultural production land for upland and paddy fields, while land capability class V is not suitable for agricultural production land. Land in class V has limiting factors that inhibit plant growth so that its allocation is for forests, grasslands, and pastures (USDA, 1961). In overcoming this, land management efforts that need to be carried out in LU 1, LU 2, and LU 5 are the addition of organic matter, during land preparation and after harvest, and during rest periods. Organic materials that can be added include cow and chicken manure because they are easy to obtain, considering that most local farmers also keep cows and chickens. Organic matter in the soil can improve soil physical properties, increase soil fertility, and build biological diversity that cannot be replaced by other means of production (Sumarno *et al.* 2009; Syamsiyah *et al.* 2023). In addition, organic matter can be used to return to soil fertility and microbiological balance (Yulianti, 2020).

At LU 6, the change of land from dry land to shrubs increased the land capability class from class V to class IV. However, land capability IV is classified as suitable for use as agricultural production land. Recommendations for land management related to this discrepancy are to choose land uses that are in accordance with the capacity of the land (Mujiyo *et al.* 2020). So that later with the potential of the land in LU 6 for agricultural production, it needs to be utilized optimally. Management efforts that can be done is the agroforestry system. Agroforestry system that applies to plant woody perennials with crops in the same area. This system diversifies the land's environment, beginning with the diversity of species, agriculture methods, and socio-economic forms (Viswanath *et al.* 2018; Weldearegay *et al.* 2021). The potential challenge faced by farmers in implementing agroforestry is re-cultivating land to plant food crops and hardwood plants which require large amounts of capital and labor. Selecting the right food commodities and annual crops is also challenging because the ultimate orientation is high economic value for farmers. Planting annual crops also requires patience because new results can be used longer than food crops such as rice, corn, soybeans, etc. The adoption of agroforestry systems is influenced by the level of profit from agricultural, forestry, and carbon prices, and research results prove that agroforestry can run well with the support of financing system policies to guarantee the risks farmers face (Abdul-Salam *et al.* 2022).

Even so, the suggestion to change agricultural land use such as rice fields and moorland into grassland and forest is a good thing in terms of land capacity. Still, there will be changes in soil characteristics, such as drainage and soil microorganisms (Kurniawan *et al*. 2023). Apart from that, this conversion activity requires a feasibility assessment and precise planning, such as what methods will be used, what types of plants are suitable for planting given the soil conditions, climate and water availability around the land, as well as what form of management is appropriate. Incompatibility of plant types with soil, drainage and climate conditions can inhibit plant growth (Anshori *et al*. 2022). Drainage that is not optimal will cause the soil to experience salinity. On a larger scale it will cause plant death, decomposition of plant organic waste and cause ecosystem imbalance, up to economic losses.

CONCLUSIONS

Land conversion in 2009-2020 covering an area of 1,308.53 ha (14.03%) with the rate of conversion of agricultural land to non-agriculture is 24 ha year^{-1} (2.88%). The largest rate of conversion of functions is found in the dry land into buildings and infrastructure (15.83 ha year^{-1}). Overall, land use change in Ngadirojo Sub-district causes changes in land capability class. In addition, the type of soil on the research land that is fixed and the land converted to function with different types has a very significant influence on the ability of the land. Management efforts that can be carried out include the addition of organic matter in LU 1, LU 2, and LU 5, and agroforestry system implementation at LU 6, while at LU 3 and LU 4 it is recommended to maintain the current land use because the condition of the land and its use is appropriate. By understanding data and information related to land use changes and their impact on land capacity, the community and stakeholders can take appropriate steps in managing land while protecting the sustainability of land ecosystems. However, it is hoped that research on land use conversion mapping and analysis of its effects can be carried out with a new one, more sophisticated methods and technology. However, research on land use conversion mapping and impact analysis is expected to be carried out using new, more sophisticated techniques and technology and perfomed over a more extended period, namely more than 1 decade, to provide a more comprehensive understanding of trends and patterns.

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WHY ADOPT SUSTAINABLE FOREST MANAGEMENT CERTIFICATIONS? MAIN DRIVERS IN ITALY AND GERMANY

SUMMARY

In recent years, sustainable forest management has proved to be a crucial issue for forestry companies becoming more and more sensitive to environmental problems. This resulted in the inherent expansion in forest certifications formally promoting long-term environmental sustainability and a wider spectrum of forest ecosystems. In this context this study aims to assess the main motivations that encourage the adoption of sustainable forest management certification for PEFC and FSC standards and how these motivations vary depending on the characteristics of the companies. Specifically, online questionnaire surveys were submitted to a sample of Italian and German forest owners and managers considering five main motivational factors offered by the economic literature: three external mechanisms represented by the market, signalling and legal mechanism and two internal mechanisms consisting of the moral and learning mechanism. Results highlighted that the main drivers are represented for both countries by the reporting mechanism, in particular certification is seen as a tool to demonstrate externally the implementation of sustainable forest management practices. Other mechanisms that guide the intentions of forest owners and managers include the legal mechanism, in particular in the Italian case, especially for publicly owned forest areas, more driven by the interest of complying with forestry regulations and the moral mechanism, in the case of Germany. The implications of this research are seen in development of forest certification, through understanding forest owners' reasons for adopting it and providing background information to improve the design of certification programs to attract greater adoption by forest companies.

Keywords: PEFC; FSC; motivations; forest owners; reasons

INTRODUCTION

A complex interplay between economic and environmental forces is common to most European countries, especially in forest sector. In particular the forest sector is undergoing a transition towards a "circular bioeconomy", on the

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basis of regulations and strategies such as “Agenda 2030” and “Clean Planet for all”, implemented by EU Member States in the context of sustainability and climate-neutral economy (Michal *et al.*, 2021). Indeed, official statistics have highlighted the growing number of forestry companies investing economic resources to improve the environmental profile of processes, products, and services (Lanfredi *et al.*, 2023). This is also highlighted by the increase in the number and complexity of forest certifications at European and in general at global level. The concept of forest certification is based on third-party auditing of compliance with established standards, principally linked to sustainability issues such as responsible or sustainable forest management, the balance between economic, social, and environmental concerns for forest management, illegal harvesting, conservation of biodiversity, timber markets (Paluš *et al.*, 2024). Regarding the latter, an important role is played by Chain of Custody (CoC) certification, which guarantees an effective system of traceability through the supply chain. There are several worldwide forest certification organizations, but Forest Stewardship Council (FSC), and Programme for the Endorsement of Forest Certification (PEFC) have become the most diffused standards at global level. These standards present some differences in relation to their origin. The FSC scheme emerged in response to the failure of national governments to address the loss of high conservation value forests, particularly in the tropics. The appearance of the FSC was followed by a concern from industry and forest owners about the cost of compliance with the different standards FSC prescriptive. So, the PEFC program was established in 1999 in response to environmental, socio-economic, political, and cultural issues of forest landowners in Europe. According to the latest statistics 390 million ha are certified worldwide (9% of the global forest surface), with FSC reporting a total certified area of 170 million ha and PEFC of 296 million ha. Both are present in the Northern hemisphere mostly, and less in tropical areas. Currently, dual certification exists in 33 countries with 86 million ha (Rocchi *et al.*, 2023). Specifically, in Europe 81 million ha of forest are certified PEFC and 56 million ha are certified FSC (FSC, 2023; PEFC, 2023). The spread of the above-mentioned certification schemes has led to an increase in scientific production in this field of research, with the aim of mainly examine attitudes and motivations of forest owners and managers to adopt forest certification. Specifically, the main categorizations of motivations are those proposed by Cashore *et al.* (2004), Overdevest and Rickenbach (2006), and Faggi *et al.* (2014). According to the non-state market-driven model (NSMD), proposed by Cashore *et al.* (2004), there are three structural factors affecting the choice to adopt forest certification: the role of the global economy in relation to the companies’ dependence on foreign markets sensitive to environmental issues; the structure of the forest sector; and the public policy agenda. Overdevest and Rickenbach (2006) considered three mechanisms: market mechanism, linked to forest companies’ economic and market interests; signalling mechanism, aimed to inform external stakeholders about the firm’s pro-environmental behaviors, and learning mechanism, which helps to transfer knowledge, skills, and practices to the enterprises. Instead Faggi *et al.* (2014), in their study, added two other relevant mechanisms, namely the moral mechanism linked to individual ethical values and the legal mechanism that concerns legal compliance with mandatory regulation. Given this background, the aim of this study is to understand the main motivations driving forest owners and managers towards PEFC and FSC certifications of sustainable forest management. The basic idea is at first to assess the main motivational drivers starting from a solid base offered by the economic literature on forest certifications and thus considering three external mechanisms represented by the market, signalling and legal mechanism and two internal mechanisms consisting of the moral and learning mechanism (Galati *et al.*, 2017; Zubizarreta *et al.*, 2021). Furthermore, the presence of possible differences and variations in these motivational drivers is assessed in relation to the characteristics of the certified companies and the stage of certification diffusion. Specifically, two parallel surveys were conducted, adopting the same methodological approach, but in different economic and territorial areas: Italy and Germany. The peculiar selection of the study areas can be traced back to the desire to investigate the phenomenon of the diffusion of forestry certifications which, although widely treated in the scientific literature (Lombardo *et al.*, 2021), has been little explored in relation to these two geographical areas. The number of studies concerning the assessment of the main drivers that induce Italian and German forest owners to adopt certification is rather scarce. Indeed, although Italy is one of the most important European countries and in the international context, in terms of number of environmental certifications, only a few studies have been conducted in this field research. Specifically, in their study Galati *et al.* (2017) mainly focused on the implementation of the FSC standard as a means of promoting responsible forest management and traceability of derived products, compared to the standard PEFC. This last scheme was analyzed in the work of Negro *et al.* (2021), as part of the project "PEFC Solidarity Chain" established after the Vaia storm. This also considering that the country has a large, certified forest area: out of a forest area of 10 982 013 ha, 85 838 ha are FSC certified, while 949 907 ha are PEFC certified. In the case of Germany, it can be emphasized, again, that in the literature, the analysis of the main motivational drivers towards forest certification is addressed in a very limited way, often in conjunction with other countries (Cashore *et al.*, 2005). In general, the main studies developed in this country refer to the adoption of FSC and PEFC certifications for the paper industry importers (Korhonen *et al.*, 2017; Dieckmann *et al.*, 2020), although Germany has certification levels above 65% of the national forest area (Maesano *et al.*, 2018), with respectively 1 553 728 ha of forest with FSC certification and 8 275 727 ha certified PEFC out of a total of 11.4 million ha of forest. So, this study aims to fill this research gap in the literature, analyzing for these countries, the main drivers towards the adoption of for PEFC and FSC SFM certifications and their dependence in relation to the characteristics of the companies.

MATERIAL AND METHODS

Survey design

The survey was conducted using a structured questionnaire in Google Forms. In the case of Italian sample this questionnaire was sent directly in Italian whereas in the case of Germany it was translated into German, after having pre-tested a sample of eight Italian companies through the administration of the questionnaire through telephone interviews. The questions were formulated according to a closed or hierarchical response scheme. The questionnaire was structured in three sections. In the first section, “General characteristics of the company/entity and forestry certifications adopted”, aspects relating to the entities interviewed were noted, such as: name; location; legal form; the type of ownership; the total area wooded and the certified forest area in ha; the type of forest species present; the main production that constitutes the core business; the number of employees; main product destination markets; main sales channels; average company turnover in euro; the types of certification adopted and the year of adoption. The second section, “Characteristics of the forest owner/manager”, provided information on the profile of the interviewees, specifically on age, educational qualification and gender, years of experience in the forestry sector. The third section "Analysis of the main motivations towards the adoption of forest certification" allowed for the detection of the motivational aspects that drive owners/managers towards the adoption of forest certification. Specifically, the statement 'I have chosen to adopt forest certification...' was followed by twenty-two items for each of which respondents were asked to indicate their degree of agreement or disagreement. For this purpose, a 5-point Likert scale (Likert, 1932) was used for the answers, where a score of 1 corresponds to 'completely disagree' and a score of 5 to 'completely agree'. The list of proposed items was developed on the basis of the main studies in this research area (Hartsfield and Ostermeier, 2003; Overdevest and Rickenbach, 2006; Jayasinghe *et al.,* 2007; Faggi *et al.,* 2014; Johansson, 2014; Mikulkova *et al.,* 2015; Galati *et al.,* 2017; Misue, 2018; Hălălişan *et al.,* 2018; Zubizarreta *et al.,* 2021; Paluš *et al.,* 2021).

Data collection

The forest owners and managers involved in the survey were identified from the international databases of FSC and PEFC including only SFM certification holders. The development of the survey was based on the methods recommended by Dillman (2007) which include an information telephone call (in the case of Italy) a pre-notification and a first and second email in order to maximize the response rate. Data were collected in September-December 2022. In the case of Italy, out of a total of 133 certified companies/bodies contacted, respectively 47 for PEFC and 86 for FSC, 83 complete questionnaires were received with a response rate of 62.4%. For Germany, there were 271 certified entities in the FSC database, but taking into account the difficulty of finding the contact details of some owners, especially in the case of private individuals, a group of 55 entities was considered (without considering all other entities within the same group certification). In the case of PEFC certification, considering the high number of certified entities, around 12 000, following the methodology applied by Jaung *et al.* (2016) and Krause and Matzdorf (2019) a sample of 400 subjects was considered, using a simple randomization sampling method, and received 71 completed questionnaires with a response rate of 15.60%.

Data analysis

The statistical analysis was performed using the SPSS (Version 25) and Stata (Version 17) software. Specifically, a univariate descriptive analysis of the surveyed variables was carried out by calculating the centrality and variability indices for quantitative variables and the frequencies for qualitative ones. The elaboration of averages and standard deviations, for responses on a Likert scale, were conducted to analyze the main motivations for forest owners/managers to become certified. To assess how motivations could be influenced by different factors, non-parametric tests were applied. This approach has previously been used by various authors analyzing similar research topics (Hălălişan et al., 2018; Paluš *et al.*, 2018; Paluš *et al.*, 2021; Zubizarreta *et al.*, 2021). Therefore, in order to verify how the motivations for Italian and German forest owners and managers varied according to type of certification (PEFC, FSC, PEFC-FSC), total forest area (up to 100 ha, from 101 to 300, from 301 to 1000, over 1000), and the year of certification (up to 2004, from 2005 to 2012, from 2013 onwards) the Kruskal-Wallis test was applied. This test is usually used when a normal distribution of the population cannot be assumed and makes it possible to determine the presence of differences in the central value (mean or median) of more than two independent groups or samples. In the case of the factor 'type of ownership' (public or private) and legal form (sole proprietorship, other form), the Mann-Withney test or U-test was used. This test, of which the Kruskal-Wallis test is an extension, is applied in the presence of two independent groups (Hălălişan *et al.*, 2019). Specifically, for Italy, where the presence of 18 properties with poplar cultivation, this test was applied to assess possible differences in motivations according to the type of forest species present (poplar or other species). The null hypothesis underlying the methodology is that all averages identified by the variables considered are compared instead with the alternative hypothesis, namely that there exists at least one pair of averages that differ from each other. For such tests, a significance level of 5%.

RESULTS AND DISCUSSION

Description of the characteristics of the entities and certified owners/managers

The analysis of the Italian context shows a distribution of the realities surveyed mainly in the regions of northern Italy. In fact results show 34% in Friuli-Venezia Giulia, followed by 28% in Veneto, 11% in Trentino-Alto Adige, 10% in Lombardy, 6% in Tuscany, 2% in Emilia-Romagna, Piedmont and Sardinia, 1% in Calabria, Lazio, Liguria and Umbria. In Germany the largest percentage of forest owners/managers is concentrated in the federal state of

Baden-Wuerttemberg (24.3%), followed by Bavaria with 15.7%, Rhineland-Palatinate with 14.3%, North Rhine-Westphalia with 11.4%, Hessen with 8.6%, Saxony-Anhalt and Lower Saxony, each with 5.7%, Brandenburg and Thuringia, each with 4.3%, Saxony with 2.9% and, finally, by Mecklenburg-Vorpommern and Saarland, each with 1%. In the German case, the sample taken includes almost all federal states except Hamburg and Schleswig-Holstein. Regarding the legal form, in the Italian case 73.5% are represented by another form (this is a very diverse sample including, for example, 22 municipalities, 2 Regole, 4 regional authorities, 2 associations of producers, 6 consortia, 3 capital companies, 6 partnerships), and the remaining 26.5% by individual enterprises. Also, in the case of Germany, the predominant legal form is other, i.e., 78.9 % (comprising in most cases municipalities, around 35, and forestry offices), while 21.1 % are sole proprietorships. Regarding the type of forest ownership, the data show that in the Italian case there is a greater presence of privately managed forest areas (59%), compared to 28.2% in Germany where 71.8% of the surveyed areas are public property. With reference to the total forest area, the Italian realities are very diversified; in fact, forest properties of up to 100 ha are mostly consisting of poplar growers and individual enterprises (32.5%) and forest areas of over 1000 ha represented mostly by public properties (34.9%). While the percentage of German forest areas of more than 1,000 ha is 52.1 %, mostly public property. In the Italian case, the certified forest area accounts for 90% of the total forest area, whereas in Germany this incidence is 95%. About forest types, Italy has a higher percentage of broadleaf forest (43%), considering that it includes poplar forests (18 entities) while in the German case the highest percentage is found for mixed deciduous and coniferous forest (47.8%). The main production types constituting the core business in Italy are timber for industry (53.0%), followed by other functions (e.g., non-timber products and tourism-recreation), amounting to 15.66%, wood for energy (3.63%). In the remaining 27.7%, the type of product is not specified. Also, in the case of Germany, the main production is wood for industry (74.7%), followed by other functions (12.7%), and energy wood (9.85%), while 2.81% are not classified. Regarding the number of employees in the business, in the Italian case is 95.2% for entities with a number of employees < 50 and 4.8% for those with a number between 50 and 250. There are no enterprises with more than 250 employees. The percentage of the number of employees in Germany has the following values: 88.7% <50 employees; 4.2% between 50 and 250; 7.05% over 250. The main destination markets for forest products for both countries are the domestic market, namely 86.7% in the case of Italy and 95.8% in the case of Germany. The remaining part for both countries is destined for foreign markets. With reference to the main distribution channels, Italy has a higher percentage in the 'direct sale' category (61.4%) followed by 'other' with 20.5% (represented by e.g., consortium sale, standing sale to forestry companies, public auction, sale on the wownature.eu portal) and 18.1% for sale to processing industries. In the case of Germany, sales to processors (56.3%) accounted for the largest percentage, followed by direct sales (28.2%), while

15.5% fell into the 'other' category. Regarding the average company turnover, most Italian companies reported a turnover of less than two million euro (79.5%), 4.8% between 2.1 and 10 million and 15.7% did not provide an answer. In Germany, the average company turnover was 76.1% below 2 million euro, 12.7% between 2.1 and 10 million euro and 11.2% between 10.1 and 50 million euro. For both countries, it emerges that many of the realities surveyed have both FSC and PEFC certification, in particular 24.1% in Italy and 28.2% in Germany. Considering the presence of dual certification, in some cases, the survey results show that 41% of Italian companies are FSC certified and 83.1% PEFC certified; in contrast, in Germany 33.8% is certified FSC, while 94.4% PEFC.

Furthermore, it should be noted that eleven of the Italian companies, in some cases within the group certification, had already extended SFM certification to ecosystem services.

About the characteristics of the respondents, it should be noted that in the case of Italy, 62.7% were male, 9.6% female (27.7% preferred not to give any answer). Furthermore, the majority of respondents are between 41 and 60 years old (47.0%), followed by 18.1% over 60 and 13.2% between 26 and 40 years old, while 21.7% of the respondents did not state their age.

As far as the level of education is concerned, 30.1% have a high school diploma, 19.3% master’s degree, 11.0% secondary school undergraduate degree, 8.4% PhD/master's degree, and 6.0% bachelor's degree, while 25.3% provided no answer. The years of experience in the forestry can be described as follows: for 23.0% the experience was between 1 to 15 years, for 27.7% from 16 to 30 years, for 14.4% over 30 years. The remaining 34.9% did not provide any answer. In the case of Germany, the majority of respondents were male (63.4%), followed by 1.4% female, while 35.2% preferred not to answer. Regarding age, the majority of respondents fall into the 41 to 60 age group (38.0%) followed by 19.7% over 60, 14.1% from 26 to 40, 2.8% from 18 to 25 (25.35% did not provide any data). In addition, 25.3% had a high school education, 18.4% a master's degree, 15.5% a bachelor's degree and 11.3% a PhD/master's degree, while 29.6% did not want to give any answer. Finally, with regard to experience in the forestry sector, it can be seen that 31.0% have more than 30 years’ experience, followed by 19.7% with experience of up to 15 years, 18.3% from 16 to 30 years, while 31.0% gave no answer.

Main drivers for the adoption of forest certification in Italy and Germany

To assess the internal consistency of the items proposed to evaluate the five mechanisms, the Cronbach's α coefficient was calculated for Italy and Germany. A reliability coefficient of 0.7 was considered as an acceptable level of consistency. In fact, in the case of Italy, a value of the α coefficient of 0.90 was recorded, as in the case of Germany where the value of α was 0.91. Figure 1 shows the comparison between the two countries in relation to the main mechanisms driving certification and Table 1 the related items attributable to each mechanism.

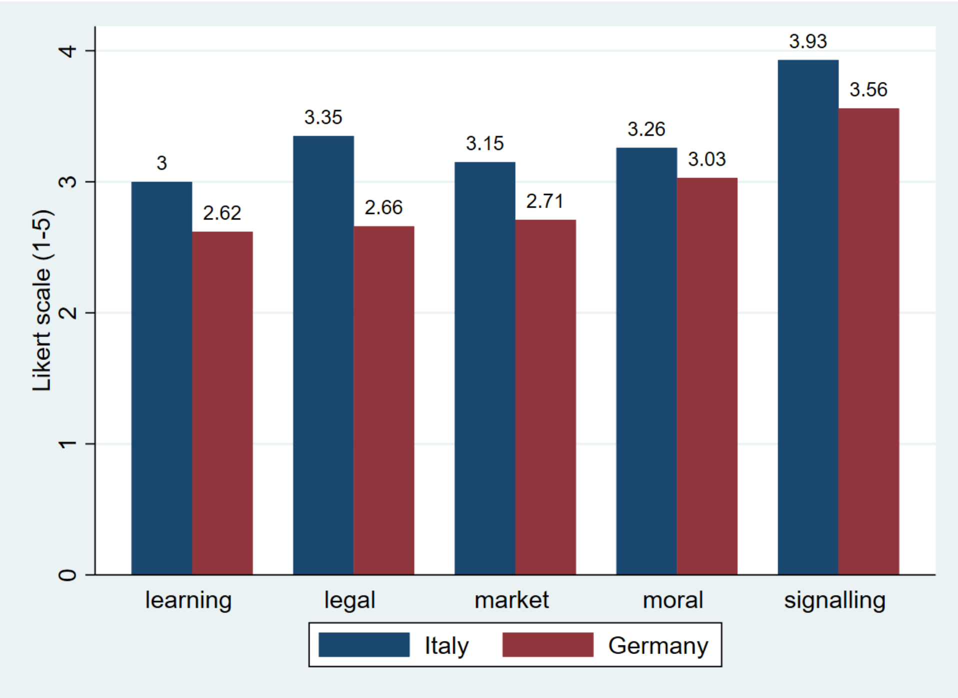

Figure 1. Mechanisms driving the adoption of forest certification.

As shown in Figure 1, the main mechanism driving both Italian and German owners towards the adoption of forest certification is represented by signalling, with an average value, respectively, of 3.93 in the Italian case and 3.56 for Germany. In particular, for Italy, as shown in Table 1, the items "To have sustainable forest management practices recognized" (4.14) and "Because certification represents a commitment to environmental responsibility" (3.94) acquire important relevance. Also, in the case of Germany, the item “To have recognition of sustainable forest management practices” has the highest average value of 3.96, followed by "To improve corporate image" with a value of 3.68. These findings are in line with those of other authors specifically focusing on SFM certification (Hartsfield and Ostermeier, 2003; Mikulková *et al.*, 2015; Lee and Chang, 2019) and highlight how forest certifications can be a useful tool to improve corporate image and to demonstrate and promote good forest management practices to external stakeholders. As for the other mechanisms, however, there are some differences between countries. In the case of Italy, in fact, the mechanism that takes, in order of importance, is represented by legal with a score of 3.35. Specifically, within this category, the item "To ensure compliance with forest policy objectives", shows the highest average value of 3.60. Although this result differs from those reported by the majority of the empirical evidence on this research topic, where more often than not, this mechanism is little analyzed however, some authors (Paluš *et al.*, 2021) nevertheless attribute a relevant score to this mechanism especially in the case of SFM certification. In fact, this result can be explained by the fact that most of the realities under study are represented by public properties that, also in view of the new national (TUFF) or European (EUTR) forestry regulations, aim at the respect and application of sustainable forest management practices at the basis of these regulations. This is also confirmed by the new National Forestry Strategy, which plans to encourage the increase of certified areas by 30% by 2025 (Romano, 2021). In the case of Germany, however, the second most important mechanism is the moral mechanism. In fact, the item “For an interest towards environmental protection” has a mean value of 3.49. This result is in line with that of other authors (Zubizarreta *et al.*, 2021) and is of particular interest, especially in the case of SFM certification. In fact, as various authors point out, forest certification has several positive effects, especially for the conservation of biodiversity (Carlsen *et al.*, 2012; Kalonga *et al.*, 2016). Again, in the German context, the moral mechanism is followed by market aspects with an average value of 2.71. Specifically, the items of greatest importance are "To meet customers' expectations" with an average of 3.37 and "To increase competitiveness in the market" with a score of 3.35. Some studies (i.e., Galati *et al.*, 2017; Misiune, 2018) report similar results (Galati *et al.*, 2017; Misiune, 2018). Less importance in both geographical contexts is found for the learning mechanism, although for both countries the item "For improving of forest management practices" has the highest score, respectively 3.54 in the case of Italy and 3.21 in the case of Germany. This is probably because forest owners/managers in both countries already adopt sustainable forest management practices based on their forestry tradition and experience and independently of certification.

Motivational factors according to the characteristics of certified enterprises

To test the relationship and influence of the adopted certifications on the motivation of forest owners to become certified, the Kruskal Wallis test was applied. In the German case, some differences emerge in relation to the adoption of one of the two certifications (FSC or PEFC) or of dual certification (FSC, PEFC). Only for four items does the test reject the null hypothesis, there is therefore for each item at least one pair that differs significantly on average. By means of pairwise comparisons (post hoc test with Bonferroni correction) such pairs were identified. The items where it is possible to reject the null hypothesis, i.e. the absence of differences in motivation between the groups identified, fall into three specific mechanisms and in particular "To reduce environmental pollution" of the moral mechanism, "To increase internal organizational efficiency" and "To improve product quality" of the learning mechanism, "compliance with forest policy objectives" of the legal mechanism.

Table 1. Motivations for adopting FSC and PEFC SFM certifications in Italy and Germany

Mechanism	*Items*	Average* It	SD It	Average Germ.	SD Germ.
Signalling	To improve corporate image	3.81	1.84	3.68	1.25
	To ensure product traceability	3.86	1.19	3.03	1.38
	To gain recognition for sustainable forest management practices	4.14	1.14	3.96	1.31
	Because certification is a commitment to environmental responsibility	3.94	1.16	3.58	1.40
Moral	For employee satisfaction	2.16	1.30	2.28	1.19
	To reduce environmental pollution	3.20	1.42	2.92	1.47
	To increase customers' awareness of environmental issues	3.77	1.31	3.44	1.42
	For an interest in environmental protection	3.92	1.25	3.49	1.44
Market	To increase the selling price of the product	3.34	1.37	3.00	1.37
	To increase market competitiveness	3.49	1.35	3.35	1.45
	To increase national market share	3.06	1.37	2.01	1.16
	To increase foreign market share	2.48	1.36	1.92	1.20
	To meet customers' expectations	3.50	1.35	3.37	1.29
	For the diversification of sales channels	3.03	1.42	2.62	1.26
Learning	To increase internal organisational efficiency (processes and procedures)	2.71	1.24	2.30	1.26
	Because certification supports learning and facilitates the exchange of experiences	2.94	1.25	2.58	1.26
	For product quality improvement	2.80	1.35	2.40	1.31
	For the improvement of forest management practices	3.54	1.28	3.21	1.39
Legal	To participate in calls for tenders	2.84	1.55	2.28	1.33
	To ensure compliance with forest policy objectives	3.60	1.29	3.44	1.42
	To ensure compliance with current environmental legislation	3.58	1.32	2.62	1.37
	To benefit from RDP measures	3.36	1.64	2.31	1.24

* With 5-point Likert scale (1= completely disagree; 5= completely agree)

In the case of the item "To reduce environmental pollution" as can be seen both from the averages shown in Table 2, and from the values of the average ranks respectively, equal to 36.77 for PEFC-certified companies, 39.40 for those with dual certification (FSC, PEFC), and 10.00 for those with FSC, it can be seen that those with FSC certification score lower on average than those with

PEFC and dual certification. This is also confirmed by the post hoc test with the Bonferroni correction (Table 3). These results show that German forest owners and managers are more driven by moral mechanisms to adopt PEFC certification or dual certification, compared to FSC certification. Other differences emerge for the item "To increase internal organizational efficiency (processes and procedures)". For this statement, there is a statistically significant difference between the PEFC and FSC, PEFC categories.

Table 2. Analysis of differences in motivation for Germany according to the certification with the K. Wallis test

Germany	***Items***	**FSC**	**PEFC**	**FSC/PEFC**	**Sig K. Wallis**
Moral	**To reduce environmental pollution**	1.00	2.98	3.15	0.026
Legal	**To ensure compliance with forest policy objectives**	2.25	3.30	4.00	0.030
Learning	**To increase internal organisational efficiency (processes and procedures)**	1.75	2.11	2.85	0.027
	For product quality improvement	1.25	2.26	2.95	0.021

Table 3. Post-hoc testing for the Kruskal-Wallis test according to certification type

Sample	**Test statistic**	**Std. Error**	**Std. Test Statistic**	**Sig**	**Adj. Sig.**
To reduce environmental pollution					
FSC-PEFC	-26.766	10.500	-2.549	.011	**.032**
FSC-FSC, PEFC	-29.400	11.042	-2.663	.008	**.023**
PEFC-FSC, PEFC	2.634	5.382	.489	.625	1.000
To increase internal organisational efficiency (processes and procedures)					
FSC-PEFC	-5.992	10.283	-.583	.560	1.000
FSC-FSC, PEFC	-19.200	10.814	-1.775	.076	.227
PEFC-FSC, PEFC	13.208	5.271	2.506	.012	**.037**
For product quality improvement					
FSC-PEFC	-15.348	10.364	-1.481	.139	.416
FSC-FSC, PEFC	-26.500	10.899	-2.431	.015	**.045**
PEFC-FSC, PEFC	11.152	5.312	2.099	.036	.107
To ensure compliance with forest policy objectives					
FSC-PEFC	-14.088	10.451	-1.348	.178	.533
FSC-FSC, PEFC	-25.025	10.991	-2.277	.023	.068
PEFC-FSC, PEFC	10.937	5.357	2.042	.041	.124

In particular, German forest owners and managers with dual certification are more driven by legal mechanisms than those with PEFC certification alone. In the case of the item "For product quality improvement", as is evident from the average ranks of 44.88 for the FSC, PEFC, 33.72 for PEFC and 18.38 for FSC, respectively, significant differences between the FSC category and the others can be seen. In other words, as confirmed by Bonferroni's correction, the motivation "product quality improvement" is not mentioned among the main reasons for adopting FSC certification.

For Italian respondents, on the other hand, the Kruskal-Wallis test accepts the null hypothesis for all items. The test for the presence of statistically significant differences in the motivational factors was also assessed for the variable “legal form” of enterprises. Two categories were identified: "individual enterprise" and "other form" and this in order to allow a better comparison between the two countries under study. Specifically, the Mann Whitney test was applied since two categories were involved. As shown in Table 4, this test in the German case allowed the rejection of the null hypothesis for the following items: "to reduce environmental pollution"; "to increase competitiveness in the market"; "to increase internal organizational efficiency (processes and procedures)"; "because certification supports learning and facilitates the exchange of experience"; "for product quality improvement"; "for the improvement of forest management practices"; "to ensure compliance with current environmental legislation"; “to benefit from RDP measures”. In other words, there are significant differences on average for these items between individual enterprises and other legal forms. These differences are positive for both variables, for almost all items, i.e., as shown by the test statistic, the category "other form" almost always scores higher, only in the case of the item "increase competitiveness in the market" the category "individual enterprise" appears to have a higher motivation. This could mean that the individual enterprise is more oriented towards market mechanisms. A similar situation is found for Italy. In fact, the Mann Whitney test made it possible to reject the hypothesis of equality of the averages for the single item "to increase foreign market share", thus as also confirmed by the value of the test statistic, individual enterprises have a higher average score than "other form" (Table 4).

The analysis was also conducted with reference to the variable “type of ownership” (public or private). For the German respondents the Mann Whitney test confirmed the null hypothesis of equality, on average, between the type of ownership for all items examined. In the Italian case, however, the U-test made it possible to reject the null hypothesis for the items: "for employee satisfaction"; "because certification supports learning and facilitates the exchange of experiences"; "to ensure compliance of forest policy objectives". With respect to these items, public properties differ significantly, on average, from private ones. In particular, public properties have on average higher scores (respectively 2.56, 3.32 and 4.00) than private ones (1.88, 2.67 and 3.33), underlining that mechanisms such as moral, learning and legal are more representative for this category. Only in the Italian case was the U-test applied, in order to check for differences in motivation with regard to the type of forest species (poplar or other species). This test allowed us to reject the hypothesis of equality, on average, only for the statements "for the diversification of sales channels" and "to benefit from RDP measures". In fact, it emerges that the category "poplar" has a higher score (respectively 3.67 and 4.33) than "other forest species"(2.86 and 3.09).

Table 4. Analysis of differences in motivation for Germany and Italy according to legal form

Germany	Items	Individual enterprise	Other form	Sig. U	Test statistic
Moral	**To reduce environmental pollution**	2.13	3.13	0.020	2.322
Legal	**For compliance with environmental regulations**	1.87	2.82	0.017	2.390
	To benefit from RDP measures	1.80	2.45	0.022	2.282
Market	**To increase market competitiveness**	4.00	3.18	0.045	-2.009
Learning	**To increase internal organisational efficiency (processes and procedures)**	1.53	2.50	0.006	2.746
	Because certification supports learning and facilitates the exchange of experiences	1.93	2.75	0.025	2.237
	For product quality improvement	1.53	2.63	0.004	2.908
	For the improvement of forest management practices	2.20	3.48	0.002	3.043
Italy					
Market	**To increase foreign market share**	2.95	2.31	0.026	-2.220

In practice, it can be deduced that the poplar growers are more interested in the possibility of enjoying the benefits of the RDP and diversification of the sales channels.

Finally, the Kruskal Wallis test was applied to detect any differences in motivations in relation to the size of the wooded total area and the year of certification. In the first case, with reference to the German sample, the null hypothesis of equality on average was accepted for all variables and no significant differences emerged between the categories of the total forest area and motivations. With reference to Italy, the Kruskal Wallis test allows the rejection of the null hypothesis for the item “To have recognition in SFM practices”; For this purpose, pairwise comparisons were made, when looking at the actual p-value, three pairs are significantly different, on average, these pairs are those from: "101 to 300 ha" vs "over 1000 ha", "101 to 300 ha" vs "101 to 1000 ha" and "up to 100 ha" vs "over 1000 ha". In any case, the correction of Bonferroni does not confirm differences between the pairs. Regarding the differences in the motivational drivers in relation to the year of certification for the FSC standard (up to 2004, 2005 to 2012, from 2013 onwards), it can be seen from Table 5 that for Germany the Kruskal Wallis test rejects the null hypothesis only for the statement “to meet customers' expectations”. As can be seen from the pairwise comparison, from the average ranks of 7.88 for the category "up to 2004", 19.93 for the category "2005 to 2012" and 13.17 for "2013 onwards" respectively, the category "up to 2004" differs significantly from the category "2005 to 2012". It can therefore be said that the adoption of certification has intensified over time in order to meet customer expectations. Also, for Italy, the Kruskal Wallis test allows to reject the null hypothesis only for the item "To meet customer expectations" (Table 5). In contrast to the German case, the category "2005 to 2012" differs significantly from the others, and in particular from the category "2013 onwards" and thus shows that "Meeting the customer expectations" was a stronger motivation for those who certified in that period (2005 to 2012).

Table 5. Analysis of differences in motivation for Germany and Italy based on year of FSC certification.

	Items	**Until 2004**	**From 2005 to 2012**	**From 2013 onwards**	**Sig K. Wallis**
Market	**To meet customers' expectations (Germany)**	2.75	3.86	3.44	0.025
Market	**To meet customers' expectations (Italy)**	2.50	4.50	3.27	0.008

With regard to the year of certification for the PEFC standard, the application of the Kruskal Wallis test allows the null hypothesis to be rejected only in the case of the German sample, for the items "Because certification represents a commitment to environmental responsibility" and "For product quality improvement".

Applying the pairwise comparison shows that the pair that differs significantly is the one "from 2013 onwards - up to 2004", in particular the first one has an average rank of 26.97, the second one of 40.20. It can therefore be deduced that German owners/managers who obtained certification before 2004 compared to those who certified after 2013, considered the certification as a tool to manifest their commitment to the environment. Regarding the item "For product quality improvement" the application of the pairwise comparison shows, also in relation to the ranks average, that the pairwise comparison which presents a significant difference on average is made up of the category "from 2005 onwards" with an average rank of 28.45 and "from 2005 to 2012" with a rank of 44.59. It can therefore be stated that those who certified "from 2005 to 2012" considered as their main motivation the possibility of improving their product in terms of quality through PEFC certification, compared to those who certified recently.

CONCLUSIONS

The aim of this study was to analyze the motivations driving Italian and German forest owners and managers towards the adoption of SFM certification. The results of the work showed that the main drivers for both countries are the reporting mechanism, in particular certification is seen as a tool to demonstrate externally the implementation of SFM practices. In addition, other mechanisms that guide the intentions of forest owners and managers include the legal mechanism, in particular in the Italian case, especially for publicly owned forest areas, more driven by the interest of complying with forestry regulations and the moral mechanism, in the case of Germany. The latter result stems from the fact that German owners show more interest in certification as an instrument for the protection of the environment, and more specifically for the conservation and protection of biodiversity. The learning mechanism, on the other hand, received less importance for both countries probably because the sustainable forestry management is now an established practice for the companies surveyed. Evaluating the motivations also on the basis of certain characteristics of the companies certified companies, it emerged that in the German case the characteristic "legal form" and specifically individual enterprises, are more motivated to certify themselves by the possibility of increasing competitiveness on the market. In the Italian case, on the other hand, some items pertaining to the moral, learning and legal mechanisms are more relevant for public properties. Finally, in the case of Italy, it emerged that poplar growers are more interested in adopting certification, compared to owners of areas characterized by other forest types, because of the possibility of benefiting from RDP measures. These results could have interesting implications for policy makers in designing policy protocols, message strategies and incentive mechanisms for encouraging more forest owners to adopt forest management certifications. In addition, these findings can provide baseline information for improving certification programs to satisfy owners ‘expectations to attract more owners’ participation. The main limitation of the research is the number of respondents, a total of 154 (71 for Germany and 83 for Italy), which, particularly in the case of Germany, is an unrepresentative number, considering the large number of certified companies. However, it should also be pointed out that the study is based on companies, and not individuals.

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MOTIVATING GENERATION Y: WHAT MILLENNIALS NEED FOR ENHANCING WORKPLACE SATISFACTION AND PRODUCTIVITY

SUMMARY

The issues surrounding motivation within Generation Y have emerged as profoundly significant in contemporary society, presenting complex challenges that demand careful resolution. Constructing an effective motivation system has emerged as a paramount determinant of job performance. Moreover, the recognition of motivation as a critical factor influencing overall employee engagement has gained prominence; however, it frequently lacks appropriate attention and consideration. Motivation is not a delegable concept; it does not manifest implicitly and does not inherently form a part of an employee's professional competency portfolio. This article researches the intrinsic beliefs and preferences among Generation Y members relating to their motivation, which once met will most likely subsequently contribute to heightened job satisfaction and increased organizational productivity. It delves into the motivational drivers and employs empirical methodologies to substantiate the relationship and positive influence of motivation characteristics specific to this generation on their effectiveness and efficiency. The findings offer valuable recommendations for enterprises, managers, and human resources departments aiming to explore the dimensions of motivation, job satisfaction, and interpersonal relationships within their organizations.

Key words: generation Y, motivation, job satisfaction, employee engagement, organizational productivity.

INTRODUCTION

In the contemporary business landscape, characterized by intense competition, organizations are grappling with the challenge of retaining high-quality talent. Irrespective of their size, technological infrastructure, or market orientation, many organizations face the pressing issue of staff retention. To harness the full potential of their existing resources and maximize employee motivation, modern companies

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recognize the necessity of fostering strong and positive relationships between employees and the organization (Sheppard, 2016).

Effective utilization of human resources is pivotal to an organization's success, as human resources constitute the driving force behind an organization's operations (Postolov, 2011). Motivation, therefore, emerges as a central concern within the realm of management. A robust motivational philosophy and practice enhances productivity, quality, and service, while fostering a harmonious and pleasant work environment amplifies employee motivation and, consequently, organizational profitability (Benegal, 2013). However, despite the recognition of motivation's significance, it often receives inadequate attention in the pursuit of numerical targets and performance indicators (Robbins, Judge, Sanghi, 2009). This paper endeavors to explore the extent to which certain preferences and factors influence the effectiveness and efficiency of Generation Y members, subsequently impacting job satisfaction and organizational productivity.

In contemporary workplaces, generational diversity is discernible, posing a managerial challenge in terms of creating conducive work conditions and managing activities that align with the values and expectations of each generation (Masic, 2009). Generation Y, in particular, presents a unique challenge, characterized by distinctive attributes that set it apart from previous generations and, at times, provoke misconceptions. Consequently, there is a compelling need for in-depth investigation.

The primary objective of this paper is to elucidate the preferences of Generation Y and its responsiveness to motivational drivers. Given that Generation Y constitutes a significant portion of the emerging workforce, adapting to their needs becomes a top priority for employers. The initial step in this process involves comprehending their characteristics, desires, and needs, along with the factors influencing their work habits and attitudes, as all of them will be explained in-depth in the theoretical background and the review of the foundational literature. Subsequently, it necessitates dispelling stereotypes and emphasizing the advantages that this generation offers to secure a competitive market position (Kultalahti, Viitala, 2015). These stereotypes, which include those that members of this Generation quickly hop from one job to the next, work to live instead of living to work, strive to find shortcuts when working, feel entitled, and similar, are unfounded and managers and business leaders analyze their signature strengths to improve the overall competitiveness of their workforce (Kultalahti, Viitala, 2015).

Generation Y exhibits a proclivity for working in environments that foster innovation, skill development, and displays a philanthropic approach to societal concerns. They are inclined toward tasks that hold essential significance for the organization's vision and goals (Howe, Strauss, 2009).

With the rising number of Generation Y members entering the workforce, organizations are increasingly compelled to bridge generational gaps, foster acceptance of new generations, and harness their potential fully. Consequently, this paper aims to lay the foundation for understanding motivation, delving into motivational theories, and exploring various motivational instigators. It will also elucidate the fundamental characteristics of Generation Y, underpinned by empirical research, which substantiates the relationship between Generation Y's unique motivators and their effectiveness and efficiency.

The outcomes of this research endeavor can serve as a cornerstone for future studies on similar themes and offer valuable recommendations for enterprises, managers, and human resources departments seeking to assess motivation levels, job satisfaction, and interpersonal dynamics within their organizations.

Concept of Motivation

The concept of motivation is multifaceted and defies a singular definition. Just as there is no one-size-fits-all job position or task, there exists no universal approach to motivation. Moreover, alongside motivation lies its counterpart, demotivation, which poses a significant challenge to organizational management (McClelland, 2009). The ability to discern early signs of demotivation is a crucial skill for effective managers, preventing dissatisfaction from culminating in open expressions of discontent among employees (Shuklev, 2013).

Human desires are inherently insatiable, with employee expectations perpetually on the rise, regardless of the organization's achievements (Yamaguchi, 2003). To align these expectations with an organization's goals, it is essential to keep employees informed about the company's objectives and actively involve them in their realization. Motivation derives its etymology from the Latin word "muovere," meaning "to move." Numerous scholars have contributed their definitions of motivation, reflecting its multifaceted nature:

- McClelland (2009) characterizes motivation as a broad force residing within individuals, driving excitement, direction, and persistent voluntary efforts to attain goals.
- Campbell and Pritchard (1976) see motivation as a set of psychological processes that induce initiative, direction, intensity, and persistence in behavior.
- Vroom (1964) defines motivation as the rationale behind human behavior and the logic guiding individuals' activity choices.
- Denhardt et al. (2008) simply state that motivation is what drives people's actions.

Two primary types of workplace motivation exist. The first involves employees seeking, finding, and engaging in work that fulfills their needs, while the second hinges on extrinsic factors such as salary increases, promotions, and rewards (Chapman, 2014).

In addition to these two types, workplace motivation can also be categorized into internal (Type 1) and extrinsic (Type 2) motivation. Internal motivation stems from self-generated factors like autonomy, skill development, and work challenge, leading individuals to perform tasks for personal satisfaction and intrinsic rewards. Extrinsic motivation, on the other hand, revolves around external rewards, including salary increases, promotions, praise, and disciplinary actions.

The most effective organizational motivation system typically combines both internal and extrinsic motivation strategies, necessitating the creation of a comprehensive framework that incorporates both types (Shannon, 2005).

One of the contributions to the motivation theory has been made by Maslow's theory of needs, which considers that motivation is the result of people's efforts to fulfill five elementary needs, including psychological needs, the need to feel safe and secure, social needs or belongingness, esteem, and eventually individual self-actualization (Taormina, Gao, 2013). Maslow's theory of human motivation is one of the landmark theories for describing human motivation, and its applicability in a wide variety of contexts has been proven multiple times besides the criticism and support expressed in current literature. In that sense, the theory claims that if an employee has met all the basic human needs, they are more likely to feel motivated and stay in their workplace (Trivedi, Mehta, 2019). Additionally, it has been found that the satisfaction of every higher-level need is influenced by the satisfaction of the need, which is just below it in Maslow's hierarchy (Taormina, Gao, 2013).

Strategies of Financial and Non-Financial Motivation

Financial Motivation

Financial incentives have historically been employed to motivate employees, as exemplified by Frederick Taylor's use of financial rewards in the late 19th century to stimulate employee productivity (Vroom, 1964). These incentives ranged from salary bonuses to innovation fees, fostering financial well-being. Direct material benefits often include salaries and bonuses, which contribute to the financial well-being of the workforce without any indirect effects (Spisakova, 2019). On the other hand, the indirect material benefits can include scholarships for additional education, study visits, and making company cars available at the disposal of the employee, which doesn't involve a direct transfer of cash (Alam, Hassan, Bowyer, Reaz, 2020). These two forms of material benefits constitute the two primary forms of financial compensation. However, the evolving landscape of employee compensation now calls for a more flexible, personalized approach.

Non-Financial Motivation

Beyond financial incentives, many managers recognize the effectiveness of non-financial rewards in enhancing employee satisfaction. Certain non-financial motivators, aligned with individual preferences, can yield high satisfaction levels. The COVID-19 pandemic has further underscored the importance of non-financial incentives in optimizing organizational costs while bolstering employee motivation (Mani, Mishra, 2020).

Non-financial motivators can be flexible, personalized, and easy to implement, offering long-term motivational benefits. Intrinsic rewards, such as meaningful work, are particularly potent in maintaining long-term motivation, while extrinsic rewards, such as money, may provide short-term impetus (Blaskova, Grazulis, 2009). Consequently, the optimal motivation strategy often involves a combination of both types.

Several landmark strategies for non-financial motivation stand out in current literature on employee motivation (Swartling, Poksinska, 2013; Kumar, Hossain, Nasrin, 2015):

- Workplace Design and Organization: The design of the workplace can significantly impact employee performance, job satisfaction, and physical and mental health.
- Flexible Working Hours: Shift work trends have increased, but attention to employee well-being during night shifts is crucial.
- Employee Involvement in Decision-Making: Employees at all levels desire participation in decision-making processes, as they often possess valuable insights into operational challenges.
- Staff Training: Continuous learning and development are essential for individuals and organizations.
- Optimal Working Conditions: Safe, well-ventilated, and ergonomically designed workplaces are critical.
- Respect for Employees: Respect and professional development are fundamental for employee performance.
- Work-Life Balance: Achieving harmony between work and personal life is essential for employee retention.
- Job Type: The nature of work profoundly affects motivation, with job characteristics aligning with Maslow's theory playing a crucial role.
- Workload Management, Job Rotation, and Job Enrichment: Increasing workload, job rotation, and job enrichment strategies can enhance motivation and skills development.

Defining the Term Generation

Generations consist of individuals born and living during approximately the same historical period. Generational classification typically includes attributes such as year of birth, a sense of belonging to the generation, common beliefs and behaviors, and shared historical experiences. Generational understanding is paramount in the context of the workplace.

Generation Y

Generation Y, often referred to as Millennials, presents a unique challenge to HR managers. They have grown up in a world dominated by technology, where instant messaging, DVDs, and mobile phones are ubiquitous. Their close connection to the digital realm results from the pervasive use of technology in education. Generation Y thrives in a non-linear, fast-paced environment, adapting effortlessly to multiple sources of information. This adaptability positions them as well-prepared for dynamic work environments.

Some of the wishes and needs that are often attributed to members of this organization include the need for a more balanced work-life relationship as well as more flexibility in terms of remote jobs or jobs that go beyond the traditional nine-to-five schedule (Du Plessis, Barkhuizen, Stanz, Schutte, 2015). They tend to be results-oriented, ambitious, and collaborative when working in teams. In other words, the social needs are more pronounced in this generation. Another habit of this generation is their frequent use of technology as one of the first massively digitally literate generations in the workplace (Buzza, 2017).

However, many Generation Y members lack certain skills sought by HR managers for specific roles within organizations. They prioritize career suitability, work-life balance, and independence over mere job acquisition, with job satisfaction outweighing monetary rewards. Understanding and catering to the needs of this generation is imperative for HR managers to successfully manage and retain talented individuals. Generation Y places a premium on access to knowledge and technology, the fulfillment of educational demands, participatory management, compensation flexibility, feedback, teamwork, negotiation readiness, technology adoption, professional use of the internet, and a conducive online presence.

In this sense, Generation Y's attributes and expectations necessitate a thorough understanding by HR managers, who must adapt their strategies to harness this generation's potential while navigating the challenges associated with its unique characteristics.

HR Managers' Role in Understanding and Motivating Generation Y

To successfully manage and retain the talent within Generation Y, HR managers must grasp their unique needs and motivations. Here are key considerations (Kultalahti, Viitala, 2015; Ray, Singh, 2016; Du Plessis, Barkhuizen, Stanz, Schutte, 2015):

Access to Knowledge and Technology. Talented workers demand access to up-to-date technology. Organizations must stay abreast of technological advancements to attract and retain Generation Y employees. This entails a continuous cycle of upgrading outdated technology to remain competitive.

Meeting Training Needs. While Generation Y may have grown up in a technologically advanced environment, it doesn't necessarily mean they possess all the skills required for their roles. HR managers must recognize the importance of ongoing learning and development in a rapidly changing world. Formal education doesn't end at a specific age; instead, it becomes a lifelong pursuit.

Participatory Management. The workforce of the future craves participation in decision-making processes. Generation Y seeks to have their voices heard and to actively contribute to organizational changes. HR managers must foster a culture of inclusion and participation.

Compensation Flexibility. Unlike the rigid compensation structures of the past, today's compensation is often more flexible and tailored to individual needs. HR managers need to adapt to this shift and offer options such as additional education benefits, parental leave, and retirement plans that suit the diverse needs of Generation Y employees.

Providing Feedback. Generation Y values feedback, often seeking affirmation for a job well done. HR managers should cultivate a feedback-rich environment that provides constructive praise and recognition.

Embracing Teamwork. This generation thrives in collaborative settings. They prefer seeking multiple opinions and working in teams. HR managers should design teams to maximize their effectiveness as a whole.

Negotiation Readiness. Generation Y resists rigid rules and seeks logical explanations for actions. They've been raised in an environment that encourages negotiation. HR managers should be prepared for questions and discussions.

Fearless Technology Adoption. This generation fearlessly adopts new technologies, but their confidence can sometimes lead to challenges. HR managers must guide and support them, addressing any overconfidence that may arise.

Managing Online Presence. Generation Y's constant use of the internet, including personal social media accounts, can pose challenges. HR managers must establish clear guidelines to ensure professional conduct online, protecting both the organization's reputation and the individual's privacy.

In navigating these considerations, HR managers can harness the potential of Generation Y, providing an environment where they can thrive while contributing to the organization's growth in the 21st century.

MATERIAL AND METHODS

The research conducted for this study utilized an electronic survey approach. The data collection period spanned from March 28, 2023, to April 12, 2023. The primary objective of the survey was to investigate the impact of motivational factors on individuals belonging to Generation Y. To achieve this, a structured questionnaire comprising a total of 12 questions was developed. The survey instrument employed a Likert scale, enabling respondents to express their level of agreement or disagreement on a five-point scale, as follows: 1 = Strongly Disagree, 2 = Disagree, 3 = Neutral (Neither Agree nor Disagree), 4 = Agree and 5 = Strongly Agree.

A total of 106 participants voluntarily took part in the survey. Of these, 48 identified as female, and 58 identified as male. The research aimed to capture a diverse sample of Generation Y individuals to ensure a comprehensive understanding of their perceptions regarding motivational factors. All the participants identified as currently employed in an organization operating on the territory of the Republic of North Macedonia.

RESULTS AND DISCUSSION

Motivational Factors for Generation Y

In this section, we present the key findings regarding the motivational drivers for Generation Y, based on the responses collected from the survey participants.

Salary as a Motivational Driver

The survey sought to assess the significance of salary as a motivator among Generation Y individuals. The results indicate that a substantial 50% of respondents consider salary to be of great importance in motivating them, while only a mere 4% do not find it important for motivation. This emphasizes the centrality of salary as a motivational factor within this generation (Fig. 1).

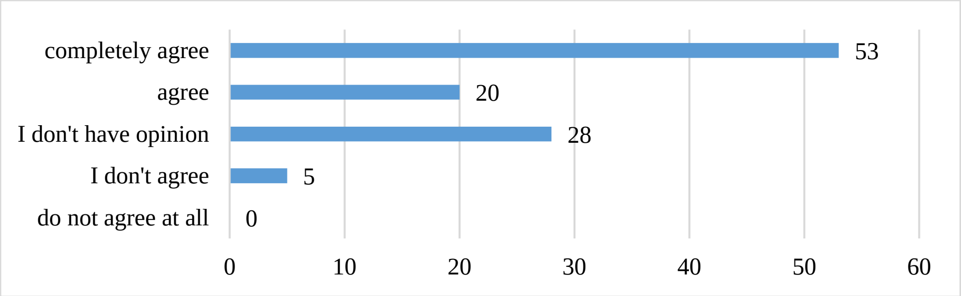


Figure 1 Salary as a motivational driver

Bonuses and Incentives as Motivational Boosters

Similar to the findings concerning salary, the responses regarding bonuses and incentives yielded comparable results. It is evident that both bonuses and incentives are significant motivational boosters for Generation Y, aligning with the importance they place on these forms of recognition and reward (Fig. 2).

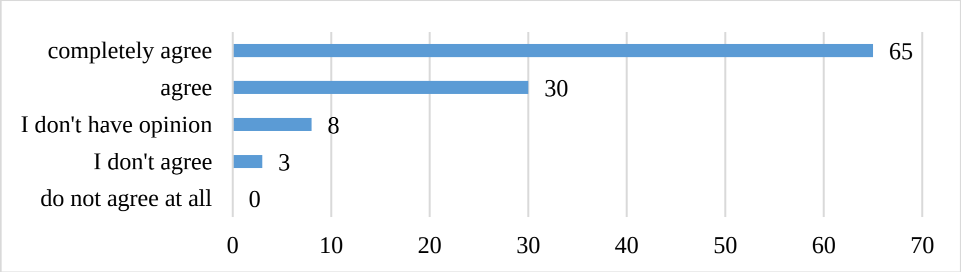


Figure 2 Bonuses and incentives as a motivational booster

Company Car as a Motivational Booster

When it comes to the provision of a company car as a motivator, respondents demonstrated a preference for this benefit (Fig. 3). Notably, having a company car not only signifies status within society but also holds importance among their peers within Generation Y.

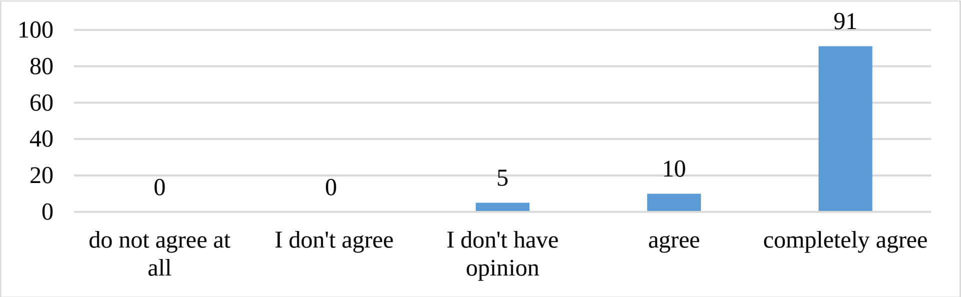


Figure 3 Company car as a motivational booster

Other Motivational Triggers

The table presents responses related to various motivational factors, such as security on paid free days (as a reward), career development, access to paid training, feedback, balance between private and professional life, and independence[3]:

Table 1 Other motivational triggers

	Do not agree at all	I don't agree	I don't have an opinion	Agree	Completely agree	Average
Answer/corresponding factor	***(1)***	***(2)***	***(3)***	***(4)***	***(5)***	*
Reward and security on paid free days	0	2	10	35	59	4,41
Development of career	0	0	0	17	89	4,84
Access and paid trainings	0	0	0	26	80	4,75
Feedback	0	0	2	36	68	4,62
Balance of the private-professional life	0	0	0	10	96	4,91
Independence	0	0	0	12	94	4,89

Source: Authors' own research

The findings from this survey underscore the significance of the listed motivational factors for Generation Y in the workplace. These motivators play a vital role in enhancing the efficiency and effectiveness of this generation. Therefore, organizations should pay special attention to these motivational triggers in their efforts to engage Generation Y more effectively.

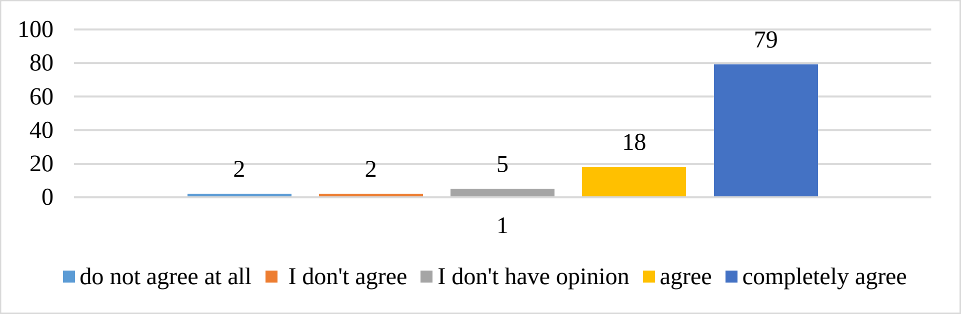
The results demonstrate a high level of consensus among Generation Y members regarding the importance of the motivational factors listed in Table 1. Their preferences align with these factors, indicating a clear inclination toward benefits and conditions that promote motivation.

Clear Responsibility as a Motivational Driver

Generation Y respondents exhibited a unanimous preference for clear responsibility as a motivational driver (Fig. 4).

An impressive 100% of the respondents expressed agreement with this notion, highlighting the importance of well-defined roles and accountabilities in their work.

[3] We weighed the answers: do not agree at all with 1, I don't agree with 2, I don't have opinion with 3, agree with 4 and completely agree with 5. Then, we calculated a mean value of the given answers, weighed by the corresponding factor.

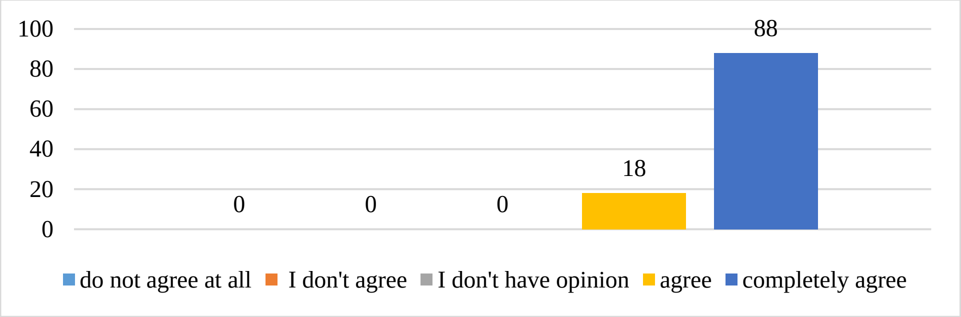


Source: Authors' own research

Figure 4 Clear responsibility as a motivational driver

Participation in Decision-Making as a Motivational Booster

In terms of participation in decision-making, Generation Y individuals indicated a strong desire to have their voices heard. The chart shows that the vast majority of respondents seek active involvement in decision-making processes (Fig.5).

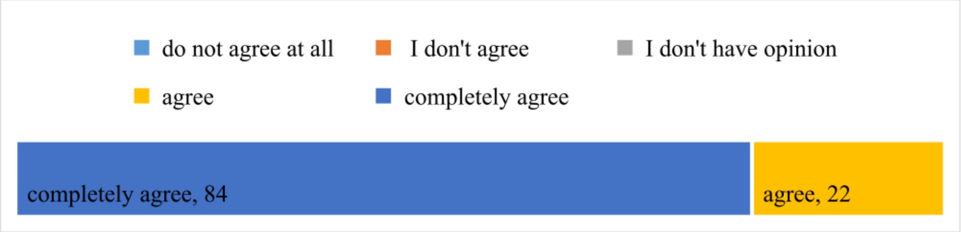


Source: Authors' own research

Figure 5 Participation in decision-making as a motivational booster

Flexible Work and Flexible Schedules

Lastly, with respect to flexible work arrangements and schedules, Generation Y demonstrates a clear preference (Fig.6).



Source: Authors' own research

Figure 6 Flexible work and flexible schedules

The graph indicates that respondents overwhelmingly agree (groups 4 and 5) with the importance of flexibility in their work arrangements.

These findings collectively shed light on the motivational preferences of Generation Y, emphasizing the importance of aligning workplace practices with their expectations and needs. Organizations that recognize and cater to these preferences are better positioned to engage and retain this dynamic generation of workers.

When the members and demographics of the workforce are profiled, HR professionals can formulate and implement specific, custom-tailored strategies for boosting employee motivation at the workplace per the principles of strategic HR management (Tessema, Tesfom, Faircloth, Tesfagiorgis, Teckle, 2022). Based on the research results, HR professionals can consider participatory strategies for motivating employees who are members of Generation Y. Bottom-up and holistic strategies, which stimulate a collaborative environment and at the same time flexible working arrangements (Kusumah, Fitriani, Satriadi, 2023), are more likely to keep this segment of the workforce more engaged and satisfied as their primary source of motivation likes in the financial motivators and their value for freedom and contribution in the organization. Thus, HR professionals and business leaders can benefit from including them and their experience-driven ideas in the decision-making processes and the formulation of the HR strategies themselves.

The study doesn’t come free of a few constraints, which at the same time are potential avenues for future research. To begin with, the chosen research context was the Republic of North Macedonia, yet the study can be further scaled up and implemented in other countries and regions to gain a cross-regional aspect of the study. Moreover, future researchers can explore how different employees who are members of Generation Y find motivation based on their years of experience in the organization, years of experience in the same job position, sector, or industry where they work, their educational background, and similar.

CONCLUSION

In the ever-evolving landscape of the twenty-first century, effective human resource management has emerged as a pivotal source of competitive advantage for organizations, possibly even surpassing other factors in long-term performance. As competition among organizations intensifies, the ability to replicate technology, production processes, and products becomes increasingly common. However, what remains challenging to emulate is the intricate realm of human resource management. Thus, human resources have assumed the role of a distinctive and unparalleled competitive edge for organizations. In the current milieu characterized by rapid changes and unique organizational conditions, the management of human resources stands as a linchpin for organizational success. Motivation, both as a managerial function and as a phenomenon within the intricate workings of enterprises operating in turbulent environments, presents a rich arena for exploring methods and their practical application in daily operations. Motivating employees in companies has evolved over the course of management's historical development. Understanding the nuances of motivating Generation Y, a demographic marked by distinct characteristics, is imperative. In this regard, it is crucial to first acknowledge their distinctive traits and then tailor strategies accordingly, such as a participatory strategy and a strategy for flexible working arrangements. One of the critical dimensions in which Generation Y diverges from other generations lies in their motivational drivers. While extensive research has explored motivation in the past, yielding various theories and motivator types, this study has cast a spotlight on how these motivators resonate with Generation Y. Born and raised in an era defined by pervasive information technology and a unique mindset, Generation Y exhibits a distinct outlook on life. Drawing from a comprehensive review of the literature, this research has identified a key priority for Generation Y concerning motivational triggers: achieving a harmonious balance between their private and professional lives. Additionally, their preferences encompass the necessity for collaborative teamwork, dynamic and diversified work responsibilities, a nuanced understanding of compensation structures, active participation in decision-making processes, and avenues for career development. Empirical evidence substantiates that the theoretical tenets concerning Generation Y's behavior with regard to motivational drivers closely align with reality. Notably, the research conducted in the Republic of North Macedonia underscores those members of Generation Y accord high importance to factors such as compensation, teamwork, the equilibrium between private and professional life, career advancement, and more. As organizations continue to adapt to the changing times, recognizing and embracing the motivational preferences of Generation Y is pivotal. Those who successfully integrate these preferences into their human resource management strategies are poised to not only attract but also retain the talents of this unique and influential generation, ensuring a competitive edge in an ever-evolving landscape.

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INFLUENCE OF SEEDING RATE ON THE PRODUCTIVITY AND QUALITY OF SOFT SPRING WHEAT GRAIN (*TRITICUM AESTIVUM* L.)

SUMMARY

The grain industry in Ukraine is the most important component of the agro-industrial complex. At the same time, spring wheat grain production remains low and unstable due to insufficient efficiency of cultivation technology. The purpose of the research was to establish optimal seeding rates for soft spring wheat (*Triticum aestivum* L.) seeds of the Shirocco variety. The study of the influence of the seeding rate on the productivity and quality indicators of soft spring wheat was carried out according to the scheme: 3.5 million pcs ha^{-1}, 4.0 million pcs ha^{-1}, 4.5 million pcs ha^{-1}, 5.0 million pcs ha^{-1}; 5.5 million pcs ha^{-1}; 6.0 million pcs ha^{-1}. As a result of the research, it was found that the highest density of standing was obtained on the variant with a seeding rate of 6.0 million pcs ha^{-1} – 522 pcs m^2 with plant preservation during the growing season of 86.8% (453.1 pcs m^2). The maximum yield was observed on the variant with a seeding rate of 6.0 million pcs ha^{-1} – 5.63 t ha^{-1}. At a seeding rate of 3.5, 4.0, 4.5, 5.0, 5.5 pcs m^2 the yield was 4.07, 4.58, 4.76, 5.00, 5.30 t ha^{-1}, respectively. According to the results of the research, it was studied that at a seeding rate of 6.0 million pcs ha^{-1}, the highest yield of spring wheat grain was obtained – 5.63 t ha^{-1}, and the maximum quality indicators of gluten and protein content at a seeding rate of 5.5 million pcs ha^{-1} – 25.5, 13.82%, respectively.

Keywords: seeding rate, standing density, productivity, yield, protein, gluten.

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INTRODUCTION

The grain industry in Ukraine is the most important component of the agro-industrial complex. Grain is the basis for food production, raw materials for many industries, fodder for animals and is an integral part of the country's food security. Grain is also the main exchange-traded food commodity (Halenko, 2017). Wheat occupies a prominent place among grain crops around the world and is the main food product for about 35% of the world's population and provides about 20% of humanity's energy needs (Demydov *et al.,* 2017a; Velimirović *et al.,* 2023). Today, on the territory of our country, the question arises of solving the scientific and technical problem of improving the regulatory provision of grain quality and harmonizing it with international standards (Khomenko *et al.,* 2018). Significant success in social and cultural development is achieved only by those countries that are able to ensure the highest quality of their products. Therefore, the grain industry of Ukraine is faced with the task of ensuring a stable growth of grain production and the achievement of certain quality characteristics, which should satisfy both all the needs of the country in the domestic market and provide an opportunity to be competitive in the foreign market (Kaflevska and Kozyar, 2013).

Spring wheat is a valuable grain crop and is not inferior in quality to winter wheat, and the emergence of new varieties of strong wheat makes it possible to obtain a higher grain quality than that of winter wheat varieties under the same soil and climatic growing conditions (Yula, 2016; Yarchuk *et al.,* 2019; Litvinov *et al.,* 2020).

Grain, which is obtained from soft varieties of spring wheat, is used to make bread, confectionery, cereals and pasta. In Ukraine, it is sown on an area of only 160–190 thousand hectares. Such a slight distribution of spring wheat is explained by the fact that it is significantly inferior in terms of grain yield of winter wheat (Zinchenko, 2016).

But in recent years, there has been an upward trend in spring wheat crops. From the status of insurance, it is gradually turning into one of the main crops, the cultivation of which is aimed at the production of high-quality grain to improve baking properties (Tanchyk *et al.*, 2019; Nazarenko *et al.*, 2023).

During the sowing process, it is imperative to recognize that augmented seeding rates do not confer a proportional increase in the yield of spring wheat. Conversely, elevated seeding rates engender heightened grain consumption, elevate susceptibility to lodging, pests, and diseases. Conversely, inadequate seeding rates contribute to delayed tillering and uneven ripening of the crop, as substantiated by the findings of Karpenko *et al.* (2020). To a large extent, the seeding rate of wheat depends on the biological characteristics of the variety. Varieties that are characterized by high bushiness and plant height, unstable to lodging, provide maximum grain yield at reduced seeding rates, and low-growing and semi-dwarf varieties – at increased ones. Therefore, for undersized and semi-dwarf varieties, it is recommended to increase the seeding rate by 0.5–1.0 million pcs ha^{-1}, compared to tall varieties. However, even varieties with a low stem at an increased seeding rate (more than 6 million pcs ha^{-1}) suffer from a deterioration in illumination in crops, an increase in nutrient and water consumption, and a higher incidence of plant diseases, resulting in a decrease in yield (Kochmarsky *et al.,* 2014).

There are recommendations for the use of thicker crops on weedy lands. The augmentation of seeding rates from 3 to 5 million grains per hectare resulted in a notable reduction in weed infestation, exhibiting a decrease ranging from 1.2 to 1.5 times. The seeding rate of spring wheat is higher than that of winter wheat, since it does not have an autumn tillering period, as a result of which it has a lower tillering coefficient (about 1.3). Under current conditions, it is recommended to sow wheat after the worst predecessors of 5.5–6.0, and after the best 5.0–5.5 million germinating grains per hectare (Hansueli and Rainer, 2015).

The purpose of the study was to determine the influence of the seeding rate on the processes of growth and development, the formation of yield and grain quality of spring wheat (*Triticum aestivum* L.).

MATERIAL AND METHODS

The study of the seeding rate of soft spring wheat was carried out in the conditions of a field stationary experiment on the territory of the educational and scientific production center of Sumy National Agrarian University. The experimental field is located in the Sumy district of the Sumy region, Ukraine, geolocation data 50°52.742N latitude, 34°46.159E longitude, 137.7 m above sea level (50°52'46.6"N 34°46'07.8"E Map date ©2023 Google). The study was conducted during 2021–2023.

The study of the impact of seeding rates on the productivity and quality indicators of soft spring wheat was carried out according to the scheme: 3.5 million pcs ha^{-1}, 4.0 million pcs ha^{-1}, 4.5 million pcs ha^{-1}, 5.0 million pcs ha^{-1}; 5.5 million pcs ha^{-1}; 6.0 million pcs ha^{-1}. The object of the study was a variety of soft spring wheat Shirocco. The technology of spring wheat cultivation is generally accepted for the conditions of the Forest-Steppe zone of Ukraine, except for the questions posed to the study. The studies were carried out after the predecessor soybean. Sowing of spring wheat was carried out with a seeder Maple – 1.5, at a soil temperature of 6–8 °C. The method of sowing is ordinary continuous, the depth of seed incorporation is 3–4 cm. During cultivation, fertilizer was applied at a dose of $N_{32}P_{32}K_{32}$ as a background fertilizer. Nitrogen-phosor-potassium complex mineral fertilizer was applied. Mass fraction: total nitrogen – 16 ± 1%, toaliy in terms of K_2O – 16% ± 1%, total phosphates – 16 ± 1%. The plots were systematically arranged one after the other, the area of 1 plot was 50 m^2 and the computing area was 30 m^2.

The dynamics of ground mass growth was determined at the main stages of growth and development by selecting 25 plants in a typical plot with two incompatible repetitions, and the yield structure by selecting test sheaves from each accounting plot (Pidoprygora and Pisarenko, 2003).

The amount of gluten was determined by manual washing in water according to STST 13586.1–68 Grain (СТСТ 13586.1–68). Methods for determining the quantity and quality of wheat gluten. The amount of protein was determined according to STST 10846–91 (СТСТ 10846–91) "Grain and products of its processing". Protein determination method.

Mathematical and statistical processing of experimental data and determination of the reliability of the results obtained was carried out according to Dospekhov (1985) using Microsoft Excel.

The soil of the test site is a typical thick heavy loamy and medium-humus chernozem, which is characterized by the following indicators: the content of humus in the arable layer (according to I.V. Tyurin) is 4.0%, the reaction of the soil solution is close to neutral (pH 6.5), the content of easily hydrolyzed nitrogen (according to I.V. Tyurin) is 9.0 mg, mobile phosphorus and exchangeable potassium (according to F. Chirikov) is 14 mg and 6.7 mg per 100 g of soil, respectively.

The average daily annual air temperature in 2021 was 9.4 °C, which is 2.0 °C higher than the long-term indicator of 7.4 °C. Its absolute maximum of 35.0 °C was recorded in June in the third decade, and the minimum was minus 24.0 °C in the second decade of January. The amount of precipitation for the reporting 2020–2021 agricultural year was 453 mm, which is 140 mm less than the long-term indicator (593 mm). Precipitation was distributed by periods of the year in the following order: autumn 2020 – 75 mm (54% of the long-term indicator of 139 mm); winter 2020–2021 – 90 mm (74% of the long-term indicator of 122 mm); spring 2021 – 119 mm (90% of the long-term indicator of 132 mm); summer 2020 – 169 mm (85% of the long-term indicator of 200 mm).

The average daily annual air temperature in 2022 was 8.7 °C, which is 1.3 °C higher than the long-term indicator of 7.4 °C. Its absolute maximum of 36 °C was recorded in June in the third decade, and its minimum in January in the second decade minus 18.0 °C. The amount of precipitation for the reporting 2021–2022 agricultural year was 604 mm, which is 11 mm more than the long-term indicator (593 mm). Precipitation was distributed by periods of the year in the following order: autumn 2021 – 96 mm (69% of the long-term indicator of 139 mm); winter 2021–2022 – 103 mm (84% of the long-term indicator of 122 mm); spring 2022 – 144 mm (109% of the long-term indicator of 132 mm); summer 2022 – 261 mm (130% of the long-term indicator of 200 mm).

The average daily annual air temperature in 2023 was 9.0 °C, which is 1.6 °C more than the long-term indicator of 7.4 °C. Its absolute maximum of 36 °C was recorded in August in the first decade, and its minimum in January in the first decade was minus 19 °C. The amount of precipitation for the reporting 2022–2023 agricultural year was 634 mm, which is 41 mm more than the long-term indicator (593 mm). Precipitation was distributed by periods of the year in the following order: autumn 2022 – 176 mm (127% of the long-term indicator of 139 mm); winter 2022–2023 – 102 mm (84% of the long-term 122 mm); spring

2023 – 83 mm (63% of the long-term figure of 132 mm); summer 2023 – 273 mm (136% of the long-term indicator of 200 mm).

In general, 2022 and 2023 were the most favorable for the formation of crop yields. Dry conditions developed in 2021, which was characterized by low rainfall and extreme deviation in air temperature during the growing season.

RESULTS AND DISCUSSION

It is well known that pre-sowing preparation of grain seeds is an important condition in increasing the yield of grain crops. By sowing seeds with high varietal and sowing qualities, it is possible to obtain a yield increase of more than 30%. Due to the increase in the germination rate of seeds, their quality also improves. It has been proven that field germination, productivity and quality are closely interrelated (Alimov and Shelestov, 1995; Sviderko *et al.*, 2004). In the studies of Sumy National Agrarian University, it was found that the field germination of spring wheat ranged from 87.0 to 89.8% (LSD_{05} (Least Significant Difference) = 0.56). The maximum indicators of field germination were noted on the variant with a seeding rate of 3.5 million pcs ha^{-1} and amounted to 89.8%, slightly lower indicators were observed at a seeding rate of 4.0 million pcs ha^{-1} – 89.4%, 4.5 million pcs ha^{-1} – 88.7%, 5.0 million pcs ha^{-1} – 88.0%, 5.5 million pcs ha^{-1} – 87.2%, 6.0 million pcs ha^{-1} – 87.0%. The standing density ranged from 314.3 pcs m^2 to 522.0 pcs m^2 (LSD_{05} = 9.45). The highest density of spring wheat was obtained at a maximum seeding rate of 6.0 million pcs ha^{-1} and amounted to 522.0 pcs m^2, a decrease in the seeding rate led to a decrease in the density of standing. Thus, the lowest density of spring wheat was obtained on the variant of 3.5 million pcs ha^{-1} and amounted to 314.3 pcs m^2 (Table 1).

Table 1. Stand density of soft spring wheat depending on the seeding rate (average for 2021–2023)

Seeding rate, million pcs ha^{-1}	Field germination, %	Standing density, pcs m^2	Plant safety during the growing season	
			pcs m^2	%
3.5	89.8	314.3	283.5	90.2
4.0	89.4	357.6	321.8	90.0
4.5	88.7	399.2	357.2	89.5
5.0	88.0	440.0	389.0	88.4
5.5	87.2	479.6	418.7	87.3
6.0	87.0	522.0	453.1	86.8
LSD_{05}	0.56	9.45	10.0	0.36

The preservation of plants during the growing season ranged from 86.8 to 90.2% (LSD_{05} = 0.36). The maximum preservation of plants was recorded on the variant with a seeding rate of 3.5 million pcs ha^{-1} – 90.2% (283.5 pcs m^{2}), and the lowest preservation was obtained using a seeding rate of 6.0 million pcs ha^{-1} –

86.8% (453.1 pcs m^2). For sowing spring wheat with a seeding rate of 4.0 million pcs ha^{-1} plant safety was 90.0% (321.8 pcs m^2), 4.5 million pcs ha^{-1} – 89.5% (357.2 pcs m^2), 5.0 million pcs ha^{-1} – 88.4% (389.0 pcs m^2), 5.5 million pcs ha^{-1} – 87.3% (418.7 pcs m^2) (Table 1).

A decrease in crop productivity is caused by a deviation of the seeding rate from the optimum. Competition between plants increases with unreasonably high seeding rates, as a result of which the productivity of individual plants and sowing as a whole decrease. An increase in the seeding rate leads, as a rule, to a decrease in the coefficient of total tillering, the mass of dry matter in the plant and the number of nodal roots. The ability to bush in wheat, as well as in other cereals, is determined by both the method of sowing and the seeding rate and is related to the area of nutrition: the number of productive stems decreases with a decrease in the area of nutrition and vice versa (Cherenkov *et al.*, 2009). According to the results of the research, the indicator of the coefficient of productive tillering in the conditions of Sumy National Agrarian University ranged from 1.23 to 1.33 (LSD_{05} = 0.56). Thus, the highest coefficient of productive tillering was obtained at a seeding rate of 3.5 million pcs ha^{-1} and was 1.33, and the lowest at the seeding rate was 6.0 million pcs ha^{-1} – 1.23 (Table 2).

A fairly important indicator in which soft wheat forms the highest yield is the density of productive stems: 400–500 plants per 1 m^2. Such plant density requires a mild seeding rate for wheat after the best and worst predecessors: 5.0–5.5 and 5.5–6.5 million pcs ha^{-1} respectively (Demydov *et al.*, 2017b). In the conditions of the educational and scientific production center of Sumy National Agrarian University, it was noted that the number of productive stems was the highest in the variant with a seeding rate of 6.0 million pcs ha^{-1} – 557.3 pcs m^2. The smallest number of productive stems was observed at a seeding rate of 3.5 million pcs ha^{-1} – 377,1 pcs m^2. At a seeding rate of 4.0 million pcs ha^{-1} of productive stems amounted to 427.8 million pcs ha^{-1}, 4.5 million pcs ha^{-1} – 453.6 pcs m^2, 5.0 million pcs ha^{-1} – 486.3 pcs m^2, 5.5 million pcs ha^{-1} – 519.2 pcs m^2 (Table 2).

Table 2. Productive tillering coefficient and number of productive stems depending on the seeding rate (average for 2021–2023)

Seeding rate, million pcs ha^{-1}	Productive tillering coefficient	Number of productive stems, pcs m^2
3.5	1.33	377.1
4.0	1.32	427.8
4.5	1.27	453.6
5.0	1.25	486.3
5.5	1.24	519.2
6.0	1.23	557.3
LSD_{05}	0.03	9.13

One of the main factors in the yield of grain crops is the weight of the plant, the weight of the ear and the length of the ear. Formation takes place during the period when plants are best provided with light, moisture, heat and other vital factors (Kalenska and Shutyy, 2015). In the conditions of the educational and scientific production center of Sumy National Agrarian University, the weight of the spring wheat plant depended on the seeding rate and ranged from 2.27 g to 2.96 g (LSD_{05} = 0.58). The highest weight of the plant was obtained at a seeding rate of spring wheat of 3.5 million pcs ha^{-1} – 2.96 g, and ear weight – 1.80 g. The lowest weight indicators were obtained at the seeding rate of 6.0 million pcs ha^{-1} – 2.27 and 1.42 g, respectively. At the seeding rate of 4.0 million pcs ha^{-1} – 2.88, 1.73 g, 4.5 million pcs ha^{-1} – 2.78, 1.70, 5.0 million pcs ha^{-1} – 2.64, 1.62 g, 5.5 million pcs ha^{-1} – 2.45, 1.51 g, respectively (Table 3).

The length of an ear of wheat is largely determined by the genotype, but it also depends on the growing conditions. The ear of wheat plants can be of different lengths: short – up to 8 cm, medium – 8–10, long – more than 10 cm. Under unfavorable conditions for the development of the growth cone, in particular, the lack of nutrients and moisture in the soil, strong thickening of plants, lack of light, spikelet tubercles in the upper part of the growth cone underdeveloped and dry out. This leads to a sharp reduction in the size of the ear. In addition, some of the spikelet’s die off in the process of growth and development. In addition to varietal characteristics and meteorological conditions, the size of the ear is also influenced by certain elements of the technology, in particular the seeding rate, with the help of which it is important to delay the time of laying the apical spikelet in the ear (Shelepov et al., 2007). According to the results of research conducted in the conditions of Sumy National Agrarian University, the length of the ear changed due to the seeding rate. Thus, the highest ear length was noted in the variant for spring wheat with a seeding rate of 3.5 million pcs ha^{-1} and was 9.2 cm, the shortest ear length was observed at a seeding rate of 6.0 million pcs ha^{-1} – 7.8 cm (Table 3).

Table 3. Weight of the plant and ear, ear length of soft spring wheat depending on the seeding rate (average for 2021–2023)

Seeding rate, million pcs ha^{-1}	Plant weight, g	Ear weight, g	Spike length, cm
3.5	2.96	1.80	9.2
4.0	2.88	1.73	9.0
4.5	2.78	1.70	8.7
5.0	2.64	1.62	8.4
5.5	2.45	1.51	8.0
6.0	2.27	1.42	7.8
LSD_{05}	0.58	0.04	0.28

The grain weight of an ear is a sign of structures such as length, the number of ears and grains in an ear, the weight of 1000 grains and is due to many genes with different types of interactions. In practice, the mass of the grain of the ear has always been given one of the central places. Ear selection is the main principle of work of many breeders (Shakalii, 2017). The maximum weight of grain per ear in the experiments of Sumy National Agrarian University varied in the range of 1.01–1.08 g (LSD_{05} = 0.02). The highest weight of grain per ear was obtained at a seeding rate of 3.5 million pcs ha^{-1} and amounted to 1.08 g, slightly lower indicators were observed at the seeding rate of 4.0 million pcs ha^{-1} – 1.07 g, 4.5 million pcs ha^{-1} – 1.05 g, 5.0 million pcs ha^{-1} – 1.03 g, 5.5 million pcs ha^{-1} – 1.02 g and 6.0 million pcs ha^{-1} – 1.01 g (Table 4).

The grain content of the ear and the total tilleriness are inversely dependent on each other, since a large number of nutrients are spent on the creation of the vegetative mass, and by the time of ear formation, their content in the most bushed plants becomes insufficient for the full formation of the crop. The number of grains per ear increases with a decrease in the seeding rate to 3.0–4.0 million pcs ha^{-1} (Lykhochvor, 2004). According to Yula and Oliinyk (2013), Macholdt and Honermeier (2017), high productivity of winter wheat crops can be achieved only if there is an optimal ratio between the components of productivity, which are laid down at the early stages of plant development and formed during the growing season. In the studies carried out by Sumy National Agrarian University, it was found that the maximum number of grains was obtained on the variant with a seeding rate of 3.5 million pcs ha^{-1} – 28.2 pcs. An increase in the seeding rate led to a decrease in the number of grains in ears of corn. Thus, the smallest amount of grains was obtained on a warrant with a seeding rate of 6.0 million pcs ha^{-1} – 27.2 pcs. At seeding rates of 4.0, 4.5, 5.0, 5.5 million the number of grains ha^{-1} was 28.0, 27.8, 27.5, 27.4 pcs., respectively (Table 4).

Table 4. Structural indicators of the spring bread wheat plant depending on the seeding rate (average for 2021–2023)

Seeding rate, million pcs ha^{-1}	Grain weight per ear, g	Number of grains per ear, pcs.	Weight of 1000 seeds, g
3.5	1.08	28.2	38.3
4.0	1.07	28.0	38.2
4.5	1.05	27.8	37.8
5.0	1.03	27.5	37.5
5.5	1.02	27.4	37.2
6.0	1.01	27.2	37.1
LSD_{05}	0.02	0.16	0.15

From a breeding point of view, the weight of 1000 grains are of great importance, which is a reliable indicator in the selection for yield (Shakalii, 2020). The weight of 1000 grains depend on both environmental factors and the seeding rate, as a result of which it can vary widely (Chetveryk *et al.*, 2021). According to the results of research in the conditions of the educational and scientific production center of Sumy National Agrarian University, the weight of 1000 grains ranged from 37.1 to 38.3 g (LSD_{05} = 0.15). Thus, the highest weight of 1000 grains were noted in the variant with a seeding rate of 3.5 million pcs ha^{-1} – 38.3. An increase in the seeding rate led to a decrease in the weight of 1000 grains and the smallest weight was observed at a seeding rate of 6.0 million pcs ha^{-1} – 37.1 g (Table 4).

Dry conditions in recent years have led to low yields of spring wheat, which did not exceed 3.52 t ha^{-1}. Modern varieties of spring wheat of foreign and domestic selection, included in the State Register of Plant Varieties of Ukraine, have a high yield potential and can provide a yield of 5–8 t ha^{-1} in production conditions (Lozinska and Fedoruk, 2017; Radchenko *et al.*, 2023). As a result of the research conducted by Sumy National Agrarian University, it was found that the yield according to the experiment options ranged on average from 4.07 t ha^{-1} to 5.63 t ha^{-1} (LSD_{05} = 0.12). The maximum yield of spring wheat was obtained at a seeding rate of 6.0 million pcs ha^{-1} and amounted to 5.63 t ha^{-1}. A decrease in the seeding rate affected the decrease in grain yield. So, at a seeding rate of 5.5 million pcs ha^{-1}, the yield was 5.30 t ha^{-1}, 5.0 million pcs ha^{-1} – 5.00 t ha^{-1}, 4.5 million pcs ha^{-1} – 4.76 t ha^{-1}, 4.0 million pcs ha^{-1} – 4.58 t ha^{-1} and at a seeding rate of 3.5 million pcs ha^{-1} – 4.07 t ha^{-1} (Table 5).

The crude gluten content in wheat grain ranges from 5 to 36%. The gluten content of wheat grains and the physical properties that characterize their quality can vary significantly (Hasanova, 2017). In the conditions of the educational and scientific production center of Sumy National Agrarian University, it was found that the gluten content depended on the seeding rate. Thus, the maximum amount of gluten was noted on the variant with a seeding rate of 5.5 million pcs ha^{-1} and amounted to 25.20%, and the smallest amount of gluten was obtained with a seeding rate of 6.0 million pcs ha^{-1} – 23.64%. Reduction of seeding rate from 5.0 to 3.5 million pcs ha^{-1} led to a decrease in the amount of gluten. Thus, it is noted that at a seeding rate of 5.0 million pcs ha^{-1} – gluten content was 24.81%, 4.5 million pcs ha^{-1} – 24.32%, 4.0 million pcs ha^{-1} – 24.05% and 3.5 million pcs ha^{-1} – 23.85% (Table 5).

Table 5. Yield and grain quality of soft spring wheat depending on the seeding rate (average for 2021–2023)

Seeding rate, million pcs ha^{-1}	Yield, t ha^{-1}	Gluten content, %	Protein content, %
3.5	4.07	23.85	13.10
4.0	4.58	24.05	13.17
4.5	4.76	24.32	13.41
5.0	5.00	24.81	13.56
5.5	5.30	25.20	13.82
6.0	5.63	23.64	12.64
LSD_{05}	0.12	0.20	0.13

In addition to yield, an important characteristic is to obtain a consistently high protein content in wheat grain, without reducing the yield level (Bilousova, 2019; Radchenko *et al.*, 2021).

If the grain contains less than 9% protein, then it is not worth talking about the satisfactory quality of flour from such a crop. The minimum protein content in bread wheat grain should be 12% to ensure satisfactory quality (Bozhko and Burdeynaya, 2010; Cherno, 2015). In the studies conducted by Sumy National University, the protein content in the grain of bread spring wheat was at the level of 12.64–13.82% (LSD_{05} = 0.13).

At a seeding rate of 3.5 million pcs ha^{-1} the protein content was 13.10%, 4.0 million pcs ha^{-1} – 13.17%, 4.5 million pcs ha^{-1} – 13.41%, 5.0 million pcs ha^{-1} – 13.56%, 5.5 million pcs ha^{-1} – 13.82%, 6.0 million pcs ha^{-1} – 12.64%. According to the results of the research, the highest protein content was obtained at a seeding rate of 5.5 million pcs ha^{-1} – 13.82% (Table 5).

CONCLUSIONS

According to the results of the research, it was found that the highest density of spring wheat plants was obtained on the variant with a seeding rate of 6.0 million pcs ha^{-1} – 522 pcs/m^2. In the experiment, it was found that the maximum preservation of plants during the growing season was 6.0 million pcs ha^{-1} and amounted to 86.8% (453.1 pcs m^2). The number of productive stems was the highest in the variant with a seeding rate of 6.0 million pcs ha^{-1} – 557.3 pcs m^2, and the smallest number of productive stems was noted at a seeding rate of 3.5 million pcs ha^{-1} – 377.1 pcs m^2.

It is noted that the maximum yield of spring wheat was obtained at a seeding rate of 6.0 million pcs ha^{-1} – 5.63 t ha^{-1}. A decrease in the seeding rate affected the decrease in grain yield. So, at a seeding rate of 5.5 million the yield was 5.30 t ha^{-1}, 5.0 million pcs ha^{-1} – 5.00 t ha^{-1}, 4.5 million pcs ha^{-1} – 4.76 t ha^{-1}, 4.0 million pcs ha^{-1} – 4.58 t ha^{-1} and at a seeding rate of 3.5 million pcs ha^{-1} – 4.07 t ha^{-1}. On average, over the years of research, the maximum amount of gluten was observed in the variant with a seeding rate of 5.5 million pcs ha^{-1} and amounted to 25.20%. According to the results of the research, the highest protein content was obtained at a seeding rate of 5.5 million pcs ha^{-1} – 13.82%.

In the soil and climatic conditions of the Sumy region (north-eastern Forest-Steppe of Ukraine), in order to obtain a yield of soft spring wheat at the level of 5.63 t ha^{-1}, it is proposed to sow with a seeding rate of 6.0 million pcs ha^{-1}, and to obtain the largest amount of gluten in the grain 25.20% with a protein content of 13.82%, reduce the seeding rate to 5.5 million pcs ha^{-1}.

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PERSPECTIVES AND CHALLENGES OF USING BREAD WITH A FUNCTIONAL COMPONENT INULIN HPX

SUMMARY

Functional nutrition is a concept of nutritional science for which it has been proven to have special health effects, so the constant innovation of functional products contributes to the development of the modern food industry. The paper examines the influence of the addition of Inulin HPX on the sensory quality of bread in order to obtain a new functional product that can improve the nutrition of the population, as well as the catering offer of the destination. Different proportions of Inulin HPX in the amount of 0%, 7.5% and 15% were used for the production of bread dough, as a substitute for part of the flour in the basic raw materials. The goal of the research was to analyze the impact of the functional component Inulin HPX on the sensory (volume, texture, color, smell and taste) and nutritional properties of bread, as well as the acceptance of the new functional product by consumers through a demand survey in the territory of the city of Kotor, Montenegro. Based on the test results, the bread with the functional component of 7.5% Inulin HPX has the best sensory characteristics and a high level of acceptance by consumers. This pilot study can serve as a basis for the marketing of a new functional product on the market of Montenegro intended for consumers who care about healthy lifestyles. One of the conclusions is that it can be a part of improving the menu of restaurants that promote healthy food, but also catering establishments within the framework of wellness and health tourism in Montenegro.

Keywords: bread, Inulin HPX, functional food, tourism, Montenegro

INTRODUCTION

In a technologically developed society, as a consequence of the modern way of life, which is characterized by a lack of free food, more and more fast

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food is consumed, food with high energy value and insufficient intake of food contaminants. This type of diet as well as reduced physical activity have led to the spread of some health problems such as: diabetes, obesity and digestive system diseases (Saka *et al*., 2021). In recent decades, consumer demands in the field of food have been changing rapidly. Consumers are becoming increasingly educated about the importance of food, the connection between food quality and health, and today food is not only intended to satisfy hunger and provide the necessary nutrients for the normal functioning of human organisms, but care is taken to prevent diseases related to nutrition but also to improve the physical and mental health of consumers (Siró *et al*., 2008). For this reason, the demand for functional food is increasing, and this can be explained by the increase in health care costs, the increase in people's life expectancy and the desire of the elderly population to improve the quality of life in their later periods of life (Siró *et al*., 2008). These facts have increased the interest in functional food, that is, to enrich food products with functional components.

When looking at the chronology of the history of human nutrition, until recently people produced food directly from natural resources. In the last hundred years, food technology began to take the main role and produce an unlimited number of food products from original foods (natural resources). The term "functional food" is a relatively new direction in the way of eating and refers to the concept of nutritional science, rather than a specific type of new food. Functional food is one of the main food categories in the global health and wellness market, and is becoming the main focus of new product development in the food industry (Khan, *et al.*, 2013). There is no single and generally accepted definition of functional food, but all definitions have in common that functional food is considered to be those foods that are characterized by a balanced ratio of nutrients that should satisfy the nutritional and health needs of consumers, while not having negative effects during consumption. The positive effect of functional food in a short period of time is difficult to observe, because the effects are measurable only after a period of several years of consumption.

The modern concept of functional food began in Japan. A major research project on the functions of food began in the early 1980s in Japan, and has led to the world's first policy on the legal approval of functional foods as "foods for specific health uses" (Shimizu, 2019). In 1991, the Ministry of Health, Labor and Social Welfare of Japan introduced regulations on functional food under the name "Food for Specified Health Uses" or FOSHU. After the introduction of functional foods, many clinically proven FOSHU products with health benefits were developed and launched on the market (Iwatani & Yamamoto, 2019).

Understanding food as medicine is not new. In ancient Greece, the philosopher Hippocrates, the father of medicine, is also known for the catchphrase "Let Food be your Medicine and Medicine be your Food" (Yoldaş İlktaç, & Hızlı, 2019; Fatić *et al*., 2020) with which he gave a unique explanation about the connection between food and health. During the 19th century, the philosophy of "food as medicine" was neglected and coincided with the great expansion of the pharmaceutical industry. In the 20th century, nutrition again assumes an important role in the prevention of some diseases and the improvement of health.

Modern society at the beginning of the 21st century is characterized by numerous challenges such as: increasing the price of medical services, prolonging human life, the development of science and new technologies, and all of this has the effect of changing the lifestyle.

When considering the enrichment of food products with functional components, the choice is on bread because it is a suitable food item, it is consumed daily in all dietary regimes and is an integral part of all catering menus (Filipović *et al.*, 2020). By replacing flour or part of flour with different functional components, which contribute to improving the nutritional composition, the final product results in a high-quality product with certain health benefits (Al-Ansi *et al.*, 2022). Rye bread play an increasingly important role in a healthy diet, is recommended in the diet of diabetics and people with high blood pressure , and rye play a very important role in providing complete functional nutrition for humans and domestic animals (Popović *et al.*, 2022).

The functional properties of bread can be improved by the proper use of added raw materials, such as Inulin HPX which is a commercial dietary fiber derived from chicory (Helianthus tuberosus). Inulin or oligofructose is a natural dietary fiber isolated from various types of plants, chicory root, dandelion, onion and garlic, artichokes, bananas, aloe. Inulin is a linear chain composed of 2 to 60 fructose units connected by β (2-1) bonds to the terminal glucose unit and has prebiotic properties. There are two types of dietary fiber, water-soluble and water-insoluble. Insoluble dietary fibers are cellulose, some fractions of cellulose, resistant starch, lignin, oligofructose, waxes, inulin, and soluble dietary fibers are of endogenous origin, some pectins, β glucans, of exogenous origin, gum arabic, guar gum, locust gum, etc. Knowing the differences between soluble and insoluble dietary fibers is useful for understanding their action in food products. Most grains contain a large percentage of insoluble dietary fiber, with the exception of oats and barley, while a large proportion of soluble dietary fiber is mainly found in fruits and vegetables (Nelson, 2001).

The modern attitude of nutritionists implies the preparation of a healthy and nutritionally valuable meal, in which grain products are the most represented. The American Dietetic Association, in addition to daily amounts of dietary fiber, also recommends an appropriate ratio of insoluble/soluble fiber, which should be 3:1. The recommendations of the Food and Drug Administration (FDA) for the daily intake of nutrients and dietary fiber are related to the average daily energy intake. For moderately physically active women, teenage girls, a daily dietary fiber intake of 25 g/8.4 KJ (2000 kcal) and 30 g dietary fiber/10.5 KJ (2500 kcal) is recommended for men, teenage boys (Guillon *et al.*, 2000).

From the aspect of tourism, in the field of catering, functional food is especially interesting for the catering menu of facilities that deal with the offer of

healthy food, and the concept of functional nutrition follows modern trends in nutrition, especially in the field of wellness and health tourism (Vujačić, 2011).
When we consider the diet of tourists, in addition to specific dietary needs, there are tourists with special individual habits related to healthy lifestyles, which are not related to medical needs. For tourists with medical needs, dietary needs increase daily depending on food sensitivities, allergies, celiac disease, diabetes or other needs related to their medical condition (Andrews *et al*., 2010). Today, in the hospitality industry, questions related to functional products in the restaurant offer are increasingly being asked, given the recognition of functional products and the knowledge of the impact of these products on health, balancing and maintaining the maximum physiological functions of consumers (Milner, 2000; Roberfroid, 2002).

In these pilot studies, we started from the basic hypothesis that functional products are an integral part of the modern way of eating and that they have their place in the catering offer of Montenegro.
The goal of the research was to analyze the impact of the functional component Inulin HPX on the sensory (volume, texture, color, smell and taste) and nutritional properties of bread, as well as the acceptance of the new functional product by consumers through a demand survey in the territory of the city of Kotor, Montenegro.

MATERIAL AND METHODS

Material

The following raw materials were used for the production of bread with a functional component: wheat flour (T-500, Danubius, Novi Sad), baker's yeast (manufacturer "Alltech-Fermin" Senta), salt (So produkt, Stara Pazova), Inulin HPX commercial product produced by "ORAFTI Active Food Ingredients", Belgium.

Bread production

The bread was mixed according to the standard AACC method (Kaluđerski & Filipović 1998). The composition of the bread dough is as follows: flour 100%, 92.5% and 85%, functional component (Inulin HPX) 0%, 7.5% and 15%, salt 2.0% and baker's yeast 2%. The experimental design is shown in Table 1.

Table 1. Experimental bread production plan with inulin HPX

	Amount of inulin HPX		
	0%	7,5%	15%
	Sample 1	**Sample 2**	**Sample 3**
Flour (g)	300	277.5	255.0
Inulin HPX(g)	0	22.5	45.0
Salt (g)	6	6	6
Baker's yeast (g)	6	6	6
Water (g)	166,2	150,3	143,4

Sensory evaluation

Sensory analysis of bread with inulin was performed according to the current standard ISO 4121:2002. Six trained evaluators identified the descriptors and evaluated the sensory characteristics of the bread using a 6-point scale (0 = unacceptable, 1 = poor, 2 = acceptable, 3 = good, 4 = very good, 5 = excellent). The list for the sensory evaluation of bread with Inulin HPX consisted of 5 descriptors, where two descriptors related to the quality of the bread (volume and texture), two descriptors to the aroma (taste and smell) and one to the color.

Nutritional composition of bread

The basic chemical composition (content of proteins, starch, lipids, minerals and cellulose) was determined according to the standard AOAC method (2000), the dietary fiber content was determined according to the AOAC method (1990).

Descriptive analysis

The results of the influence of Inulin HPX on the nutritional quality of bread was performed by statistical analysis of variance ANOVA, and the significance of the difference was tested by Tukey's test using the StartSoft Statistica 10 statistical program.

Standard rating

The standard rating or "Score" analysis uses maximum and minimum measurement parameters for the normalization of the response values which transfers responses from their dimensional system to a new dimensionless system that enables further mathematical processing of different types of response. (Jayalakshmi & Santhakumaran, 2011). The maximum value of the normalized score represents the optimal value of all the combined analyzed responses and indicates the optimal amount of added inulin in the bread formulation.

$$S_{ki} = \frac{\left(x_{ki} - x_{k\,\min}\right)}{\left(x_{k\,\max} - x_{k\,\min}\right)} \quad \text{k= 1-9}$$

Where the X_k values are: volume, texture, color, smell, taste, proteins, minerals, cellulose, total fiber.

$$S_{ni} = 1 - \frac{(y_{ni} - y_{n\,\min})}{(y_{n\,\max} - y_{n\,\min})} \text{ k=1-3}$$

Where the *Y*ni values are: starch, lipids, energy value.

The normalized responses give a "Score" or "Quality Score", where the highest value S represents the optimal value of all the analyzed sensory and nutritional parameters of the bread and indicates the optimal amount of inulin in the bread.

$Si = 0.12 \cdot S1 + 0.18 \cdot S2 + 0.06 \cdot S3 + 0.18 \cdot S4 + 0.06 \cdot S5 + 0.1 \cdot S6 + 0.06 \cdot S7 + 0.02 \cdot S8 + 0.02S9 + 0.04S \cdot 10 + 0.08S \cdot 11 + 0.08 \cdot S12$

$\max[S_i] \rightarrow optimum$

Consumer survey

Acceptability of bread with a functional component on the market was examined by a consumer survey. Consumers in the territory of the city of Kotor, Montenegro rated two types of bread: bread with 0% Inulin HPX and bread with 7.5% Inulin HPX. Based on a random sample, consumers answered the questions with "yes" and "no" (Table 3), how they liked the new bread with 7.5% inulin HPX. The consumer test was conducted by distributing bread samples to randomly selected consumers (300) of different socio-demographic characteristics, of which four questions were of a general nature and three questions were specific, referring to bread with 7.5% Inulin HPX.

RESULTS AND DISCUSSION

Sensory evaluation is an important parameter for defining the quality of the finished product because it contributes to the assessment of the degree of acceptability of the new product by consumers, which reduces the risk of failure in terms of product acceptance on the market and its placement in catering facilities. Figure 1 shows the sensory evaluation of bread with the addition of a functional component. The parameter volume and texture of the bread in the control sample (1) were evaluated with almost the highest score "excellent" (4.77 and 4.67), while the addition of Inulin HPX as a functional component in the amount of 7.5% in bread (sample 2) tends to decrease the quality of bread, volume and texture compared to the sample 1, but it was rated as "very good" (3.87 and 4.23), which indicates that the bread has good quality despite the addition of the functional component. The addition of 15% to inulin to bread leads to a significant decrease in quality in terms of volume and texture of bread by about 2.5 quality scores in (2.0 and 1.8) compared to the control sample. The addition of Inulin HPX in the amount of 7.5% and 15% does not visually change the color of the bread, it even leads to an increase in the brightness of the bread compared to the control sample, which is a desirable contribution. The results of the analysis of the taste and smell of bread with a functional additive showed that the addition of 7.5% and 15% of inulin HPX to bread has a positive effect on the taste and smell of bread, contributes to a slight increase in these parameters compared to the control sample (1). This shows that the added functional component does not change the characteristic taste and smell of the bread.

Based on the chemical composition of bread with the addition of a functional component, there is a statistically significant decrease in the content of protein and starch compared to the control sample. This is a direct consequence of replacing part of the flour with a functional component in the raw material composition of the bread dough (Table 2). The Tukey test shows that increasing the amount of Inulin HPX (7.5% and 15%) does not result in a statistically significant change in the content of lipids, mineral substances and cellulose, while there is a statistically significant increase in the content of dietary fiber. These data also indicate that the addition of the functional component (Inulin HPX) changes the nutritional composition of the bread, i.e. that there is a decrease in energy value from 8% to 15% and an increase in dietary fiber content, which is in accordance with the recommendations of nutritionists and the World Health Organization (WHO 2003) that nutrition should be enriched with dietary fiber and reduce the energy value of products. In order to assess the impact of the addition of the functional component on the sensorial and nutritional properties of the bread, the amount of the additive was optimized (Figure 1 and Table 2) and based on the standard score, bread with 7.5% Inulin HPX has the best score(0,62).

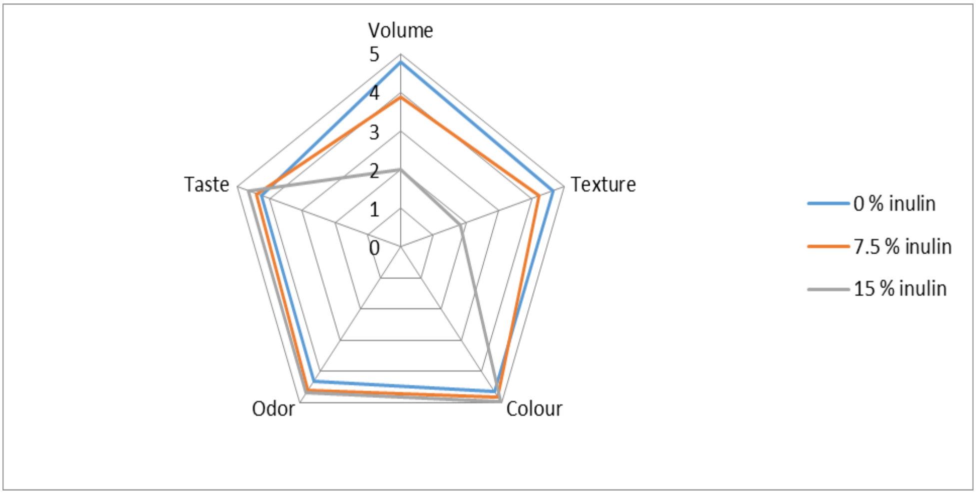

Figure 1. Sensory properties of bread with a functional component

Based on the highest score (table 3), the acceptance of bread with 7.5% Inulin HPX by consumers of different socio-demographic characteristics, who answered the questions anonymously through a survey, was examined. Based on the analyzed results (table 3), it can be seen that more than half of the respondents pay attention to the nutritional (58%) and energy (67%) value of the product, which indicates that they take care of what they buy and consume. 83% of respondents are ready to consume bread with a functional component if it has a positive effect on health and 61% of respondents are ready to compromise on the quality of the food product if it contributes to improving health. These analysis data on functional food show us that the respondents take care of their health and are aware of the importance of nutrition and health which provides the opportunity for both technologists to create new products and catering facilities to market these products. 77% of the analyzed respondents have a positive attitude about the likeability of bread with Inulin, and 75% of the respondents would buy this bread. 64% would be willing to pay a higher price for bread if it is beneficial for health. The high percentages on the likeability of bread with 7.5% Inulin HPX show us that this bread can be found on the market as well as in restaurants and hotels and is intended for people who care about nutrition and health.

Table 2. Nutritional composition of bread with a functional component

	Amount of inulin		
	0%	7,5%	15%
Chemical composition			
Protein content (%s.m)	12,23±0,44^{c}	11,04±0,18^{b}	10,02±0,31^{a}
Starch content (%s.m)	76,16±0,97^{c}	67,65±0,69^{b}	60,51±0,47^{a}
Lipid content (%s.m)	0,76±0,07^{a}	0,77±0,01^{a}	0,74±0,02^{a}
Mineral matter content (%s.m)	0,70±0,06^{b}	0,69±0,05^{a}	0,74±0,06^{a}
Cellulose content (%s.m)	0,28±0,08^{a}	0,39±0,01^{a}	0,38±0,01^{a}
Total fiber content (%d.m)	0,41±0,02^{a}	4,43±0,33^{b}	9,26±0,51^{c}
Nutritional composition			
Increase in fibers compared to the control sample	-	7,06	14,11
Fiber content in 100g of bread	0,69±0,21	12,06±1,0	24,10±1,27
Energy of 100g of bread (J)	1536,3	1401,3	1303,1
Total score	0,41	0,62	0,60

^{abc} Different letters in the exponent in the same row of the table indicate a statistically significant difference between values, at a significance level of $p<0.05$ (based on post-hoc Tukey's HSD test)

Table 3. Consumer attitudes regarding bread with a functional component

Question number	**Question type**	**Questions**	**Yes (%)**	**No (%)**
P 1	**Functional effect**	**Is it important to you that bread has improved nutritional value?**	58	42
P2		**Is the energy value of the product important?**	67	33
P 3		**Would you consume bread with a functional component if it positively affects your health?**	83	27
P 4		**Are you ready to compromise on the quality of bread with a functional component if you know it has other positive effects?**	61	39
P 5	**Bread with a functional component**	**Do you like bread with HPX inulin?**	77	24
P 6		**Would you buy bread with inulin HPX?**	75	25
P 7		**Are you willing to pay a higher price for bread with inulin if it is good for your health?**	64	36

CONCLUSIONS

Based on the results of the pilot research on the impact of Inulin HPX as a functional component on the quality of bread in order to improve the health and tourism of Montenegro, it can be concluded that the addition of the functional component affects the volume and texture of bread, improves the nutritional composition and reduces the energy value of bread by 8.8% and 15.2%. The average consumption of 250 g of bread with 7.5% Inulin HPX (which has the highest standard rating of 0.62) provides 30.5 g of fiber, which meets the daily human needs for dietary fiber in a daily meal. This indicates the optimal amount of supplements and the positive impact of the applied functional supplement. Based on the results of a consumer test in the city of Kotor, Montenegro, this bread has a high degree of likeability (77%) and acceptance (75%), so it can be placed both on the market and in the catering industry of Montenegro. These pilot studies can be the basis for placing a new functional product on the market, especially interesting for consumers who take care of healthy lifestyles, and it can also be part of improving the menu of restaurants that promote healthy food, as well as catering facilities within the wellness and health tourism of Montenegro.

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TEXTURAL AND SENSORY PROPERTIES OF YOGURT OBTAINED BY BIOCONVERSION OF MILK-LIQUID WHEY MIXTURE AND ARONIA (ARONIA MELANOCARPA) SUPPLEMENTATION

SUMMARY

Yogurt is a fermented dairy product of great importance worldwide because of its nutritional and health benefits. The objective of this study was the bioconversion of a milk-liquid whey mixture into yogurt and Aronia (*Aronia melanocarpa*) supplementation. *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* were used as microorganisms for control and functional yogurt production. Samples were analyzed in terms of syneresis index and textural and sensory properties throughout cold storage. The syneresis index was determined using the drainage method, and the textural parameters were obtained from the texture profile analysis of samples. Sensory evaluation was used to assess consumer acceptance and responses to the sensory properties of functional yogurt. Functional yogurt expressed differences in syneresis index during storage and was significantly higher than control yogurt on day 21. The textural properties of the samples were similar during storage. Higher values of cohesiveness and springiness and lower values for hardness, adhesiveness, and gumminess were observed in functional yogurt. The results showed that color and taste had no statistically significant effect on yogurts. Only the level of whey separation differed considerably on the last day of storage, according to an evaluation of yogurt qualities. The bioconversion of liquid whey into functional yogurt provides a technology for whey valorization that promotes human health and environmental sustainability.

Keywords: bioconversion, liquid whey, yogurt, Aronia, sensory properties

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INTRODUCTION

Cheese whey is the yellow-green colored liquid portion of milk, also called serum, obtained after separation of curd (Minj and Anand, 2020). The main nutrients found in whey are mineral salts, lipids, soluble proteins, and lactose (León-López *et al.*, 2022). The manufacturing of cheese and casein yields whey, a byproduct that is quite important in the dairy sector because of its high yield and nutritious contents (Mollea *et al.*, 2013). The improper disposal of whey by the dairy industry is seen as an environmentally damaging practice due to its significant potential for pollution (Schaefer *et al.*, 2023). This clarifies why the disposal of whey is highly regulated and why large-scale dairy enterprises must find other ways to dispose of it. (Flinois *et al.*, 2019). The dairy industry is very concerned about managing dairy waste to reduce pollution to the environment by using whey as the main waste material in various food and non-food items (Hameed *et al.*, 2023). North Macedonia's dairy industry faces ongoing whey discharge concerns. Wasted components can be removed from dairy products utilizing several extraction processes, such as traditional homogenization, ultra-high-pressure homogenization, ultrafiltration, reverse osmosis, and nanofiltration (Hameed *et al.*, 2023). However, bioconversion of whey in liquid form without further processing has numerous advantages. Dairy products are at the top of consumers' choices for functional foods, as they are considered the best host for functional ingredients (Dimitrellou *et al.*, 2020). Yogurt is a semi-solid, fermented dairy product that is rich in numerous nutrients and minerals. Its consumption has expanded globally given its nutritional benefits and ease of digestion (Plessas *et al.*, 2023). As yogurt is an excellent source of proteins, vitamins and minerals, it plays a significant part in a healthy diet (Gómez *et al.*, 2020). Using liquid whey (LW) as an ingredient in yogurt production, along with Aronia, gives a novel functional yogurt with increased nutritional content. However, it can increase the risk of whey separation, as indicated by the syneresis index (SI). The primary causes of whey separation are (i) a higher whey protein concentration than casein, (ii) lower total solids concentrations, and (iii) changes in the organic acids produced by living lactic acid bacteria (LAB) during storage (Plessas *et al.*, 2023). According to Cais-Sokolińska and Walkowiak-Tomczak (2021), adding fruit (juice, pulp, or pureè) to yogurt improves its functional and nutritional properties, shelf life, and consumer appeal. Aronia is high in anthocyanins, minerals, antioxidants, and vitamin C, all of which assist to boost the immune system (Cușmenco and Bulgaru, 2020). Aronia melanocarpa's polyphenolic components have numerous health benefits, including anti-inflammatory, anticancer, antibacterial, antiviral, antidiabetic, antiatherosclerotic, hypotensive, antiplatelet, and antioxidant properties (Jurendić and Ščetar, 2021). Buffalo yogurt's high nutritional value, mixed with the bioactive phenolic compounds present in Aronia fruits, provides a way to create naturally functional food that is beneficial to people's health (Zheleva *et al.*, 2023). According to Cușmenco and Bulgaru (2020), adding Aronia fruits, strawberries, raspberries, and peaches greatly increases the biological value and quality indexes of goat milk yogurt with fruits. More and more importantly, in today's climate, individuals are increasingly willing to experiment with new flavor combinations, especially those that contain chemicals with possible health advantages (Plessas *et al.*, 2023).

To the best of our knowledge, this is the first study to make yogurt using both liquid whey and Aronia (*Aronia melanocarpa*) supplements. As a result, the study focused on the bioconversion of the milk-liquid whey mixture into yogurt and Aronia (*Aronia melanocarpa*) supplementation for improved health benefits, as well as the syneresis index, textural, and sensory qualities under refrigeration.

MATERIAL AND METHODS

Raw materials and yogurt samples

Yogurt was produced with milk, whey, and a starter culture containing *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* YoFlex ® Premium 3.0, Chr. Hansen (Copenhagen, Denmark) for DVS inoculation (provided by Vemilk, a milk processing company in Republic of North Macedonia). White sugar and frozen strawberries (stored at –18°C) were acquired from a local market in Bitola, Republic of North Macedonia.

The milk was pasteurized for 5 minutes at 90 ± 2°C and cooled to 4°C. Whey (0.15% fat) was pasteurized at 90 ± 1°C for 25 minutes before cooling to 45 ± 2°C. Frozen Aronia berries were melted and pasteurized at 65 to 70 °C for 15 minutes. The fruits were then cooled to between 25 and 30 °C. Later, milk (3.05% fat) was heated to 43.8 °C and 4% sugar was added. Milk and milk with 25% whey were inoculated with 0.04% and 0.05% active cultures, respectively (Hiseni, 2023). The pH of the milk and milk with whey were 6.54 and 6.39, respectively, prior to fermentation. After three hours of fermentation at 43 °C, the pH of conventional and functional yogurt was 4.64. Yogurt samples were cooled to an ambient temperature of 25 to 30 °C before being blended with Aronia berries (12%) for 5 minutes. Finally, (CYA) conventional yogurt with Aronia and (FYA) functional yogurt with Aronia samples were stored at 4 to 8°C for 21 days.

Syneresis index

The yogurt syneresis index (SI) was determined using the drainage method (Isanga and Zhang, 2007). SI was measured in one replicate.

Textural profile analysis

Textural parameters were extracted using texture profile analysis (TPA) (CT3-10kg, Amtek Brookfield, USA) using a cylindrical-shaped probe (38.1mm diameter). The tests were performed at temperatures ranging from 20 to 25°C, whereas the samples were collected at 4 to 8°C. Yogurt samples were kept between 10 and 12°C. TPA was applied to yogurt samples in their original 110-mL container, which was placed underneath the probe with a 70% deformation test target at 1 mm/s (Hiseni, 2023). Hardness, adhesiveness, cohesiveness, gumminess, and springiness were determined. The analyzer runs on a PC using TexturePro CT V1.8 software.

Sensory evaluation

The sensory analysis of the yogurts was conducted using the corrected five-point scoring system (scoring with 25 points), a commonly used ranking approach for dairy samples (Radovanović and Popov-Raljić, 2000/2001; Makarijoski, 2018). It was used to assess consumer acceptance of products. According to the international standards (ISO 22935-1:2009; ISO 22935-2:2009; ISO 22935-3:2009), sensory descriptive analysis of yogurts was conducted on 14 attributes: the distribution of the ingredients (Aronia); the presence of mold; the whey separation; the aroma of the milk; the fermentation aroma; the intensity of the flavor; and the taste of the yogurt—sweet, bitter, sour, Aronia, milky, whey, yogurt, and aftertaste (Hiseni, 2023). Each quality was scored using an ascending scale of 1 (not present), 2 (very weak), 3 (moderate), 4 (intense), and 5 (extremely intense).

Statistical analysis

Mean comparison was done by student's t-test where statistical significance was shown at P<0.05, using Minitab 18 (Minitab Inc, USA). All figures were drawn using OriginPro 2021b (OriginLab Corporation, Northampton, MA).

RESULTS AND DISCUSSION

SI of yogurt samples during storage is plotted in Figure 1. The use of LW in yogurt with Aronia resulted in a lower SI only on day 1. SI in CYA and FYA was 45.35%, respectively, 36.46% on day 1, 55.06%, and 78% on day 21. The products with LW showed higher stability during the first week of storage, and then the increase in SI in FYA was more evident compared to CYA.

The negative impact on the SI in FYA on day 21 was due to the addition of LW. SI may become significantly higher in the case of low-fat yogurts or yogurts produced with the addition of fruit juices (Dimitrellou *et al.*, 2020). Literature shows a range of SI values depending on the evaluation method. The SI in our study for CYA and FYA was higher except for day 1 than those reported by Dimitrellou *et al.* (2020) in which the SI of yogurts with Aronia juice on day 1 and 21 were 38% and 37%, respectively. Predescu *et al.* (2022) analyzed control yogurt (0%) and yogurt with 5% black chokeberry purée and reported a continuous increase of syneresis during storage from 33.31% on day 1 to 37.81% on day 15. The SI in our study for CYA and FYA is significantly higher compared to these results, which is due to the higher fruit percentage and also the whey addition in the last product. Other authors reported that the addition of other types of fruit juices, like concentrated grape juice or carrot juice, to yogurt samples with a 0.5% stabilizer resulted in a significant (P<0.05) increase in syneresis (Öztürk and Öner, 1999; Kiros *et al.*, 2016).

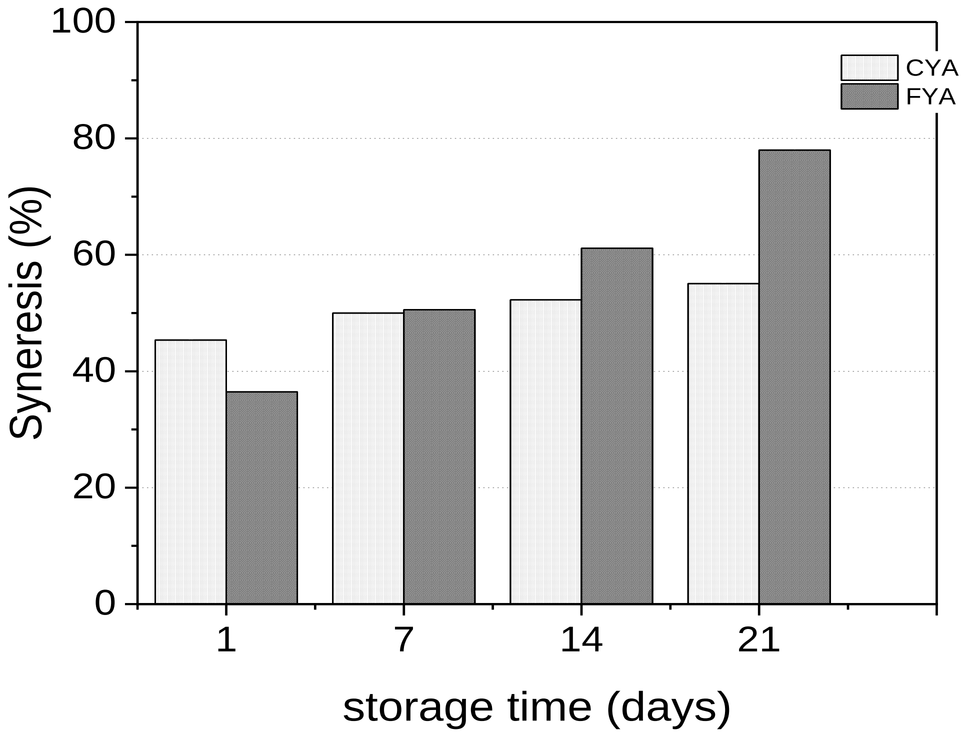

Figure 1. Comparison of syneresis index of yogurt samples. CYA = conventional yogurt with Aronia (control); FYA = functional yogurt with Aronia

Texture profile of yogurts during storage

The TPA test, a double compression test, evaluates perceived sensory qualities by imitating the first two bites of a complex oral processing action (Jonkers, 2021). The TPA test employed in this investigation yielded the following texture parameters: hardness, adhesiveness, cohesiveness, gumminess, and springiness, as shown in Figure 2. The hardness of a product is determined by the amount of force required to compress it (Jonkers, 2021). According to Figure 2, adding whey to the yogurt formulation did not affect the hardness when compared to the control sample. The hardness of CYA and FYA during storage was similar, with a slight increase during storage. The highest level of hardness in samples with whey FYA was (484 ± 127 g) and the lowest was (230 ± 159 g). CYA results on day 21 showed a very high SD. This was due to Aronia`s presence. Hardness results were not statistically significant during storage (P>0.05). Cais-Sokolińska and Walkowiak-Tomczak (2021) observed lower outcomes on day one for yogurt with restructured elderberry juice. Adhesiveness is defined as the effort required to remove food that adheres to the mouth (generally the palate) during the normal swallowing process, which is the force required to separate the material that sticks to the teeth during eating and has an inverse relationship with yogurt eating quality (Park *et al.*, 2020; Mousavi *et al.*, 2019). FYA had lower adhesion values than CYA. When compared to the control samples, the inclusion of whey significantly reduced the adhesiveness of FYA. There was a significant difference between CYA and FYA on days 1 and 21 (P<0.05). On day 21, CYA had the highest levels of adhesiveness (11.15 ± 0.21 mJ), while FYA had the lowest level on day 7 (0.8 ± 0.14 mJ). Ziarno and Zareba (2020) found higher adhesiveness values (15.1 mJ on day 28) in yogurt made with skim milk powder. Cohesiveness refers to the strength of internal links that comprise the food's body (Park *et al.*, 2020). Yogurt samples with the addition of whey (FYA) demonstrate higher levels of cohesiveness than CYA (Figure 2). However, a significant difference between CYA and FYA was observed only on day 21 (P<0.05). The highest cohesiveness level was FYA on day 14 (0.7 ± 0.07) and the lowest level was CYA on day 21 (0.35 ± 0.007).

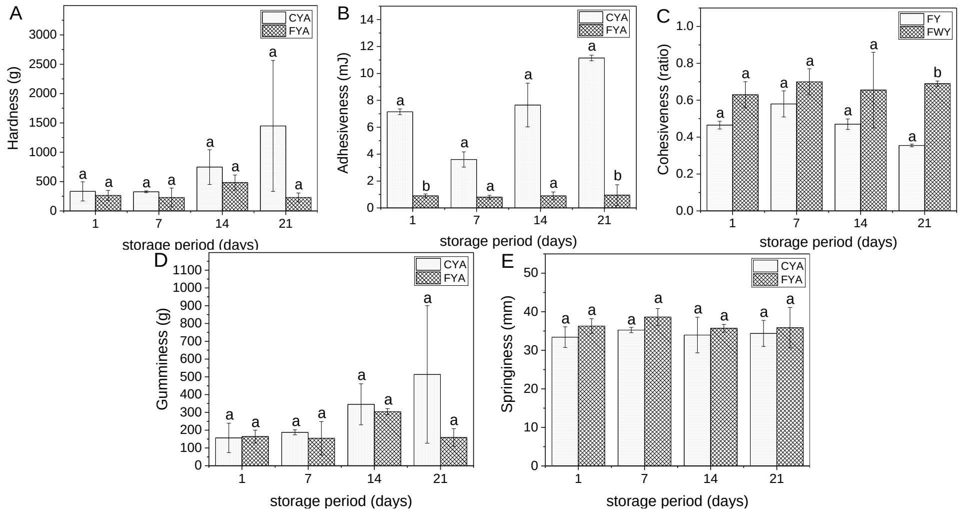

Figure 2. Comparison of yogurt samples for hardness (A), adhesiveness (B), cohesiveness (C), gumminess (D) and springiness (E). CYA = conventional yogurt with Aronia (control); FSY = functional yogurt with Aronia. The mean in the graph indicated by different letters (a–b) is significantly different (P<0.05) from the other product. Error bars represent the standard deviation (SD) of the mean of duplicate experiments

Similar results have been obtained for the contribution of liquid milk whey to the yogurt formulation (without fruits), which increased the cohesiveness of yogurt samples (Gauche *et al.*, 2009). Gumminess is the amount of energy required to break up a semisolid food before it is swallowable, and it is determined by firmness and cohesiveness (Mousavi *et al.*, 2019; Khorshidi *et al.*, 2021). The gumminess of yogurt with whey FYA was comparable to that of CYA. Adding whey did not result in a statistically significant difference in gumminess compared to control samples after storage. Gumminess levels demonstrated a correlation with hardness values. Overall, the FYA mean outcomes were lower. Similarly, a previous study found that adding liquid milk whey to the yogurt (without fruits) formulation reduced the gumminess in yogurt samples (Gauche *et al.,* 2009). Springiness is the rate and extent to which a deformed material returns to its original condition after the force is withdrawn, and it is determined by elements such as heat treatment, protein interaction, flexibility, and protein unfolding (Delikanli and Ozcan, 2017). Figure 2 shows that there is no substantial difference between CYA and FYA in terms of springiness. FYA has higher mean values than CYA. However, the lowest springiness level was associated with CYA (36.41 ± 2.68mm). Variable interactions between milk protein particles, as well as variances in the mineral makeup of different types of milk powders, might alter the textural features of fat-free set-type yogurt with diverse types of milk proteins (Peng *et al.,* 2009).

Sensory evaluation

Figure 3 shows the average scores for sensory qualities that are assessed for customer acceptance. FYA results were compared to CYA results during storage. The score of sensory attributes (color and taste) based on the five-point scoring system method of CYA and FYA was not statistically significant during cold storage ($P>0.05$). FYA appearance and aroma score differences were statistically significant on day 21, whereas consistency was consistent on days 14 and 21 ($P<0.05$). FYA was evaluated as having better aroma and taste on day 7 and less consistency during storage, especially on day 21.

Kim *et al.* (2019) reported that the sensory attributes of yogurt decreased with the addition of *Aronia melanocarpa* (black chokeberry) powder (0.5%, 1.0%, 1.5%, and 2.0%). Overall acceptability is calculated by giving each sensory attribute a coefficient of importance, as shown in Figure 4. Taste, aroma, and consistency received the greatest scores (8/20 points, 6/20 points, and 3/20 points, respectively). CYA and FYA had higher overall weighted acceptance during the first week ($P>0.05$). In the following weeks, CYA improved its score, but FYA decreased with a statistically significant difference ($P<0.05$). On the final day of storage, the overall acceptability of CYA and FYA on a five-point scale was 4.61 ± 0.38 points and 3.94 ± 0.51 points, respectively. Kim *et al.* (2019) found that the overall acceptability scores for yogurt with *Aronia melanocarpa* (black chokeberry) powder (0.5%, 1.0%, 1.5%, and 2.0%) varied from 4.2 to 3.7 points, whereas the control (0%) scored 4.3. Nguyen and Hwang (2016) demonstrated that the addition of 3% Aronia juice to yogurt had no harmful impacts on flavor, mouthfeel, thickness, or overall acceptance, despite the fact that Aronia has a bitter or astringent taste.

The addition of Aronia up to 12% in CYA and FYA had no adverse effect on the taste of yogurt, whereas in CYA during storage, it increased the overall acceptability.

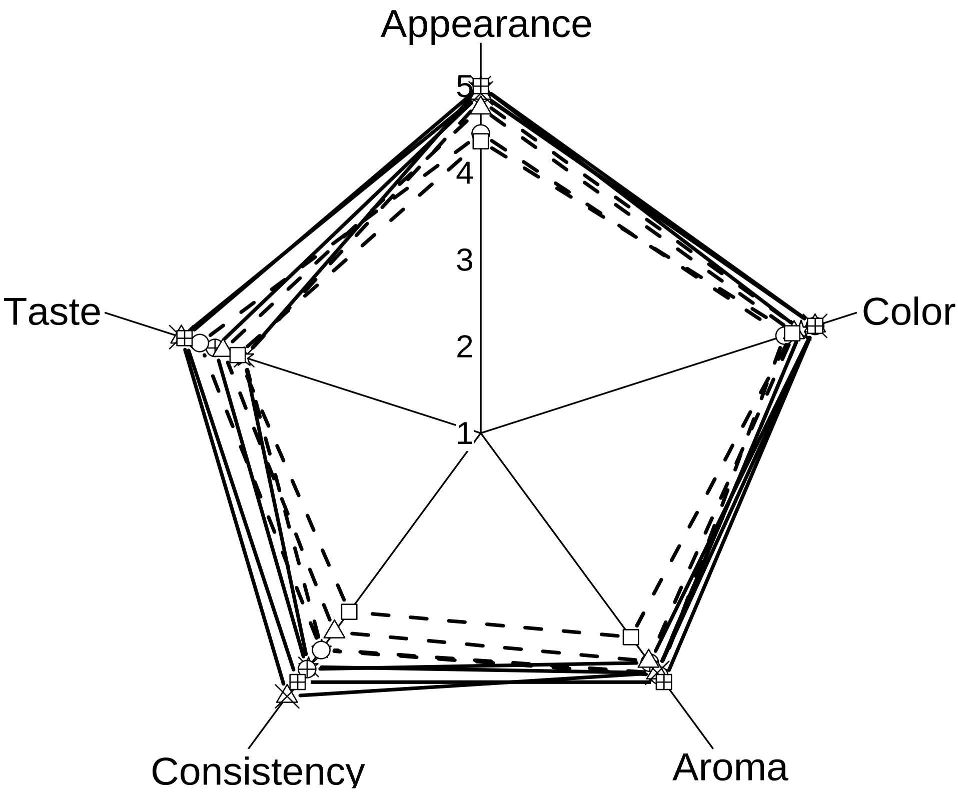

Figure 3. Sensory attributes of yogurts during storage: CYA = conventional yogurt with Aronia (, day 1; , day 7; , day 14; , day 21); FYA = functional yogurt with Aronia (, day 1; , day 7; , day 14, , day 21)

Figure 5 shows descriptive sensory evaluation. Distribution of ingredients scored higher on FYA on days 7 and 14 (P>0.05). Aronia contains anthocyanins, which contribute to the purple color of yogurt (Nguyen and Hwang, 2016).

It remained stored without developing any mold. Whey separation in FYA was more obvious on day 21 and there was a significant difference (P<0.05) compared to CYA. This demonstrates that the sensory panel showed strong syneresis, indicating a deteriorated structure on the last day of storage. Milk odor was present at a very weak intensity during storage because the Aronia has a strong flavor (P>0.05). Fermentation odor was very weak to moderate intensity in CYA and FYA (P>0.05). During storage, flavor intensity, sweetness taste, bitter taste, sour taste, milky taste, and Aronia taste obtained identical scores for CYA and FYA samples (P>0.05). Whey was tasted only on day 7 by one assessor (P>0.05).

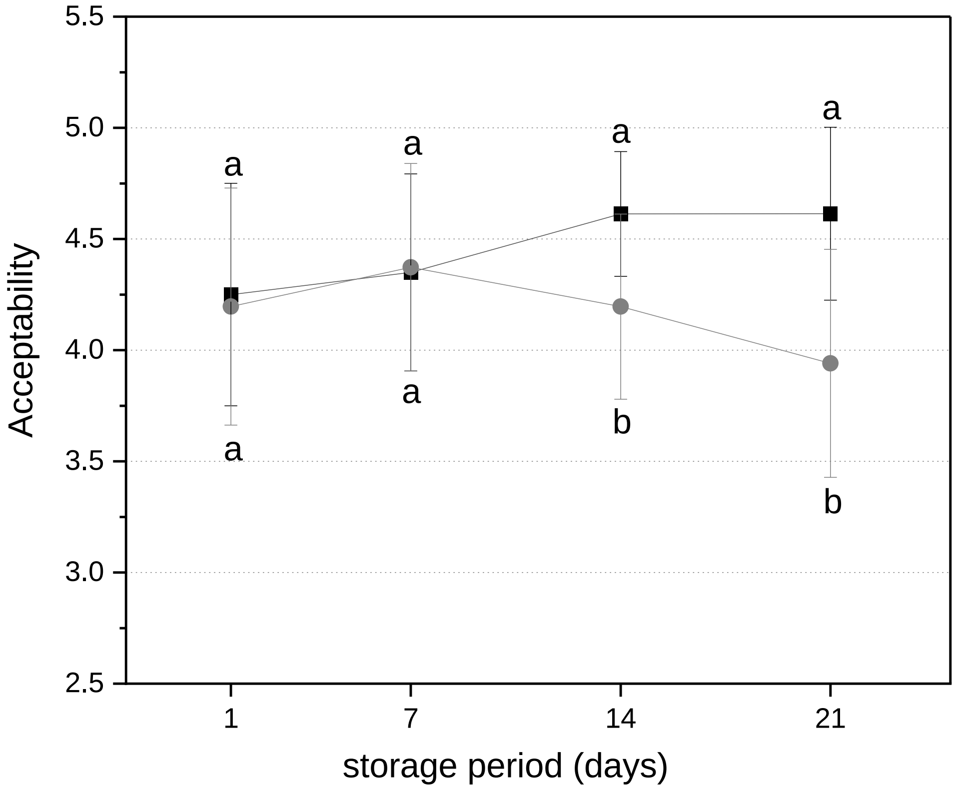

Figure 4. The overall weighted acceptability of yogurts (means ± SD). CYA = conventional yogurt with Aronia (■); FYA = functional yogurt with Aronia (●). The mean values in the graph indicated by different letters indicate significant differences (P<0.05). Error bars represent the standard deviation (SD) of the mean of twelve assessors (N = 12)

Volatile phenols generated through the metabolic processes of probiotic or yogurt cultures can contribute to an enriched flavor and aromatic profile (Plessas *et al.*, 2023).

The yogurt taste score was higher for CYA (P>0.05). FYA's aftertaste score was higher in the first week and decreased in the last week. These findings suggest that liquid whey addition is appropriate for producing functional yogurt with Aronia without the user being aware of the whey inclusion.

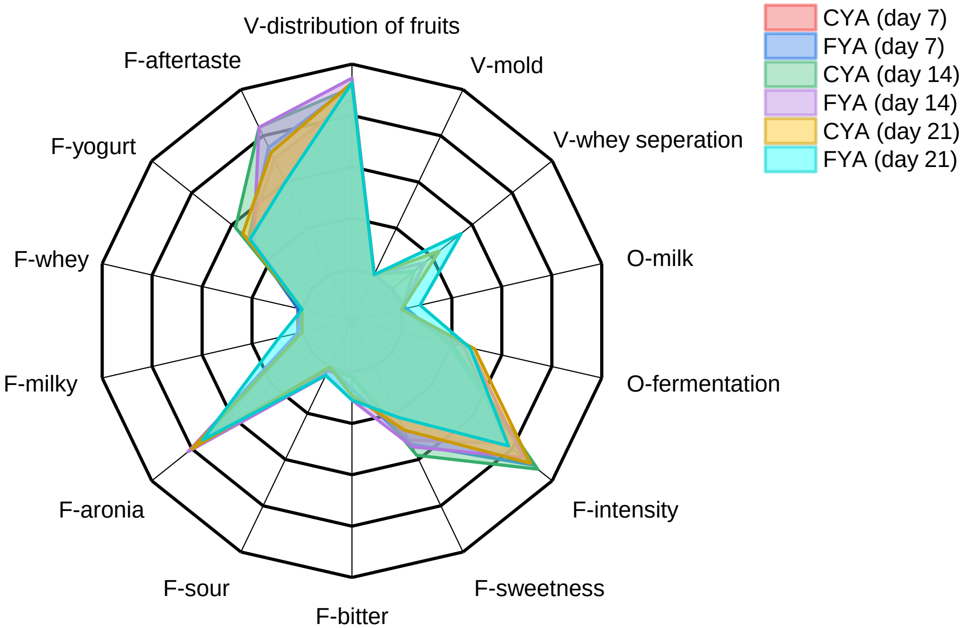

Figure 5. Sensory results from the quantitative descriptive analysis of yogurt samples: CYA = conventional yogurt with Aronia; FYA = functional yogurt with Aronia of 14 major attributes. V = visual; O = odor; F = flavor

CONCLUSIONS

Yogurt produced through the bioconversion of milk-liquid whey mixture and Aronia (*Aronia melanocarpa*) supplementation is a promising beneficial dairy product. On the final day of storage, whey supplementation had an impact on the syneresis index. Except for adhesiveness on days 1 and 21, and cohesiveness on day 21, the textural parameters of functional yogurt samples did not differ significantly from ordinary yogurt during storage. The addition of whey did not affect the flavor or color of the functional yogurt. Other qualities, such as appearance, aroma, and consistency, differed from ordinary yogurt, particularly on the final day of storage. On the last day of storage, functional yogurt with Aronia received consumption acceptance ratings of 3.94 points on a five-point scale. The findings provide valuable insights for the utilization of whey into functional yogurt with Aronia, which can offer health benefits beyond its nutritional value. In addition, in vivo studies would also be helpful to delineate the potential health benefits.

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The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of Универзитет „Св. Климент

Охридски“—Битола, Технолошко—технички факултет Велес (protocol code 10-428/2 approved on 2 December 2020).

Informed consent was obtained from all subjects involved in the study.

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ASSESSING THE AGRICULTURAL TRADE COMPLEMENTARITY OF THE CZECH REPUBLIC AND COUNTRIES IN WESTERN BALKAN

SUMMARY

The article interrogates the complementarity of agrarian trade between the Czech Republic and Albania, Bosnia and Herzegovina, Montenegro, North Macedonia and Serbia applying the Trade Complementarity Index. Analyzing the last decade, the results indicate two-way complementarity of agrarian trade between the Czech Republic and North Makedonia and Montenegro. Due to the changes in the exports and import structure during the period under scrutiny, Serbia and Albania have also become complementary to the agrarian trade of the Czech Republic and vice versa. The Czech Republic's agrarian exports are also complementary to Bosnia and Herzegovina's agrarian imports. Generally, the results support the ongoing liberalization process between the EU and the Western Balkans and the rationale for the cooperation of the Czech Republic with Western Balkan countries and taking advantage of existing economic resources in the segment of agrarian products. However, results also indicate existing barriers to developing more intensive agrarian trade between the Czech Republic and countries of the Western Balkans.

Keywords: Economic integration, Foreign trade, Trade complementarity index, Agrarian trade, the Western Balkans

INTRODUCTION

Economic and trade integration became a vital component of the economic development of countries in the Western Balkans. It is mostly the regional CEFTA agreement as well as trade agreements with EU countries that are forming the shape of Western Balkan countries' trade (Matkovski et al., 2022).

Current geopolitical dynamics following Russia's aggression against Ukraine have led to a strengthening of the strategic interaction and enhanced European Union's (EU) engagement with the Western Balkans. Currently, the EU

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has reiterated that is fully committed to the integration of the Western Balkans (EC, 2023). All countries of the Western Balkans have applied for EU accession (Croatia has already become an EU member state in 2013). North Macedonia has got the status of candidate country since 2005, Montenegro since 2010, Serbia since 2012, Albania since 2014, and Bosnia and Herzegovina since 2022 (EC, 2023). Although all countries formally have candidate status, they are in different stages of the negotiation process. There are geopolitical, security and other motivations for such integration; nonetheless the economic determinants of economic integration play fundamental role.

The agrarian trade is natural and important part of this dynamic. Agriculture sectors play a vital role in economic, employment and trade structures in all Western Balkan countries, and are contributing to economic as well as social stability.

The European Union is already playing a vital role in Western Balkan countries' trade as well as is playing an important role in the further integration of the Western Balkan region into the economic and political structures of the European region.

In this context, it is important to analyse welfare effects linked to this liberalization process and evaluate the rationale for the cooperation between Western Balkan countries and European Union and its member states. Against this backdrop, there is still gap in literature dealing with trade complementarity of Western Balkan countries and EU member states. Also, agrarian trade is typically a sensitive part of negotiations because of the potential impacts on food security and on rural areas.

This article aims to analyse the level and changes in agrarian trade complementarity of countries in Western Balkan (those that are potential EU member states) and the Czech Republic in the last decade. This study uses the example of the Czech Republic as an EU member state and EU candidate states from Western Balkan to shed light on the reasoning and effects of this specific economic cooperation.

Agricultural trade in the Western Balkans

Most countries of the Western Balkans (except Albania) became independent after the collapse of the former Socialist Federal Republic of Yugoslavia (SFRY), at the beginning of the 90s of the XX century. The countries of the former Yugoslavia, which had a long history of trade exchange within the same state, continued to trade with each other. The establishment of the Central and Eastern Europe Free Trade Agreement (CEFTA) also contributed to the maintenance of mutual trade (Ćejvanović et al., 2014) between countries that used to be part of the same country and are now part of CEFTA.

Volk et al. (2012) claim that most the Western Balkans have quite high but unused potential for agriculture. It is worth noting that, as pointed out by Mizik (2012), the agrarian sectors in the Western Balkans face many challenges as unbalanced sectoral production, fragmented structure of farms, relatively low yields and generally low level of agricultural productivity, and unfavourable export structure because a gap when compare hygiene and quality controls to the EU standards.

The analysis of agrarian trade for the mentioned countries in Western Balkan was done by numerous authors (Ćejvanović et al., 2014; Hodo, 2014; Jovanović and Despotović, 2014; Jovanović et al., 2015; Braha et al., 2017; Matkovski et al., 2017; Matkovski et al., 2018; Milovanović et al., 2018; Marković et al., 2019; Mrdalj et al., 2019; Brkić et al., 2021; Matkovski et al., 2021) applying different methodologies, and calculating different indices of foreign trade exchange, mainly those related to determining the competitiveness of an individual country in foreign trade in agricultural and food products.

Matkovski et al. (2017) analysed the effects of trade liberalization on the performances of foreign trade in agrarian products and found that liberalization had a positive effect on the intensification of Serbia's foreign trade with other CEFTA and EU countries. Matkovski et al. (2021) concluded that all Western Balkan countries, except Albania, have comparative advantages in exporting agrarian products. They suggest that all Western Balkan countries should improve positions of their agrarian products on the EU market already during pre-accession negotiations for EU membership and increase the level of competitiveness of these products in the EU common market. However, despite positive effects of liberalization of the agrarian trade, all Western Balkan countries have a lower level of competitiveness when compared to the EU countries. Matkovski et al., (2018) show that changes in partial productivity in agriculture have a positive impact on the comparative advantage of Western Balkan countries.

Braha et al. (2017) suggest that despite its huge agrarian potential, Albania has achieved trade deficit in the production and trade of agrarian commodities. Ćejvanović et al. (2014) concluded that foreign trade has an impact on the agricultural sector in Bosnia and Herzegovina. Brkić et al. (2021) found low intensity and vertical nature of the agrarian intra-industry trade of Bosnia and Herzegovina with the EU countries. Milovanović et al. (2018) found that Bosnia and Herzegovina's agrarian exports and imports have been increasing recently, while the volume of agrarian exports have a higher growth rate than of imports. Mrdalj et al. (2019) analysed the agrarian trade of poultry, pork and beef meat between Bosnia and Herzegovina and the rest of the world. All these meat categories revealed comparative disadvantage. Jovanović and Despotović (2014) have found prominent role of agrarian trade in Montenegro's economy due to the high share of the trade deficit in GDP, and high share of agrarian imports in total GDP as well as its high contribution to the total trade deficit. Jovanović et al. (2015) analysed the competitiveness and changes in agrarian foreign trade of the Montenegro from 2006 to 2013. They found that level of self-sufficiency was the lowest one in Montenegro, followed by Albania and Bosnia and Herzegovina.

Natos et al. (2014) studied the extent of agricultural trade complementarity between Western Balkan and EU countries between 2007 and 2012 applying the

Regional Hirsmann index, Sectoral Hirsmann index and the Michaely Index. They conclude lack of agrarian trade complementarity between EU member states in relative geographical proximity to WB and Western Balkan countries, while North-Western EU countries like Finland, Germany, UK or France are displaying grater potentials as future exporting markets for the Western Balkans agrarian exports.

MATERIAL AND METHODS

The analysis of the changes in agrarian foreign trade between the Czech Republic and countries in the Western Balkan (Bosnia and Herzegovina, Serbia, Montenegro, North Macedonia and Albania) is using data from UNCTAD database (UNCTAD, 2023). Kosovo is not included in the analysis because the database UNCTAD does not provide trade data for this country. Croatia is also not included because the country is already an EU member state. The analysed time series covers the period 2013 – 2022. The focus is on the current period of the agrarian trade dynamic from the post-crisis recovery period after the Great Recession and it also includes the sub-period (2020-2022) when the pandemic of COVID-19 and economic turbulences appeared. The individual agrarian sectors (product groups) are defined according to the Standard International Trade Classification (SITC) Revision 3. Similarly to Hoang (2018), the analysis was carried out with a 3-digit data code, i.e. for 61 different commodity groups of agrarian foreign trade (SITC 0 + 1 + 2 + 4 - 232 - 251 - 266 - 267 - 269 - 27 - 28). The sum of these product groups defines the total agrarian trade in this study. It is therefore rather a broader definition of agrarian trade. The nominal values of the trade flows are in current prices in USD.

The Trade Balance Index (TBI) was employed to analyze the current development, position stages, and dependencies of agrarian trade. The index analyses whether a nation has specialization in export (as net-exporter) or import (as net-importer) for a specific group of products (Verter et al., 2021):

$$TBI_j^i = \frac{x_j^i - m_j^i}{x_j^i + m_j^i} \tag{1}$$

Where, TBI denotes the trade balance index of country i for product j; x and m represent exports and imports of product products j by nation i, respectively. The values of the index range from -1 to +1. Exceptionally, the TBI equals -1 if a nation only imports. On the other hand, the TBI equals +1 if a nation only exports. the country is a “net exporter” of a given food product if the value of TBI is positive. Inversely, the country is a “net importer” of agrarian products if the value is negative.

Complementarity of trade and its measurement

Traditional trade theories suggest that liberalization of trade leads to welfare-improving trade creation, because the removal of trade barriers eliminates the domestic sourcing by firms and consumers in some industries in favour of imports that are more efficiently produced in other countries. Contrasting to these generally accepted trade theories, Viner (1950) concluded that the impact of preferential trade liberalization effort is a combination of trade creation effects (welfare gain implications for both partners involved) and of trade diversion effects (reducing the importer country's welfare). Following this argument, the Natural Trading Partners hypothesis is linked to attempts to identify characteristics of states that lead to more trade creation than trade diversion and thus ensure net welfare gains as a result of preferential liberalization agreements.

Proponents of the hypothesis (Lipsey, 1960; Wonnacott and Lutz, 1989; Krugman 1991; Frankel et al., 1995) suggest that natural trading partners are countries (1) significantly trading with each other prior to the agreement and countries close each other and/or (2) the more proximate they are, the less transport costs are limiting the trade flows. Trade agreement among such countries is less likely to be trade-diverting and a preferential trading agreement is more likely to benefit its members. Such an approach has also received criticism as Bhagwati and Panagariya (1997) and Panagariya (1997) questioned such a reasoning, because the larger the initial level of trade between the partners or the closer countries are geographically, the more they will lose from a preferential trading agreement. Krugman (1991) pointed out that the distance and transport costs already does not play such a role due to the technological progress in transport and communication.

Important contribution was made by Schiff (2001). He argued that the definition of natural trading partners should be changed to a situation characterized by the complementarity of countries in trade rather than by substitutability and competition in trade to maintain the theory's predictions. In other words, where a country tends to import what the other country exports. Similarly, Chandran (2010) points out that for the success of any regional trade agreement, it is necessary that the individual economies have complementary trade structures to be exploited for mutual benefit. Complementarity is used to define the extent to which countries have dissimilar resources and patterns of production, and they are likely to trade intensively with each other (Drysdale, 1969).

There are various empirical tools and procedures that are used in trade studies to assess trade complementarity as Export similarity index (Finger and Kreinin, 1979), association between trade competitiveness indices (Jayawickrama and Thangavelu 2010; Hoang 2018), Michaely index (Michaely, 1996), Regional Hirschman index (Mikic and Gilbert, 2009) and Trade complementarity index (TCI) proposed by Drysdale (Drysdale, 1969). Each of these tools assesses the complementarity slightly in a different manner and accents different aspect of complementarity. In this study, we use the Trade complementarity index (Drysdale, 1969). The reason is that this index allows us to assess complementarity both ways (e.g. the Czech Republic's exports to

Albania's imports and vice versa). To assess complementarity, the index also uses not only the export structures but also the import structures.

The main idea of TCI is to measure the extent to which one country's export structure matches another country's import structure more closely that it matches the structure of the world imports.

$$TCI_{ab} = \sum_j^n \left(\frac{X_a^j}{X_a} \times \frac{M_W - M_a}{M_W^j - M_a^j} \times \frac{M_b^j}{M_b} \right) \tag{2}$$

where M_a^j and M_b^j are imports of commodity j by the countries a resp. b, M_a and M_b are total agrarian imports of countries a resp. b, M_w^j is the world import of commodity j and M_w is the total world agrarian import.

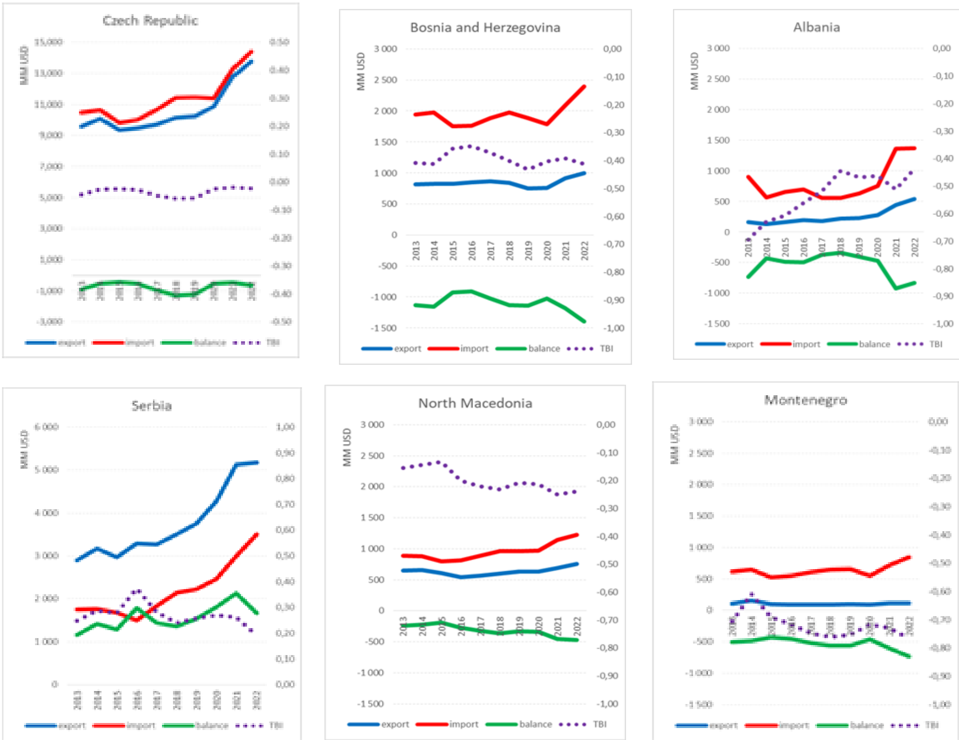
The TCI value of unity indicates that the export and import specializations are similar to the world economy specialization and the existence of comparative advantage cannot explain the bilateral trade (Hoang, 2018). The value of TCI greater then (smaller) than unity points to the existence of strong (weak) complementarity between the export specializations of country a and the import specialization of country b.

RESULTS AND DISCUSSION

Agrarian trade of analyzed countries

The agrarian sector of the Czech Republic is positioned in the structure of the national economy as is typical for developed economies, i.e. with a small share of GDP and the labour force, as the economic centres of gravity are located in other sectors. The Czech Republic’s agrarian trade have been characterized by changes since the liberalization after 1992. Important changes in the structure and volumes of traded agrarian products into/from markets of other European countries have also occurred, especially after the Czech Republic joined the European Union in 2004 (Smutka et al., 2018). In 2022, the agrarian exports of the Czech Republic have reached 13.8 billion US dollars, and the agrarian imports 14.4 billion US dollars. The Czech Republic’s balance of agrarian trade is negative, nonetheless, the Trade balance index score reached -0.03, indicating that the Czech Republic is a net importer in agrarian trade. However, agrarian exports cover a significant part of the value of agrarian imports. The main markets of Czech agrarian trade are mostly other member states of the European Union (EU is around 90% of exports), specifically Slovakia, Germany, Poland, Italy, Austria and Hungary. These countries represent around 80% of the Czech Republic's agrarian exports to the European Union.

In absolute values, the largest exporter of agrarian products in the Western Balkan region is Serbia (5.2 billion US dollars in 2022), followed by Bosnia and Herzegovina (1.0 billion US dollars), North Macedonia (0.8 billion US dollars), Albania (0.5 billion US dollars), and Montenegro (0.1 billion US dollars). Bosnia and Herzegovina (TBI: -0.39 on average from 2013 to 2022), North Macedonia (TBI: -0.20), Albania (TBI: -0.53), and Montenegro (TBI: -0.72) are positioned as net importers in agrarian trade. The negative TBI indicates a relatively high dependence on agrarian imports. Serbia is positioned as net exporter.



Source: Authors' construct based on data from UNCTAD (2023)

Figure 1. Dynamics of total agrarian trade of the Czech Republic and particular countries from Western Balkan (2013-2022); export, import balance, TBI; millions USD

The structure of Serbia's agrarian export mostly consists of S044, S058, S122, S057, and S421 (5 most exported products on average from 2013 to 2022). The structure of agrarian imports mostly consists of S057, S098, S071, S122, and S081. These products contribute 43.5% to the value of Serbia's agrarian exports and 32.1% to the value of agrarian imports. The structure of Bosnia and Herzegovina's agrarian export mostly consists of S248, S421, S245, S022, and S058. The structure of agrarian imports mostly consists of S098, S112, S048, S081, and S111. These products contribute 51.6% to the value of Bosnia and Herzegovina's agrarian exports and 31.7% to the value of agrarian imports. The structure of North Macedonia's agrarian export mostly consists of S121, S054, S048, S112, and S057. The structure of agrarian imports mostly consists of S012, S098, S048, S421, and S057. These products contribute 61.7% to the value of North Macedonia's agrarian exports and 33.5% to the value of agrarian imports. The structure of Albania's agrarian exports mostly consists of S054, S037, S058, S057, and S292. The structure of agrarian imports mostly consists of S048, S041, S057, S111, and S012. These products contribute 61.2% to the value of Albania's agrarian exports and 34.9% to the value of agrarian imports. The structure of Montenegro's agrarian export mostly consists of S112, S248, S016, S246, and S017. The structure of agrarian imports mostly consists of S012, S098, S111, S048, and S022. These products contribute 62.8% to the value of Montenegro's agrarian exports and 39.0% to the value of agrarian imports. This overview points to a relatively high level of specialization in agrarian exports especially in North Macedonia, Albania, and Montenegro as the five most imported agrarian products contribute more than 60% to agrarian exports.

Complementarity of agrarian trade between the Czech Republic and Western Balkan

On average 2013-2022, Western Balkan country's agrarian export structure matches the Czech Republic's agrarian import structure in an almost similar way as it matches the structure of the world agrarian imports. Nonetheless, the TCI scores are steadily increasing during the period reaching 1.15 in 2022. This suggests that agrarian exports from Balkan countries are getting more complementary to the agrarian imports of the Czech Republic than to the world agrarian imports (table 1).

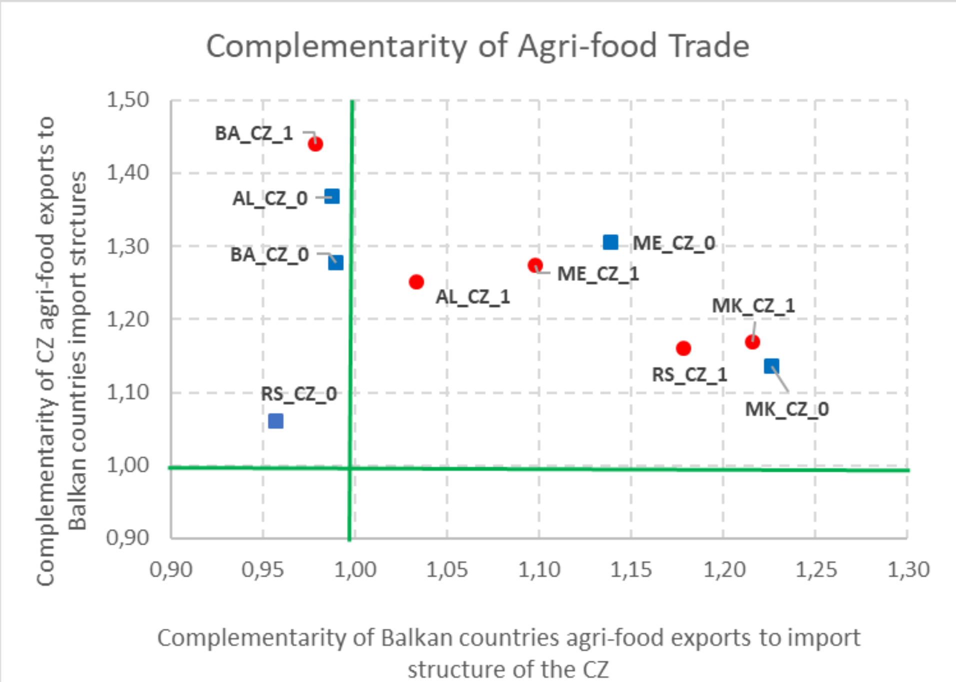
Table 1. Scores of TCI index for country pairs, 2013-2022

From	To	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	13-22
Western Balkan	**Czechia**	1.01	1.06	1.01	1.03	1.07	1.04	1.04	1.05	1.09	1.15	**1.05**
Czechia	**Western Balkan**	1.23	1.28	1.33	1.33	1.31	1.28	1.25	1.28	1.23	1.24	**1.27**
Albania	**Czechia**	0.99	1.03	0.98	0.92	0.95	0.96	0.91	0.96	1.04	1.03	**0.98**
Bos. and Herz.		0.99	1.00	0.95	0.97	0.93	0.91	0.95	0.94	0.99	0.98	**0.96**
Montenegro		1.14	1.40	1.09	1.03	1.03	1.03	1.06	1.08	1.24	1.10	**1.12**
N. Macedonia		1.23	1.28	1.19	1.13	1.21	1.14	1.12	1.21	1.13	1.22	**1.18**
Serbia		0.96	1.02	0.99	1.03	1.09	1.05	1.05	1.05	1.10	1.18	**1.05**
Czechia	**Albania**	1.37	1.34	1.35	1.46	1.29	1.30	1.25	1.19	1.26	1.25	**1.30**
	Bos. and Herz.	1.28	1.26	1.28	1.28	1.27	1.26	1.31	1.31	1.31	1.44	**1.30**
	Montenegro	1.30	1.25	1.29	1.27	1.29	1.25	1.23	1.26	1.26	1.27	**1.27**
	N. Macedonia	1.14	1.17	1.19	1.19	1.16	1.15	1.15	1.16	1.13	1.17	**1.16**
	Serbia	1.06	1.12	1.10	1.12	1.06	1.15	1.16	1.19	1.13	1.16	**1.12**

Source: Authors' construct based on data from UNCTAD (2023)

When assessing the agrarian trade complementarity between the Czech Republic and analysing five countries in Western Balkan as a block, the Czech Republic's agrarian exports match even more closely agrarian imports of the Balkan countries as a block, reaching the score of 1.27 on average between 2013 and 2022. These results support the conclusion that the Czech Republic and Bosnia and Herzegovina, Serbia, Montenegro, North Macedonia and Albania as a block are natural trading partners in agrarian trade.

When assessing the complementarity between the Czech Republic and Western Balkan countries on the bilateral level, there is complementarity of the Czech Republic's agrarian export structure to the agrarian import structure of each of these countries. On average between 2013 and 2022, the highest TCI scores reveal the Czech Republic with Albania and Bosnia and Herzegovina (1.30), followed by Montenegro (1,27) North Macedonia (1.16) and Serbia (1.12). The analysis of the change in complementarity shows that in the case of Serbia and Bosnia and Herzegovina, the complementarity has increased. On the other hand, in the case of Albania the complementarity has decreased and remained about the same in the case of Monte Negro and North Macedonia.



Source: Authors' construct based on data from UNCTAD (2023)
Note: AL – Albania, BA – Bosnia and Herzegovina, ME – Montenegro, MK – North Macedonia, RS – Serbia; 0 – year 2013, 1 – year 2022

Figure 2 Complementarity of agrarian trade between the Czech Republic and specific countries from the Western Balkans (avg, 2013-2022); TCI

The second important result is that, on average, the results suggest that Albania's and Bosnia and Herzegovina's agrarian export structures match the world agrarian import structures more than the Czech Republic's agrarian import structures. However, here is important to point out that the TCI scores of Albania and Bosnia and Herzegovina are close to unity and scores are higher than unity in some of the years. On the other hand, Montenegro's (TCI: 1.12 on avg. 2013-2020) and North Macedonia's (TCI: 1.18 on avg. 2013-2020) agrarian export structures are complementary to the Czech Republic's agrarian import structures. In the case of Serbia, the TCI scores are increasing during the period. Analysis of the complementarity of the agrarian export and import structures between the Czech Republic and Serbia (and vice versa) is signalling improved reciprocal complementary of Serbian and the Czech Republic's agrarian trade structures.

The reciprocal complementarity of agrarian trade structures between the Czech Republic and analysed Western Balkan countries is presented in figure (figure 2).

It compares the pair complementarity scores between the beginning of the period and the end of the period. If the reciprocal TCI scores are both higher than unity, it represents win-win situation for both the Czech Republic and particular Balkan country (quadrant right-up). Some of pair complementarity scores are present in left-up quadrant. This represents complementarity of the Czech Republic's agrarian exports structures to agrarian import structure of the Balkan country (TCI score y axis is greater than unity), but the analysed Balkan country agrarian export structure is rather more complementary to the World agrarian import structure than to the Czech Republic's agrarian imports structures (TCI score on x axis is smaller than unity).

Since so far there have been no researches and published papers based on them that specifically deal with the complementarity of foreign trade exchange between the countries of the Western Balkans and specifically the Czech Republic (but only general foreign trade exchange between the Western Balkans and the EU), it was not possible to compare the obtained results with the results of other analysis on the same topic.

Agrarian Trade between the Czech Republic and Western Balkan Countries

In 2022, the Czech Republic's agrarian exports to these five Western Balkan countries reached about 102 million USD and the agrarian imports 66 million USD. On average from 2013 to 2022, this trade exchange consists of only 0.4% of the Czech Republic's agrarian exports and 0.3% of the Czech Republic's agrarian imports. Among these Western Balkan countries under scrutiny, the Czech Republic mostly exports to Serbia (51.9% of the Czech Republic's agrarian exports to these Western Balkan countries), and to Bosnia and Herzegovina (29.5%). The Czech Republic mostly imports from Serbia (56.7%), and North Macedonia (26.0%). The Czech Republic mostly exports S098, S022, S112, S048, S247 and these products consist 59.5% of agrarian exports of the Czech Republic to the analysed Western Balkan countries. Analysed Western Balkan countries export products as S057, S054, S081, S058, S421 to the Czech Republic. These five products consist of 55.5 of the exports of these countries to the Czech Republic. The Czech Republic reveals a positive agrarian trade balance with Serbia, Bosnia and Herzegovina, and Montenegro. On the other hand, the Czech Republic reached a negative agrarian trade balance with North Macedonia and since 2017 also with Albania.

Table 2 Bilateral agrarian trade, 2013-2022, export from the Czech Rep. to WB countries and import to the Czech Republic from WB

	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	%
	Czech Republic's export to (1000 USD)										
Albania	1,658	1,445	2,200	3,603	2,036	940	1,371	2,138	2,479	8,373	5.2
Bos. and Herz.	5,123	8,706	10,605	9,088	11,592	15,135	17,574	16,868	17,048	37,297	29.5
Montenegro	513	785	738	976	830	766	978	673	1,010	1,182	1.7
N. Macedonia	6,512	5,149	4,753	4,258	4,476	4,888	6,158	5,956	6,554	10,141	11.7
Serbia	20,666	20,400	16,245	18,576	20,992	24,184	29,229	31,266	34,967	45,477	51.9
Suma	**34,472**	**36,485**	**34,541**	**36,502**	**39,857**	**45,912**	**55,310**	**56,901**	**62,067**	**102,470**	**100.0**
	Czech Republic's import from (1000 USD)										
Albania	1,485	1,343	1,036	1,291	3,898	3,728	4,894	7,202	8,847	10,629	11.4
Bos. and Herz.	1,549	1,247	1,100	1,898	1,449	1,896	2,548	3,026	3,429	4,432	5.8
Montenegro	66	75	31	46	33	53	75	37	47	48	0.1
N. Macedonia	13,411	10,958	10,013	8,684	6,333	9,392	9,987	11,425	9,373	11,144	26.0
Serbia	19,243	17,907	14,143	15,764	14,349	18,104	19,133	24,859	36,772	39,690	56.7
Suma	**35,753**	**31,530**	**26,323**	**27,682**	**26,062**	**33,172**	**36,637**	**46,549**	**58,467**	**65,943**	**100.0**

Source: Authors' construct based on data from UNCTAD (2023)

CONCLUSIONS

This article interrogates the agrarian trade complementarity of countries in the Western Balkans and the Czech Republic to assess the ongoing integration and liberalization processes and the rationale for the economic cooperation.

Agrarian trade is an important part of the development's dynamic of agrarian and food sectors in countries of the Western Balkans and plays a vital role in economic, employment and trade structures in these countries. Countries of the Western Balkans have a long tradition of mutual trade, nonetheless the establishment of the Central and Eastern Europe Free Trade Agreement (CEFTA), trade with EU member states and various trade agreements led to the strengthening of the role of the agrarian trade on the changes in agrarian sectors of Western Balkan countries. This has implications to economic as well as social development and/or stability as countries of the Western Balkans are net importers (except of Serbia) of agrarian products. The international trade is an important mechanism how these countries partially solve their food security. This of course has its potential benefits as well as risks, depending on the dynamic of international agrarian markets. The deepening of political and economic integration with the European Union will further increase the intensity of the agrarian trade. It can be expected that the growing agrarian trade intensity will bring additional set of factors influencing the agrarian sectors in the region of the Western Balkans, similar to the experience of countries in Central and Eastern Europe joining EU in the past two decades. Also, the increase in intensity of Croatian agrarian trade after EU accession can be taken as a benchmark. The further liberalization of trade with EU member states can help Western Balkan countries to increase their export of agrarian commodities and food (while these countries will of course also open its markets to agrarian imports).

We used the Czech Republic as an example to interrogate complementarity of agrarian trade between Western Balkan countries and EU member states. The analysis of agrarian trade complementarity indicates two-way complementarity of agrarian trade for the Czech Republic and North Makedonia and Montenegro. After the changes is the exports and imports structure during the period under scrutiny, Serbia and Albania have also become complementary to the agrarian trade of the Czech Republic and vice versa. The Czech Republic agrarian exports are complemented to agrarian imports of Bosnia and Herzegovina, however, Bosnia and Herzegovina rather match with structure of the world agrarian import than with the one of the Czech Republic. Generally, these results support the ongoing liberalization process and the rationale for the cooperation of the Czech Republic with Western Balkan countries (and vice-versa) and taking advantage of existing economic resources exists in the segment of agrarian products. These results are in line with previously published study from Natos et al. (2014).

The analysis of the actual agrarian trade exchange between the Czech Republic and particular countries of the Western Balkans shows rather low values despite existing complementarities. Although these potential trade opportunities exist, there is probably further room for improvement and promotion of mutual agrarian trade. Besides specialized trade and investments agencies, one specific form how the Czech Republic is supporting and promoting mutual agrarian trade with other countries are the dedicated positions of agrarian diplomats. Since 2016, the Czech Republic has placed its agrarian diplomat also in Serbia; the diplomat is also involved in promoting trade with other countries in the region as Montenegro, Bosnia and Herzegovina, and North Macedonia. In general, the task of the agrarian diplomats is mainly to strengthen and support the business cooperation of Czech food producers, farmers and entrepreneurs with partners from the respective countries. Results of this study provide justification and support of this particular policy measure. All Western Balkan countries, except Montenegro, have diplomatic missions in the Czech Republic, and the Czech Republic has diplomatic missions in all WB countries. The most countries of the Western Balkans are small in terms of economic strength, and have a limited number of staff in their embassies (newly established after the collapse of SFRY). The Western Balkan countries have diplomats in charge of strengthening economic cooperation and trade only in large countries, but almost nowhere they have persons exclusively in charge of agrarian trade.

Despite existing effort of the Czech Republic to facilitate and promote the agrarian trade with Western Balkan countries, the results suggest existing barriers to the trade and the nature of these barriers should be the subject of further research.

There are also limitations of our research. The trade complementarity in this paper is defined as extent to which one country's export structure matches another country's import structure more closely than it matches the structure of the world imports. There are other empirical tools and procedures that are used in trade studies to assess trade complementarity. This gives opportunity for further research by applying these methods and derives more robust results, points of view and policy recommendations.

This article's focus on the agrarian trade complementarity is using the trade between the Czech Republic and EU candidate countries in the Western Balkans as an example. Current strengthening of the strategic interaction and enhanced European Union's engagement with the Western Balkans, and the potential accession of countries in the Western Balkans to the EU increases the importance of this theme of trade complementarity.

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MORPHOLOGY AND ANATOMY OF INDIGENOUS RICE LANDRACES IN KHON KAEN, THAILAND

SUMMARY

This study comprised collecting and determining the morphology and anatomy of leaf blade and leaf sheath of indigenous rice landraces in Khon Kaen, Thailand. A total of twenty-seven indigenous rice landraces were discovered, while, only 12- plant and grain samples were collected: Khao Plong Aew, Khao Jao Leuang, Khao San Pla Tong, Khao Gam Poon, Khao E-Leuang Noi, Khao Niaw dang, Khao Lao Taek, Khao E-Tom Kao, Khao Kam Pai, Khao Kao Gon, Khao Nang Nuan and Khao Mun Ped. Gross morphology was investigated and descriptions made. The anatomical characteristics of leaf blade and leaf sheath were studied using peeling and paraffin methods. The results demonstrated that the morphological and anatomical characters of 12 indigenous rice landraces are similar with a slight difference in some characters. Morphology differed in the color of collar, auricle and ligule as well as the shape and color of brown rice. The main distinguishing morphological characteristics for identifying indigenous rice landraces are grain features. For the anatomy of leaf blade and leaf sheath, the results revealed that there were only differences in the presence of prickles and the shape of anticlinal wall of epidermal cell on the lower surface of leaf sheath. Even thought, the anatomical characters of leaf blade and leaf sheath were not appropriated to identify the indigenous rice landraces in Khon Kaen. However, Khao Jao Leuang was distinguished from other indigenous rice landraces by the absence of prickles on both leaf blade surfaces. Moreover, Khao Jao Leuang is the only landrace in which the shape of the anticlinal wall of the epidermal cell on lower surface of leaf sheath, is undulate, others are sinuate.

Keywords: indigenous rice, anatomy, morphology, leaf, grain

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INTRODUCTION

At the present, Thailand is developing in industry and the adoption of modern technology. However, rice cultivation remains Thailand 's principal industry and priority crop; maximizing yields relies on farmers' knowledge and understanding of ecology and environment, on societal and cultural factors, and the ability to adapt agricultural technology to increase rice production (Polthanee, 2010). Nowadays, modern technology has enabled changes in rice cultivation, while economic and environmental factors often necessary novel approaches. New rice cultivars are able to grow more abundantly, offering high tillering and yield, and good grain quality. Thus, rice breeding programs require the collection and survey of rice germplasm as a genetic resource. In the past, human collected grains and carried them as a food during migration. This resulted in the transfer of rice grains from one locality to another. The presence of particular rice landraces in different regions leads to the rice diversity in each locality. As a result, the name of rice landraces posses distinctive traits or a point of origin, becoming indigenous rice.

Thailand is an important source of rice germplasm; indigenous rices are genetic diverse. These Thai indigenous rice germplasms are useful for future rice breeding programs. Department of Agriculture collected over 2000 samples of rice germplasm from various provinces across Thailand for conservation, consisting of indigenous rice, good rice cultivars, foreign rice and wild type rice. Since 1937, the indigenous rice germplasms were collected in Thailand and between 1946-1950, there was the first phase for rice collection and selection from Thai farmers in term of rice government. In 1982, Rice Research Institute established a gene bank in Thailand as a center for Thai rice germplasm collection, which was supported by the Japanese government. Unfortunately, many indigenous rice landraces have been degraded, and Thai farmers also prefer to cultivate high yield rice cultivar. This has led to the rapid disappearance of Thai indigenous rice. Consequently, nowadays, rice germplasm collection aims to conserve rice grains as a rice genetic resource with potential utilization in various fields including rice selection and breeding for industrial processing. This allows the development of Thai indigenous rice landrace to increase their diversity and unique characteristics in terms of value, taste and health benefits.

Between 1995-1999, the Rice Department found and collected 5,928-Thai indigenous rice landraces (Wuttiyano, 2000) and in the Northeast area, 1,564-Thai indigenous rice landraces were found (Jaidee and Thongpitak, 1988). At the present, there were a number of Thai indigenous rice landraces because of the development of a rice breeding program and a growing rice trade leading to export of Thai indigenous rice landraces (Alternative agriculture network in the Northeast, 2003). In 2000, the collection of Thai indigenous rice across 76 provinces of Thailand, there was 101-Thai indigenous rice landraces in Khon Kaen province (Plant varieties protection office, 2000). Additionally, 15-Thai indigenous rice landraces in Khon Kaen were collected by the Center for Promoting Rice Production (Kaewkaenkoon et al., 2017). In other provinces of

Thailand, the indigenous rice landraces were Hom Udom, San Patong, Nang Nguan, and Keetom in Keang subdistrict, Mahasarakham province. However, in earlier study, 20-indigenous rice landraces had been discovered from Mahasarakham (Kijtewachakul et al., 2008). The field survey in Thale Noi Basin, Phatthalung province by Panomjan and Amornviriyachai (2011) exhibited 7-indigenous rice landraces, namely; Dawk Pa-Yawm (uplandrice), Sang Yod, Leb Nok, Khem Tong, Niaw Dam (glutinous rice), Chiang Phatthalung and Hawm Jan. And 17-indigenous rice landraces were found in Emad Esai, Banrai district, Uthaithani province (Khunhan, 2018).

In addition, morphological characters are used as a basic information for classifying rice varieties and rice landraces. Anatomical details can also be utilized to supply additional information for rice classification. However, this is dependent on plant species (Taia, 2005). Further, the morphological and anatomical characteristics can be used to classify plants at different levels, for example, some characteristics may be classified at species level, whilst some may be classified at infraspecific level. The database of Thai indigenous rice, was informed by the study of rice diversity in different areas of Thailand (Promsomboon and Promsomboon, 2016) and reports comparing the different Thai indigenous rice landraces from some areas of Thailand by using morphology and anatomy. Such Thummavongsa et al. (2012) showed the different morphology and anatomy of different rice grain cultivated in Northeast Thailand. Boonrueng and Jampeetong (2016) studied the morphology of grain and the anatomy of leaf blade and leaf sheath in rice landrace *Bue Po Lo,* an upland indigenous rice landrace. In addition, indigenous rice landraces in Southern Thailand, such as Sang Yod, Chaw Jam Pah, Niaw Look Pueng, Niaw Dawk Yong, and Khao Nang exhibited various seed coat color including straw and brown which could classify them as different indigenous rice landraces (Panomjan and Tongkiaw, 2011). Besides seed coat color, the shape and color of brown rice were also classified for the different indigenous rice landraces (Panomjan and Amornviriyachai, 2011; Panomjan and Tongkiaw, 2011). In analyzing the function of anatomy characteristics of leaf blade and leaf sheath were useful for identifying indigenous rice landraces. These characters might relevant to plant adaptation, such as the leaf blade occurring papillae in upper and/or lower epidermis was a plant against pathogen invasion by modification of cell wall to form a physical barrier (Underwood, 2012). Moreover, unicellular hair appearing on leaf sheath was found to be a simple structure which usually does not have glands. It plays a role in resisting extreme environmental conditions such as drought, high salt, UV, pathogen and insects (Han et al., 2022). Thus, these anatomical characteristics of leaf blade and leaf sheath were useful for identifying indigenous rice landraces.

As mentioned above, the database of indigenous rice collection is informed by old data and the survey area only covers some parts of Khon Kaen province. Also, the morphological and anatomical database of Khon Kaen indigenous rice landrace is limited. This resulted in lack an updated database and germplasms bank for Khon Kaen indigenous rice landraces, which would better enable classifying, conservation and breeding programs. Thus, this study aimed to contribute a collection and a database of Khon Kaen indigenous rice diversity for conservation by collecting and determining the morphological and anatomical characteristics of indigenous rice in Khon Kaen province. In addition, the obtained data was used for classifying and also selecting high potential Khon Kaen indigenous rice landraces for a rice breeding program.

MATERIAL AND METHODS

Survey and sample collection

Indigenous rice samples were surveyed and collected in Khon Kaen province. Rice samples were obtained from the farmer and took them for studying morphological and anatomical characteristics; a reference specimen (voucher specimen) was kept in Khon Kaen University Herbarium. For the anatomical characteristics study, rice samples were preserved with 70% ethanol.

Morphological study of Indigenous rice landraces

The morphological characteristics of Indigenous rice samples were described, such as leaf size, leaf blade color, leaf sheath color, grain size and grain color. The description of general morphology was compared among diverse different indigenous rice landraces.

Anatomical study of Indigenous rice landraces

The comparative leaf and leaf sheath anatomy of Indigenous rice samples were studied. Leaf and leaf sheath samples were preserved with 70% ethanol at least 24 h and then epidermal features were studied using the leaf epidermal peeling technique, and the transverse section was studied using the paraffin method. Leaf and leaf sheath epidermal peeling was stained with 1% safranin in 70% ethanol, washed with tap water and then, permanent slides were made by the dehydration with ethanol, soaked in 70%, 95%, 100%, 100% ethanol:xylene (ratio 1:1) and xylene for 5 min, respectively. After that, the slides were mounted with DPeX. A transverse section of the leaf was prepared by paraffin methods according to Johansen (1940). All anatomical characters of leaf epidermis and leaf cross section of 12 indigenous rice landraces were recorded under a light compound microscope.

RESULTS AND DISCUSSION

Indigenous rice diversity in Khon Kaen province

Base on the survey and collection of indigenous rice in 16 districts of Khon Kaen province, Thailand, 27-landraces were found as show in Table 1 and Figure 1. Only plant and grain samples of 12-landraces were collected as show in Table 1 and Figure 1, namely, Khao Plong Aew, Khao Jao Leuang, Khao San Pla Tong, Khao Gam Poon, Khao E-Leuang Noi, Khao Niaw dang, Khao Lao Taek, Khao E-Tom Kao, Khao Kam Pai, Khao Kao Gon, Khao Nang Nuan and Khao Mun Ped. The results indicated a slight decline in the diversity of indigenous rice varieties from Year 2017, when 15 landraces were reported by Center for Promoting Rice Production, Khon Kaen (Kaewkaenkoon et al., 2017). Furthermore, the diversity sharply decreased compared to Year 2000, when 101 landraces were reported by the Plant varieties protection office (2000). The reduction in the numbers of Khon Kaen indigenous rice landraces may be caused by the undesirable texture (such as hard grain) of some cultivars or some landraces, leading to rejection by consumers. Nowadays, Khon Kaen farmers tend to cultivate commercial varieties such as KDML105 and RD6, which are famous by customers and hold strong market demand, command high cost, and also offer good quality, with a good texture and taste. Thus, slight reduction in Khon Kaen indigenous rice landraces found by this study suggested a need to conserve Khon Kaen indigenous rice landraces in order to maintain a store of rice germplasms and evolution knowledges for the future.

Table 1. Indigenous rice landraces in 16 districts of Khon Kaen province

No.	Indigenous rice landraces	Location
1	Khao Plong Aew*	Ban Phai district; Ubolratana district
2	Khao Jao Leuang*	Phon district
3	Khao San Pla Tong*	Waeng Yai district; Ubolratana district
4	Khao Gam Poon*	Non Sila district; Phra Yuen district; Nong Na Kham district
5	Khao E-Leuang Noi*	Non Sila district
6	Khao Niaw dang*	Non Sila district; Ban Haet district
7	Khao Lao Taek*	Manchakhiri district
8	Khao E-Tom Kao*	Non Sila district
9	Khao Kam Pai*	Waeng Yai district; Chum Phae district; Phu Pha Man district
10	Khao Kao Gon *	Chum Phae district; Phu Pha Man district
11	Khao Nang Nuan*	Non Sila district
12	Khao Mun Ped*	Khao Suan Kwang district
13	Khao Keetom Yai	Mueang Khonkaen district
14	Khao Keetom Klang	Mueang Khonkaen district
15	Khao E-Kao Yai	Waeng Yai district
16	Khao Jaw Loy	Waeng Noi district; Sam Sung district
17	Khao Kao Bai Rong	Phon district
18	Khao Jaw Dang	Non Sila district
20	KhaoE-Dang Pla Look Krok	Chum Phae district
21	Khao Ta Dee	Chum Phae district
22	Khao Lung Ma	Sam Sung district
23	Khao E-Tum	Ban Phai district
24	Khao Kam	Phra Yuen district
25	Khao Niaw Dum	Phon district
26	Khao Niaw E-Tia	Phu Wiang district
27	Khao Kao Lum	Phu Pha Man district

* indicates the landraces that plant and grain samples can be obtained for this study.

In addition, our findings showed that 27-indigenous rice landraces were found in Khon Kaen province. While, in other province of the Northeastern region such as in Keang subdistrict, Mahasarakham Province, Kijtewachakul et al. (2008) found 20-indigenous rice landraces in the past. However, many landraces were still cultivated in the Northeastern region, namely Hom Udom, San Patong, Nang Nguan, and Keetom. Two indigenous rice landraces, namely Nang Nguan, and

Keetom, were similarly found in Non Sila district and Mueang Khon Kaen district, Khon Kaen province, respectively.

In the other regions of Thailand, a field survey of indigenous rice landraces in the Southern region in Thale Noi Basin, Phatthalung province found seven indigenous rice landraces, including Dawk Pa-Yawm (uplandrice), Sang Yod, Leb Nok, Khem Tong, Niaw Dam (glutinous rice), Chiang Phatthalung and Hawm Jan (Panomjan and Amornviriyachai, 2011). While, in the Central region, in the community of Emad Esai, Banrai district, Uthaithani province, approximately 17-indigenous rice landraces were found (Khunhan, 2018). In ASEAN country such as Lao PDR, there was 49-rice varieties from 6 villages, 3 districts of Luang Prabang province (Xiongsiyee et al., 2018). Overall, these findings suggested that the indigenous rice diversity from the same region or different regions may be similarly name of some indigenous rice landraces.

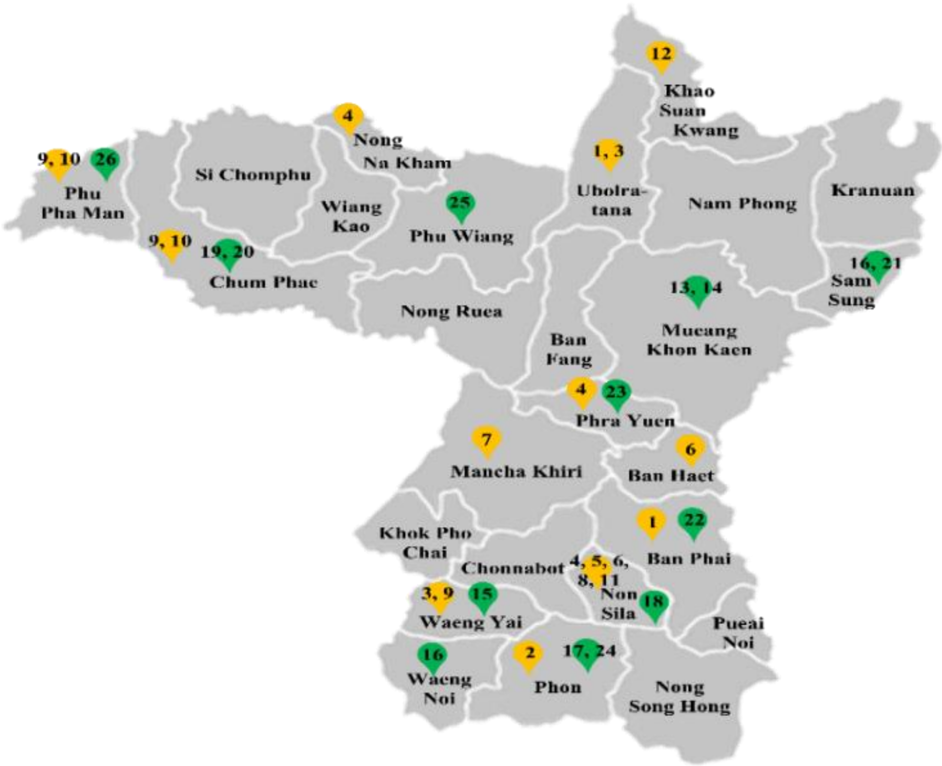

Figure 1. 27-indigenous rice landraces are found in 16 districts of Khon Kaen province, Thailand. The number represents indigenous rice name, including 1) Khao Plong Aew, 2) Khao Jao Leuang, 3) Khao San Pla Tong, 4) Khao Gam Poon, 5) Khao E-Leuang Noi, 6) Khao Niaw dang, 7) Khao Lao Taek, 8) Khao E-Tom Kao, 9) Khao Kam Pai, 10) Khao Kao Gon, 11) Khao Nang Nuan, 12) Khao Mun Ped, 13) Khao Kee Tum Yai, 14) Khao Kee Tum Klang, 15) Khao E-Kao Yai, 16) Khao Jaw Loy, 17) Khao Kao Bai Rong, 18) Khao Jaw Dang, 19) Khao E-Dang Pla Look Krok, 20) Khao Ta Dee, 21) Khao Lung Ma, 22) Khao E-Tum, 23) Khao Kam, 24) Khao Niaw Dum, 25) Khao Niaw E-Tia and 26) Khao Kao Lum. The yellow pin indicates the present plant and grain samples collecting for this study and the green pin indicates plants reported in the past.

Morphology of Indigenous rice landraces in Khon Kaen province

The leaf morphology of 12 indigenous rice landraces are shown in Table 2 and Figure 2. Plant height was determined in all rice and the highest and the lowest of plant height was found in Khao Plong Aew (120-170 cm) and Khao E-Leuang Noi (80-105 cm), respectively. The color of leaf blade, leaf sheath and node were green in all landraces. However, different colors of collar, auricle and ligule were found. The collar color in Khon Kaen indigenous rice varied between white, yellow, light green, green, and purple. The auricle color in Khon Kaen indigenous rice varied in white, yellow, light green, purple and brown. In addition, the ligule color in Khon Kaen indigenous rice varied between white and purple. Some indigenous rice landraces such as Khao E-Tom Kao, Khao Kam Pai and Khao Kao Gon was not determined because their leaf organs such as collar, auricle and ligule were damaged or incomplete.

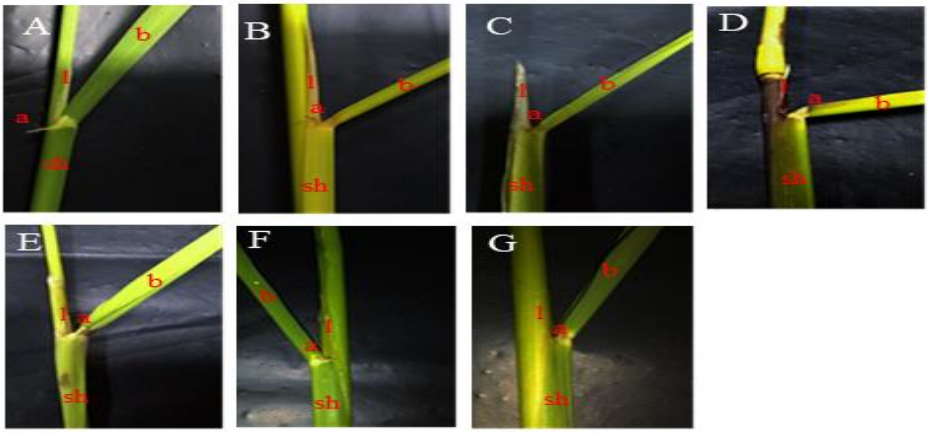

Figure 2. Leaf morphological characters (blade; b, auricle; a, ligule; l and sheath; sh) of some indigenous rice landraces in Khon Kaen province such as Khao Plong Aew (A), Khao Jao Leuang (B), Khao San Pla Tong (C), Khao Kam Poon (D), Khao E-Leuang Noi (E), Khao Lao Taek (F) and Khao Mun Ped (G).

The grain morphology of 12 indigenous rice landraces are shown in Table 3 and Figure 3. The size of grain and brown rice was similar in all rice landraces. However, the color of seed coat of grain and shape and color of brown rice showed variation in all rice landraces. The color of seed coat of grain in Khon Kaen indigenous rice varied between yellow, purple and brown. This result was quite similar to indigenous rice landraces identified in the Southern Thailand, such as Sang Yod, Chaw Jam Pah, Niaw Look Pueng, Niaw Dawk Yong, and Khao Nang, their seed coat color was straw and brown. However, only purple color was unrepresented in rice from the Southern Thailand (Panomjan and Tongkiaw, 2011). The shape of brown rice varied between long round, long narrow, long flat, short round and short flat. Moreover, the color of brown rice in Khon Kaen indigenous rice varied between white, white with yellow and yellow with green. Meanwhile, the shape of brown rice in the Southern Thailand differed in being slender (Sang Yod, Chaw JamPah, and Niaw Dawk

Yongvarieties) and medium (Niaw Look Pueng, and Khao Nang), while the brown rice color was distinguished by a red color compared to Khon Kaen indigenous rice (Panomjan and Tongkiaw, 2011).

Thus, our studies suggested that leaf and grain morphology such as colors of collar, auricle, ligule, seed coat of grain and brown rice and also shape of brown rice in Khon Kaen indigenous rice showed distinctive characteristic in each landrace. The results revealed that the morphological and anatomical characters of 12 indigenous rice landraces were similar with a slight difference in some characteristics. The different morphology was color of collar, auricle and ligule as well as shape and color of brown rice. The most distinguished morphological characteristics to identify indigenous rice landraces was grains feature. This study accorded to Panomjan and Amornviriyachai (2011) showed the highly distinctive grain morphology to identify indigenous rice landraces was seed coat color and shape and color of brown rice.

Figure 3. Grain morphological characters of indigenous rice such as Khao Plong Aew (A), Khao Jao Leuang (B), Khao San Pla Tong (C), Khao Gam Poon (D), Khao E-Leuang Noi (E), Khao Niaw dang (F), Khao Lao Taek (G), Khao E-Tom Kao (H), Khao Kam Pai (I), Khao Kao Gon (J), Khao Nang Nuan (K) and Khao Mun Ped (L).

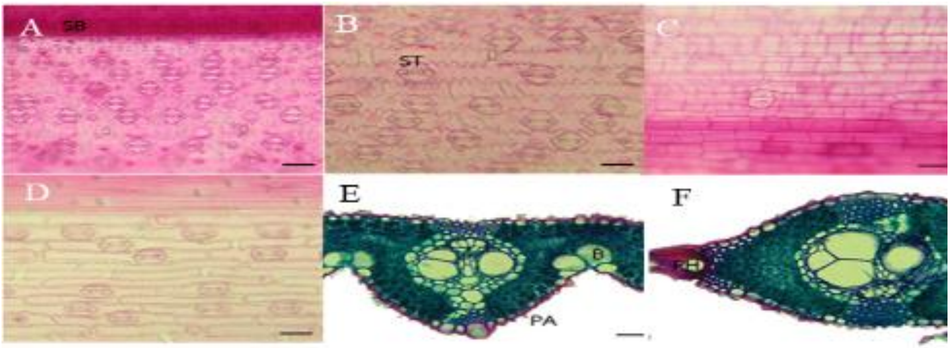

Figure 4. Leaf anatomical characters of Khao Jao Leuang: upper epidermis (A), lower epidermis (B), upper epidermis of leaf sheath (C), lower epidermis of leaf sheath (D), x-section of leaf blade (E) and x-section of leaf margin (F) (B = bulliform cell, FG = fibrous group, PH = prickle hair, PA = Papillae, SB = silica bead and ST = stomata), Scale bar = 20 µm.

Table 2. Leaf morphological characters of 12 indigenous rice landraces in Khon Kaen province, Thailand.

No.	Indigenous rice landraces	Height (cm)	Leaf blade		Leaf sheath color	Collar color	Node color	Auricle color	Ligule	
			Width × Length (cm)	Color					Length (cm)	Color
1	Khao Plong Aew	120 - 170	1.00 × 25 - 55	green	green	light green - white	green	light green - white	1.5 - 1.7	white
2	Khao Jao Leuang	100 - 145	1.00 × 20 - 40	green	green	light green	green	light green - white	0.5 - 1.5	white
3	Khao San Pla Tong	115 - 140	1.00 × 20 - 50	green	green	light green - yellow	green	Brown-white	1.2 - 2.0	white
4	Khao Gam Poon	85 - 100	1.00 × 15 - 40	green	green	purple	green	purple	1.0 - 1.5	purple
5	Khao E-Leuang Noi	80 - 105	1.00 × 25 - 40	green	green	light green	green	yellow	0.5 - 1.5	white
6	Khao Niaw dang	82 - 120	1.00 × 30 - 55	green	green	light green	green	white	1.0 – 3.0	white
7	Khao Lao Taek	100 - 130	1.00 × 25 - 55	green	green	light green - white	green	white	0.5 - 2.5	white
8	Khao E-Tom Kao	95 - 125	1.00 × 35 - 48	ND	ND	ND	ND	ND	1.5 - 3.0	ND
9	Khao Kam Pai	135 - 140	1.00 × 35 - 52	ND	ND	ND	ND	ND	2.0 - 2.5	ND
10	Khao Kao Gon	125 - 135	1.00 × 25 - 45	ND	ND	ND	ND	ND	1.0 - 2.0	ND
11	Khao Nang Nuan	85 - 105	1.00 × 30 - 48	green	green	green	green	purple	0.5 – 2.0	purple
12	Khao Mun Ped	105 - 185	1.00 × 30 - 50	green	green	green-white	green	white	1.5 - 2.0	white

Noted: ND = not determine

Table 3. Grain morphological characters of 12-indigenous rice landraces in Khon Kaen province, Thailand.

No.	Indigenous rice landraces	Grain			Brown rice			100-grain weight (g)
		Width × Length (cm)	Awn	Seed coat color	Width × Length (cm)	Shape	Color	
1	Khao Plong Aew	0.30 × 1.00	present	yellow	0.20 × 0.70	long round	white	2.67
2	Khao Jao Leuang	0.30 × 1.00	present	yellow	0.20 × 0.60	short round	white	2.52
3	Khao San Pla Tong	0.30 × 0.80	present	yellow	0.20 × 0.70	long round	white	2.61
4	Khao Gam Poon	0.30 × 1.00	present	purple	0.30 × 0.60	short flat	purple	1.27
5	Khao E-Leuang Noi	0.25 × 1.00	present	yellow	0.20 × 0.70	long narrow	yellow with green	1.34
6	Khao Niaw dang	0.25 × 1.00	present	brown	0.20 × 0.70	long round	yellow with green	1.61
7	Khao Lao Taek	0.30 × 1.00	present	yellow	0.20 × 0.70	long round	white with yellow	3.67
8	Khao E-Tom Kao	0.30 × 0.90	present	yellow	0.20 × 0.70	long flat	white	3.09
9	Khao Kam Pai	0.30 × 1.00	present	yellow	0.30 × 0.70	long flat	white	3.23
10	Khao Kao Gon	0.30 × 0.90	present	yellow	0.30 × 0.70	long flat	white	3.31
11	Khao Nang Nuan	0.30 × 0.80	present	yellow	0.20 × 0.60	short round	white	2.36
12	Khao Mun Ped	0.30 × 1.00	present	yellow	0.20 × 0.70	long round	white with yellow	7.14

Table 4. Leaf blade anatomical characters of 12-Indigenous rice landraces in Khon Kaen province, Thailand.

No.	Indigenous rice landraces	Epidermal cell				Stomata type		Trichrome type		Vascular tissue type
		Shape		Anticlinal wall of epidermis						
		Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	
1	Khao Plong Aew	rectangular	rectangular	sinuous	sinuous	P	P	PA	PH, PA	CB
2	Khao Jao Leuang	rectangular	rectangular	sinuous	sinuous	P	P	PA	PA	CB
3	Khao San Pla Tong	rectangular	rectangular	sinuous	sinuous	P	P	PH, PA	PH, PA	CB
4	Khao Gam Poon	rectangular	rectangular	sinuous	sinuous	P	P	PA	PH, PA	CB
5	Khao E-Leuang Noi	rectangular	rectangular	sinuous	sinuous	P	P	PH, PA	PH, PA	CB
6	Khao Niaw dang	rectangular	rectangular	sinuous	sinuous	P	P	PH, PA	PH, PA	CB
7	Khao Lao Taek	rectangular	rectangular	sinuous	sinuous	P	P	PA	PH, PA	CB
8	Khao E-Tom Kao	rectangular	rectangular	sinuous	sinuous	P	P	PH, PA	PH, PA	CB
9	Khao Kam Pai	rectangular	rectangular	sinuous	sinuous	P	P	PH, PA	PH, PA	CB
10	Khao Kao Gon	rectangular	rectangular	sinuous	sinuous	P	P	PH, PA	PH, PA	CB
11	Khao Nang Nuan	rectangular	rectangular	sinuous	sinuous	P	P	PH, PA	PH, PA	CB
12	Khao Mun Ped	rectangle	rectangular	sinuous	sinuous	P	P	PH, PA	PH, PA	CB

Noted: P = paracytic stomata, PA= papillae, PH = prickle hair and CB = collateral bundle.

Table 5. Leaf sheath anatomical characters of 12-Indigenous rice landraces in Khon Kaen province, Thailand.

No.	Indigenous rice landraces	Epidermal cell				Stomata type		Trichrome type		Vascular tissue type
		Shape		Anticlinal wall of epidermis						
		Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	
1	Khao Plong Aew	rectangular	rectangular	straight	sinuous	P	P	-	UN	CB
2	Khao Jao Leuang	rectangular	rectangular	straight	undulate	P	P	-	-	CB
3	Khao San Pla Tong	rectangular	rectangular	straight	sinuous	P	P	-	-	CB
4	Khao Gam Poon	rectangular	rectangular	straight	sinuous	P	P	-	-	CB
5	Khao E-Leuang Noi	rectangular	rectangular	straight	sinuous	P	P	-	-	CB
6	Khao Niaw dang	rectangular	rectangular	straight	sinuous	P	P	-	-	CB
7	Khao Lao Taek	rectangular	rectangular	straight	sinuous	P	P	-	-	CB
8	Khao E-Tom Kao	rectangular	rectangular	straight	sinuous	P	P	-	UN	CB
9	Khao Kam Pai	rectangular	rectangular	straight	sinuous	P	P	-	UN	CB
10	Khao Kao Gon	rectangular	rectangular	straight	sinuous	P	P	-	UN	CB
11	Khao Nang Nuan	rectangular	rectangular	straight	sinuous	P	P	-	UN	CB
12	Khao Mun Ped	rectangular	rectangular	straight	sinuous	P	P	-	UN	CB

Noted: P =paracytic stomata, UN= unicellular hair, CB = collateral bundle and - = absent

Anatomical characteristics of Indigenous rice landraces in Khon Kaen province

The anatomy of leaf blade and leaf sheath of 12 indigenous rice landraces is shown in Table 4 and 5, respectively. Most of the leaf blade anatomical characters in all Khon Kaen indigenous rice landraces were the same as follows (Table 4):

Upper and lower epidermis were: 1) rectangular epidermal cells, 2) shape of anticlinal wall of epidermal cell was deep sinus, 3) elongated- and shorted-epidermal cells arranged alternately along leaf blade, 4) epidermal cells were oval-shape with smooth cell wall, 5) paracytic stomata, 6) silica beads present, 7) some trichomes were papillae type and 8) most trichomes were prickle hair. *Mesophyll* were: 1) mesophyll cells were oval-shape chlorenchyma with rough cell wall that could not classify as palisade and spongy mesophyll and 2) bulliform cells arranged alternately with vascular tissue.

Vascular tissues were: 1) three sizes of vascular bundle were large, medium, and small, 2) collateral bundle arranged as the same radius with xylem, 3) bundle sheath cells were parenchyma cell type, 4) fibrous tissue placed on upper and lower of or only upper of vascular bundle and 5) parenchyma tissue arranged along with epidermis.

However, leaf blade of one indigenous rice landrace, Khao Jao Leuang, was different from others by absence prickle hair in both upper and lower epidermis. Meanwhile, papillae only occurred in both upper and lower epidermis (Table 4 and Fig. 4F). This suggested that Khao Jao Leuang was found in Phon district where the climate was relatively low rainfall and drought condition compared to other districts. That resulted in Khao Jao Leuang was sensitive to environmental stress such as drought and also pathogen. Thus, it may modify the cell wall to be physical barrier, as papillae, against pathogen invasion (Underwood, 2012).

The anatomy of leaf sheath in all Khon Kaen indigenous rice landraces was described as follows: 1) epidermal cell in upper epidermis was rectangular, 2) shape of anticlinal wall of upper epidermal cell was straight, 3) epidermal cell in lower epidermis was rectangular, 4) shape of anticlinal wall of lower epidermal cell was deep sinus excluding Khao Jao Leuang that was undulate (Table 5), 5) paracytic stomata in both upper and lower epidermis and 6) trichome absent in upper epidermis but unicellular hair occurred in lower epidermis in Khao Plong Aew, Khao E-Tom Kao, Khao Kam Pai, Khao Kao Gon, Khao Nang Nuan and Khao Mun Ped (Table 5). This unicellular hair has a simple structure and usually does not have glands. It can play an important role to resist drought and high salt and to prevent UV and biological invasion (Han et al., 2022). Because these indigenous rice landraces were found in various climate condition, such as high rainfall (Khao Plong Aew, Khao Kam Pai, Khao Kao Gon), low rain fall (Khao E-Tom Kao, Khao Nang Nuan and Khao Mun Ped), high salt (Khao E-Tom Kao and Khao Nang Nuan). However, the anatomical characteristics of leaf blade and leaf sheath cannot be used for identifying indigenous rice landraces.

CONCLUSIONS

In summary, we found 27-landraces, while, only 12-plant and -grain samples were collected in Khon Kaen province, namely; Khao Plong Aew, Khao Jao Leuang, Khao San Pla Tong, Khao Gam Poon, Khao E-Leuang Noi, Khao Niaw dang, Khao Lao Taek, Khao E-Tom Kao, Khao Kam Pai, Khao Kao Gon, Khao Nang Nuan and Khao Mun Ped. The morphological characteristics of the vegetative organs such as color of leaf blade, leaf sheath, auricle and ligule and of the reproductive organs such as shape and color of brown rice can be used to identify indigenous rice landraces. Particularly, the shape and color of brown rice were the most effective diagnostic characteristics of indigenous rice landraces. The anatomical characters of leaf blade and leaf sheath such as upper and lower epidermis, mesophyll cells and vascular tissue were similar in all 12 indigenous rice landraces. Thus, the anatomical characteristics were not appropriated to identify the indigenous rice landraces. However, Khao Jao Leuang was distinguished from other indigenous rice landraces by the absence of prickles on both leaf blade surfaces. Moreover, Khao Jao Leuang was the only landrace in which the shape of anticlinal wall of epidermal cell on lower surface of leaf sheath was undulate while the other were sinuate. Consequently, the database of 12-Khon Kaen indigenous rice landraces such as the collection sites, and the characteristics of leaf and grain morphology and anatomy will benefit the conservation indigenous rice seeds, providing a rice genetic resource, and evolution knowledges. Classifying rice landraces enables the selection of high potential indigenous rice landraces for rice breeding program.

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INFLUENCE OF DIFFERENT METHODS OF WEED CONTROL ON THE VINEYARD WEED SYNUSIA IN PODGORICA SUBREGION

ABSTRACT

The study of the impact of different methods of weed control in vineyard was carried out in 2015 in the Podgorica sub region. Six different variants of weed control were tested: control, mechanical control, glyphosate (one treatment), glyphosate (two treatments), flazasulfuron and flazasulfuron+glyphosate. A total of 13 weed species from nine families were identified. The *Asteraceae* family was the most widespread with four species (31%), followed by *Poaceae* with two (15%), while all other families participated with one weed species each (8%).

The dominant weed species in the experimental vineyard were *Ambrosia artemisiifolia, Amaranthus retroflexus, Chenopodium album, Sorghum halepense, Heliotropium europaeum* and *Xanthium strumarium*. Annual thermophilic, heliophilic weeds dominated the weed synusia of the vineyard (77%), while perennial species participated with 23%. All applied methods of weed control showed a satisfactory level of efficiency, reducing the number of weed plants and the weed mass per unit area. The best effect in weed control between rows of the vineyard was demonstrated by the variants glyphosate, applied twice (92.7%), flazasulfuron, applied once (92.0%) and glyphosate+flazasulfuron (91.7%). The combination of glyphosate+flazasulfuron (100%) showed the highest efficiency in controlling weeds between the vines in a row.

Keywords: vines, weeds, weed control, Podgorica vineyards

INTRODUCTION

Weeds represent a very complex and diverse group of plants that grow against human's will together with cultivated plants, and are mainly the result of

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agricultural activities (Čekić and Kovačević, 2015; Hulina, 2005). Weeds are characterized by certain biological and ecological characteristics, which are the result of long-term adaptation to elevated anthropogenic effects (Stefanović *et al*., 2011). Their vitality and resilience come from the ability to adapt to different conditions and influences, high level of plasticity, wide ecological adaptability, production of huge amount of seeds, cosmopolitanism, etc. (Kojić & Šinžar, 1985). Weeds are a regular companion of the vine, and their abundance and biomass depend primarily on climatic and soil conditions, but also on the soil cultivation techniques applied during vineyard maintenance (Gago *et al*., 2007; Bešlić, 2019).

Weed plants cause multiple damages to the vine, primarily by depriving it of water and nutrients (Fredrikson, 2011). In addition, they significantly complicate soil cultivation, disrupt the water-air regime of the soil, and increase air humidity, which creates more favourable conditions for the development of fungal diseases. They are also hosts of numerous disease-causing agents and pests (Korać, 2011; Štefanac, 1988; Cvrković, 2009; Filippin *et al*., 2009; Agustí-Brisach *et al*., 2011; Cvrković *et al*., 2011; Atanasova, 2015). Certain weed species significantly affect the reduction of vine vigour and wine quality (Saayman & Huyssteen, 1983; Karoglan - Kontić *et al*., 1999; Hulina 1998; Dujmović-Purgar & Hulina, 2004). Finally, to a lesser or greater extent, they also reduce grape yield, and increase the price of grape production (Savić, 2006).

Due to all mentioned above, the control of weeds within and between row spaces must be carried out continuously, primarily by regular autumn, spring and summer tillage, destroying weeds on the surrounding surfaces, preventing flowering and seed formation, using natural or synthetic mulch and by using herbicides (Mirošević & Karoglan-Kontić, 2008; Fredrikson, 2011).

For a long period of time, multiple cultivations of the soil (deep and surface layers) were the main method of weed control in vineyards. However, this measure of physical weed control often favours the survival of certain annual weed species, and significantly contributes to the spread of perennial, especially rhizome weed species (Mirošević & Karoglan-Kontić, 2008; Gago *et al*., 2007; Fredrikson, 2011). Due to the unsatisfactory efficiency of the mechanical method of weed control, the increased lack of manpower, as well as the increased costs of purchasing, using and maintaining mechanization for tillage, herbicides have been more used in viticulture in recent years. The effectiveness of chemical weed control has been confirmed in a large number of studies, which is why herbicides are so widely used in grape production. Nowadays, in conventional viticulture, herbicides are mostly used to control weeds between the vines in the row, while weed control within row is performed with the combined use of agrotechnical and chemical measures (Konstantinović, 1999; Ostojić, 1999; Marković, 2012). Unlike agrotechnical measures that have a one-time effect and destroy weeds at the time of application, the use of herbicides provides more effective weed control over a longer period of time (Dolijanović *et al*., 2017).

The floristic composition of the weed community significantly depends on the agro-ecological conditions prevailing in the wine-growing regions, and therefore the application of herbicides in each locality is strictly specific. For the proper selection of herbicides, one of the most important prerequisites is knowledge of the weed flora in a given vineyard, because the selection of preparations for their control is made based on the weed species present. Only in this way is it possible to make a correct choice of the type and amount of herbicide, the method and time of application, which will enable the achievement of maximum effects in controlling weeds and elimination of possible negative consequences to the greatest extent for the cultivated plant, the environment, domestic animals and humans (Jovović *et al*., 2013).

The weed flora and vegetation of vineyards in the territory of Montenegro has been relatively modestly researched. Most of the research so far is restricted to the influence of the methods of cultivation and soil maintenance in vineyards (Ulićević *et al*., 1991) on weed suppression and vine productivity, while a very small number of works dealt with issues related to weed flora and vegetation. For these reasons, this research was designed with the aim of establishing the dominant weed species in the vineyard of the Biotechnical Faculty in Podgorica and detecting the most effective ways to control them. In addition, the aim of this work was to study the effectiveness of the new Chikara herbicide, which has not been used in Montenegro so far.

MATERIAL AND METHODS

The study of the effectiveness of different methods of weed control on the weediness of Vranac cultivar was carried out in 2015. The research was carried out in the experimental vineyard of the Biotechnical Faculty in Podgorica, planted in 2005 with a planting distance of 2.4 x 1 m (42°26′54″N, 19°12′19″E). The cultivation form is a two-rods horizontal cordon with a stem of approximately 80 cm height. Data on applied herbicides, amount and time of application are given in table 1.

Evaluation of weediness was carried out twenty days after the last treatment with herbicides, using the method of quantitative-qualitative determination, in permanent squares with an area of $1m^2$. Determination of weediness was done in the within row space and in the space between rows. By analysing the samples, the species and number of weed plants were determined and the effectiveness of the studied herbicides (EH) was calculated for the number of weeds and their biomass (fresh and air-dried) according to the following formula:

$$HE\ (\%) = \frac{NWC - NWH}{NWC} \times 100$$

HE – efficiency of herbicides (%)
NWC – number of weeds in control variant
NWH – number of weeds in variant with herbicides applied

The average annual air temperature was 17.2°C in Podgorica in 2015, while the average vegetation temperature was 23.2ºC. During the year, 1176.0 mm of rain fell, i.e. 438 mm during the vegetation period (Monstat, 2016).

Table 1. Data on applied methods of weed control

Variant		Active substance	Preparation	Content of active substance	Preparation amount per hectare	Time of application
K		Control variant (no weed control)				
MO		Variant with mechanical weed control				Several times during the growing season
Variants with herbicides applied	H_1	Glyphosate	Glifosav 480 SL	$480\ g\ l^{-1}$	$4\ l\ ha^{-1}$	End of April
	H_2	Glyphosate	Glifosav 480 SL	$480\ g\ l^{-1}$	$4\ l\ ha^{-1}$	End of April
		Glyphosate	Glifosav 480 SL	$480\ g\ l^{-1}$	$4\ l\ ha^{-1}$	End of June
	H_3	Flazasulfuron	Chikara 25 WG	$250\ g\ kg^{-1}$	$0{,}2\ kg\ ha^{-1}$	Before weed germination
	H_4	Flazasulfuron	Chikara 25 WG	$250\ g\ kg^{-1}$	$0{,}2\ kg\ ha^{-1}$	Before weed germination
		Glyphosate	Glifosav 480 SL	$480\ g\ l^{-1}$	$4\ l\ ha^{-1}$	End of April

The soil of the experimental field is carbonate-free in the surface layer 0-30 cm, in the layer 30-60 cm $CaCO_3$ is found in traces (1.62%), while in the layer 60-90 cm $CaCO_3$ is present in larger quantities (4.39%). Based on the pH value in KCl, the soil has an acidic to slightly acidic reaction. It is well supplied with humus (2.26%) and easily accessible potassium (28.19 mg/100g of soil), and poor with easily accessible phosphorus (5.32 mg/100g of soil).

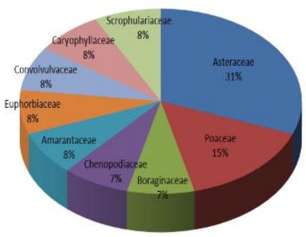
Statistical data analysis was done using the analysis of variance (ANOVA), and the evaluation of differences between mean values was performed using the LSD test.

RESULTS AND DISCUSSION

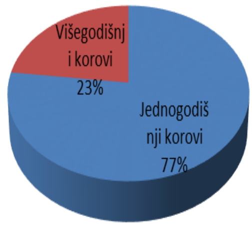
In the vineyard weed community a total of 13 weed species from 9 families were recorded in 2015 (graph 1). The majority of weed species - four, i.e. 31% belong to the *Asteraceae* family, two weed species (15%) belong to the *Poaceae* family, while the other families participated in the total weediness of the vineyard with one weed species each (8%).

The analysis of the represented weed species established the dominance of annual thermophilic, heliophilic weeds (77%), while perennial weeds were represented by 23% (graph 2).

The dominant group is represented by 6 species: *Ambrosia artemisiifolia, Amaranthus retroflexus, Chenopodium album, Sorghum halepense, Heliotropium europaeum* and *Xanthium strumarium*. In addition to them, other weed species such as: *Convolvulus arvensis*, *Euphorbia maculata*, *Digitaria sanguinalis*, *Sonchus asper*, *Sonchus asper* subsp. *glaucescens*, *Stellaria media* and *Veronica chamaedrys* were detected. Bagi and Bodnar (2012) came with similar results. The most common weed species in their research were: *Ambrosia artemisiifolia*, *Amaranthus retroflexus*, *Chenopidium album*, *Convolvulus arvensis*, *Digitaria sanguinalis*, *Stellaria media*, *Sorghum halepense* and *Xanthium strumarium*.



Graph 1. Representation of weed species



Graph 2. Representation of annual and perennial weeds in the vineyard

The results presented in table 2, show that in the control variant in within row space, the dominant weed species are *Chenopodium album* 22% (49 units/m^2), *Ambrosia artemisiifolia* and *Amaranthus retroflexus* with a share of 18% each (40 units/m^2), *Sorghum halepense* 13% (29 units/m^2), *Heliotropium europaeum* 11% (24 units/m^2), and *Xanthium strumarium* 10% (21 units/m^2). Other weed species were represented by 8% (17 units/m^2). The lowest weediness was measured in the H4 variant, where only two weed species were registered, *Chenopodium album* with the participation of 15 units/m^2 (94%) and *Ambrosia artemisiifolia* with single unit (6%).

Table 2. The number of weed species in the inter-row space of the vineyard

Variant	Weed species														Total
	Sorghum halepense		*Heliotropium europaeum*		*Ambrosia artemisiifolia*		*Chenopodium album*		*Xanthium strumarium*		*Amaranthus retroflexus*		Other weed species		
	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No
K	29	13	24	11	40	18	49	22	21	10	40	18	17	8	209.7
MO	8	8	9	8	23	22	17	16	11	10	26	25	12	22	107
H1	3	6	4	9	9	19	9	20	11	24	6	13	4	9	41.6
H2	2	12	4	23	3	18	3	18	4	23	0	0	1	6	16
H3	3	18	1	6	4	23	1	6	7	41	1	6	0	0	16.7
H4	0	0	0	0	1	6	15	94	0	0	0	0	0	0	17.3

	LSD 0.05	*LSD 0.01*
Dominant weeds	*25.787*	*36.151*
Total	*23.256*	*32.603*

Rotim (2016) states that in the Herzegovinian and South Dalmatian vineyards, among the perennial weeds, *Sorghum halepense* and *Convolvulus arvensis* are the most abundant, and among the annual weeds, *Amaranthus retroflexus*, *Stellaria media* and *Chenopodium album*, which is partly in agreement with our results. Janjić (1985) mentions the high efficiency of Glyphosav in controlling the weed species *Amaranthus retroflexus*.

The highest total number of weeds in the inter-row space (table 2) was recorded in the control - 209.7 units/m^2 (52%), followed by the variant with mechanical weed control 107 units/m^2 (26%), while the lowest weediness recorded in the variant with two-time application of Glyphosav (H2) - 16 units/m^2 (4%). Statistical data analysis revealed a significantly higher number of weed individual plants in the control and variants with mechanical control compared to all variants with the application of herbicides. A significant difference in weediness was also determined by comparing the variants H2, H3 and H4 with the variant H1, which had 41.6 units/m^2 (10%).

Table 3. The number and percentage of weeds between and within the rows

Variant	Weed species														Total
	Sorghum halepense		*Heliotropium europaeum*		*Ambrosia artemisiifolia*		*Chenopodium album*		*Xanthium strumarium*		*Amaranthus retroflexus*		Other weed species		
	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No
K	29	34	10	11	8	9	18	21	5	6	11	13	5	6	86.7
MO	16	41	5	13	4	10	6	15	2	5	3	8	3	8	38.3
H1	5	23	2	9	3	14	4	18	2	9	3	14	3	14	21.3
H2	2	28	0	0	2	29	0	0	2	29	0	0	1	14	8.6
H3	6	43	5	36	0	0	0	0	3	21	0	0	0	0	14.3
H4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

	LSD 0.05	*LSD 0.01*
Dominant weeds	*17.903*	*25.099*
Total	*18.554*	*26.012*

From the data provided in table 3. it can be concluded that the dominant weed species on the control variant, between the rows, were *Sorghum halepense* with 29 units/m^2 (34%), *Chenopodium album* with 18 units/m^2 (21%), *Amaranthus retroflexus* with 11 units/m^2 (13%), *Heliotropium europaeum* with 10 units/m^2 (11%) and *Ambrosia artemisiifolia* with 8 units/m^2 (9%). *Xanthium strumarium* and other weed species participated in the total weediness of the control with five plants each (6%). The highest prevalence of weeds between the vines in the row was also the highest in the control variant - 86.7 units/m^2 (51%). Higher weediness was also noted in the variants MO 38.3 units/m^2 (23%) and H1 21.3 units/m^2 (13%). The difference in the number of weeds in the control variant and all other weed control methods was statistically very significant. The differences in the number of weeds between the variant with double application of Glyphosav (H2) - 8.6 units/m^2 (5%) and the variant Chikara+Glifosav (H4) - 0 units/m^2, compared to the variant with mechanical control, were rated as very significant. All other differences in the total number of weeds between rows were without statistical significance.

Data on the fresh and dry biomass of weeds in the inter-row space and the space between the vines in the row are presented in Table 4. The highest fresh biomass of weeds in the inter-row space was measured on the control (1304.7 g) and mechanically treated variants (914.3 g), while the lowest values for this parameter were measured in variants H4, H3 and H2 (88.3, 92.3 and 95.7 g, respectively). Compared to the other variants, these three variants had a statistically very significant reduction in the fresh biomass of weeds. The dry biomass of weeds in the inter row space was also the highest in the control (466.7 g), while the lowest was measured in the variants H3, H2 and H4 (41, 43 and 46 g, respectively). Statistical data analysis showed a very significant increase in the dry biomass of weeds in the control compared to all other studied methods of weed control. A significant increase in weediness was also noted in the variant with mechanical control (220 g) compared to the variants H3, H2 and H4.

Table 4. Fresh and dry biomass of weeds between and within rows

Variant	Between rows		Within rows	
	Fresh (g)	Dry (g)	Fresh (g)	Dry (g)
K	1304.7	466.7	636.7	236.0
MO	914.3	220.0	467.0	167.7
H1	370.0	141.0	182.0	69.0
H2	95.7	43.0	58.3	24.0
H3	92.3	41.0	52.0	24.0
H4	88.3	46.0	0.0	0.0

	Fresh biomass		*Dry biomass*	
	LSD 0.05	*LSD 0.01*	*LSD 0.05*	*LSD 0.01*
Between rows	*100.63*	*141.07*	*99.162*	*139.02*
Within rows	*148.93*	*208.79*	*34.003*	*47.670*

The lowest fresh biomass of weeds between the vines in the row (table 4) was measured in the herbicide treatments H4, H3 and H2 (0, 52 and 58.3 g, respectively), while the highest was in the control (636.7 g) and the variant with mechanical treatment (467.0 g). By comparing the differences in the fresh biomass of the weeds in the control and variants with mechanical processing with the variants with the application of herbicides, statistically very significant differences were noted. A very significant increase in the dry biomass of weeds between rows was determined by comparing the control variant (236.0 g) and all other methods of weed control, as well as by comparing the variant with mechanical treatment (167.7 g) and the variants on which weeds were controlled using herbicides.

The efficiency of the studied methods of weed control is presented in table 5. From the results shown, variants H2, H3 and H4 (92.7%, 92% and 91.7%, respectively) showed a very high efficiency in controlling weeds in the inter-row space, the variant H1 had high efficiency (80.0 %), while the effectiveness of weed control using mechanical measures (MO) was very low (49.3%). Statistical data analysis revealed a very significant increase in efficiency on variants H2, H3 and H4 compared to the variant on which Glyphosav (H1) was applied once and the variant with mechanical control (MO).

The variant with the combined application of the Chikara+Glyfosav herbicide - H4 (100%) and the variant where the herbicide Glyfosav was applied twice - H2 (90%) showed the highest effectiveness in controlling weeds between the vines in the row. The differences in effectiveness between these two tretments and all other weed control methods were marked as statistically highly significant. The lowest efficiency was on plots with the application of mechanical measures (55%). Compared to the studied herbicides, this method of weed control exhibited statisticaly significantly lower performance.

Table 5. Effectiveness of the studied methods of weed control in reducing the number of weed individual plants

Variant	Between rows (%)	Within rows (%)
MO	49.3	55.0
H1	80.0	74.3
H2	92.7	90.0
H3	92.0	71.3
H4	91.7	100.0

	LSD 0.05	*LSD 0.01*
Between rows	*6.9282*	*9.7128*
Within rows	*9.7349*	*13.648*

Along with the reduction in the number of individual weed species, all applied herbicides had a very significant effect on the reduction of fresh biomass of weeds in the inter-row space compared to mechanical treatment (table 6). A very significant increase in efficiency was also determined by comparing variants H4, H3 and H2 (94%, 93% and 92.7%, respectively) with variant H1 (72%). Herbicides H3, H2 and H4 (90%, 89%, and 87.7%, respectively) showed the best performance in reducing the dry biomass of weeds in the inter-row space. Treatments with a single application of Glyphosav and mechanical weed control showed a rather unsatisfactory effect in this respect.

The treatment with the application of the herbicide combination Chikara+Glifosav (100%) showed the greatest efficiency in the reduction of fresh weed biomass between the vines in the row (tab. 6). The H2 variant, on which Glyphosav was applied twice (87.3%) and the H3 variant (85.7%), showed high efficiency, while the weakest effect had the H1 variant, on which the Glyphosav herbicide was applied once (72.0%), as well as the variant with mechanical weed control (26.7%). All studied methods of weed control showed a significantly higher efficiency in the reduction of fresh biomass of weeds compared to the variant with the application of mechanical weed control. Treatments H4, H2 and H3 showed significantly higher efficiency compared to variant H1.

Table 6. The effectiveness of the studied methods of weed control in the reduction of weed biomass

Variant	Between rows		Within rows	
	Fresh (%)	Dry (%)	Fresh (%)	Dry (%)
MO	29.0	47.3	26.7	28.3
H1	72.0	64.0	72.0	70.3
H2	92.7	89.0	87.3	91.3
H3	93.0	90.0	85.7	87.3
H4	94.0	87.7	100.0	100.0

	Fresh biomass		*Dry biomass*	
	LSD 0.05	*LSD 0.01*	*LSD 0.05*	*LSD 0.01*
Between row	*7.3470*	*10.300*	*19.752*	*27.691*
Within rows	*7.8558*	*11.013*	*7.7658*	*10.887*

In the reduction of dry biomass of weeds between the vines in the row, the best results were shown by the variant H4 (100%), while the variant with a double application of the herbicide Glyphosav (91.3%) and a single application of the herbicide Chikara (87.3%) showed a very satisfactory effect. The variant with mechanical weed control showed very poor efficiency (28.3%). Statistical data analysis showed a very significant reduction in the dry biomass of weeds on all studied varieties compared to mechanical control.

CONCLUSIONS

Based on the conducted studies, the following conclusions can be drawn:

1. A total of 13 weed species systematized into nine families were found in the sample vineyard.
2. The dominant group of weed species consists of: *Ambrosia artemisiifolia*, *Amaranthus retroflexus*, *Chenopodium album*, *Sorghum halepense*, *Heliotropium europaeum* and *Xanthium strumarium.*
3. Annual thermophilic, heliophilic weeds dominate the weed synusia of the vineyard (77%), while perennial weeds accounted for 23% of the total weeds.
4. The highest total number of weed plants both within and between row spaces was recorded in the control variant (209.7 units/m^2; 86.7 units/m^2), while the lowest presence of weed plants in the inter-row space was in the variant with two applications of Glyphosav - H2 (16 units/m^2), and in the space between the vines in the row in the variant with the combined application of the Chikara+Glyfosav herbicide, where the presence of weed plants was not recorded.

5. Fresh and dry biomass of weeds both within and between rows was the highest in the control variant and the variant with mechanical control.
6. The best effect on weeds within the rows of the vineyard was demonstrated by the herbicide Glyphosav with two applications (92.7%), and the combination of herbicide Chikara + Glyphosav (100%) was the most effective between the vine rows. The least effective in controlling weeds was the variant with mechanical control (49.3% in the inter-row space, i.e. 55% between the plants in the row).
7. The efficiency coefficient based on the total fresh and dry biomass of weeds within and between row spaces was the highest with the Chikara+Glifosav herbicide combination, while the lowest was recorded with the variant with mechanical control.

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THE SIGNIFICANCE OF REAL FINANCIAL REPORTING OF AGRICULTURAL MECHANISM IN RELATION TO THE MAKING OF MANAGEMENT DECISIONS OF INDIVIDUAL FARMS AND MEDIUM-SIZED AGRICULTURAL ENTERPRISES

SUMMARY

The importance of realistic financial reporting depends on the consideration of numerous factors by the decision maker. In this paper, the authors focused on a comprehensive analysis of agricultural machinery in use by individual farms and medium-sized agricultural enterprises. The main conclusion reached by the authors would be that there are significant differences regarding the use of agricultural machinery in relation to its age, value, fuel consumption and its maintenance ($p<0.0005$*) in relation to the two forms of organizing agricultural production. Medium-sized agricultural enterprises use younger, more expensive agricultural machinery, as well as the fact that the owner's satisfaction with it is somewhat lower with lower fuel consumption and lower maintenance costs of agricultural machinery. The value of agricultural mechanization can be predicted both for individual farms based on the mentioned factors ($F=262.901$, $p<0.0005$) and for medium-sized agricultural enterprises ($F=161.229$, $p<0.0005$).

Key words: financial reporting, agricultural machinery, individual farms, agricultural enterprises

INTRODUCTION

The growing observation of real financial reporting in numerous studies has been observed from several aspects, but it is basically aimed at meeting the demand

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of heterogeneous decision-makers (Bakmaz *et al.*, 2017; Duc *et al.*, 2021; Arnautović *et al.*, 2022; Jakubowska and Sadílek, 2023).

The reporting of owners and top management largely depends on the quality and anatomy of the professional staff who are involved in compiling valid reports (Bowden and Liddle, 2018; Alibegović *et al.*, 2018; Finžgar and Brezovnik, 2019; Shi *et al.*, 2021; Jordan *et al.*, 2023).

The organization of agricultural production first of all depends on the clearly made business decisions of the decision-maker, and these in turn depend on the quality of the established security of reporting to them from all parts of agricultural production, sectors and parts of lower organizational structures (Bourne, 2011; Mijić & Popović 2016; Biščak & Benčina 2019; Bieńkowska *et al.*, 2020; Kwon & Han 2020; Filipović *et al.*, 2021).

Agricultural production viewed as low accumulative depends to a large extent on the establishment of mechanisms, primarily in the sense of eliminating risks, with which it will be possible to improve the process of comprehensive decision-making in all forms of agricultural organization (Kunesch, 1996; Duhovnik, 2007; Popović, 2014; Bjelica *et al.*, 2017; Lartey *et al.*, 2020; Lenggenhager, 2021; Oyewo and Akinsanmi, 2021; Tomas-Miskin *et al*., 2022a).

Processes of essential business improvement in agriculture can be viewed through the prism of numerous factors, although essential business improvements can be made in the short term through internal factors of the organization (Novaković *et al.*, 2018; Popović *et al.*, 2018; Trung and McMillan, 2021; Zhang and Colak, 2022; Milunović *et al.*, 2022; Lakić *et al.*, 2022; Burić *et al.*, 2023; Stevanović *et al.*, 2023; Sekulić *et al.*, 2023; Stupar *et al.*, 2023; Rajičić *et al.*, 2023; Kosev *et al.*, 2023a; 2023b; Vasileva *et al.*, 2023).

The value of individual segments in the organization of agrarians, and therefore of agricultural mechanization, should be observed continuously and realistically in order to make valid business decisions regardless of the form of organization of agricultural production (Vitomir *et al.*, 2020; Popović *et al.*, 2021; 2022; Uyar *et al.*, 2022; Wang *et al.*, 2022; Xu *et al.*, 2022; Stevanović *et al.*, 2023).

The aim of the study was to examine two forms of organizing agricultural production. Very much is an important of realistic evaluation of agricultural mechanization, because its use can achieve an increase in productivity on the one hand, and on the other, give a realistic assessment of the value of agricultural mechanization and bring a key business decision on the economic justification of the purchase of new machinery or its overhaul.

MATERIAL AND METHODS

The authors of the study conducted research in two forms of organizing agricultural production, which have the most participants in organizing agricultural production. This was the basis for making the decision to carry out the research in the mentioned forms in order to obtain valid conclusions regarding the real importance of reporting to the holder decision-making in the aforementioned forms of organizing agricultural production.

The main focus of the author was on the observation of legal entities that correspond to the established criteria of agricultural holdings or that are registered as medium-sized agricultural enterprises. Therefore, the authors did not provide details in the research regarding, for example, how much land they cultivate, whether they are focused on arable or vegetable crops and other peculiarities. They essentially focused on the research of the mentioned two forms of agricultural production organization with the aim of examining the importance of realistic reporting regarding the factors that were the focus of the author's research.

The concept of financial reporting is somewhat different in the mentioned two forms of organizing agricultural production. In medium-sized agricultural enterprises, it is mandatory once a year in the form of final accounts, but in the case of agricultural holdings, this obligation would be somewhat lower and would apply only to those holdings that are in the VAT system. However, the research was also done in farms that are not in the VAT system. With such entities, financial reporting is done with respect to their assessment, for example, of the value of agricultural machinery. The authors found the justification for this in the fact that the owners of agricultural holdings have no reason to incorrectly evaluate their, for example, agricultural machinery, because in the case of obtaining, for example, a loan from a bank, such reports are subject to the control of experts hired by banks (appraisers), and they would never correspond to incorrect reports on, for example, the value of agricultural machinery.

In addition, is an importance of realistic evaluation of agricultural mechanization in a country, because its use can achieve an increase in productivity on the one hand, and on the other, a realistic assessment of the value of agricultural mechanization as a basis for reporting in both observed forms it will be brought a key business decision on the economic justification of the purchase of new machinery, its overhaul or some other decision related to doing business with agricultural machinery.

The research was conducted on the territory of the Republic of Serbia in the period from 01.12. to 31.12.2023.

Used agricultural machinery that is unfortunately used in agricultural production is very old. However, in order to realistically express the value of agricultural machinery, the surveyed subjects used the international accounting standard IRS 41. This is of great importance because it allows the value of any equipment to be expressed at its real value both in business books and in reporting to banks, owners and other.

The research examined the existence of differences between individual farms and medium-sized agricultural enterprises in relation to: age of use of agricultural machinery, its value in business books, evaluation of the owner's overall satisfaction with agricultural machinery and equipment in use, fuel consumption and machinery maintenance in relation to the analyzed six types of agricultural mechanization. The t-test of independent samples was used to examine the differences between individual farms and medium-sized agricultural enterprises.

The survey included 355 documents that were created in individual farms and the same number of documents in medium-sized agricultural enterprises, all with the aim of making a comparison between the two types of agricultural production organization. The authors guaranteed the participants of the survey anonymity and that after the survey, the data obtained will be used exclusively for scientific purposes and the writing of this paper.

The value of the agricultural machinery that was used in the processing and analysis was based on the statement of the owner of the farm about the real value of the machinery, that is, on the basis of the data obtained from the business books in which the agricultural machinery was kept and that in relation to the values that were in the last final account companies (31.12.2022).

Agricultural machinery was analyzed, namely: its age, values of agricultural machinery, fuel consumption, maintenance costs and satisfaction of the owner, i.e. the top management managing the agricultural machinery, i.e. at the end, an analysis of the total score for the mentioned factors was done. This was done based on the evaluation of the respondents. The interval ranged from 1 to 10, so that 1 was evaluated as a weak influence of the factor on the business, and 10 represented a strong influence on the business of the respondents.

The goal of the research was to examine the existence of possible differences between individual farms and medium-sized agricultural enterprises in relation to the mentioned factors. The stated propositions were strengthened using the t test of independent samples.

Finally, a multiple linear regression was performed for agricultural farms and for medium-sized agricultural enterprises in relation to the mentioned factors and in relation to the possibility of predicting the value of the use of machinery in use.

Statistical data processing was performed using the IBM SPSS (Statistical Package of Social Science) version 25 software, and the threshold value where 0.05.

RESULTS AND DISCUSSION

The obtained results point to the existence of significant differences between individual farms and medium-sized agricultural enterprises, which the authors presented in Tables 1 to 5, and everything was strengthened by using the t test of independent samples.

Presentation of differences in relation to the age of agricultural machinery in use.

The obtained results of the author, which are shown in table 1, indicate that there is a statistically significant difference for all types of analyzed mechanization. This difference relates to the age of use of agricultural machinery. In addition, it can be observed that medium-sized agricultural enterprises use somewhat younger machinery in their work compared to individual farms.

Table 1. Differences in the age of use of agricultural machinery

Analyzed agricultural machinery	Individual farms	Medium-sized agricultural enterprises	t	p
	Middle value			
Tractor (N=52)	1983.81 ± 15.21	1986. 32 ± 14.96	-5.908	<0.000*
Harvesters (N=53)	1995.31 ± 9.05	1997.38 ± 8.87	-5.325	<0.000*
Water systems (N=49)	1980.34 ± 11.34	1986.06 ± 9.52	-10.920	<0.000*
Transport trailer N=57)	1976.77 ± 6.22	1982.22 ± 6.96	-17.992	<0.000*
Motocultivators (N=47)	1996.05 ± 21.91	2000.45 ± 19.17	-10.454	<0.000*
Small machinery (N=50)	1986.44 ± 11.41	1992.68 ± 10.94	-13.825	<0.000*

Source: authors' calculation (2024); *Statistical level of significance at the level of 0.05;

The obtained results are significant in all forms of agricultural organization, which can be seen in numerous works such as works (Filipović et al., 2023) because in real agricultural production, above all, agricultural mechanization is used, which has different ages and which can dominantly affect the organization of agricultural production.

Presentation of differences in relation to the agricultural machinery value in use.

Based on the results shown in table 2, it can be seen that for all types of mechanization there is a statistically significant difference in the value of agricultural mechanization that is used every day, but it should be emphasized that medium-sized agricultural enterprises operate with more expensive agricultural mechanization compared to individual farms.

The results obtained in the study indicate the importance of evaluating equipment, because frequent evaluation of equipment leads to real data about the real value, which is already expressed to a large extent in already published works by authors such as (Radović *et al.*, 2023) who pointed to the importance of the evaluation process of the equipment and therefore of the agricultural mechanization that was analyzed in this study.

Presentation of the differences in relation to the owner's satisfaction with the agricultural machinery in use

Based on the results shown in table 3, there is a statistically significant difference in the overall satisfaction rating for all types of agricultural mechanization, with a note that medium-sized agricultural enterprises have a lower overall satisfaction in using agricultural mechanization compared to individual farms, except for combine harvester owners. The essential satisfaction of the owner is focused on the reliability of the use of the equipment, on its technical-technological capabilities, which comes to the fore in soil cultivation, but also within the overall use of the equipment.

Table 2. Differences in the value of machinery in use

Analyzed agricultural machinery	Individual farms	Medium-sized agricultural enterprises	t	p
	Middle value			
Tractor (N=52)	3907.54 ± 2558.53	4925.28 ± 3059.96	-10.582	<0.0005*
Harvesters (N=53)	22893.61 ± 4864.39	41808.51 ± 17810.15	-8.669	<0.0005*
Water systems (N=49)	5495.91 ± 2139.70	7693.87 ± 3172.49	-13.672	<0.0005*
Transport trailer (N=57)	864.15 ± 424.98	1246.98 ± 604.36	-15.340	<0.0005*
Motocultivators (N=47)	4963.92 ± 4624.39	6130.58 ± 5375.69	-10.655	<0.0005*
Small machinery (N=50)	371 ± 230.45	541 ± 242.87	-18.323	<0.0005*

Source: authors' calculation (2024); *Statistical level of significance at the level of 0.05

Table 3. Differences in owner satisfaction with agricultural machinery in use

Analyzed agricultural machinery	Individual farms	Medium-sized agricultural enterprises	t	p
	Middle value			
Tractor (N=52)	7.03 ± 0.70	4.67 ± 1.13	24.195	<0.0005*
Harvesters (N=53)	7.02 ± 0.60	9.02 ± 0.60	-21.919	<0.0005*
Water systems (N=49)	5.30 ± 0.74	3.79 ± 0.40	13.781	<0.0005*
Transport trailer (N=57)	4.84 ± 0.74	4.07 ± 0.58	13.329	<0.0005*
Motocultivators (N=47)	6.78 ± 2.71	6.00 ± 2.68	13.484	<0.0005*
Small machinery (N=50)	4.80 ± 0.69	3.44 ± 1.14	19.833	<0.0005*

Source: authors' calculation (2024). *Statistical level of significance at the level of 0.05

At the same time, it is observed that there is a lower level of satisfaction in medium-sized agricultural enterprises compared to individual farms. The reason is that they operate with agricultural equipment that is old (Table 1), they are not satisfied with their performance, but basically there is the impossibility of acquiring both the necessary spare parts and the impossibility of purchasing equipment that would correspond to the size of the legal entity engaged in agricultural production . As a solution to overcome that problem, the authors pointed out that the obtained results coincide with already published works (Vitomir *et al.*, 2023; Dragosavac *et al.*, 2023) that pointed to the importance of internal control in legal entities that would indicate specific problems, for example in the use of agricultural of equipment, all of which can greatly affect the owner's satisfaction regarding the use of agricultural equipment with which they operate.

Display of differences in relation to fuel consumption of agricultural machinery in use

Based on the obtained results shown in table 4, it can be seen that for all types of mechanization there is a statistically significant difference in real fuel consumption, however, medium-sized agricultural enterprises have lower fuel consumption compared to individual farms. At the same time, these results are such that they were based on the analysis of very similar agricultural mechanization used in both forms of agricultural production organization, with the fact that in medium-sized agricultural enterprises, very similar mechanization is somewhat younger (Table 1).

In addition, the value of agricultural machinery was created based on the real application of International Accounting Standard 41, that is, the reassessment of the value of the mentioned equipment was done based on a comparison with the asking value of the same or similar machinery. Essentially, both forms operate with very similar equipment that should be kept in business books in order to be able to make valid management decisions based on the use of real documents, which the authors emphasized (Tomas-Mishkin *et al.*, 2022b) and which generally coincides with obtained results based on predictions (Table 4).

Table 4. Differences in fuel consumption

Analyzed agricultural machinery	Individual farms	Medium-sized agricultural enterprises	t	p
	Middle value			
Tractor (N=52)	6.66 ± 0.73	5.24 ± 1.65	9.369	<0.0005*
Harvesters (N=53)	6.95 ± 0.99	8.34 ± 2.02	-8.364	<0.0005*
Water systems (N=49)	5.95 ± 0.19	4.12 ± 0.43	34.429	<0.0005*
Transport trailer (N=57)	4.20 ± 0.40	2.20 ± 0.40	15.152	<0.0005*
Motocultivators (N=47)	6.21 ± 2.71	5.01 ± 2.70	19.063	<0.0005*
Small machinery (N=50)	5.00 ± 0.00	3.00 ± 0.00	16.120	<0.0005*

Source: authors' calculation (2024). *Statistical level of significance at the level of 0.05

Presentation of the differences in relation to the maintenance of agricultural machinery in use

Based on the results shown in Table 5, it can be concluded that for all types of mechanization there is a statistically significant difference in the valuation of mechanization maintenance, however, medium-sized agricultural enterprises have lower maintenance costs compared to individual farms, except for combine harvester owners.

This kind of monitoring of real business indicators is similar to the already stated views (Radović *et al.*, 2021), especially if the observation focuses on internal control factors in business decision-making processes.

Table 5. Differences in the maintenance of agricultural machinery

Analyzed agricultural machinery	Individual farms	Medium-sized agricultural enterprises	t	p
	Middle value			
Tractor (N=52)	6.28 ± 1.16	3.43 ± 1.11	24.014	<0.0005*
Harvesters (N=53)	6.82 ± 0.73	8.17 ± 1.61	-7.123	<0.0005*
Water systems (N=49)	5.63 ± 0.48	2.95 ± 0.19	39.498	<0.0005*
Transport trailer (N=57)	4.20 ± 0.40	2.00 ± 0.00	39.252	<0.0005*
Motocultivators (N=47)	5.76 ± 3.58	5.29 ± 3.88	6.667	<0.0005*
Small machinery (N=50)	4.64 ± 0.48	2.00 ± 0.00	38.500	<0.0005*

Source: authors' calculation (2024); *Statistical level of significance at the level of 0.05

Display of differences in the total age of agricultural machinery, total value, total owner satisfaction with agricultural machinery, total fuel consumption and total agricultural maintenance

Based on the results shown in Table 6, it can be seen that there are significant differences between individual farms and medium-sized agricultural enterprises.

Table 6. Differences in total age, value of machinery, owner's satisfaction with equipment, total fuel consumption and total maintenance of machinery

Analyzed agricultural machinery	Individual farms	Medium-sized agricultural enterprises	t	p
	Middle value			
Tractor (N=52)	1986.86 ± 15.00	1990.62 ± 13.91	-20.357	<0.0005*
Harvesters (N=53)	6317.83 ± 7558.48	9973.63 ± 14679.51	-8.456	<0.0005*
Water systems (N=49)	6.07 ± 1.56	5.08 ± 2.14	13.211	<0.0005*
Transport trailer (N=57)	5.98 ± 1.51	4.76 ± 2.33	18.037	<0.0005*
Small machinery (N=50)	5.65 ± 1.80	3.90 ± 2.58	19.982	<0.0005*

Source: authors' calculation (2024); *Statistical level of significance at the level of 0.05

However, the total value of agricultural machinery is higher in medium-sized agricultural enterprises, while the owner's satisfaction with agricultural machinery, fuel consumption and its maintenance is higher in individual farms.

Forecasting the value of agricultural machinery in the business of individual farms

Multiple linear regression was performed to examine the relationship between age of machinery, owner satisfaction, fuel consumption and machinery maintenance in relation to predicting the value of agricultural machinery for individual farms.

The regression analysis yielded a coefficient of determination of 0.376, on the basis of which it can be seen that the obtained model describes 37.6% of the total variance. The value of agricultural mechanization can be predicted since the model is statistically significant ($F=262.901$, $p<0.0005$).

Table 7. Prediction of the value of agricultural machinery at individual farms

Parameter	Beta	t	p
A constant	-	-4.782	<0.0005*
Age of mechanization	0.210	4.545	<0.0005*
Equipment owner satisfaction	0.026	0.264	0.792
Fuel consumption	0.312	2.946	0.003*
Maintenance of machinery	0.188	1.788	0.075

Source: authors' calculation (2024). *Statistical level of significance at the level of 0.05

Based on the results shown in Table 7, it can be seen that the age of machinery and fuel consumption have a significant impact on predicting the value of agricultural machinery in the business of individual farms.

Forecasting the value of agricultural mechanization in the business of medium-sized agricultural enterprises

Multiple linear regression was performed to examine the relationship between machinery age, equipment owner satisfaction, fuel consumption and machinery maintenance in relation to predicting the value of agricultural machinery for medium-sized agricultural enterprises.

The regression analysis yielded a coefficient of determination of 0.648, on the basis of which it can be concluded that the obtained model describes 64.8% of the total variance.

The value of the machinery can be predicted based on the age of the machinery, satisfaction of the equipment owner, fuel consumption and maintenance of the machinery as the model is statistically significant ($F=161.229$, $p<0.0005$). It clearly coincides with the views already given (Popović *et al.*, 2021) regarding the importance of the reality of decision-making in the organization of the agrarian economy.

Table 8. Prediction of the value of mechanization at medium-sized agricultural enterprises

Parameters	Beta	t	p
A constant	-	-0.897	0.370
Age of mechanization	0.024	0.689	0.491
Equipment owner satisfaction	0.307	3.768	<0.0005*
Fuel consumption	0.326	4.275	<0.0005*
Maintenance of machinery	0.194	1.896	0.059

Source: authors' calculation (2024); *Statistical level of significance at the level of 0.05

Based on the results shown in Table 8, it can be seen that the satisfaction of owners of agricultural machinery and fuel consumption have a significant impact on predicting the value of agricultural machinery in the business of medium-sized agricultural enterprises

In the end, the authors point out that this study achieved its justification because it highlighted the importance of research in two forms of organizing agricultural production, as well as the importance of discovering possible differences in making business decisions among decision makers in the mentioned forms of agricultural production in the Republic of Serbia. In the opinion of the accountant, this research can be extended to other countries of the region, because it indicates the importance of a realistic presentation of equipment valuation, as well as other factors that can influence decision-making regarding the purchase of a new one, sale of the same, overhaul, etc.

CONCLUSIONS

The results obtained in this study indicate the existence of significant differences in terms of the use of agricultural machinery in relation to the analyzed factors, in relation to: its age, value, fuel consumption and its maintenance ($p<0.0005$*) both in individual farms and in to the operations of medium-sized agricultural enterprises. In addition, the study shows that medium-sized agricultural enterprises use younger, more expensive agricultural machinery, and that owner satisfaction is somewhat lower with lower fuel consumption and lower maintenance costs of agricultural machinery. In the end, the results show that the value of agricultural mechanization can be predicted in both aspects of the organization of agricultural production. The prediction value for individual farms based on the analyzed factors is ($F=262.901$, $p<0.0005$), while the prediction value for medium-sized agricultural enterprises is slightly lower and amounts to ($F=161.229$, $p<0.0005$). In the end, it can be concluded that both obtained values for forecasting the value of agricultural mechanization are significant and possible for both analyzed forms of agricultural production organization.

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GEOMATICS ASSESSMENT OF COASTAL EROSION VULNERABILITY: A CASE STUDY OF AGADIR BAY, MOROCCO

SUMMARY

Coastal zones are characterized by a complex interaction between continental and marine ecosystems. They are often subject to anthropogenic and natural pressures such as urbanization, pollution, erosion, storms, and the impact of climate change. It is an environment that requires sustainable management to preserve its biodiversity and ecosystem services. This study focuses on assessing coastal vulnerability to erosion in the Bay of Agadir, one of Morocco's main tourist attractions. The methodology adopted is based on geomatic science such as GIS and geospatial remote sensing. This approach involved the preparation of a database for the study area, relating mainly to geomorphological parameters, topography, sea level rise, and shoreline change data. The latter was assessed using DSAS (Digital Shoreline Analysis System) tools and then all data were integrated into a GIS software to evaluate the Coastal Vulnerability Index (CVI). The first result shows that the EPR (endpoint rate) index reveals an erosion trend over the 8.3 km studied area. Over the 262 transects analyzed, the variations show a predominant erosion (92%) with rates of up to -13.5 m/year over 46 years. This justifies the use of the LRR (Linear Regression Rate-of-Change) index rather than the EPR for kinematic analysis of the coastline. The results of the CVI index show that 2.58 km of the coastal zone, and 1.68 km of the Bay are successively highly and very highly vulnerable to coastal erosion (20-60%). This study can be considered as a basic document, providing crucial information to guide decision-makers and planners in the implementation of effective coastal conservation strategies.

Keywords: Coastal erosion, Geomatics, Vulnerability index, Agadir Bay, Morocco.

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INTRODUCTION

The climate of Morocco is diverse, ranging from arid desert to humid north. Climate change can exacerbate erosion phenomena (Sabri et al., 2022), especially along coastal areas, by intensifying storms, raising sea levels, and altering precipitation patterns, thereby endangering fragile ecosystems and coastal infrastructure.

Coasts constitute dynamic environments shaped by various physical factors such as geology, geomorphology, sedimentology, and oceanic forcing, including wave action and sediment transport. With approximately 21% of the world's population residing in coastal areas (Brooks et al., 2006), these regions are increasingly impacted by a significant concentration of anthropogenic activities and socio-economic challenges.

The costs associated with climate change and larger natural disasters, whether economic, social, or ecological, are expected to further burden affected communities and government administrations (Thouret and Leone, 2003; Fairbank and Jakeways, 2006). Erosion induced by water constitutes a challenge with far-reaching environmental and socioeconomic implications across diverse global regions (Bouayad et al., 2023). Coastal erosion due to storm waves is a significant problem threatening economic stability (Martzikos et al., 2021), given its impact on tourism and major infrastructure (Thouret and D’ercole, 1996). It is considered one of the main factors in the modification of the coastal landscape (Arabadzhyan et al., 2021).

Beaches are not inevitably destined to disappear; they can shift inland by rolling upon them unless impeded by obstacles. These obstacles may be natural, such as an escarpment or a dormant cliff, or anthropogenic, like man-made constructions. In the latter scenario, where migration becomes impracticable, erosion accelerates, ultimately leading to the disappearance of the beach.

In recent years, the utilization of databases and Geographic Information Systems (GIS) in coastal scientific studies has led to a significant increase in the development of vulnerability indices for coastlines (Ariffin et al., 2023; Furlan et al., 2021; Subraelu et al., 2021). This is due to the intense development of coastal areas and the impact of climate change, which have heightened flooding risks. (Aitali et al., 2020). This approach can be explored by integrating the multi-criteria to enable better identification of areas with high vulnerability to sea-level rise and would enable better implementation of guidelines for the management of this coastline (Bagdanaviciute et al., 2015).

The Coastal Vulnerability Index (CVI) is the most common index for assessing coastal vulnerability to physical climate change and sea level rise (Rocha et al., 2023; Roy et al., 2023). All CVI methods are based on an index that simplifies a number of complex and interactive parameters and is widely used to measure coastal vulnerability on a global scale (Khouakhi et al., 2013; Koroglu et al., 2019). The choice of coastal variables is very delicate.

Previous studies from the late twentieth century demonstrate significant variation in the number of variables included in published Coastal Vulnerability

Index (CVI) methodologies (Rocha et al., 2023). At the time, former researchers believed that the more variables there were, the more reliable the result would be. The overall objective of this research is to assess the vulnerability of Agadir Bay to the threat of coastal erosion. The site was chosen because of its geographical location on the coast and its susceptibility to various coastal hazards: flooding, tsunamis (Fajri et al. 2021), based on the "CVI" index, which integrates geomorphological and physical parameters in order to generate a map of coastal vulnerability against erosion.

The main goal of this study is the utilization of the Coastal Vulnerability Index (CVI) as a crucial metric for assessing vulnerability. By leveraging geomatics tools, we aim to provide a comprehensive understanding of the region's susceptibility to coastal erosion. The findings of this analysis are anticipated to contribute valuable insights for coastal management and resilience strategies in the Bay of Agadir. This paper thus serves as a vital contribution to the ongoing discourse on coastal vulnerability assessment and management practices.

MATERIAL AND METHODS

Study area. The study area is part of Agadir Bay in the Souss region of Morocco (Ambroggi, 1963), a country that is in Northern Africa, bordering the North Atlantic Ocean and the Mediterranean Sea, between Algeria and Mauritania. This position gives it a role of economic relay through which all north-south flows transit and it also has a strategic role in economic and socio-cultural terms (Aouiche, 2016a).

Agadir Bay's Geographic location is presented in Figure 1.

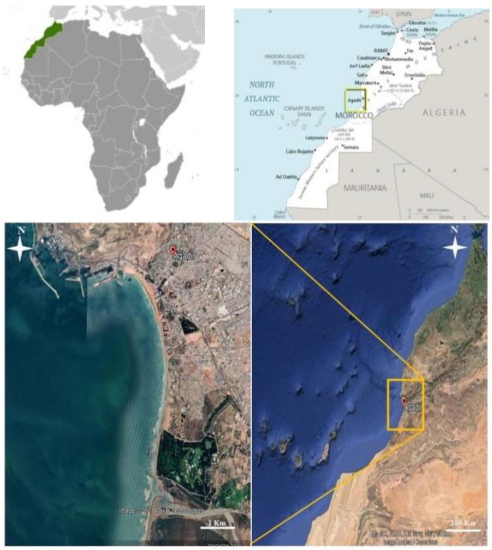

Fig 1: Study area, Agadir Bay's Geographic location
(Source: The World Factbook and Google Earth)

MATERIAL

Geomorphology

The Bay of Agadir is made up of three morphological zones:

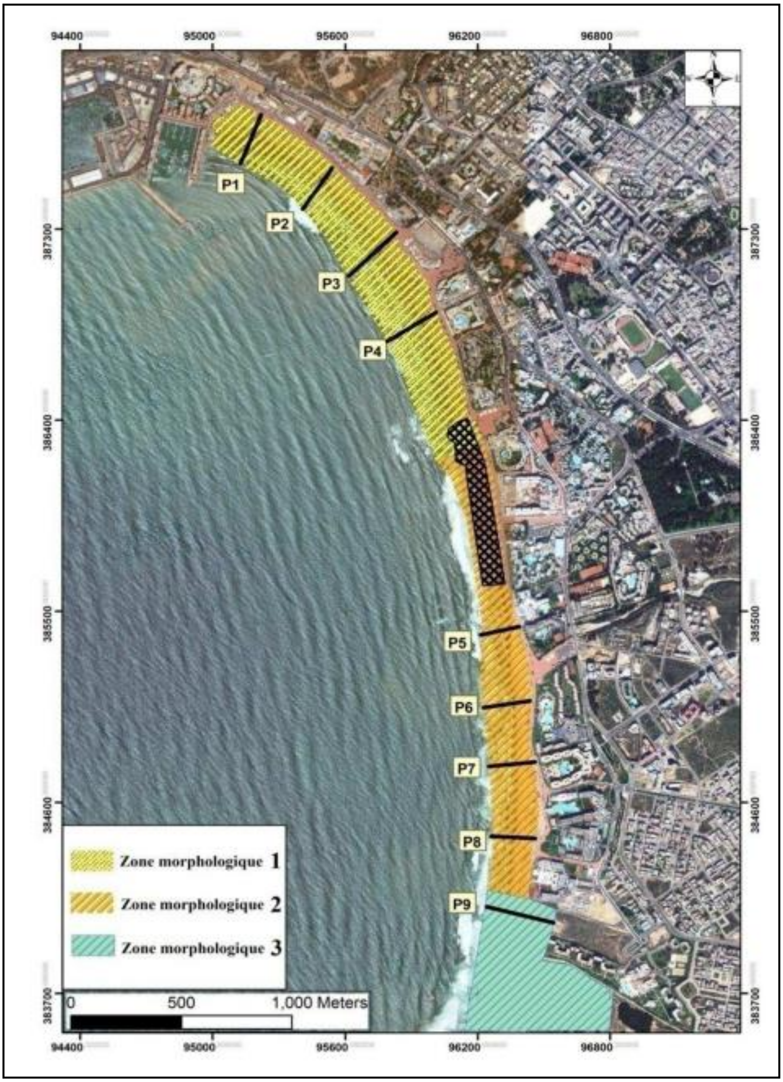

Figure 2: Location of topographical profiles and morphological zones of the beach in April 2015 (Aouiche, 2016a).

Morphological zone 1: Located to the north of the study area (Fig.2), it is characterized at the top of the beach by a vast platform almost 150 m wide with a very gentle slope. Approaching the mid-foreshore we observe a very marked break in slope.

Morphological zone 2: Located in the extreme south of the study area (Fig.2), it represents a different morphology compared to the north. The platform detected in the north has disappeared; the shape of the foreshore is much narrower, steeper, and smoother.

Morphological zone 3: Located in the extreme south of the study area (Fig.2), it represents what remains of the dune system of the Bay of Agadir. The foreshore is quite smooth and less steep. The top of the beach is distinctive with the presence of a dune of 13m/Zh altitude.

The first two morphological zones 1 and 2 are marked by the presence of a promenade dike at the top of the beach, built in 2012. In the 1980s, this portion of the bay of Agadir was formed by a massive dune (12 to 14 m) and a gently sloping beach that extended to the underwater beach.

Coastal slope

According to the topographic data measured (Tab.1) according to the profiles (Fig.2):

–***Morphological zone 1:***

In this zone, 4 transverse profiles were produced P1 to P4.

–***Morphological zone 2:***

In this zone, 4 transverse profiles were made from P5 to P8.

–***Morphological zone 3:***

A single transverse profile was carried out in this zone P9.

Table 1: Measurement of the coastal slope of Agadir Bay

Morphological zone	Profiles	Average slope (degrees)
Zone1	P1	0.96
	P2	1.39
	P3	1.45
	P4	1.45
Zone2	P5	2.44
	P6	2.60
	P7	2.73
	P8	2.60
Zone3	P9	2.49

According to (Aouiche, 2016a).

Erosion and accretion at the coastline

For monitoring the coastline, we chose to work with:

–Panchromatic images from Landsat (Tab.2), with a projection of “WGS_1984_UTM_Zone_29 N”

–The topographic map (1976) of the city of Agadir 1/50,000.

Results are presented in the Table 2.

Table 2. Satellite images used in monitoring the coastline

Satellites/sensor	Date	Resolution
Landsat 7 ETM	09/18/2000	15m
Landsat 8 OLI	09/09/2011	15m
Landsat 8 OLI	05/11/2022	15m

Relative sea level change (mm/yr)

Using the Climate Change Knowledge Portal (World Bank Climate Change Knowledge Portal) which is the hub of climate-related information, data, and tools for the World Bank Group (WBG), it was possible to extract Morocco's sea level values in mm over 21 years (Fig.3):

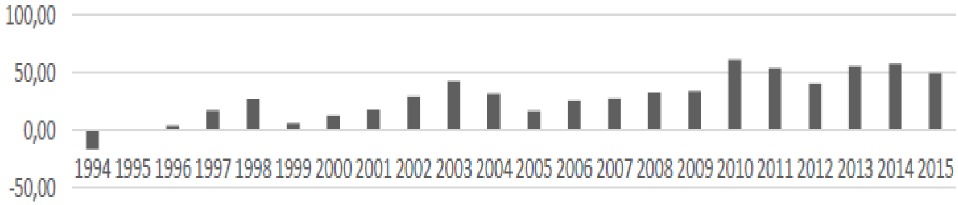

Figure 3: Relative sea level change in Morocco in mm, 1994-2015 (World Bank Climate Change Knowledge Portal).

Mean Tide (m)

Mean Tide (m) refers to the average height of the water level observed over a specific period, typically measured over several tidal cycles. This measurement helps to establish a baseline reference for tidal behaviour. It represents the middle point between the high tide and low tide levels, providing a standard measure of tidal variation. The tide in Agadir is semi-diurnal with a period of 12h25 min.

Mean Significant Wave Height (m)

Mean Significant Wave Height (m) refers to the average height of the highest one-third of waves in a given sea state. It is a statistical measure used to describe the typical height of waves over a specific period, usually measured over an extended duration to account for variations in wave height. MSWH is a crucial parameter in oceanography, providing valuable information about wave conditions and their potential impact on coastal areas, offshore structures, and marine activities. We used data (Fig.4) from the SIMAR database point 1040022(" puertos.es," n.d.), this dataset covers a period of twenty years (2002-2021):

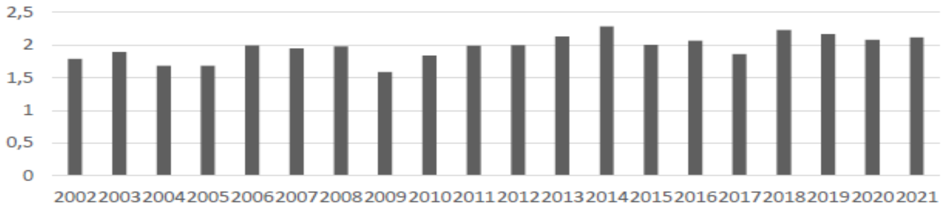

Figure 4: The average of the significant wave height, 2002-2021

CVI methodology and parameters

Geomorphology and Coastal slope. The bay of Agadir is a sandy beach.

In order to use the topographic data on (Tab.1), it is necessary to convert it from degree to percentage (Tab.3):

Table 3: Measurement of the coastal slope of the Bay of Agadir (in %)

Morphological zone	Profiles	Average slope(degrees)	Average slope %
Zone1	P1	0.96	1.68%
	P2	1.39	2.43%
	P3	1.45	2.53%
	P4	1.45	2.53%
Zone2	P5	2.44	4.26%
	P6	2.60	4.54%
	P7	2.73	4.77%
	P8	2.60	5.54%
Zone3	P9	2.49	4.35%

According to (Aouiche, 2016a)

Erosion and accretion at the coastline

Digital Shoreline Analysis System (DSAS) Statistical Calculations. The digitization work was carried out on a GIS environment, from which statistical calculations of the rate of evolution of the coastline are carried out using the DSAS 5.1 extension. Indeed, DSAS is an extension developed by the USGS (United States Geological Survey) available free of charge that allows you to make calculations on the deviations of the coastlines already digitized from the selected images (Zonkouan et al., 2022).

The general principle of this tool is to measure the differences between the coastlines of the same series but also to calculate statistics on the rates of change (in m/year). To do this, the use of the tool requires rigorous formatting of the data in a personal geodatabase, the creation of a baseline and equidistant transects, an estimation of the uncertainty related to the method as well as the choice of statistics for calculating the rates of change.

Pre-calculation operations. At the very beginning, you have to create a personal Geo-database composed essentially of two entities: A first entity made up of already digitized coastlines called shorelines; A second entity containing one or more row(s) of references named baseline. For example, the buffer shoreline buffer stabilizes the linear space in which the coastlines have been digitized.

Shorelines. The shorelines represent the coastlines of the years 2000, 2011, and 2022 that are already digitized in the Geodatabase depending on the version of the extension used. These are the coastlines to which the baseline must be parallel and must be used as a measurement in the DSAS environment. Thus, to be able to perform an index calculation (End Point Rate (EPR), Linear Regression Rate (LRR)) the shorelines must be at least two entities of different dates or times.

Choosing the Reference Line. More than a dozen reference lines show the position of the coastline (Robin, 2002; Boak and Turner, 2005), for our study we used the high tide as a reference choice by Google Earth Pro and the topographic map of Agadir.

The Baseline. It involves digitizing an imaginary baseline from which DSAS creates transects that intersect the different coastlines. Thus, all transects are perpendicular to the baseline, which must also be parallel to the coast. However, sometimes the transects are distorted due to the irregularity of the coast. This sometimes results in aberrant or intersecting transects before they intersect on coastlines. In this way, they can be corrected, straightened, or deleted.

Buffer shoreline. To perform a calculation on DSAS, it is necessary to define the segment on which the measurements of variation in the evolution of the coastline will be carried out. It is the buffer zone that allows you to define on which side of the baseline the profiles will be drawn. Indeed, the buffer zone gives two possibilities of the position of the baseline (on the sea side or on the land side) and this depends on the direction of the baseline, defined by the start and end vertices. But in this process, all baselines were digitized from the boundary of the land side of the buffer zone with a distance of 500m separating it from the shorelines.

Calculated indices. This step was first done by the creation of transects, which are profiles perpendicular to the baseline that make it possible to measure the variation in the rates of evolution of the coastlines, then by the calculation of the indices and the graphical representation of the attribute tables of the indices calculated according to their relevance.

When all input parameters are correctly entered, DSAS automatically generates transects perpendicular to the coastal lines according to the defined measurement step, measures the deviations between the coastlines, and calculates the average displacement rates along each transect. Various statistics (Tab.4) are provided by the software to assess the dynamics of the coastline. We mainly used the following indices (EPR) regression statistics: a rate calculated by dividing the distance of the coastline change by the time elapsed between the oldest and newest coastline; LPR Point Change: is the slope of the regression line positioned in the scatter plot formed by the distance measurements between all the intersection points of each transect and the comparative coastlines.

Based on the previous study of coastline kinematics and the classification of the (Pendleton et al, 2004), the results of the RPA could be categorized: (Accretion:1.0-7.2; Stabilization: -1.0 to 1.0; Erosion: 1.0 to -23.3)

Table 4: Statistics calculated by DSAS

Abbreviation	Statistics
NSM	Net Shoreline Movement
SCE	Shoreline Change Envelope
EPR	End Point Rate
ECI	Confidence of End Point Rate
LRR	Linear Regression Rate
LES	Standard Error of Linear Regression
LCI	Confidence Interval of Linear Regression
WLR	Weighted Linear Regression Rate
WSE	Standard Error of Weighted Linear Regression
WR2	Standard Error of Weighted Linear Regression
LMS	Least Median of Squares

Relative sea level change (mm/yr). A certain alternation of sea level rise and fall with the lowest value -16.16mm in 1994 and the highest of 61.61mm in 2010 are recorded. To include this parameter in our index, it is necessary to calculate the average by dividing the sum of all the values over the number of years of twenty-one years, for which we gave an average of 30.28 mm/year. The relative sea level change of 30.28mm/year is classified as a very high level of vulnerability.

Mean Tide (m). The Bay of Agadir is characterized by an average spring water of 2.9 amplitude (m) with a medium vulnerability.

Mean Significant Wave Height (m). The average SWH of the Bay of Agadir (Fig.33) was between 1.76 m in 2009 and 2.11 m in 2014 when the area experienced many storms. The calculated average is 1.96m with a very high vulnerability.

The investigation into vulnerability to coastal erosion was conducted utilizing the Coastal Vulnerability Index (CVI). The specific equation employed for calculating the CVI is outlined as follows:

$$CVI = \sqrt{\left(\frac{axbxcxdxexf}{6}\right)}$$

where: (a) is geomorphology, (b) shoreline erosion and accretion rate (m/yr), (c) coastal slope (percent), (d) relative sea-level rise rate (mm/yr), (e) mean tidal range (m), and (f) mean wave height (m).

RESULTS AND DISCUSSION

Cartographic results and statistical analysis. The DSAS calculation was utilized to determine and extract the following parameters: A map model was generated based on the calculation of the (EPR) between three dates.

After the mapping of the coastline (Fig.5) for the years 1976, 2000, 2011, and 2022, and the calculation of the DSAS, it was possible to extract the first EPR index which expresses the distance between the oldest and most recent coastline. Variations in the position of the high tide line during these 46 years (1976-2022) show a regressive trend along the 8.3 km of the coastline studied. Indeed, of the 262 transects analyzed, 240 (92%) are in erosion and 29 (8%) are in accretion. With a visible change at the mouth of Oued Souss.

For its spatial analysis, we superimposed the curve and the End Point Rate map to build our first cartographic model (Fig.6), we noticed: Accretion after the first and second wave breezes that can reach from 7.2 m/year to 0.5 m/year; Strong erosion after the spur and at the level of the boom from -23.3 to -4 m/year.

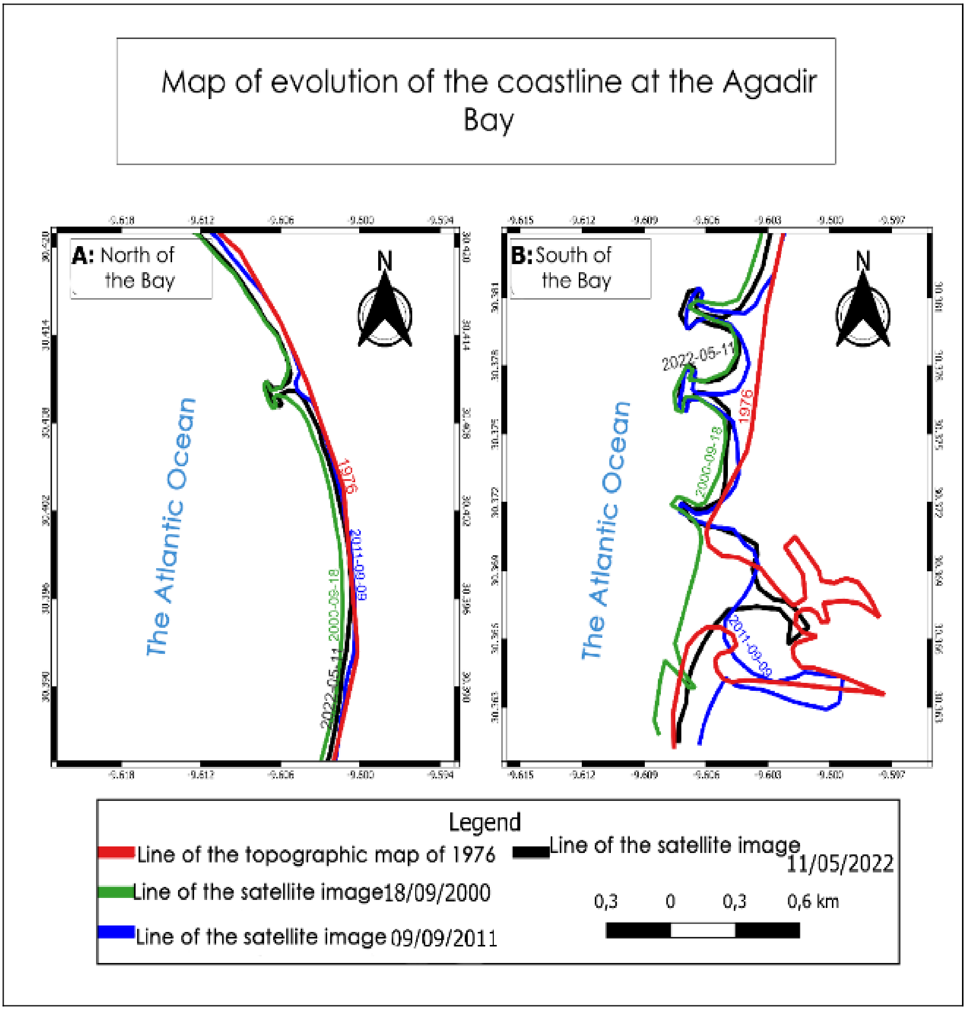

Figure 5: Map of the evolution of the coastline at the Agadir Bay (1976-2022)

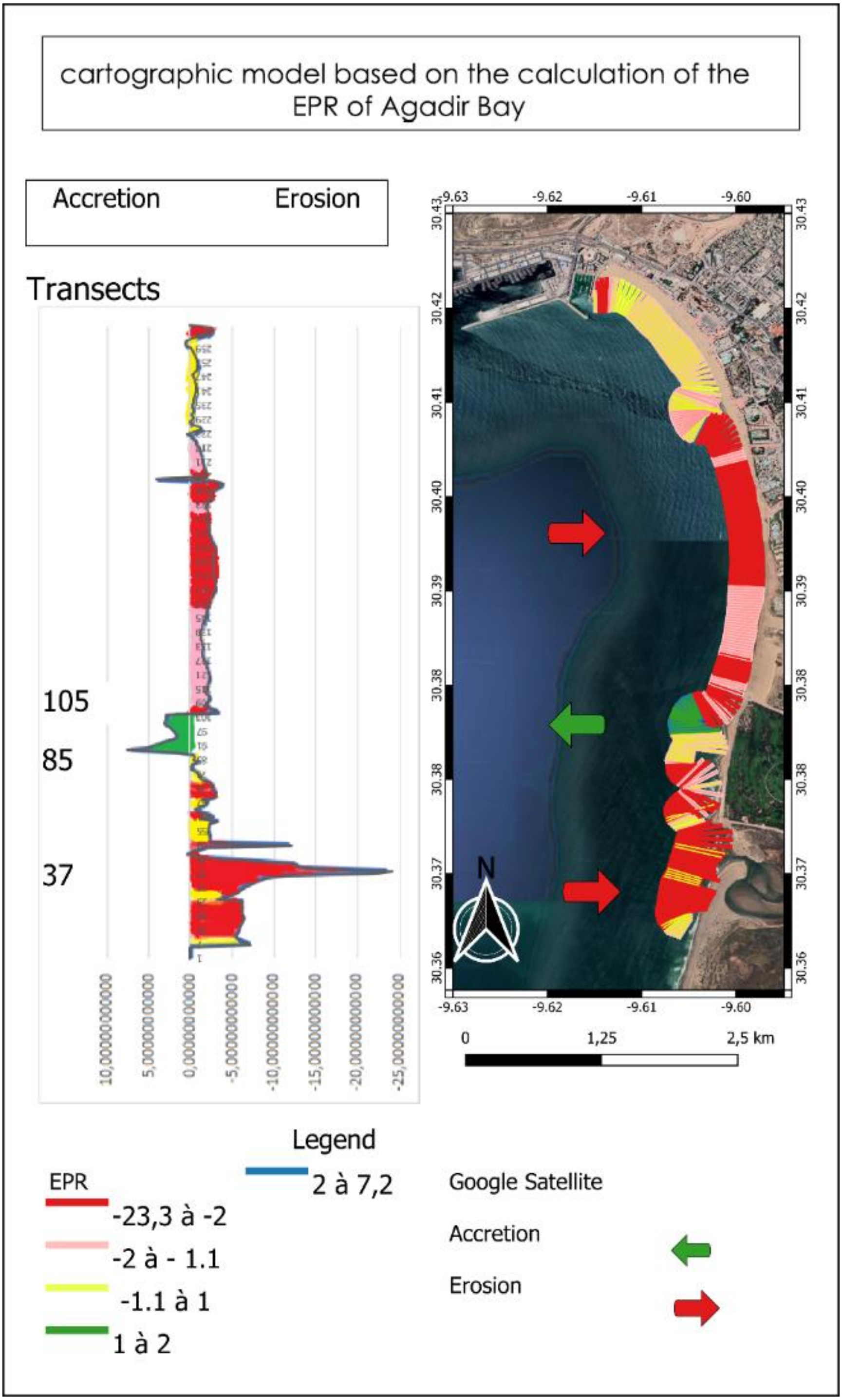

Figure 6. Cartographic model based on the EPR of Agadir Bay

The map model based on the calculation of the LRR (Linear Regression Rate-of-Change) between three dates. The Linear Regression Rate-of-change (LRR) is the slope value of a linear regression line positioned in the scatter plot formed by the distance measurements between all the intersection points of each transect and the compared dimension lines. This attribute, which also reflects the annual rate of change of the reference line along each transect, is interesting for analyzing coastal kinematics over more than three dates, as the calculation method takes into account the evolution of the coastline over the entire period considered, as shown by the results of the study by (Emran et al., 2019) in Bangladesh. Figure 8 illustrates the use of this attribute for a case study based on three shorelines.

Figure 8 illustrates an accretion of 3 to 7.2 m/year just after the second wave breeze; alternating erosion between -13.5 and -1.1m/year and accretion up to 3 m/year.

As a deduction from (Fig.8): LRR is more adequate in the littoral kinematic analysis for more than two dates as in our case, which indicates the choice of using the Linear Regression Rate-of-change (LRR) index instead of the EPR as the main parameter for our study.

Figure 7. Study area photograph (Source: Spalevic, 2018)

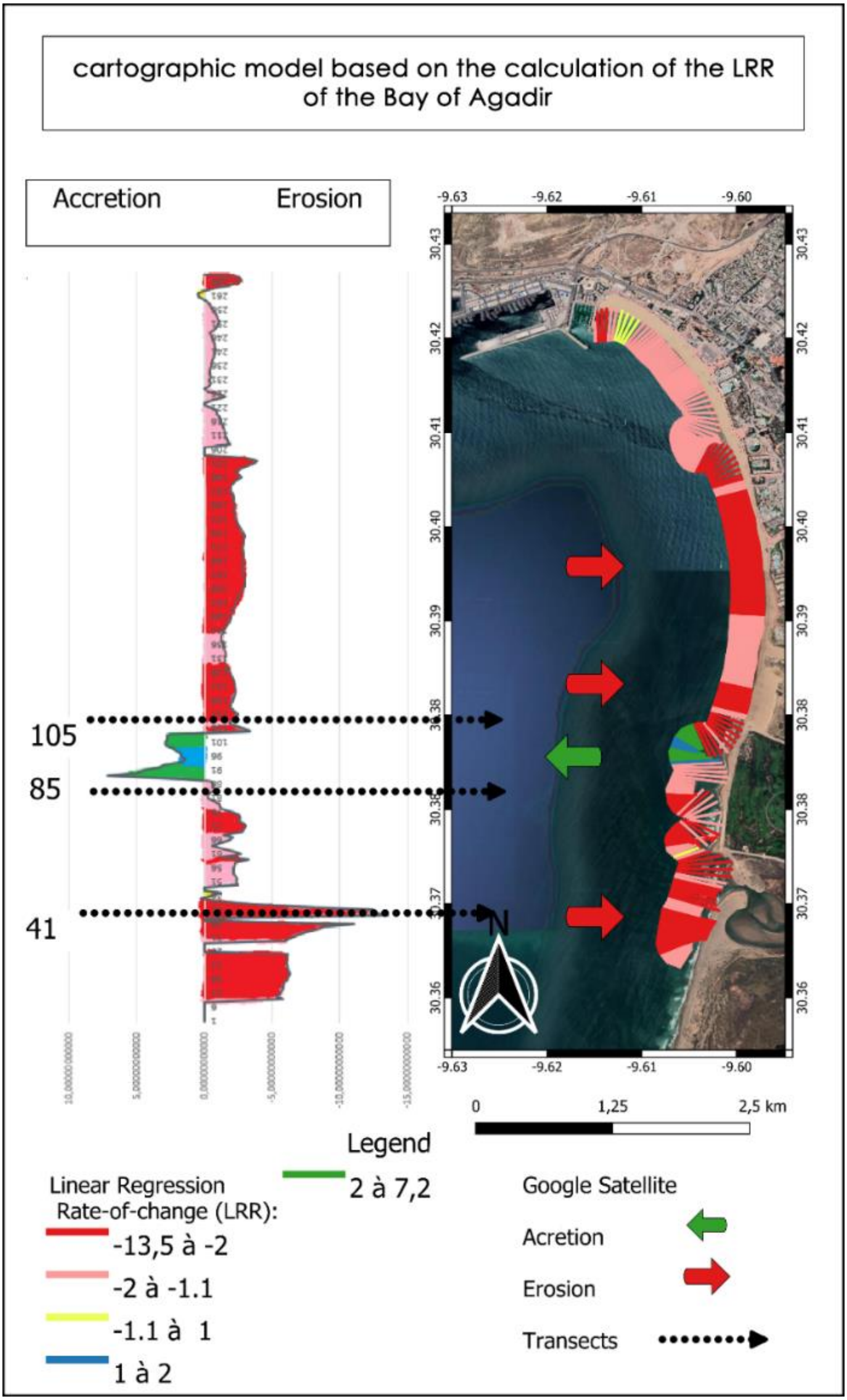

Figure 8. Cartographic model based on the LRR of Agadir Bay

Coastal Vulnerability Index (CVI) Mapping Result. The study of the Coastal Vulnerability Index (Fig.9) has identified: that 1.68km of the coastal zone has a very high vulnerability; 2.58 km of the bay has a high coastal vulnerability.

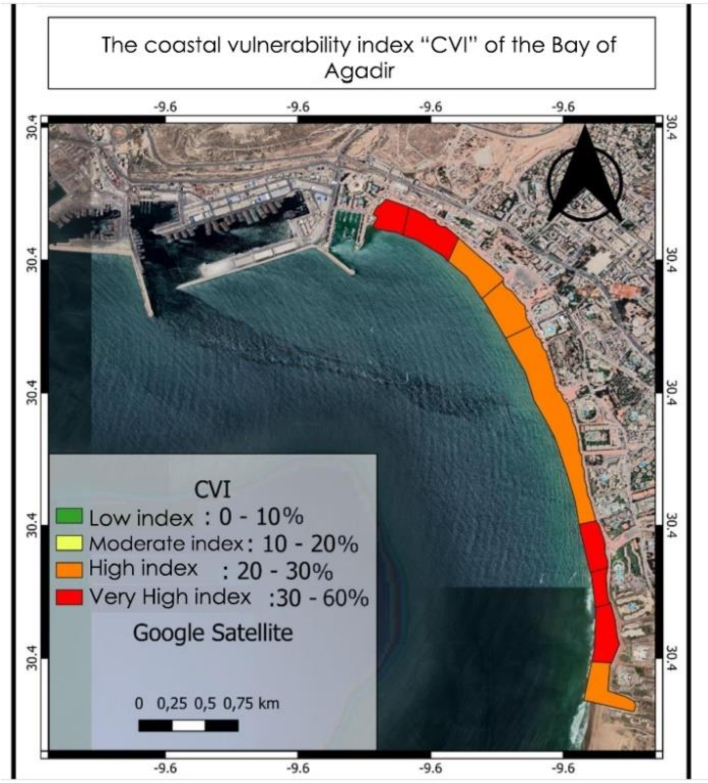

Figure 9. Map of the coastal vulnerability index "CVI" of the Bay of Agadir

The retrospective study of the morphological evolution of the coastline showed that this coastline has been heavily stressed, for several decades (Aouiche et al. 2016b), by development activities, particularly urban and tourist, with an increase in land for buildings and green space. These activities interfered with the hydro-sedimentary dynamics of the coastal system and caused strong erosion of the beaches, considered as an economic capital of prime importance for seaside tourism in the region (Aouiche et al. 2016c), and as an essential buffer reserve for the readjustment of the seaside profiles beach in a state of erosion.

This study, the objective of which was to assess coastal vulnerability to the hazard of coastal erosion on the coast of Agadir Bay in Morocco, revealed a strong socio-economic vulnerability of this coastline. The prospective study was based on the analysis of a large number of available data and information, the fragmentary and sometimes imprecise nature of which must not be concealed. However, despite these weaknesses and the margins of uncertainty contained in the established evolution scenarios, the work made it possible to assess vulnerability to accelerated sea level rise. The analysis highlighted that the southern coast of this coastline was generally more vulnerable to flooding phenomena, due to the low altitude of the coastal fringe.

Indeed, according to the report by (Stern, 2015), the benefits of firm and early action far outweigh the economic costs of inaction. It is, therefore, possible to avoid, or at least reduce these impacts, by acting in a global context, and by implementing anticipatory, ecologically acceptable and financially feasible adaptation and mitigation measures. These options should be reassessed regularly due to: changes that may affect coastal dynamics and therefore vulnerability, scientific understanding of processes, developments in technology, etc. The majority of the scientific community considers that global warming is the major issue of the 21st century. We must therefore act quickly because adopting the scenario of indifference would have very serious consequences. Only general and concerted awareness among socio-economic actors will enable the success of such strategies, as well as the implementation of relevant coastal conservation measures as a guarantee of protection of coastal populations and infrastructure against potential impacts—climate change.

The study of vulnerability to coastal erosion was carried out using the coastal vulnerability index “CVI”. Indeed, the established maps show a high to very high coastal vulnerability of 20% to 60%.

CONCLUSION

Risk, defined as the probability of damage, is a reality for exposed companies, but which only materializes through events considered random. The existence of natural risks for a society obliges us to seek to reduce the possible impacts of these events.

The prospective study was based on the analysis of a large number of available data and information, the fragmentary and sometimes imprecise nature of which must not be concealed. However, despite these weaknesses and the margins of uncertainty contained in the established evolution scenarios, this study nevertheless made it possible to assess the vulnerability of the coastline studied.

The work carried out on the Bay of Agadir has certainly provided important and unprecedented results on the vulnerability of this coastline to coastal erosion, it nevertheless remains perfectible. It requires more in-depth studies to improve knowledge and to remove certain uncertainties using high-resolution drone images for coastline monitoring. Furthermore, it opens the way to additional research, which must be carried out in collaboration with other specialists, particularly in the following areas:

- The development of territorial planning and evacuation strategies based on information relating to the maximum wave height contained in the model.
- Economic evaluation, using cost-benefit analyses, of adaptation options
- and damages caused in the event of a “do nothing” policy
- Raising awareness of risks via web mapping to popularize information on risks on a large scale.

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THE AGRONOMIC RESPONSE OF *CARTHAMUS TINCTORIUS* TO SULPHUR FERTILIZERS

SUMMARY

Sulphur fertilizer can be used as a soil amendment in semi-arid region. The current field trial aimed to study the effect of different levels of various sulphur fertilizers (control: no sulphur application, utilization of 25 or 50 kg S from single superphosphate: $P+S_{25}$ or $P+S_{50}$, 25 or 50 kg S from the elemental form: S_{25} or S_{50}, 25 or 50 kg S from zinc sulfate: $Zn+S_{25}$ or $Zn+S_{50}$) on growth and qualitative proprieties of safflower in west of Iran. The application of sulphur increased vegetative growth components such as plant height, first capitulum height, stem diameter, and the number of lateral branches. The highest rate of vegetative growth was obtained with the application of zinc sulfate. Chlorophyll content increased by 21-24% with zinc sulfate compared to the control. The widest canopy was recorded under the conditions of the use of $Zn+S_{50}$ and $P+S_{50}$ (a 53% increase). Regardless of the amount of consumption, the highest plant dry weight was obtained with the use of Zn+S and S. Utilization of the high level of zinc sulfate and elemental sulphur by increasing the number of capitula, number of achenes in capitulum and diameter of capitulum improved achene yield (by 17%). The highest amount of achene oil was achieved by S_{50} and the application of $P+S_{50}$ was in the second rank. However, consumption of all levels of S or Zn+S and $P+S_{50}$ caused a significant increase in achene protein content. The highest amount of oleic acid, stearic acid, and palmitic acid was obtained with the use of Zn+S. Altogether, under farmyard applied conditions, elemental sulphure and zinc sulphate significantly improved safflower performance due to its high S content, slow S releasing property and low leaching. Application of elemental sulphure and zinc sulphate in semi-arid areas should be seriously considered.

Keywords: achene protein content, number of achenes, single superphosphate, soil amendment, zinc sulfate

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INTRODUCTION

Safflower (*Carthamus tinctorius* L.) is a member of the Composite family with a very long history of cultivation and is domesticated in the semi-arid regions of West Asia. Safflower is a multi-purpose plant that has been cultivated to produce colored petals, medicinal uses, bird feed, forage, and for extracting oil from Achene (Emongor and Emongor 2023). Owing to the extensive root system and shoot anatomy, this plant has a relatively good tolerance against water scarcity and can be considered a crop option in areas with low water resources (Sarto *et al*., 2018). However, during the past decades, this plant has not had an acceptable place in crop rotations and has been less interested or concerned by farmers. However, the amount of safflower annual production in the world is about 996,000 t harvested from 1.2 million hectares of cultivated area. Most of its production is in Asia, North America, and South America. Despite the numerous potentials of safflower and its compatibility with semi-arid areas, it has remained a minor, underutilized, and neglected crop (Emongor 2010) so its cultivated area in Iran is very limited and estimated at 4,000 ha, and about 5,000 t of safflower seed is harvested from these areas (FAOSTAT, 2022). However, safflower compared to other oilseed crops has not fully achieved its rightful position and the potential of this plant can still be used to produce oil and protein. The absence of technical and scientific information about agronomic management, the weak promotion process regarding the introduction of this crop among the farmers, the lack of promoted cultivars, and the absence of a continuous breeding process for this crop are among the things that have caused the reduction of the cultivated area of safflower (Knowles 1955). The oil extracted from safflower achene has a balanced fatty acid profile, and with several monounsaturated and polyunsaturated fatty acids, is noteworthy in terms of oil quality and its effect on various aspects of health (Katkade *et al*., 2018).

The soils of semi-arid regions face serious restrictions such as low organic matter, high pH, and the unavailability of elements for the root system of plants due to special climatic conditions, low rainfall, and irregular distribution of precipitation (Roozitalab *et al*., 2018). Sulphur (S) as one of the essential elements and macronutrients ranks fourth after NPK according to the importance and amount required. Sulphur plays a key role in the structure and biosynthesis of proteinaceous amino acids such as cysteine, methionine, and chlorophyll, as well as in the structure of defense molecules such as glutathione, sulpholipids in the chloroplast membrane, some vitamins such as biotin and thiamine, coenzymes involved in the synthesis and oxidation of fatty acids and molecules involved in trans-sulphuration such as S-Adenosyl methionine (Narayan et al., 2023). However, sulphur deficiency is evident in sandy soils or soils that have little organic matter. In addition to providing plant needs, supplying sulphur fertilizer can play a role as a soil amendment. Sulphur application in calcareous soils can improve plant nutrition. Sulphur has the potential to reduce soil pH, at least on a small scale around its particles. Due to sulphur ability to oxidize and produce sulphuric acid, if sulphur fertilizer particles are present in the rhizosphere environment, it can be effective in dissolving insoluble food compounds and releasing essential nutrients (Sharma *et al*., 2024). In West Bengal, by supplying sulphur from single superphosphate, elemental sulphur and zinc sulphate (zero, 20, 40 and 60), it was determined that the consumption of 40 kg of sulphur through zinc sulphate resulted in the highest plant height, growth rate, accumulation of dry matter and also it resulted in the highest achene yield (Divya 2019). Therefore, it seems that the use of sulphur-containing fertilizers as acidifying substances can affect the ability to absorb other food elements. It should be noted that the biological oxidation of sulphur in the soil is mainly carried out by some bacteria, such as Thiobacillus, and the population of these bacteria is strongly influenced by the amount of organic matter in the soil, and to increase the effectiveness of sulphur, it is necessary to improve the organic matter of the soil (Malik *et al*., 2021). Therefore, it appears that using sulphur fertilizers on the soil surface, conjoining them with animal manure, incorporating them into the soil to the depth of root expansion, and then providing the moisture through irrigation can accelerate the action of sulphur oxidation by bacteria. In addition, the use of manure improves the permeability and water-holding capacity of the soil. The application of sulphur in the calcareous soils of Iran along with bio-sulphur (biological sulphur fertilizer) increased seed yield and oil percentage in *camelina sativa* (Rostami *et al*., 2022). Compared to other crops, oilseed crops require more sulphur due to the formation of oil bodies and the need for more acetyl-CoA and other sulphur-containing compounds and enzymes. In the nutritional management of oilseed crops, a special categorizer for sulphur application should be opened. Sulphur also plays a role in improving the effectiveness and efficiency of consumption of other food elements such as nitrogen, phosphorus, and micronutrients as well as has synergistic effects on some nutrients (Narayan *et al.*, 2023). The soils of semi-arid regions face many physical, moisture and chemical limitations. It appears that the biological oxidation of sulphur produces H_2SO_4 which decreases soil pH and solubilizes $CaCO_3$ in alkaline calcareous soils of semi-arid region and in addition to supplying the sulfur needed by the plant it may provide more advantageous conditions in rhizospher for plants growth (Sharma *et al*., 2024). However, there is no comprehensive information regarding the comparison of different sources of sulphur-containing fertilizers in mesic active Calcixerepts soil in semi-arid regions of Iran. The present experiment is designed to explore the impact of different sources of sulphur-containing fertilizers (single superphosphate, elemental sulphur and zinc sulphate) on the agronomic characteristics and oil quality of safflower grown in the west of Iran.

MATERIAL AND METHODS

Soil and climate characteristics

The achenes of safflower (*Carthamus tinctorius* L.) cv. "ZYS" was kindly provided by the Agricultural Research Station of Kurdistan Province. This variety has been genetically improved in China and has an acceptable range of compatibility with the semi-arid conditions of Iran, and its root system is relatively deep. To evaluate the effect of different sources of sulphur fertilizers on the growth and achene yield of safflower, a field trial was conducted in the northwestern region of Kurdistan province and near the Iraqi border during 2021-2022. The height of the experimental field was 1650 above sea level and the amount of precipitation during the growing season was 149 mm, the average annual temperature was 16.4 centigrade, the maximum annual average temperature was 29.4 centigrade and the minimum annual average temperature was 9.7 centigrade and the average and the annual relative humidity was 48%. The texture of the soil was sandy clay loam. The combined soils were sampled before crop sowing. Then some chemical properties and nutrient concentrations in the soil were evaluated. The amount of absorbable potassium estimated by the method with Ammonium acetate one normal (Stanford and English, 1949). The amount of phosphorus was calculated by Olsen's method (Olsen, 1954). The amount of organic carbon calculated by digestion method (Walkley and Black, 1934), and crude pH evaluated in 1:1 water to soil suspension (Mclean, 1982), electrical conductivity (EC) measured in 1:1 water to soil solution calcium carbonate evaluated by neutralization with acid and titration with soda (Richards, 1954). The amount of soil sulfate was measured by the monocalcium phosphate method (Singh *et al*., 1995). Overall soil properties were pH: 7.68, organic carbon: 0.51%, total nitrogen: 0.26%, $CaCo_3$: 17%, EC: 2.15 ds m^{-1}, SO_4^-: 3.42, P: 16.28 mg kg^{-1}, K: 625 mg kg^{-1}.

Treatments implement

The initial tillage of the field was done by moldboard plow in February 2021. Before plotting and dividing the land, 10 t ha^{-1} of cow manure was added to the land and integrated with the soil through the tandem disk. The plotting of the land was done in February 2021, and sulphur fertilizers were applied on the soil surface according to the dimensions of the plot and integrated with the soil through a shovel, and the field surface was prepared with a pattern of ridges and furrows. The 7 treatments examined in this research were 1) control: no sulphur application, 2, 3) utilization of 25 or 50 kg S from single superphosphate: $P+S_{25}$ or P+S50, 4, 5) application of 25 or 50 kg S from elemental form: S_{25} or S_{50}, 6, 7) use of 25 or 50 kg S from zinc sulfate: $Zn+S_{25}$ or $Zn+S_{50}$. The experimental plots with a size of 4 × 4 meters and with three replications were based on an arbitrary complete block design. Between the experimental plots, 0.5 m was considered as a margin or boundary to prevent the leakage or leaching of fertilizers to the adjacent plots. 50 cm inter-row spacing with 5 cm intra-row spacing was considered and planting was done manually on March 27, 2022. Watering was performed with the *irrigation drip tape* rolls and the water from the pool was delivered by polyethylene pipes to the *tapes* by a pressure pump. The first irrigation was done immediately after planting and the subsequent irrigations were done at intervals of 5-7 days according to climatic conditions and plant requirements. During the growing stages of saffron, weeds were manually weeded. Due to the absence of pests, pesticides and herbicides were not used.

Assessment of growth and achene yield

To evaluate the chlorophyll as the main photosynthetic pigment, a Soil Plant Analysis Development (SPAD) chlorophyll meter (502-PLUSE, Japan) was used at the stage of appearance of the capitulum in the main branch. At the end of the stages of capitula formation in the secondary branches, the width of the canopy was measured from the tip of the branches on the right side to the tip of the branches on the left side using a meter. In the physiological maturity stage, 10 plants were randomly selected and harvested from each plot, and growth characteristics and achene yield components such as stem diameter, height, number of capitulum per plant, the diameter of the capitulum, number of lateral branches, number of achenes per capitulum and percentage of wrinkled and unfilled achenes were calculated and counted. To calculate the achene yield per unit area, A quadrat ($1m^{-2}$) was randomly used to sample plants, the plants were cut from the surface of the ground and dried by placing them in an electric oven at a temperature of 60 °C for 24 h and by weighing them biological yield was obtained, then by threshing capitula, achene yield was acquired.

Oil Extraction

The harvested achene samples were desiccated at 45°C for 45 h under vacuum to reach the low moisture content (≤7%) and then pummeled to the ideal particle size by a mortar. The oil mining was done by Soxhlet extractor for 8 h using cold industrial hexane as a solvent, according to the AOCS method Ba 3-38 (AOCS 1993). For the assessment of fatty acids profiles, the gas chromatography methods were used. The preparation of methyl ester of fatty acids was done based on the instruction described by Ortega et al., (2004). Finally, the prepared samples were measured by a gas chromatography device (Agilent 6890N, USA), fortified with a FFAP-TC capillary column with a length of 30 meters, a diameter of 0.32 mm, and a thickness of the thin layer inside the tube (phase constant) of 0.25 μm was applied. Detector the FID type device was at 250 ˚C and the carrier gas was nitrogen.

For the statistical analysis of the data, the normality of the data was evaluated by SAS software before subjecting the data to the analysis of variance. Correlation and decomposition into main components were done with Minitab software. The comparison of average data was done using an LSD test at a 5% level. Box plots were drawn with Statistica software.

RESULTS

The results of the analysis of variance indicated that the application of sulphur had a significant effect on the diameter of the stem and regardless of the amount of application, the consumption of zinc sulfate and elemental sulphur increased this component by 4% and 26% compared to the control (Table 1). The thickest stems were recorded for plants grown under the application of $Zn+S_{50}$.

The number of lateral branches increased with the application of sulphur fertilizer. The highest number of branches was obtained with the application of high levels of single superphosphate, low levels of elemental sulphur and both levels of zinc sulfate (Table 1).

Table 1- The effect of application of different levels of sulphur fertilizers on the growth characteristics and yield components of safflower in the western region of Iran

Sulphur treatments	SD	SBN	CD	SNP	TSW	SS	CNP
control	7.33^{e}	7.00^{d}	28.64^{c}	32.22^{cd}	28.26^{c}	5.11^{c}	11.44^{c}
$P+S_{25}$	8.33^{d}	8.81^{c}	30.82^{ab}	32.89^{bcd}	29.57^{bc}	5.33^{ab}	12.88^{bc}
$P+S_{50}$	8.77^{cd}	9.71^{abc}	30.63^{ab}	34.44^{abcd}	31.37^{a}	5.88^{a}	15.00^{a}
S_{25}	9.40^{bc}	9.92^{ab}	32.07^{ab}	31.77^{d}	30.11^{ab}	4.55^{c}	14.55^{ab}
S_{50}	9.14^{cd}	9.37^{bc}	32.29^{a}	36.44^{ab}	31.57^{a}	5.33^{ab}	15.66^{a}
$Zn+S_{25}$	10.13^{ab}	10.14^{ab}	30.55^{b}	35.88^{abc}	31.13^{a}	5.66^{ab}	15.00^{a}
$Zn+S_{50}$	10.40^{a}	10.66^{a}	32.24^{a}	37.33^{a}	30.47^{ab}	5.22^{abc}	15.88^{a}
LSD	0.906	1.04	1.67	3.71	1.53	0.684	2.02
CV	5.61	6.28	9.03	16.83	7.09	12.24	8.34

Control: no-sulphur application, $P+S_{25}$: application of 25 kg ha^{-1} sulphur by utilization of single superphosphate, $P+S_{50}$: application of 50 kg ha^{-1} sulphur by utilization of single superphosphate, S_{25}: application of 25 kg ha^{-1} sulphur by utilization of elemental sulphur, S_{50}: application of 50 kg ha^{-1} sulphur by utilization of elemental sulphur, $Zn+S_{25}$: application of 25 kg ha^{-1} sulphur by utilization of zinc sulfate, $Zn+S_{50}$: application of 25 kg ha^{-1} sulphur by utilization of zinc sulfate. SD: stem diameter (mm), SBN: number of branches, CD: capitulum diameter (mm), SNP: achene number per plant, TSW: thousand achene weight (g), SS: unfilled and wrinkled achene (%), CNP: number of capitula per plant. LSD: least significant difference, CV: coefficient of variation. In each attribute, the means with the same letters do not have statistically significant differences.

The lowest effect of sulphur application on the number of branches was observed with low levels of single superphosphate (25%) and the highest effect was observed with the application of ZN (52%) compared to the control.

The diameter of the capitulum was greatly increased with the application of high levels of elemental sulphur and zinc sulfate, and the plants grown under the mentioned conditions had larger layers (13%) compared to the control (Table 1). The number of achenes in capitulum, as one of the important components of the achene yield, was affected by the level and type of sulphur fertilizer source at a statistical level of 5%. The application of high levels of single superphosphate and other sulphur sources could increase this component.

The highest number of achenes in the capitulum was recorded in the plants grown with the application of $Zn+S_{50}$, which was 15% higher than the control condition (no sulphur consumption

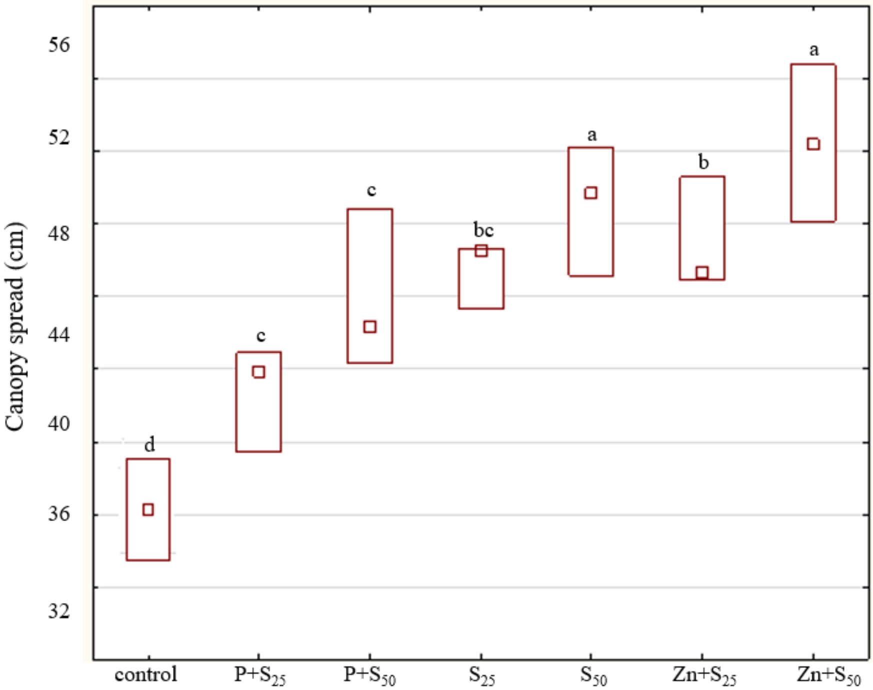

Figure 1. Investigating the effect of applying different levels of sulphur from different fertilizer sources on the growth and canopy size of safflower in the western region. Control: no-sulphur application, $P+S_{25}$: application of 25 kg ha^{-1} sulphur by utilization of single superphosphate, $P+S_{50}$: application of 50 kg ha^{-1} sulphur by utilization of single superphosphate, S_{25}: application of 25 kg ha^{-1} sulphur by utilization of elemental sulphur, S_{50}: application of 50 kg ha^{-1} sulphur by utilization of elemental sulphur, $Zn+S_{25}$: application of 25 kg ha^{-1} sulphur by utilization of zinc sulfate, $Zn+S_{50}$: application of 25 kg ha^{-1} sulphur by utilization of zinc sulfate. Boxes with different letters have a statistically significant difference at the 5% level.

The examination of the canopy spread indicated that the lateral growth and width of the canopy were strongly affected by the investigated treatments. The widest canopies were observed in plants grown using high levels of zinc sulfate and elemental sulphur (Figure 1). However, low levels of zinc sulfate and elemental sulphur improved canopy width by 34% and 27%.

Evaluation of chlorophyll content showed that the application of zinc sulfate at both levels of 25 and 50 kg ha^{-1} led to the highest chlorophyll content in the safflower leaves. The application of elemental sulphur was in the second position of influence on this component. Application of low and high levels of single superphosphate could increase the chlorophyll content by 5 and 11%, respectively (Figure 2).

The evaluation of the number of capitula in the plant indicated that this component increased with the application of sulphur-containing fertilizers.

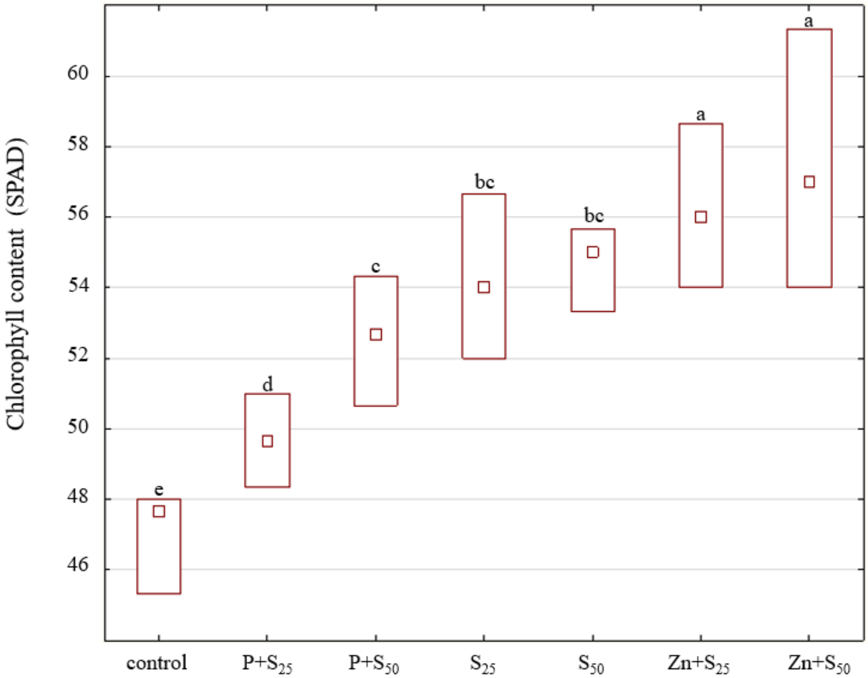

Figure 2- Comparison of chlorophyll content of upper leaves of safflower grown at different sulphur levels. Control: no-sulphur application, $P+S_{25}$: application of 25 kg ha^{-1} sulphur by utilization of single superphosphate, $P+S_{50}$: application of 50 kg ha^{-1} sulphur by utilization of single superphosphate, S_{25}: application of 25 kg ha^{-1} sulphur by utilization of elemental sulphur, S_{50}: application of 50 kg ha^{-1} sulphur by utilization of elemental sulphur, $Zn+S_{25}$: application of 25 kg ha^{-1} sulphur by utilization of zinc sulfate, $Zn+S_{50}$: application of 25 kg ha^{-1} sulphur by utilization of zinc sulfate. Boxes with different letters have a statistically significant difference at the 5% level.

Application of $P+S_{50}$ (31%), S_{50} (36%), $Zn+S_{25}$ (31%) and $Zn+S_{50}$ (38%), increased compared to the control. The consumption of $P+S_{25}$ did not have much effect on this important component of achene yield.

The application of sulphur at a statistical level of 1% affected the achene yield. The utilization of $P+S_{25}$ was associated with a slight increase in achene yield (82 kg ha^{-1}). However, the consumption of $Zn+S_{50}$ improved the achene yield by more than 200 kg ha^{-1}.

The use of $P+S_{50}$, S_{25}, $Zn+S_{25}$, and S_{50} $Zn+S_{50}$ fertilizers could increase the yield by 8%, 10%, 12% and 14%, respectively, compared to the no sulphur applied condition (Figure 3). The results indicated that high levels of zinc sulfate and elemental sulphur led to the highest achene yield.

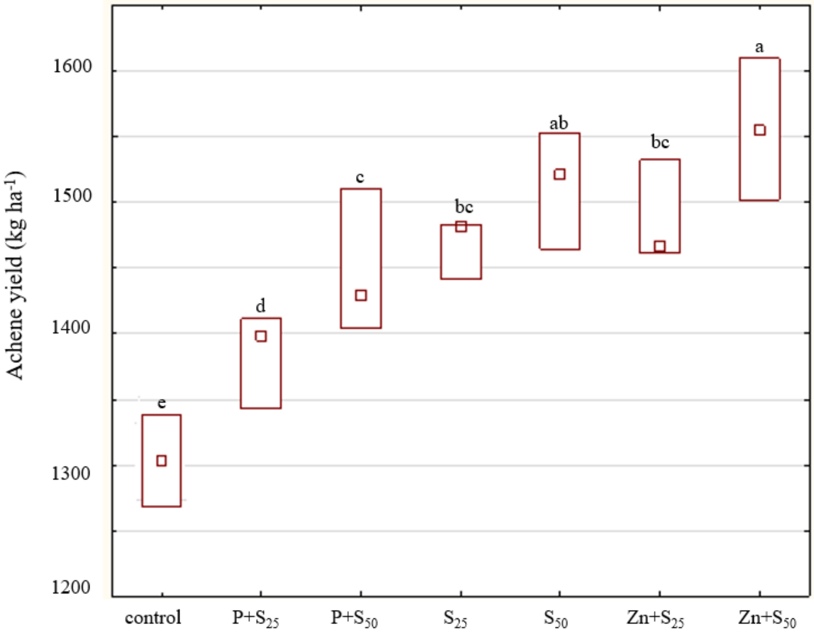

Figure 3- The effect of utilization of different levels of sulphur from different fertilizer sources on safflower achene yield grown in the western region. Control: no-sulphur application, $P+S_{25}$: application of 25 kg ha^{-1} sulphur by utilization of single superphosphate, $P+S_{50}$: application of 50 kg ha^{-1} sulphur by utilization of single superphosphate, S_{25}: application of 25 kg ha^{-1} sulphur by utilization of elemental sulphur, S_{50}: application of 50 kg ha^{-1} sulphur by utilization of elemental sulphur, $Zn+S_{25}$: application of 25 kg ha^{-1} sulphur by utilization of zinc sulfate, $Zn+S_{50}$: application of 25 kg ha^{-1} sulphur by utilization of zinc sulfate. Boxes with different letters have a statistically significant difference at the 5% level.

DISCUSSION

The obtained results showed that both vegetative growth characteristics, yield components, and oil quality were affected by sulfur treatments. The evaluations of the soil in the region indicated that the soil is facing serious limitations and soil amendment should be placed in the priorities of agronomic management. Although Organic fertilizers such as cow farm yard manure contain low levels of total sulfur, our results showed that the application of sulfur along with animal manure has a great effect on growth. Undoubtedly, the simultaneous application of animal manure and fertilizers containing sulfur leads to the acceleration of microbial oxidation and release of sulfuric acid in the rhizosphere environment, and at least on a small scale, they can cause chemical modification of the soil and improve the availability of nutrients (Salih, 2021). Among the examined fertilizers, the most improving effect was observed in zinc sulfate and elemental sulfur fertilizers. It seems that combined fertilizers containing micronutrients, while affecting the soil properties and modifying the chemical aspects of the soil, have been able to meet the need for this element by supplying sufficient amounts of zinc. Zinc plays a role in many key plant enzymes as a cofactor and is also essential for the activation of plant hormone biosynthesis pathways (Castillo-González et al., 2018). The application of sulfur along with organic fertilizer improves plant nutrition and can lead to an increase in soil fertility. Although all the sulfur-containing fertilizers increased the yield of achene, the greatest increase (20%) was obtained with the application of high levels of zinc sulfate. It appears that in the current experiment, the increase in vegetative growth with the application of sulfur caused a significant alteration in source-sink patterns with the increase in the size of the source and more supply of the photoassimilates. Consequently, high amounts of photosynthetic products can convert a larger number of reproductive primordia into achene yield components. With sulfur application, canopy width, chlorophyll content, plant height, and number of lateral branches increased, all of which indicate an increase in source size and activity. Evaluation of the achene yield components and quality characteristics of the extracted oil showed that the application of sulfur not only improves photoassimilate partitioning between different reproductive organs, but also changes the allocation of photoassimilates between different biochemical pathways such as fatty acid and protein biosynthesis. The use of zinc sulfate and elemental sulfur showed the highest fatty acids content, oil, and protein. Although one of the effects of sulfur application is increasing the solubilization of phosphorus in the soil and increasing its availability for the root system (Sugiura et al., 2021), in this experiment the application of composite phosphorus + sulfur had not prominent improving effects compared to other sulfur fertilizers, which can be attributed to the nature of the used composition or its slow phosphorus release.

Our findings confirmed the results of Kaya et al. (2020) as they reported that the simultaneous application of organic fertilizer and sulfur improved the yield of corn under phosphate deficiency conditions by improving vegetative growth components, relative water content, chlorophyll content, and antioxidant enzyme activity. The results obtained in this study showed that Sulfur-enriched soil amendments should be considered as one of the agricultural techniques in semi-arid areas. The evaluation of the quality characteristics of safflower oil showed that to improve the quantity and quality of the oil, it is necessary to use zinc sulfate or elemental sulfur in high amounts. Considering the low of oil production in the country and the high need of oilseed crops for sulfur, the consumption of sulfur, especially along with micronutrients such as zinc, should be an integral part of nutritional management. In addition to the roles that sulfur has in soil chemical modification, sulfur by forming disulfide bonds can play a role in fine regulation and activating and deactivating enzymes or modulating gene expression (Koprivova and Kopriva 2014). Continuous use of traditional chemical NPK fertilizers leads to soil acidification in the long term and reduces soil carbon content and soil fertility (Xun et al., 2016). The obtained results showed that according to the existing restrictions in the soils of the studied area, the use of sulfur along with other chemical or organic fertilizers will be very fruitful. The results of correlation between traits showed that under sulfur applied conditions, the number of capitula per plant, the number of achenes per capitulum, the content of linoleic acid, linolenic acid, the total oil content and the percentage of achene protein increased with the improvement of the canopy width, the increase of the leaf area and the supply of photoassimilates. Our finding revealed that sulphur deficiency is more prevalent in semi-arid region and safflower production system in west of Iran. Especially under farm yard applied condition elemental sulphur and zinc sulphate can affect both vegetative and qualitative characteristics of safflower. Sulphur deficiency is becoming more common in soil with low organic material and Promotion and incentive policies for farmers to use sulphur should be on the agenda.

CONCLUSIONS

This research is unique in terms of evaluating the effect of various sulphur fertilizer sources on the quantitative and qualitative characteristics of safflower. The soil of the investigated area was faced with severe deficiencies of organic matter and some nutrients. The obtained results showed that the use of all types and levels of sulphur-containing fertilizers caused a significant increase in vegetative growth compared to the control. However, the application of zinc sulfate and elemental sulphur had the best effect on reproductive growth components and achene yield. Our finding showed that application of zinc sulfate and elemental sulphur affected safflower oil. Fatty acide profile revealed that from qualitative prospect its oil has high nutritional value and has high potential to use oil in agro-food industries. However, the interaction of compound fertilizers containing other primary plant nutrients is not well known and needs further investigation. The obtained results showed that the application of zinc sulfate or elemental sulphur is one of the correct and necessary nutrient management options in pre-planting soil amendments. The use of sulphur could increase the release of elements from animal manure and improve their availability for the plant roots system and subsequently increase the amount of oil and protein in achenes. This eliminates the need to use high levels of other chemical fertilizers to a large extent. Providing subsidized sulphur fertilizers in semi-arid areas with small-scale farmers and those with low socioeconomic status can significantly improve safflower production. Studying the molecular aspects of the effect of sulphur can provide valuable information for safflower breeding processes.

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COMPARATIVE GEOMORPHOMETRIC ANALYSIS OF DRAINAGE BASIN USING AW3D30 MODEL IN ARCGIS AND QGIS ENVIRONMENT: CASE STUDY OF THE IBAR RIVER DRAINAGE BASIN, MONTENEGRO

SUMMARY

Geomorphometric analysis provides crucial insights into the hydrological characteristics by delineating the land-surface features of a drainage basin. The study focused on analyzing the geomorphometric parameters of the Montenegrin segment of the Ibar River drainage basin using the ALOS Global Digital Surface Model 30 m (AW3D30). Geomorphometric parameters, covering linear and areal parameters, were computed using standard mathematical formulas in LibreOffice Calc software and hydrology tools in commercial GIS software ArcGIS, as well as open-source software QGIS with SAGA GIS modules. Results reveal a dendritic pattern in the stream network, with an inverse relationship between stream length and order, and an elevated bifurcation ratio indicating heightened vulnerability to flooding, influenced by geological, geomorphological, and climatic factors. Furthermore, examination of diverse areal morphometric parameters, such as drainage density, stream frequency, form factor, circularity ratio, and elongation ratio, unveils the hydrological dynamics of the Ibar basin. This characterization illustrates the region as possessing high permeability and dense vegetation cover, suggesting vulnerability to erosion and consequent effects on water and sediment discharge. Additionally, this study underscores the significance of user-defined parameters in geomorphometric modeling, particularly in selecting algorithms within analysis software, which significantly impact drainage basin parameters.

Keywords: drainage basin, Montenegro, geomorphometry, DEM, AW3D30.

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INTRODUCTION

Geomorphometry is a scientific discipline that deals with the quantitative analysis of the earth's land-surface (Pike *et al.*, 2009). However, drainage basins occupy a large part of the Earth, so the analysis has become dominant in process-oriented implementations of general geomorphometry (Rodríguez-Iturbe & Rinaldo, 1997, Shit *et al*., 2022).

Analyzing the geomorphometrics of a drainage basin proves valuable in establishing effective systems for land and water management and protection. It plays a crucial role in assessing natural disaster risks such as floods, erosion, wildfires and landslides (Lukić *et al*., 2018; Spalević *et al*., 2020; Durlević *et al*., 2019, 2021, Vujačić *et al*., 2023, Nikolić *et al*., 2023). Additionally, it aids in identifying optimal locations for constructing water and other infrastructure facilities (Valjarević *et al*., 2020, 2023).

Analyses of drainage basin using manual methods from topographic maps in quantitative analyses were initiated by Horton (1932) and Strahler (1952). Traditional approaches have been replaced by computer-graphic methods such as "Surface and distance measuring," "River basins," "Intensity of Erosion and Outflow model," and "Web-based Intensity of Erosion and Outflow model," as indicated in the studies conducted by Spalević *et al*. (1999, 2000, 2011, 2017, 2019).

Over three decades ago, the modern method for analyzing terrain geomorphometric characteristics began to emphasize the use of Digital Elevation Models (DEMs) and the progress of Geographic Information Systems (GIS). Among commercial software options, ArcGIS, developed by Environmental Systems Research Institute (ESRI), is the predominant choice for geomorphometric analysis in GIS environment (Bogale, 2021). Open-source software and free geospatial data are becoming very popular in the field of GIS and remote sensing. Namely, they give users the possibility and rights to use, study, change and distribute them. Quantum GIS (QGIS) is the most popular free open-source software in the world. It belongs to the Open-Source Geospatial Foundation (OSGeo). Among the main advantages of QGIS are the possibility of embedding tools for spatial analysis through plugins, and the user community of developers and users is constantly growing (Graser, 2016, Šiljeg, 2018).

DEM is a digital statistical terrain model with a series of known x, y and z coordinates within an arbitrarily chosen system (Miller & Laflamme, 1958). Data sources for generating DEM have been developing rapidly. From ground surveys and existing topographic maps to passive remote sensing methods and active sensors such as LiDAR and RADAR (Marić *et al*., 2021; Šiljeg *et al*., 2023). During the last two decades, several open-access global DEMs models with moderate resolution, including TanDEM-X, SRTM, NASADEM ASTER, AW3D30, MERIT, and EU-DEM for Europe, have been released utilizing RADAR sensors. This has notably enhanced geomorphometric analyses (Uuemaa, *et al*., 2020; Nikolić *et al., 2024*).

Manufacturers and users of DEMs typically overlook the need to verify their accuracy, disregarding the impact of user-defined parameters and demonstrating inadequate awareness of their significance (Wechsler, 2003, Šiljeg, 2018).

Numerous researchers have conducted geomorphometric analysis of drainage basins, utilizing open-source DEM data and GIS across various geographical regions. This combination has proven to be a valuable instrument with distinct advantages and disadvantages in producing quantitative data for characterizing drainage basins (Ascione *et al*., 2008; Hlaing *et al*., 2008; Javed *et al*., 2009; Rai *et al*., 2018; Asfaw & Workineh, 2019; Różycka & Migoń, 2021; Bogale, 2021; Derakhshani *et al*., 2023)

Thus, the objective of this study is to examine the geomorphometric attributes of the Ibar river basin in Montenegro utilizing the AW3D30 model via SAGA GIS modules within QGIS software, alongside hydrological analysis tools in ArcGIS software.

MATERIAL AND METHODS

Study Area

The Ibar River begins in northeastern Montenegro at Mount Hajla, then travels through southwestern Serbia and the northern part of Kosovo, before finally joining the West Morava River near Kraljevo in central Serbia. The Ibar drainage basin covers 8,059 km², with 413 km² situated within Montenegro's territory (Figure 1).

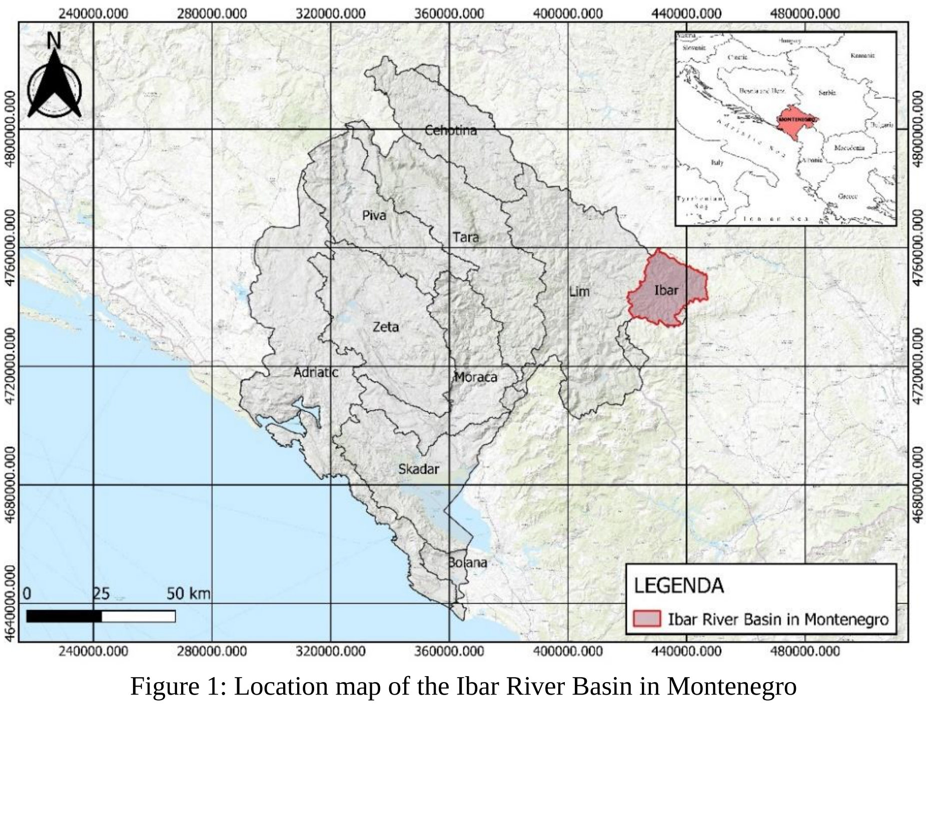

Figure 1: Location map of the Ibar River Basin in Montenegro

The area in Montenegro encompasses hilly-mountainous terrains and upper valleys along the Ibar River, with elevations ranging from 784 m asl in the Draga region to 2,382 m asl at the summit of Rusolija Mountain (Figure 2a). The upper reaches of the Ibra River are located in Montenegro, specifically in the municipality of Rožaje. This region is situated in the Inner Dinarides and falls under the "Durmitor" tectonic zone (Bešić, 1983). Interpreter of the Geological Map of Montenegro (1:200,000 scale) indicate a prevalence of Mesozoic limestones and dolomites (T, K) in terms of geological composition. Additionally, the area features diabase-hornblende formations (J2+3), Neogene deposits comprising clay, marl, sand, and coal, as well as glacial and glacial-fluvial deposits (Mirković *et al.*, 1985). The landscape is characterized by numerous surface and subsurface karst landforms. The Ibar River, along with its tributaries, serves as the primary hydrological network in this region, representing the upper course of the river Ibar. Forests represent the pivotal form of plant communities and vegetation cover in the area (Figure 2b).

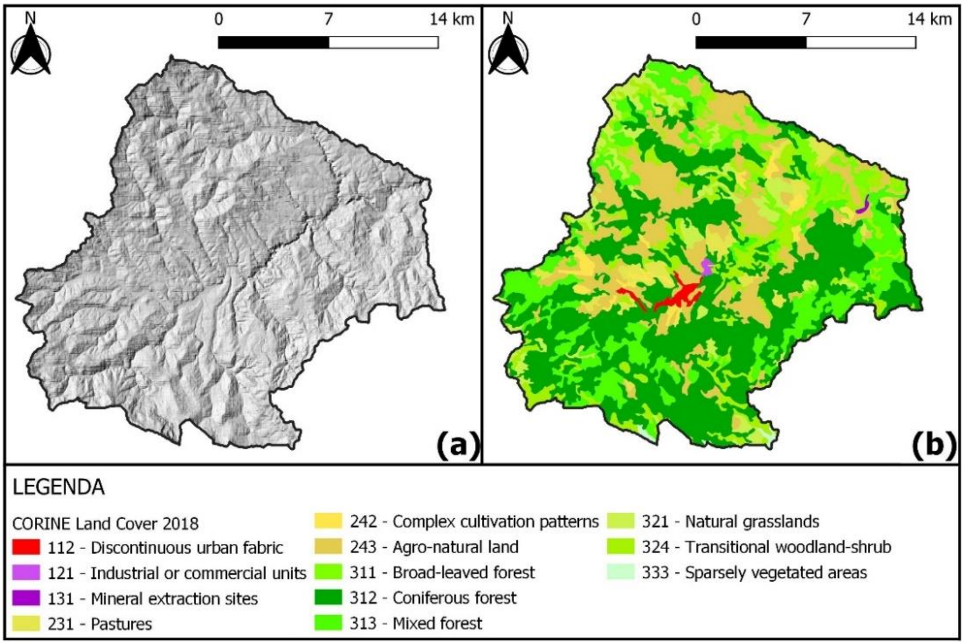

Figure 2: (a) Hillshade of the Ibar River Basin in Montenegro using the EU-DEM model (https://www.copernicus.eu/en/use-cases/eu-dem); (b) Land cover of the Ibar River Basin in Montenegro using the Corine Land Cover 2018 (https://land.copernicus.eu/en/products/corine-land-cover)

According to the Köppen classification, the area is classified as cold temperate D climate (Burić *et al.*, 2012). This climate is characterized by cold winters and mild summers, with an average annual air temperature of 6.3˚C and an annual precipitation of 920 mm. It falls under the pluvio-nival regime, specifically the moderate-Mediterranean subtype as identified by Dukić & Gavrilović (2006).

The perennial flow of HS Rožaje is documented at 2.51 m^3/s for the period 1968-2003 (https://www.meteo.co.me/page.php?keyword=reports). Unfortunately, reliable data for HS Bać is lacking due to interruptions in the station's operation.

Data Acquisition

The ALOSWorld3D 30 m Digital Elevation Model (AW3D30; version 3.1) was created utilizing a vast collection of images captured by the panchromatic optical sensor (PRISM) aboard the Advanced Land Observing Satellite (ALOS), operated by the Japan Aerospace Exploration Agency (JAXA). These stereoscopic images were obtained in nadir, backward, and forward views with a spatial resolution of 2.5 m. Initially introduced in 2016, AW3D30 has undergone subsequent updates to enhance absolute/relative height accuracies through additional calibrations and void filling. The most recent version, utilized in this study, was released in April 2020 (Takaku, *et al*., 2020). This aligns with previous findings indicating that the vertical Root Mean Square Error (RMSE) remained below 5 m in flat areas, while it increased to 12 or 14 m in regions with more complex terrain. The AW3D30 model exhibited the highest accuracy and the least uncertainty compared to other global DEM models such as ASTER, SRTM and NASADEM (Uuemaa, *et al*., 2020). The data was obtained by downloading from the JAXA Geoportal, which provides geospatial data collected through satellites and other space missions (https://www.eorc.jaxa.jp/ALOS/en/index_e.htm).

Methodology in QGIS

The data were acquired at a resolution of 30 meters in the WGS 84 Geographic Coordinate System and then transformed into the Mercator Universal Transverse Projection (UTM 34N) Projected Coordinate System, utilizing the WGS 84 rotating ellipsoid (EPSG: 32634). For the purposes of geomorphometric analysis, the border of Ibar River drainage basin is defined in the administrative borders of Montenegro. Geomorphometric analysis is based on complex algorithms and other features that can be done with SAGA GIS modules for Terrain Analysis in QGIS 3.6.3. software (https://www.qgis.org/en/site/forusers/download.html). In the beginning, Clip tool was used to define AW3D30 model within the borders of Montenegro. Fill Sink tool was used to fill sink on DEM. Basin boundary, stream orders, number of stream segments, and lengths were obtained using Channel Network and Drainage Basins tool (Threshold: 5). Area, perimeter and length were calculated using formulas in Field Calculator. While the other linear and areal parameters were obtained based on formulas (Table 1) in software LibreOffice Calc 7.3 (https://www.libreoffice.org/download/download-libreoffice/).

Methodology in ArcGIS

The same border of the Ibar river basin in Montenegro and reprojected DEM data as in QGIS were used. The complete GIS methodology was carried out using ArcGIS 10.4.1 (https://desktop.arcgis.com/en/quick-start-guides/10.4/arcgis-desktop-quick-start-guide.htm) software, making use of the Hydrology toolset found within the Spatial Analyst toolbox module in ArcMap. The Fill tool is employed to fill sink areas. Subsequently, the Flow Direction and Flow Accumulation tools come into play. Following this, a threshold value of 350 is set for the flow accumulation model. Once the Flow Direction model is obtained, a drainage basin is delineated and selected using tool Basin. The Strahler classification is then conducted using the Stream Order tool based on Flow direction model with threshold value. Following classification, the resultant raster models are converted into vector formats for final analysis and estimation of geomorphometric parameters. Area, perimeter, and length are determined using the Calculate Geometry tool. While the other linear and areal parameters were obtained based on formulas (Table 1) in software LibreOffice Calc 7.3 (https://www.libreoffice.org/download/download-libreoffice/).

Table 1: Analyzed geomorphometric parameters in GIS and formulas

S. no	Parameter	GIS Analaysis/Formula	Unit	Reference
1.	Stream order (So)	GIS analysis	km	Strahler (1952)
2.	Stream number (Nu)	GIS analysis		Strahler (1952)
3.	Stream length (Lu)	GIS analysis	km	Horton (1945)
4.	Basin perimeter (P)	GIS analysis	km	Horton (1945)
5.	Basin length (Lb)	GIS analysis	km	Horton (1945)
6.	Basin area (A)	GIS analysis	km^2	Horton (1945)
7.	Mean stream length (Msl)	$L_{sm} = \frac{L_u}{N_u}$	km	Horton (1945)
8.	Bifurcation ratio (Rb)	$R_b = \frac{N_u}{N_{u+1}}$		Schumm (1956)
9.	Mean bifurcation ratio (Mrb*)*	$R_{bm} = \frac{\sum R_b}{n \times u}$		Schumm (1956)
10.	Drainage density (Dd)	$D_D = \frac{\sum L_u}{A}$	km/km^2	Horton (1945)
11.	Stream frequency (Fs)	$F_s = \frac{\sum Nu}{A}$	km/km^2	Horton (1945)
13.	Form factor (Rf)	$R_f = \frac{A}{Lb^2}$		Horton (1945)
14.	Circulatory ratio (Rc)	$R_c = \frac{4\pi A}{P^2}$		Miller (1953)
15.	Elongation ratio (Re)	$R_e = \frac{2\sqrt{\frac{A}{\pi}}}{Lb}$		Schumn (1956)

RESULTS AND DISCUSSION

Linear parameters

Table 2 contains the analysis outcomes for linear parameters processed using QGIS software, while Table 3 presents the analysis results processed through ArcGIS software. Figure 3 illustrates the cartographic depiction of linear parameters based on Strahler's classification.

Table 2: Results of analysis of linear parameters in QGIS environment

Stream order	No. of segments	Stream length	Mean Stream length
QGIS			
1st order	377	272.57	0.72
2nd order	72	134.70	1.87
3rd order	17	69.22	4.07
4th order	6	34.54	5.76
5th order	1	23.34	23.34
Total	473	534.37	
Bifurcation ratio			
1st/2nd	2nd/3rd	3rd/4th	4th/5th
5.24	4.24	2.83	6.00
Mean	4.58		

Table 3: Results of analysis of linear parameters in ArcGIS environment

Stream order	No. of segments	Stream length	Mean Stream length
ArcGIS			
1st order	509	309.60	0.61
2nd order	98	144.65	1.48
3rd order	23	79.26	3.45
4th order	6	43.71	7.29
5th order	1	23.25	23.25
Total	704	600.47	
Bifurcation ratio			
1st/2nd	2nd/3rd	3rd/4th	4th/5th
5.19	4.26	3.83	6.00
Mean	4.82		

Stream order, a fundamental parameter in hydrological analysis, refers to the hierarchical classification of stream segments. Originally pioneered by Horton (1945), stream sorting techniques were later refined by Strahler (1952). The arrangement of the stream network in the Montenegrin section of the Ibar River indicates a dendritic pattern typical of terrain where channels align with the slope. Analysis reveals a reduction in the number of segments with increasing stream order, influenced significantly by geological factors and other physical-geographical conditions (Dukić & Gavrilović, 2006).

Stream length is also one of the most potential parameters used to understand hydrological characteristics. The mean length of the stream is a parameter from the group of derived linear parameters and shows the characteristic size of the component basin. In this study, stream length shows an inverse relationship with stream order. The higher stream order, the lower is stream length. Streams of lower order and shorter stream lengths are located on terrains with a steep slope and a fine texture of the basin. This indicates the geological consistency of the basin, as well as the strong control of drainage network characteristics in the moving water.

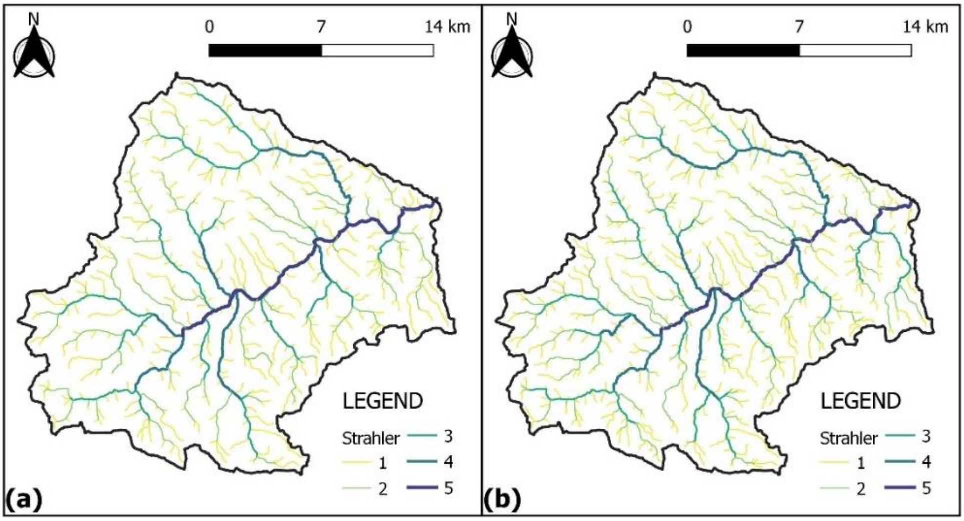

Figure 5: (a) Map of linear parameters in QGIS (b) Map of linear parameters in ArcGIS

Bifurcation ratio, as defined by Schumm (1956), represents the relationship between the quantity of channels within a specific order and the quantity of channels in the subsequent higher order. In the context of the study, the drainage basin exhibits heightened susceptibility to flooding, attributed to its elevated bifurcation ratio. This suggests that the basin is impacted by both geology and geomorphology, and when coupled with climatic conditions, it results in the occurrence of floods.

Areal parameters

Table 4 presents areal parameters that have been analyzed using both QGIS and ArcGIS software. Drainage density is the ratio of total stream length of all the orders per unit basin area (Horton 1945). The density of drainage is influenced by geology, geomorphology, climate, vegetation and soil characteristics. Moreover, this parameter serves as an indicator of soil infiltration capacity (Radulović, 2000) and contributes to the discharge of water and sediment from the drainage basin, as well as indicating susceptibility to erosion (Spalević *et al*, 2020; Vujačić *et al.*, 2023). The drainage density indicates that the basin is highly permeable and has a fairly well-developed vegetation cover. The stream frequency of a basin is characterized as the quantity of streams per unit area (Horton, 1945). Previous studies show that current frequency is positively related to drainage density.

Table 4: Areal parameters of the Ibar River Basin in Montenegro

Areal Parameters	**QGIS**	**ArcGIS**
Basin perimeter (P)	117.60 km	116.35 km
Basin length (Lb)	28.29 km	27.99 km
Basin area (A)	404.77 km^2	403.90 km^2
Drainage density (Dd)	1.32 km/km^2	1.40 km/km^2
Stream frequency (Fs)	0.93 km/km^2	1.74 km/km^2
Form factor (Rf)	0.51	0.52
Circulatory ratio (Rc)	0.37	0.37
Elongation ratio (Re)	0.80	0.81

Form factor is a dimensionless ratio of the area of a drainage basin to the square of its maximum length (Horton, 1945). The form factor serves as an indicator for the formation and movement of floods, the extent of erosion, and the transport capacities of sediment loads within a river basin. The form factor ratio in this area indicates a lower form factor value. Consequently, the basin is characterized by a lower peak flow and an extended duration, attributed to its elongated shape.

As per Miller (1953), the circularity ratio is defined as the ratio of the basin area to the area of a circle with an equivalent perimeter to that of the basin. For the Ibar basin, this parameter indicates similar characteristics to other areal parameters (Table 2).

Elongation ratio is described as the proportion of the diameter of a circle with an equivalent area to that of the basin, relative to the maximum length of the basin (Schumm 1956). According to the results in Table 4, the study area is classified as an oval type characterized by a steep slope and high altitudes in combination with other physical-geographical factors.

ArcGIS vs. QGIS: A Comparison

Table 5 shows the percentage deviation between the linear parameters generated in QGIS and ArcGIS, while Table 6 shows the percentage differences for the areal parameters. Tools in the open-source software QGIS, incorporating SAGA GIS modules, and the commercial software ArcGIS utilize different algorithms for deriving geomorphometric parameters of drainage basins. QGIS offers users a choice among multiple algorithms for Fill Sinks, such as Wang & Liu, QM of ESP, and Planchon/Darboux, 2001, whereas ArcGIS provides only one method. Once the Fill Sinks algorithm is applied in QGIS, the entire process of deriving the modelling of streams and basins is automated through the Channel Network and Drainage Basins algorithm, whereas in ArcGIS, each step needs to be executed separately. Although both software utilize the 8D (Eight Direction) method within the Flow Direction algorithms, crucial modeling algorithms such as

Fill Sink, Flow Accumulation and Basin exhibit slight variations. Furthermore, in QGIS, algorithm Channel Network and Drainage Basins automatically sets the threshold for the flow accumulation algorithm, offering the option to define the threshold as needed. In contrast, in ArcGIS, this step must be done manually. The research results confirmed the significance of this user-defined parameter, regulating stream order, number of segments, and length, emphasizing notable differences between the linear parameters obtained in QGIS and ArcGIS. Regarding the areal parameters, the disparities between these two software environments are relatively minor.

Table 5: Comparison of linear parameters in ArcGIS and QGIS

Stream order	**No. of segments**	**Stream length**	**Mean Stream length**
QGIS vs ArcGIS			
1st order	-25.93%	-11.96%	18.03%
2nd order	-26.53%	-6.88%	26.35%
3rd order	-26.09%	-12.67%	17.97%
4th order	0.00%	-20.98%	-20.99%
5th order	0.00%	0.39%	0.39%
Total	-32.81%	-11.01%	
Bifurcation ratio			
1st/2nd	2nd/3rd	3rd/4th	4th/5th
0.96%	-0.47%	-26.11%	0.00%
Mean	-4.98%		

Table 6: Comparison of areal parameters in ArcGIS and QGIS

Areal Parameters	**Difference**
Basin perimeter (P)	1.07%
Basin length (Lb)	1.07%
Basin area (A)	0.22%
Drainage density (Dd)	-5.71%
Stream frequency (Fs)	-46.55%
Form factor (Rf)	-1.92%
Circulatory ratio (Rc)	0.00%
Elongation ratio (Re)	-1.23%

Constraints in Establishing User-Defined Parameters

Throughout the process of computing geomorphometric parameters for the drainage basin, the user identifies several factors that impact the output result variably. This collection of numerous parameters, crucial for the accuracy of the output result, which the user can adjust, is referred to as user-defined parameters (Barada, 2017).

One of the primary and crucial user-defined parameters is selecting the appropriate DEM. Selecting the appropriate DEM can improve the reliability and accuracy of morphometric analyzes for the drainage basin. The accuracy of both horizontal and vertical data of the DEM holds significant importance in geomorphometric analyses of drainage basins. Generally, according to previous studies, it can be inferred that higher-resolution DEMs offer enhanced accuracy (Shekar & Mathew, 2023). Even with similar resolutions, DEMs datasets like SRTM, NASADEM, ASTER, AW3D30, MERIT, and EU-DEM might yield varied results when generating geomorphometric parameters for drainage basins. The AW3D30 model showed the highest accuracy and lowest uncertainty compared to other global DEM models in previous studies (Uuemaa, *et al.*, 2020; Shekar & Mathew, 2023).

Another significant user-defined parameter in geomorphometric modeling of drainage basins involves selecting algorithms within geomorphometric analysis software. As outlined in the implementation study, various tools within both QGIS and ArcGIS software yield disparate outcomes for drainage basin parameters. Areal parameters exhibit significantly lower deviations compared to linear parameters, which are contingent on user-defined factors like the threshold for flow accumulation.

In future studies on this subject, it would be beneficial to conduct a geomorphometric analysis of the drainage basin comparing global DEMs data with supplementary relevant sources such as topographic maps, orthophoto maps, or LIDAR technology. Exploring the impact of user-defined parameters when utilizing DEMs of varying resolution and quality, and their effects on the output results, would be a valuable endeavor.

CONCLUSIONS

Examining stream order, stream length, and bifurcation ratio offers valuable insights into the hydrological and geomorphological features of the investigated drainage basin. These parameters illustrate the intricate relationship among physical-geographical variables, which collectively shape the behavior of the drainage network and its vulnerability to flooding. The analysis of drainage density, stream frequency, form factor, circularity ratio, and elongation ratio provides valuable insights into the hydrological and geomorphological characteristics of the studied basin, reflecting its permeability, vegetation cover, flood potential, and erosion susceptibility. This approach contributes to gaining insights into river basin hydrology, facilitating the prioritization of river basin, executing efficient soil and water conservation initiatives, overseeing natural resource management, and conducting analyses for hydrology disasters. The accuracy and reliability of geomorphometric analyses for drainage basins are notably impacted by the selection of suitable user-defined parameters, especially concerning the choice of DEM and algorithms within geomorphometric analysis software.

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ASSESSMENT OF THE QUALITY OF THE WORK OF TRACTOR AGGREGATES DURING BASIC TILLAGE

SUMMARY

A plow is a tool used to perform basic cultivation of soil in order to restore the arable soil layer. The quality of its execution is one of the most important factors that ensures the stable production of plant crops. With a well-executed basic tillage, the same plot is of the appropriate structure, which implies minimal energy consumption for pre-sowing preparation.

The research was conducted in field conditions, and it included basic parameters that were an indicator of the productivity of machine and human work. The Zetor Forterra tractor has an installed power of 100.02 kW and is in operation for the first time.

The goal of the research is to examine the possibility of adjusting basic parameters in order to increase the quality and productivity of work.

The results obtained in this research indicate that despite the good conditions of exploitation of tractor aggregates, the effects that should be provided by new, modern mechanization during intensive production have not been achieved in practice.

It is necessary to pay special attention to parameters such as: coefficient of utilization of the work scope, shift efficiency, and productivity of machine and human work.

Keywords: Plow, basic tillage, tractor, productivity, application in practice

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INTRODUCTION

As with other agrotechnical operations, more efficient use of resources is necessary during basic tillage. Plowing is done with ploughs, which can be: field plows, disc plows, rotary plows, vibration plows, etc. The most widely used in practice is the field plow, which cuts and crushes the soil horizontally and vertically with the help of a plowshare and colter.

When using a field plow, the cut strip (furrow or plastic) moves along the plow board and twists due to the movement of the plow, and eventually falls due to its own weight, causing the soil to overturn, mix and crush. Of all agrotechnical measures, plowing consumes 50-55% of total energy and time, which makes basic tillage the most demanding operation, both in terms of the required power and the resources invested (Fanigliulo et al. 2016; Jugovic et al. 2020). The increased resistance of the plow is influenced by negative resistance components whose forces often lead to deviations from the linear movement of the tractor and changes in its trajectory (Troyanovskaya et al. 2017).

Key technological changes in today's global agriculture include: mechanization, seeding, and increased use of synthetic fertilizers and pesticides (Gathorne-Hardy 2016). According to statistical estimates, 792,000 hectares of agricultural land, 576,000 hectares of arable land could potentially be cultivated in Republika Srpska, and the rest consists of orchards, vineyards and meadows (RS development strategy, 2021). There are around 377,819 ha of agricultural land in the Republika Srpska, of which 201,428 ha or 53.31% are under arable land (SGRS, 2022). From the above mentioned, the need arises for the maximum optimization of the operating parameters of the plow unit in order to maximize performance and quality of work.

A prerequisite for optimal use of the tractor user has both economic and technical foreknowledge. Emphasis on the tractor is given for a reason since the tractor is main source of energy for working with attachment machines and tools and also has great universality of application, so that economic justification depends on it (Nadykto et al. 2016). Tractor fuel consumption is a significant parameter which shows performance of the tractor in a plowing operation (Troyanovskaya, 2014).

One of the problems of using a tractor in modern agricultural production is the lack of research and scientific results that are related to the way of optimal utilization of traction energy potential of the tractor in the conditions of exploitation (Nadykto et al. 2017). Optimization of the necessary power in a plowing operation can be achieved by improvement of adhesion of the tractor to the soil which is achieved by adjusting the plow aggregate using the „push-pull“ principle (Bulgakov et al. 2017).

Although the development of plows has been going on for hundreds of years, there is still room for improvement in exploitation. The goal of the research was to determine and show the basic operating parameters of the plow unit which consists of a tractor „ZETOR Fortera 135/13441.23“ and rotary plow „Akpil KM 80“.

MATERIAL AND METHODS

The plowing was done on 21st March of 2022 on the plot of the experimental-educational center of the Faculty of Agriculture in Aleksandrovac, which is located between the Banja Luka-Gradiška highway and the M16 road. The size of the plot was 16,83 ha, and geographical location coordinates (44°58′21,17″ N i 17°18′09,44″ E). Corn was used as a previous crop on the plot, there were not many harvest residues on the surface. The soil was moderately moist, the average temperature was 4oC and did not drop bellow 0 °C and wind gusts reached 8 m/s.

Table 1.Technical specifications of tractor

ENGINE	Engine type, stage III A	Zetor 1605
	Power, ISO 2000/25 (*kW/KS*)	100,2/136
	Number of cylinders	4
	Nominal number of revolutions (*o/min*)	2200
	Engine displacement (cm^3)	4156
	Maximum torque (*Nm*)	581
TRANSMISSION	Number of gears (*forward/reverse*)	24/18
	Maximum movement speed (*km/h*)	40
	RPM PVT (*o/min*)	540/1000
HYDRAULICS	Lifting powers on levers (*kN*)	77
	Pump capacity (*l/min*)	70
	Number of connections	6+1
DIMENSIONS	Mass (*kg*)	5950
	Wheel base (*mm*)	2490
	Fuel tank volume (*l*)	180

Figure 1. Tractor ZETOR Forterra 1605

Figure 2. Plow turner Akpil KM80

Table 2. Technical specifications of the plow

Number of plow bodies	3
Type	turner
The distance between the plow bodies (*cm*)	102
Frame height (*cm*)	82
Working engagement of plow bodies (*cm*)	35/50
Mass (*kg*)	1080

The tractor ZETOR Forterra 1605 with 135 horsepower, i.e. 100.02 kW (power according to ISO 2000/25) was used as the traction-drive unit (Fig.1, Tab.1). The tractor is equipped with front 420/70R24 and rear 520/70R38 tires. The weight of the tractor is 5950 kg. A three-bladed plow turner manufactured by Akpil-Poland, model KM80, was used as a working attachment. The plow is set to a working reach of 135 cm, i.e. 45 cm per plow body (Fig.2, Tab.2). The tractor and the plow are owned by the faculty, the tractor was used for the first time (1 MČ), i.e. it was purchased for the needs of the experimental educational center Aleksandrovac. The plot was processed by one worker-operator of the unit in one shift that lasted 10 hours. The compaction of the soil was analyzed based on the collection of data on the resistance of the cone penetration into the soil, volumetric mass and current soil moisture (Molnar et al. 2015, Ćirić et al. 2012, Ćirić et al. 2016). Soil compaction was measured using an electric penetrometer Eijkelkamp Penetrologgers with a diameter of 11.28 mm, the tip of which is at an angle of 60^{o}. Recording of soil compaction was performed in 10 repetitions at two depths 0.05 m and 0.10 m and was expressed in Pa. Compacting the soil results in an increase in the volume of the soil, which leads to greater resistance of machines during soil cultivation and resistance to the growth of the root system, which absorbs food and water more poorly, which has a negative impact on the yield (Simikić et al. 2018).

The power is expressed according to ISO 2000/25 kW/HP the gearbox is mechanical with Power Shift technology, 24/18 multi-speed synchro -24 forward and 18 reverse speeds.

RESULTS AND DISCUSSION

The productivity of the machine unit largely depends on the coefficient of utilization of the work engagement, so the actual work engagement was measured in three passes. Based on the measurements, the values were obtained, the first passage was 1.59 m, the second 1.33 m, and the third 1.25 m. From the mentioned measurement, the average actual working reach (Br) is obtained, which is 1.39 m, from the set 1.65 m (Bt), it is smaller by 0.26 m, it can be seen that it varies by an average of 0.17 m that is 12.233 %.

The coefficient of utilization of work capacity (β) is 0,903.

The unit operator monitored the working speed during plowing (ν) in the tractor cabin and it is 9.70 km/h. The idle time (Ttp or T21) is 34.988% with a variation of 1.543 %. Turning time (T212) is 5 % with a variation of 15.28 %. Uptime (Tr or T1) is 91 % with a variation of 9.104 %. Downtime (Tto or T22) 4 % with a variation of 200 %. The above data are shown in the Figure 3.

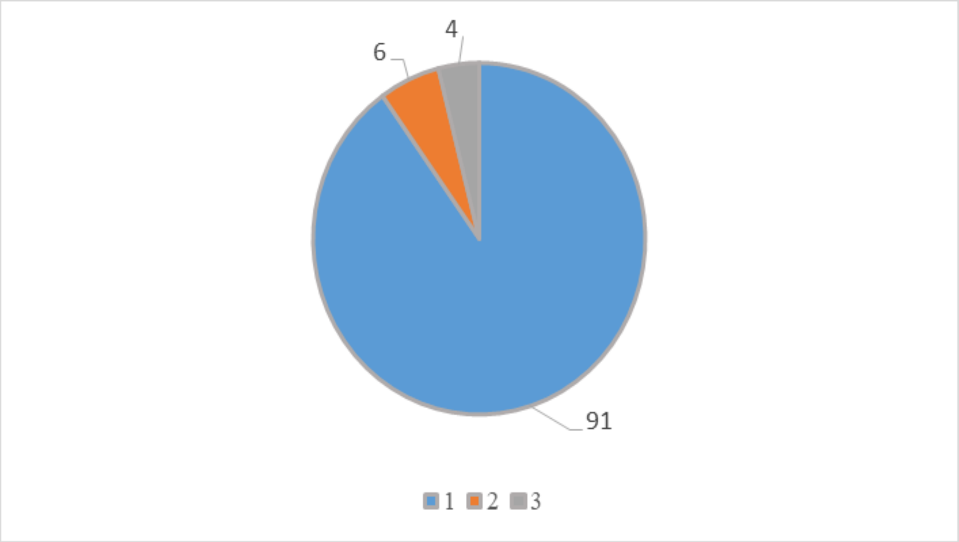

Figure 3. Turning time (1), Uptime (2), Downtime (3)

The results obtained by this research show that the training of the operator of the aggregate can significantly contribute to the improvement of the coefficient of the utilization of working time, throughthe reduction of the time spent for turning the aggregate at the gates of the plot.

Table 3. Research results

	Worth	**Deviation**
Time utilization ratio ($\tau_{pr} ili \tau_{02}$)	0,92	
Shift utilization coefficient ($\tau_{sm} ili\ \tau_{03}$)	0,92	
Shift performance ($W_{t(sm)}$)	12,413 ha/shift	
Daily performance (W_d)	12,413 ha/shift	
Fuel consumption per hour (G_r)	23,45 l/h	
Fuel consumption per area (q_g)	17,325 l/ha	
Productivity of mechanical work (M_{ha})	79.208 kWh/ha	
Productivity of living labor (H_{ha})	1,241 h/ha	
Depth of work (*a*)	0,27 m	0,018 m (7,212 %)
Soil compaction at depth of 0,05 *m*	104 Pa	52,237 (52,817%)
Soil compaction at depth of 0,10 *m*	135 Pa	43,591 (33,891%)

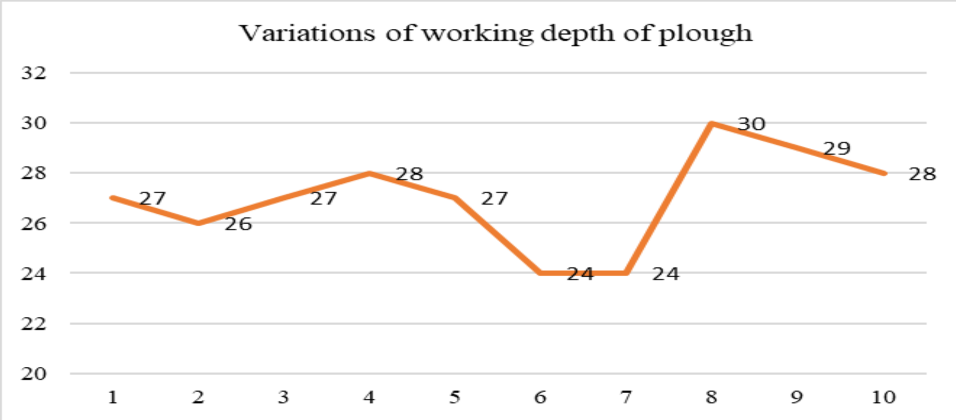

Figure 4. Variations of working depth of plought

Based on the research the results shown in table 3 were obtained. The data were obtained by measuring in three passes, and based on the measurements, the time utilization coefficient is obtained (τpr or τ02) 0.92, shift time utilization coefficient (τsm or τ03) 0.92, shift output (Wt(sm)) 12.413 ha/sm, daily performance (Wd) 12.413 ha/day. Hourly fuel consumption (Gr) was obtained by monitoring the display in the tractor cabin, and it amounted 23.45 l/h. Fuel consumption per unit area is (qg) 17.235 l/ha.

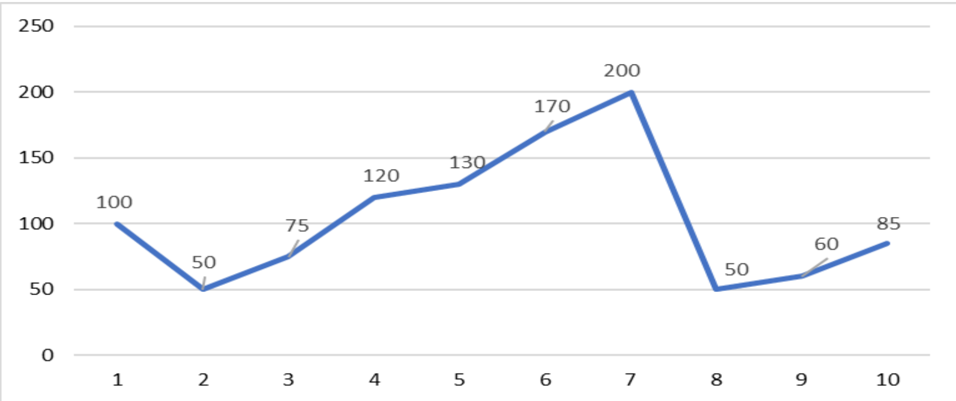

Figure 5. Soil compaction at depth 0.05 m

Based on the measured data shown in Table 3, the productivity of machine work (M ha) was calculated as 79.208 kWh/ha, as well as the productivity of live work (Hha) of 1,241 h/ha.

According to Kovačević et al. (2019) the time utilization coefficient is obtained 0.91, shift time utilization coefficient 0.91, shift output 15.365 ha, daily performance 15.365 ha, which is in line with the results of these researches.

The depth of work (a) was 27 cm, with a variation of 1.827 cm or 7.212 %. Data on the depth of work were obtained by measuring ten values at different locations. Variations of depth work are shown in the Figure 4.

Soil compaction was measured at a total of 20 positions on the plot, including measurements at two depths 5 and 10 cm (10 times each). Soil compaction varied significantly and was 104 Pa with a variation of 52.237 Pa, i.e. 52.817% at a depth of 5 cm.

At a depth of 10 cm, soil compaction is slightly more pronounced and amounts to 135 Pa, and it varied by 43.591 Pa. i.e. 33.891%. The results obtained by measuring on the plot are shown in Figure 5 and 6.

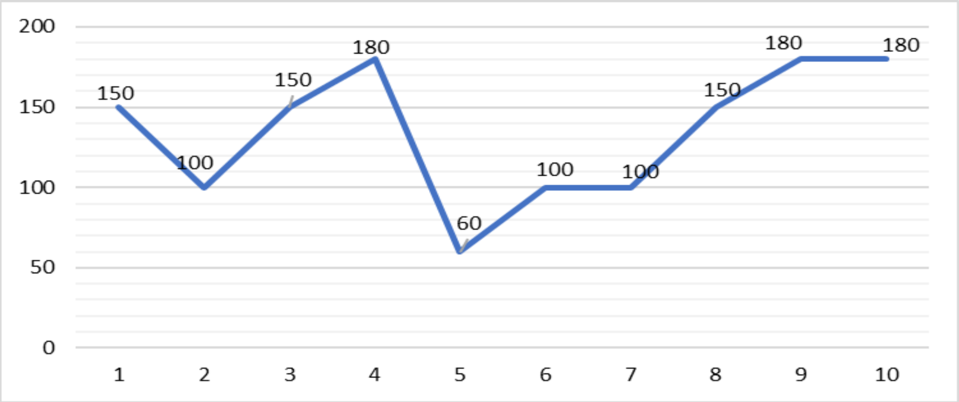

Figure 6. Soil compaction at depth 0.1 m

Soil compaction was measured in 10 repetiotions at two depths, 0,05 m and 0,10 m and was expressed in Pa.

CONCLUSIONS

The use of heavy machinery in agricultural production is necessary due to the use of the power of the tractor on the one hand, while on the other hand it negatively affects the state of soil compaction. One of the indicators of the set-up of the aggregate is the coefficient of utilization of the work engagement, which in this research was 0.903. The achieved performance is significantly affected by the working speed, which during the research is 9.70 km/h. It is to be expected that the fuel consumption tends to decrease according to the cultivated area after a certain number of engine hours, and the data obtained by measurement are 17.325 l/ha.

Examination of the quality of the unit’s operation during the exploitation of the rotary plow showed good results in terms of the shift time utilization coefficient. The obtained results indicate that despite the solid conditions for the exploitation of aggregates, the effects that should be provided by new, modern mechanization during intensive production have not been achieved. The coefficient of utilization of the shift time is significantly influenced by the training of the unit operator, since it is a new tractor, which is in use for the first time, the results achieved can be considered good. Further testing of the unit is recommended in order to optimize the operating parameters and better adapt and train the operator unit.

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IMPACT OF CHILLING ON GROWTH AND HORMONAL HOMEOSTASIS OF *TRITICUM AESTIVUM* AND *TRITICUM SPELTA* DURING INITIAL STAGE OF VEGETATION AND AFTER RECOVERY

SUMMARY

The effect of short-term chilling (+4°C, 2 h) on hormonal homeostasis and growth parameters was investigated in 14-day-old stressed and 21-day-old recovered plants of *Triticum aestivum* cv ‘Podolyanka’ and *T. spelta* cv. ‘Frankenkorn’. Short-term chilling didn't impact the linear indicators of wheat and spelt shoots and roots but stimulated shoot and root fresh and dry weight growth, while reducing root fresh weight. Spelt plants exhibited better post-stress recovery. Chilling led to a 396% increase in ABA content in wheat and a 74% increase in spelt. Stressed and recovered plants exceeded control plants in ABA content. Wheat exhibited three times higher constitutional SA content than spelt, while spelt exceeded wheat in gibberellins (GA_3 and GA_4) content. After chilling, SA content increased by 17.3% (wheat) and 18.7% (spelt), while gibberellins decreased by 32.4% (wheat) and 24.4% (spelt). Wheat dominated spelt in constitutional IAA content. Following chilling, IAA content decreased by 62% (wheat) and 72.2% (spelt). Our findings revealed both common characteristics and organ- and species-specific traits in phytohormones accumulation and balance in wheat and spelt plants during the rapid adaptation to chilling and subsequent recovery period. This will contribute to the understanding of how these related wheat species respond to chilling in the early stages of growth. Analyzing differences in constitutional and stress-induced endogenous phytohormone balances may be useful for developing screening methods for resilient genotypes and environmentally friendly technology of stress resistance induction.

Key words: *Triticum aestivum*, *Triticum spelta*, phytohormones, chilling, growth, recovery

Abbreviations: ABA, Abscisic acid; IAA, indole-3-acetic acid; GA_3 and GA_4, gibberellins; ROS, reactive oxygen species; SA, salicylic acid

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INTRODUCTION

Abiotic stresses exert adverse effects on the growth and productivity of cereal crops and account for approximately 50% of the crop losses, with around 7% attributed to low temperature (Kajlaa et al., 2015). Chilling adversely impacts both vegetative and reproductive growth in wheat, causing delayed seed germination and partial plant death due to embryo development disturbances. In seedlings, reduced water and nutrients uptake rates result in cell dehydration, disrupted nutritional processes, and crop losses ranging from 10 to 30% (Ji et al., 2017; Wu et al., 2023). Under chilling, alterations occur in photosynthesis, respiration, and substance transport in wheat plants (Hassan et al., 2021; Yadav, 2010). Chilling triggers the generation of reactive oxygen species (ROS) (Foyer et al., 2002) and lipid peroxidation (Thakur et al., 2010). Within the initial hours of exposure to chilling, a reorganization of the photosynthetic and energy apparatus takes place (Babenko et al., 2019).

Phytohormones play a pivotal role in acquiring cold resistance, serving as components of intricate signaling cascades that regulate growth, initiate morphological and molecular adaptive changes (Eremina et al., 2016; Santner et al., 2009; Tian et al., 2022). The isoprenoid phytohormone abscisic acid (ABA) orchestrates numerous physiological, biochemical, and molecular processes, governing plant growth and development under both normal and stressful conditions (Kishor et al., 2022). Plant tissues experience rapid ABA content elevation under diverse abiotic factors. The hormone interacts with receptors, instigating signaling cascades that trigger plant adaptive response (Rehman et al., 2021; Sah et al., 2016. Mehrotra et al., 2014). ABA's participation in environmental signal integration, in conjunction with other phytohormones, has been established (Parwez et al., 2022). Salicylic acid (SA), a signaling compound governing plant growth and development, plays a pivotal role in fostering plant tolerance to abiotic stresses (Arif et al., 2020; Hu et al., 2022). Fluctuations in endogenous SA dynamics and distribution, along with exogenous application results, indicate the hormone's involvement in mitigating low-temperature effects *via* ABA-dependent or independent pathways, Ca^{2+} signaling pathways, mitogen-activated protein kinase pathways, and reactive oxygen and nitrogen species pathways. Activation of these pathways prompts antioxidant production, osmolyte accumulation, cold-responsive protein synthesis (such as LEA and dehydrins), and adjustments in hormonal balance (Miura, Tada, 2014; Saleem et al., 2021). Gibberellins (GAs), a class of hormones crucial in regulating growth and development processes under shifting external conditions, also play a vital role in abiotic stress response formation. Cold, salt and osmotic stress decreased GA biosynthesis and signaling, which leads to growth inhibition, fostering resistance, while enhanced biosynthesis of GA contributes to resistance to shading and submergence (Colebrook et al., 2014). While the role of auxins, particularly indole-3-acetic acid (IAA), in regulating plant growth and development under optimal conditions is extensively studied, their involvement in acquired resistance to abiotic stresses remains less explored (Rahman, 2013).

The homeostasis of endogenous auxins under stressors is determined by changes in hormone transport flow direction and intensity along with metabolic conversions (Korver et al., 2018). Hormonal changes accompanying the response of *Triticum aestivum* plants to cold stress (+4°C) during the “alarm” phase involve increased ABA levels and decreased bioactive auxin and gibberellin contents (Kosova et al., 2012). Additionally, endogenous ABA and H_2O_2 form a positive feedback loop to mediate SA-induced freezing tolerance in wheat (Wang et al., 2018). Previously, we analyzed the influence of short-term heat stress (+40°C, 2 h) and moderate soil drought (4 days without watering) on growth and endogenous hormonal homeostasis in 14- and 18-day-old plants of winter wheat cv. ‘Podolyanka’ and spelt wheat cv. ‘Frankenkorn’, both during and after a 21-day recovery (Kosakivska et al., 2022; 2023). It is worth noting that majority of scientists now support the idea that whole-grain hexaploid common wheat, *Triticum aestivum* L., is derived from hulled spelt wheat, *Triticum spelta* L. (Faris J. 2014; Luo et al., 2007). This study aims to explore the impact of short-term chilling on growth and hormonal homeostasis of these related wheat species, identifying common and specific features in their response during the initial stages of growth. In our study, we proceeded from the hypothesis that the impact of chilling on wheat and spelt during early growth stages induces specific alterations in the accumulation and distribution of four phytohormone classes. These changes activate stress-protective systems, shaping an adaptive strategy.

MATERIALS AND METHODS

Plant material Fourteen- and 21-day-old plants of winter wheat (*T. aestivum* L.) cv. ‘Podolyanka’ and spelt (*T. spelta* L.) cv. ‘Frankenkorn’ were investigated. These plants were cultivated in laboratory conditions during 2021-2022 years at the M. G. Kholodny Institute of Botany of the NAS of Ukraine (Kyiv). The ‘Podolyanka’ wheat variety is known for its winter and drought resistance, high yield, and adaptability. The ‘Frankenkorn’ spelt variety exhibits frost resistance and ecological flexibility. Wheat and spelt seeds were sterilized with an 80% ethanol for 5 min, rinsed with distilled water, soaked in water in cuvettes for 3 hours, and then germinated at a temperature of +24°C for 21 hours. Sprouted seeds were then planted in 2-liter containers filled with calcined river sand. Plants were grown at a temperature of +20°C, light intensity of 190 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 16/8 h (day/night) photoperiod, and relative air humidity of 65±5%. Substrate humidity was maintained at 60% of full moisture content, and daily watering was carried out using Knop's solution at a rate of 50 ml per container.

Chilling stress induction and sampling Fourteen-day-old plants in the 2-3 leaf stage were divided into two groups. One group was subjected to a temperature of +4°C and light intensity of 190 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 2 hours (LT-plants), while the other group served as a control (C-plants), continuing growth under the initial experimental conditions. For recovery, plants were grown under controlled conditions until the 21st day, reaching the 3-4 leaf stage. Shoots and roots of 14-

and 21-day-old LT- and C-plants were examined. Morphometric parameters of both species were promptly measured following a short-term chilling.

Extraction and analysis of phytohormones Samples of shoots and roots (1,5 g) were frozen and ground in liquid nitrogen using 10 ml of extraction solution – methanol, distilled water, and formic acid in a ratio of 15:4:1. The homogenate was incubated at +4°C for 24 hours in the dark. The extracts were obtained by 30 min centrifugation at 15,000 RPM and +4°C and separation of the supernatant. The precipitate was resuspended in 5 ml of extraction solution. The suspension was incubated for 30 minutes and centrifuged again. Internal chemical standards were added to the combined supernatants for identification and calculation of losses (1.6 ng of each substance per microliter). The combined supernatants were evaporated to an aqueous residue under reduced pressure in a vacuum evaporator at +40°C.
Further purification was performed on two SPE cartridges: C18 Sep-Pak Plus, Waters and Oasis MCX, 6 cc/150 mg, Waters. Elution of IAA, ABA, GA_3, GA_4 and SA was performed with 100% methanol. This obtained fraction was evaporated to dryness in concentrator flasks using a vacuum rotary evaporator at a temperature not exceeding +40°C. Each dry residue was dissolved to 200 μl with 45% methanol before analysis.

The aliquots were analyzed through high-performance liquid chromatography using an Agilent 1200 LC/MS series instrument (USA), equipped with a diode-array detector G1315B and single quadrupole mass-detector G6120A. Chromatographic separation was carried out using an Agilent ZORBAX Eclipse Plus C18 column 4.6×250 mm with a lipophilic-modified sorbent, particle size 5 μm (reverse phase chromatography). Column ZORBAX Eclipse Plus C18 SS 3.0×150 mm with a sorbent particle size of 3.5 μm was used for the analysis of SA. Agilent OpenLAB CDS ChemStation Edition chromatograph software (rev. C.01.09) was employed for quantitation of the analytes The content of analytes in the samples was monitored using a mass-detector in the combined mode (electrospray and chemical ionization at atmospheric pressure) with ionization of molecules of analytes in negative polarity during analysis. Detailed chromatographic conditions are described in Kosakivska et al., 2020.

Statistical analysis The experiments were conducted with three biological and three analytical replicates. The results were statistically processed using Statistix v. 10.0. Univariate analysis of variance was applied, and differences between mean values were assessed using the ANOVA criterion. Significance was considered at $P \leq 0.05$. Our comparative analysis involved stressed 14-day-old plants versus their respective controls, as well as 21-day-old recovered plants versus 21-day-old control plants. Additionally, during result discussion, comparisons were made between 14-day-old stressed plants with 21-day-old recovered plants.

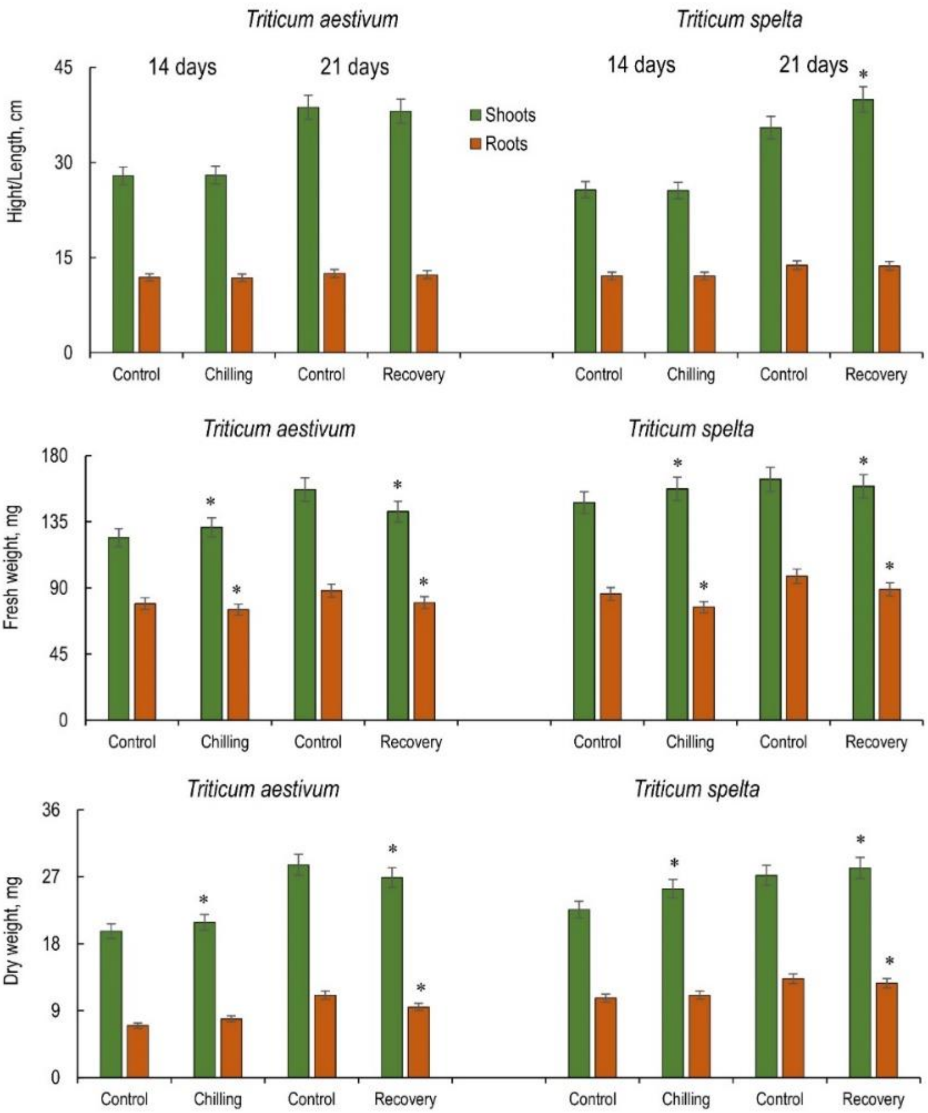
RESULTS

Growth parameters of wheat and spelt plants after chilling The short-term exposure to low positive temperature had minimal impact on the linear parameters of shoots and roots in 14-day-old ‘Podolyanka’ winter wheat and ‘Frankenkorn’ spelt wheat plants. Conversely, shoot fresh weight (FW) and dry weight (DW) of stressed wheat plants increased by 5.6% and 6.1%, respectively, while root FW decreased by 5.1% and DW increased by 12.9%. During the recovery phase, the linear parameters of both shoots and roots for 21-day-old stressed wheat plants returned to levels close to unstressed control plants. However, shoot and root FW were 9.6% and 9.3% lower, and DW was reduced by 5.9% and 14.4%, respectively, compared to non-stressed 21-day-old plants (Fig. 1). In stressed ‘Frankenkorn’ spelt plants, shoot FW and DW experienced increases of 6.1% and 12,4%, respectively, while root FW decreased by 10.5%, and DW has not changed. In the case of 21-day-old spelt wheat plants after recovery, shoot height saw a 12.4% increase the FW and DW of shoots decreased by 3% and 3.6%, respectively, (within the margin of error). Meanwhile, the FW of the roots decreased by 9.2%, and the DW decreased by 4.5%, also within the margin of error (Fig. 1).

Overall, both species displayed considerable resistance to chilling, with spelt plants exhibiting a better recovery.

ABA accumulation and distribution after chilling The distribution of endogenous ABA between shoots and roots of 14-day-old winter wheat ‘Podolyanka’ and spelt wheat ‘Frankenkorn’ C- plants exhibited similarities. The hormone was predominant in the shoots of both species. By day 21, ABA levels in the shoots of wheat and spelt C- plants increased by 1.6 and 1.3 times, respectively, while changes in hormone content in roots remained within the range of statistical error (Fig. 2). ABA continued to dominate in the shoots of all tested samples of ‘Podolyanka’ wheat and ‘Frankenkorn’ spelt. Under the influence of chilling, ABA levels in wheat and spelt LT- plants shoots increased by 4.3 and 1.9 times, respectively. In the roots of wheat, hormone content increased by 5.9 times, while in spelt, it rose by 1.4 times. After the recovery period, ABA levels in both species decreased, yet hormone content in the shoots and roots of wheat remained 1.7 and 3.8 times higher than that of 21-day-old C-plants. In spelt ABA content approached control values (Fig. 2).

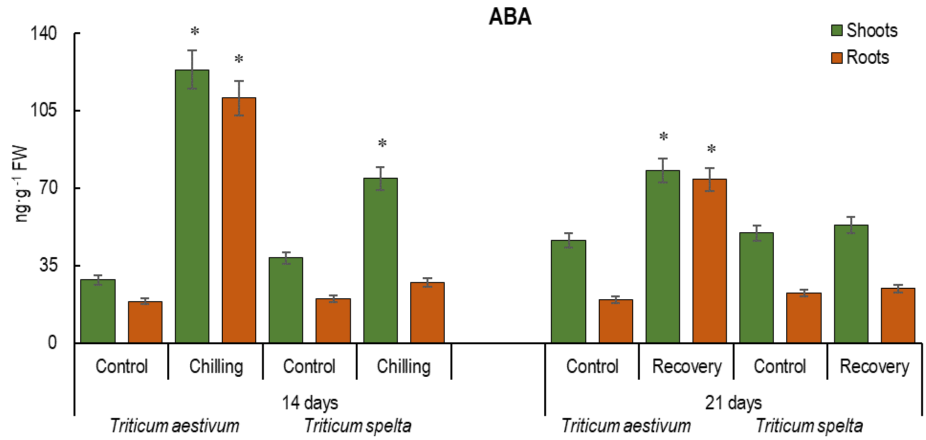
The overall ABA content of spelt C- plants was higher than that of wheat C-plants, and this predominance persisted in stressed and recovering wheat plants. The total ABA content in 14-day-old stressed wheat plants saw a 396% increase, totaling 234.2±11.7 ng g^{-1} FW. By day 21 of recovery, hormone levels decreased by 35% compared to 14-day-old LT- plants, measuring 151.9±7.6 ng g^{-1} FW; yet, it remained 130% higher than levels observed in 21-day-old C- plants. Following exposure to chilling, the total ABA content in 14-day-old spelt plants rose by 74.1% to 101.7±5.1 ng g^{-1} FW. The ABA level in 21-day-old recovered plants reached 77.9±3.9 ng g^{-1} FW, which was 23.4% lower than levels found in 14-day-old LT- plants, but 8.2% higher than those observed in 21-day-old control non-stressed plants.



Note * – significant at $P \leq 0.05$ compared to control at these stages of vegetation; data are the mean ± SE, n = 40

Fig. 1. Effect of chilling (+4°C, 2 h) on growth parameters of 14-day-old *Triticum aestivum* L. cv 'Podolyanka' and *Triticum spelta* L. cv. 'Frankenkorn', and on 21-day-old plants after recovery.

SA accumulation and distribution after chilling The endogenous SA content in the shoots and roots of 14-day-old winter wheat C- plants exceeded that in spelt C- plants organs by 2.3 and 2.7 times, respectively. The pattern of hormone accumulation in the studied cereal organs on the 21st day of growth exhibited distinct differences. A 1.2-fold increase in SA content was observed in the shoots of wheat plants, whereas a 1.3-fold decrease hormone concentration was noted in the shoots of spelt plants and in the roots of both species (Fig. 3).



* – significant difference at P ≤ 0.05 vs. control; data are the mean ± SE, n=9

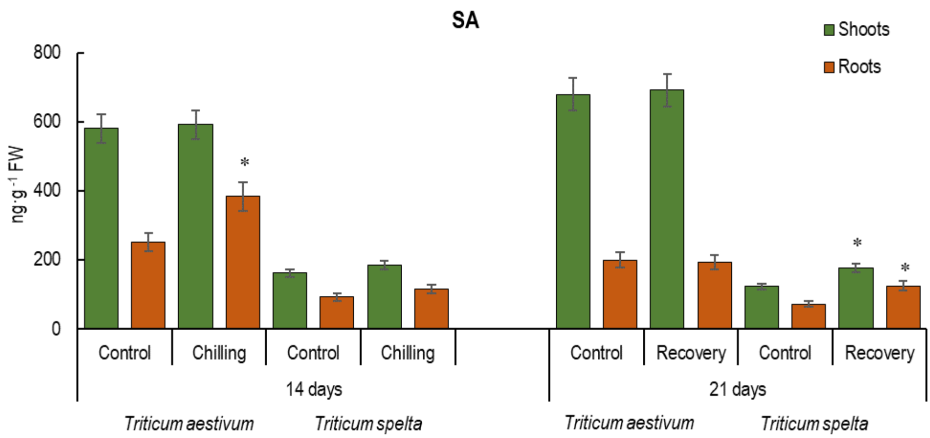
Fig. 2. Accumulation and distribution of endogenous abscisic acid in *Triticum aestivum* L. cv. 'Podolyanka' and *Triticum spelta* L. cv. 'Frankenkorn' plants under chilling (+4°C, 2 h) and during the recovery period (ng g^{-1} FW)

Under the influence of chilling, the SA content in the roots of winter wheat and spelt wheat LT- plants increased by 1.5 and 1.3 times, respectively, while the changes in the shoots remained within the range of statistical error. Following recovery, in 21-day-old plants, SA levels rose in wheat shoots to 691.0±34.6 ng g^{-1} FW and in spelt roots to 124.0±6.2 ng g^{-1} FW, marking increases of 16.8% and 6.7%, respectively, compared to control unstressed plants. Conversely, in the roots of wheat and shoots of spelt, SA content decreased by 2 and 1.1 times, respectively, during the recovery period. In wheat roots, SA content stayed within the control range at 192.6±9.6 ng g^{-1} FW, whereas in spelt shoots it was 1.4 times higher than that of non-stressed plants, measuring 175.1±8.8 ng g^{-1} FW (Fig. 3).

In essence, the SA content in the organs of winter wheat significantly exceeded that of spelt wheat. The preferred site of hormone accumulation was observed in the shoots of both species. The total SA content in 14-day-old 'Podolyanka' wheat plants increased by 17.3% under the influence of chilling, reaching 975.0±48.8 ng g^{-1} FW. After recovery, the hormone concentration decreased by 9.4% to 883.6±44.1 ng g^{-1} FW, well within the parameters of control unstressed 21-day-old plants. For 14-day-old 'Frankenkorn' spelt plants, exposure to cold stress resulted in an 18.7% elevation in total SA content, registering at 300.9±15.2 ng g^{-1} FW. While no further changes in hormone levels were observed after recovery, this indicator remained 53.9% higher than that of 21-day-old control unstressed plants.

GAs accumulation and distribution after chilling The total content of GA_3+GA_4 in 14-day-old 'Frankenkorn' spelt C- plants exceeded that in

'Podolyanka' wheat. By the 21st day of growth, the content of GA in the shoots of wheat C- plants remained virtually unchanged at 32.0±1.6 ng g^{-1} FW, while in the roots it decreased by 1.2 times, amounting to 50.8±2.5 ng g^{-1} FW. In spelt shoots and roots on the 21st day, the accumulation of GA_4 was observed, with its levels being 1.7 and 3.2 times lower than those of GA_3. Conversely, GA_4 was found only in trace amounts in wheat (Fig. 4).



* – significant difference at P ≤ 0.05 vs. control; data are the mean ± SE, n=9

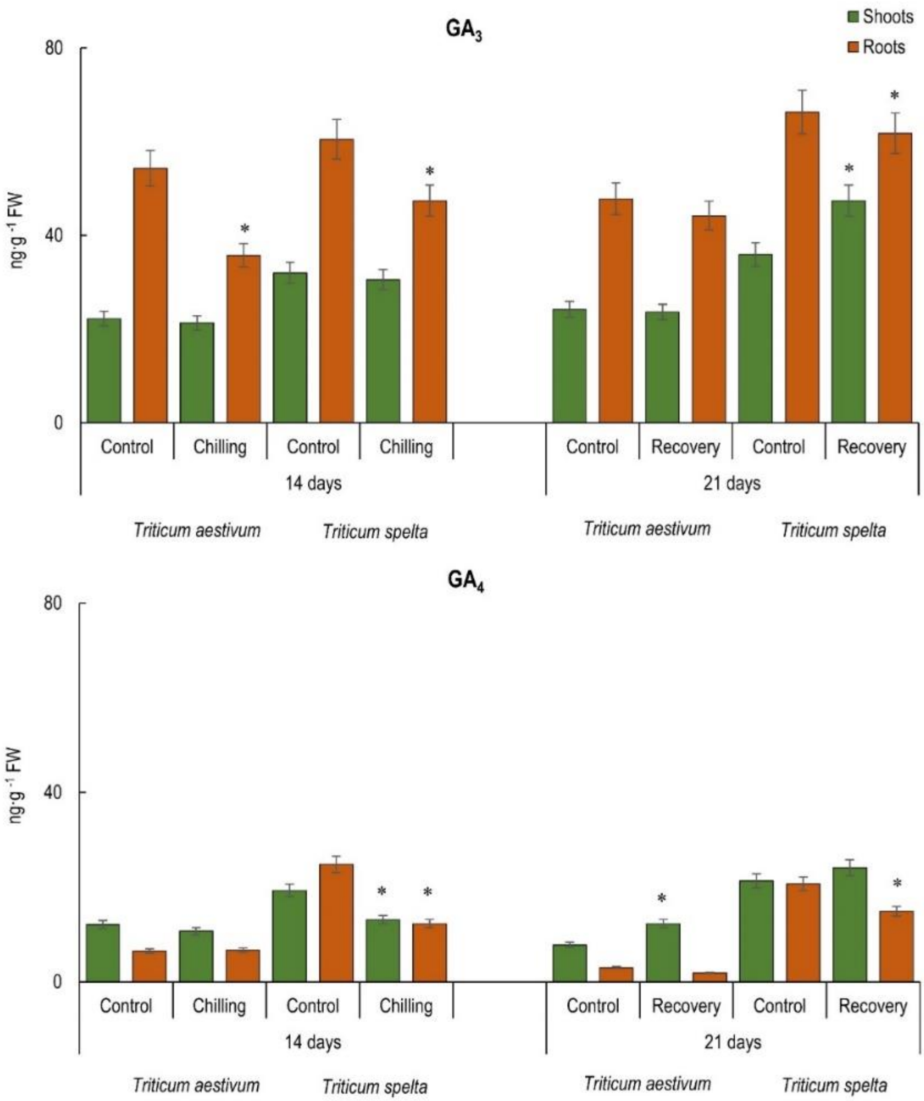
Fig. 3. Accumulation and distribution of endogenous salicylic acid in *Triticum aestivum* L. cv. 'Podolyanka' and *Triticum spelta* L. cv. 'Frankenkorn' plants under chilling (+4°C, 2 h) and during recovery period (ng g^{-1} FW)

Following exposure to cold stress, the total gibberellin content in 14-day-old 'Podolyanka' wheat plants decreased by 32.4%, reaching 64.4±3.2 ng g^{-1} FW. Upon recovery by the 21st day, the gibberellins concentration increased by 27.2%, amounting 81.9±4.1 ng g^{-1} FW, nearly approaching the values of control unstressed plants. In case of 'Frankenkorn' spelt, chilling led to a 24.4% decrease in total GA content, measuring 103.3±5.2 ng g^{-1} FW. In recovered plants, hormone levels rose by 43.5%, reaching 148.2±7.4 ng g^{-1} FW, a 2.8% increase compared to the 21-day control unstressed plants. GA_4 was primarily accumulated in the shoots of both species, and GA_3 was predominantly stored in the roots (Fig. 4).

In general, GA_3 remained dominant across all experimental samples, with gibberellin accumulation favoring the roots. Under the effects of stress and during the recovery period, more pronounced changes were observed in the accumulation of GA_3 in wheat plants, whereas active fluctuations in GA_3 and GA_4 levels were observed in spelt plants.

IAA accumulation and distribution after chilling The content of endogenous IAA in both shoots and roots of 14-day-old 'Podolyanka' winter wheat C- plants exceeded that in 'Frankenkorn' spelt. Notably, the hormone dominated in wheat roots and spelt shoots. In particular, the IAA level in wheat roots was 3.3 times higher than that in spelt roots, whereas the IAA content in spelt shoots was

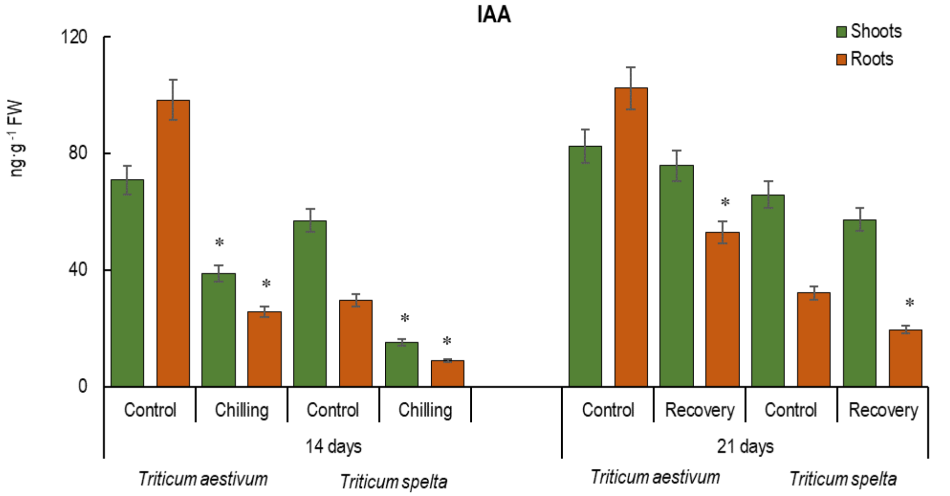
1.2 times lower compared to wheat. By the 21st day of growth, the hormone content exhibited an increase, maintaining a similar quantitative distribution between the shoots and roots as in the 14-day-old wheat and spelt C- plants (Fig. 5).



* – significant difference at $P \leq 0.05$ vs. control; data are the mean ± SE, n=9

Fig. 4. Accumulation and distribution of endogenous gibberellins GA_3 and GA_4 in *Triticum aestivum* L. cv. 'Podolyanka' and *Triticum spelta* L. cv. 'Frankenkorn' plants under chilling (+4°C, 2 h) and during recovery period (ng g^{-1} FW)

Upon short-term exposure to chilling, the IAA level decreased by 1.8 and 3.8 times in the shoots and roots of wheat, respectively, and by 1.9 and 3.3 times in spelt. On the 21st day of recovery, IAA levels increased by 3.8 and 2.2 times in spelt shoots and roots, respectively, and by 2.0 and 2.1 times in wheat. During the recovery period, the endogenous IAA content in the shoots of both wheat and spelt was approached control values, while in the roots it remained 1.9 and 1.6 times lower (Fig. 5).



* – significant difference at P ≤ 0.05 vs. control; data are the mean ± SE, n=9

Fig. 5. Accumulation and distribution of endogenous indole-3-acetic acid in *Triticum aestivum* L. cv. 'Podolyanka' and *Triticum spelta* L. cv. 'Frankenkorn' plants under chilling (+4°C, 2 h) and during recovery period (ng g^{-1} FW)

The total IAA content in 14-day-old 'Podolyanka' wheat plants experienced a 62% decrease after cold stress, amounting 64.4±3.2 ng g^{-1} FW. By the 21st day recovery, the IAA levels increased by 99.7%, and reaching 128.6±6.4 ng g^{-1} FW, which was 30.4% lower, then in control non-stressed plants. Similarly, in 14-day-old spelt plants cold stress resulted in a 72.2% reduction in the total IAA content, measuring 24.0±1.2 ng g^{-1} FW. Recovery brought a remarkable 220% increase in hormone content, totaling 76.8±3.8 ng g^{-1} FW, albeit remaining 26.6% less than the IAA content in 21-day-old control unstressed plants. Throughout all experimental variations, 'Podolyanka' wheat plants consistently demonstrated higher IAA content.

DISCUSSION

The combination of winter warming and late spring frosts significantly diminishes the growth and survival of wheat seedlings, causing inhibition of photosynthetic activity, reduced ear count, and grain loss. Early spring frost exposure, however, fosters better survival rates, elevated photosynthetic activity, and increased resistance to subsequent frosts, thus limiting yield loss (Li et al., 2014). The transition from grain-reserve nutrition to external nutrient assimilation through the root system marks a crucial juncture in wheat ontogenesis, particularly evident at the three-leaf stage. One detrimental outcome of global climate change is the rising temperature during of autumn, often accompanied by brief nocturnal frosts. This phenomenon negatively impacts agricultural crops growth and productivity, resulting in significant yield losses. Low-temperature stress induces plant damage, evident at biochemical and ultrastructural levels (Babenko et al., 2019: Ritonga, Chen, 2020). Winter cereals exhibit high cold tolerance and typically necessitate prolonged vernalization to for spring reproduction (Deng et al., 2015). However, sudden chilling during early seedling development poses significant risk, leading to membranes integrity loss, ion leakage, photosynthesis and respiration inhibition, reduced enzymatic activity and carbohydrate metabolism, impaired water and nutrients absorption, assimilate transport disruption, and oxidative stress (Hassan et al., 2021). While the optimum temperature range for growth falls between 16-22^{o} C, germination and tillering can occur at temperature below 5^{o} C (White et al., 1990). Nevertheless, even short-term exposure to low temperatures in unhardened seedlings can be deleterious, resulting in death or hindering subsequent plant development (Kolupaev et al., 2019). The previous work on *Triticum aestivum* L. cv. Yatran 60, a short-stemmed, moderately intensive cultivar resistant to lodging, heat and drought, analyzed the effect of short-term chilling on endogenous phytohormone content and distribution. Results indicated active accumulation of free ABA and IAA in the roots of 7-day-old seedlings, following short-term chilling (+2°C, 2 h) (Kosakivska et al., 2014). Similarly, the impact of short-term chilling (+2°C, 2 h) on hormonal homeostasis was investigated in plants with different ecological strategies, such as *Festuca pratensis*, *Rumex patientia* × *R. tianshanicus*, *Brassica campestris*, and *Amaranthus caudatus* (Kosakivska et al., 2012; 2013). Subsequent studies confirm that two-hour chilling period serves as a stressor for plants. Upon short-term exposure to chilling (+4°C), linear parameters of the shoots and roots in 14-day-old winter wheat ‘Podolyanka’ and spelt wheat ‘Frankenkorn’ plants remained unchanged, whereas biomass was affected. Spelt plants exhibited more pronounced changes, with root DW decreasing under chilling and subsequently increasing after recovery on the 21st day. Conversely, 21-day-old wheat plants experienced significant decreases in FW and DW, implying an extended impact of chilling on growth parameters (Fig. 1). A similar reduction in root length, biomass, branching, and surface area was reported in various crop plants by Hussain et al. (2018). Maize plants displayed slower growth and alterations in primary root morphology under low temperature stress (Hussain et al., 2020), with diminished root biomass accumulation (Frey et al., 2020), and reduced branching angles between primary and lateral roots (Nagel et al., 2009). Cold-sensitive rice genotypes exhibited lower DW, shorter and finer root hairs, negatively impacting root area (Rativa et al., 2020). Wu et al. (2023) demonstrated that cold priming (10°C/6°C, 1 day) enhanced cold resistance of wheat seedlings, with the most robust resistant phenotype observed nine days after priming. Stress memory persisted for 6-12 days in 3-4 leaves following recovery, gradually waning over time.

Changes in growth dynamics paralleled alterations in the balance of endogenous phytohormones. Extensive physiological and genetic studies have illuminated the diverse roles of different hormone classes, revealing intricate signaling pathway crosstalk and interaction (Vanstraelen, Benková, 2012). Among these hormones, abscisic acid serves as principal regulator of plant resistance to abiotic stresses, orchestrating an array on responses related to adaptation and adjustment. (Sreenivasulu et al., 2012). A pivotal role for ABA in inducing cold tolerance has emerged. Guo et al. (2023) highlighted that barley plants exhibited disrupted chloroplast ultrastructure, altered starch and sucrose metabolism, reduced antioxidant enzymes activity, and changes in the hormonal regulatory network upon ABA deficiency during early stages (3-leaf phase) exposure to low temperature (0°C, 24 hours). In cold-resistant barley varieties, exposure of chilling (+5°C, one day) significantly increased endogenous ABA content (Ahres et al., 2022).

In response to chilling, the endogenous ABA content exhibited a significant increase in 14-day-old wheat plants, whereas the hormone accumulation in spelt plants was comparatively pronounced. ABA dominated in the shoots of both studied species (Fig. 1). The intensive accumulation of endogenous ABA in the shoots of winter and spring wheat under the influence of low (+4°C) temperature was previously reported by Kosova et al. (2012). This study highlighted that the response of winter wheat was more rapid and prominent than that of spring wheat. Alongside the rise in ABA levels, the content of the protective protein dehydrin WCS120 also increased. However, with prolonged exposure to stress (3-7 days), the ABA concentration diminished.

By the 21st day after recovery, the hormone content in both shoots and roots of both wheat and spelt had decreased. Yet, it surpassed the levels observed in control plants, implying a lasting impact of cold priming on ABA accumulation (Fig. 2). Throughout the experimental observations, the endogenous ABA content was consistently twice as high in wheat plants compared to spelt plants, irrespective of the control or experimental conditions. This trend remained consistent even though both genotypes exhibit winter hardiness and frost resistance.

As a crucial stress-related signaling compound, salicylic acid exerts direct or indirect influence on diverse plant growth and development processes. SA plays an important role in the induction of cold tolerance in wheat by regulating ROS formation of (Wang et al., 2018). In ABA-deficient mutant barley, chilling resulted in reduced SA content, albeit still higher than the wild type (cv Steptoe) (Guo et al., 2023). The endogenous SA content in winter wheat leaves increased significantly after extended cold exposure (+4° C, 3 days) (Kosova et al., 2012). However, in seedlings of both tolerant and sensitive barley varieties (*Hordeum vulgare* L), the endogenous SA content decreased following prolonged cold stress (+5/7° C, 3 days) (Mutlu et al., 2015). Exogenous SA application was found to alleviate the adverse effects of chilling (4°C) by triggering the antioxidant system and elevating proline levels in winter wheat plants (Ignatenko et al., 2019). In 14-day-old wheat and spelt plants, the endogenous SA content exhibited an increase approximately 17-19% in response to short-term cold stress. Notably, constitutive and stress-induced SA levels in wheat were consistently higher compared to spelt. The most prominent shifts due to chilling were observed in the roots of the plants (Fig. 3). During the recovery period after cold stress, the SA content in 21-day-old wheat plants returned to levels comparable to control non-stressed plants. Conversely, no notable changes in hormone content were observed in spelt, however, this indicator significantly surpassed that of the control non-stressed plants (Fig. 3).

Gibberellins play a pivotal role in orchestrating plant responses to signals from other phytohormones activated by abiotic stressors (Achard et al., 2006). Notably, the expression of genes encoding enzymes catalyzing main stages of GAs synthesis, such as soluble GA 20-oxidases, typically hinges on environmental cues. Consequently, the endogenous GA content becomes exquisitely responsive to alteration in the external milieu (Colebrook et al., 2014). Our study discerned that chilling engendered a reduction endogenous GA_3 and GA_4 levels in 14-day-old plants of both studied species, with more pronounced changes emerging in the roots. Remarkably, spelt plants exhibited elevated levels of GA_3 and GA_4 comparison to wheat plants. During the recovery period on the 21st day, the hormone content displayed a more distinct elevation in spelt plants, eventually reaching levels akin to unstressed control plants. GA_3 remained dominant across all experimental variations (Fig. 4). Kosova et al. (2012) unveiled that low temperature caused a decline in biologically active GAs in the shoots and roots of winter and spring wheat, with a concomitant rise in inactive hydroxylated forms. Cold stress also triggered a reduction in endogenous GA_4 and GA_7 levels in rice anthers, while their precursor GA_{12} remained unaffected (Sakata et al., 2014). When exposed to chilling, wheat leaf growth was impeded, concurrently prompting the accumulation of active GAs, which in turn stimulated cell elongation. This circumstance corresponded to heightened sensitivity thresholds to GA action (Tonkinson *et al*., 1997). In the quest to bolster stress resilience in agricultural crops, regulation of endogenous GA levels and the employment of synthesis inhibitors stand as pivotal strategies.

Short-term cold stress induced a decrease in IAA content of 14-day-old wheat and spelt plants. The decrease in IAA level was more pronounced in wheat roots (by 73.9%), while in spelt, the converse, was observed in shoots (by 73.5%). The hormone's level in wheat organs was almost threefold higher than that in spelt (Fig. 5). A parallel pattern of changes was noted in leaves and roots of both spring and winter wheat (*Triticum monococcum*) during cold stress (Vanková et al., 2014). A similar decrement in auxin content in the leaves of winter and spring wheat upon initial exposure to chilling (+4°C) was reported by Kosová et al. (2012). In barley mutants deficient in ABA, IAA content increased under chilling, whereas in the wild type (cv Steptoe), it underwent a notable decrease (Guo et al., 2023). After recovery, endogenous IAA accumulated in the shoots and roots of 21-

day-old across both species under study, with spelt plants revealing more pronounced variations (Fig. 5). During the adaptation phase (21 days) of single-grain wheat Vanková et al. (2014), documented the accrual of endogenous auxins alongside an elevation in phenolic compounds, integral for stabilizing hormone levels. Garbero et al. (2012) demonstrated that short-term cold stress (5°C) led to growth retardation and reduced IAA content in the sensitive *Digitaria eriantha* variety during the recovery period, whereas the resistant variety exhibited heightened hormone levels.

CONCLUSION

Our research has contributed to comprehending the hormonal systems response in winter wheat and spelt wheat during the initial stage of vegetative growth under chilling stress. These species were chosen due to their genetic relationship, with spelt considered a possible predecessor of wheat. The selected genotypes did not significantly differ in cold resistance. Short-term chilling didn't impact the linear indicators of wheat and spelt shoots and roots but stimulated shoot and root FW and DW growth, while reducing root FW. Spelt plants exhibited better post-stress recovery. Differences manifested in constitutional hormones content and stress-induced changes. Spelt wheat demonstrated higher constitutional gibberellin content, while winter wheat exhibited elevated SA and IAA content. In terms of stress-induced changes, wheat showed more pronounced alterations in ABA and IAA content. Our study revealed that following recovery, the hormonal balance in shoots and roots of stressed plants differed from that in control non-stressed plants, suggesting long-term effects of chilling.

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NEW MEMBER OF THE FAMILY HADZIIDAE S. KARAMAN, 1932 FROM ALBANIA, FINGERHADZIA ZORAE, GEN. NOV., SP. NOV. (CONTRIBUTION TO THE KNOWLEDGE OF THE AMPHIPODA 334)

SUMMARY

The new genus and species of the family Hadziidae, *Fingerhadzia zorae*, gen. and spec. nov., is described and figured from the subterranean waters of the river Shushica, tributary of Vjosa River, S. Albania (Adriatic Sea drainage system). The problem of recognition of genera within "Hadziid" Group of genera is discussed, and genus *Fingerhadzia* differs from other "Hadziid" genera by partially reduced palpus of left maxilla 1, dilated conus excretorius near antenna 2 and very short inner ramus of uropod 3.

Keywords: Amphipoda, Hadziidae, *Fingerhadzia, zorae*, taxonomy, new genus, new species, subterranean, Albania.

INTRODUCTION

The subterranean Amphipoda fauna of Albania is still only partially known. Stanko Karaman described (1929) first subterranean amphipod from Ohrid Lake (divided between N. Macedonia and Albania), Niphargus ohridanus, sp. nov. (Fam. Niphargidae) from N. Macedonian part of Ohrid Lake (up to 100 m depth), later cited several times from the same lake [Karaman, S., 1937; 1943; 1960; Schellenberg, 1943; Karaman, G., 1963; 1972; 1974]. G. Karaman (2011) described second new subterranean Niphargus species from Albania, N. tomori from Tomor Mts.

Thanks to Prof. Christian Griebler from the University of Wien, Austria, I have a possibility to study the subterranean amphipods collected in Albania in 2021 and 2023 during the realization of the APPEAR project VjoSusDev, of the Austrian Development Cooperation implemented by the OeAD, and a part of these studies is presented in this work.

METHODS AND MATERIAL

The material of this study was collected in the subterranean waters in southern Albania during the realization of the APPEAR project VjoSusDev

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Notes: The author declares that he has no conflicts of interest. Authorship Form signed online.
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(2021, 2023), and sent me at disposition for study. APPEAR is a program of the Austrian Development Cooperation implemented by the OeAD.

The studied material was preserved in 70-95% ethanol. Specimens were dissected using a WILD M20 microscope and drawn using a camera lucida attachment. All appendages were temporarily submersed in a mixture of glycerin and water for study. All illustrations were inked manually. After the end of the study, the dissected body-parts were fixed in Liquid of Faure and covered by cover glass as permanent slides. Some setal formulae follow G. Karaman`s terminology (Karaman, G., 1969b) for the third mandibular palpus article [A= A-setae on outer face; B= B-setae on inner face; C= additional C-setae on outer face; D= lateral marginal D-setae; E= distal long E-setae].

The terms “setae” and “spines” are used based on their shape, not origin. The investigations are provided based on morphological, ecological and zoogeographical studies.

In References are mentioned the presence and number of figures in various papers, what is very important and helpful in taxonomical determination of this species.

TAXONOMICAL PART

Order AMPHIPODA

Suborder SENTICAUDATA Lowry & Myers, 2013
Infraorder HADZIIDA S. Karaman, 1932
Family HADZIIDAE S. Karaman, 1943

GENUS *FINGERHADZIA* gen. nov.

DIAGNOSIS: Body *Metahadzia*-like, epimeral plates pointed. Urosomal segment 1 with ventroposterior strong spine near basis of uropod 1-peduncle. Antenna 1: peduncular articles 1-3 progressively shorter, main flagellum with numerous articles, accessory flagellum 2-articulated; conus excretorius ovoid, broad. Labrum broader than long. Labium without inner lobes. Mandible incisors and lacinia mobilis left and right asymmetric, toothed, palpus 3-articulated, article 3 much longer than articles 1 and 2, bearing lateral D- and distal E-setae only. Left and right maxilla 1 with inner and outer plate symmetric; inner plate pointed distally, along mesial margin with row of setae, outer plate with distal toothed spines. Left maxillar palpus weak, 2-articulated, partially reduced, with 1-3 distal setae; right palpus 2-articulated, strong, with distomesial strong spine-like teeth. Maxilla 2 both plates bearing distal group of setae, inner plate with oblique row of facial setae. Maxilliped: both plates and palpus well developed.

Coxae 1-4 relatively short, coxa 4 unlobed, coxa 5 nearly as long as coxa 4. Gnathopods 1-2 nearly *Metahadzia*-like, article 5 without lateral dilatation, dactylus of gnathopods with one mdian seta at outer margin. Pereopod 7 article 2 without distinct lobe, dactylus with short nail nail and mesial seta near basis of the nail. Pleopods 1-3 well developed, with 3 retinacula, pleopod 3 modified in males. Uropod 1 peduncle with ventrolateral median strong spine. Uropod 3 inner ramus very short, outer ramus 2-articulated, spinose, second article very short. Telson incised nearly to the basis, with distal spines only. Coxal gills with long peduncle (stalk), appear on gnathopod 2 to pereopod 6; oostegites narrow, setose, appear on gnathopod 2 to pereopod 5.

Differing from *Hadzia, Metahadzia, Liagoceradocus and Metaniphargus* distinctly by different left maxilla 1, conus excretorius and uropod 3.

Typus generis: *Fingerhadzia zorae*, sp. nov.

Taxa: monotypic.

FINGERHADZIA ZORAE, SP. NOV.

Figures 1-9

MATERIAL EXAMINED: ALBANIA:

AL 5 (Vjo 003-Shus 17): left bank of the Shushica River between the new and old bridge of Gjorm (Ura e Gjormit), 2.5 km north of the city of Gjorm, Latitude 40.3384000249207, Longitude 19.6387849655002, one female and 5-6 body fragments; mixed with one juv. exp. of *Niphargus* sp., June 2021, (leg.: C. Karwautz, G. Rasch & C. Griebler).

AL 7 (Vjo 020- Shus 19): right bank of the Shushica River at Himare, 3 km southeast of the village Kallarat. Latitude 40.19856075724803, Longitude 19.773140649349333, (leg.: C. Karwautz, G. Rasch & C. Griebler), two gnathopods only.

AL 23 (Vjosa Shus 23): Shushica River, 26.4.2023 (leg. C. Griebler et al.) 1 exp. mixed with *Niphargus* sp.

AL 28 (Vjosa Shus 24), Shushica River, 26.4.2023 (leg. C. Griebler et al.), 1 exp.

AL 27 (Vjosa Shus 26), Shushica River, 26.4.2023 (leg. C. Griebler et al.), 1 exp. male 4.0 mm, mixed with *Salentinella angelieri* and *Niphargus* sp.

AL 26 (Vjosa Shus 27), Shushica River, 26.4.2023 (leg. C. Griebler et al.); 5 exp. very damaged;

AL 24 (Vjosa Shus 28), Shushica River, 26.4.2023 (leg. C. Griebler et al.) 1 exp. mixed with *Niphargus* sp.

DIAGNOSIS: With the characters of the genus; males differs from females by modified pleopod 3 and shape of gnathopods 1-2.

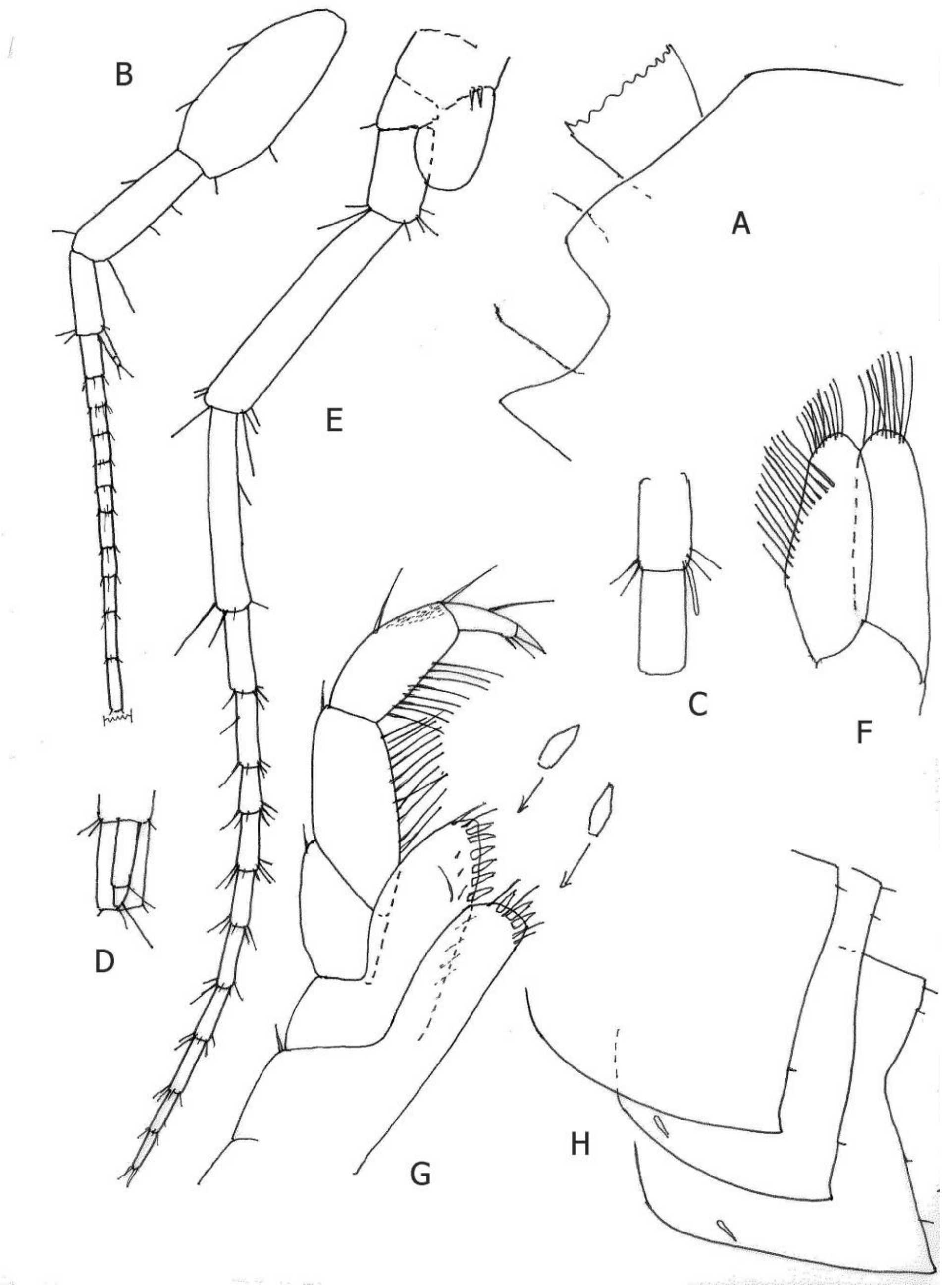

Fig. 1. *Fingerhadzia zorae*, gen. nov. sp. nov., AL 5 (Vjo 003 = Shus 17): left bank of the Shushica River; Gjorm, Albania, female 4.9 mm: A= head; B= antenna 1; C= aesthetasc; D= accessory flagellum; E= antenna 2; F= maxilla 2; G= maxilliped; H= epimeral plates 1-3.

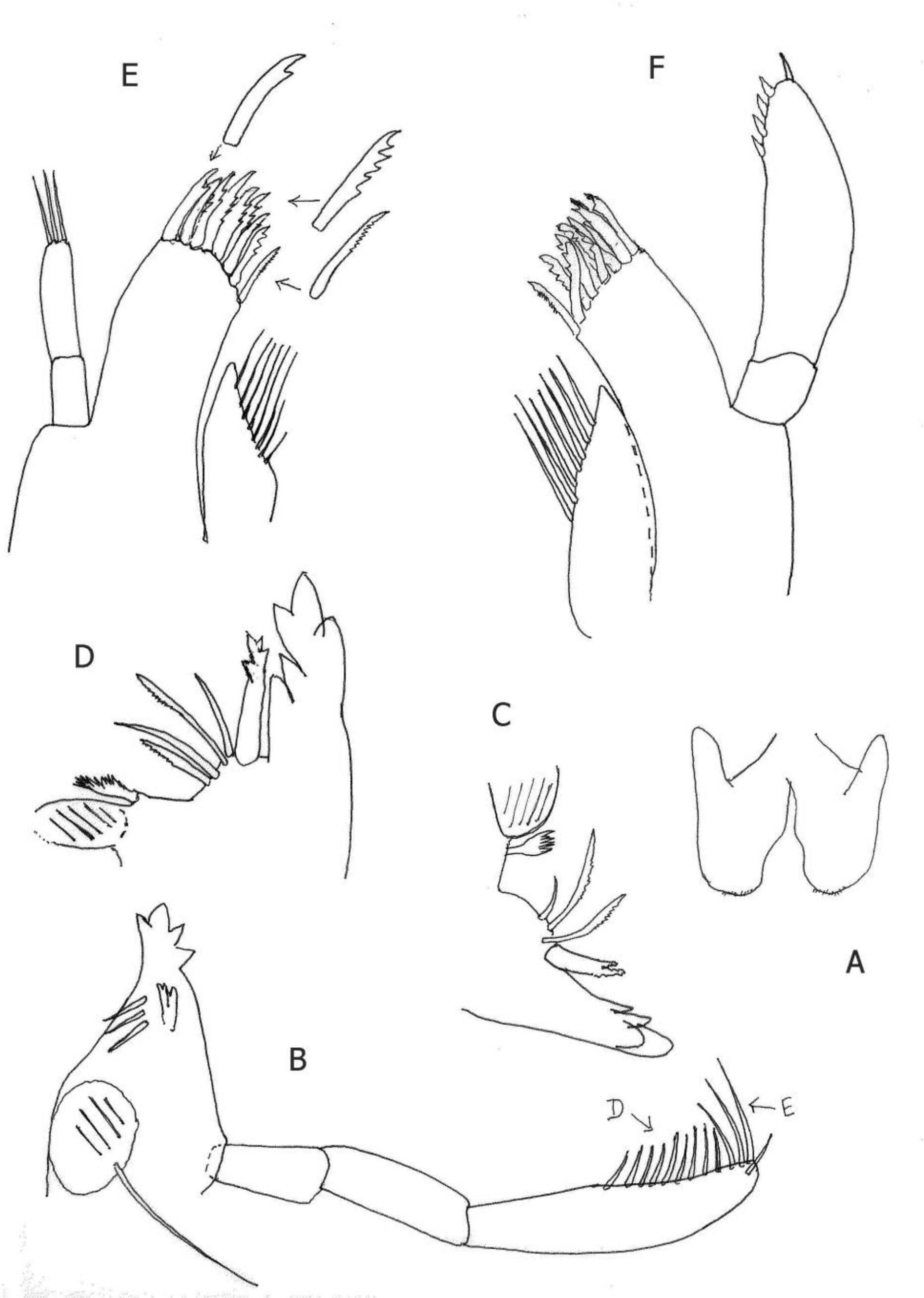

Fig. 2. *Fingerhadzia zorae*, gen. nov. sp. nov., AL 5 (Vjo 003 = Shus 17): left bank of the Shushica River, Gjorm, Albania, female 4.9 mm: A= labium; B= right mandible with molar; C= tip of right mandible with incisor, lacinia mobilis and rakers; D= tip of left mandible with lacinia mobilis and rakers; E= left maxilla 1; F= maxilla 2.

Fig. 3. *Fingerhadzia zorae*, gen. nov. sp. nov., AL 5 (Vjo 003 = Shus 17): left bank of the Shushica River; Gjorm, Albania, female 4.9 mm:: A= gnathopod 1; B= distal part of gnathopod 1-propodus; C= gnathopod 2; D= distal part of gnathopod 2-propodus.

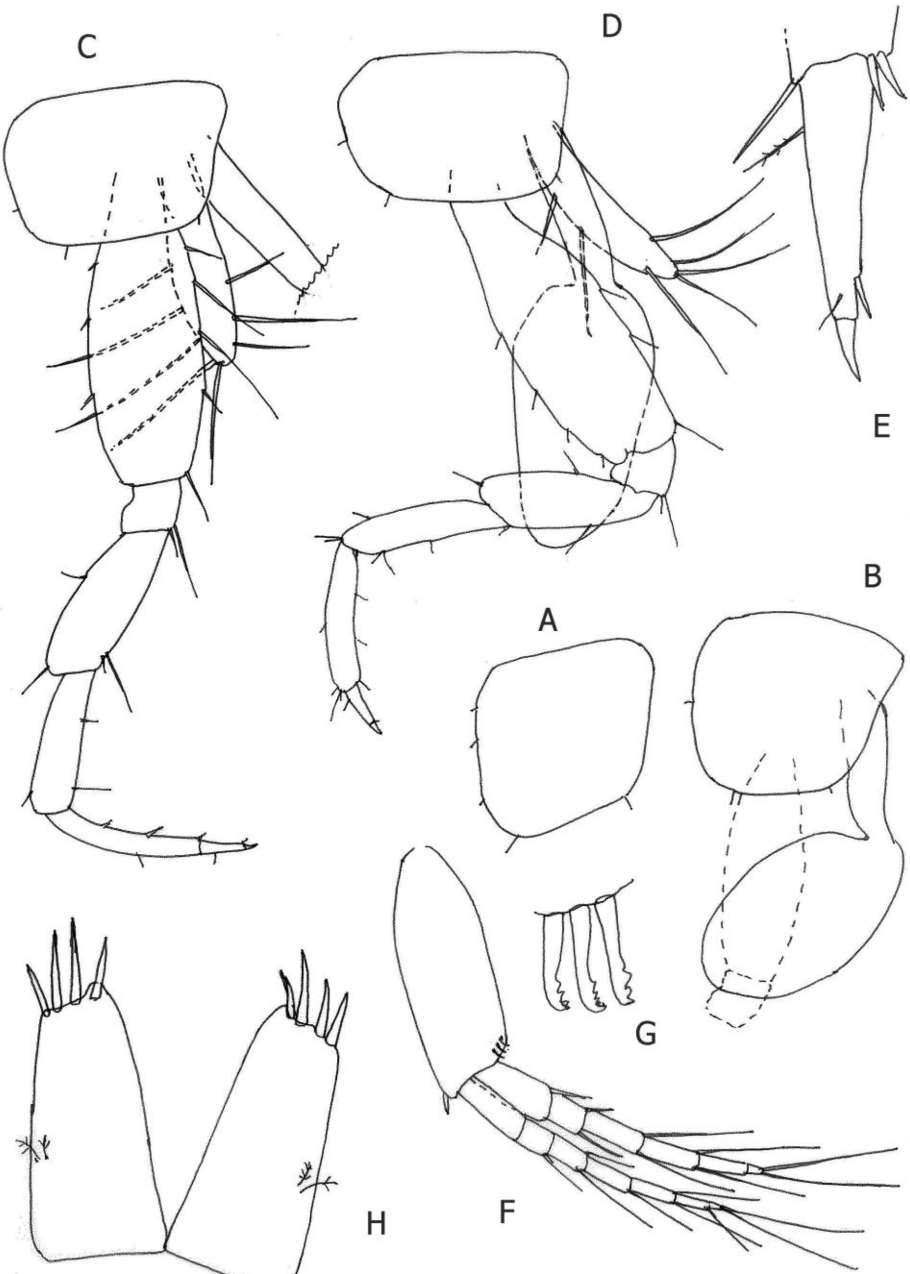

Fig. 4. *Fingerhadzia zorae,* gen. nov. sp. nov., AL 5 (Vjo 003 = Shus 17): left bank of the Shushica River; Gjorm, Albania, female 4.9 mm: A= coxa 1; B= coxa 2 with coxal gill; C= pereopod 3; D= pereopod 4; E= pereopod 4-dactylus; F= pleopod 3; G= retinacula.; H= telson.

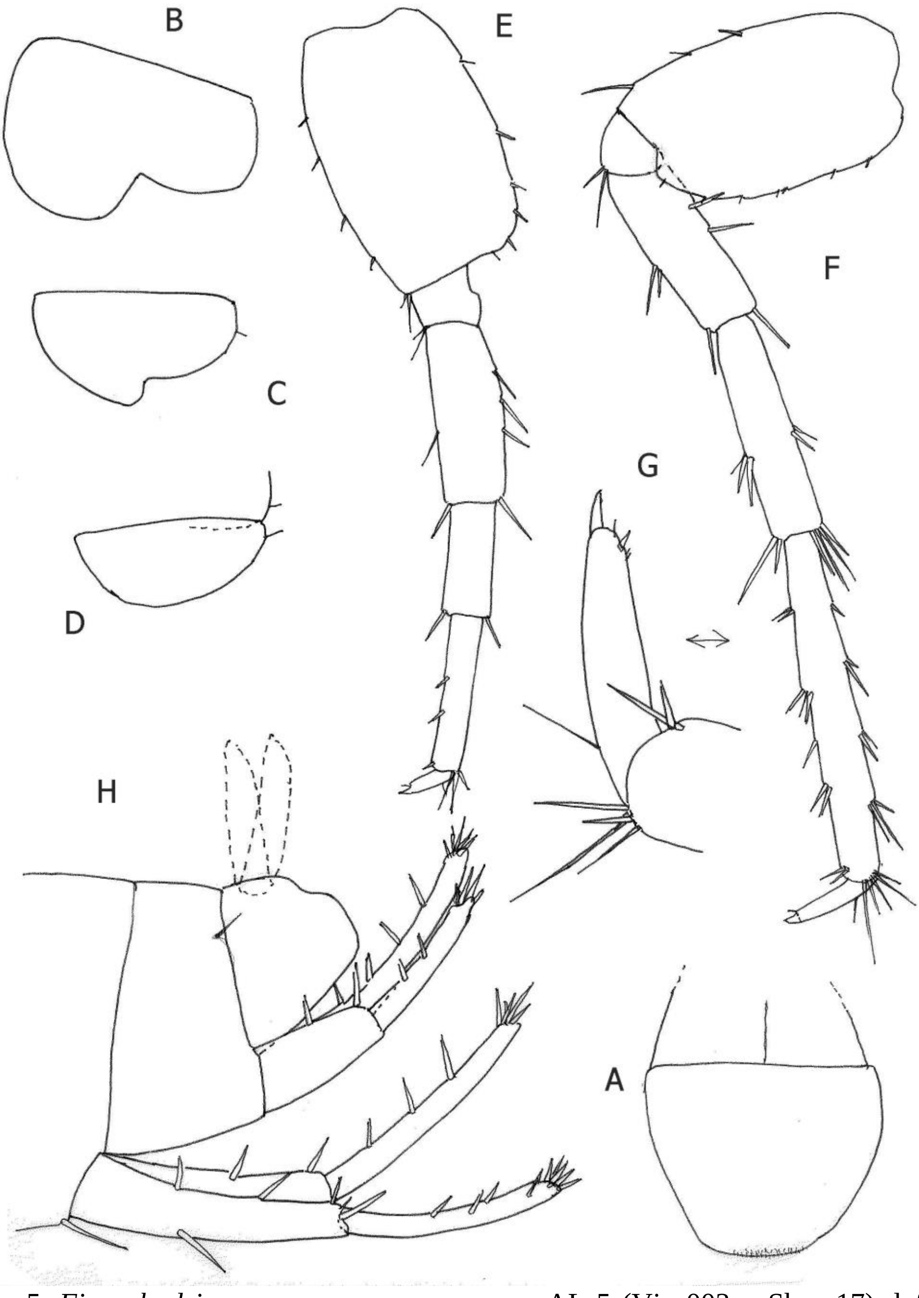

Fig. 5. *Fingerhadzia zorae*, gen. nov. sp. nov., AL 5 (Vjo 003 = Shus 17): left bank of the Shushica River; Gjorm, Albania, female 4.9 mm: A= labrum; B= coxa 5; C= coxa 6; D= coxa 7; E= pereopod 6; F= pereopod 7; G= pereopod 7 dactylus. H= urosome with uropods 1-2.

DESCRIPTION: **Female with setose oostegites 4.9 mm (Vjo 003-Shus 17, holotype)**: Body moderately slender, mesosomal segments naked, metasomal segments with 3-4 posterior dorsomarginal setae (fig. 1H); urosomal segment 1 naked dorsally, but with one strong ventroposterior spine near basis of uropod 1-peduncle (fig. 5H); urosomal segment 2 on each dorsolateral side with one stronger seta, urosomal segment 3 naked. Epimeral plates 1-2 with ventroposterior pointed tip, epimeral plate 3 distinctly acute (fig. 1H). Epimeral plates 2 and 3 with one submarginal ventral spine.

Head with short, nearly angular lateral cephalic lobes, eyes absent (fig. 1A).

Antenna 1 remarkably exceeding half of body, peduncle consisting of 3 articles gradually shorter (ratio: 56:38:23) covered with several mainly short setae each; first article twice wider than second one (fig. 1B); main flagellum consisting of 11+ articles (missing distal part) scarcely setose, some articles with one aesthetasc (fig. 1C). Accessory flagellum 2-articulated, reaching nearly half of last peduncular article-length (fig. 1D), second article short but distinct.

Antenna 2 shorter than antenna 1, peduncular article 3 slightly longer than broad, with 4-6 distal setae; peduncular articles 4-5 narrow; article 4 longer than 5 (ratio: 69:55) with several distal setae; article 5 more narrow than article 4, with one lateral and 3-4 distal setae. Flagellum moderately slender, rather longer than peduncular articles 4-5 combined, composed of 11 articles bearing several short setae each (fig. 1E). Conus excretorius broad, rounded (fig. 1E).

Mouthparts well developed. Labrum rather broader than long, distally rather convex (fig. 5A), with carinate epistome. Labium rather broader than long, outer lobes broad, subrounded distally, inner lobes absent (fig. 2A).

Mandibles with triturative molar; on right mandible with long distolateral seta (like these in *Gammarus*), and incisor with nearly 4 teeth and 3 pectinate rakers; lacinia mobilis toothed; near basis of molar appear one small serrate lamella (fig. 2B).

Left mandible: molar without long distal plumose seta, on basis with small serrate lamella, incisor with 5 (?) teeth and 4 pectinate rakers; lacinia mobilis serrate (fig. 2D).

Mandibular palpus similar in left and right mandible, 3-articulated, articles of different length (ratio: 32:40:80); first and second article naked; third article rather subfalciform, with 9 D-setae and 4 distal E-setae, A and B setae absent (fig. 2B).

Right maxilla 1: inner plate triangular, with row of 7 strong lateral setae (fig. 2F); outer plate with 9 distal spines in two rows, bearing 1-5 lateral teeth each (the number of lateral teeth increasing from outer margin towards inner (mesial) margin of maxilla). Palpus 2-articulated, strong and rather dilated, exceeding distal tip of outer plate-spines; first article short, naked, second article bearing 4 distomesial teeth and one distal narrowed spine-like tooth (fig. 2F).

Left maxilla 1: inner and outer plate like these in right maxilla, but palpus weak, 2-articulated: not dilated, almost reaching basis of outer plate-spines; first article rather exceeding half of distal article (ratio: 20:32), naked, second (distal) article with 3 distal setae (fig. 2E).

Maxilla 2 longer than broad; inner and outer plate of the similar length, with numerous distal setae; inner plate provided with oblique row of facial setae (fig. 1F).

Maxilliped relatively long; inner plate nearly reaching outer tip of first palpus article, bearing 3 distal pointed spines mixed with 3-4 setae; outer plate relatively short, tapering distally, bearing 6 distomesial marginal spines and 3-4 distal setae, along mesial side with several very small submarginal spine-like setae. Palpus 4-articulated, article 2 with row of longer setae at mesial margin; article 3 with row of setae at inner (mesial) margin and 2 setae at outer margin; a field of very small dense setulae present in distal part of article itself. Article 4 with short nail and 2 ventral setae near basis of the nail, along outer margin with one long median seta (fig. 1G).

Coxae relatively short. Coxa 1 nearly as long as broad, quadrate, with subrounded ventroanterior corner and 4-5 marginal short setae (fig. 4A).

Coxa 2 rather broader than long (ratio: 55:49), with 4-5 marginal short setae (fig. 4B). Coxa 3 broader than long (ratio: 59:41), with 3 marginal short setae (fig. 4C). Coxa 4 broader than long (ratio: 60:40), with 3 short marginal setae, ventroposterior lobe not developed (fig. 4D).

Coxa 5 bilobed, broader than long (ratio: 68:46), anterior lobe nearly as long as coxa 4 (fig. 5B). Coxa 6 bilobed, smaller than coxa 5, broader than long (ratio: 55:30), with one posterior short seta (fig. 5C). Coxa 7 entire, much broader than long (ratio: 51:23), with one posterior marginal seta (fig. 5D).

Gnathopods 1-2 dissimilar to each other. Gnathopod 1: article 2 inflated medially, with 3 short setae at anterior side, 5-6 long setae at posterior margin; article 3 short, with distoposterior bunch of 3 short setae (fig. 3A); article 4 with distoposterior bunch of setae and 2 spine-like setae at inner margin. Article 5 triangular, narrow, not dilated, at posterior margin with 2 transverse rows of setae, at anterior margin with one short median seta and distal row of longer setae (fig. 3A). Propodus almost ovoid, longer than broad (ratio: 73:38), as broad as article 5, at posterior margin with 3 setae; palm very inclined, convex, reaching almost to the half of propodus-length, bearing 5-6 submarginal spines mixed with several setae (fig. 3B). Dactylus rather exceeding half of propodus-length, at inner margin with distoventral small tooth and short seta near basis of the nail, at outer margin with one median seta.

Gnathopod 2 rather longer than gnathopod 1; article 2 inflated medially, with 3 short anterior marginal setae, at posterior margin with nearly 10 long setae; article 3 short, with distoposterior bunch of setae; article 4 rather smaller than that of gnathopod 1, with distoposterior bunch of setae (fig. 3C); article 5 narrow, elongated, but without distoposterior lobe; along anterior margin with one bunch of setae, along posterior margin with nearly 7 bunches of long setae. Propodus almost as long as article 5, longer than broad (ratio: 85:31), but rather more narrow than article 5, at anterior margin with 4 groups of spines and spine-like setae, along posterior margin with 3 groups of setae (fig. 3D). Palm very inclined, rather shorter than half of propodus-length, with 5 spines and several long strong setae. Dactylus much shorter than half of propodus-length, at inner (mesial) margin with distoventral tooth near basis of the nail, at outer margin with one median seta.

Pereopods 3-4 rather similar to each other. Pereopod 3: article 2 elongated, inflated in the middle, with 3-4 short setae along anterior margin and 5 longer setae along posterior margin; article 3 short, with long distoposterior seta. Articles 4-6 of different length (ratio: 46:39:40); bearing single setae at both margins, article 6 with 3 single spines at posterior margin (fig. 4C). Dactylus much shorter than article 6 (ratio: 15:40), tapering distally, with one spine near basis of the nail, at outer margin with one median plumose seta; nail strong, much shorter than pedestal.

Pereopod 4 similar to pereopod 3; articles 4-6 of different length (ratio: 40:40:39) (fig. 4D); pilosity and dactylus like these in pereopod 3; nail shorter than pedestal (ratio: 17:70) (fig. 4E).

Pereopod 5 missing.

Pereopod 6 (?aberrant): Article 2 dilated, but not distinctly lobed, longer than broad (ratio: 73:45), along anterior margin with 6 spine-like setae, along posterior slightly convex margin with 6 setae (fig. 5E). Articles 4-6 of different length (ratio: 47:32:42), along both margins with single spines or setae; dactylus remarkably shorter than article 6 (ratio: 15:42), at inner margin with short spine-like seta near basis of the nail, at outer margin with one median plumose seta; nail short.

Pereopod 7: article 2 elongated, ovoid, longer than broad (ratio: 75:44) with developed ventroposterior lobe, anterior margin with 3 spine-like setae, posterior convex margin with 6 short setae (fig. 5F); article 3 short, with distoanterior group of setae. Articles 4-6 of different length (ratio: 47:60:94), articles at both margins with single or groups of spines or spine-like setae. Dactylus much shorter than article 6 (ratio: 26:94), with spine and 3 minute setae at inner margin near basis of the nail, at outer margin with one median seta; nail strong and shorter than diameter of dactylus itself (fig. 5G).

Pleopods 1-3: peduncles almost naked, with 3 retinacula each (fig. 4G), rami well developed. Pleopod 3 inner ramus with 6 articles, outer ramus with 7 articles (fig. 4F).

Uropod 1: peduncle with dorsoexternal and dorsointernal row of strong spines, as well as with one median lateral spine at outer margin (fig. 5H). Inner ramus nearly as long as peduncle, with row of 3 lateral and 5 unequal distal spines; outer ramus rather shorter than inner ramus, with 4 lateral and 5 distal spines.

Uropod 2: peduncle short, with dorsal spines; inner ramus distinctly longer than peduncle, bearing 3-4 lateral and 5 distal spines; outer ramus rather shorter than inner ramus, with 2-3 lateral and 4 distal spines (fig. 5H).

Uropod 3 (female 3.1 mm) biramous, inner ramus short, scale-like, with distal spine; outer ramus 2-articulated, second article very short; first article elongated, with spine-like setae along both margins, long plumose setae absent (fig. 6D).

Telson nearly as long as broad, lobes incised to the bottom; each lobe with 4 distal short spines; a pair of short plumose setae attached near the middle of each lobe (fig. 4H).

Coxal gills attached on gnathopod 2 till pereopod 6, consisting of long narrow peduncle almost as long as ovoid dilated part of gill (fig. 4B, C, D). Oostegites attached on gnathopod 2 till pereopod 5, bearing long plumose marginal setae (fig. 4C, D).

MALE 4.0 mm, paratype (Shus 26):

Rather similar to the females. Metasomal segments 1-3 with 2 dorsomarginal setae (fig. 7E), urosomal segments 1-2 like these in females (fig. 8E), including strong ventroposterior curved spine near basis of uropod 1 peduncle. Epimeral plates acute, like these in females but without subventral spines (fig. 7E).

Antenna 1 rather shorter than body-length, main flagellum with 27 articles, some of them with aesthetascs almost as long as article itself. Flagellum of antenna 2 with 10 articles, conus excretorius like that in female.

Mouthparts like these in female. Mandibular palpus article 2 naked, article 3 with 7 D setae and 3-4 distal E-setae, A and B setae absent.

Maxilla 1 inner plate triangular, with 6 marginal setae, outer plate with 8-9 serrate spines. Left palpus similar to that in female, weak, 2-articulated, not reaching basis of outer plate-spines, and bearing only one distal seta (fig. 7F). Right maxilla 1 like that in female.

Maxilliped inner plate with 3 distal spines mixed with setae, outer plate ovoid, reaching half of palpus article 2, along mesial margin with 8 spines; palpus article 4 with one seta at inner margin near basis of the nail.

Coxae short, Coxa 1 broader than long (ratio: 44:38), with subrounded margins bearing 4-5 marginal setae (fig. 7A). Coxa 2 rather broader than long, (ratio: 45:39), subrounded margin with 2-3 setae (fig. 7C). Coxa 3 more broader than long (ratio: 45:35), with 3 short marginal setae (fig. 6E). Coxa 4 remarkably broader than long (ratio: 50:30), with 3 marginal setae, ventroposterior lobe absent (fig. 6F).

Coxa 5 bilobed, nearly as long as coxa 4, broader than long (ratio: 57:35), anterior lobe rather shallow, with 2 marginal setae (fig. 6G). Coxa 6 rather smaller than coxa 5, broader than long (ratio: 34:19), anterior lobe very shallow (fig. 8A). Coxa 7 entire, much broader than long (ratio: 37:15), with one posterior seta (fig. 8B).

Gnathopod 1 rather shorter than gnathopod 2, article 2 along posterior margin with 2 median and one distal seta, anterior margin nearly naked; article 3 with 3 distoposterior setae; article 4 with 2 distoposterior strong setae; article 5 triangular, poorly longer than broad, without posterior dilatation, bearing distoanterior numerous marginal setae and distoposterior bunch of setae (fig. 7A).

Propodus (article 6) rather longer than article 5 (ratio: 40:36), nearly ovoid, longer than broad (ratio: 93:50), along posterior margin with 3 setae; at anterior margin with 3 distal setae; palm convex, exceeding half of propodus length, with nearly 9 unequal spines and 2 setae (fig. 7B). Dactylus slender, remarkably longer than diameter of propodus, at outer margin with one short plumose seta, inner (mesial) margin naked (fig. 7B).

Gnathopod 2: article 2 at anterior margin with one short distal seta, along posterior margin with numerous setae; article 3 with one distoposterior seta; article 4 with 3 distoposterior setae (fig. 7C); article 5 elongated, triangular, without posterior dilatation, along posterior margin with 5 groups of setae, along anterior margin with one distal group of setae. Propodus rather elongated, longer than article 5 (ratio: 51:46), much longer than broad (ratio: 100:44), at anterior margin with one mediodistal group of setae, along posterior margin with 3 group of long setae. Palm very oblique, slightly convex, rather shorter than half of propodus-length, with nearly 5 short palmar spines and 2 setae, at palmar corner with one stronger spine (fig. 7D). Dactylus exceeding half of propodus-length, at outer margin with one median short plumose seta, inner (mesial) margin naked (fig. 7C).

Pereopods 3-4 like these in female (fig. 6E). Pereopod 5 missing.

Pereopod 6 elongated (fig. 8A). Article 2 much longer than broad (ratio: 60:35), with only rather convex margins; along anterior margin with 5-6 stronger setae, along posterior margin with only 5 short setae, ventroposterior lobe not distinctly developed. Articles 4-7 of different length (ratio: 50:53:75:25); articles 4-6 along margins with single or pair of spines of various length. Article 2 is shorter than article 6 (ratio: 60:75). Dactylus slender, at inner margin with one seta, at outer margin with one median short plumose seta, nail short and strong (fig. 8A).

Pereopod 7 rather shorter than pereopod 6 (fig. 8B); article 2 rather similar to that of pereopod 6, longer than broad (ratio: 61:35), along anterior margin with 5 stronger setae, along posterior rather convex margin with 5 short setae, ventroposterior lobe not distinctly developed. Articles 4-7 of different length (ratio: 40:50:78:23). Articles 4-6 along both margins with single or pairs of spines. Article 2 is shorter than article 6 (ratio: 61:78). Dactylus slender, at inner margin with one stronger seta near basis of the nail, at outer margin with one median short plumose seta, nail short and strong (fig. 8C).

Peduncle of pleopods 1-3 with 3 retinacula each. Pleopods 1-2 biramous with rami consisting of several articles like these in female. Pleopod 3 modified: outer ramus well developed, with 7 articles bearing long setae; inner ramus modified into curved protrusion, probably in function of reproduction (fig. 8D).

Uropod 1: peduncle with dorsointernal median and distal spine and with dorsoexternal row of spines; at ventroexternal margin with one strong median curved spine (fig. 8E).

Uropod 2 like that in female. Uropod 3 missing.

Telson nearly as long as broad, incised nearly to the basis, lobes pointed distally, provided with 3-4 short distal spines each (fig. 8F).

Coxal gills with long peduncle like these in female (figs 6E, F, G; 7C).

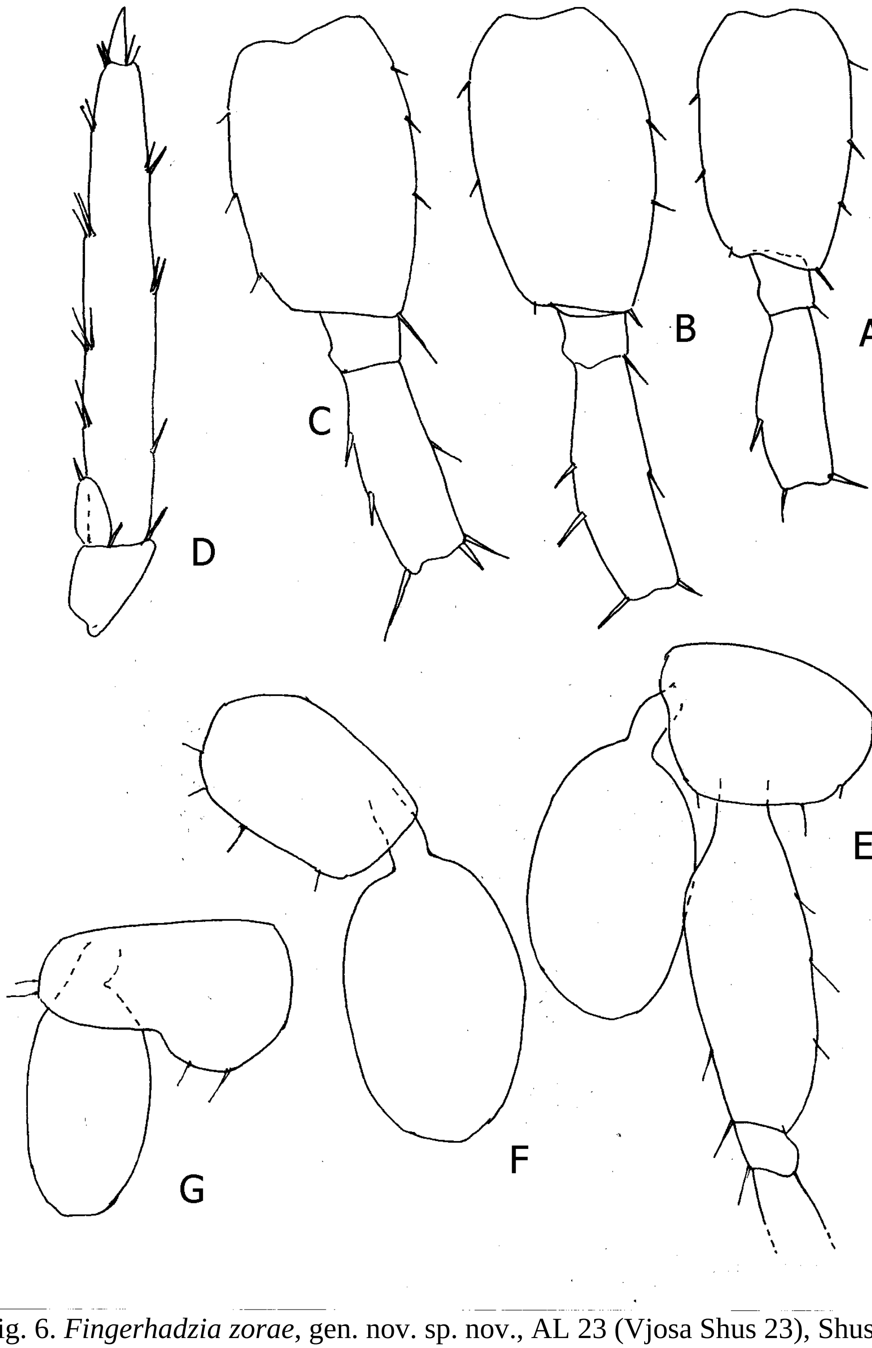

Fig. 6. *Fingerhadzia zorae*, gen. nov. sp. nov., AL 23 (Vjosa Shus 23), Shushica River, female 3.1 mm: A= pereopod 5' B= pereopod 6; C= pereopod 7; D= uropod 3.

Male 4.0 mm: AL 27 (Vjosa Shus 26), Shushica River: E= pereopod 3; F= pereopod 4; G= pereopod 5.

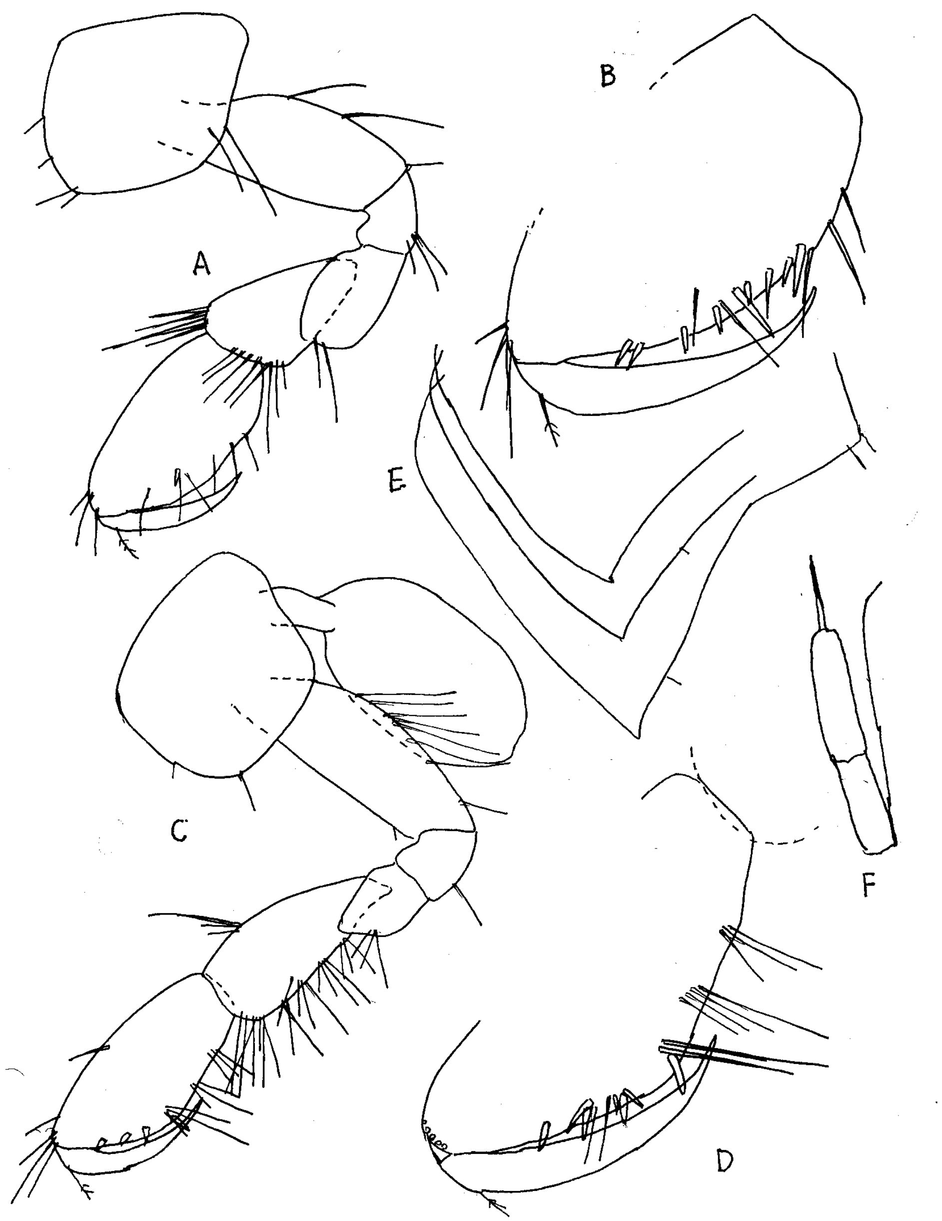

Fig. 7= *Fingerhazdia zorae*, gen. nov., sp. nov., AL 27 (Vjosa Shus 26), Shushica River, male 4.0 mm: A-B= gnathopod 1; C-D= gnathopod 2; E= epimeral plates 1-3; F= palpus of left maxilla 1.

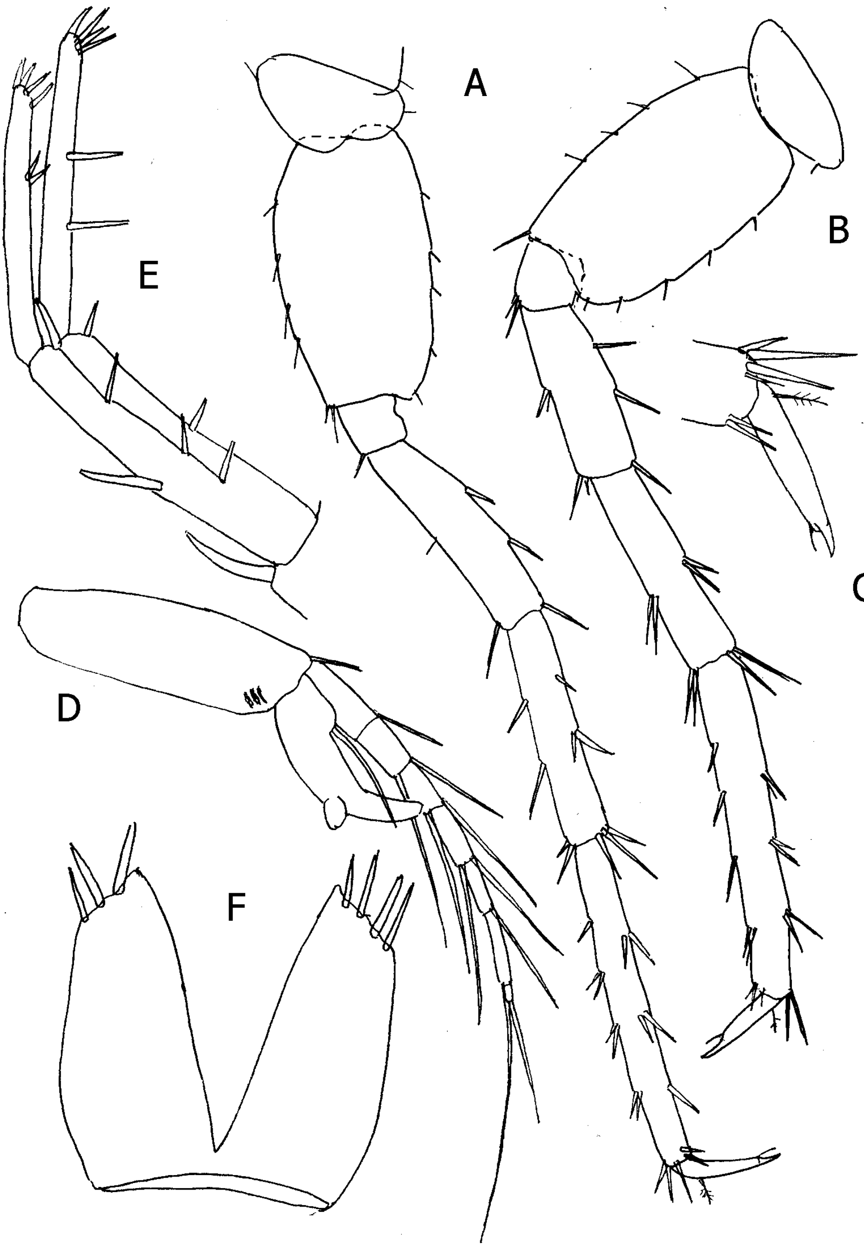

Fig. 8= *Fingerhazdia zorae*, gen. nov., sp. nov., AL 27 (Vjosa Shus 26), Shushica river, male 4.0 mm: A= pereopod 6; B= pereopod 7; C= dactylus of pereopod 7; D= pleopod 3; E= uropod 1; F= telson.

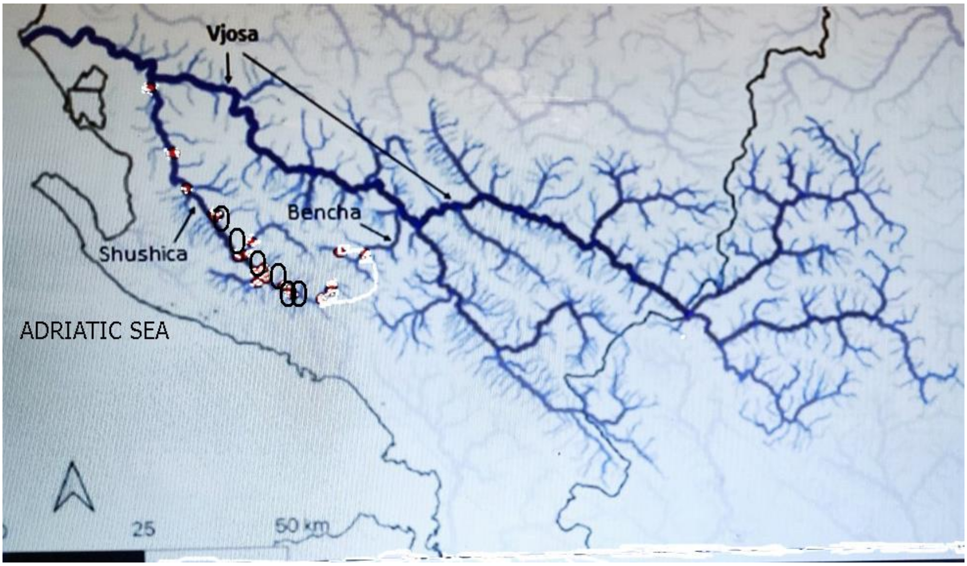

Fig. 9. Distribution of *Fingerhadzia zorae*, gen. nov. sp. nov. in Albania [0= collecting localities].

VABIABILITY. Pereopod 5 is missing in male and females, except in female 3.1 mm from AL 23 (Vjosa Shus 23) where pereopods 5-7 was partially broken (fig. 6A, B, C). Uropod 3 in male missing. Palpus of left maxilla 1 weak, with 1-3 distal setae.

The pereopod 6 of holotype female is maybe aberrant (based on figured pereopod 6 of male).

HOLOTYPE: Female 4.9 mm (Vjo 003-Shus 17) with setose oostegites, and paratype male 4.0 mm (Shus 26) are deposited in Museum of Natural History in Wien, Austria.

LOCUS TYPICUS: Subterranean waters of the river Shushica, tributary of Vjosa River, S. Albania.

DERIVATIO NOMINIS: This taxon is dedicated to my deceased mother **Prof. Dr Zora Karaman,** entomologist**,** from the University of Skopje, Northern Macedonia.

The name *Fingerhadzia* derived from the reminding of maxilla 1 left palpus to the finger.

REMARKS AND AFFINITIES

The new taxon *Fingerhadzia zorae*, gen. nov., spec. nov., rather belongs to the Hadziid group of taxa within the family Hadziidae S. Karaman, 1943 [typus familiae: *Hadzia* S. Karaman, 1932], but differs from all species of this family by reduced palpus of left maxilla 1 and broad conus excretorius; uropod 3 like that in *Melita* species.

It is very interesting that almost all Hadziid species are with 2 retinacula on pleopods [*H. acuta* (Andres, 1978) is with 2-3 retinacula; *Fingerhadzia* 3 retinacula].

Fingerhadzia zorae is rather close to genus *Metahadzia* by narrow article 5 of gnathopod 2 bearing numerous setae at posterior margin, but differs from this genus by reduced palpus of left maxilla 1, dilated conus excretorius and very short inner ramus of uropod 3. From the morphological point of view, established differences are of generic level, conducting us to create a new genus *Fingerhadzia* [see diagnosis above].

Within the family Hadziidae there are recognized nearly 27 genera (World Amphipoda Database, 2024). In Europe there are recognized genera: *Hadzia* S. Karaman, 1932, *Metahadzia* Stock, 1977 and *Liagoceradocus* Barnard, J.L., 1965.

The genus *Liagoceradocus* Barnard, J.L.1965 [typus generis: *Liagoceradocus pusillus* Barnard, J.L., 1965], was submersed into genus *Hadzia* as synonym (Vigna-Taglianti, 1988, etc.), or recognized as a distinct genus (Ronde-Broekhuizen & Stock, 1987, etc.).

Genus *Metahadzia* [typus generis: *Hadzia tavaresi* Mateus, A. & Mateus, E., 1972] seems to be limited on north-central and western region of Mediterranean Sea (Italy, Greece, Spain, Portugal). consisting in Europe of 5 species: *M. helladis* Pesce, 1980 [loc. typ.: Assos, Cephalonia Island, Greece]; *M. minuta* (Ruffo, 1947) [loc. typ.: cave L`Abisso, Castromarina, Lecce, S. Italy]; *M. adriatica* Pesce, 1979 [loc. typ.: Mola di Bari, Italy]; *M. tavaresi* (Mateus, A. & Mateus, E., 1972) [loc. typ.: wells in Tavira, Algavre, SE Portugal] and *M. uncispina* Notenboom, 1988 [loc. typ.: Sevilla, Los Pajares, Cantillana, S. Spain].

Genus *Hadzia* [typus generis: *Hadzia gjorgjevici* S. Karaman, 1932] is consisting of nearly 20 known taxa, presented in Europe by 6 taxa: *H. gjorgjevici* S. Karaman, 1932 [loc. typ.: Skopje (=Skoplje), Northern Macedonia]; *H. fragilis fragilis* S. Karaman, 1932 [loc. typ.: Vjetrenica Cave, Bosnia & Herzegovina]; *H. fragilis stochi* G. Karaman, 1989 [loc. typ.: cave near La Peschiera del Timavo, NE Italy]; *H. crispata* G. Karaman, 1969a [loc. typ.: Podgorica (= Titograd), Montenegro]; *H. drinensis* G. Karaman, 1984 [loc. typ.: Drina River near Brod na Drini, Bosnia & Herzegovina], and (?) *H. acuta* (Andres, 1978) [loc. typ.: Canary Islands (Lanzarote, Jameos del Agua, Spain]; [some of them with unknown male].

All these taxa settled area near Adriatic and Ionian Seas, except one in Atlantic (Lanzarote island, Spain]. Outside this region is known *Hadzia pachypoda* Ruffo, 1982, from Somalia [loc. typ.: Uadi Nogal, N. Somalia], as well as several species from western Pacific region till Hawaii (Sawicki, Holsinger & Iliffe, 2004, etc.).

Discovery of genus *Metaniphargus* Stephensen, 1933 (typus generis: *Metaniphargus curasavicus* Stephensen, 1931) from Curacao Island in Caribbean

Sea, Atlantic, and numerous different species later discovered in adjacent regions of West India, made the problem of recognition of various genera and subgenera rather problematic, especially because *Metaniphargus* was rather similar to genus *Metahadzia* differing mainly by the length of inner ramus of uropod 3 [*Metahadzia curasavicus* Stephensen, 1933 with uropod 3 inner ramus reaching 1/3 of outer ramus]

Just because of not quite clear differences between species and combination of taxonomic characters of genera *Hadzia*, *Metahadzia, Liagoceradocus and Metaniphargus,* , various authors mentioned different status of these genera and corresponding species in it.

Stock and Nijssen (1965) and G. Karaman (1969a) considered genus *Metaniphargus* as synonym of genus *Hadzia*. Later Stock (1977; 1983) considered *Metaniphargus* as a valid genus, and established (1977) a new genus *Metahadzia*.

Andres described (1978) *Liagoceradocus acutus*, sp. nov. [loc. typ.: Jameos de Agua, Lanzarote, Canarian islands, Atlantic, Spain].

Pesce (1979) considered *Metahadzia* as a good genus and proposed (1980) a new diagnosis of this genus.

Ruffo (1982) and G. Karaman (1984) considered *Metahadzia* and *Liagoceradocus* as synonyms of genus *Hadzia*.

Barnard, J.L & Barnard, C.M. (1983) mentioned that *Metahadzia* differs from *Hadzia* and *Metaniphargus* by various characters.

Stock (1983) based on certain cladogram, considered *Liagoceradocus* as a distinct genus, as well as genera *Metaniphargus, Hadzia* and *Metahadzia,* with unclear position of *Hadzia pachypoda*.

Ronde-Broekhuizen & Stock (1987) recognized genera *Metahadzia* and *Liagoceradocus* as distinct genera.

Vigna-Taglianti (1988) consider genera *Liagoceradocus* and *Metahadzia* as synonyms of *Hadzia*, describing a new genus and species *Parhadzia sbordonii*, gen. et spec. nov. from Turkey [Mustan Ini Cave, S. Anadolia], rather similar to *Metahadzia* by gnathopod 2, but characterized by uropods 1-2 covered with plumose setae, modified uropod 2 in males, extremely long pereopods 5-7, coxa 4 with well developed posterior lobe.

Notenboom (1988) consider *Metahadzia* as a distinct genus, as well as *Hadzia* and *Metaniphargus*, but regarding genus *Liagoceradocus* he remains questionable.

Bradbury, J.H. & Williams, W.D. (1996) mentioned *Liagoceradocus* as distinct genus, describing two new species from Western Australia [*L. subthalassicus* n. sp. (Barrow Isl.) and *L. branchialis* n. sp. (Northwest Cape), both from anchialine habitats].

Sawicki, Holsinger & Iliffe (2004) revised genus *Hadzia* (sensu lato), put *Liagoceradocus* as synonym of genus *Hadzia*, and preserved genera *Hadzia*, *Metahadzia* and *Metaniphargus* as distinct genera. They mentioned that "these three genera are derived from a recent common ancestor, probably very similar to the *Hadzia*". Describing several new species of the genus *Hadzia* from western Pacific, they concluded that–*Hadzia* is with remarkably broader distribution, probably reached the Hawaiian Islands from other part of the Pacific, while the distribution area of genus *Metaniphargus* is mainly on West Indian region and Hawaii, where species of both genera are present, proposing also alternative possibility that *Metaniphargus* evolved "independently in the Pacific from the putative ancestor common to *Hadzia* and *Metaniphargus".*

Vonk (1991) suggested possibility that *Metaniphargus* have expanded their distribution from Caribbean westward into the eastern Pacific prior to the closing of the Isthamians corridor in the late Miocene, following opinion of Rosen (1985) that the closing of the Isthmian corridor in the late Miocene, trigger separation and isolation of tropical Atlantic and tropical Pacific marine organisms.

The so large variability of the recognition of genera of Hadziid-group in Europe indicates the necessity of detailed revision of all known taxa of these genera from Europe and another part of the World. On the other hands, there are not clear taxonomical characters differing Hadziids from Melitids.

KEY TO THE MENTIONED HADZIID TAXA

1. Maxilla 1 left palpus much shorter and partially reduced (pleopods with 3 retinacula, male pleopod 3 modified; uropod 3 inner ramus scale-like)
FINGERHADZIA ZORAE

----Maxilla 1 left palpus as long as right palpus …………………........…………2

2. Uropods 1-2 covered with plumose setae (coxa 4 with well developed ventroposterior lobe) ……………………………..…...*PARHADZIA SBORDONII*

---Uropods 1-2 without plumose setae …………………………………..……3

3. Article 5 of gnathopod 2 lobed posteriorly, bearing submarginal (facial) setae..…4

---.Article 2 of gnathopod 2 not lobed posteriorly, bearing marginal setae only…………………………………………………………….…..……10

4. Mandibular palpus article 3 with 2 distal setae only (telson with distal, mesial marginal and outer marginal spines)……..……………… *HADZIA PACHIPODA*

--- Mandibular palpus article 3 with distal E and lateral D-setae (telson with or without spines at outer margins)…… ……………………………………..…....5

5. Gnathopod 2 propodus in female with excavated palm…..…………..……..6

---Gnathopod 2 propodus in female with convex entire palm…………………….7

6. Uropod 3 with broader rami; mandibular palpus article 2 without setae; pleopod 3 peduncle without posterior marginal setae.…..*HADZIA GJORGJEVICI*

--- Uropod 3 with narrow rami; mandibular palpus article 2 with 1 seta; pleopod 3 peduncle with 2 posterior marginal setae…………..……..…*HADZIA. CRISPATA*

7. Dactylus of pereopod 7 with several setae along outer margin (accessory flagellum 2-articulated)……………………………………….*HADZIA ACUTA*

---Dactylus of pereopod 7 with one median seta at outer margin (accessory flagellum 1-articulated or indistinctly 2-articulated)…………….……………….8

8. Lobes of telson with one distal spine (accessory flagellum 2-articulated, peduncle of pleopods with 2 retinacula without one spiniform seta)…………..………… *HADZIA FRAGILIS STOCHI*
---Lobes of telson with 2 -4 distal spines (accessory flagellum 1-2 articulated)………………...…9
9. All pereopods and antennae very long and slender; dactylus of pereopods 5-7 long and slender, lobes of telson with 2, occasionally 3 distal spines; accessory flagellum indistinctly or more distinctly 2-articulated)…........
HADZIA. FRAGILIS FRAGILIS
---All pereopods and antennae shorter and stouter. Dactylus of pereopods 5-7 shorter and stouter, lobes of telson with 3-4 distal spines (exceptionally one left lobe with one outer marginal spine); accessory flagellum 1-articulated.....
HADZIA DRINENSIS
10. Telson with mesial marginal spines…………….……………..…………11
---Telson with outer marginal spines…………………..………….…………12
11. Palm of gnathopod 2 in females excavated. Peduncle of uropod 2 in males with distal processus………..…………..…………… *METAHADZIA TAVARESI*
---Palm of gnathopod 2 in females not excavated. Peduncle of uropod 2 in males without distal processus............…………….……… *METAHADZIA. MINUTA*
12. Lobes of telson with 5-6 spines at mesial margin.*METAHADZIA ADRIATICA*
---Lobes of telson with 2-3 spines at mesial margin……………..……..……13
13. Lobes of telson long and narrow, 2.5 times longer than broad, bearing 4-5 mesial spines……....…………………………………*METAHADZIA HELLADIS*
---Lobes of telson shorter and broader, 2 times longer than broad, bearing 2 mesial spines…………………........………..……*METAHADZIA UNCISPINA*

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ANALYSIS OF THE PROMOTION OF SMALL WINE PRODUCERS IN WINE REGIONS OF MONTENEGRO AND THE PERSPECTIVE OF WINE TOURISM IN COOPERATION WITH LOCAL TOURISM ORGANIZATIONS

SUMMARY

Grape and wine production has a long tradition in Montenegro. Its potential is not used to its utmost considering that a large number of small wine producers exists in this agricultural production segment. This study analyses the production and promotion of small wine producers in Montenegro and their cooperation with local tourism organisations. The aim is to analyse the representation of small wine producers in the domestic market and the participation of local tourism organisations in promoting wine as one of the most critical Montenegrin agricultural products. A survey was conducted among small wine producers and regional tourism organisations. The survey determined that most Montenegrin small wine producers sell their products in their vineyards in the domestic market. Only 24.2% of the surveyed wine producers exported their wine. Regarding wine promotion, 75.8% think they need to promote their wines more. The most effective and cost-effective promotional activities worldwide are underrated and underused in Montenegro. Small wine producers' promotions through festivals and other cultural events were used by 42.4% of survey participants, whereas only 46.5% use digital marketing for promotional purposes for their wines. There is a similar trend in local tourism organisations. Regarding their overall promotional strategies, only 38% fell under digital marketing (website), 8% social networks and 46% fell under festivals and other cultural events. While considering survey results and based on determined deficiencies in the wine promotion of small wine producers in the domestic market, measures are observed and suggested to both small wine

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producers and tourism organisations at local and national levels that could be carried out to increase tourist and domestic consumption.

Keywords: wine production, enotourism, agriculture, consumption, wine promotion.

INTRODUCTION

Grape and wine production as a traditional and significant agricultural activity is important in the South-Eastern European economy, i.e., the Montenegrin economy. Many organisations and individual producers in this respective activity are linked directly or indirectly with other economic activities, such as trade, catering, and the beverage industry (Perović, 2014). The increase in wine consumption in Montenegro is associated with expanding wine production and quality, adequate awareness of wine consumers, and various types of wine education through electronic and print media (Savić, 2013).

This is confirmed by data on the export of various agricultural and food products, on the basis that Montenegro could be considered a “wine country” (Perović and Ristanović, 2019). Montenegro registers a considerable increase of vineyard area as well as the trend of increase of wine production with small producers (Pajović-Šćepanović et al., 2013). Precisely this increase of vineyards and grape production in last few decades resulted in the increase of types and numbers of Montenegrin wines. Thus, for production of most famous Montenegrin white wines, there are autochthonous types Krstač and Chardonnay, whereas for red/black wines, these are Kratošija and Vranac (Pajović et.al., 2016). Precisely from these autochthonous grape types, wines of exceptional property and very good quality are produced by which Montenegro is recognized worldwide (Sošić et al., 2023). Apart from mentioned grape types, significant research on yield and quality of table grape Cardinal was carried out by the authors (Popović et al., 2023).

In addition, in previous years in Montenegro, a growing number of small wine producers faced problems such as determining prices for their wines, where to market and sell them, and what model to use to promote their wines.

The importance of the research of wine is also imposed due to the fact that it is one of rare products which has been on the list of export agricultural products of Montenegro for many years. Having in mind the fact that, in comparison to other Western Balkan countries, Montenegro, as well as Northern Macedonia, has a surplus in foreign trade exchange (Monstat, 2022; Basha et al., 2022), the emphasis is put in this paper on the research of the status of small wine producers and their contribution to the development of agricultural tourism, i.e., wine tourism, which is considerably developed in other wine countries worldwide.

Wine tourism or enotourism represents an essential component for the development and success of many small vineries. Many countries worldwide have considerable economic development and growth depending on the tourism industry. This is particularly true with the much-needed expansion of the countries’ local economies. Enotourism has emerged as a promising and sustainable type of tourism that can benefit local, regional, and national economies (Figueroa and Rotarou, 2018). The UNWTO recognized wine tourism as a critical element of gastronomy tourism, further enhancing sustainable rural development by combining destination promotion, the preservation of natural resources, and income generation. Consequently, specific forms of tourism have been developed to provide maximum benefits to a particular region. One of these is wine tourism. According to (Quintela et al., 2023) wine tourism is seen as a strategic product that enhances the attractiveness of a destination as it positions itself as a unique selling proposition and serves as a tourist attraction.

A nation's tourism industry benefits from wine tourism in three ways. It is a draw for travellers, a tactic that helps places develop and market a reputation and facilities associated with the wine industry, and a chance for wineries to offer their goods to customers directly (Mitchell and Hall, 2006). The wine routes are vital for the economic development of rural communities (Ingrassia et al., 2022; Festa et al., 2020).

Wine tourism provides a unique experience to visitors of the winemaking regions through organized or individual visits to vineyards and wineries, participation in wine festivals and wine-tasting events (Vukojević *et al*., 2022). Wine tourism in rural territories, sometimes organized along wine routes, increasingly attracts visitors, tourism agents, wine producers and those responsible for developing rural regions (Kastenholz *et al*., 2022).

According to (Sekhniashvili and Bujdosó, 2023), marketing and promotion of a destination are part of its management and assist in the positioning of wine regions. The same authors state that wine tourism plays a vital role in the positioning strategies of wine destinations. (Savić, 2013) states that wine tourism also implies an increase in sales of wine and other domestic products and services at one’s doorsteps and an increasing positive image and identity of local family wineries.

One of the methods of promoting small wine producers is organizing various fairs and dedicated festivals because festivals allow the tastes of tourists and wine lovers to be determined for specific wines and activities related to wine production. Celebrating wine, consuming food and wine and introducing local customs and products are the essence of wine festivals.

According to Savić (2013), experience shows that many domestic and foreign wine lovers visit such events. Many participants sell a certain quantity of wine during festival days. Additionally, the importance, information sharing and experience of online wine tourism confirms the popularity of social networks among wineries and their efforts to communicate in such a way with their buyers (Haller et al., 2021; Szolnoki *et al*., 2018). Promotion through the internet and web presentations is compelling and accessible worldwide with an internet connection and a click away. For example, (Andrlić *et al*., 2011), studied that the Internet provides numerous possibilities for wine tourism, including cost reduction, timing of information, better communication, booking options, unifying participants in the wine tourist market and viral marketing.

Namely, the paper analyses the participation and representation of small wine producers on domestic i markets and measures Montenegro implements in promoting this tourism activity. That is, the paper analyses the participation of local tourism organisations in promoting Montenegrin wine as one of the most important Montenegrin products as part of their local promotion and tourist offer. By analysing the survey results and based on the deficiencies noted in the promotion of wine by small wine producers in domestic and foreign markets, measures are proposed that the state, through tourism organisations on a local and national level, can carry out to increase visibility and tourist and domestic consumption of this critical Montenegrin product.

This study aims to identify directions for developing sustainable enotourism among small wine producers and tourism organisations and their relationship in promoting wine tourism in destination countries.

Apart from this, small producers do not have enough potential either to improve their living standards through their wine production and sales or to promote wine tourism and Montenegro as “wine country”. Additionally, in past period with more and more climatic changes, more importance is given precisely to this agricultural sector and growing grapes in the areas with tradition, fertile land and favourable climatic conditions (MIA, 2022).

MATERIAL AND METHODS

For this study, much research was conducted by reviewing the literature, such as papers and reports discussing wine tourism and the correlation between small wine producers and national promotional strategies in tourism, and wine tourism in particular. Further, primary data were used based on a survey conducted for this study (direct communication with interviewees by phone and email with tailor-made questionnaires for both small wine producers and tourism organizations, respectively). Finally, secondary resources such as the National Statistics Office (Monstat, 2022) were used. The findings were further structured based on analytical and comparative methods, and the results and discussion are presented in the tables and graphs.

According to data from the Montenegrin Vineyard Register of the Ministry of Agriculture and Rural Development, there are 500 registered grape producers with a total area of 3301 ha of registered vineyards and 85 registered wine producers. However, direct communication with interviewees determined that a certain number of registered vine/wine producers went out of business or were only producing grapes to make other types of drinks (e.g., brandy-rakija). Considering the above, with the exclusion of large and medium wine producers, 50% of interviewees participated in the tailor-made survey and provided feedback for further analysis.

Based on a study area of the viticulture zoning of wine-growing areas, the following are zoned areas in Montenegro (Official Gazette of Montenegro, 2017; Basha et al., 2022). The wine-growing region of the Montenegrin basin of Skadar Lake (Crnogorski basin Skadarskog jezera) (Figure 1a) includes seven sub-regions, Wine-growing region Montenegrin coastal area (Figure 1b), Small wine-growing area (region Nudo), (Figure 1c) and Wine-growing region Montenegrin north (Figure 1d),

Figure 1a. Wine-growing region Montenegrin basin of Skadar Lake

Figure 1b. Wine-growing region Montenegrin coastal area (Source: Basha et al., 2022)

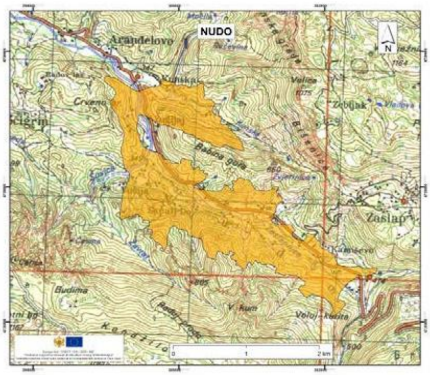

Figure 1c. Small wine-growing area Nudo

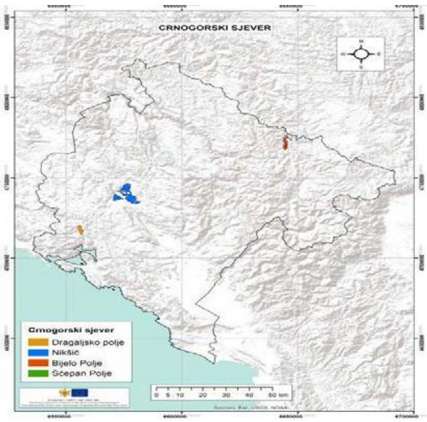

Figure 1d. Wine growing region Montenegrin north (Source: Basha et al., 2022)

The survey contained questions that, apart from basic information (name of producers, annual yield, etc.), also covered the sales, promotion, and distribution of their wine, as well as questions of respective support at the local and national levels.

The survey was conducted electronically, using online surveys, and by phone.

Apart from the above-mentioned survey of small wine producers, a survey of local tourism organizations was also conducted. Of the 23 tourism organizations in Montenegro, 13 (56.5 %) participated in the survey. The reason for this is that the focus was placed on those tourism organizations located in the areas defined as zones of grape and wine production, that is, the remaining 43.5% of local tourism organizations still need wine producers in their respective areas. The survey contained questions about the annual programs of tourism organizations, their cooperation with small wine producers, and their engagement and support in promoting their wines at the local and national levels. Figure 2 shows the areas in which local tourism organizations were surveyed.

Figure 2. Marked municipalities whose local tourism organizations participated in the survey (Source:https://uom.me/ (accessed on 31 August 2023).

RESULTS

In order to obtain necessary information from small wine producers and to analyse the situation in terms of promotion and sales of their products, as well as cooperation with local tourism organizations', a survey was carried out online or in direct phone communication in the period May – July 2023. Table 1 shows that out of the total surveyed small producers, 33.4% produce up to 3,000 l of wine annually, whereas 30.3% each produce 5,000–10,000 l and more than 10,000 l of wine, respectively. The survey further showed that the majority of wine producers (72.7%) secure grapes for their production from their vineyards, whereas only 27.3% purchase grapes to reach their production.

Table 1. Small Wine Producers' Survey Data.

Wine production (l)	Share in total responses
500 – 1000	3%
1001 - 3000	33.4%
3001 - 5000	3 %
5000 – 10000	30.3%
Above 10000	30.3%
Do you obtain this from your own vineyards?	
No	27.3%
Yes	72.7 %
Do you sell wine outside Montenegro (export)?	
No	75.8%
Yes	24.2%
Are you a member of Wine Producers Association?	
No	42.4%
Yes	57.6%
Do you sell your wine through one trademark with other small producers?	
No	100 %
Yes	0 %
Do you think you promote enough your product (wine)?	
No	75.8%
Yes	24.8 %

Source: Data processed by the authors

In terms of wine export, out of all surveyed, 24.2% export their wine and only 10-20% of their total annual production. Considering the smaller quantities they produce, the number of exports would be higher if they sold their wine under one brand, although all surveyed participants replied that they sold their wines exclusively under their own brand, and that more than half (57.6%) were members of some wine producer associations. When asked how they promoted their wines (Figure 3), with the possibility of marking more categories, most of the respondents (46.5%) stated that it was via the Internet (website, email, etc.).

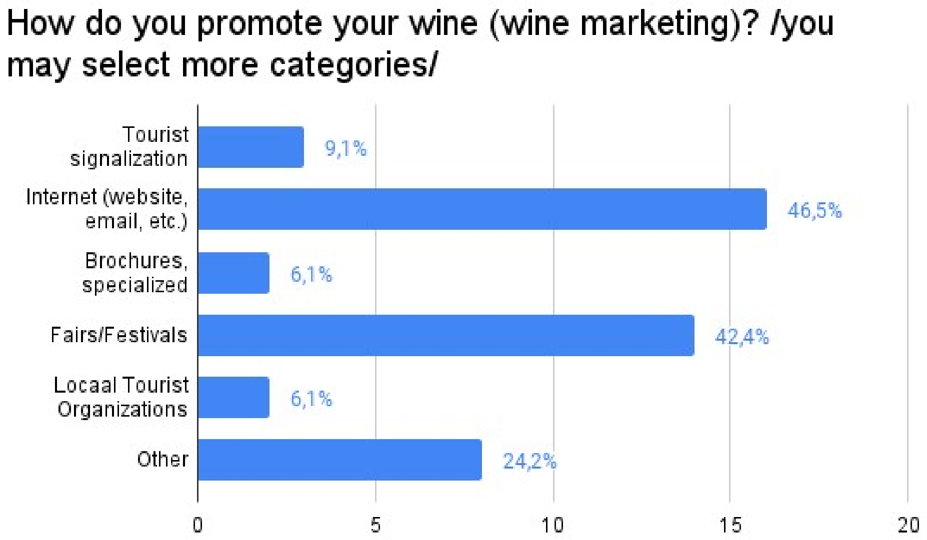

Figure 3. Models of wine marketing used by Montenegrin small wine producers.

Communication via websites and social media can contribute considerably to an increased flow of information, better visibility, and wine tourism promotion (Alonso *et al*., 2013; Szolnoki *et al*., 2021). The importance and experience of online wine tourism are growing (Marzo-Navarro and Pedraja-Iglesias, 2021; Simeon and Sayeed, 2011; Scorrano *et al*., 2019), which confirms the popularity of social networks among wineries and their efforts to communicate with their consumers in this way (Haller et al., 2021; Szolnoki *et al*., 2018). A somewhat lower percentage of surveyed wine producers (42.4%) said that they visited wine-related fairs and festivals. One positive example of wine promotion is the "Festival of Wine and Bleak", which occurs every year in December in Virpazar (the Montenegrin Basin of Skadar Lake). The winner and the best wine are promoted in media (free advertisement, additions, and reports in printed media, etc.), which in most cases increases the sales of the winner's wine (Savić, 2013).

Apart from advertising and promotion via the Internet, fairs, and festivals, very few stated that they promote themselves via personalized brochures, specialized wine magazines, and tourist signalization (2–3%), whereas 2% of the interviewees said that they promote themselves via local tourism organizations, which is problematic and opens the door for further analysis.

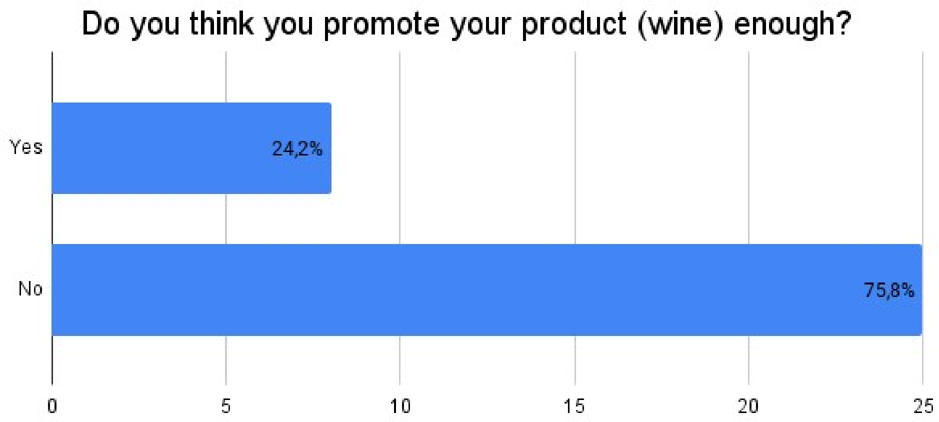

Figure 4. Evaluation of Montenegrin small wine producers' self-promotion.

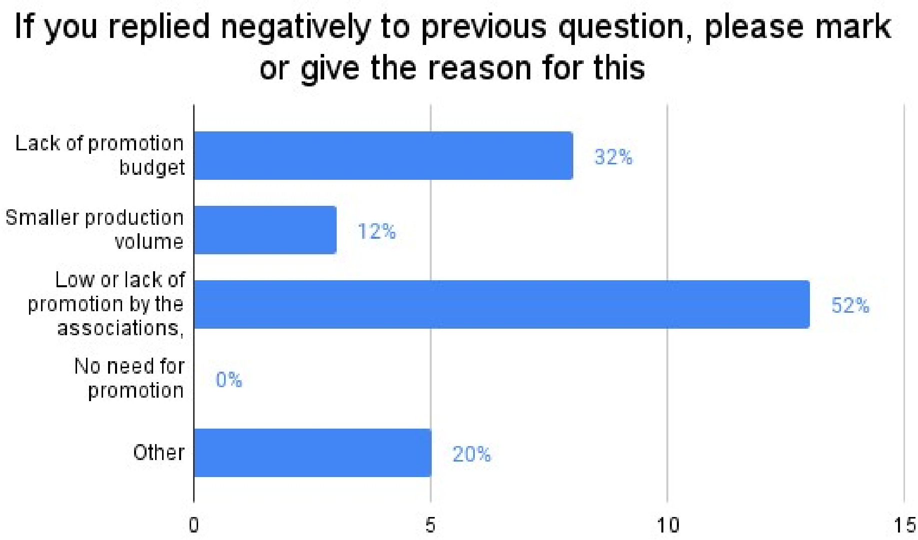

Figure 5. Reasons for lack of promotion by Montenegrin small wine producers.

Regarding the question of whether they think they promote their wine sufficiently, only 24.2% said "yes", whereas 75.8% said they do not promote enough (Figure 4). Of those who answered negatively (Figure 5), 52% stated that they do not have enough support from associations, local tourism organizations, and the state; 32% mentioned a lack of promotion budget as the reason because they are small wine producers managing their vine yards with high production and sales costs. Finally, regarding the promotion of wine through distribution channels, the interviewees gave one of the proposed reasons (Figure 6).

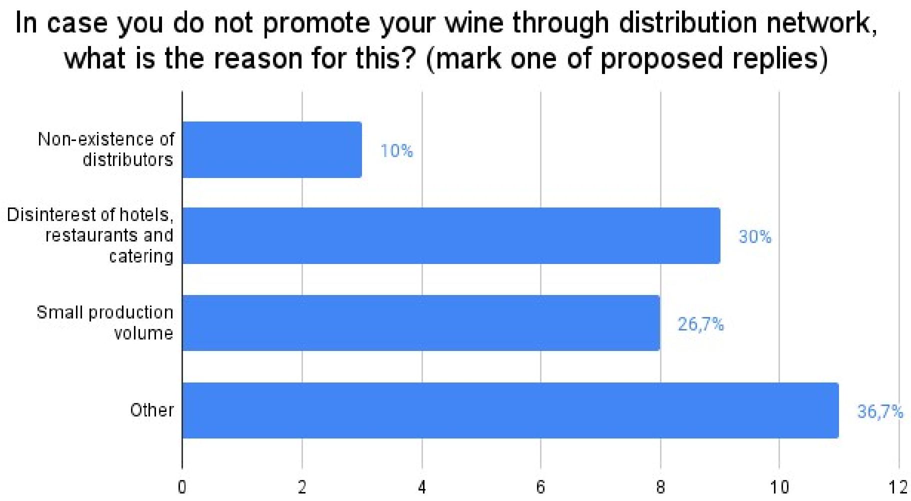

Figure 6. Reasons for not promoting the product through distribution networks.

Furthermore, 36.7% stated “other reasons,” which were clarified in direct communication with small wine producers as a lack of or low support and cooperation in promotion and distribution by the state, relevant associations, and national and local tourism organizations. Approximately 30% of those surveyed stated that neither hotels, restaurants, nor caterers showed great interest in including domestic small wine producers in their offers. In addition, 26.7% of respondents mentioned small production volumes as ae reason. Regarding local tourism organizations and the survey in which they participated, some of the selected replies with yes/no answers are given in Table 2.

They considered that the survey focused on local tourism organizations with small wine producers in their municipalities; only 53.8% stated that they had projects or activities to promote local wine producers in their work programs. Local tourism organizations mentioned some of the activities in their work programs that are promoting small wine producers: making new catalogues for their respective municipalities; planned events such as “Wine-coloured Danilovgrad”, where the best and highest quality producers shall be presented in this municipality, including wine producers in gastronomic events; Podgorica Wine Salon; traditional festivals related to wine and other local products; organization of visits to small wineries; printed promotional material; promotion via official tourism organization websites, etc.

Regarding whether they were in direct communication with local wine producers, 38.5% said no, whereas 61.5% of regional tourism organizations said yes. The same percentage (61.5 %) supports the promotion of small wine producers in their municipalities, and the same rate suggests that their tourism organization is not promoting enough local wine producers.

Table 2. Local Tourism Organizations ' Survey Data

Share in total responses
Do you have projects or activities of promotion of local wine producers in your work programmes?
No 46.2 %
Yes 53.8 %
Are you in direct communication with local wine producers?
No 38.5%
Yes 61.5 %
Do you support their wine promotion?
No 38.5%
Yes 61.5 %
Do you think your tourist organization is doing enough in promoting local wine producers?
No 61.5 %
Yes 38.5 %
Do you think there is enough quantity in the offer of local wine producers?
No 46.2 %
Yes 53.8 %
Do you think that domestic small wine producers should promote themselves more independently
No 7.7%
Yes 92.3%

Source: Data processed by the authors

More than half of those surveyed (53.8%) thought that small wine producers needed more wine in their offerings. Most surveyed tourism organizations (92.3%) stated that they believe domestic small wine producers should promote more independently, whereas only 7.7% have the opposite opinion.

The Surveyed local tourism organizations that stated that they provide support to small wine producers in their municipalities (Figure 7) further listed the type of support they provide (Figure 8), where the largest number of surveyed

(54%) are doing so by organizing various events or festivals related to wine and food for local inhabitants where small wineries' products are promoted as well. (Yuan et al., 2006) studied that visitors to wine festivals would be interested in visiting other tourist attractions or the place or region where the festival occurs. Wine festivals are solid catalysts for wine tourism (Yuan et al., 2006; Mitchell and Hall 2006). Further, (Newey, 2012) believes that wine festivals attract a broader public than the visitors of wineries. Whereas (Yuan et al., 2005) explain that wine festivals are also visited by tourists, who generally do not visit wineries. In other words, they were not considered to be wine tourists. Only 23% said that they organized target group visits to local wine producers. In contrast, 15% replied that they do this by making wine route maps and promotional material, because the practice until now was for wine producers to organize themselves and finance promotional materials for their wineries. Regarding marketing models used to promote small local wine producers, the most significant number of the surveyed 46% (Figure 9) replied that these were promotional events (targeted events or festivals).

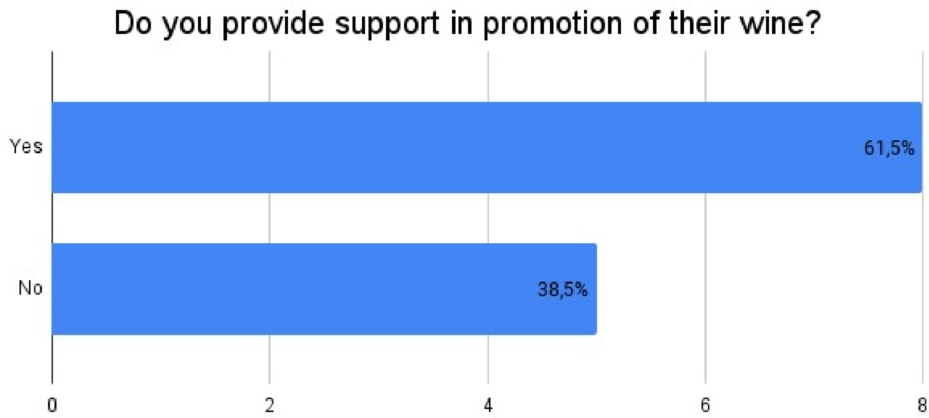

Figure 7. Tourism organization support in small wine producers' promotion.

In addition, 38% of respondents use marketing activities through digital marketing (websites), which was confirmed by visiting the official websites of local tourism organizations.

Most provide information and promotional activities for local wineries in this way. Only 8% used printed media (fliers, brochures) to inform tourists about the available offers and promotions of small local wine producers and their products. Marketing messages via fliers and brochures significantly promote wine tourism and its attractions (Sellitto, 2005; Brown and Getz, 2005; Dodd, 1995; Carlsen and Dowling, 1998). When asked if they do enough for the promotion of local wine producers, 61.5% of local tourism organizations said "no” and 38.5% said that they promote them enough (Figure 10).

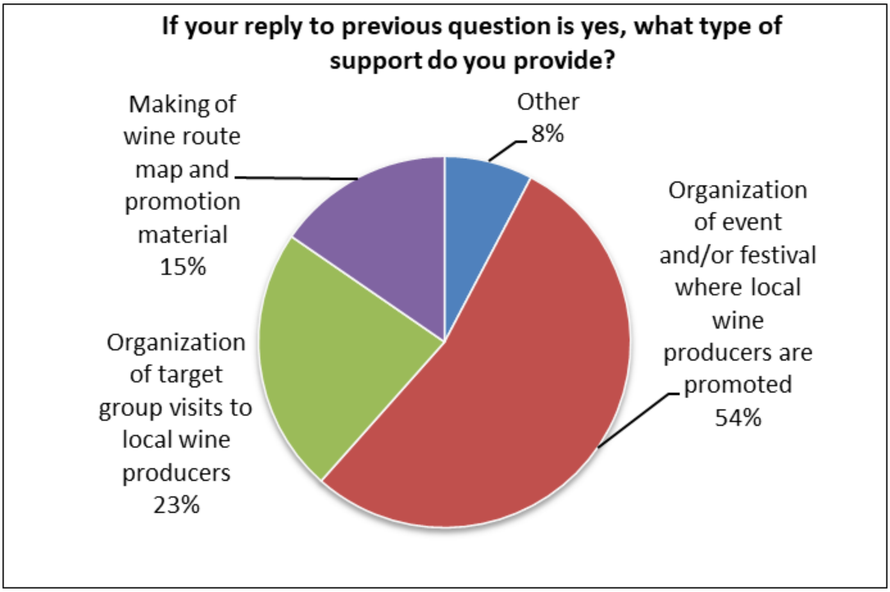

Figure 8. Type of support provided by tourism organizations.

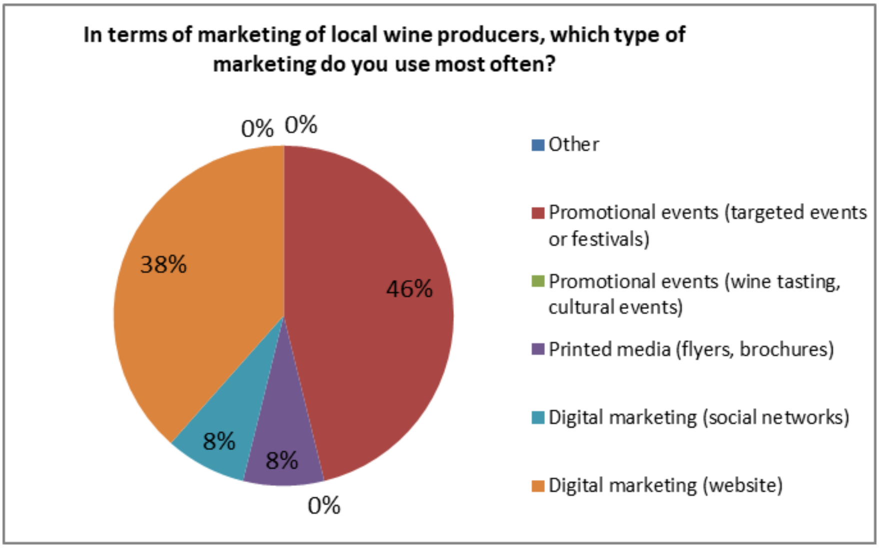

Figure 9. Marketing tools used by tourism organizations.

Those who replied negatively gave reasons for this (Figure 11). Approximately 37% stated that producers were not interested.

However, the majority (27%) mentioned other reasons without providing specifics (Figure 11). Considering that they only 18% mention the lack of funds for promotion, it can only be assumed that they do not see wine tourism as an important part of the tourist offer, or may even reflect the overall approach to this type of tourism by local communities (municipalities) and even the state.

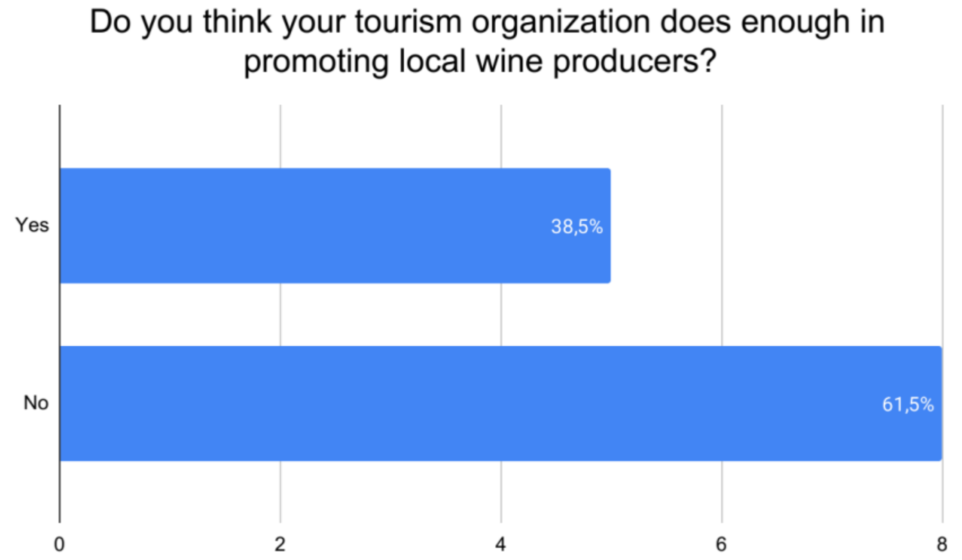

Figure 10. Tourism organizations' perspective on their engagement in small wine producers' promotion.

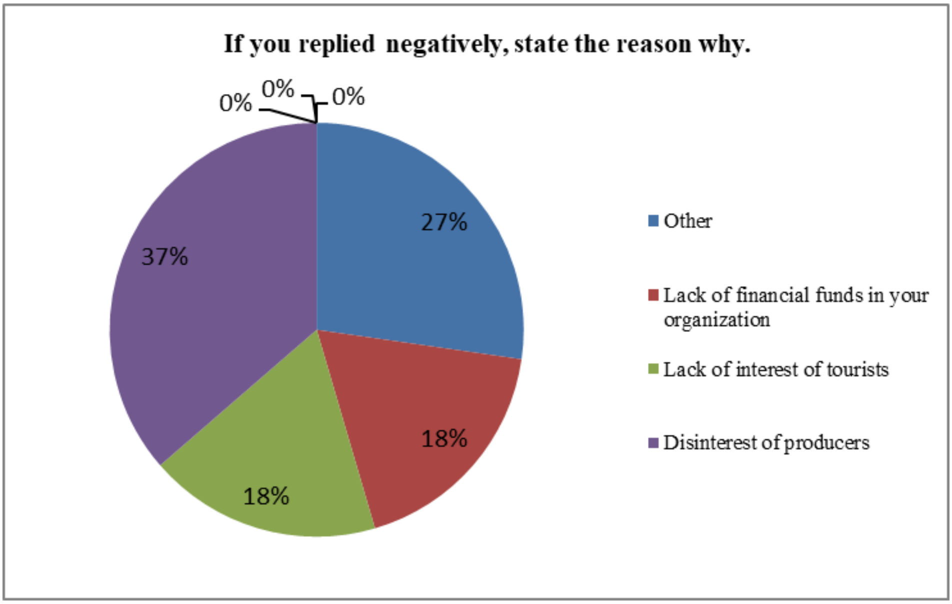

Figure 11. Reasons for lower small wine producers' promotion by local tourism organizations.

Finally, slightly more than half of the surveyed (53.8%) think that small producers do not have enough wine quantity in their offer. Most of the surveyed organizations (92.3%) stated that domestic small wine producers should promote themselves more in a dependent manner, whereas only 7.7% are of the opposite opinion (Table 2).

DISCUSSION

The survey determined that most small wine producers sell their wine exclusively in wineries in the domestic market. Only 24.2% of the surveyed small wine producers exported their wine. By contrast, others sell them in their wineries or distributors to hotels, restaurants, or shops in the domestic market.

However, small wine producers' low promotional capacities and activities are of great concern to the wine industry in Montenegro and its wine tourism development. The survey showed that many small wine producers believed that they must promote their wine more (75.8%). The main reasons for this poor visibility and promotion were the need for more support from governmental institutions (52%) and the need for a promotion budget (32%). By analyzing the survey results, it is determined that small wine producers in Montenegro do not use, to the fullest extent, promotional activities that require a low or no promotional budget.

For example, considering ways of promoting their wines, less than half of the surveyed small wine producers (42.4%) participate in festivals, using this as one of their primary methods of promoting their wine. This means that more than half (57.6 %) did not use this method, which is quite the opposite. Considering the limited and sometimes non-existent budget for promotion used by large wine producers in the market, the focus of all small wine producers in Montenegro should be on increasing participation in thematic fairs as exhibitors, advertising during some cultural or seasonal events, festivals, participation of producers in so-called “wine routes," ethno-tourism, promotion of domestic products of households producing wine, etc.

Wine and food festivals are cheap ways for wineries to promote their brands to new customers (Hall *et al.*, 2000). As (Carlsen and Getz, 2006) noted, wine festivals can offer critical impulses to wineries and wine regions and their competitiveness to better position themselves in the market. The authors state that wine events and festivals represent an essential segment of marketing activities in branding processes and promotion, so all more essential wine regions and wineries worldwide organize them.

However, this type of promotion of small wine producers is closely linked with national and local tourism organizations and their promotional activities to create visibility and attractiveness of the region to tourists.

Since enotourism is a crucial economic industry and the most significant contributor to GDP, national and local tourism organizations should increase their involvement in developing and promoting more specific tourist offers. In this case, they should raise their participation in promoting wine tourism as a particular Montenegrin tourist offer. However, the survey showed that wine tourism needs to be explored more and has not resulted in a specific tourist offer. Even though more than half of local tourism organizations (53.8%) state that they have wine tourism in their annual work programs, 61.5% are in communication with small wine producers, and the same percentage (61.5%) state that they support wine producers, it is evident that their involvement in the promotion of small wine producers and thus wine tourism as a specific tourist offer is not in their primary focus. Only 38.5% of tourism organizations think they must do more to promote local wine tourists, 27% do not give specific reasons, whereas some state there is a lack of interest among small wine producers (37%). Interestingly, the lack of financial funds of local tourism organizations or the disinterest of tourists was given with 18% as a reason for low wine tourism promotion in their respective regions.

Additionally, almost all tourism organizations (92.3%) think that small wine producers should promote themselves more independently. In contrast, tourism organization involvement is mainly focused on promotion through participation in organized festivals, which, according to tourism organizations, represents 46% of their promotional activities for small wine producers. Although this seems like considerable support, there are only two official wine festivals throughout the year where small wine producers can promote their wines to a broader audience. Other, less-promoted festivals refer to local products. Festivals where wine is one of many advertised local products.

Getz and Brown (Getz and Brown, 2006) suggest three main perspectives on wine tourism: the wine producers, the tourism agencies (representing the destination), and the consumers. Apart from festival promotion for small wine producers, another vital advertising segment has low or no costs for small wine producers and is used worldwide. It is digital marketing. However, looking at the survey results, Montenegrin small wine producers need to use this free and effective promotional tool to the maximum. Less than half of surveyed small wine producers (46.5%) use some form of digital marketing (website, email, etc.). This raises the question of their digital skills or even their awareness that using social networking profiles can be exceptionally useful. They can create a "personal experience" of their product by uploading photos, videos, and news. Additionally, by using the profiles of their end users, who tend to grade consumed products, they can present their experiences to a broader audience, resulting in a significant multiplication effect.

Nowadays, the world population exceeds 8 billion people. Of this number, 5.3 billion are internet users, i.e., 65.7% of the global population (Statista, 2023). There are 4.95 billion social media users worldwide, or 61.4 percent of the global population [Data Reportal). This is why less traditional and more digital promotion and marketing are present throughout the industries. So, it is no surprise that the same applies to the wine industry. Wine marketing is considered information-intensive (Stricker *et al*., 2007; Koval *et al*., 2019). Communication has become almost instant. Due to the rapid development of e-commerce technologies and business process (Quinton and Harridge-March, 2003; Soloviova *et al*., 2022; Trachenko *et al*., 2021) and global wine consumption (Lazarova *et al*., 2023; Barber *et al*., 2006), small wine producers use direct marketing channels and strategic flexibility (Arsawan *et al*., 2022). Digital tools and applications enable small wine producers to be more competitive globally (Nesenenko, 2022.)

(Lalicic and Gindl, 2018) presented data from the obtained research showing that social media positively impacts clients' relations, increases brand awareness and enables online comments, posts. Considering the price, social media presents a good communication and promotion channel for small wineries and that more and more wineries are aware of the importance of social media. However, very few know how to include them in their marketing strategies as younger managers are more likely to use these networks.

(Andrade-Viana, 2016) states that the wineries should use their content to interact with consumers who share their experiences on their social accounts and indirectly strengthen the brand with the target audience. Consumers use social media to network, shop, and have fun but also save money and time without intermediaries. Simultaneously, they create content, discuss products and services, and promote various brands by disseminating their content. However, the same applies to both national and local tourism organizations. Their websites do not have specific pages dedicated to wine tourism, available wine routes, contact information, content information, or links to small wine producers' websites and social media accounts. As presented in the survey, only 38% of promotional activities carried out by local tourism organizations fall under digital marketing (website) and 8% social networks. Again, this shows either a lack of digital skills or awareness of digital marketing and promotion's overall impact on attracting tourists worldwide.

This means NTOs and LTOs (local tourism organizations) can no longer sustainably use traditional means of marketing in an exceptionally globalized world. Although digital marketing has been well established in the developed world, it still seems that Montenegrin national and local tourism organizations must catch up. Digital marketing is cost-effective, flexible, and reaches a global audience without geographic barriers and constraints. It provides instant availability worldwide, with promotions being adjusted daily to reach and target profitable niche segments (Vargas-Sánchez et al., 2009).

Montenegrin NTOs and LTOs should use digital marketing to engage with customers, thus managing a customer database to create promotional events and build and strengthen brand image and loyalty. A click can promote new products and changes through a customer database. Further, Montenegrin NTOs and LTOs can enter new geographical markets with their existing products and offers with low online advertising costs, thus considerably decreasing traditional marketing costs, which require a lot of resources to set up and manage.

CONCLUSIONS

This study recognizes that the developing South-Eastern European wine-producing countries, with traditional agricultural activity and solid export results, have yet to have wine tourism as one of their major brand products in their overall tourism promotional strategy. If appropriately developed by all relevant stakeholders, it could add value to promoting Montenegro as a wine-producing country.

The development of wine tourism is based on small wine producers. In cooperation with and support of NTOs and LTOs, a survey was conducted to determine deficiencies in currently available promotional activities and propose possible measures to increase visibility, brand creation, and increased tourist consumption of this critical Montenegrin product. The survey showed that although some of the causes of such a state of wine tourism in Montenegro should be found in the minimal economic and commercial capacities of family wineries, high investments, and high prices per product and service, the results of the survey show that major promotional activities, such as festivals and other promotional events, as well as digital marketing, are underrated and underused. In both cases, less than half were promoted through festivals (42.4%), and 46.5% used digital marketing for wine promotion.

To turn these observed weaknesses into strengths, both small wine producers and NTOs and LTOs should invest time and effort in utilizing available promotional tools while not burdening the available financing resources too much. Regarding promotions through festivals and other cultural events, awareness should be raised with tourism organizations and small wine producers about the impact of cultural events on tourism development and growth. Both wine producers and tourism organizations currently have low expectations from the festivals; 42.4% of wine producers attend festivals, whereas festivals are 54% of the overall promotion support provided by tourism organizations. Creating festivals and similar promotional events, especially during off-season periods, should be joint efforts with the additional involvement of all other stakeholders. Product development responsibility, as well as developing and helping to organize new festivals and events to attract visitors during low seasons, falls on local and national tourist organizations, as this impacts brand creation and reputation for the region as a festival and event destination.

Regarding digital marketing, awareness and utilization of available digital tools should be raised among small wine producers in Montenegro, who use them to set up, manage, and constantly update their websites and social media pages, as only 46.5% of surveyed wine producers use them in their daily business. These present a perfect opportunity to make an excellent first impression by showing their wineries, tasting rooms, vineyards, and cellars, and trying to convey the beauty and promise of an incredible personal experience. Conversely, NTOs and LTOs dedicate only 38% of their marketing activities to digital marketing. This needs to be changed considerably, that is promotion should be intensified through digital marketing activities. This would strengthen and intensify cooperation and communication with the local wine producers.

Ultimately, the most significant economic benefit from wine tourism could be for the local family wineries involved in wine production and the entire local community, as it attracts tourists, sells a destination, and facilitates direct sales to consumers. Montenegro must follow the trends in the region and be more comprehensive if it wants to be competitive and increase its tourist offer. In particular, tourism is the most prominent industry on which Montenegro bases its growth and development.

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APPLICATION OF STATISTICAL FUNCTIONS FOR RAINFALL DISTRIBUTION MODELLING AND SPI CALCULATION IN MEDITERRANEAN WATERSHED

SUMMARY

Drought indices are one of the most widely used methods for drought monitoring because of their ease of application and interpretation. The most commonly used drought index method is the Standardized Precipitation Index (SPI) method, which uses precipitation as an input. The performance of the Standardized Precipitation Index (SPI) is affected by the choice of an incorrect probability distribution function, which can distort index values, exaggerating or minimizing drought severity. This study aims to test the suitability of the statistical distribution functions proposed by the Bootstrap model, which estimates the closest probability distribution for calculating the SPI at time scales (TS) of 3, 6, 9, and 12 months. Daily rainfall data collected at the Dar Chaoui meteorological station were used for the period 2000-2021. Distribution function parameters were estimated using the maximum likelihood (ML) method and the Kolmogorov-Smirnov method and then confirmed using the Bootstrap method. The results show several extremely dry picks, especially in the 9- and 12-month time scales.

Keywords: Rainfall distribution modelling, SPI calculation, Drought indices, Mediterranean, Bootstrap model, Kolmogorov-Smirnov method

INTRODUCTION

Like other natural phenomena closely linked to climate change, drought is increasingly affecting all throughout the world, more than other forms of disasters

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(Zarei et al. 2021) and is one of the most expensive natural disasters globally. It is often described as a slowly unfolding phenomenon (Sylla et al. 2016) due to its gradual onset as a natural hazard. Delayed identifications of natural hazards, which often take longer to manifest and have an impact, typically result in delayed or more expensive reactions compared to interventions made during the initial phase following timely identification (Sestras et al., 2023; Spalevic, 2011). Drought impacts vary across regions, depending on their unique climatic characteristics and socio-economic environments, as stated by Liu et al. (2012).

In relation to climatology and meteorology, drought is characterized by a significantly prolonged and severe lack of water, which falls below normal levels, resulting in adverse consequences for plants, animals, and society (Quenum et al., 2019). Higher temperatures, increased water evaporation, and decreased vegetation cover all contribute to exacerbating the phenomenon of drought, although occasional droughts have always been a part of Earth's natural phenomena (Ojha et al., 2021; Sabri et al., 2022).

There is no single globally accepted definition of drought (Wilhite and Glantz, 1985), as drought can be analyzed and interpreted from different angles and different perceptions (Liu et al. 2018). This is typically defined based on the circumstances in each specific area.

Drought monitoring requires a variety of approaches because of differences in local rainfall, seasonal cycles, and types of rainfall. This complexity in the accurate description of the phenomenon led researchers to define drought indexes, ranging from the simplest to the most complex. These indicators enable the characterization of droughts by their intensity, duration, spatial extent, probability of recurrence (Spinoni et al., 2014), and, as highlighted by Zhang and Li (2020), their detection at various stages of evolution, including location, time of occurrence, and termination.

A variety of drought indicators is in use, including the Palmer Drought Index (PDSI: Palmer, 1965), the Standardized Precipitation and Evapotranspiration Index (SPEI: Vicente-Serrano et al., 2010), and the Standardized Precipitation Index (SPI: McKee et al., 1993). The selection of these indicators depends on the specific impact to be evaluated within the framework of monitoring and comprehending changes in vulnerability to the phenomena. The Standardized Precipitation Index (SPI) is endorsed by the World Meteorological Organization as a standard for meteorological drought characterization (Hayes et al., 2011) due to its distinct advantages. It is flexible enough to be applied across various timescales (Fotse et al., 2024). It is applicable to all climate regimes and exhibits good spatial consistency, enabling comparison across different areas subject to varying climatic conditions (Pieper et al., 2020). Due to these exceptional advantages, the index has been demonstrated to be effective in detecting various historical drought events in numerous regions worldwide (Ndayiragije et al., 2022).

Promoters of the SPI have suggested using a gamma distribution to fit cumulative precipitation in the calculation of this index, but many studies have shown the limitations of this distribution (Touma et al. 2015; Blain et al. 2018), and researchers have shown that the applicability of theoretical distributions to describe cumulative precipitation is inconsistent across different regions and climates (Raziei 2021). For this reason, in this study, we applied the Bootstrap model to find the closest distribution to the precipitation series, and then conducted tests to decide which the appropriate distribution is. We also applied extreme value theory to find the return period over the next 100 years.

MATERIAL AND METHODS

Study area. Morocco, officially known as the Kingdom of Morocco, is situated in the north-western part of Africa within the historically significant Maghreb region (Amraoui et al., 2023; Bouayad et al., 2023). The study area is the Tangier region (Figure 1), which is one of the twelve regions of Morocco and is located in its northernmost part (Ouallali et al., 2024; Badda et al., 2023). This part is known for its rich geological and environmental diversity.

The region exhibits a diverse geological landscape, with coastal areas featuring distinctive formations influenced by maritime processes. Tangier and Tetouan boast unique geological formations shaped by coastal erosion and sedimentation. In contrast, the mountain ranges, exemplified by Chefchaouen, exhibit distinct geological features formed through tectonic activity and erosion processes over millennia. The environmental diversity of the region is primarily attributed to its varied climate. Ranging from humid Mediterranean to sub-humid, the climate of the northern region of Morocco is influenced by its geographical location and proximity to both the Mediterranean Sea and the Atlantic Ocean.

Data used. Monthly precipitation data ranging from 2000 to 2021 were obtained from the database of the Loukkos Hydraulic Basin Agency (LHBA). They are from Dar Chaoui meteorological stations located in the Tangier region in northern Morocco. The geographical positions of this station and the topography of the domain are shown in Figure 1.

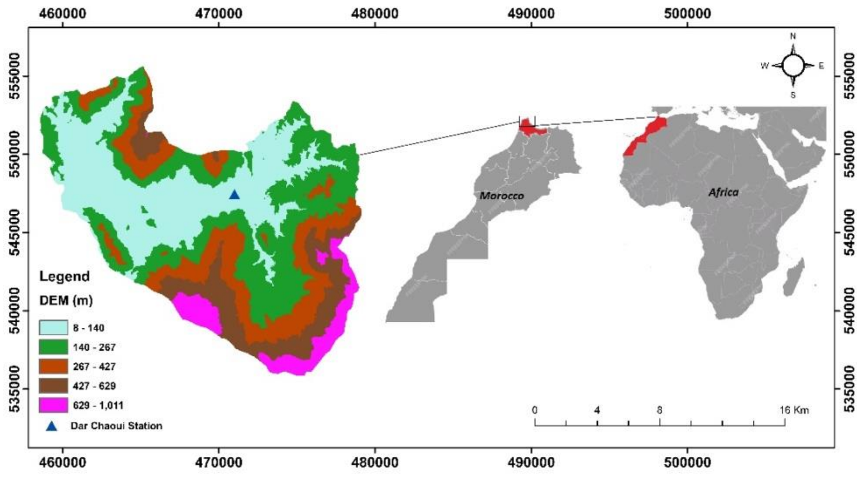


Figure 1: Study area with the geographical location of the station.

Computation of the Standardized Precipitation Index (SPI). To calculate SPI values, suitable probability density functions are fitted to frequency distributions of rainfall data. These distributions are aggregated for all selected timescales (3, 6, 9 &12 months) and subsequently transformed into standardized normal distributions (Raziei, 2021). The maximum likelihood (ML) estimation method was employed to determine the optimal parameters of distribution functions for testing purposes. Subsequently, the Kolmogorov-Smirnov (K-S) test was conducted to select the most suitable distribution from the bootstrapped functions (Raziei, 2021). The distribution with the lowest K-S statistic was identified as the best-fitting distribution. Subsequently, this distribution was utilized to construct the Cumulative Distribution Function (CDF). The CDF was transformed into normalized random variables and subsequently converted into Standardized Precipitation Index (SPI) values.

The duration of the Standardized Precipitation Index (SPI) varies depending on the specific type of drought under analysis and the intended applications (Gebremichael et al., 2022). Thus, the interpretation of the SPI indicates analyzing anomalies, which denote deviations from the average total rainfall observed within each specific period. High positive SPI values indicate excessively wet conditions, whereas high negative SPI values signify severe drought conditions. In the classification system proposed by McKee et al. (1993), various categories of drought are defined based on the SPI values, as illustrated in Table 1.

Table 1: Drought classification by SPI scores

SPI Value	Sequence of drought
SPI > 2	Extremely humid
1.5 < SPI < 1.99	Very humid
1 < SPI < 1.49	Moderately humid
-0.99 < SPI < 0.99	Near normal
-1 < SPI < -1.49	Moderately dry
-1.5 < SPI < -1.99	Very dry
SPI < -2	Extremely dry

Source: McKee et al, 1993; Cancelliere et al. 2007

The ML method, as introduced by Streit and Luginbuhl (1994), facilitates the estimation of parameters in a regression model, assuming knowledge of the true distribution law of these parameters. It involves maximizing the likelihood function, also known as the joint density function, with respect to the parameters for a given sample. The objective is to identify the parameter that has a high probability of reproducing the observed values of the sample, thereby closely matching the true values (Streit & Luginbuhl, 1994).

In simpler terms, the ML method seeks to determine the most likely value of a parameter for a population, based on a given sample (Horvath, 1993). When applied to a dataset, it identifies the distribution parameter value that maximizes the likelihood function (Meng et al., 2014).

Random sample is $X_1,X_2,X_3,\ldots,X_n$ from a distribution $F(x;\theta_1,\theta_2,\ldots,\theta_p)$, where $\theta_1,\theta_2,\ldots,\theta_p$ are parameters of the distribution. The maximum likelihood (ML) estimators, denoted as $\hat{\theta}_1, \hat{\theta}_2, \ldots., \hat{\theta}_p$, are obtained as the solutions to the system of p equations:

$$\frac{\partial \mathrm{L}(\theta_1,\theta_2,\ldots.\theta_p)}{\partial \theta_r} = 0 \qquad (1)$$

For r = 1,2,…,p, where the likelihood function is defined as:

$$\mathrm{L}(\theta_1, \theta_2, \ldots.\theta_p) = \prod_i^n f\left(X_i, \theta_1, \theta_2, \ldots.\theta_p\right) \qquad (2)$$

Maximizing the log of the likelihood function is often preferred as it simplifies the calculations. Both methods, maximizing the probability function and maximizing its logarithm, lead to the same maximum value because the logarithm is a monotonically increasing function. Therefore, we maximize the log-likelihood function:

$$ln\, \mathrm{L}(\theta_1, \theta_2, \ldots.\theta_p) = \prod_i^n ln\, f\left(X_i, \theta_1, \theta_2, \ldots.\theta_p\right) \qquad (3)$$

This simplifies the maximization process and still yields the same maximum likelihood estimators.

the maximum likelihood (ML) method is widely regarded as an efficient estimator due to several favorable properties it exhibits.

Firstly, it typically yields estimators with lower variance compared to other methods, making it desirable for statistical inference. This property contributes to the precision and reliability of the estimates produced.

Moreover, the ML method tends to produce even more satisfactory results when applied to large datasets, particularly those with a sample size greater than 100 ($n > 100$). With larger sample sizes, the estimates tend to converge more closely to the true population parameters, enhancing the accuracy of the estimation process.

Furthermore, the ML estimator possesses several desirable properties of a good estimator. Firstly, it is consistent, meaning that it tends to converge to the true value of the parameter (θ) as the sample size increases. Additionally, the ML estimator is asymptotically unbiased, implying that the expected value of the estimator approaches the true parameter value as the sample size tends to infinity. Lastly, the ML estimator is asymptotically efficient, suggesting that it achieves the lowest possible variance among all consistent estimators, making it highly desirable for statistical inference (Horvath, 1993). These favorable properties contribute to the widespread use and popularity of the ML method in statistical analysis and modelling.

Bootstrap model application. The main advantage of the bootstrap resampling approach is that good estimates can be obtained regardless of the complexity of the data processing. In the context of density, the bootstrap method can be effectively used to estimate statistics such as skewness and kurtosis to explore density functions that closely represent the underlying reality of the data (Delignette-Muller and Dutang 2015). The following steps are typically taken to estimate skewness and kurtosis using Bootstrap:

Resample: Randomly sampling observations with replacement from the original data set to create multiple bootstrap samples.

Estimation: Compute skewness and kurtosis for each bootstrap sample.

Aggregation: Calculate the average skewness and kurtosis over all bootstrap samples.

By using bootstrapping in this way, obtained more reliable estimates of skewness and kurtosis help to better understand the shape and distribution of the data, leading to a more accurate density estimate that is closer to the real world scenario.

A general description of the basic principle of bootstrap methods are as follows: Suppose we are interested in estimating some parameter δ, and suppose we have observations $Y_1 ..., Y_n$ from a distribution F that depends on δ.

Furthermore, we have a method for finding an estimate $\widehat{\delta}$ of δ, say $\hat{\delta}$ = T $(Y_1,...,Y_n)$. The estimator T can be as simple as computing the skewness or kurtosis of the observations.

The main idea of bootstrapping is to replace the distribution F in the above study by the empirical distribution function $\widehat{F}$.

We will show that sampling from a distribution means sampling by replacement from $Y_1,..., Y_n$. A bootstrapped sample has the same size as the original sampled data. It consists of the original observations, some of which may appear more than once, while others may not be included. We then apply the estimate T to each of them and obtain bootstrapping estimates $\widehat{\delta}_1 ,..., \widehat{\delta}_B$ of δ . To get an idea of the error and bias of T, or more generally of its sample distribution, we can then examine these bootstrap estimates.

Statistical distributions used to fit data

Gamma's law. Several studies have been carried out on the gamma law, and in particular (Choi and Wette, 1969) treat the gamma law in great detail. The X random variable follows a gamma distribution if its probit probability density functional (PDF) is:

$$f(x) = \frac{1}{\beta^{(\alpha)}\Gamma(\alpha)} x^{(\alpha-1)} \exp(-\frac{\alpha}{\beta}) \quad (4)$$

Proceed as follows to obtain the cumulative gamma function:

$$F(x) = \int_0^x f(x) \frac{1}{\beta^{(\alpha)}\Gamma(\alpha)} \int_0^x x^{(\alpha-1)} \exp(-\frac{\alpha}{\beta}) \quad (5)$$

With: α > 0 is the parameter of shape

β > 0 is the parameter of scale

Γ is the mathematical gamma function

α and β are given by the ML method in the following way:

$$\begin{cases} \hat{\alpha} = \sqrt{1 + \frac{4A}{3}} \\ \hat{\beta} = \frac{-x}{\hat{\alpha}} \\ A = \ln(-x) - \frac{\Sigma \ln(x)}{n} \end{cases} \qquad (6)$$

Where n is the number of years of observation. Note that this function is undefined for x=0, and its modified cumulative function has the form:

$$H(x) = q + (1 - q)F(x) \qquad (7)$$

where q is the probability of zero precipitation at each station over the entire period under consideration.

Lognormal's law. If the logarithm of the random variable is normally distributed, then a positive random variable x follows a lognormal distribution. The PDF of a lognormal distribution is defined as (Mage and Ott 1984):

$$f(x) = \frac{1}{x\sigma\sqrt{2\pi}} exp\left[\frac{-(\ln x - \mu)^2}{2\sigma^2}\right] \qquad (8)$$

where $x > 0$, $\sigma > 0$ and $-\infty < \mu < +\infty$

μ is a scale parameter, stretching or shrinking a distribution, and σ2 is a shape parameter, affecting distribution shape. These can be estimated using the ML estimator method in the following way:

$$\begin{cases} \hat{\mu} = \frac{1}{n}\sum_{i=1}^{n} \ln x_i \\ \hat{\sigma}^2 = \frac{1}{n}\left(\sum_{i=1}^{n} \ln x_i - \hat{\mu}\right)^2 \end{cases} \qquad (9)$$

Weibull's law. According to Panahi and Asadi (2011), the PDF of a Weibull distribution for a random positive variable X is:

$$f(x, \alpha, \beta) = \alpha\beta x^{\alpha-1}\exp(-\beta x^{\alpha}) \qquad (10)$$

Wu (2002) provides a detailed explanation of the shape and scale parameters derived by the ML approach mentioned above. Since there are no closed-form formulations for the parameters α and β, they are estimated by maximizing the equation's log-likelihood expression (Panahi and Asadi, 2011). Its complementary cumulative distribution function is a stretched exponential function, and its explicit form is provided by:

$$F(x) = 1 - \exp(-(\frac{x}{\alpha})^{\beta} \qquad (11)$$

Gumbel's law. A random variable X is distributed according to a Gumbel law (Cooray 2010), also called a double exponential law or extreme value law, if its PDF is given by:

$$f(x) = \frac{1}{\beta} exp\left[-\exp(-\frac{x-\mu}{\beta})\right]\exp(-\frac{x-\mu}{\beta}) \qquad (12)$$

With: $\mu > 0$ is the position or mode parameter

$\beta > 0$ is the non-zero scale parameter, positive or negative

-∞ < x < +∞

The ML method is used to estimate the terms μ and β. Their cumulative distribution function is given by:

$$F(x) = exp\left[-\exp(-\frac{x-\mu}{\beta})\right] \tag{13}$$

The maximum and minimum of a number of samples of normally distributed data is represented by Gumbel's law.

Exponential's law. The distribution of a random variable X is exponential if its PDF is defined as follows:

$$f(x) = \frac{1}{\beta}\frac{exp[-(x-\mu)]}{\beta} \tag{14}$$

Rahman and Pearson (2001) define x ≥ μ and β > 0 as the location and scale parameters, respectively. Commonly referred to as the constant failure rate, the scaling parameter is $\lambda = \frac{1}{\beta}$. In this way, the PDF of the exponential rule can be represented as follows:

$$f(x) = \lambda \exp[-(x-\mu)]^{\lambda} \tag{15}$$

It is designed to distribute the following:

$$F(x) = 1 - exp(-(x-\mu))\,\lambda \tag{16}$$

A random and independent sample is used to estimate the parameters μ and λ. By taking the derivative of the logarithm of the likelihood function of the exponential law, the ML estimator is determined:

$$\hat{\lambda} = \frac{1}{\bar{x}}$$

Where $\bar{x} = \frac{1}{n}\sum_{i=1}^{n} x_i$

Logistic's law. If the PDF of a random variable X is given by (Pérez-Sánchez and Senent-Aparicio 2018), then the random variable X follows a logistic law:

$$f(x) = \frac{exp\frac{-(x-\alpha)}{\beta}}{(\alpha)(1+exp\frac{-(x-\alpha)}{\beta})^2} \tag{17}$$

-∞ < x < +∞, where α is the shape parameter and β is the scale parameter that is nonzero and positive. Their cumulative distribution function is:

$$F(x) = \frac{1}{1+exp\frac{-(x-\alpha)}{\beta}} \tag{18}$$

The ML approach predicted the parameters α and β, which were used as the initial values of the program (α = 0 and β = 1).

Burr's law. Burr's XII distribution is a continuous and widely known distribution, as it incorporates the characteristics of several well-known distributions, such as the Weibull and Gamma distributions (Pérez-Sánchez and

Senent-Aparicio 2018). A random variable X is said to follow a distribution of type XII of the Burr or Burr type if its PDF is:

$$f(x) = \frac{\alpha\gamma}{\lambda}\left(\frac{x}{\lambda}\right)^{\alpha-1}\left(1 + \left(\frac{x}{\lambda}\right)^{\alpha}\right)^{-\gamma-1} \quad (19)$$

Where:

$x > 0$

$\lambda > 0$ the scale parameter

$\alpha > 0$ the shape parameter

$\gamma > 0$ the shape parameter

ML is most often used to estimate these parameters (Ghitany and Al-Awadhi 2002). Their cumulative distribution function has the form:

$$F(x) = 1 - \left(1 + \left(\frac{x}{\lambda}\right)^{\alpha}\right)^{-\gamma} \quad (20)$$

The K-S fit test. This test is inspired by the (Kolmogorov 1933) distribution fitting statistic, as mentioned by (Stephens 1970). It is a measure of the extent that the data Xi (i=1,...n) follow a specific distributional rule. K-S-Test is a nonparametric test for comparing a sample to a reference probability distribution or for comparing two samples (Mitchell, 1971). It can be used for comparing a sample with a reference probability distribution or for comparing two samples (Mitchell, 1971). This difference is negligible, and the distribution of observations fits a pre-defined distribution according to the H_0 hypothesis. The better the law fits the data, the weaker the K-S test for a given data set and distribution. Thus, a law must have a significantly lower K-S test than the others for it to be the best. The K-S test is a measure of the difference between the empirical distribution function of the sample and the cumulative distribution function of the reference distribution, or between the empirical distribution functions of two samples. The statistic (K-S) was defined by Stephens (1970) as follows:

$$D_n = max_x \|F_n(x) - F(x)\| \quad (21)$$

with $-\infty < x < +\infty$, and by means of the Glivenko-Cantelli theorem (Dehardt 1971):

$$F_n(x) = \frac{1}{n}\sum_{i=1}^{n} I_{(-\infty,\mathrm{x})}(x_i) \quad (22)$$

With: n appears the observation parameter in population x.

$F_n(x)$ represents the empirical cumulative distribution function.

$I_{(-\infty,\mathrm{x})}$ is the indicator function for the event x.

$F(x)$ shows the theoretical cumulative distribution function.

Return Period Based on Extreme Value Theory. Extreme events play a crucial role in various natural processes. Knowledge of extreme events is required for the design and management of human activities in the environment. Therefore, to be able to make conclusions about extreme values of large magnitude associated with low probabilities of occurrence, the statistical modelling of extreme values is carried out. In order to refer to this type of value, the concept of a return period (T) has been introduced. The recurrence interval T

(Meylan et al. 2008) is the average length of time that, from a statistical point of view, an event of the same intensity occurs again. In hydrologic terms, the average time interval between two events of a certain intensity. There is a simple relationship between the probability of an event occurring and its return period.

Let X be the random variable associated with the precipitation series and "p" the probability of an extreme occurrence, p = P (X >=xT). The average time between two successive occurrences of the event (X =xT) is the return period T of the event. In the case of a yearly period, the return period T is related to this probability as p=1/T. Thus, the probability that an extreme event will not occur given a year is given by:

$$P\ (\mathrm{X} < x_T) = 1 - p = 1 - \frac{1}{\mathrm{T}} \tag{23}$$

The probability of the design rain not occurring for N years, the duration of our study, is:

$$P\ (\mathrm{X} < x_T) = \left(1 - \frac{1}{\mathrm{T}}\right)^{\mathrm{N}} \tag{24}$$

We define the return level zp as the distribution of this model given by the following equations:

$$GEV(z_p) = 1 - p \tag{25}$$

This will result in:

$$1 - p = \begin{cases} exp\left[-(1 + \varepsilon\frac{z_p - \mu}{\sigma})^{\frac{-1}{\varepsilon}}\right] & si\ \varepsilon \neq 0 \\ exp\left[-\exp(-\frac{z_p - \mu}{\sigma})\right] & si\ \varepsilon = 0 \end{cases} \tag{26}$$

We can then derive the expression for zp as follows:

$$z_p = \begin{cases} \mu - \frac{\sigma}{\varepsilon}[1 - \{-\ln(1-p)\}^{-\varepsilon}] & si\ \varepsilon \neq 0 \\ \mu - \sigma ln[-\ln(1-p)] & si\ \varepsilon = 0 \end{cases} \tag{27}$$

Our choice of p is small (unlikely value). By substituting the maximum likelihood estimators for the three model parameters in the formula, we obtain the maximum likelihood estimate of zp (likelihood invariance).

RESULTS

Determining Appropriate Distribution Functions

Bootstarp method. The unbiased estimation of skewness and kurtosis values is necessary for better decision making when a given observed sample is assumed to estimate the population distribution. In particular, these characteristics can be very useful to guide the choice of the most appropriate parametric distributions, since "a non-zero skewness reveals a lack of symmetry in the empirical distribution, while the kurtosis value quantifies the weight of the tails compared to the normal distribution, for which the kurtosis is equal to 3".

As a first step, we applied a bootstrap method to consider the uncertainty of the estimated values of kurtosis and skewness from the observed data. Bootstrapping is an efficient resampling technique used to estimate the variance of statistics, especially when the underlying data distribution is unknown or complex (for example, (DiCiccio and Efron 1996). The idea is to use the observed sample to estimate the population distribution by computing the descriptive parameters of an empirical distribution, and to provide a skewness-kurtosis plot that has the square of the skewness on the x-axis and the kurtosis on the y-axis. The plot includes a point corresponding to the empirical distribution of the collected sample. It also includes bootstrapped values derived from random resampling.

To aid in the selection of distributions to fit the data, comparisons are made with values for various common distributions. For certain distributions such as normal, uniform, and logistic, where there is only one possible value for skewness (indicated by points with zero skewness) and kurtosis, these distributions are represented by distinct points on the graph. Other distributions exhibit ranges of possible values, depicted by lines (as seen with gamma and lognormal distributions) or larger areas (as observed with beta distributions). The Weibull distribution is often considered a close approximation of gamma and lognormal distributions.

In this paper, the "descdist" function of R, with boot = 1000, is employed to generate Cullen and Frey plots for the analyzed approaches, as depicted in Figure 2. Both observed and bootstrapped values exhibit notable deviations from the points representing symmetric distributions across all approaches. Consequently, to narrow our focus towards other potential distributions, we exclude symmetric distributions from consideration for fitting. Specifically, we focus on beta, log-normal, gamma, Weibull, and Burr distributions as candidate models.

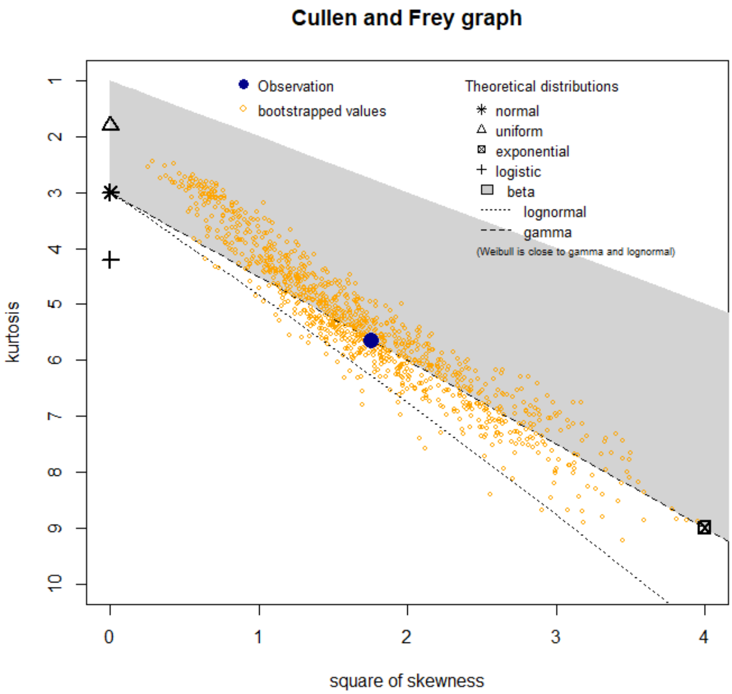
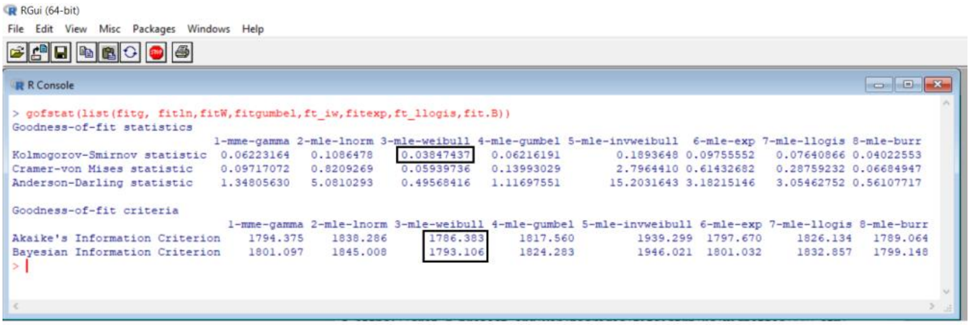


Figure 2: Determination of suitable distribution functions with Bootstrap model

Theoretical comparison. As we mentioned in the methodology section, the best fit law must have a low K-S test value, a low Akaike information criterion value, and a low Bayesian information criterion value. Figure 3 shows that the law with the lowest values of these goodness of fit tests is the Weibull law with K-S=0.03, AIC=1786.38 and BIC=1793.10; it is followed by Burr's law with K-S=0.04, AIC=1789.06 and BIC=1799.14; and Gumbel's law with K-S=0.06, AIC=1817.56 and BIC=1824.28.

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> gofstat(list(fitg, fitln,fitW,fitgumbel,ft_iw,fitexp,ft_llogis,fit.B))
Goodness-of-fit statistics
                             1-mme-gamma 2-mle-lnorm 3-mle-weibull 4-mle-gumbel 5-mle-invweibull  6-mle-exp 7-mle-llogis 8-mle-burr
Kolmogorov-Smirnov statistic  0.06223164   0.1086478    0.03847437   0.06216191        0.1893648 0.09755552   0.07640866 0.04022553
Cramer-von Mises statistic    0.09717072   0.8209269    0.05939736   0.13993029        2.7964410 0.61432682   0.28759232 0.06684947
Anderson-Darling statistic    1.34805630   5.0810293    0.49568416   1.11697551       15.2031643 3.18215146   3.05462752 0.56107717

Goodness-of-fit criteria
                               1-mme-gamma 2-mle-lnorm 3-mle-weibull 4-mle-gumbel 5-mle-invweibull 6-mle-exp 7-mle-llogis 8-mle-burr
Akaike's Information Criterion    1794.375    1838.286      1786.383     1817.560         1939.299  1797.670     1826.134   1789.064
Bayesian Information Criterion    1801.097    1845.008      1793.106     1824.283         1946.021  1801.032     1832.857   1799.148
>
```

Figure 3: Theoretical determination of suitable distribution functions

Graphical comparison. Figure 4 displays a graphical comparison of all cumulative distribution functions for empirical rainfall alongside each of the test distribution functions. The results are presented for the study station and for a 12-month time series (TS). The findings corroborate those obtained from the theoretical comparison, indicating that the Weibull distribution provides the best fit, followed by the Burr and Gumbel distributions. The test comparing empirical and theoretical cumulative distribution functions (CDFs) reveals that the distributions best fitting the precipitation series and exhibiting nearly perfect alignment are Weibull's, Burr's, and Gumbel's distribution, respectively.

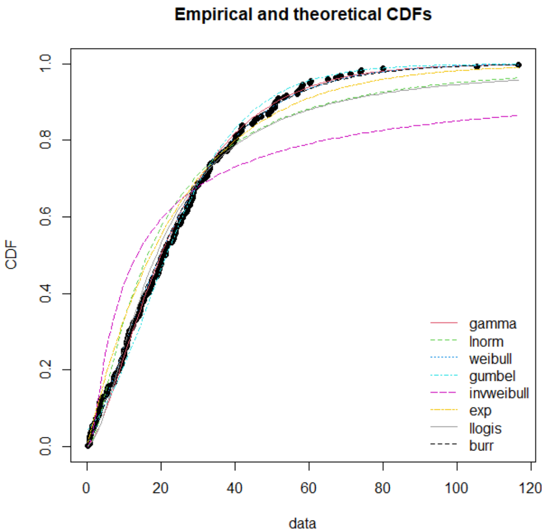

Figure 4: Graphical determination of suitable distribution functions

Weibull distribution fitting. To test hypotheses, we begin by stating a null hypothesis and an alternative hypothesis. For example, if we want to compare rates between two groups, we might say that the null hypothesizes that the rates will be the same, and the alternative hypothesizes that the rates will vary.

To test the truth of the null hypothesis, we then collect data. Specifically, the data allow us to calculate the p-value, which is defined as "the probability, under a particular statistical model, that a statistical summary of the data would be equal to or more extreme than its observed value" (Wasserstein and Lazar 2016), and is in effect a reflection of the degree of consistency of the data with the null hypothesis.

Usually, we reject the null hypothesis and accept the alternative hypothesis if the p-value is less than the 0.05 significance level. In our case, the P-value is equal to 0.89 (Figure 5), which is a very large value at 0.05. Therefore, the H0 hypothesis is accepted.

Also, the results obtained by fitting the Weibull distribution according to the equations cited in the Methodology section show that the value of shape = 1.232 and the value of scale = 26.586 (Figure 5).

From Figure 6, it is clear that the empirical and theoretical densities are very close to each other, and also that the empirical and theoretical probabilities, as well as the empirical and theoretical quantiles of the observations, are well aligned to the right. Thus, we can confirm that the Weibull distribution fits our data very well.

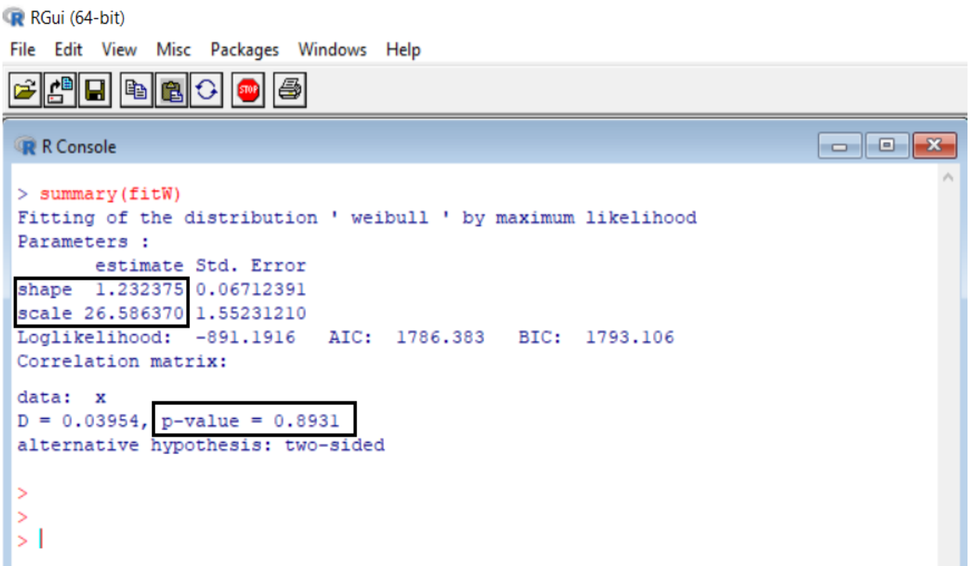

Figure 5: Summary results of Weibull's fit

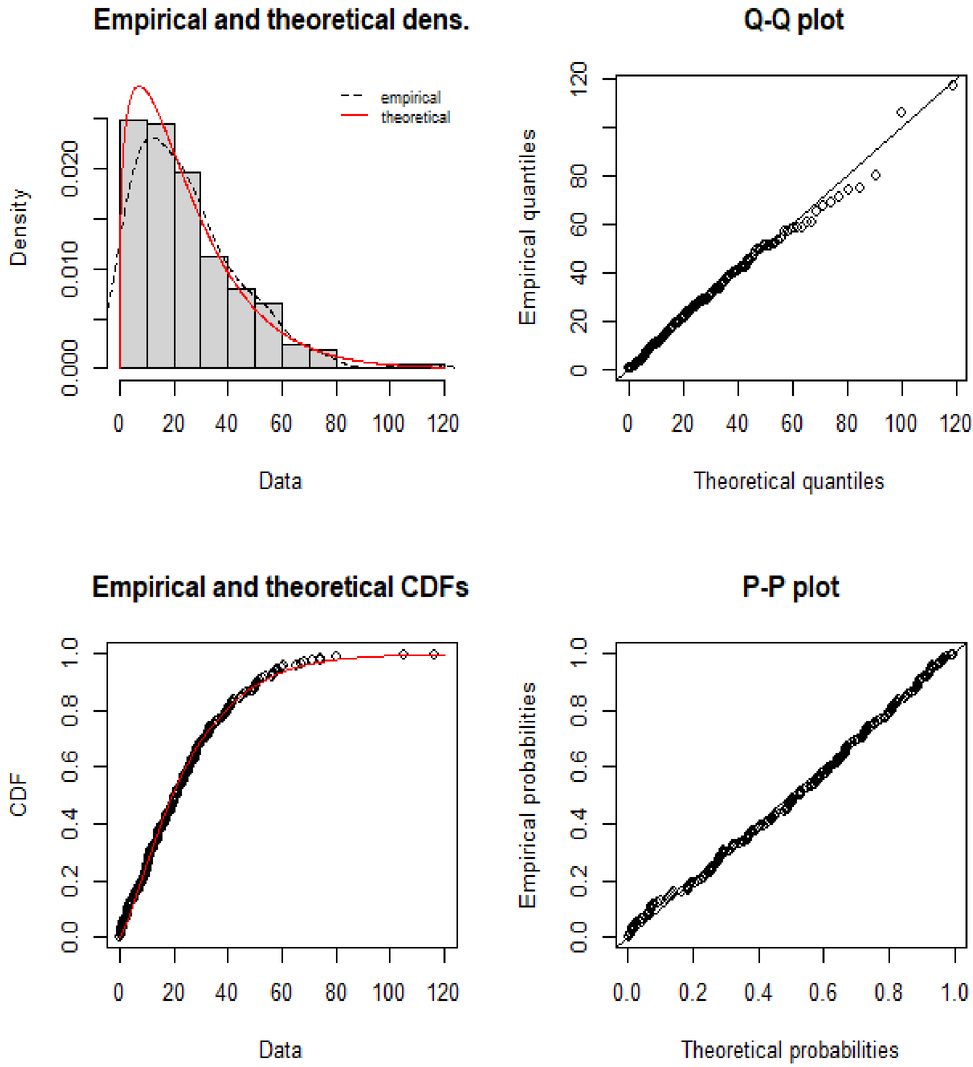

Figure 6: Empirical and theoretical CDFs of Weibull

Confirmation of Weibull Distribution Function with Bootstrap method

The results obtained by bootstrapping shape and scale values based on simulations of observed data without the intervention of distribution laws confirm the results obtained using Weibull distribution with shape value near 1.25 and scale value around 26.5 (Figure 7). Furthermore, all observations are within 95% confidence limits, which support our choice of distribution (Figure 8).

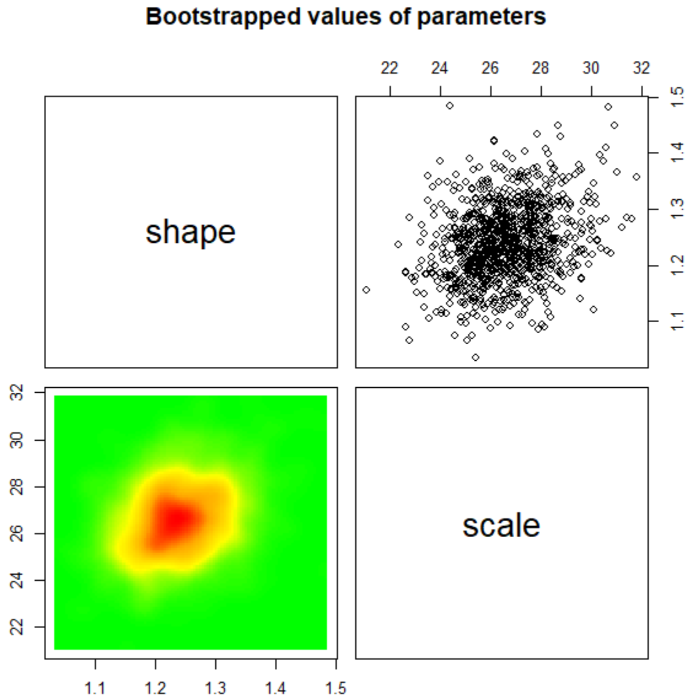

Figure 7: Shape and Scale of Weibull Distribution

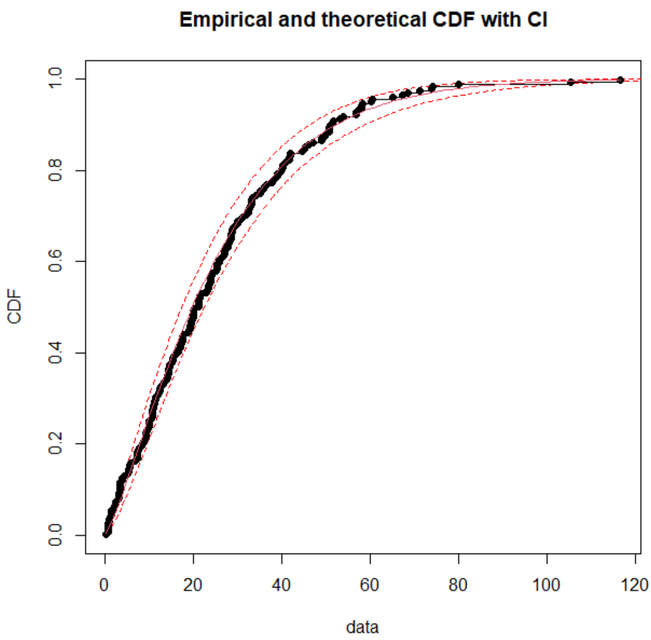

Figure 8: Weibull distribution with 95% confidence interval

Analysis of computed SPIs with adequate distributions. SPI time series were computed using the distribution most suitable for the station, and the results are illustrated in Figures 9 and 10. The 3-month SPI (Figure 9) exhibits a high frequency of drought episodes, ranging from mild to extreme. In the case of the 6-month SPI (Figure 9), there are 6 episodes of extreme drought observed. Analysis of the 9-month SPI (Figure 10) reveals 5 episodes of very severe drought and 1 episode of extreme drought recorded at the station. Furthermore, for the 12-month SPI index (Figure 10), the station experienced 3 episodes of very severe drought and 1 episode of extreme drought. Notably, the dramatic drought episodes in the years 2000 and 2021 are evident across all-time series, with an observable increase in drought duration, particularly from the 2010s onwards

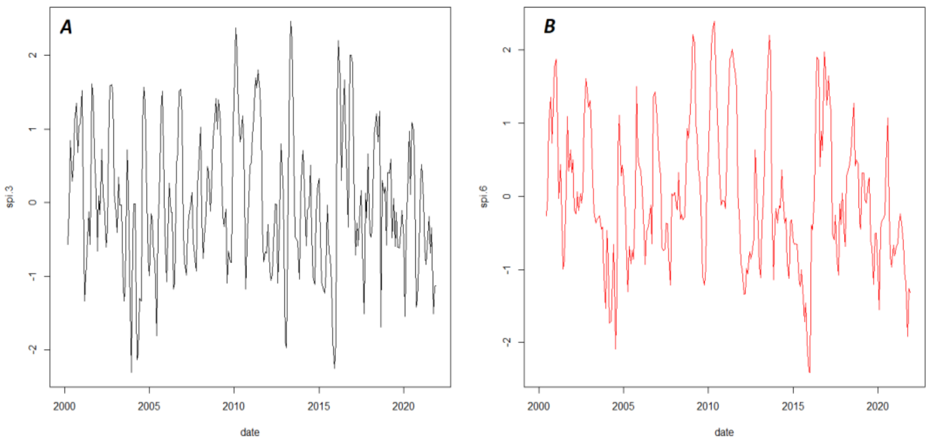

Figure 9: (A) SPIs at 3-month TS; (B) SPIs at 6-month TS

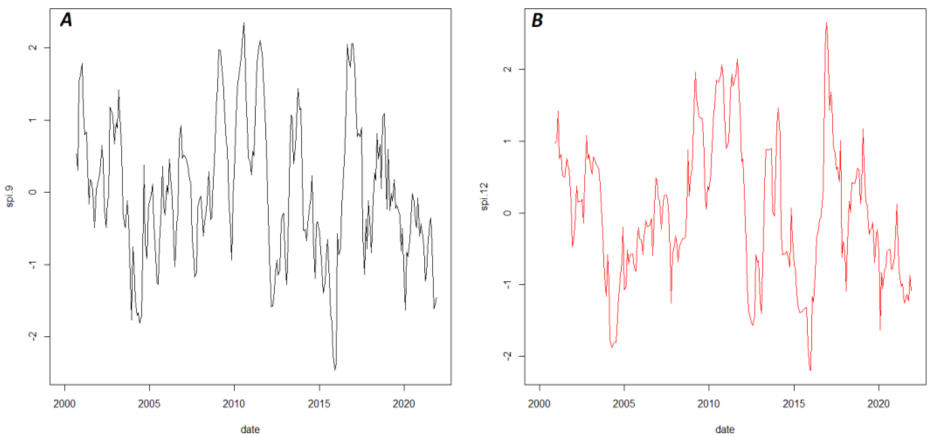

Figure 10: (A) SPIs at 9-month TS; (B) SPIs at 12-month TS

Return Period with Gumbell distribution. The station's Q-Q plot was created, and the fit of the distribution to the observed data was determined using the RMSE. The main aim of fitting the probability distribution here is to represent low-probability extreme events as accurately as possible. A Q-Q plot is used to study the level of fit of the extreme right tail (Alam et al. 2018). Any perfect match with the observed data points would fall on the [1:1] line. In Figure 11(A and B), the GEV distribution matches the data well, with the right tail close to the [1:1] line. The densities of the empirical and modelled data are very close to each other (figure 11(C)). Moreover, all observed values are within the 95% confidence interval (figure 11(B)).

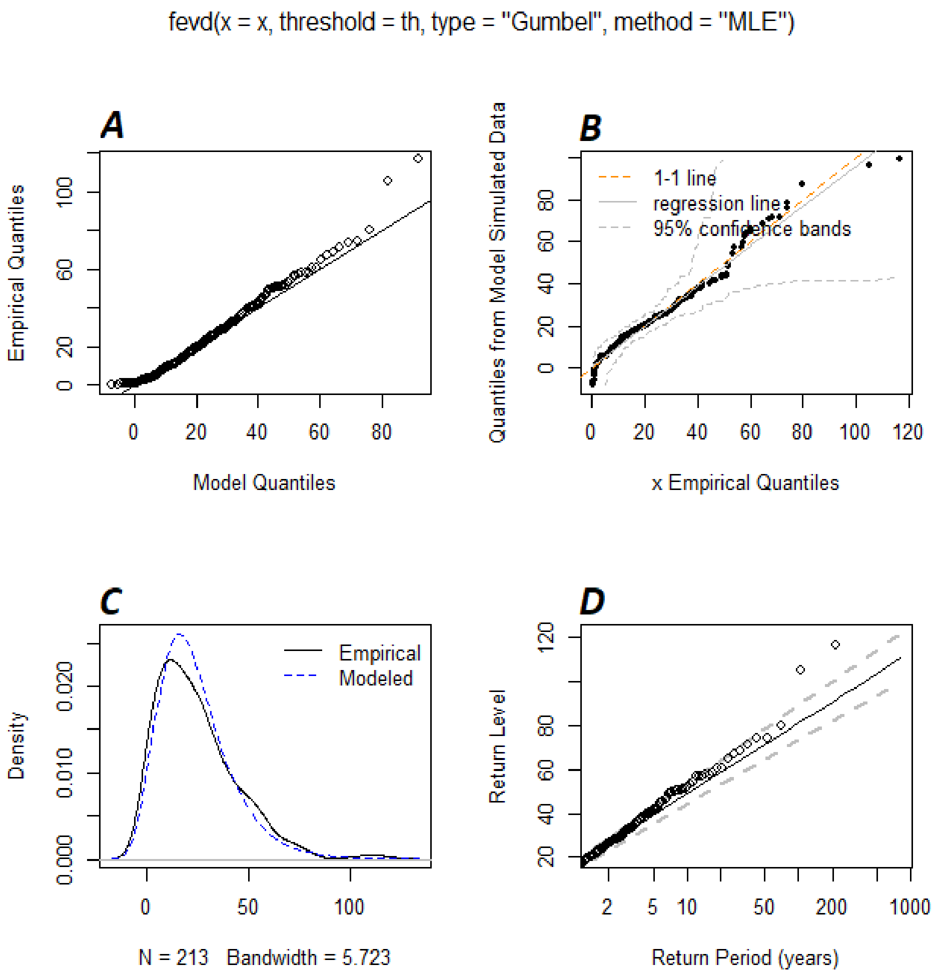

Figure 11: (A) Empirical and Model Quantiles; (B) Quantiles from model simulated data with 95% confidence interval; (C) Empirical and Modelled density; (D) Return Period.

DISCUSSION

Gamma is selected by default as the best fit without comparison with other distributions in most studies of SPI. In this study, we found that new functions (Weibull) are able to better fit the data in the station when a larger number of distribution functions are used. Therefore, depending on the geographical location of the station and the TS under consideration, the choice of the appropriate distribution function is important. These results are corroborated by our present study, which shows different distribution functions. The findings confirm those of (Fotse et al. 2024), who suggested that it is not possible to recommend a single, optimal distribution because the ratio of skewness and the coefficient of variation of the data rainfall could be the indicator for the choice of the most appropriate distribution for a particular region. Furthermore, (Angelidis et al. 2012) and (Stagge et al. 2015) considered that the appropriate probability distribution was associated with the TS of the rainfall data to be fit.

In a comparison of seven probability distributions, (Stagge et al. 2015) concluded that the gamma distribution produces the best fit for precipitation with long accumulations (> 6 months TS), while the Weibull distribution consistently performs the best for precipitation with short accumulations (1-3 months TS). In this study, weibull distribution gives best fit for rainfall with short time accumulation (3months TS), and longer time accumulation (>6months TS).

Significant variations in average precipitation patterns have a pronounced impact on the frequency and severity of droughts in the context of climate change particularly that attributed to global warming. This study shows that both intensity and duration of droughts show an increasing trend over different time scales. This observed phenomenon is likely due to reduced precipitation levels, a consequence attributed to climate change, as posited by scientific works such as those by (Beroho et al. 2020) and (Boulahfa et al. 2023). Specifically, temperature emerges as a key determinant of water availability dynamics, mainly by regulating evapotranspiration rates.

CONCLUSION

This research contributes to the improvement of mathematical methods for drought modelling, which is particularly relevant given its hazardous nature and the challenges associated with adaptation, especially in developing countries such as Morocco. In this context, the Standardized Precipitation Index (SPI) serves as a central drought indicator, prompting this study to investigate the efficacy of using alternative probability distribution functions to fit and characterize observed precipitation data - a crucial initial stage in the SPI calculation.

In this study, eight different statistical distribution functions were examined to determine the optimal fit for data from the Tangier region station, spanning the 2000-2021-time domain, at different time scales (TS) of 3, 6, 9, and 12 months. The Maximum Likelihood (ML) method was used to estimate the parameters of these distribution functions. The Kolmogorov-Smirnov (K-S) statistic served as a discriminating metric to identify the distribution functions that best fit the observed station data, which were subsequently used in the SPI calculation. The results of this analysis were used to identify patterns of drought occurrence and to quantify discrepancies resulting from the use of mismatched distribution functions. The choice of an optimal distribution function for precipitation data depends on both the geographic location of the station and the temporal scope of the analysis, as defined by the number of months in the time series (TS). In particular, the Weibull probability distribution consistently demonstrated superior performance across all TS durations.

This investigation underscores the importance of conducting a careful preliminary assessment aimed at identifying the most appropriate distribution functions for data fitting, and then using them in the SPI calculation. Such an approach is critical to reducing error and improving the accuracy of results in drought modelling and assessment.

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CLIMATE CHANGE INFLUENCE ON THE OCCURRENCE OF EXTREME DRY-WET PERIODS IN BOSNIA AND HERZEGOVINA

SUMMARY

The air temperature in Bosnia and Herzegovina is increasing (0.3 - 0.6 °C per decade), while rainfall events are more extreme. Additionally, there is an increased variability in weather conditions across all seasons, marked by rapid shifts from extremely cold to warm weather, as well as transitions from periods of exceptionally high rainfall to exceedingly dry periods. Droughts and floods pose the most significant risks. However, Bosnia and Herzegovina faces a lack of adequate information and analyses of extreme climate events, especially regarding their timing, intensity, magnitude, duration, and spatial extent. Therefore, this study aims to address these questions using the precise drought index SPEI. The identified wet years were: 1969, 1970, 1976, 1978, 1999, 2001, 2010, 2013, and 2014, while the dry years were 1961, 1971, 1983, 1990, 2000, 2003, 2007, 2011, 2012, and 2020. In the northern and western regions of BiH, the longest wet period in terms of duration and spatial extent occurred from May 2014 to July 2015. In the central and southern parts of BiH, the longest wet period extended over 19 months from February 1969 to August 1970. The longest drought with the largest spatial extent was the drought from August 2011 to July 2013. The obtained data shows a significant shift towards drier weather, as dry months have increased by 15% compared to wet months. At the same time, there has been a decrease in near-normal conditions and an increase in extremely wet months.

Keywords: SPEI, Drought, Flood, Climate change, extreme weather conditions

INTRODUCTION

Many studies on climate change in Bosnia and Herzegovina (BiH) agree that air temperatures are rising (0.3 - 0.4°C per decade), with the most

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pronounced increases occurring during the summer (up to 0.6°C per decade)(Čadro et al., 2023; Popov, 2020; Popov et al., 2018a; Popov et al., 2018b; Trbic et al., 2017). On the other hand, precipitation trends are not consistent (Čadro et al., 2020; Čadro et al., 2023; Popov et al., 2019; Popov et al., 2017; PSP-BiH, 2023), and in most cases, there are no significant changes in annual precipitation totals (FNC, 2021; TNC, 2016). However, these studies consistently emphasize that due to the increased occurrence of heavy rainfall in the total precipitation, the risk of floods has heightened, particularly in the northeastern region of Bosnia and Herzegovina (FNC, 2021; NAP, 2021).

According to regional climate models (RCM), under the Representative Concentration Pathway RCP8.5 or “worst-case scenario”, the projected change in mean daily temperature for the near future (up to 2035) in BiH, varies between 0.5 to 1.5^{o}C, while for the last period temperature increase ranges from 2.5 to 5^{o}C. Notably, there is a particular emphasis on the rise in maximum daily temperatures during the June-July-August (JJA) season (FNC, 2021). Additionally, JJA is the period with the most significant reduction in precipitation, which for the last period of the prediction may be as much as a 30% decrease, particularly in the southern part of the country (FNC, 2021). Such a situation primarily contributes to the increasing occurrence of extreme weather conditions, such as heatwaves, spring frosts, and intense rainfall leading to floods, and droughts.

During the last 20 years, nearly every year in BiH was characterized by extreme weather conditions. Specifically, 2001, 2003, 2004, 2009, 2010, 2014, and 2019 are classified as flood years, while 2000, 2003, 2007, 2011, 2012, 2013, 2015, 2016, and 2017 are categorized as years of droughts and heatwaves (FACP, 2014; NAP, 2021; PSP-BiH, 2023; TNC, 2016; UNDP, 2020). Floods and droughts are one of the main natural causes of agricultural, economic, and environmental damage (Vicente-Serrano et al., 2010).

Given this situation, significant effort has been invested globally in developing a unified approach to drought monitoring. Currently, the most widely used indices worldwide are the Palmer Drought Severity Index – PDSI (Palmer, 1965), the Standardized Precipitation Index – SPI (McKee et al., 1993), and more recently, the Standardized Precipitation Evapotranspiration Index – SPEI (Vicente-Serrano et al., 2010). SPEI is defined as a drought index. However, similar to the SPI, it has a multi-scalar character (Christidis & Stott, 2021) and it can also be utilized to represent wet periods. It requires more input data compared to the SPI but provides a more realistic depiction as it incorporates the evapotranspiration factor (Pei et al., 2020), i.e., the difference between precipitation and evapotranspiration: PRCP-ET_o (Čadro et al., 2017).

If basic climate data is available, such as air temperature (T_{min} and T_{max}), it may be sufficient for estimating evapotranspiration using methods like Thornthwaite (1948) or Hargreaves and Samani (1985). However, if there are data on relative humidity (RH_{min} and RH_{max}), wind speed (u), and sunshine duration (n), more accurate methods like standardized FAO56-PM (Allen et al., 1998) can be used to obtain more precise results.

Responsibility for climate data collection necessary for Early Warning (EW) and Decision Support Systems (DSS) is divided among the BiH entities, for the Federation of Bosnia and Herzegovina (FBiH) the Federal Hydrometeorological Institute BiH (FHMI) and for the Republika Srpska (RS) the Republic Hydrometeorological Service of Republika Srpska (RHMS). Monitoring involves daily collection of basic climate data, calculating reference evapotranspiration (Hargreaves & Samani, 1985), measuring water levels and flows of major rivers, monitoring soil temperature, and various phenological characteristics (FHMZ, 2022, 2023; RHMZ, 2023). Drought is determined using the Standardised Precipitation Index – SPI (McKee et al., 1993), with a spatial representation of moisture conditions by FBiH and RS entities, along with SPI_2 indicating the moisture condition of the last two months and the forecasted SPI_1 for the next 30 days. FHMI and RHMS are more focused on weather forecasts and drought alerts, while river basin agencies, such as the Agency for the Sava River Basin (AVP Sava) and Waters of Republika Srpska (JU VS), focus on hydrology and floods. These institutions use a hydrological-hydraulic forecasting platform and a Water Information System for stream monitoring (ISV) as part of the flood early warning system. Their responsibility is to inform relevant institutions in Bosnia and Herzegovina, as well as the public, about activities related to the protection and rescue of people and property (AVP, 2021).

Based on numerous national plans and strategic documents (NAP, 2021; TNC, 2016; Trbic et al., 2018; UNDP, 2013, 2020; WBG, 2021), BiH is committed to supporting a wide range of climate change adaptation measures including the implementation of improved water resources management systems, investments in new irrigation-drainage systems (WB, 2020), development of an early warning system for meteorological and climatic extremes with special focus on floods and droughts, support for the application of Smart Agriculture practices (drones, remote sensing, software, sensors as well as automatization and digitalization), and new farming systems more appropriate for hotter and more arid climates. Although these activities are fully aligned with European objectives and the Sofia Declaration (RCC, 2021) that Bosnia and Herzegovina signed on November 10, 2020, their implementation is delayed due to the insufficiently robust institutional, policy, and legislative framework for risk management and adaptation to climate change. Additionally, sustainable financial mechanisms for their implementation are needed (FNC, 2021; MAWFFiB, 2022).

However, BiH faces a lack of adequate information and analyses of extreme climate events, primarily floods and droughts. Often, it is not possible to get answers to a large number of questions such as: Which years in BiH were dry and which were wet? What was the strongest and longest such event? Which events had the greatest spatial extent? Are these events more frequent today than before? What is the best method for their determination? Is this process valid compared to recorded droughts and wet spells? Can this determination method be used for automatic monitoring of these occurrences and integrated into future early warning systems? Hence, the aim of this study is to address some of these questions. Specifically, using the precise drought index – Standardized Precipitation Evapotranspiration Index (SPEI), it aims to analyze the occurrence, intensity, magnitude, duration, and frequency of wet and dry periods in BiH, considering various locations (Bihać, Livno, Tuzla, Sanski Most, Sarajevo and Mostar) to analyze the spatial extent of these events. Additionally, through these analyses, the study aims to verify the credibility of the obtained data in comparison to recorded extreme floods and droughts across BiH.

MATERIAL AND METHODS

Study area and data availability

For this study, six weather stations (WS) were selected to represent all significant regions in BiH: Bihać, Tuzla, and Sanski Most in the north/west part of BiH and Sarajevo, Livno, and Mostar in the central/south part. From these WS, a dataset of continuous monthly values for the period from 1961 to 2020, covering 60 years or a total of 720 months. These data include maximum (T_{max}), average (T_{mean}), and minimum (T_{min}) air temperatures, monthly precipitation totals (PRCP), average relative air humidity (RH_{mean}), monthly insolation averages (n), and wind speed (u). The data was provided by the Federal Hydro-Meteorological Institute of BiH. The basic geographical characteristics of this location are given in Table 1.

Table 1. Basic geographical and climate characteristics of research locations

Weather station	°E	°N	Altitude (m)	PRCP (mm)	T_{mean} (ºC)	Köppen–Geiger
Bihać (BI)	15°51'	44°48'	246	1341	11.0	Cfb x“s
Tuzla (TU)	18°41'	44°32'	305	906	10.4	Cfb x“s
Sanski Most (SM)	16°40'	44°46'	158	1039	10.5	Cfb x“s
Sarajevo (SA)	18°25'	43°52'	630	940	9.9	Cfb x“s
Mostar (MO)	17°47'	43°20'	99	1493	15.0	Csa sx“
Livno (LI)	17°00'	43°49'	724	1151	9.4	Cfb x“s

Note: °E – longitude; °N – latitude; PRCP – precipitation; T_{mean} – mean air temperature; Csa sx“ – Mediterranean climate; Cfb x“s – temperate warm and humid climates

In terms of altitude, the lowest WS is located in Mostar (99 m), while the highest is in Livno (724 m). All locations fall under the classification of a temperate warm and humid climate (Cfb), except for Mostar which exhibits a Mediterranean climate, and thus has distinctive climatic characteristics, including higher average air temperature (15.0°C) and a greater total precipitation (1493 mm). However, the locations differ in terms of annual precipitation totals and average air temperatures. Precipitation ranges from 906 mm in Tuzla to 1314 mm in Bihać, and average air temperatures range from 9.4°C in Livno to 11.00°C in Bihać.

Reference evapotranspiration (ET_0) and index (SPEI) calculation

Reference evapotranspiration (ET_0) was calculated using FAO56-PM (Allen et al., 1998):

$$ET_0 = \frac{0.408\Delta(R_n - G) + \gamma \frac{900}{T_{mean} + 273} u_2 (e_s - e_a)}{\Delta + \gamma(1 + 0.34u_2)}$$

where ET_0 is the reference evapotranspiration (mm/day), R_n the net radiation at the crop surface (MJ/m^2/day), G the soil heat flux density (MJ/m^2/day), T_{mean} the mean daily air temperature at 2 m height (^{o}C), u_2 the wind speed at 2 m height (m/s), es the saturation vapor pressure (kPa), e_a the actual vapor pressure (kPa), e_s - e_a the saturation vapor pressure deficit (kPa), Δ the slope of the vapor pressure curve (kPa/^{o}C) and γ is the psychrometric constant (kPa/^{o}C).

The calculation was performed based on all necessary parameters (air temperature, air humidity, solar radiation, and wind speed). The absence of solar radiation (R_s), required for R_n calculation, was compensated by using insolation data (n) and the Ångström equation (Ångström, 1924):

$$R_s = \left(a_s + b_s \frac{n}{N}\right) R_a$$

where R_a is the extraterrestrial radiation (MJ/m^2/day), N is the maximum possible duration of sunshine or daylight hours (h), a_s is the regression constant, expressing the fraction of extraterrestrial radiation reaching the earth on overcast days (n = 0) and a_s + b_s is the fraction of extraterrestrial radiation reaching the earth on clear days (n = N). Values of a_s = 0.25 and b_s = 0.5 were used as suggested by (Allen et al., 1998). Monthly values ET_0 following previously mentioned equations were calculated using the SRCLET tool available at: https://meet.motherlandia.org/srclet/.

To assess extreme wet and dry periods or spells standardized precipitation evapotranspiration index or SPEI (Vicente-Serrano et al., 2010) is calculated for a longer 12 months' time scale ($SPEI_{12}$). $SPEI_{12}$ is a long-term wet or dry period influencing groundwater storages and the hydrological regime in general, considered a hydrological drought.

SPEI can have both positive and negative values. Positive values indicate a wet period, while negative values indicate a dry one. Based on the intensity of the value, dry and wet events are categorized. Values between -1 and 1 are defined as "Near normal" conditions, indicating a period of typical weather conditions for the given area (McKee et al., 1993; Vicente-Serrano et al., 2010). In this study, "Near normal" is divided into two categories: values from 0.0 to 1.0, which we defined as "Near normal wet", and values from 0.0 to -1.0, which we defined as

"Near normal dry". This was done to allow for a more appropriate comparison of wet and dry periods. The following table provides the classification of SPEI based on these criteria (Table 2). Additionally, a color has been assigned to represent each value. Blue indicates a wet period, while red indicates a dry one.

Table 2. Dry and wet month categories based on SPEI values

SPEI category	SPEI value	Abbreviation
Extremely wet	> 2.00	EW
Very wet	1.50 - 2.00	VW
Moderately wet	1.00 - 1.50	MW
Near normal wet	0.00 - 1.00	NNW
Near normal dry	0.00 - -1.00	NND
Moderately dry	-1.00 - -1.50	MD
Severely dry	-1.50 - -2.00	SD
Extremely dry	< -2.00	ED

Each dry or wet event can be defined by its duration, which is calculated as the number of months from the moment when the index value was 1 or higher (wet period) or -1 or lower (dry period). When the value reaches the "near normal" level (from -0,99 to 0,99), this event comes to an end. The sum of accumulated SPEI values for such an event is defined as Drought magnitude – DM (McKee et al., 1993). Peak wet-dry event intensity or drought severity is defined as the value for each month following the SPEI classification.

In this study, $SPEI_{12}$ is calculated to assess wet-dry events on an annual basis and determine wet and dry years. The number of wet-dry months is analyzed by categories, along with their percentage concerning the entire period (720 months). Furthermore, the duration, magnitude, and severity of wet-dry events are defined. To determine the influence of climate change on the occurrence of such events, the entire analysis period (1961 - 2020) is divided into two periods: the period from 1961 - 1990, defined as the “reference climatic period”, and the period from 1991 - 2020, defined as the "current state of the climate".

$SPEI_{12\ December}$, takes into account the state of the previous 12 months, considering December as the last month of that period. This allows it to be used to describe a specific year within the period of 1961 - 2020 with a single SPEI value. The values of this index are analyzed, and a linear trend is calculated to determine the impact of climate change on the occurrence of extremely wet and extremely dry periods. Additionally, the #ShowYourStripes principle is applied (Hawkins, 2023).

RESULTS AND DISCUSSION

Precipitation (PRCP) and reference evapotranspiration (ET_0)

Before we analyze wet-dry periods, it is important to examine the parameters used for their determination, namely precipitation (PRCP) and reference evapotranspiration (ET_0). Table 3 provides the average values of monthly, seasonal, and annual sums of PRCP and ET_0 for each of the six analyzed locations.

Table 3. Monthly, seasonal, and annual average sums of PRCP and ET_0 for the period 1961 - 2020.

	LO	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	DJF	MAM	JJA	SON	*Veg.*	*Ann.*
PRCP (mm)	BI	95	97	101	111	120	106	99	96	132	122	148	120	311	332	301	**402**	*664*	*1346*
	LI	96	88	92	95	82	83	52	66	98	115	154	132	316	268	201	**368**	*475*	*1153*
	TU	58	54	60	74	97	114	95	77	70	67	71	71	184	231	**286**	208	*527*	*909*
	SM	70	68	77	89	99	103	86	81	99	90	93	89	227	265	269	**282**	*556*	*1042*
	SA	69	67	69	76	85	89	78	67	80	84	90	87	223	229	234	**253**	*474*	*939*
	MO	154	140	134	125	97	74	49	66	117	151	194	180	**474**	357	189	462	*528*	*1482*
ET_0 (mm)	BI	16	23	46	72	100	118	**132**	114	74	46	26	17	57	218	**364**	146	*610*	*785*
	LI	16	26	46	69	98	116	**136**	122	81	49	27	17	59	214	**375**	157	*623*	*805*
	TU	14	22	43	67	95	110	**125**	114	75	46	24	15	50	206	**348**	145	*586*	*749*
	SM	14	23	46	70	99	115	**131**	116	76	47	25	15	52	215	**362**	148	*607*	*777*
	SA	15	23	47	70	98	114	**129**	117	79	49	26	16	54	215	**361**	154	*608*	*784*
	MO	32	42	67	87	120	144	**179**	163	110	69	39	33	107	273	**486**	218	*803*	*1084*

Note: LO – Location name; DJF – December, January, and February or winter; MAM – March, April and May or spring; JJA – June, July, and August – or summer; SON – September, October, and November or autumn; Veg. vegetation period; Ann - Annual; PRCP – Total precipitation in mm; ET_0 – Reference evapotranspiration in mm; BI – Bihać, LI – Livno; TU – Tuzla; SM – Sanski Most; SA – Sarajevo, MO - Mostar

Annually the highest sums of PRCP (1482 mm) and ET_0 (1084 mm) are recorded at the Mostar (MO) location. Following Mostar, the highest PRCP values are observed in Bihać (1346 mm) and Livno (1153 mm), while in Sanski Most (SM), Tuzla (TU), and Sarajevo, the values are similar, ranging around 1000 mm. The sum of ET_0, except for Mostar, ranges narrowly from 749 to 805 mm. When observing the seasons, only in the Tuzla location the highest amount of precipitation occurs during the summer period (June - August). In Mostar highest PCP is in winter (December – February) while in all other locations, the majority of the PRCP occurs in autumn (September - November).

Monthly, there are significant differences among the six research locations. In Mostar, which has the highest amount of PRCP, the smallest amount falls during the summer, whereas June in Tuzla (114 mm), Sanski Most (103 mm), and Sarajevo (89 mm) is one of the rainiest months of the year. In the locations of Bihać and Livno, autumn is by far the rainiest period, with September being the wettest month, averaging 148 mm in Bihać and 154 mm in Livno. The month with the highest ET_0 values at all locations is July, with its value ranging from 114 mm in Tuzla to 163 mm/month in Mostar.

Standardized precipitation evapotranspiration index (SPEI)

In this study, the Standardized Precipitation Evapotranspiration Index (SPEI) is computed using $SPEI_{12}$ and extracting $SPEI_{12\ December}$ to evaluate wet and dry events on an annual scale, enabling the identification of wet and dry years. This analysis also allows for the characterization of the duration (DU), magnitude (MA), and severity (PV) of these events.

$SPEI_{12}$

Given that any month with a SPEI value above 1 is considered a wet period, and a value below -1 is considered a dry period (table 1.), the following table provides the average values of all such months for $SPEI_{12}$, as well as the peak values for all six locations.

Table 4. The average and peak (max) values of $SPEI_{12}$ for all wet and dry months in the period from 1961 to 2020.

	Location	BI	TU	SA	SM	MO	LI	BiH
WET	Average	0.82	0.80	0.79	0.79	0.79	0.83	*0.80*
	Max	3.30 *10.2014*	2.74 *03.2015*	2.50 *06.1978*	2.94 *03.2015*	2.38 *12.2010*	2.33 *11.2010*	*3.30* *10.2014*
DRY	Average	-0.79	-0.79	-0.85	-0.81	-0.85	-0.81	*-0.82*
	Max	-2.62 *01.2004*	-2.39 *07.2012*	-2.33 *05.1983*	-2.54 *11.2011*	-2.15 *06.1989*	-2.31 *05.1989*	*-2.62* *01.2004*

Note: BI – Bihać, LI – Livno; TU – Tuzla; SM – Sanski Most; SA – Sarajevo, MO - Mostar

Based on the average values of $SPEI_{12}$ for all wet and dry months, the location with the highest values in the wet interval (1 >) is Livno (0.82), and the lowest values are in Sarajevo, Sanski Most, and Mostar (0.79). This indicates that Livno is the location where the changes in the number of wet months are most pronounced. On the other hand, the values in the dry interval (< -1) are most pronounced in Sarajevo and Mostar (-0.85), while the least pronounced are in Bihać and Tuzla (0.79). The average for BiH indicates greater changes in the direction of dry intervals (-0.82) than in wet (0.80). Moreover, if we examine the peak values, the SPEI of 3.30 is the highest value and occurred in Bihać in October 2014. Bihać also had the highest value for the dry period, which is -2.62, and occurred in January 2004. The lowest peak values were recorded in Livno and Mostar. According to the analysis of all $SPEI_{12}$ months, the highest values for wet conditions occur in the years 1978, 2010, 2014, and 2015. However, the lowest SPEI values, meaning the driest months, occurred in 1983, 1989, 2004, 2011, and 2012. Interestingly, in both the wet and dry periods, these are mostly years after 1991.

The following table presents the percentage distribution of months classified under $SPEI_{12}$ from extremely wet (SPEI >2) to extremely dry (SPEI <-2) for all research locations, as well as the average for BiH. The data is divided into two climatic periods, 1961 - 1990 and 1991 – 2020, and the difference in percentage values between these two periods is also provided (Table 5).

Based on the analysis of the percentage distribution of months in different $SPEI_{12}$ categories, which was conducted separately for two climatic periods (Table 5), and the calculated differences between them, it can be noted that there has been an increase in extremely wet months at all locations except Sarajevo, and a decrease in near normal conditions at all locations, with a particularly significant decrease in Tuzla, where the number is lower by 24%.

Table 5. The percentage distribution of months in different $SPEI_{12}$ categories

	1961 - 1990							1991 - 2020							Difference						
	B	T	S	SA	M	L	BiH	B	T	S	SA	M	L	BiH	B	T	S	SA	M	L	BiH
EW	1	1	3	0	1	0	1	4	4	0	4	1	2	2	**4**	3	-3	**4**	0	2	2
VW	2	3	6	6	7	9	5	3	7	4	2	5	4	4	1	4	-2	-4	-1	-4	-1
MW	9	6	10	11	14	11	10	13	9	11	8	6	9	9	3	3	1	-3	-8	-3	-1
NNW	36	47	37	36	38	32	38	31	23	33	36	32	32	31	-5	**-24**	-4	0	-6	0	-6
NND	38	31	30	34	27	36	33	33	39	29	33	34	33	33	-5	8	-1	-1	7	-4	1
MD	10	4	7	9	7	7	8	8	11	15	8	13	12	11	-3	**7**	**8**	-1	6	5	4
SD	3	5	5	3	6	2	4	6	5	7	7	8	8	7	2	0	2	4	2	6	3
ED	1	3	2	2	1	2	2	3	2	1	3	0	1	2	2	0	0	1	0	-1	0
WET	47	57	56	52	60	52	54	51	43	48	49	44	47	47	4	**-15**	**-8**	**-4**	**-15**	**-5**	**-7**
DRY	53	43	44	48	40	48	46	49	57	53	51	56	53	53	-4	15	8	4	15	5	7

Note: BI – Bihać, LI – Livno; TU – Tuzla; SM – Sanski Most; SA – Sarajevo, MO – Mostar; EW - Extremely wet; VW - Very wet; MW - Moderately wet; NNW - Near normal wet; NND - Near normal dry; MD - Moderately dry; SD - Severely dry; ED - Extremely dry

Essentially, the changes in dry months are less pronounced than the changes in wet months. There's a general increase in the number of dry months, particularly in the category of severely dry, and this increase is most noticeable in Tuzla and Sarajevo, where it's 7% and 8% respectively. The data indicates a marked shift towards drier weather conditions, with a general increase in dry months in contrast to wet months, except Bihać. The change is most pronounced in Tuzla and Mostar, where dry months have increased by 15% compared to wet months. Additionally, there has been a decline in near-normal weather conditions and a rise in extremely wet months. This could have a range of potential impacts in the future, such as changes in agriculture and water management, increased risk of flooding, and socio-economic implications for affected communities.

The following table presents the analysis of the magnitude (MA), duration in months (DU), and peak value (PV) of wet-dry periods based on $SPEI_{12}$ for each research location. The following table displays only the first 5 wet-dry periods ranked highest in magnitude (MA) value.

The data presented in Table 6, namely the wet and dry periods, are ranked according to their magnitude (MA), which is calculated as the sum of SPEI values from the beginning to the end of a given wet-dry.

In the overall analyzed period, the wettest period with the highest magnitude of 31.11 occurred in Bihać, lasting for 13 months from 07.2014 to 07.2015. The next wettest periods were in Sarajevo, with a magnitude of 30.48 (02.1969 - 08.1970), and in Sanski Most with a magnitude of 30.05 in the same period as Bihać. The longest recorded wet period (DU) was observed in Sarajevo and Sanski Most (02.1969 - 08.1970) when the monthly value of $SPEI_{12}$ remained above 1 for a remarkable 19 months.

Table 6. Magnitude (MA), duration in months (DU), and peak value (PV) of wet-dry periods based on $SPEI_{12}$

	WET					**DRY**				
	RA	Period	DU	MA	PV	RA	Period	DU	MA	PV
BI	1	***07.2014 - 07.2015***	13	31.11	3.30	1	***09.2011 - 10.2012***	14	-27.12	-2.41
	2	***01.1994 - 07.1994***	9	12.26	1.65	2	***08.2003 - 08.2004***	13	-24.55	-2.62
	3	***05.2010 - 12.2010***	8	15.43	2.78	3	*05.1990 - 02.1991*	10	-16.94	-2.27
	4	*01.1977 - 07.1977*	7	12.22	2.02	4	***04.2007 - 10.2007***	7	-11.94	-1.97
	5	***12.1999 - 05.2000***	6	8.71	1.70	5	*07.1971 - 03.1972*	9	-11.89	-1.59
TU	1	*02.1970 - 03.1971*	14	23.82	2.30	1	***08.2011 - 07.2013***	24	-42.93	-2.39
	2	***07.2001 - 08.2002***	14	23.42	2.25	2	*07.1971 - 06.1972*	12	-23.07	-2.23
	3	***05.2010 - 03.2011***	11	19.54	2.24	3	*06.1990 - 04.1991*	11	-19.64	-2.36
	4	***01.2015 - 06.2015***	6	12.91	2.74	4	***06.2007 - 04.2008***	11	-15.37	-2.12
	5	***06.2005 - 12.2005***	7	11.15	1.97	5	*11.1988 - 06.1989*	8	-12.54	-1.96
SM	1	***07.2014 - 07.2015***	13	30.05	2.94	1	***08.2011 - 01.2013***	18	-33.59	-2.54
	2	***05.2010 - 02.2011***	10	17.55	2.48	2	*05.1988 - 06.1989*	13	-18.46	-2.10
	3	*10.1974 - 09.1975*	12	16.72	1.89	3	***07.2000 - 05.2001***	11	-17.93	-2.01
	4	*07.1976 - 05.1977*	11	16.47	1.78	4	*07.1971 - 06.1972*	12	-17.77	-1.83
	5	*10.1972 - 07.1973*	10	15.78	1.86	5	*05.1990 - 02.1991*	10	-16.88	-2.34
SA	1	*02.1969 - 08.1970*	19	30.48	2.30	1	***12.2019 - 12.2020***	13	-21.55	-2.28
	2	*02.1978 - 04.1979*	15	28.52	2.50	2	***11.1992 - 01.1994***	15	-21.43	-2.10
	3	***01.2010 - 12.2010***	12	16.42	1.76	3	*07.1990 - 06.1991*	12	-21.21	-2.18
	4	***08.2005 - 01.2006***	6	8.78	1.67	4	*08.1982 - 08.1983*	13	-20.94	-2.33
	5	***02.2005 - 06.2005***	5	6.23	1.44	5	*09.1973 - 05.1974*	9	-12.00	-1.69
MO	1	***02.2010 - 02.2011***	13	23.65	2.38	1	*01.1989 - 01.1991*	25	-41.02	-2.15
	2	*03.1978 - 04.1979*	14	22.85	2.12	2	***11.2011 - 10.2012***	12	-18.89	-1.94
	3	***03.2013 - 01.2014***	12	21.65	2.16	3	***02.1993 - 12.1993***	11	-17.04	-2.01
	4	*12.1976 - 11.1977*	12	20.96	2.22	4	***10.1994 - 07.1995***	10	-16.19	-1.94
	5	*12.1969 - 08.1970*	9	14.22	1.91	5	***12.2006 - 11.2007***	12	-15.85	-1.73
LI	1	*02.1969 - 08.1970*	19	28.04	1.90	1	*05.1990 - 04.1991*	12	-21.39	-2.30
	2	***07.2014 - 06.2015***	12	21.68	2.13	2	***11.2011 - 09.2012***	12	-18.38	-2.03
	3	***03.2010 - 03.2011***	13	20.81	2.33	3	***09.1997 - 08.1998***	12	-16.26	-1.63
	4	*05.1978 - 04.1979*	12	17.7	1.77	4	***01.1993 - 10.1993***	10	-14.74	-1.95
	5	*11.1964 - 09.1965*	11	17.56	1.85	5	***07.2003 - 02.2004***	8	-13.94	-2.05
Bihać (BI)			43	79.74	2.29			53	-92.44	-2.17
Tuzla (TU)			52	90.84	2.30			66	-113.55	-2.21
Sanski Most (SM)			56	96.57	2.19			64	-104.63	-2.16
Sarejevo (SA)			57	90.43	1.93			62	-97.13	-2.12
Mostar (MO)			60	103.33	2.16			70	-108.99	-1.95
Livno (LI)			67	105.79	2.00			54	-84.71	-1.99

Note: BI – Bihać, LI – Livno; TU – Tuzla; SM – Sanski Most; SA – Sarajevo, MO – Mostar; RA – Rank; DU – Duration in months; MA – Magnitude; PV – Peak value

Summing up the values of the first five wet periods for each location points to the longest wet periods in Livno (105.79) and Mostar (103.33). However, the months with the highest SPEI values are in Tuzla (2.30) and Bihać

(2.29). It is also interesting to note that of 30 highest magnitude wet periods, 17 occurred after 1991. The wet period in 2010 and 2011 varied in duration but is present at all research locations. The wet period in 2014 and 2015 is present at all locations except in Sarajevo and Mostar. Other notable wet periods include 1976 - 1977, 1978 - 1979, and 2001 - 2002.

Based on the obtained magnitude values (MA), we can see that droughts are more pronounced than wet periods in BiH. Tuzla is the location where the highest drought magnitude of -42.93 was recorded, referring to a dry period that lasted for an extensive 24 months from 08.2011 to 07.2023. Following this drought in Tuzla, a high-magnitude drought period is observed in Mostar, with a value of -41.02. This dry period lasted even longer, spanning 25 months from 01.1989 to 01.1991. The drought in Bihać during 2003 and 2004 had the highest peak value (PV) of -2,62.

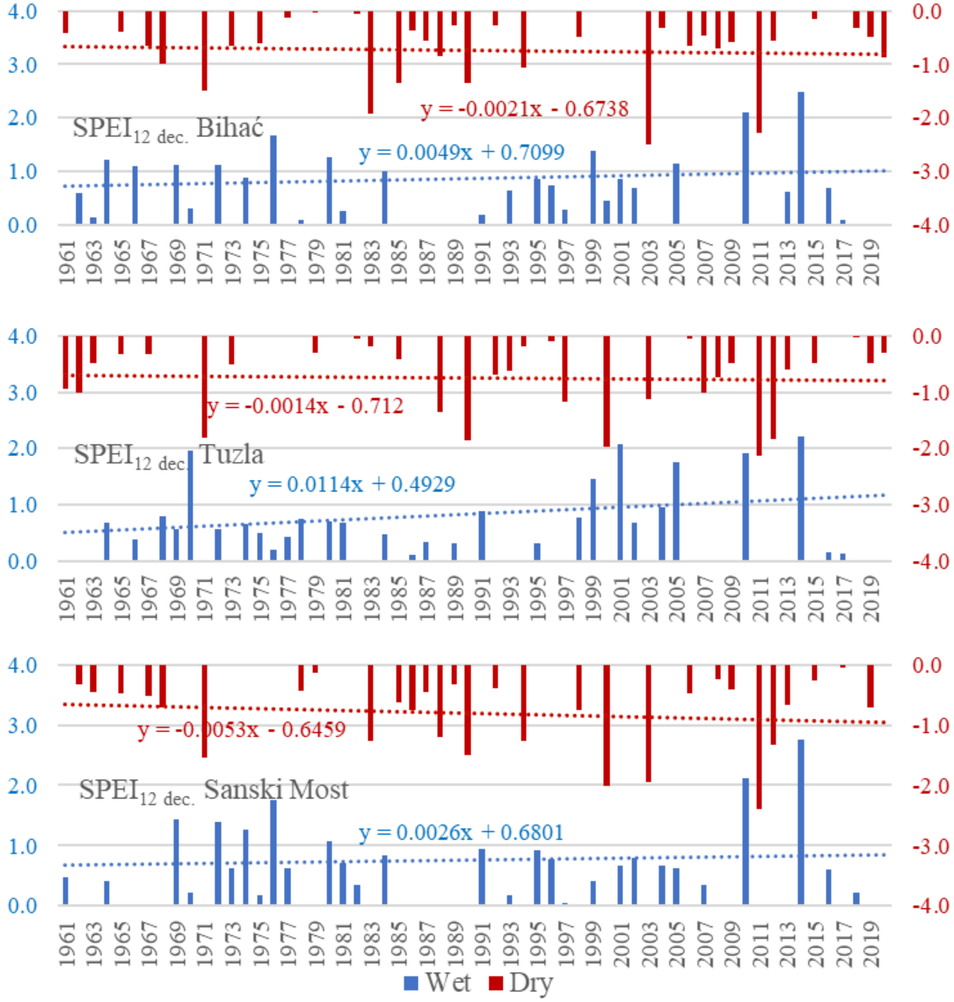
By summing up the values of the five most pronounced droughts for each location individually, the droughts with the highest magnitude are present in Tuzla (-113.55), where we also have the highest peak values (-2.21), followed by Mostar (-108.99) and Sanski Most (-104.63). Out of these 30 analyzed drought periods, similar to wet periods, 17 of them occurred after 1991. This clearly indicates a trend towards more extreme values of this indicator as a result of climate change.

The period from May 1990 to no later than June 1991, emerges as a significant drought period at all locations, with magnitudes ranging from -16.94 to -41.02. Additionally, the drought in 2011 and 2012 is present at all locations except in Sarajevo. Other notable droughts occurred in the following periods: 1971 - 1972, 1988 - 1989, 1993 - 1994, 2000 - 2001, 2003 - 2004, 2007 - 2008, and 2019 - 2020.

$SPEI_{12\ December}$

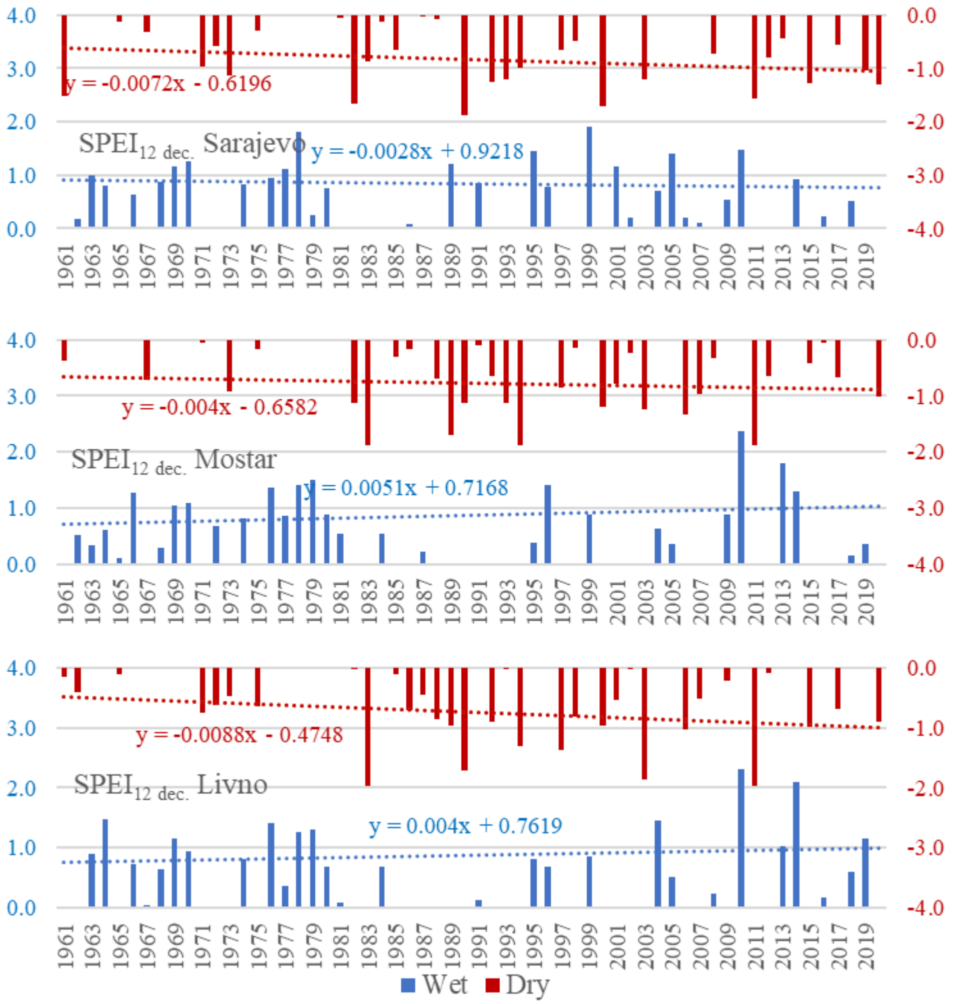
The results for $SPEI_{12\ December}$, are presented in the following graphs 1 and 2. This index considers the conditions of the preceding 12 months, with December as the concluding month of this period. Consequently, it can be used, to sum up a specific year within the timeframe of 1961 - 2020 using a singular SPEI value. Additionally, trend analysis can be applied to these data to assess the influence of climate change on the future occurrence of wet and dry years. Graph 1 illustrates the $SPEI_{12\ December}$ status for the first three research locations, namely: Bihać, Tuzla, and Sanski Most.

It can be observed that after the year 1991, there are years with higher SPEI values, indicating an increased occurrence of severe and extreme years, both wet and dry. The trend analysis of wet years indicates a positive trend, most pronounced in the Tuzla area. Similarly, the trend of dry conditions shows an increase in all locations, with the most pronounced in Sanski Most. Across these three locations, there are certain differences. Firstly, the trend in wet conditions shows an increase in Mostar and Livno, but a decrease or rather a fairly stable state with no significant changes in Sarajevo. It is interesting to note that the unstable period with many wet years during the 1970s was replaced by a stable condition up until the year 2000, after which we have fewer wet years but with high index values. Regarding drought, similar to the previous three locations, here we also observe a positive trend. More intense drought years are becoming increasingly common. The trend is particularly pronounced in the Livno area. It can be noted that we can see a fairly stable condition until the year 1983, after which there were regular drought years of strong intensity.



Graph 1. The values of $SPEI_{12\ December}$ and the trend of wet and dry conditions in Bihać, Tuzla, and Sanski Most.

Graph 2 illustrates the $SPEI_{12\ December}$ status for the second three research locations, namely: Sarajevo, Livno, and Mostar.

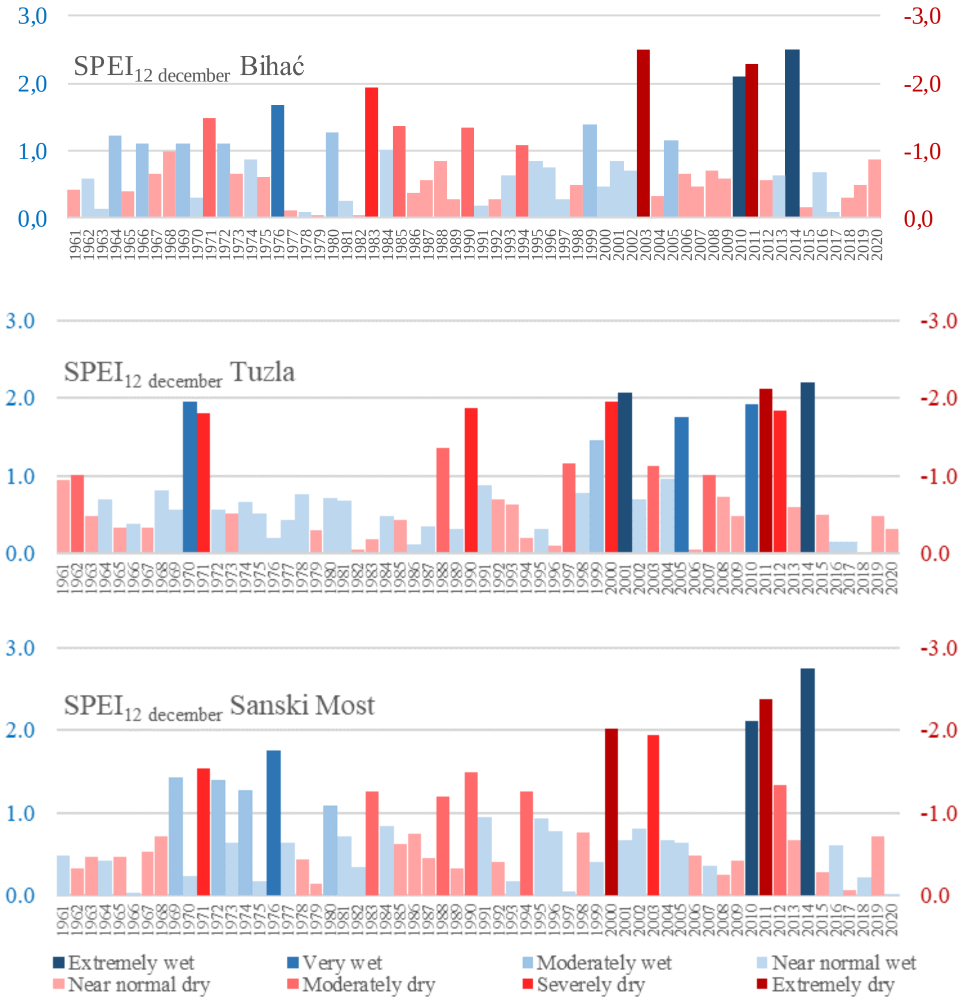


Graph 2. The values of $SPEI_{12\ December}$ and the trend of wet and dry conditions in Sarajevo, Livno and Mostar.

To illustrate all wet and dry years based on their intensity or categories, we applied the Ed Hawkins "ClimateSTripes" or "#ShowYourStripes" principle (Hawkins, 2023), specifically using Drought Stripes. Graph 3 displays drought stripes for the locations of Bihać, Tuzla, and Sanski Most. From Graph 3, wet and dry years as well as their intensity are clearly visible, represented by the intensity of the blue and red colors.

All extreme wet or dry years in all three locations have occurred in the last 20 years of the analyzed period (from 2000 – 2020). At each location, we have two extremely wet years. In Bihać and Sanski Most, these are 2010 (2.10 - 2.11) and 2014 (2.20 - 2.75), while in Tuzla, they are 2001 (2.07) and 2014 (2.02). In Bihać and Sanski Most, we have one severely wet year, which is 1976 (1.67 -

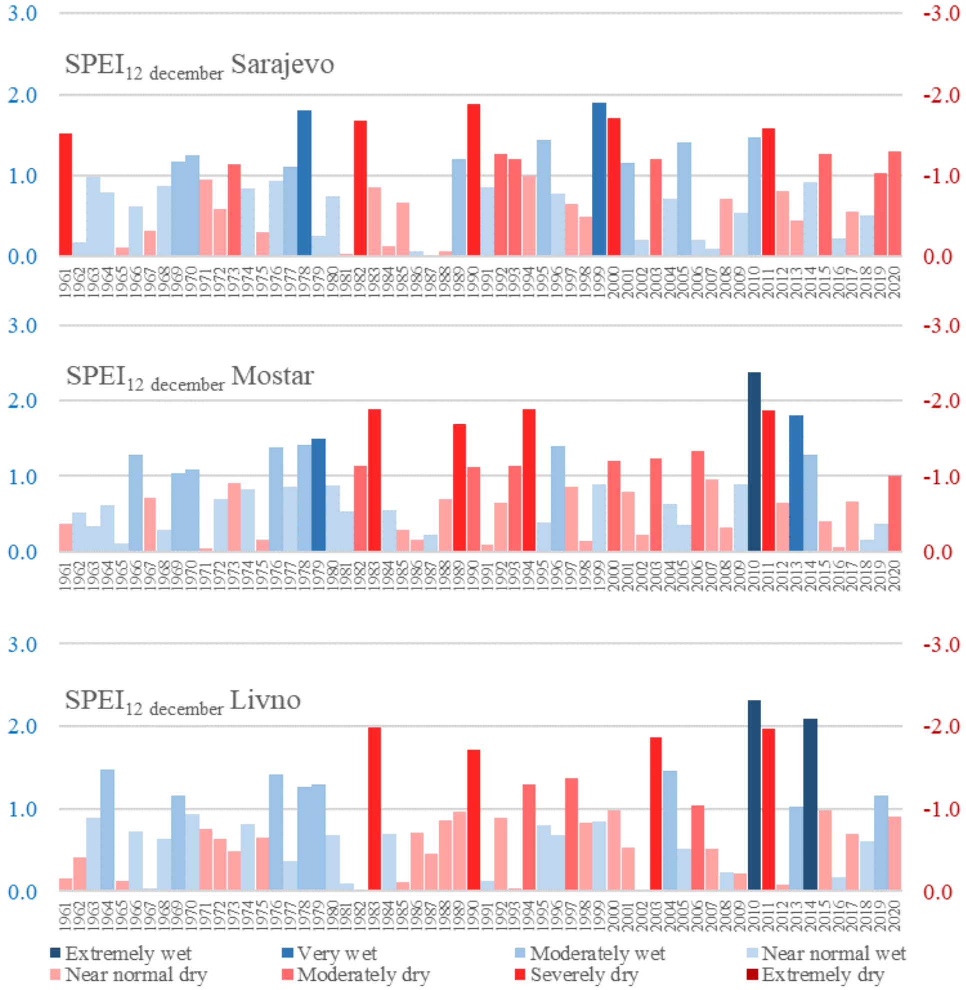
1.76), while in Tuzla, we have as many as three: 1970 (1.95), 2005 (1.75), and 2010 (1.91).



Graph 3. #DroughtStripes for Bihać, Tuzla, and Sanski Most locations

When it comes to dry years, extremely dry periods were observed in Bihać in 2003 (-2.50) and 2011 (-2.29), in Tuzla only in 2011 (-2.12), while in Sanski Most in 2000 (-2.01) and 2011 (-2.38). Additionally, in Bihać, we had one severely dry year in 1983 (-1.92). In Tuzla, there were as many as four: 1971 (-1.81), 1990 (-1.86), 2000 (-1.96), and 2012 (-1.83). In Sanski Most, two years stood out: 1971 (-1.53) and 2003 (-1.93). It is interesting to note a higher intensity of extreme conditions in the period after the 1990s. This is particularly noticeable at the location of Tuzla, where from 1972 to 1987, we have very consistent conditions with index values not exceeding an absolute value of 1.

Graph 4 displays drought stripes for the locations of Sarajevo, Livno and Mostar.



Graph 4. #DroughtStripes for Sarajevo, Livno and Mostar locations

Extremely wet conditions were not recorded at the Sarajevo location. In Mostar, an extremely wet year was 2010 (2.37), while in Livno, there were two such years: 2010 (2.31) and 2014 (2.09). Nevertheless, in Sarajevo, two severely wet years were found: 1978 (1.79) and 1999 (1.89). Similarly, in Mostar, two such years were observed: 1979 (1.50) and 2013 (1.80). Severely wet years were not recorded in Livno.

Graph 4 shows that the period until 1981 is mostly characterized by wet conditions in most cases. Afterward, a period of drought followed, with the occurrence of extremely wet years after 2010.

Notably, Sarajevo experiences neither extremely wet nor extremely dry years. Among the locations analyzed, it appears to be the least sensitive to the influence of climate change in terms of extreme weather conditions. Similarly, Mostar and Livno do not have extremely dry years. However, all three locations do have a notable number of severely dry years. In Sarajevo, these years are: 1961 (-1.52), 1982 (-1.66), 1990 (-1.88), 2000 (-1.70), and 2011 (-1.57). In Mostar, four years exhibit this characteristic: 1983 (-1.88), 1989 (-1.70), 1994 (-1.88), and 2011 (-1.87). In Livno, there are also four such years: 1983 (-1.97), 1990 (-1.72), 2003 (-1.86), and 2011 (-1.97).

Based on the comprehensive analysis of extremely wet periods, it can be concluded that the north and west parts of BiH (Bihać, Tuzla, Sanski Most) experienced exceptionally wet years in 1970, 1976, 2001, 2010, and 2014. The central and southern parts of BiH (Sarajevo, Mostar, and Livno), due to their climatic characteristics, geographical location, and hydrological network, exhibit a higher degree of resilience to floods. In this region, exceptionally wet years were observed in 1969, 1978, 1999, 2010, 2013, and 2014. To confirm the wet years 1969, 1970, 1976, and 1978, we do not have adequate data sources. However, for 1999, 2001, 2010, 2013, and 2014, we can confirm they were years of significant floods in this area of BiH. The floods in June 2001, with rainfall ranging from 50 to 100 l/m^2, severely impacted Northern BiH (Posavina, Tuzla, Zenica-Doboj), resulting in extensive agricultural damage. In 2010, BiH experienced substantial floods that inflicted significant harm on agricultural production, with the most substantial destruction recorded in Tuzla, Zenica-Doboj (central), Posavina (north), Bosnia-Podrinje (east), and Herzegovina-Neretva Canton (south) (WBIF, 2019). Additionally, in late April and early May, as well as the beginning of August 2014, floods displaced hundreds of families, inundated numerous buildings, and damaged thousands of hectares of agricultural land. The worst situation was in the flooded areas of Zenica-Doboj, Tuzla, Brčko district, Semberija, and Posavina (CMBH, 2014; FACP, 2014).

Extremely dry periods in north and west BiH (Bihać, Tuzla, Sanski Most) were confirmed in the following years: 1971, 1983, 1990, 2000, 2003, 2007, 2011, and 2012. In the southern part of BiH, we have the exact same intensely dry years, and in the central part, in addition to the ones mentioned, 1961 and 2020 can also be considered very dry years. Given this situation, it can be stated that droughts have a larger spatial extent compared to floods. Just like with floods, these data align perfectly with the recorded droughts in BiH. Indeed, the drought in 2000 affected around 60% of agricultural production. In the summer of 2003, some regions in BiH faced a drought that resulted in over 2 billion euros in agricultural damage and impacted nearly 200,000 people. In the summer of 2007, drought destroyed more than 40% of the country's agricultural production and led to forest fires covering approximately 250 hectares of land (Čaušević et al., 2020; PSP-BiH, 2023; UNDP, 2020).

CONCLUSIONS

Considering the established aim of this study, which was to accurately determine which years in BiH can be characterized as extremely wet and which as extremely dry, when such an extreme event was most intense, lasted the longest, and covered the largest area, using the SPEI index, the wet years were identified as 1969, 1970, 1976, 1978, 1999, 2001, 2010, 2013, and 2014, while the dry years were 1961, 1971, 1983, 1990, 2000, 2003, 2007, 2011, 2012, and 2020.

In the period after 1991, fewer years are categorized as "Near Normal" or "Moderately” wet-dry, and there is an increasing number of severely dry years (2000, 2003, 2011, and 2012), often accompanied by the occurrence of extremely wet years (2010 and 2014). Given the established trend of SPEI, an intensification of this state can be expected at all locations. As a result of climate change, droughts will become more frequent and more intense, while wet years will lead to catastrophic floods, especially in the northwestern part of BiH. The obtained data shows a significant shift towards drier weather, as dry months have increased by 15% compared to wet months. At the same time, there has been a decrease in near-normal conditions and an increase in extremely wet months.

The period from 2010 to 2014 can be categorized as a period of extreme weather conditions, characterized by alternating extremely wet and extremely dry years. Historic climate trends, as well as climate projections, indicate the possibility of the recurrence of such periods, potentially even more intensified.

In the northern and western regions of BiH, the longest wet period in terms of duration and spatial extent occurred from May 2014 to July 2015. On the other hand, in the central and southern parts of BiH, the longest wet period extended over a total of 19 months from February 1969 to August 1970. The longest drought with the largest spatial extent was the drought from 2011 to 2012, which lasted up to 24 months in certain locations, such as Tuzla.

Taking into account the previous 12 months ($SPEI_{12}$), the month with the highest moisture intensity was October 2014 in Bihać, with an SPEI of 3.30. Conversely, the month with the lowest cumulative water content was also in Bihać, in January 2004, with an SPEI of -2.62.

The obtained SPEI index values align perfectly with recorded flood and drought years, indicating the potential for using this index in predictive models and early warning systems as crucial measures for climate change adaptation, enabling users to select a time interval and view the current state, as well as short-term and long-term forecasts for a specific location or on the interpolated map. Also, the next step would involve utilizing Regional Climate Models (RCM) for various Representative Concentration Pathway (RCP) scenarios to predict and analyze droughts and floods. Such analyses could be done using SPEI or similar indices.

Given the increasing occurrence of years with both droughts and floods, attributed to the intensified rainfall, such occurrences tend to be masked when analyzing $SPEI_{12}$ for the entire year. Therefore, future research should focus on analyzing shorter intervals of SPEI, especially for months where the most significant temperature changes are observed (June, July, and August) as well as precipitation levels (September and October).

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POPULATION EXPANSION OF THE BADGER *MELES MELES* IN SW POLAND DURING THE YEARS 1981-2020

SUMMARY

Based on hunting bags records from the years 1981–2020, distribution, numbers and population dynamics of the badger was analysed in in SW Poland (29 358 km^2, including 8411 km^2 forests). Before 1999, the badger was harvested in SW Poland only occasionally. From 1999 till 2020, there was a steady increase from c. 200 in 1999 to c. 1100 in 2019. This increase in harvesting was a result of a parallel increase in numbers of badgers between 1996 (1000 individuals) to 2013 (c. 6500 individuals). Crude population density of the badger in SW Poland in 2001-2020 was everywhere below 1 ind./1000 ha of the total area. The ecological density, however, ranged from 0.2 to 6.1 ind./100 ha of total wooded area.

Keywords: wildlife ecology, population dynamics, introductions.

INTRODUCTION

The European badger *Meles meles* is a representative of the family Mustelidae, widespread and common all over Europe up to Vola River. According to Lariviere & Jennings (2009) it is replaced further to the east by another congeneric species, the Asian badger *Meles leucurus*, which range extends to the Pacific Ocean from Vladivostok in the north to Hong Kong in the south. Japan islands are occupied by another species, the Japanese badger *Meles anakuma*. The Asian and Japanese badgers have been, however, considered conspecific by most authorities (e.g. Nowak 2005).

The European badger (hereafter referred to as the badger) is highly adaptative, generalist forage capable of exploiting a wide range of habitats. It prefers forests close to open fields, but it also occupies riparian habitats, waste lands and farmlands. In recent decades it even began to occupy rural and urban environments in many countries, including Poland (Roca et al. 2014). In forests the badger plays a role of an ecosystem engineer and as such it is useful. Unfortunately its closer than ever contact with human often results in damages caused to properties and cultivated plants, and increased road causalities. It is

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therefore often a subject of persecution. Even so, for a long time it is also a priced game animal hunted in most (69%) European countries (Kurek et al. 2022). However, in UK, Ireland, Denmark, Netherlands, Belgium, Luxemburg, Portugal, Belarus and Albania it is protected all year round. In 19 European countries it is also listed in Red Lists (Kurek et al. 2022).

The badger can be regarded rather as a harvester than a hunter. Its main food consists of vegetables and small and easily captured animals, such as worms and insects (Davison et al. 2006). In Central Poland, the mean number of young per breeding female was estimated at three, and the annual recruitment of young at 0.68 per adult animal. The average family size was 3.5 (young and adults) or 2.1 (only adults) individuals (Goszczyński & Skoczyńska 1996).

Controlling the badger populations, and especially the badger-human conflicts became therefore an important part of game management. Crucial for this management is a knowledge on its distribution, abundance, habitat preference. This may be regionally varied, and may also change with time. It is therefore important to monitor its population on a regular basis. In this paper, the badger population was studied in SW Poland over the last 40 years. The aim was to map its distribution and abundance on high-resolution maps, as well as to trace its year-to-year changes in its the distribution and abundance.

STUDY AREA

The study area comprised two provinces (actual voivodships) in south-western Poland, i.e. Opole Province (województwo opolskie) and Lower Silesia Province (województwo dolnośląskie). These include the following hunting regions (former voivodships in the years 1975–1999): Opole, Wrocław, Legnica, Wałbrzych and Jelenia Góra. Nowadays, the Opole hunting region is entirely located within Opole Province, while the four other hunting regions are located within the Lower Silesia Province. Opole, Wrocław and Legnica hunting regions are basically lowlands, while there are mountains in the southern parts of the Wałbrzych and Jelenia Góra hunting regions (Fig. 1).

The total surface area of such defined study area is 29 358 km^2, which constitutes 9.4% of the Poland's surface area. The land is located almost entirely within the Odra drainage system. Forests occupy 8411 km^2, i.e. 28.6% of the study area (Fig. 1). There are 42 districts, 240 counties (gminas), 127 towns and 3406 villages. The number of people living in this area was 3.87 mln in 2020.

Each hunting region is covered with a net of hunting districts (Fig. 1). Although all hunting districts include both forested and arable grounds, the proportion between them is varied. There are also meadows and pastures, human settlements (towns and villages), rivers and water bodies, waste and industry areas in each hunting district. The average annual air temperature in the lowlands in SW Poland is 10.6˚C, for Sudeten Mts 9.0˚C (the average for Poland is 9.9˚C). This average has increased form 7.6˚C in 1981–1990 to 9.3˚C in 2020 (0.29˚C per 10 years) (IMiGW 2021). The long-term (1901–2000) average precipitation for Wrocław is 583 mm per annum (in Sudeten Mts. the average is doubled). The amount of rainfall may greatly vary from year to year (318–892 mm) (Dubicka et al., 2002). In the first half of the 20th century, in most decades (except for 1901–1910) the rainfall was above the long-term average; while in the second half of 20th century, in most decades (except for the years 1971–1980) the rainfall was below the long-term average (583 mm) (Dubicka et al., 2002). In SW Poland, snow cover lasts for 30–40 days per year in lowlands, 40-50 days in uplands for, and 70 – 80 days in mountains. During the years 1981–2020 the most snowy winters were in 2005/2006 and 2009/2010, whereas the least snowy winters were in two successive winters 1988–1990 and 2006–2008 (Czarnecka 2012).

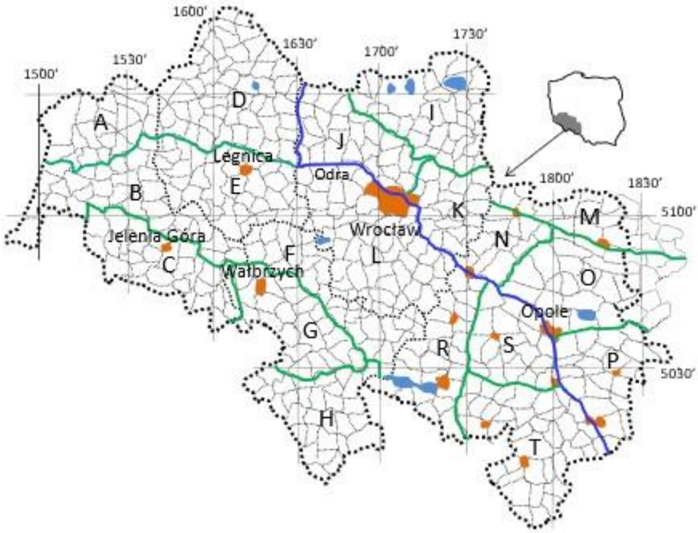

Fig.1. The study area, SW Poland, divided into hunting districts, 5 hunting regions and 19 ecoregions.

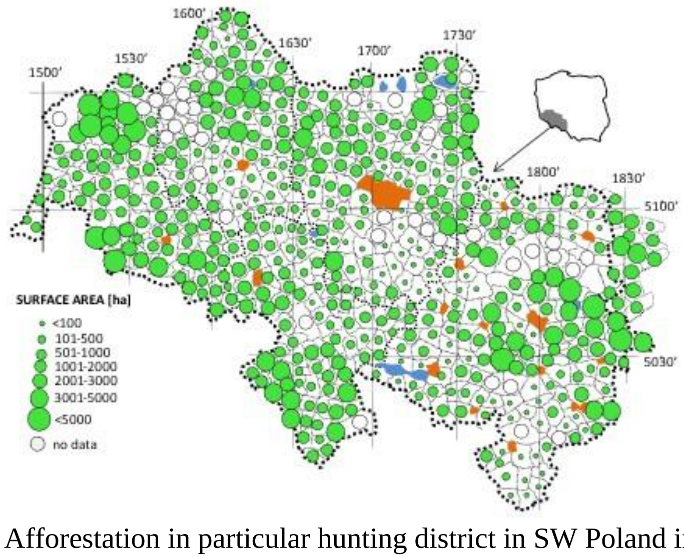

Fig. 2. Afforestation in particular hunting district in SW Poland in 2020.

Table 1. Population densities (individuals per 1000 ha) of the badger (average from 2001–2020)

#	Region	Hunting districts	Surface area [ha]		%	Popul. density	
			general	forests	forests	ecol.	crude
	Jelenia Góra Hunting Region						
A	Lower Silesian Forests	5, 7, 12, 15, 16, 20	33211	27782	83.7	0.4	0.4
B	Silesian-Lusatian Lowland	31, 33, 39, 40, 43, 52	25500	6438	25.2	1.6	0.4
C	West Sudeten Mts.	54, 55, 66, 71, 78, 80	28332	20249	71.5	0.5	0.3
	Legnica Hunting Region						
D	Northern (lowland) part	1, 2, 17, 18, 33, 35	22795	8467	37.1	2.3	0.8
E	Southern (hills) part	62, 67, 69, 71, 72, 78	29400	3754	12.8	6.1	0.5
	Wałbrzych Hunting Region						
F	Sudeten Upland	6, 7, 21, 31, 38, 39	26700	3730	14.0	1.7	0.1
G	Middle Sudeten Mts.	10, 18, 23, 25, 28, 30	26715	10576	39.6	0.7	0.2
H	East Sudeten Mts.	54, 67, 69, 70, 72, 82	16191	9491	58.6	0.7	0.4
	Wrocław Hunting Region						
I	Barycz Valley and Trzebnica Hills	2, 7, 8, 13, 15, 16	30127	10091	33.5	0.3	0.1
J	Głogów-Milicz Depression	10, 30, 32, 45, 47, 59	27803	9090	32.7	0.4	0.1
K	Oleśnica Plain	71, 85, 86, 95, 96, 107	27283	9154	33.6	1.3	0.4
L	Wrocław Plain	67, 79, 90, 100, 113, 116	28938	1884	6.5	3.0	0.2
	Opole Hunting Region						
M	Northern part of the Opole Province	3, 7, 12, 14, 15, 16	32497	4375	13.5	1.0	0.1
N	Brzeg Land	17, 19, 20, 21, 50, 51	33704	11738	34.8	0.7	0.3
O	Stobrawa Forests	28, 33, 34, 35, 36, 39	38926	32444	83.3	0.2	0.2
P	East-central part of the Opole Province	82, 83, 91, 123, 126, 129	41259	20721	50.2	0.2	0.1
R	Nysa Land	74, 76, 78, 114, 120, 122	34320	2258	6.6	1.9	0.1
S	Niemodlin Forests	47, 59, 64, 67, 96, 101	41259	20721	50.2	0.3	0.2
T	Głubczyce Plateau	105, 109, 132, 133, 138, 146	34320	2258	6.6	5.7	0.2

* Symbols in the first column (A, B, C…) refer to these in Fig. 1.
Ecological density refers to the number of harvested badgers/1000 ha of forest, whereas the crude density refers to the number of harvested badgers/1000 ha of the total surface area.

MATERIAL AND METHODS

In the case of badgers, estimates are almost invariably based on a secondary index of abundance, notably sett surveys, latrine use or game-bag data from the hunting grounds (Griffiths & Thomas 1993, Tuyttens et al. 2001). In larger areas, the badger numbers can also estimated through a questionnaire (Matyáštík & Bičík 1999, Nadolska 2002, Nadolska & Bartmańska 2003).

This study is based on records from the years 1981–2020 kept by the Polish Hunting Association Research Station in Czempiń near Poznań. Records refer to the number of badgers harvested (hunting bags) and the number of these estimated (quotas) for each hunting district (hunting ground, management area) located in SW Poland, i.e. in five hunting regions (HR): Opole, Wrocław, Wałbrzych, Legnica and Jelenia Góra.

According to Polish Hunting Code, badgers can be hunted from 1 September to 30 November (Dz. U. 2020.1683).

For each hunting district the following parameters were calculated: the total surface area (including towns, villages, roads), the percentage of arable ground coverage and the percentage of forest coverage. These calculations were made by the Polish Hunting Associations and were continually updated if any changes in the land use structure took place.

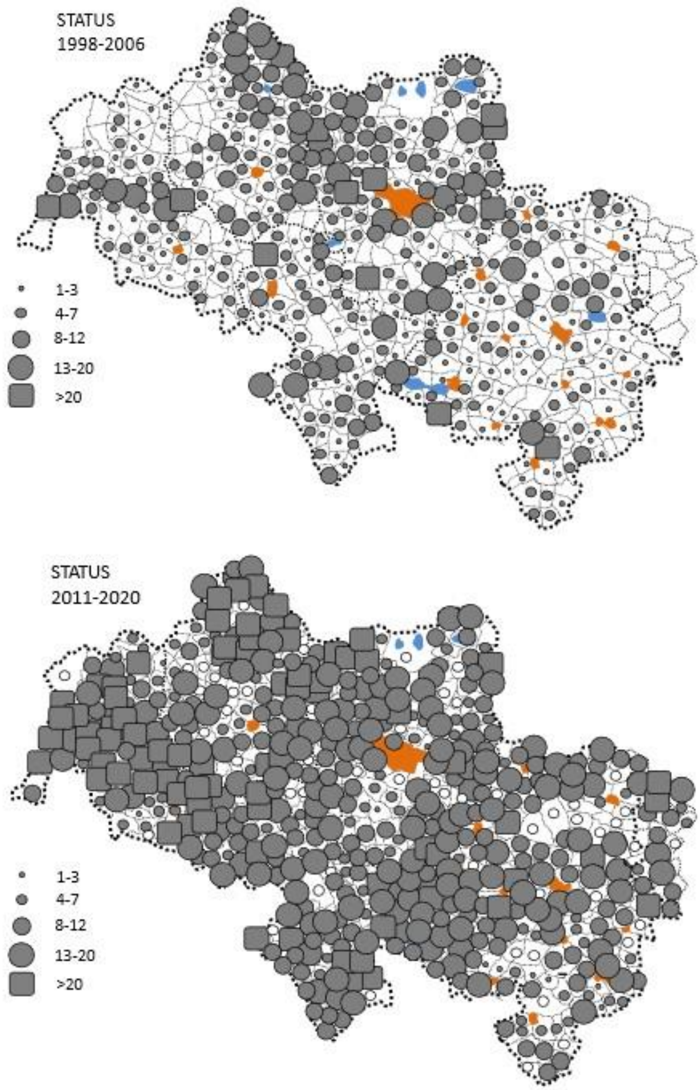

Fig. 3. Estimated mean numbers of the badger in particular hunting districts in SW Poland during the years 1998-2006 and 2011-2020.

In the winter of each year members of a hunting club of a given hunting district and staff of forest districts located within this hunting district attempt to estimate numbers of badgers and other game mammals in their respective hunting district (Kopij 2022, 2023a, 2023b). Numbers of badgers in particular hunting district were estimated by den counts. In the entire period 1980-2020, estimations were based on the same rules (Zalewski et al. 2018).

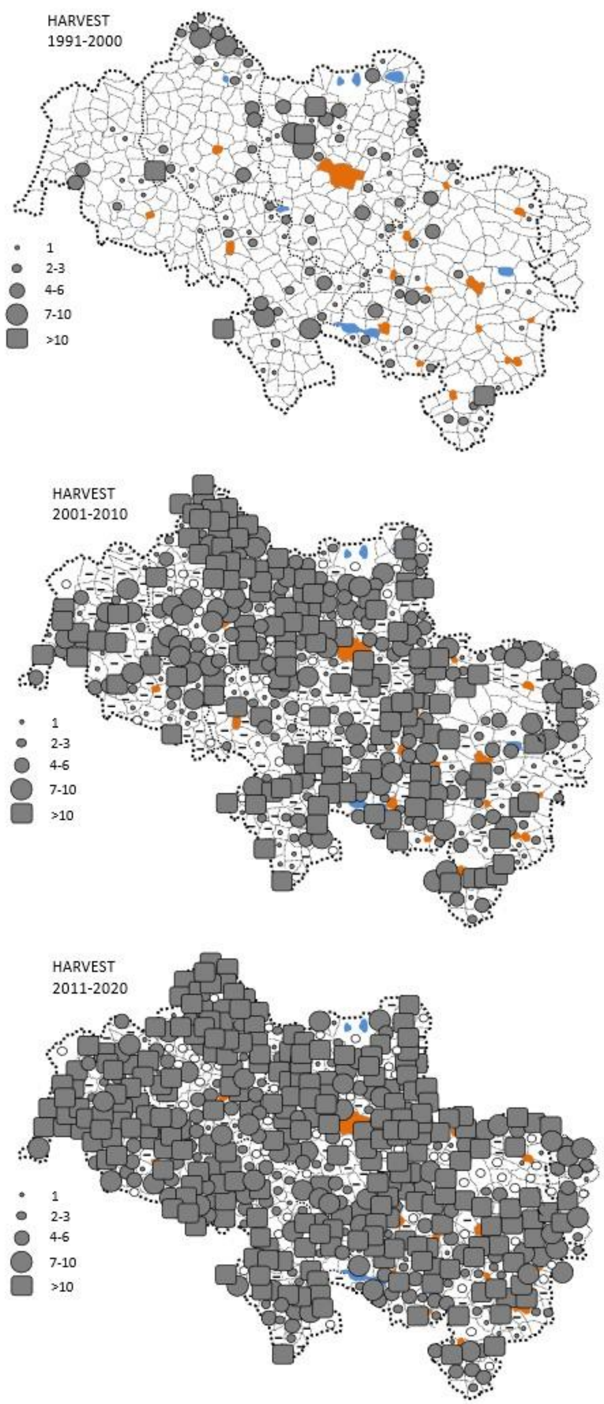

Fig. 4. The number of harvested badgers in particular hunting districts in SW Poland during the years 1991-2020.

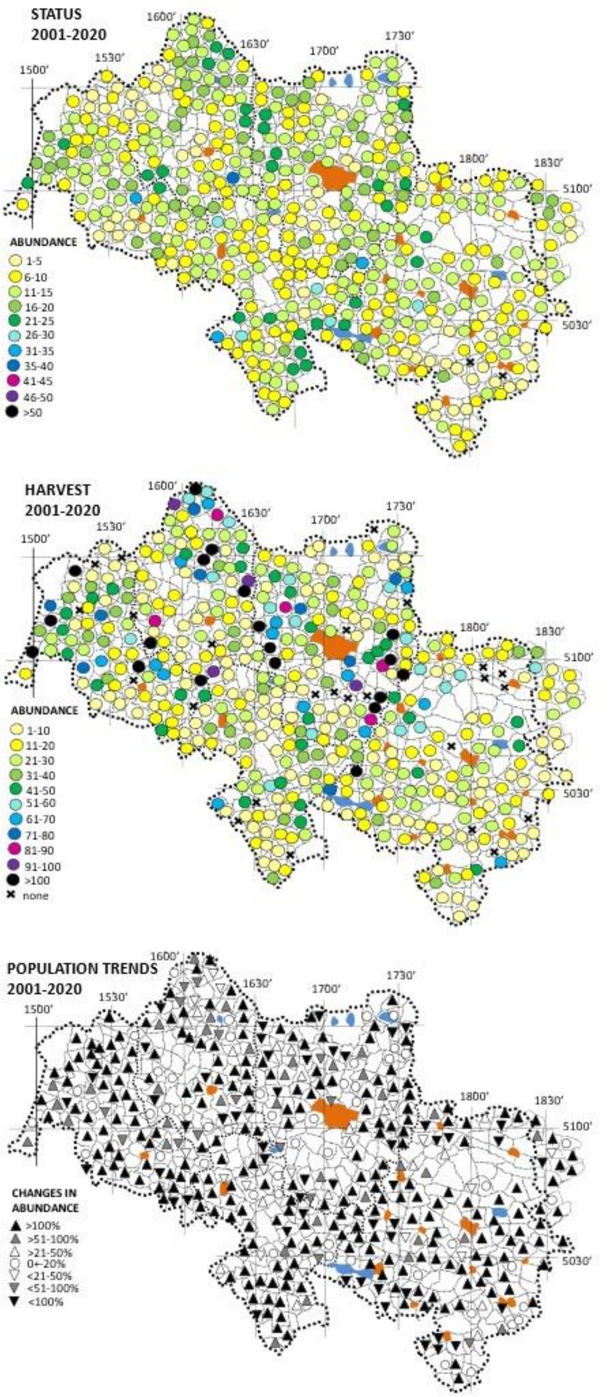

Fig. 5. An overall abundance and population trends of the badger in particular hunting districts in SW Poland during the years 2001-2020.

Counting dens is the most efficient and most often used method (Zalewski et al. 2018). Dens are searched for throughout the year, but mostly in autumn, and plotted on a map. Each den is revisited in early spring to record whether it is occupied or not; and again in late spring in order to determine whether it is occupied by breeding animals (the presence of young animals, their excrements left around the den, presence of prey remnants and playing grounds). Each den occupied in early spring is an equivalent of 2-2.5 individuals; dens with young animals are used to calculate a density of breeding groups. Setts were classified into 3 categories: permanent (main) if cubs were present almost on yearly basis, with (3)-4 entrances within an area larger than 100 m^2; subordinate with cubs present in some years only, with 2-4 entrances within an area of 10-100 m^2; temporary (irregular), without cubs, occupied between October and April only, with 1-2 entrances only within an area smaller than 10 m^2 (Matyáštík & Bičík 1999).

Harvested numbers are expressed as the total number of badgers shot in a given hunting district in a given hunting season. Each hunting season begins on 1st of April and ends on 31st of March of the next year. For each ecoregion (Fig. 1), six hunting districts were randomly selected to calculate mean population density in each ecoregion. Population density is expressed as the mean number of red foxes harvested per one hunting season and per total surface of a hunting district (crude density) or per the surface of afforested area within the hunting district (ecological density). The densities are expressed as the number of individuals harvested per 1000 ha. The mean value (long-term average) is based on data from 20 years (2001-2020). The ratio between the crude density and ecological density was calculated by dividing the ecological density by the crude density.

Harvested numbers are expressed as the total number of badgers shot in a given hunting district in a given hunting season. Each hunting season begins on 1st of April and ends on 31st of March of the next year.

Both crude and ecological densities was calculated for all ecoregions distinguished in SW Poland (Fig. 1). If crude density (number of individuals harvested per 1000 ha of total surface area) is lower than 1, it is regarded low, 1-10: moderate, >10: high (Griffiths & Thomas 1993). If ecological density (number of individuals harvested per 1000 ha of wooded area) is lower than 1, it is regarded very low, 1-2: low, 2-4: moderate, 4-6: high (good), >6: very high (very good) (Štollmann 1967).

RESULTS

In 1998-2006, the northern part of Dolnośląskie Province constituted the stronghold of the badger population in SW Poland. However, in 2011-2020, the population was more evenly distributed across the whole region (Fig. 3).

Harvesting in 1991-2000, in most hunting districts, the badger was not harvested at all. Only in 5 hunting districts (including one in Opole HR) more than 10 badger were shot in that period, and only in 6 other 7-10 badgers were harvested. However in the subsequent decade (2001-2010), it was harvested in most hunting districts, and in nearly half of these districts more than 6 individuals were harvested. In 2011-2020, the badger was hunted in all except 14 hunting districts in Dolnośląskie Province and 9 hunting districts in Opolskie Province (Fig. 4).

In 2001-2020, the badger was the most numerous on the border of Opole and Dolnosląskie provinces, in the northern parts of Wroclaw HR and Opole HR and in Sudety uplands. The lowest density numbers were recorded in SE part of Opole HR, SW Wrocław HR, northern part of Wałbrzych HR, S part of Legnica HR and Bory Dolnośląskie (Fig. 3).

In 2001-2020, harvesting was higher in Dolnośląskie than Opole Province. It was the highest in the Odra river valley and in Sudety Uplands (Fig. 4).

In 2001-2020, an increase in harvesting (>50%) was recorded in 261 hunting districts including 77 in Opole HR, 50 Wrocław HR, 38 Legnica HR, 52 Jelenia Góra HR and 44 Wałbrzych HR. An decrease in harvesting (>50%) was recorded in 61 hunting districts (20 in Opole HR, 16 in Wrocław HR, 13 in Legnica HR, 7 in Jelenia Góra HR and 5 in Wałbrzych HR), while stable numbers were recorded in 49 hunting districts (Fig. 5).

Before 1999, the badger was harvested in SW Poland only occasionally. From 1999 till 2020, there was a steady increase from c. 200 in 1999 to c. 1100 in 2019. This increase in harvesting was a result of a parallel increase in numbers of badgers between 1996 (1000 individuals) to 2013 (c. 6500 individuals). Although in subsequent years, the population growth levelled off, the harvesting was still in increase until 2019. This population increase between 1996 and 2013 was recorded in Opole HR and Wrocław HR. In Jelenia Góra HR the population continue to grow until 2019, while in Legnica HR and Wałbrzych HR, it begun to slightly decrease after the year 2013. Harvesting was on an increase between 1996 till 2019 in all hunting regions, but a decrease was recorded in Wrocław HR in the same period (Fig. 6).

Crude population density of the badger in SW Poland in 2001-2020 was everywhere below 1 ind./1000 ha of the total area. The ecological density, however, ranged from 0.2 to 6.1 ind./100 ha of wooded area. It was the lowest (0.2-0.4) in most afforested ecoregions (Stobrawa Forests, Niemodlin Forests, Barycz Valley), the highest – in most deforested regions such as southern part of Legnica HR (6.1) Głubczyce Plateau (5.7), Wrocław Plain (3.0).

DISCUSSION

In Poland, including the south-western part, the badger was regarded as rare (Pax 1925, Kopij 1996, Kowalczyk et al. 2000, 2003; Nadolska 2002; Nadolska, Bartmańska 2003; Roca et al. 2014). The major finding of this study is that the badger once a rare game species in the south-western part of this country, became common throughout the region as a result dramatic increase in the years 2000-2020. In Britain badgers live in clans comprising on average 6 adult animals (both males and females) with home ranges (50-150 ha), without territories. However, in many other countries in continental Europe, including Poland, badgers live usually single or in pairs holding much larger territories. For example, in the Białowieża Primeval Forest, mean territory size was 1300 ha, ranging from 800 to 2600 ha (Kowalczyk et al. 2003). Also the population density is much higher in Britain than in the continental Europe. While in UK, on average 94 individuals /1000 ha or 15 setts per 1000 ha were recorded; in continental Europe 6.3 individuals (1.6-5.2) per 1000 ha or 1.7 setts (0.4-6.5) per 1000 ha was the average value (Kowalczyk et al. 2000, Byrne et al. 2013). In Białowieża Forests in 1996-1999 there were 1.6 ind./1000 ha (0.4 setts per 1000 ha). It was even lower in Białowieża National Park (10900 ha), with only three established territories (Kowalczyk et al. 2004). Population density is negatively correlated with forest cover, probably because biomass of earthworms is higher in open habitats (meadows, pastures) than in forests.

In 1993, in the northern Maravia (11 067 km^2), bordering with SW Poland, the population density was 1.2 ind./1000 ha of the total area; and 3.1 ind./1000 ha of the wooded area. It was the highest in Ostrava district (8.3), and the lowest in Bruntal district (0.8). In Jesenik and Opava districts it was 1.3. The presence of the badger was conformed in 383 out of 789 hunting districts (48.5%), with permanent occurrence reported from 310 districts (39.3%); there were 789 setts (4.5 per 1000 ha), including 195 permanent and 218 subordinate (Matyáštík & Bičík 1999). In southern Moravia (15028 km^2), the population density in 1996-1999 doubled that in northern Moravia (crude: 2.2 ind./1000 ha; ecol.: 7.6 ind./1000 ha; and 0.8 and 2.8 setts/100 ha respectively); the badgers were recorded in 657 out of 964 (68.2%) hunting districts; and the total population was estimated at 3366 individuals (Bičík et al. 2000). In Hungary, population density was estimated at 1-3 ind./1000 ha, or 3-11 setts per 100 ha (Kozak & Heltai 2006).

In countries such as France, Benelux, Estonia and the former Yugoslavia, the overall badger population density (country-wide scale) was low (<1 ind. per 1000 ha); in Germany, Austria, Czech Republic, Bulgaria, Latvia, Lithuania, Finland, the density was 3-4 ind./1000 ha; while in Sweden and Britain it was higher than 10 ind./1000 ha (Griffith & Thomas 1997). Against this background, the population density in SW Poland in 2001-2020 was low (crude density below 1 ind./1000 ha in all ecoregions). It was similar to an overall density in Poland in mid-1990's (Griffith & Thomas 1997) and in the northern Moravia in 1993 (Matyáštík & Bičík 1999). However, it should be emphasized that the numbers from SW Poland refer to the number of harvested badgers. The real numbers could have been 2-3 times higher. If so, the crude population density would be higher than 1 ind./1000 ha in most ecoregions distinguished in SW Poland.

Long-term data on badger population dynamics are scarce (van Apeldoorn et al. 2006). In Poland, such data are available only from one small study area (89 km2) near Rogów in Central Poland; during a 16-year period (1979-1995), the density of badgers increased there from 1.6 individuals to 2.6 ind. per km2 of wooded area (Goszczyński & Skoczyńska 1996). In the Czech Republic, in the northern Maravia (11 067 km^2), bordering with SW Poland, 737 individuals were counted in 1983, whereas in 1993 the number increased to 1306 individuals. The numbers therefore almost doubled over 15 years (Matyášták & Bičík 1999). In southern Moravia, 2.5-fold increase in badger numbers was recorded between the years 1983 and 1996-1999 (Bičík et al. 2000). In Hungary, badger population increased by 60 % between 1987 and 2000, and their area of occurrence has also expanded with occupying new habitats (Heltai et al. 2001). In Fennoscandia, the badger has expanded its, range up to the Arctic Circle during the years 1950-1990 (Bevanger & Lindström 1995). In the Netherlands, near Utrecht, in a mixed farmlands (c. 100 km^2, increasing maize cultivation; pastures) and forest (Van Apeldoorn et al. 2006); continuous increase has been recorded from 4 individuals in 1983 to 41 individuals in 2001. However, in Belarussian part of the Białowieża Forest in 1946-61 the density was 1.3 ind./1000 ha (0.3 setts per 1000 ha), but in 1979-99 it was even lower: 0.6 ind. (0.2 setts) per 1000 ha (Kowalczyk et al. 2000). In SW Poland, the increase in badgers numbers over the last 23 years (1997-2019) was 5-6-fold (Fig. 4), much faster than in Central Poland (Goszczyński & Skoczyńska 1996) or in Moravia bordering in the south (Matyášták & Bičík 1999, Bičík et al. 2000).

Factors governing badger's distribution and abundance are those favouring both sett location (soil type, slope, vegetation cover), forest type, human activity, abundance and availability of earthworm, competitors and predators, parasites and diseases (Mysłajek et al. 2012, Roca et al. 2014). Badgers prefer ecotone zone (forest/open fields with pasture, meadows etc. with the presence of sandy places for the establishment of dens). In Moravia, Czech Republic, out of 499 setts: 33.1% were found in mixed forests, 26.1% in coniferous forests, 16.2% in deciduous forests, and 11% quarry (Matyášták & Bičík 1999). The ecotone is important habitat requirement, as in the humid forest, earthworms constitute its staple food, while in farmlands both earthworms and cultivated plants (especially maize). Insects, snails, rodents, frogs, lizards, mushrooms, berries and other plants supplement the diet (Zabala et al. 2002, Zabala & Zuberogoitia 2003, Kauhala & Ihalainen 2014). In farmlands with forest fragments in SW Poland maize is becoming increasingly important component of the badger diet (Kochan et al. 2011; G. Kopij, own observ.), as it is in the case of other game mammals (Kopij 2022, 2023a; Kopij & Panek 2016).

The hedgehogs *Erinaceus europaeus*, can be an important prey of the badger, and a strong negative spatial relationship has been shown between hedgehogs and badgers (Young et al. 2006). The fox *Vulpes vulpes*, and racoon dog *Nyctereutes procyonoides* are potential badger's food competitors (Mysłajek et al. 2012). In SW Poland the fox is by the order of magnitude more numerous than the badger (Kopij et al. 2015, Kopij 2023b), but the overlap between badger and fox diet was proved low (Canova, Rosa 1993). Also the racoon dog, although an alien species, is in SW Poland more common than the badger (Kopij 2017). Raccoon dog diet was in all study sites more diverse than badger diet. Both diet composition of these carnivores and their habitat preferences do not indicate severe competition. Overlap of diets between these two carnivores was the smallest in the most diverse area and highest in a managed area with fields and industrial forests (Kauhala & Kowalczyk 2011, Kauhala & Ihalainen 2014).

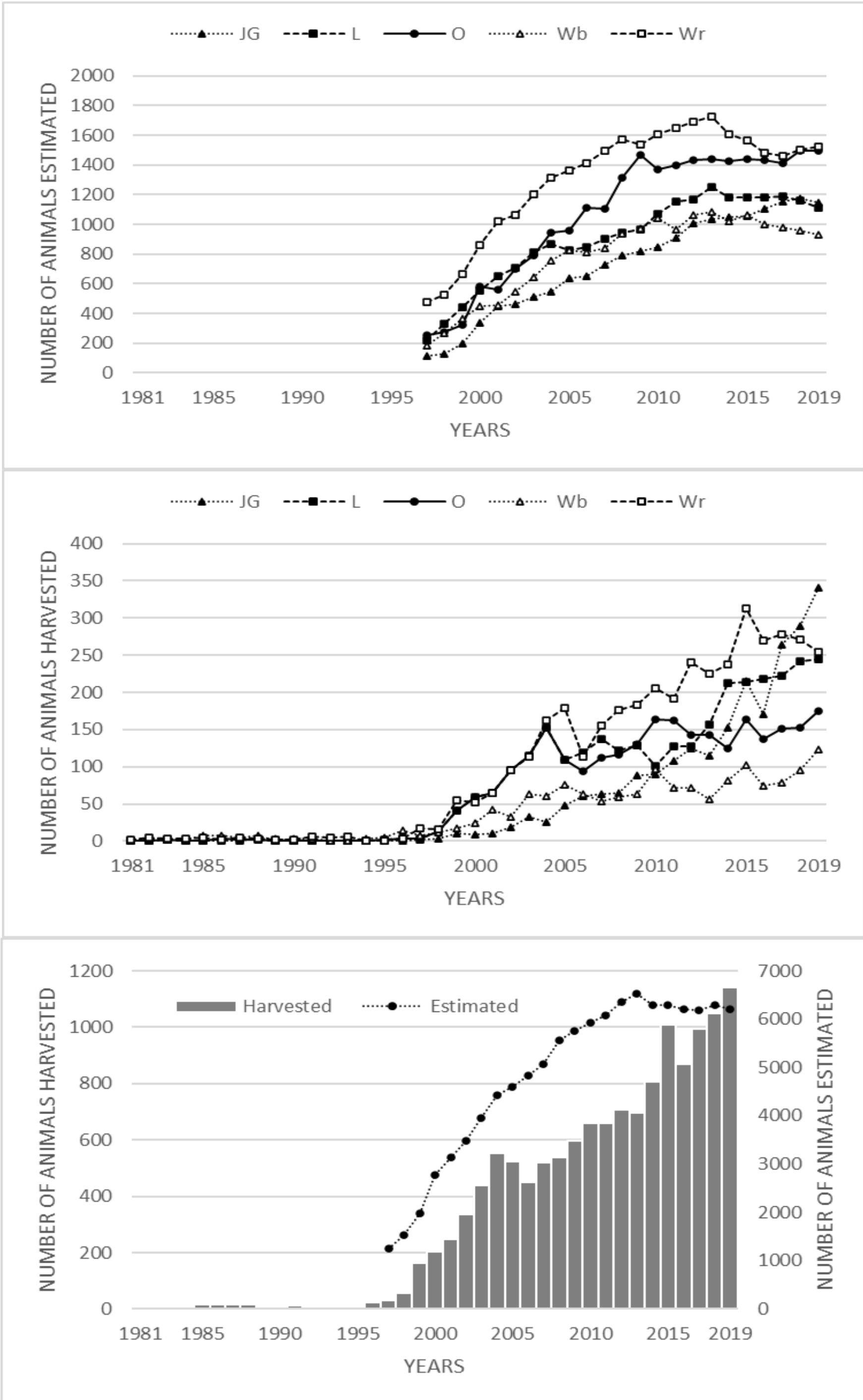

Fig. 6. Changes in the number of estimated and harvested badgers in particular hunting regions and overall in SW Poland during the years 1981-2020.

The badger is a vector of bovine tuberculosis (bTB). This multi-host zoonosis is caused by *Mycobacterium bovis*. Recent field studies demonstrated significant benefits of Bacillus Calmette–Guérin (BCG) vaccination on reducing badger susceptibility to infection. The badger suffers quite often from rabies, possible transmitted by foxes. Collision with vehicles is another important and increasing cause of badger mortality (Griffith & Thomas 1997).

Selective culling may increase badger ranging, reducing local scale badger genetic relatedness and increasing *Mycobacterium bovis* prevalence (exacerbating cTB) (Bielby et al. (2014). Culling (in order to prevent disease spread) may induce disturbance to badger social structure, facilitating wider cTB dissemination. It has been suggested that even at very low population density level, selective culling may cause similar deleterious effects by increasing ranging of individuals and greater mixing between social groups (Byrne et al. 2013, Allen et al. 2022). It is therefore advisable, to not intensify culling in the presence of cTB and other contiguous diseases.

CONCLUSIONS

In the years 2000-2020, the badger has rapidly increased in numbers (almost 5-fold) in SW Poland, possible as a result of increased areas of maize cultivation (corn may constitute its important food) and climate change (milder and shorter winters). This increase was much faster than in the neighbouring Moravia, Czech Republic, and Central Poland. The crude population density remained relatively low everywhere (1 ind./1000 ha of the total area), but the ecological density ranged from 0.2 to 6.1 ind./1000 ha of wooded area.

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ESTIMATES OF SOIL LOSSES DUE TO WATER EROSION IN THE AMAZON BIOME

SUMMARY

Located in the Brazilian Amazon biome, the Chico Mendes Extractive Reserve, Acre state, is an important area for the conservation of biodiversity and ecosystem services in the region. Despite its importance, it faces challenges such as illegal deforestation, mining, and forest fires, which increase water erosion processes and generate environmental and socioeconomic negative impacts. The need to understand these impacts motivate this research, with the objective of evaluating the influence of forest fires on water erosion and quantify soil losses at this site. For this purpose, we employed the Revised Universal Soil Loss Equation (RUSLE), utilizing parameters obtained from scientific literature and remote sensing data, such as the Normalized Difference Vegetation Index (NDVI), enabling a temporal analysis of vegetation cover. Our results indicate low variations in average soil loss rates, ranging from 3.00 to 3.74 Mg ha^{-1} yr^{-1} from 2019 to 2021. In 2021, an increase in soil loss rates was observed due to a higher incidence of forest fires, especially in pasture areas. It is concluded that the preservation and adequate management of vegetation cover are essential for the protection of natural resources. The need to adopt and develop conservation and sustainable management strategies through public policy should contribute to the mitigation of environmental impacts. Furthermore, the results obtained can highlight the importance of environmental conservation.

Keywords: Soil Degradation, Forest Fires, Land Use and Land Cover, MapBiomas Project, Remote Sensing.

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INTRODUCTION

The global climate crisis is evident through the increase in temperatures and extreme weather events (Aghakouchak *et al.*, 2020). The Amazon biome spans 419,694,300 hectares, covering 40% of Brazil's territory, and plays a fundamental role in climate and rainfall regulation. However, it has been continually impacted due to illegal activities such as deforestation, mining, and forest fires, which contribute to widespread environmental and soil degradation (Gatti *et al.*, 2021) and the climate changes (Silva *et al.*, 2019).

The creation of protected areas by law within the Amazon biome is essential to its protection, covering 27.56% of the biome (IBGE, 2023) and contributing to the resilience of local ecosystems (Campos-Silva *et al.*, 2021). These protected areas safeguard biodiversity, maintain ecosystem functions, and serve as carbon sinks against climate change (Paiva *et al.*, 2020; Franco *et al.*, 2021). Despite Brazil's robust environmental laws, their effectiveness in practice is often compromised by challenges in implementation, supervision, and enforcement (Raftopoulos and Morley, 2020), proving insufficient to contain the environmental impacts of illegal human actions.

In Brazil, the Chico Mendes Reserve is a protected area categorized as Conservation Unit of Sustainable Use (Brasil, 2000; Brasil, 2006), which aims to keep the balance between environmental conservation and the well-being of local communities (Roberts *et al.*, 2020). However, also the Reserve is subject to environmental degradation resulting from deforestation, advance of urban areas, changes in land use and land cover (LULC), forest fires and water erosion (Mascarenhas *et al.*, 2018; Marengo *et al.*, 2022), emphasizing the need for urgency of protection (Silva *et al.*, 2019).

Monitoring and addressing the phenomena rely on the assistance of environmental technologies that help in the mitigation measures of environmental degradation, such as geotechnologies tools, that provide a comprehensive spatiotemporal view of the patterns of change in landscape (Avtar *et al.*, 2020). In Brazil, owing to its vast territorial expanse, these tools become pivotal for environmental diagnostics and prognostics (D'Andrimont *et al.*, 2021; Lense *et al.*, 2021; INPE, 2023).

In this way, the use of Geographic Information Systems (GIS) makes it possible to estimate soil loss rates caused by water erosion. Thus, the Revised Universal Soil Loss Equation - RUSLE (Renard *et al.*, 1997) is widely used to estimate these rates in large areas and river basins. Several studies have evaluated the effectiveness of erosion prediction models in Brazilian soils, including research conducted by Nachtigall *et al.* (2020), Lense *et al.* (2021), and Macedo *et al.* (2021).

Considering that forest fires alter vegetation cover, we chose to emphasize factor C, which considers the impact of soil management, vegetation cover and residual biomass in estimating soil loss due to water erosion (Bertol *et al.*, 2019). The C factor can be obtained from experimental plots (Wischmeier and Smith, 1978) or vegetation index, such as the Normalized Difference Vegetation Index -

NDVI (Durigon *et al.*, 2014). From this perspective, the preservation and sustainable management of vegetation cover are pivotal for the conservation of natural resources, particularly concerning soil and water. Given the aforementioned context, our objectives were to quantify forest fires in the Reserve from 2019 to 2021 and evaluate their influence on the C factor and soil losses due to water erosion.

MATERIAL AND METHODS

Study area. The Chico Mendes Extractive Reserve covers 970,570 hectares and is situated in the south-eastern part of Acre, accounting for 3.14% of the Legal Amazon (Figure 1). Its establishment occurred through Decree No. 99,144, dated March 12, 1990 (Brasil, 1990).

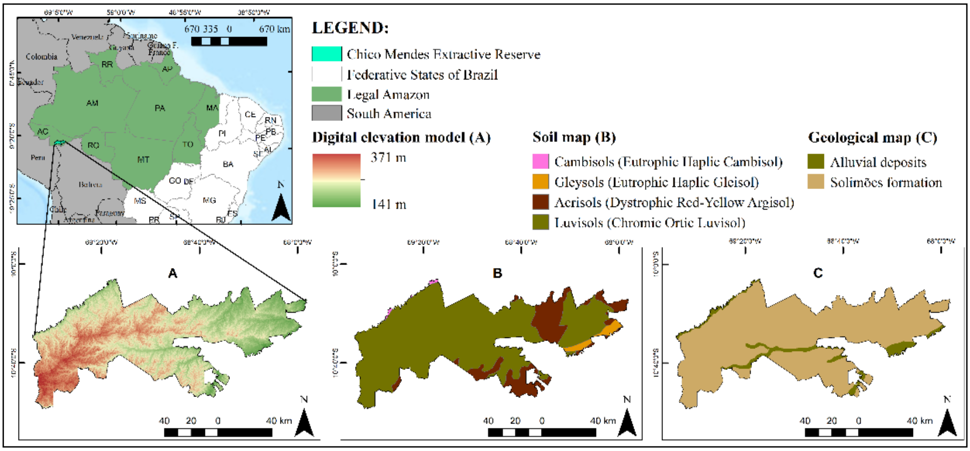

Figure 1. Location map of the Chico Mendes Extractive Reserve
(A) Copernicus mission's Digital Elevation Model (DEM) with a 30 m resolution (ESA, 2023); (B) Soil map at a 1:5,000,000 scale (Santos *et al.*, 2018); (C) Geological map at a 1:1,000,000 scale (CPRM, 2021).

The main economic activity of the population in the Chico Mendes Extractive Reserve is nut and rubber extraction (Silva *et al.*, 2019). The Reserve is predominantly covered by dense forest formations with large trees, spanning 851,324 hectares, which accounts for 91.4% of its territory (Brasil, 2006).

The climate, according to the Köppen classification (Köppen, 1936), is the Am type (tropical monsoon climate), characterized by high temperatures and a well-defined rainy season. The average annual temperature is 27°C, and the precipitation is 2,000 mm (Alvares *et al.*, 2013).

The area is composed of sedimentary rocks represented by sandstones, siltstones, mudstones, and conglomerates. The coarser textures tend to have lower erodibility, as figure 1C (CPRM, 2006; Salgado *et al.*, 2019).

The geomorphology is characterized by low relief diversity, with altitudes varying from 141 to 371 m (Figure 1A) (Cavalcante, 2005; Salgado *et al.*, 2019).

The hydrography covers the rivers Acre, Iaco and Xapuri, flowing from west to east (ANA, 2023).

According to Santos *et al.* (2018) and correlated to the IUSS (2015), the Reserve soils are Chromic Ortic Luvisoils (Luvisols) (81.5%); Dystrophic Red Yellow Argisoils (Acrisols) (16.1%); Haplic Eutrophic Gleysoils (Gleysols) (2.1%) and Haplic Eutrophic Cambisoils (Cambisols) (0.3%) (Figure 1B). There is a predominance of Luvisols. These soils offer high fertility and good water retention.

Methodological procedures. The study involved four steps, as in Figure 2.

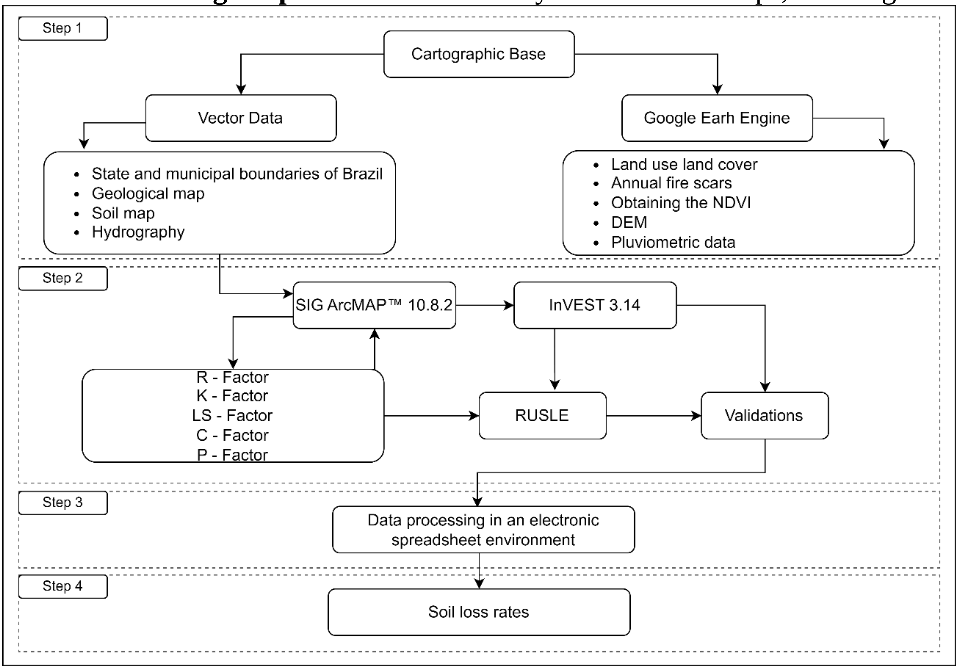

Figure 2. Flowchart of the research development

In step 1, we acquired the cartographic base in shapefile format, which includes the territorial limits used in this study (Ibge, 2022), the geological map of Brazil at scale 1:1,000,000 (Cprm, 2021), the soil map of Brazil at scale 1:5,000,000 (Santos *et al.*, 2018), hydrographic data (Ana, 2023) and the Digital Elevation Model (DEM) of the Copernicus mission with 30 m resolution (Esa, 2023). We process the files using ArcMap™ 10.8.2 (Esri, 2021).

We included data on fire scars and precipitation from 2019 to 2021. Data on fires and LULC were obtained from the MapBiomas Fire Project database - Collection 2 (Projeto Mapbiomas, 2022) and Collection 8 of the Annual Series of LULC Maps of the Brazil (Projeto Mapbiomas, 2023) in raster files. We processed the files in Google Earth Engine toolkit (Gorelick *et al.*, 2017) and converted to shapefiles in ArcGIS 10.8.2 (Esri, 2021).

We obtained precipitation data from the Climate Hazards Group InfraRed Precipitation (CHIRPS 2.0) satellite (Funk *et al.*, 2015), given the lack of operational hydrometeorological stations (ANA, 2023).

In step 2, we obtained the RUSLE factors (Renard *et al.*, 1997), described in Equation 1 (Table 1).

The R factor was obtained from the global rain erosivity map with a spatial resolution of 1 km, derived from 3,625 rain gauge stations (Panagos *et al.*, 2017; 2023). After downloading, the raster file was resized to a resolution of 30 m using the resample tool (ESRI, 2021).

The K factor was adapted from Mannigel *et al.* (2002), Cabral *et al.* (2005), and Farinasso *et al.* (2006). We inserted the K values into the attribute table (ESRI, 2021) and generated the soil map following McBratney *et al.* (2003), based on Santos *et al.* (2018).

The LS was obtained in the Digital Elevation Model (DEM) in the GIS System for Automated Geoscientific Analyzes (SAGA) (Pilesjö and Hasan, 2014), according to the method of Desmet and Govers (1996).

For factor C, we used the NDVI, according to Equation 2 (Rouse *et al.*, 1974) of Table 1, following Durigon *et al.* (2014) (factor Cr) and Macedo *et al.* (2021) (factor Cr2) (Table 1).

The method of Macedo *et al.* (2021) is an adaptation of Durigon *et al.* (2014) which considers effects of seasonality and precipitation in vegetation cover. To this end, the variables Pptx (accumulated precipitation in the 3 months prior to the first scene of the quarter to calculate the NDVI) and Lv (average accumulated precipitation in the 3 months following the first scene) are used. Thus, when Lv is less than or equal to Pptx, there is less presence of dry vegetation with low reflectance. In this case it is necessary to obtain the Cr2 factor (Equation 4). Therefore, if Lv is greater than Pptx, drier vegetation is expected due to seasonality. In this case, the CPC factor (Equation 5) is used to increase the NDVI values based on precipitation, allowing the reclassification of dry vegetation targets considered as bared soil.

The calculation of the NDVI C factor was based on Sentinel 2, Multispectral Instrument (MSI), Level-2A, orbit/point 002/067, 002/068 and 003/067, with a resolution of 10 m, including geometric data and atmospheric corrections, cloud and shadow mask (ESA, 2015). We obtained quarterly average NDVI values, processed in a script in Google Earth Engine (GEE). Thus, 1,374 scenes were processed in total, averaging 458 per year, to 2019, 2020 and 2021, providing comprehensive intra-annual spectral information. Seasonality influences NDVI values, with higher values during rainy months and lower values during dry months. This seasonality is consistent with the relationship between NDVI and soil water availability (Pettorelli *et al.*, 2005; Teixeira *et al.*, 2023).

We obtained P values from the literature, being 0.01 for forest formation and 0.5 for pastures and other temporary crops (Bertoni and Lombardi Neto, 2014).

Table 1. Equations for calculating soil loss rates and obtaining C factors.

	Equations	Variables	Reference
(1)	$A = R \times K \times LS \times C \times P$	A is the average annual soil loss estimate (Mg ha^{-1} yr^{-1}); R is the rainfall erosivity factor (MJ mm ha^{-1} h^{-1} yr^{-1}); K is the soil erodibility factor (Mg ha^{-1} MJ^{-1} mm^{-1}); LS is the topographic factor (dimensionless); C is the soil cover management factor (dimensionless); P is the soil conservation practice factor (dimensionless).	Renard *et al.* (1997)
(2)	$NDVI = \frac{NIR - R}{NIR + R}$	NDVI: Normalized Difference Vegetation Index (dimensionless); NIR: Near-Infrared surface reflectance (dimensionless); R: Red surface reflectance (dimensionless).	Rouse Jr. *et al.* (1974)
(3)	$Cr = \frac{(-NDVI + 1)}{2}$	-	Durigon *et al.* (2014)
(4)	$Cr2 = \frac{(-NDVI + z)}{2z}$	z is the variable representing the maximum NDVI pixel value.	Macedo *et al.* (2021)
(5)	$CPC = Cr2\left(\frac{Pptx}{Lv}\right)^{H}$	H is the percentage of the pixel area with low NDVI due to seasonality (Equation 7).	
(6)	$H = \frac{(NDVIPC - NDVI)}{100}$	NDVIPC is the precipitation correction (Equation 8).	
(7)	$NDVIPC = NDVI\frac{Lv}{PPtx}$	Lv is the leveling variable, equal to the average accumulated precipitation over x days in the studied historical series (mm). Pptx is the accumulated precipitation in the x period prior to the date of the image used in the NDVI calculation (mm).	

The modelling of soil losses by RUSLE does not differentiate between the fraction deposited on the ground and that which reaches water bodies. To overcome this and validate soil loss rates, we integrated the model with the Sediment Delivery Ratio tool (Sharp *et al.*, 2018) in the software InVEST 3.14, which uses the same input data for RUSLE calculations, according Vigiak *et al.* (2012), Cavalli *et al.* (2013) and López-Vicente *et al.* (2013), Borselli *et al.* (2008). Additionally, the variation of the sediment delivery rate was calculated according to the two C factors used.

RESULTS AND DISCUSSION

LULC changes. The main LULC changes occurred in the pasture, which increased by 14.35% in 2020 compared to 2019. In 2020, forest formation lost 1% of its area, equivalent to 9,200 ha. The temporary crops showed an increase of 22.27% in 2021 compared to 2020. The quantification of LULC data from 2019 to 2021 is presented in Table 2 and Figure 3.

Table 2. LULC classes in ha and percentages (Projeto Mapbiomas, 2023).

Classes	2019		2020		2021	
	Hectares	%	Hectares	%	Hectares	%
Forest formation	854,128	91.97	844,920	90.98	851,324	91.46
Pasture	64,007	6.90	73,195	7.88	68,652	7.38
Temporary crops	2	0.00	2	0.00	2	0.00
Rivers and wetlands	10,525	1.13	10,543	1.14	10,822	1.16

From 2019 to 2021, the LULC differences remained practically unchanged throughout the analyzed period.

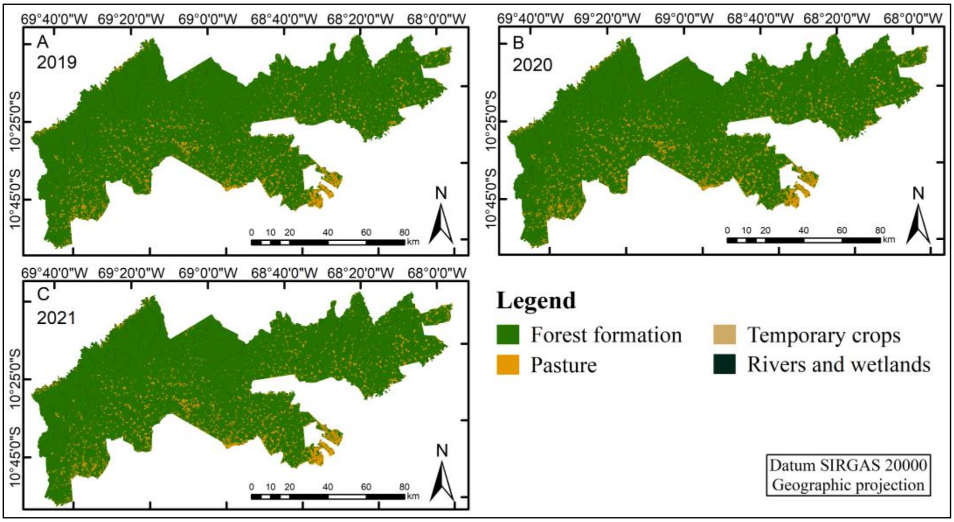

Figure 3. LULC map of the Chico Mendes Extractive Reserve (A) 2019; (B) 2020; (C) 2021.

Forest fires. In the temporal series examined, there were larger fires in the Reserve, covering 16,769 hectares or 1.72%, 9,757 hectares or 1.00% and 16,986 hectares or 1.75%, to 2019, 2020 and 2021, respectively (Figure 4).

The intensity of fires is influenced by climatic variables, deforestation, and proximity to roads, since these areas close to roads are more susceptible to the phenomenon due to easy human access, agricultural activities and inadequate disposal of flammable materials (Ferreira and Féres, 2020; Melo and Rocha, 2023).

According to Zemp *et al.* (2017), Murad and Pearse (2018) and Leite-Filho (2021), there are a significant relationship between the extent of deforested areas and the incidence of fires. All this process results in the loss of vegetation cover, intensification of water erosion and compromise of water resources (Silva Junior *et al.*, 2018; Karamesouti *et al.*, 2023).

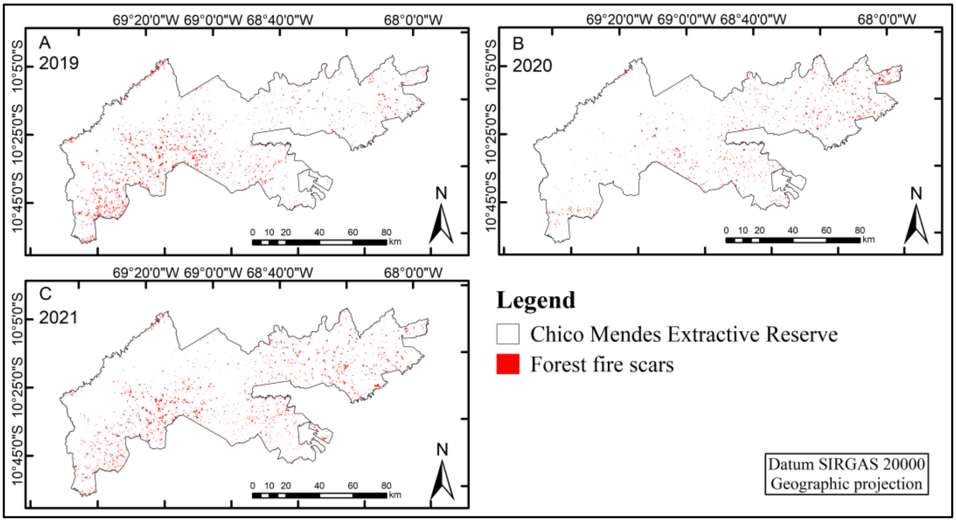

Figure 4. Forest fire scars in the Chico Mendes Extractive Reserve (A) 2019; (B) 2020; (C) 2021.

During a historical series, a class that had a higher incidence of forest fires was pasture, due to criminal management practices. In details, 2019, that had the largest burned area was pastures with 13,787 ha or 21.54%, followed by forest formation with 9,982 ha or 0.35%. While the temporary crops class did not show fire scars. By this way, in 2020 there was a reduction in burned areas, with pastures covering 8,164 ha or 11.15% and forest formation with 1,593 ha or 0.19%. Lastly, there was a significant increase in the area burned in 2021 for the forestry class, with 7,171 ha or 0.84%, and pastures, with 9,815 ha or 13.86%.

Erosivity, erodibility and topography. The R factor ranged from 7,923 to 9,739 MJ mm ha^{-1} h^{-1} yr^{-1}, with lower values in the eastern part. Erosivity ranged from medium to high (Mello *et al.*, 2013) (Figure 5A).

The reserve presents medium erodibility (Figure 5B). The highest values are observed in the Cambisols, which occupy only 0.3% of the area (Figure 5B). Luvisols and Acrisols cover, respectively, 81.5% and 16.1%, while Gleysols cover 2.1%. Luvisols and Cambisols are characterized by low depth and fragiles, making them more susceptible to erosion processes. The absence of a thicker, more resistant surface layer makes them vulnerable to the removal of particles by the impact of rainwater and runoff, leading to the loss of soil and nutrients. However, Gleysols, due to their high base saturation and the presence of highly active clay, have good natural fertility. Acrisols have their high water and nutrient retention capacity. Both are frequently used in agricultural crops. Nonetheless, their clayey texture and compacted structure can make them more susceptible to water erosion processes (IUSS, 2015).

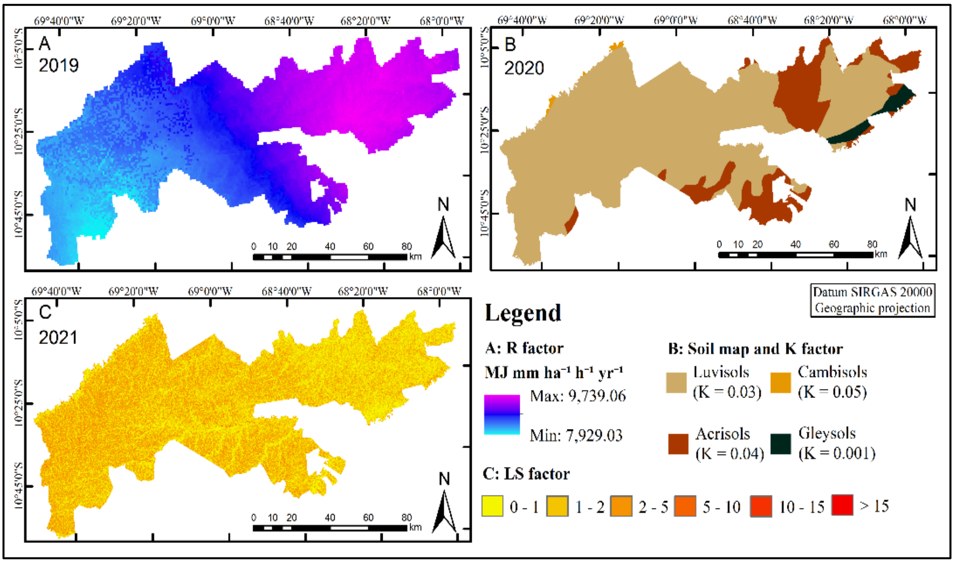

Figure 5. RUSLE factors: (A) R; (B) K; (C) LS from the Reserve.

Regarding to the LS factor, the intervals reveals that 99.4% of the area presents values below 5 and 0.03% presents values above 10 (Figure 5C). These values indicate, respectively, low to moderate vulnerability to water erosion (Beskow *et al.*, 2009) (Table 3).

Table 3. LS factor intervals for the Chico Mendes Extractive Reserve

Intervals	%
0-1	39.31
1-2	29.61
2-5	30.48
5-10	0.57
10-15	0.01
>15	0.02

The highest rainfall rates begin in September and end in May. 2019 presented the highest precipitation, surpassed only by the month of February 2021 (Figure 6).

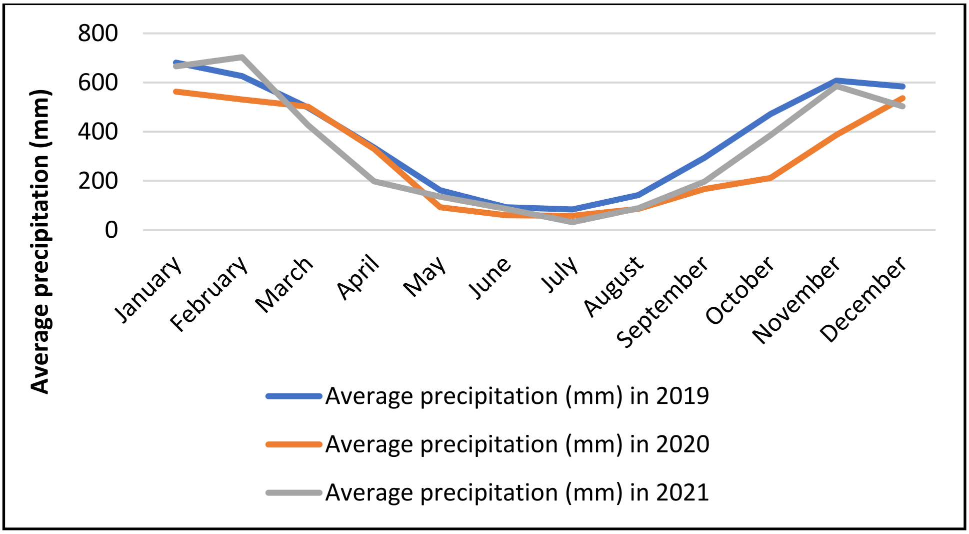

Figure 6. Chico Mendes Extractive Reserve quarterly average precipitation

C factor. The NDVI-derived C factors stand out for their ability to capture phenological and seasonal variations in vegetation cover, encompassing events such as droughts and forest fires. This effectiveness is attributed to the intrinsic ability of NDVI to identify changes in vegetation density within the same category (Rouse, 1974; Almagro *et al.*, 2019) (Table 4).

Table 4. Values of the soil cover management factor (Factors C_r and C_{r2})

Classes	2019		2020		2021	
	C_r	C_{r2}	C_r	C_{r2}	C_r	C_{r2}
Forest formation	0.157	0.128	0.139	0.100	0.148	0.113
Pasture	0.206	0.182	0.195	0.163	0.212	0.183
Temporary crops	0.210	0.187	0.215	0.184	0.235	0.208

The average values of the C_r and C_{r2} factors were slightly higher in 2019 and 2021, indicating that forest fires were also the cause of the elevation of these NDVI values. The largest fire scars are those with warmer colors on the maps, showing a growing trend of clandestine forest fires within the reserve's boundaries (Figures 7E and 7H).

The C indices obtained with NDVI identify the classes most impacted by seasonal effects or forest fires, given the variability of values. These approaches allow for more precise analyses, where lower NDVI values are directly proportional to higher C values (Durigon *et al.*, 2014; Macedo *et al.*, 2021).

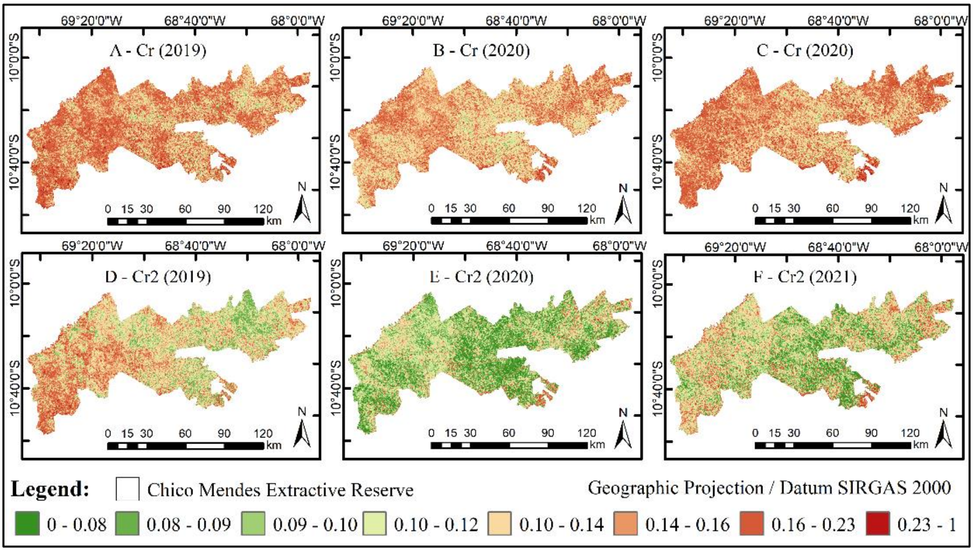

Figure 7. Maps of the Cr (A) 2019; (B) 2020; (C) 2021 and Cr2 (D) 2019; (E) 2020; (F) 2021 factors for the Chico Mendes Extractive Reserve.

Assessment of the influence of fires on C factor on soil loss rates. The estimated soil loss rates in Reserve ranged from 3.50 to 3.74 Mg ha^{-1} yr^{-1} using the C_r index and from 3.00 to 3.16 Mg ha^{-1} yr^{-1} with C_{r2} (Table 5), with slightly higher rates in 2021, likely due to a higher occurrence of forest fires. Among the LULC classes, pastures showed the highest average soil loss rates based on the used C factors (Table 5).

Table 5. Soil loss estimates in Chico Mendes Extractive Reserve from 2019 to 2021 using different obtained C factors.

LULC classes	**2019**		**2020**		**2021**	
	C_r	**C_{r2}**	**C_r**	**C_{r2}**	**C_r**	**C_{r2}**
	Mg ha^{-1} yr^{-1}					
Forest formation	0.84	0.72	0.79	0.57	0.82	0.63
Pasture	38.92	33.80	37.33	31.06	39.99	34.59
Temporary crops	26.16	22.27	27.69	23.77	23.98	21.01
Average loss	3.50	3.02	3.70	3.00	3.74	3.16

The percentages of mean soil loss rates per LULC class showed non-significant variations considering the two methods of obtaining the C factor. For pasture, using the C_r factor, there was a 4.08% decrease from 2019 to 2020 and an increase of 7.12% from 2020 to 2021. With the C_{r2} factor, there was a decrease of 8.10% from 2019 to 2020 and an increase of 11.36% from 2020 to 2021. These fluctuations likely reflect variations in deforestation rates and the occurrence of forest fires (Kumar *et al.*, 2022). The variations in soil loss rates, considering the

C_r and C_{r2} indices, demonstrated the same effectiveness in identifying areas most affected by water erosion.

We obtained the lowest soil loss rates with the C_{r2} factor, as it considers precipitation in the equation, thereby weighting spectral influences caused by seasonality (Macedo *et al.*, 2021). Thus, the C_{r2} factor better reflects the reduction in forest fires and, consequently, the increased soil protection provided by vegetation cover (Gwapedza *et al.*, 2021; Castro *et al.*, 2022).

Estimates of soil loss rate and sediment delivery rate. Our study indicated that areas with more fragile soils, steep terrain, low vegetation cover, and without conservation practices exhibit higher soil loss rates, as also observed by Liu *et al.* (2020) and Lense *et al.* (2021). The spatial distribution of areas most susceptible to water erosion is illustrated in Figure 7, following the intervals defined by Avanzi *et al.* (2013).

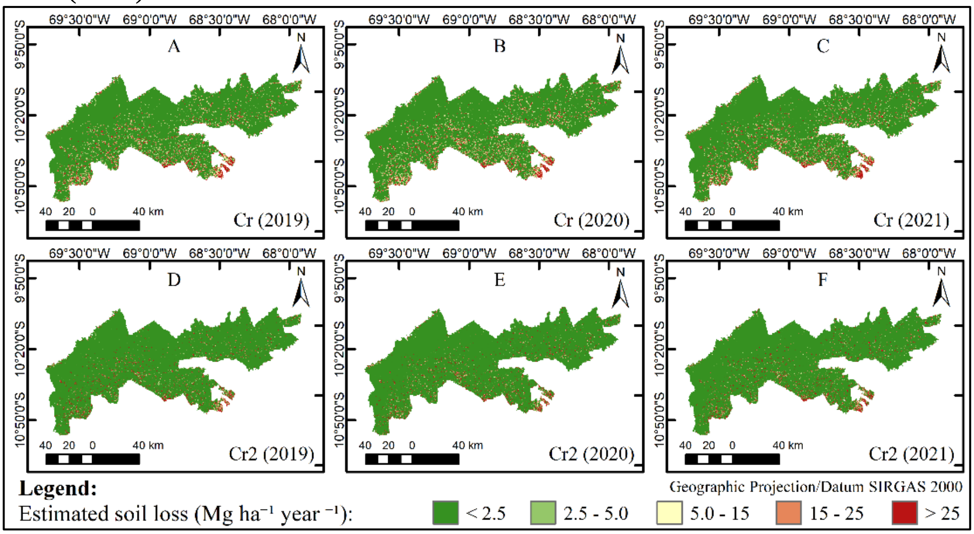

Figure 7. Soil loss estimates for C_r (A) 2019; (B) 2020; (C) 2021 and Cr2 (D) 2019; (E) 2020; (F) 2021 in Chico Mendes Extractive Reserve.

The southern margin is characterized as an area prone to the expansion of agricultural, resulting in illegal deforestation and forest fires as management practices. These activities increase environmental vulnerability (Azevedo, 2021). Therefore, this area exhibits the highest soil loss rates and lacks mitigating measures against soil degradation.

The validation of soil loss rates based on sediment delivery ratio using InVEST, for both C factors used, is presented in Table 6 (Sharp *et al.*, 2018). It is important to emphasize the effectiveness of this approach, supported by recent studies assessing erosive processes and sediment delivery rates (Hamel *et al.*, 2015; Bouguerra and Jebari, 2017; Matomela *et al.*, 2022). We estimate that, on average, only 0.10% and 0.06% of eroded sediments reach river channels, with the C_r and C_{r2} factors, respectively.

Forest fires are unsustainable and common management practices, especially during the dry season (Mascarenhas *et al.*, 2018). In early years, forest fires may initially increase soil nutrient levels, but they decline shortly after (Agbeshie *et al.*, 2022).

Table 6. Sediment delivery rate estimated by InVEST and error between C factors in Chico Mendes Extractive Reserve.

Year	**Estimation of soil loss rates (Mg ha^{-1} yr^{-1})**		**Estimation of sediment delivery rate (Mg ha^{-1} yr^{-1})**		**C_r factor error (%)**	**C_{r2} factor error (%)**
	C_r	**C_{r2}**	**C_r**	**C_{r2}**		
2019	3.50	3.02	0.025	0.020	0.09	0.06
2020	3.70	3.00	0.026	0.019	0.10	0.06
2021	3.74	3.16	0.027	0.021	0.10	0.07

Soil degradation resulting from forest fires intensifies hydrological erosion processes, leads to reduced water infiltration, increases soil loss rates, consequently affecting ecosystem services (Depountis *et al.*, 2020; Riquetti *et al.*, 2022), and contributes to greenhouse gas emissions (Friedlingstein *et al.*, 2020). Therefore, to promote sustainability and achieve the legal objectives of the Chico Mendes Extractive Reserve conservation unit (Brasil, 1990; 2000), it is essential to adopt sustainable soil and management practices, in addition to greater supervision, to effectively combat deforestation and illegal activities in the area.

CONCLUSION

As estimated average soil loss rates in Chico Mendes Extractive Reserve from 2019 to 2021 ranged from 3.00 to 3.74 Mg ha^{-1} yr^{-1}, with pasture areas experiencing the highest soil losses.

According to the validation, on average, only 0.10% and 0.06% of eroded sediments reach river channels with the Cr and Cr2 factors, respectively.

The estimation of sediment delivery rates by InVEST validated the soil loss rates estimated by RUSLE, with an average percentage error of 22.66% over the period.

Areas affected by forest fires exhibit the highest soil loss rates, characterized by vegetation indices based on NDVI. Therefore, the preservation and proper management of vegetation cover are essential for protecting natural resources and their services.

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NITRIC OXIDE: FUNCTIONAL INTERACTION WITH PHYTOHORMONES AND APPLICATIONS IN CROP PRODUCTION

SUMMARY

This review considers the action of nitric oxide (NO) as a signaling molecule of plant cells, the effects of which can be modulated in crop production practice through the use of nitric oxide donors. The current understanding of nitric oxide synthesis in plants is briefly described. The characterization of synthesized and natural compounds that can be nitric oxide donors is given. The main molecular mechanisms of nitric oxide action in plant cells are characterized: post-translational modification of proteins under the action of NO, its influence on the content of other cellular mediators, in particular, reactive oxygen species and calcium ions. Considerable attention is paid to the functional interaction of nitric oxide with individual phytohormones and new groups of physiologically active substances of plants. Modern ideas on the mechanisms of nitric oxide action on seed germination and plant resistance to adverse abiotic factors are presented. Data on the effects of priming seeds with nitric oxide donors on their germination under optimal and stress conditions are discussed.

Keywords: nitric oxide, reactive oxygen species, phytohormones, seed germination, priming, abiotic stresses, plant resistance

INTRODUCTION

Nitric oxide (NO) is one of the most widely studied gasotransmitters, small gaseous molecules that are involved in signal transduction in plants, animals, and microorganisms. NO is a lipophilic radical molecule with an unpaired π-orbital electron, capable of easily penetrating cell membranes and rapidly interacting

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with its molecular targets (Mur *et al.*, 2013; Allagulova *et al.*, 2023; Khan *et al.*, 2023a). Because of its ability to enter into direct chemical reactions with specific groups of proteins, as well as functional interactions with other signaling mediators and phytohormones, nitric oxide is involved in the regulation of multiple functions of the plant organism: seed germination, cell wall lignification, flowering, pollen formation, fruit maturation and senescence, as well as different types of symbiosis and responses to biotic and abiotic stressors (Dmitriev, 2004; Turkan, 2017; Sami *et al.*, 2018; Venkatesan *et al.*, 2020; Singhal *et al.*, 2021). The accumulation of knowledge about nitric oxide functions in plants has been very intense. A Google Scholar search reveals approximately 35 000 publications in the last decade alone.

So far, the main pathways of nitric oxide synthesis in plants have been identified: oxidative (arginine-dependent) and reductive (nitrate-dependent) (Khan *et al.*, 2023a). However, enzyme systems that ensure NO synthesis in plants, especially through the oxidative pathway, remain a matter of debate (Verma *et al.*, 2020). The mechanisms of NO synthesis through minor pathways also remain unclear, as well as how these pathways contribute to NO signaling functions (Saha *et al.*, 2015; Kumar, Ohri, 2023). In recent years, much attention has been paid to the functional interaction of nitric oxide with phytohormones, which is associated with its participation in transmitting hormonal signals to the genetic apparatus, as well as its ability to induce the formation of signals that activate the synthesis of some phytohormones (Singhal *et al.*, 2021; Shang *et al.*, 2022; Kolupaev *et al.*, 2023b; Ullah *et al.*, 2024). The mechanisms of such phenomena are still poorly understood and information about them is scattered. At the same time, the widening spectrum of compounds that perform hormonal and regulatory functions in plants contributes to the search for functional relationships of NO with compounds such as melatonin, gamma-aminobutyric acid, and other "plant neurotransmitters" (Akula, Mukherjee, 2020). However, knowledge of the functions and mechanisms of action of these compounds in plants is still far from being a coherent system.

At the same time, new compounds with hormonal activity (e.g. polyamines, melatonin, strigolactones, gamma-aminobutyric acid, etc.) have already been recognized as effective for practical application in inducing plant resistance to stress factors, regulating dormancy, and other functions (Sako *et al.*, 2020; Singhal *et al.*, 2021; Kolupaev *et al.*, 2022a; Kosakivska *et al.*, 2022; Raza *et al.*, 2022). On the other hand, the efficiency of practical application of various nitric oxide donors in crop production, primarily as stress-protective agents, has already been shown (Kolupaev *et al.*, 2022b; Khan *et al.*, 2023a; Ullah *et al.*, 2024). Considering the above-mentioned functional interaction effects of nitric oxide and many compounds with hormonal activity, the question arises about the synergistic effects of these compounds and the possibility of their combination in practical use (Kolupaev *et al.*, 2018; Karpets *et al.*, 2021). However, experimental data on these questions are still insufficiently analyzed and generalized, which motivated the writing of this review.

NITRIC OXIDE SYNTHESIS IN PLANTS

The process of NO synthesis occurs in various compartments of the plant cell, including chloroplasts, mitochondria, peroxisomes, as well as apoplast and plasma membrane (Khan *et al.*, 2023a). There are two main pathways of NO synthesis in plants: reductive, based on the reduction of nitrite to NO, and oxidative, associated with the oxidation of molecules containing amino groups.

The existence of the reductive pathway of nitric oxide synthesis in plants has been convincingly proved (Figure 1). One of the key enzymes in NO synthesis is considered to be nitrate reductase (NR), a multifunctional enzyme involved in nitrogen assimilation and metabolism. It is responsible for the first limiting step of nitrate assimilation by catalyzing the reduction of nitrate to nitrite using NADH or NADPH as an electron donor. The active enzymatic homodimeric complex requires the presence of molybdopterin, heme, and FAD as cofactors (Astier *et al.*, 2018). Besides its primary activity, nitrate reductase exhibits nitrite: NO reductase activity (Mur *et al.*, 2013). Under normal conditions, this activity accounts for only 1% of the nitrate-reducing capacity of NR (Astier *et al.*, 2018). However, NO formation involving nitrate reductase is facilitated by factors such as acidic or oxygen-free environments. Despite such specific conditions, the essential contribution of NO production by nitrate reductase in plant physiology has been convincingly demonstrated using both pharmacological and genetic approaches (Mur *et al.*, 2013).

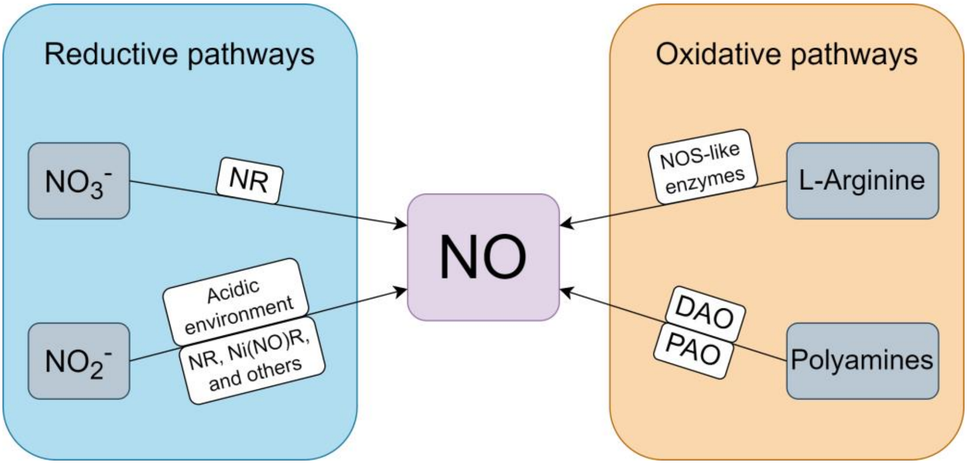

Figure 1. Nitric oxide synthesis in plants. NR – nitrate reductase; Ni(NO)R – nitrite:NO reductase; NOS – NO synthase; DAO – diamine oxidase; PAO – polyamine oxidase.

In addition to nitric oxide formation involving nitrate reductase, several minor pathways are also considered (Figure 1), which may be related to the activity of other molybdenum-containing enzymes, in particular xanthine oxidoreductase (EC: 1.17.3.2), aldehyde oxidase (EC: 1.2.3.1), and sulfite oxidase (EC: 1.8.3.1). These enzymes perform their specific functions while having the potential ability to catalyze the formation of NO from nitrite (Allagulova *et al.*, 2023). However, their contribution to NO formation in plants and to the manifestation of its physiological functions is still very poorly understood.

The oxidative pathway of nitric oxide synthesis is considered to be as important as the reductive pathway, although the nature of the enzymatic systems that provide this pathway in higher plants has been a matter of debate for nearly three decades (Farnese *et al.*, 2016). NO synthase (NOS) proteins and NOS-producing genes have been found in prokaryotes, unicellular eukaryotes, invertebrates and vertebrates, including mammals; however, NOS enzymes have not yet been identified in higher plants (Khan *et al.*, 2023a). Nevertheless, numerous studies show that the enzymatic oxidation of L-arginine to form citrulline and NO is possible in leaf peroxisomes, and chloroplasts of green algae and vascular plants (Hancock, Neill, 2019). This enzyme activity has been called NOS-like (Figure 1) because, like its animal counterpart, it has been reported to be strictly dependent on the presence of arginine and NADPH as well as several NOS cofactors (NADPH, FAD, FMN, Ca^{2+}, and calmodulin) (Corpas, Barroso, 2014; Farnese *et al.*, 2016). However, molecular genetic evidence for the existence of the corresponding protein in higher plants is still lacking (Astier *et al.*, 2018). At present, it has been hypothesized that there are polypeptides with redox-active domains that can be combined into a single enzymatic complex that catalyzes the reactions of arginine-dependent NO formation in higher plants (Kolbert *et al.*, 2019). In this regard, the identification of specific enzymes that catalyze arginine oxidation reactions leading to NO formation remains one of the most important fundamental tasks in the field of nitric oxide biology in plants. (Allagulova *et al.*, 2023).

It is assumed that oxidative NO formation in plants can occur not only from arginine but also from polyamines and hydroxylamine with the involvement of di- and polyamine oxidases, which are predominantly localized in cell walls (Saha *et al.*, 2015) (Figure 1). However, the exact mechanism underlying this process is still unknown (Khan *et al.*, 2023a).

In addition to the enzymatic pathways of nitric oxide synthesis described above, the non-enzymatic generation of NO in plants from nitric acid is considered to be proved (Khan *et al.*, 2023a). For example, it has been shown that the aleurone layers of *Hordeum vulgare* rapidly produce NO when nitrite is added to the incubation medium (Bethke *et al.*, 2004). NO production requires apoplast acidification, which can occur through the action of gibberellin. The addition of phenolic compounds to the medium can also enhance NO production. It has been suggested that apoplastic NO production may be important for grain germination and root formation in cereals (Bethke *et al.*, 2004).

NITRIC OXIDE DONORS USED IN EXPERIMENTAL PLANT BIOLOGY AND CROP PRODUCTION PRACTICES

Nitric oxide synthesis in plants increases in response to stress factors of various nature, in particular, high and low temperatures, drought, salinity, and heavy metals. It is also possible that nitric oxide synthesis and endogenous nitric oxide content increase at certain stages of ontogenesis, for example, during seed germination (Zhang *et al.*, 2023a) and in response to hormonal signals (Ullah *et al.*, 2023).

In experimental practices, molecules that are NO donors are used to increase nitric oxide content in plants (Venkatesan *et al.*, 2020; Ullah *et al.*, 2023). There are one and a half dozen classes including more than 300 compounds that can act as nitric oxide donors (Wang *et al.*, 2002; Oliveira *et al.*, 2018). Sodium nitroprusside (SNP), nitroglycerin, spermidine- or diethylamine-NONOate, S-nitroso-N-acetylpenicillamine, S-nitrosoglutathione (GSNO) are the most commonly used of them (Mur *et al.*, 2013; Oliveira *et al.*, 2018).

SNP is currently the most widely used NO donor for both research and practice (Plohovska *et al.*, 2019; Karpets, 2019; Kolomiiets *et al.*, 2021). The mechanisms of nitric oxide release from the SNP molecule are not yet fully understood. It is known that SNP in aqueous solution under normal conditions in a sealed container and without access to light can be preserved for years (Leeuwenkamp *et al.*, 1984), since the decomposition reaction is reversible and limited by solution saturation with its products. Intensive SNP decomposition with NO release occurs in the presence of NO acceptors and/or cyanide residue (Bates *et al.*, 1991). Also, SNP decomposition in aqueous solutions under aerobic conditions is accelerated in light and at elevated temperatures (Singh *et al.*, 1995; Karpets, 2019). On the other hand, in biological systems, NO release from SNP is known to be much faster in the presence of reducing agents (NADH, NADPH, thiols, and possibly ascorbate) (Wang *et al.*, 2002) and/or membrane-bound enzymes (probably NADPH oxidase, etc.) (Wang *et al.*, 2002; Grossi, D'Angelo, 2005; Diniz *et al.*, 2017).

In order to prove the physiological effect of SNP as a nitric oxide donor and not as a complex compound, experiments are carried out with NO scavengers that offset the physiological effects of SNP (Krasylenko *et al.*, 2012; Mur *et al.*, 2013; Plohovska *et al.*, 2019). One of the disadvantages of SNP as a nitric oxide donor is the formation of cyanide during its decomposition, which can have a significant side effect on physiological processes in plants (Mur *et al.*, 2013).

Along with SNP, the so-called NONOates, which consist of a diolate group [N(O-)N=O] linked to a primary or secondary amine or to a polyamine via a nitrogen atom, are also commercially available (Miller, Megson, 2007). NONOates spontaneously cleave at physiological pH and temperature to produce two NO molecules.

In separate studies on the induction of plant resistance to stressors, L-arginine (Barand *et al.*, 2015) and nitrate (Kumar *et al.*, 2021) are used as sources of NO formation. Thus, it was shown that 2-h incubation of wheat roots in a 5 mM L-arginine solution caused an increase in their nitric oxide content from about 50 to 80 nmol/g, while the same time exposure to 20 mM sodium nitrate increased nitric oxide content more than 3-fold (Karpets *et al.*, 2018). Both exposures caused activation of the enzymatic antioxidant system of seedlings and increased their survival after damaging heating.

Recently, potassium and magnesium nitrates have been used for seed priming to improve germination under stress conditions (Kumar *et al.*, 2021). Their effects are at least partly associated with acting as NO sources (see below).

Polyamines can also be considered as potential nitric oxide donors, which are known as stress metabolites capable of inducing the development of plant resistance to various stress factors when applied exogenously (Kolupaev *et al.*, 2022b). Some studies have shown a causal relationship between NO formation in plants under the action of exogenous polyamines and their ability to activate plant stress-protective systems. For example, treatment of wheat seedlings with putrescine caused a diamine oxidase activity-dependent increase in nitric oxide synthesis in roots, while under the action of the diamine oxidase inhibitor guanidine, the effect of putrescine on nitric oxide content, antioxidant enzyme activity, and heat tolerance of seedlings was eliminated (Kolupaev *et al.*, 2021).

SUMMARY OF THE MOLECULAR MECHANISMS OF NITRIC OXIDE ACTION

To date, much data has accumulated indicating that nitric oxide can directly interact with many cellular proteins and alter their functional activity, as well as influence the state of other important mediators of the signaling network.

The first mechanism has sufficiently clear experimental evidence: it is the interaction of NO with certain functional groups of proteins. Three types of post-translational modification (PTM) of proteins by nitric oxide are distinguished: S-nitrosation, tyrosine nitration, and nitrosylation of metal-containing proteins (Mishra *et al.*, 2021; Kolupaev *et al.*, 2022b).

S-nitrosation is a reversible process that can alter the functional activity of target proteins. Many cellular processes in plants, including those involved in responding to environmental factors and immune function, are considered to depend on protein S-nitrosation (Corpas, Barroso, 2015; Mukherjee, Corpas, 2023). Protein S-nitrosation occurs without the involvement of enzymes (Arora *et al.*, 2016). However, this process is highly specific, as it depends not only on the proximity between NO and the target protein, but also on the amino acid sequence and protein conformation (Lamotte *et al.*, 2015). In Arabidopsis plants, a site-specific nitrosoproteomic approach identified 926 proteins as targets for S-nitrosylation (Hu *et al.*, 2015). A nitrosoproteomic study of tomato plants under medium alkalinization (sodic alkaline) stress revealed 334 S-nitrosated proteins. Among them are proteins involved in the regulation of calcium homeostasis, NO and reactive oxygen species (ROS) content (Wei *et al.*, 2022).

Another mechanism of post-translational modification of proteins under the influence of NO is tyrosine nitration. This process is mainly carried out by peroxynitrite ($ONOO^-$) and nitrogen dioxide radical ($^{\bullet}NO_2$) (Sánchez-Vicente *et al.*, 2019). Tyrosine nitration in proteins has traditionally been considered an irreversible mechanism and a marker of nitrosative stress (Corpas, Barroso, 2013). However, the existence of tyrosine denitrase, which reduces 3-nitrotyrosine in mammalian cells, indicates a possible role for tyrosine nitration in NO-mediated signaling processes in these cells (Valderrama *et al.*, 2019). Some proteins can undergo both S-nitrosation and tyrosine nitration. For example, the important plant antioxidant enzyme ascorbate peroxidase is activated by S-nitrosation at Cys32 (Correa-Aragunde *et al.*, 2013), but inhibited by nitration at Tyr5 and Tyr235 (Begara-Morales al., 2013).

Another NO modification of proteins – nitrosylation of metal-containing proteins – occurs when nitric oxide interacts with transition metal ions that are part of metalloproteins, leading to the formation of metal nitrosyl complexes. Such processes cause reversible conformational changes in proteins and alter their structure and/or functional activity (Arora *et al.*, 2016).

However, in general, specific proteins with known functions whose activity is regulated by various PTMs under the action of nitric oxide are still poorly understood. Although the list of plant proteins whose activity is controlled by nitric oxide is constantly expanding. Examples of such proteins that are important for plant adaptation to stress factors are given in recent reviews (Kolupaev *et al.*, 2022b; Mukherjee, Corpas, 2023).

With its ability to induce PTM of proteins, nitric oxide is at the center of the signaling network (Singhal *et al.*, 2021). In many cases, such interactions lead to the activation of signaling processes involving other mediators, primarily ROS and hydrogen sulfide. Thus, the same proteins can be targets of S-nitrosation and persulfidation (hydrogen sulfide-induced PTM) (Aroca *et al.*, 2018). These include, for example, enzymes that both degrade (catalase, ascorbate peroxidase) and generate ROS (NADPH oxidase) (Mukherjee, Corpas, 2023).

The mechanisms of functional interaction between NO, H_2S, and ROS associated with their mutual influence on each other's synthesis are extremely complex to study and interpret the results. Such effects are related both to the direct modification by active molecules of functional groups of enzyme molecules synthesizing signaling mediators and to the mediated influence of these molecules on the expression of genes encoding the corresponding enzymes. The fundamental knowledge accumulated in recent years in this area is beyond the scope of this review, but it has been analyzed in a number of recent summaries (Kolupaev *et al.*, 2022b; 2023a; Mukherjee, Corpas, 2023).

In general, ROS, nitric oxide, and hydrogen sulfide form a complex signaling network that ensures the triggering of appropriate adaptive responses. Another integral component of such a network is calcium as a universal intracellular messenger (Neill *et al.*, 2008). Calcium ions are able to activate enzymes that synthesize both nitric oxide (in particular nitrate reductase) (Gao *et al.*, 2011) and other related mediators such as ROS (NADPH oxidase) (Baxter *et al.*, 2014) and hydrogen sulfide (L-cysteine desulfhydrase) (Li *et al.*, 2012; Valivand *et al.*, 2019). In turn, nitric oxide, ROS, and hydrogen sulfide can contribute to the opening of calcium channels of different types. Thus, it is known about activation of voltage-dependent calcium channels under the action of ROS (Mori, Schroeder, 2004). Under the influence of NO, calcium channel proteins can undergo S-nitrosation, which also leads to increased calcium entry into the cytosol (Laxalt *et al.*, 2016). Hydrogen sulfide can also contribute to increased Ca^{2+} concentration in the cytosol (Jin *et al.*, 2013). Thus, nitric oxide, ROS, hydrogen sulfide, and calcium are key signaling mediators that functionally interact with each other (see reviews: Kolupaev *et al.*, 2022b; 2023; Mukherjee, Corpas, 2023). These mediators are key elements of the plant cell signaling network that ensures plant adaptation to a variety of stress factors. At the same time, this network is also involved in the realization of mechanisms of hormonal regulation of physiological processes in plants (Ullah *et al.*, 2023).

NITRIC OXIDE ROLE IN THE SIGNAL TRANSDUCTION OF STRESS PHYTOHORMONES

To date, the involvement of nitric oxide in the signal transduction of almost all known plant hormones has been established (Shang *et al.*, 2022). We briefly review the data on the functional relationships of nitric oxide with the most studied and important phytohormones for stress adaptation (abscisic acid, salicylic acid, jasmonic acid, and brassinosteroids), as well as with melatonin and γ-aminobutyric acid, compounds conventionally named plant neurotransmitters (Akula, Mukherjee, 2020). Their hormonal activity in plants has been studied only in recent years.

Abscisic acid (ABA) has long been recognized as a classical stress hormone that plays a key role in plant resistance (Wilkinson, Davies, 2002; Wang, Song, 2008). The effects of ABA are realized through a number of signaling mediators. A large amount of data has been obtained indicating the role of calcium, ROS and nitric oxide in the realization of ABA-induced physiological reactions that lead to increased plant resistance to drought, salinity, and stress temperatures (Agarwal *et al.*, 2005; Kwak *et al.*, 2006; Petrov, Breusegem, 2012; Bartoli *et al.*, 2013). Meanwhile, nitric oxide is known to be involved in the induction of ABA synthesis (Dmitriev, 2004). In particular, it was shown that NO synthase inhibitors suppressed the accumulation of ABA in leaf cells, while NO donor promoted an increase in its content (Xing *et al.*, 2004; Bajguz, 2014).

One of the most studied effects of ABA, important for plant adaptation to drought and salinity, is the closure of stomata. An increase in the content of endogenous ABA under stresses leads to the opening of anion/K^+-channels of guard cells that transport potassium to the outer side of membranes, which causes stomatal closure (Kwak *et al.*, 2006). Important signaling mediators in the induction of this process by ABA are ROS, calcium ions, and nitric oxide. The ABA-induced increase of nitric oxide content in the guard cells is believed to be a consequence of an increase in the amount of hydrogen peroxide in them (He *et al.*, 2005; Neill *et al.*, 2008) (Figure 2). It has been found that ABA-induced NO generation in Arabidopsis guard cells causes S-nitrosation of SnRK2.6/OST1 (open stomata 1) protein kinase at Cys137, leading to closure of the stomata (Wang *et al.*, 2015; Khan *et al.*, 2022).

In general, most of the accumulated experimental data testify to the synergistic action of ABA and nitric oxide in plant adaptation to abiotic stresses. This is also indicated by the results of studies of the combined effect of exogenous NO and ABA on plant resistance to extreme influences. Such an effect was shown, for example, in the study of the combined effect of NO and ABA on wheat resistance to heat stress (6-hour exposure to 40°C for 15 days). It was found that NO donor and ABA separately significantly mitigated stress-induced oxidative damage, and increased the activity and gene expression of antioxidant enzymes such as SOD, catalase, ascorbate peroxidase, and glutathione reductase (Iqbal *et al.*, 2022). However, these effects of NO and ABA were significantly stronger when used together. Nevertheless, the synergistic interaction between ABA and nitric oxide, including the mutual enhancement of their synthesis, is not evident in all objects. In particular, they have antagonistic effects on seed germination (see below).

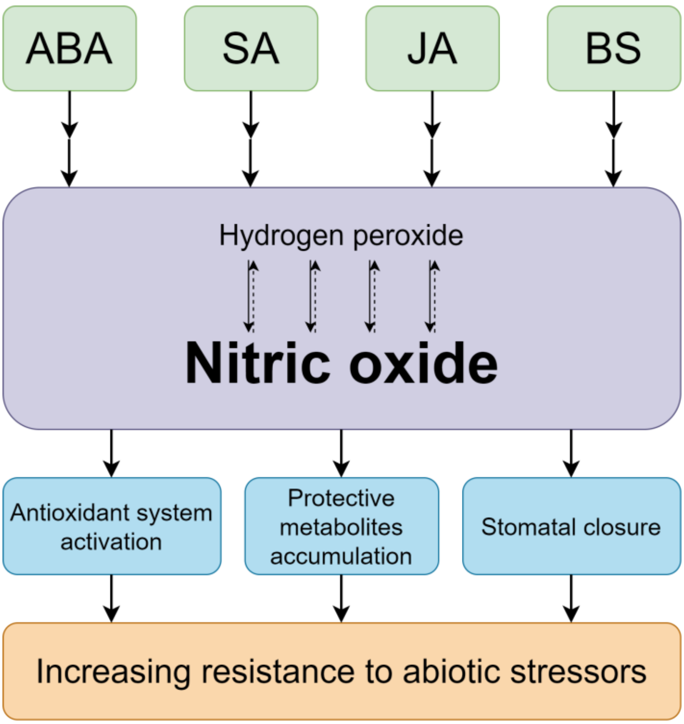

Figure 2. Involvement of nitric oxide in the realization of stress-protective effects of phytohormones. ABA – abscisic acid; SA – salicylic acid; JA – jasmonic acid; BS – brassinosteroids. Other explanations in the text.

Salicylic acid (SA) is currently considered as an endogenous polyfunctional bioregulator of phenolic nature, which participates in cell signaling, growth processes, and formation of adaptive responses of plants (Borsani et al, 2001; Kolupaev *et al.*, 2012; Isoda *et al.*, 2021; Saleem *et al.*, 2021). There are complex relationships between nitric oxide and SA as signaling molecules, the nature of which remains largely unclear. On the one hand, nitric oxide, along with ROS, is considered as a mediator in the realization of SA physiological effects (Mur *et al.*, 2006) (Figure 2). At the same time, the realization of some nitric oxide effects as a signaling molecule can also be mediated by SA. The effect of increasing SA content in tobacco cells under the influence of exogenous NO has been known for quite a long time (Durner *et al.*, 1998). It has also been shown that salicylate-deficient tobacco plants transformed with the gene of bacterial salicylate hydroxylase (*NahG*) did not develop resistance to tobacco mosaic virus under the influence of NO donor (Song, Goodman, 2001).

On the other hand, nitric oxide seems to be an important participant of SA signal transduction. An increase in NO amount in response to SA action was found in soybean (Klepper, 1991) and ginseng plants (Tewari, Paek, 2011). A convincing example of the participation of NO in the realization of physiological effects of SA is provided by the results obtained when studying the effect of stress concentrations of CO_2 on *Camellia sinensis* L. plants. It was found that this stress factor caused an increase in the content of SA, nitric oxide, and flavonoids in plants (Li *et al.*, 2019). Exogenous SA and the NO donor SNP also caused flavonoid accumulation. The SA synthesis inhibitor paclobutrazol and the NO scavenger PTIO (2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl 3-Oxide) prevented flavonoid accumulation in response to the action of elevated carbon dioxide concentrations. At the same time, treatment of plants with paclobutrazol relieved the effect of increased NO content, whereas NO binding by PTIO had no effect on SA content. Thus, NO mediates SA-dependent flavonoid accumulation in *C. sinensis* plants (Li *et al.*, 2019).

It is possible that the SA-induced increase in nitric oxide content in plant cells is mediated by an increase in ROS. Thus, it was shown that the increase in nitric oxide content in wheat coleoptiles caused by SA treatment was eliminated by the action of the hydrogen peroxide scavenger dimethylthiourea (Karpets *et al.*, 2016). At the same time, SA increased the heat tolerance of coleoptile cells, but this effect was eliminated by antagonists of both nitric oxide and hydrogen peroxide.

The induction of salt tolerance in rice by exogenous SA was accompanied by an increase in endogenous NO content. At the same time, the nitric oxide scavenger hemoglobin eliminated the SA-induced activation of plant antioxidant system (Mostofa *et al.*, 2015). Dong *et al.* (2015) investigated the effects of SNP, SA, and their combination on salt tolerance of cotton seedlings. Both SNP and SA reduced the level of lipid peroxidation and hydrogen peroxide content in cotton leaves under salt stress. However, the stress-protective effect of SA and

SNP was more pronounced when applied together. Similar results were obtained when studying the effects of SNP and SA on salt tolerance of *Vigna angularis* (Ahanger *et al.*, 2020). When treated with these compounds separately, the manifestation of growth-inhibitory effect of salt stress was mitigated, the indices characterizing the development of oxidative stress were reduced, and at the same time, the activity of the antioxidant enzyme complex, as well as the content of sugars and glycine betaine increased. Also, nitric oxide donor and phytohormone promoted the maintenance of relative water content and photosynthetic pigments pool close to the control under stress conditions. However, when SA and SNP were applied together, all the above-mentioned protective effects were more noticeable.

In our work, we studied the combined effect of pre-sowing seed treatment with SA and foliar treatment with SNP of wheat plants on their tolerance to soil drought (Kolupaev *et al.*, 2018). It was shown that pre-sowing seed treatment with 10 or 100 μM SA solutions reduced growth inhibition and increased the activity of antioxidant enzymes in 14-day-old plants under soil drought. The same effect was induced by spraying 0.5 or 2 mM solutions of SNP before drought. The protective effect was enhanced when seed treatment with 10 μM SA was combined with spraying plants with 0.5 mM sodium nitroprusside, and was weaker when they were combined at high concentrations. Thus, exogenous nitric oxide can modify the stress-protective effect of SA on plants.

Nitric oxide, along with hydrogen peroxide and calcium ions, may be involved in SA-induced closure of stomata. It is known that NO, like SA, rapidly accumulates in plants upon infection or elicitor treatment, activating defense genes and, in some cases, inducing a hypersensitivity response (Durner *et al.*, 1998; Delledonne *et al.*, 2001). The involvement of NO in salicylate-induced closure of stomata in *Vicia faba* was demonstrated by an inhibition method. Later, using direct methods, it was found that SA caused an increase in NO in guard cells of Arabidopsis (Hao *et al.*, 2010). This effect was completely eliminated by the nitric oxide scavenger PTIO, and partially by inhibitors of animal NO synthase (L-NAME – N^G-nitro-L-arginine methyl ester) and nitrate reductase (sodium tungstate). Khokon *et al.* (2011) investigated the role of the functional interaction between ROS and nitric oxide in SA-induced closure of stomata in Arabidopsis. The authors showed that a peroxidase activity-dependent (but not NADPH oxidase activity-dependent) increase in ROS generation preceded the subsequent increase in nitric oxide content. The peroxidase inhibitor salicylhydroxamic acid relieved this effect.

Jasmonic acid (JA) is a phytohormone involved in the regulation of plant resistance to biotic (primarily necrotrophic pathogens and insect pests) and many abiotic stressors (Wasternack, 2007; Santino *et al.*, 2013; Wasternack, Hause 2013; Kolupaev *et al.*, 2023b).

There seems to be a lot of overlaps in the signaling pathways of nitric oxide and JA as one of the stresses phytohormones. A number of experimental data indicate a role of NO in JA synthesis (Sami *et al.*, 2018). It has also been shown that the expression of key genes involved in JA synthesis was enhanced by exogenous NO (Wendehenne *et al.*, 2004; Mur *et al.*, 2013; Verma *et al.*, 2020). UV-B irradiation of *Panax quinquefolius* caused enhanced formation of nitric oxide, jasmonic acid, and the triterpene glycoside ginsenoside in roots. The UV-B-induced increase in ginsenoside content was eliminated by the NO scavenger PTIO, the NO synthase inhibitor L-NAME, and the JA synthesis inhibitor salicylhydroxamic acid. Treatment with NO antagonists inhibited the UV-B-induced accumulation of JA, suggesting that NO is upstream of the JA signaling pathway (Zhou *et al.*, 2019).

In *Sophora flavescens*, NO treatment induced an increase in lipoxygenase activity and JA level in cells. In turn, exogenous application of JA stimulated NO formation associated with the oxidation of L-arginine (Xu, Dong, 2008). Thus, a possible mutual enhancement of JA and NO synthesis in *S. flavescens* cells was shown.

Experimental data have also been obtained indicating the involvement of NO in the transduction of JA signals into the genetic apparatus (Figure 2). For example, it has been shown that JA and NO are involved in the control of allantoin synthesis in sugar beet plants, which is important for their adaptation to saline-alkaline soils (Zhang *et al.*, 2023b). Inhibition of JA biosynthesis completely abolished exogenous allantoin-induced plant tolerance to saline-alkaline and NO accumulation. Also, the NO donor-induced increase in salt tolerance was not evident upon suppression of JA synthesis. On the other hand, inhibition of NO biosynthesis attenuated allantoin-induced saline-alkaline tolerance, JA accumulation, and JA-induced saline-alkaline tolerance in plants (Zhang *et al.*, 2023b). These data provide experimental support for the assumption of different levels of functional interaction between JA and NO. They are manifested in the influence of JA and NO on each other's synthesis (the presence of a self-reinforcing feedback loop), and in the involvement of signal transduction.

Treatment of wheat coleoptiles with JA induced the development of their heat resistance (Karpets *et al.*, 2016). This effect was accompanied by an almost simultaneous and transient increase in nitric oxide and hydrogen peroxide content. Treatment of coleoptiles with nitric oxide antagonists and antioxidants eliminated the development of heat tolerance, indicating the involvement of ROS and RNS in the signal transduction inducing the development of heat tolerance of wheat coleoptile cells. Treatment of cucumber fruits with methyl jasmonate and the nitric oxide donor SNP reduced their oxidative damage during low-temperature storage (Liu *et al.*, 2016). This effect was accompanied by an increase in *CAT1* and *CAT3* genes expression, and total catalase activity, which was responsible for the reduction of hydrogen peroxide content in fruits. At the same time, inhibitors of endogenous NO accumulation L-NAME and PTIO eliminated the development of cold tolerance induced by methyl jasmonate. On the other hand, inhibitors of JA synthesis ibuprofen and salicylhydroxamic acid had no effect on SNP-activated cold tolerance of fruits. In this regard, the authors suggest that NO mediates the methyl jasmonate signaling pathway that activates cold tolerance in cucumber (Liu *et al.*, 2016).

The effects of jasmonic acid on the state of the stomatal apparatus are mediated by calcium ions, ROS and nitric oxide (Munemasa *et al.*, 2011). The effects of increased NO and ROS content in closure cells in *Vicia faba* and *Arabidopsis thaliana* plants upon treatment with jasmonic acid or methyl jasmonate have been shown (Liu *et al.*, 2005; Munemasa *et al.*, 2007). In our experiments, we found that the reduction in the number of open stomata and in the stomatal aperture size in Arabidopsis caused by treatment with methyl jasmonate solutions was almost completely abolished by pretreatment of the epidermis with the nitric oxide scavenger PTIO and partially by the NO synthase inhibitor L-NAME and the nitrate reductase inhibitor sodium tungstate (Yastreb *et al.*, 2018). Thus, to varying degrees, jasmonate-induced closure of stomata depends on nitric oxide formation via both oxidative and reductive pathways. Nitric oxide is thought to be the final mediator of the phytohormone signal transduction that induces stomatal closure. NO by S-nitrosylation modifies the corresponding proteins and leads to changes in the activity of potassium and anion channels in guard cells (Fancy *et al.*, 2017).

Apparently, the effects of synergistic interaction between nitric oxide and jasmonic acid can also be utilized in the practical application of these compounds as inducers of plant resistance. Ahmad *et al.* (2018) investigated the combined effects of exogenous jasmonic acid and nitric oxide donor SNP on growth, antioxidant metabolism and osmolyte accumulation in tomato plants under salt stress. Treatment with both SNP and jasmonic acid promoted the preservation of relative water content and chlorophyll pool under stress conditions, reduced the manifestation of oxidative damage, increased the activity of SOD, catalase, ascorbate peroxidase and glutathione reductase, and enhanced the synthesis of flavonoids, proline and glycine betaine. At the same time, all these effects were more pronounced in the combined treatment with SNP and jasmonic acid.

Brassinosteroids (BSs) are a class of plant polyhydroxy steroids structurally related to steroid hormones of vertebrates and insects. BSs play a key role in maintaining normal plant growth both under optimal conditions and when exposed to unfavorable environmental factors (Ahammed *et al.*, 2020; Bartwal, Arora, 2020; Mohammadi *et al.*, 2021). High biological activity in plants was recorded mainly for such BSs as brassinolide, 24-epibrassinolide (24-EBL) and 28-homobrassinolide (Bajguz, 2014).

Like other phytohormones, all major signaling mediators, including ROS and NO, are involved in BSs signaling (Figure 2). It has been shown that in cucumber plants, NO antagonists relieved the effects of BS-induced increase in resistance to the oxidative stress agent paraquat, leveled the enhancement of antioxidant gene expression and an increase in their activity, which indicates the role of nitric oxide as a mediator in the realization of stress-protective effects of BSs (Cui *et al.*, 2011). Treatment of Bentham tobacco plants with brassinolide caused an increase in their salt tolerance (Zhu *et al.*, 2016). This effect was accompanied by an increase in nitric oxide content in leaves and was leveled by treatment with the NO scavenger PTIO and the nitrate reductase inhibitor sodium tungstate.

One of the mechanisms of stress-protective action of BSs may be their induction of stomatal closure (Jiroutova *et al.*, 2018). However, it has been shown that this process is associated with an increase in ethylene synthesis, which, in turn, affects the condition of stomata by enhancing the synthesis of ROS and nitric oxide (Shi *et al.*, 2015).

It is likely that nitric oxide is also involved in the induction of heat tolerance in plants. In our experiments, it has been shown that after treatment with 24-EBL there was a transient increase in nitric oxide generation by wheat coleoptiles (Karpets, Kolupaev, 2018). At the same time, pretreatment of coleoptiles with nitrate reductase and NO synthase inhibitors partially relieved the effect of the increase in NO content caused by the action of 24-EBL. Nitric oxide antagonists also prevented the development of heat tolerance in coleoptile cells induced by 24-EBL treatment. Thus, NO appears to play a role in the transduction of the brassinosteroid signal that induces the development of heat tolerance in plant cells.

Experimental evidences for the involvement of nitric oxide in the induction of cold tolerance in *Medicago truncatula* plants by brassinolide action has also been obtained (Arfan *et al.*, 2019). Inhibitor methods showed that the NO synthesis, mediated by the increase in ROS content, is necessary for the activation of alternative oxidase gene expression under the action of brassinolide, which is important for the development of cold tolerance.

The role of nitric oxide, formed with the participation of nitrate reductase, in the realization of stress-protective effects of BSs is also indicated by the data obtained in experiments with pepper plants exposed to cadmium toxicity. It was shown that 24-EBL-induced NO formation was eliminated when plants were treated with the nitrate reductase inhibitor sodium tungstate (Kaya *et al.*, 2020a). At the same time, the nitrate reductase inhibitor also leveled the development of defense reactions induced by 24-EBL: activation of antioxidant enzymes, increase in the content of ascorbic acid and glutathione, proline accumulation.

Data have also been obtained on the enhancement of stress-protective effects of brassinosteroids and nitric oxide when applied together. Thus, it was shown on rapeseed plants that treatment with a combination of 24-EBL and SNP had a more significant positive effect on shoot growth compared to the effects of each compound separately (Gupta *et al.*, 2017). Also, under the combined effect of brassinosteroid and NO donor, higher protein and proline content was observed in plants under salt stress.

The study of the combined effect of 24-EBL and SNP on the heat tolerance of wheat seedlings showed that the nature of the effects depended on the range of concentrations of these compounds. Combined treatment with 20 nM 24-EBL and 0.2 mM SNP caused a more significant protective effects compared to the treatment with each compound separately (Karpets *et al.*, 2021). At the same time, the combined effect of high concentrations (200 nM 24-EBL and 2 mM SNP) reduced the heat tolerance of seedlings. It was shown that the enhancement of the protective effect of 24-EBL and SNP on wheat seedlings when applied together at low concentrations was largely due to the stabilization of the pro/antioxidant balance (Karpets *et al.*, 2021).

Melatonin (N-acetyl-5-methoxytryptamine) is the major indolamine in plants. It has been described as a multifunctional stress metabolite (Fan *et al.*, 2018; Arnao, Hernández-Ruiz, 2019; Khan *et al.*, 2023b). Meanwhile, a growing body of experimental evidence suggests its involvement in the functioning of signaling and hormonal networks in plants, their stress-protective systems, including antioxidant systems (Nawaz *et al.*, 2021; Karpets *et al.*, 2023). There are emerging ideas that melatonin effects may be manifested at the level of gene expression, protein status, as well as through the involvement of other hormones and non-hormonal mediators in the signal transduction system (Zeng *et al.*, 2022).

Data on the links between melatonin and signaling mediators in the manifestation of its effects in plant cells are still insufficient, but they are dynamically accumulating. For example, there are data indicating the involvement of calcium ions and ROS in the realization of protective effects on watermelon plants under cold stress (Chang *et al.*, 2021). A number of data have also been obtained indicating the involvement of NO as a signaling mediator in the realization of physiological effects of melatonin. Thus, the induction of heat tolerance in tomato by melatonin was accompanied by a decrease in the manifestation of oxidative stress and the membrane damage index. At the same time, melatonin treatment of plants caused an increase in nitric oxide content. The authors suggest that NO is a necessary mediator for the development of heat tolerance in plants under the action of melatonin (Jahan *et al.*, 2019).

The protective effect of melatonin on wheat plants exposed to cadmium toxicity was also accompanied by an increase in NO synthesis and eliminated by the nitric oxide scavenger PTIO (Kaya *et al.* 2019). The involvement of nitric oxide as a mediator was also demonstrated in a study on the effects of melatonin on cadmium stress tolerance in Catharanthus roseus (Nabaei, Amooaghaie, 2019). Melatonin treatment of plants increased proline content and antioxidant enzymes activity in roots. These melatonin-induced responses were suppressed by the nitric oxide scavenger cPTIO. Also, nitric oxide may mediate the induction of salt tolerance in rapeseed by exogenous melatonin. Treatment of rapeseed plants with melatonin caused an increase in NO content. At the same time, the nitric oxide scavenger PTIO eliminated the stress-protective effect of melatonin (Zhao *et al.*, 2018).

In addition, it was shown that the induction of salt tolerance in rapeseed by melatonin action is mediated by nitrate reductase-dependent increase in NO synthesis. The authors believe that S-nitrosylation of target proteins is an obligatory component of the melatonin-triggered signaling pathway leading to increased salt tolerance (Zhao *et al.*, 2018).

A nitric oxide-dependent manifestation of the stress-protective effect of melatonin was also found in pepper plants subjected to salt or iron deficiency stress. The positive effect of melatonin on plants under such conditions was eliminated by treatment with the NO scavenger PTIO (Kaya *et al.*, 2020b). In general, the effects of melatonin on stress-protective systems, particularly antioxidant systems, are thought to be mediated by nitric oxide-induced post-translational modifications of target proteins (Martínez-Lorente *et al.*, 2022).

On the other hand, nitric oxide can act as a signal that induces melatonin synthesis. It has been shown that NO, mediated by cGMP, can activate the expression of genes of melatonin synthesis enzymes – *TDC*, *T5H*, *SNAT* and *COMT*, resulting in an increase in endogenous melatonin content (Wang *et al.*, 2022). The NO scavenger cPTIO disrupts Cd-induced melatonin synthesis by decreasing the expression of *TDC* and *COMT* genes in rice (He, He, 2020). Thus, it is likely that there are mechanisms for the mutual enhancement of melatonin and nitric oxide synthesis in plants.

γ-aminobutyric acid (GABA), a non-proteinogenic four-carbon amino acid found in many prokaryotic and eukaryotic organisms, has only recently been listed as an important physiologically active substance in plants (Seifikalhor *et al.*, 2019). At present, new knowledge about the regulatory functions of GABA in plants is dynamically accumulating (Suhel *et al.*, 2023), and the influence of exogenous GABA on the resistance of plants of different taxonomic groups to various types of stress factors is intensively investigated. The identified phenomena are also related to plant resistance to low and high temperatures, drought, and salinity (Kolupaev *et al.*, 2024). The relationship between GABA and nitric oxide as signal-regulatory molecules in the formation of plant adaptive responses is still poorly understood. Nevertheless, there are results that indicate the possibility of inducing GABA synthesis under the action of nitric oxide. For example, the NO donor SNP enhanced GABA biosynthesis in soybean seedlings under UV-B irradiation (Suhel *et al.*, 2023). Treatment of banana fruits with exogenous NO increased glutamate decarboxylase activity, which led to GABA accumulation (Wang *et al.*, 2016).

At the same time, the physiological effects of GABA are probably also realized with the participation of nitric oxide as a signaling mediator. Thus, it was found that the stress-protective effect of GABA on melon plants exposed to soda salinity was accompanied by an increase in the activity of the NO synthesis enzymes, such as nitrate reductase and NO synthase, as well as in nitric oxide content (Xu *et al.*, 2021). Meanwhile, treatment of plants with the nitric oxide scavenger cPTIO eliminated the increase in NO content induced by GABA treatment and its stress-protective effect on plants. The authors conclude that NO is a component of GABA signaling that is necessary for stimulation of the antioxidant system and regulation of ion homeostasis under stress conditions (Xu *et al.*, 2021).

Treatment of wheat plants with GABA increased photosynthetic productivity and reduced salt stress-induced oxidative damage by improving ascorbate-glutathione cycle function and proline metabolism (Khanna *et al.*, 2021). Treatment with the NO scavenger cPTIO eliminated the positive effects of GABA on plant growth and photosynthesis under salt stress, indicating that the action of GABA is mediated by NO.

FUNCTIONAL INTERACTION OF NITRIC OXIDE WITH PHYTOHORMONES IN THE REGULATION OF SEED GERMINATION

It is known that the processes of seed emergence from dormancy and germination initiation are caused by an increase in the level of water content in the axial parts of the embryo and changes in the balance of phytohormones, primarily such as ABA, gibberellins, auxins, and ethylene. It has been shown that cell cycle activation, which is required for embryo germination, is induced by increased content of ethylene and gibberellins (especially gibberellic acid 3 – GA3) and decreased ABA content (Kepczynski *et al.*, 2017). According changes may be induced by signaling mediators, particularly nitric oxide, including exogenous application (Liu *et al.*, 2011). Attempts have been made to investigate the relationship between the generation of endogenous nitric oxide in seeds and their ability to germinate. Thus, it has been shown that seeds of chickpea varieties that generated more NO germinated faster than seeds of varieties with weak NO synthesis (Pandey *et al.*, 2019).

To date, the general molecular mechanisms of nitric oxide effects on the hormonal balance of germinating seeds have been elucidated (Arc *et al.*, 2013). These effects are largely related to S-nitrosation and tyrosine nitration of target proteins. Thus, an increase in the content of tyrosine nitrated proteins was recorded in sorghum germ axes during germination (Jasid *et al.*, 2008). In Arabidopsis, it has been shown that tyrosine nitration leads to the inhibition of the Mo cofactor of sulfurase, the enzyme of the last step of ABA synthesis (Lozano-Juste *et al.*, 2011). It is suggested that inactivation of ABA synthesis by this mechanism may promote seed germination (Rajjou *et al.*, 2012) (Figure 3). Also, under the influence of NO, S-nitrosation of a number of proteins involved in ABA signal reception and transduction occurs, thereby inhibiting the transmission of this signal (Signorelli, Considine, 2018). Finally, it is known that NO entails an increase in ABA catabolism, probably through induction of ABA 8'-hydroxylase gene expression (Arc *et al.*, 2013). In addition, it has been reported that nitric oxide can enhance ROS generation in plant cells through complex mechanisms involving both post-translational modifications of target proteins and influence on many components of the signaling network (Kolupaev *et al.*, 2023a). Moderate enhancement of ROS formation usually promotes seed germination. One of the reasons for this phenomenon may be the ability of ROS, primarily H_2O_2, to enhance ABA degradation processes (Bailly, 2004). Together, this eliminates the regulatory effects of ABA that condition seed dormancy (Figure 3). At the same time, nitric oxide leads to the activation of enzymes of GA3 (Signorelli, Considine, 2018) and ethylene synthesis (Kolbert *et al.*, 2019), which can activate germination. In particular, it has been shown that interruption of apple seed dormancy by the action of exogenous NO induced ethylene production and was eliminated by inhibitors of its synthesis (Kolbert *et al.*, 2019). Induction of Amaranthus retroflexus seed germination by NO donors was also preceded by an increase in ethylene synthesis (Kepczynski *et al.*, 2017). It has been found that the treatment of seeds with NO can directly alter the activity of key enzymes in the ethylene synthesis process – S-adenosyl-L-methionine methylthioadenosine lyase (ACS) and 1-aminocyclopropane-1-carboxylic acid oxidase (ACO) (Gniazdowska *et al.*, 2010).

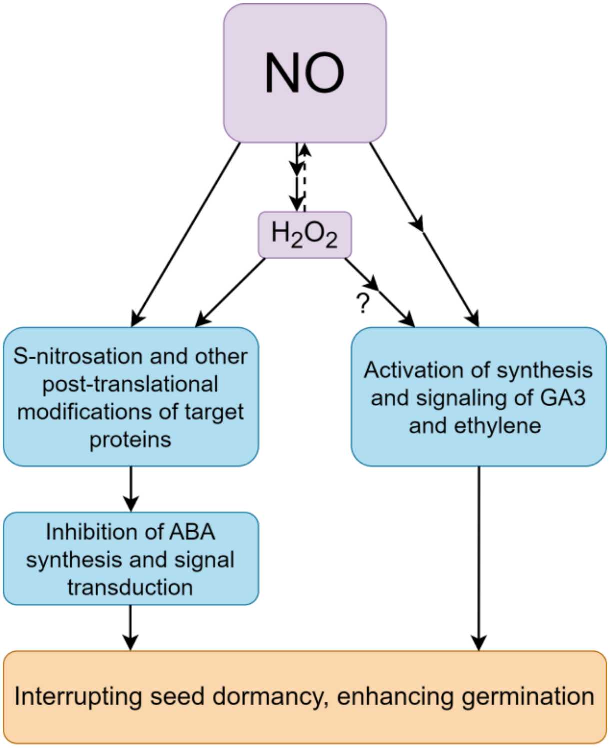

Figure 3. Mechanisms of nitric oxide effects on seed germination.

In addition to gibberellins and ethylene, two other major classes of phytohormones, auxins and cytokinins, seem to be involved in the action of nitric oxide donors. Using seed germination of different plant species as an example, it has been shown that SNP treatment promotes lateral root development and primary root elongation (Nejadalimoradi *et al.*, 2014; Ullah *et al.*, 2024). These effects are attributed to the interaction between auxin and nitric oxide, as treatment with nitric oxide donors can affect auxin content and stimulate auxin-induced root development responses (Ullah *et al.*, 2024).

At the same time, treatment of seeds with exogenous nitric oxide stimulates shoot growth in many species. This effect is attributed to the positive interaction of NO with cytokinins, which affect cell elongation in the shoot apical meristem (Zhao *et al.*, 2020).

The effect of nitric oxide donors on seed germination is particularly pronounced under stress conditions (Ciacka *et al.*, 2022). It is well known that almost all unfavorable effects on plants lead to the disturbance of pro/antioxidant balance and the development of oxidative stress (Hasanuzzaman *et al.*, 2020). It has also been found that increased ROS formation is an indispensable attribute of seed germination process (Kranner *et al.*, 2010). At the same time, as mentioned above, ROS may be involved in stimulating seed germination, including by enhancing the degradation of ABA. However, excessive ROS generation during seed germination under stress conditions can cause oxidative damage to proteins and membrane lipids, thereby reducing germination and/or retarding seedling growth (Janmohammadi *et al.*, 2012). Thus, agents that mitigate oxidative stress by activating the antioxidant system could potentially promote seed germination. Nitric oxide, at least at physiological concentrations, activates many antioxidant enzymes both by PTM of protein molecules and by affecting gene expression of antioxidant enzymes (Kolupaev *et al.*, 2023a). In this regard, it is believed that one of the components of the potentially positive effect of nitric oxide donors on seed germination under stress may be the activation of their antioxidant system (Ciacka *et al.*, 2022). This component of NO action may explain the described phenomena of more effective influence of nitric oxide donors on seed germination under stress conditions compared to normal conditions (Shams *et al.*, 2018).

A number of studies have reported the enhancement of seed germination of various crops at reduced temperatures by nitric oxide donors. Such effects were observed, for example, in wheat (Bibi *et al.*, 2020) and tomato seeds (Amooaghaie, Nikzad, 2013). Soaking rapeseed seeds in SNP solutions also increased seed germination indicators under low temperature and drought conditions (Zhu *et al.*, 2021). At the same time, under the influence of SNP treatment, a change in the hormonal balance and an increase in the activity of antioxidant enzymes in seedlings under stress conditions were observed.

Priming of maize seeds with NO donor also enhanced antioxidant defense of seedlings under heat stress conditions. Increase in SOD, catalase and peroxidase activities and decrease in content of hydrogen peroxide and LPO product (MDA) were observed in seedlings of seeds primed with SNP (Kaur, Kaur, 2018). Similarly, priming of *Arachis hypogaea* L. seeds with SNP enhanced their germination under model drought conditions. It increased the activity of antioxidant enzymes, sugar and soluble protein content, and decreased MDA content (Sepehri, Rouhi, 2016).

It has also been shown that NO mitigates the decrease in germination percentage, germination index, viability index and water absorption rate in wheat seeds subjected to salt stress (Duan *et al.*, 2007). According to the authors, this effect is largely due to an increase in β-amylase activity. Treatment of quinoa (*Chenopodium quinoa*) seeds with SNP also reduced the adverse effects of salt stress on α-amylase and β-amylase activity and seed germination rate (Hajihashemi *et al.*, 2020). A similar effect of SNP was also shown in the germination of *Brassica chinensis* L. under salt stress (Ren *et al.*, 2020). The authors showed an increase in the indicators of seed germination and vigor under the influence of the NO donor, as well as an increase in the activity of antioxidant enzymes. Moreover, SNP pretreatment significantly increased K^+ content and decreased Na^+ content in roots and shoots, resulting in an increase in the K^+/Na^+ ratio (Ren *et al.*, 2020).

A number of studies have reported the enhancement of seed germination by nitric oxide donors in the presence of heavy metals. Thus, it was shown that SNP treatment attenuated Cd-induced inhibition of seed germination and growth of rice seedlings (He *et al.*, 2014). The authors attributed this effect to the stimulation of antioxidant enzyme complex activity and proline accumulation by the nitric oxide donor. Soaking lettuce (*Lactuca sativa* L.) seeds in SNP solutions reduced the toxic effect of copper, although in the absence of stress the NO donor did not affect the germination of seeds of this species (Shams *et al.*, 2018). SNP pretreatment of Indian mustard (*Brassica junea* L.) seeds also improved their germination against the toxic effect of copper (Rather *et al.*, 2020). Under the influence of NO, the antioxidant defense system was enhanced (SOD, glutathione reductase and ascorbate peroxidase activities increased) and lipid peroxidation decreased in seeds exposed to copper. Seeds pretreated with NO donor also retained higher amylase activity (Rather *et al.*, 2020).

CONCLUSIONS

Nitric oxide is a key regulatory molecule of plant cells. Its diverse effects on almost all functions of the plant organism are due to its ability to selectively interact with many protein targets, and thus influencing the formation or inactivation of other signaling molecules, as well as the synthesis and degradation of phytohormones. Many of the effects of nitric oxide are associated with its involvement in the subtle processes of redox regulation of cellular functions and are related to both the enhancement of ROS generation and the activation of the antioxidant system. These processes are very important for plant resistance to stress factors. At the same time, these processes together with the hormonal system are also involved in the regulation of seed germination. Thus, nitric oxide can be considered as a subtle tool in the regulation of plant resistance to stress factors and seed germination processes. The availability of relatively non-toxic and cheap nitric oxide donors (e.g., the most popular sodium nitroprusside) makes such a tool available for practical application. However, the effects of nitric oxide donors are dose-dependent. The range between concentrations that have stress-protective or stimulating effects and concentrations that cause toxic or inhibitory effects is not so wide. In this regard, for the practical application of nitric oxide donors, special studies are needed to select optimal concentrations and treatment methods for each new object, taking into account its species and even varietal characteristics. The practical application of nitric oxide donors in crop production is also hampered by the fact that the currently accumulated knowledge is mainly in the field of physiology and biochemistry and is largely empirical in nature. It is hoped that the use of molecular genetic tools, as well as proteomics and metabolomics, will make it possible to develop theoretical models that more thoroughly explain the mechanisms of nitric oxide influence on growth and stress-protective processes in plants. This, in turn, should allow for a clearer prediction of the effects of exogenous nitric oxide, which is necessary for its application in crop production technologies.

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