University of Montenegro
Biotechnical faculty

Univerzitet Crne Gore
Biotehnički fakultet

Agriculture and Forestry
Poljoprivreda i šumarstvo
1

Agriculture and Forestry, Vol.65. Issue 1: 1-258, Podgorica, 2019

ISSN 0554-5579; E-ISSN 1800-9492; DOI: 10.17707/AgricultForest
COBIS.CG-ID: 3758082 www.agricultforest.ac.me

Agriculture and Forestry - ***Poljoprivreda i šumarstvo***
PUBLISHER - ***IZDAVAČ***
University of Montenegro – Univerzitet Crne Gore
Biotechnical faculty (BTF), Podgorica - Biotehnički fakultet, Podgorica
Bul. M. Lalića 1, 81000 Podgorica, Crna Gora (Montenegro), P.Box 97,
Tel.: +382 20 268434; +382 20 268437; Fax: +382 20 268432
Web: www.agricultforest.ac.me; E-mail: agricultforest@ac.me

Indexed in (by alphabethical order): AGRICOLA, AGRIS, CAB Abstracts, CAB Direct, CABI full text, COBISS, CrossRef, DOI, DOAJ, EBSCO (currently included in EDS EBSCO Discovery Service), Elektronische Zeitschriftenbibliothek EZB (Electronic Journals Library), Genamics JournalSeek, Global impact factor (GIF), Google Scholar, HathiTrustt, Index Copernicus, International Scientific Indexing (ISI), Israel Union List of Electronic Journals (ULE), JournalRate (Academic journal search tool), Journals Impact factor (JIF) by Global Society for Scientific Research (GSSR), KoBSON, MALMAD, National Agricultural Library – USA, Open Academic Journals Index (OAJI), ProQuest, Research bible, RoMEO, Regional Information Center for Science and Technology (RICeST) – Iran, Rubriq database, The Open University Library, The Rudjer Bošković Institute Library, Ulrichsweb, Universal Impact Factor, WorldCat, XML, Zeitschriftendatenbank (ZDB), Zoological Record of the Clarivate Analitics Master Jouurnal List, ...

The journal "Agriculture and Forestry" is funded by the Biotechnical faculty, Co-funded by the Ministry of Science & the Ministry of Agriculture and Rural Development of Montenegro

CIP – Каталогизација у публикацији
Централна народна библиотека Црне Горе, Цетиње
ISSN 0554-5579
COBIS.CG-ID 3758082

CONTENT

DOI: 10.17707/AgricultForest.65.1.01

Aleksandar SIMIĆ*, Željko DŽELETOVIĆ, Savo VUČKOVIĆ, Muamer BEZDROB, Marija ĆOSIĆ, Gordana ANDREJIĆ, Hakan GEREN [1]

HEAVY METAL UPTAKE BY GRASSLANDS DEVELOPED IN A DEGRADED SOIL IN CENTRAL BALKANS

SUMMARY

Extensively managed natural grasslands are predominant in Central Balkan countries (Montenegro, Bosnia and Herzegovina, and Serbia) and the productivity of these communities is extremely low in general. Beside main limitations in grassland productivity, such as strongly acidic soils, organic matter fluctuations, high variability in phosphorus content, some soils were developed on bedrocks with potentially high heavy metal content. The researches were conducted in 2016 and 2017 on representative grasslands in three countries, mainly on *Agrostietum capillaris* type of community. There were five study sites in Montenegro, two in Bosnia and Herzegovina, and six in Serbia in mountainous region.

We analysed nutritive status of the topsoil samples collected in summer in each study site, as well as possible presence of heavy metals (Ni, Cd, Pb and Cr) in the soil. The experimental fields were cut once in the time of inflorescences formation of the dominant grasses and the total concentrations of heavy metals (Pb, Ni, Cd and Cr) in the samples of plant material were determined. In all study sites soil pH was acidic, with low P content, except in a certain site in Bosnia. Generally, the soils were low productive, but according to Regulation of tolerant amount of hazardous and toxic materials in soil, there were not surpassed maximum permissible concentrations of Ni, Cd, Pb and Cr in Montenegro and Bosnia, while in some sites in Serbia very high concentrations of Ni and Cr were observed. Although some elements exceeded maximum permissible amount for soil and water, the ability of plants collected from the *Agrostietum capillaris* communities to accumulate heavy metals was generally low. It could be explained by the physiology of dominant plant species (grasses), which influenced relatively low uptake and generally low accumulation of micronutrients.

Keywords: *Agrostietum capillaris*, Central Balkans, degraded soil, heavy metals.

[1]Aleksandar Simić*(corresponding author: alsimic@agrif.bg.ac.rs), Savo Vučković, Marija Ćosić, University of Belgrade, Faculty of Agriculture, Nemanjina 6, Zemun-Belgrade, SERBIA; Željko Dželetović, Gordana Andrejić, University of Belgrade, Institute for Application of Nuclear Energy, Belgrade, SERBIA; Muamer Bezdrob, University of Sarajevo, Faculty of Agriculture and Food Sciences, Sarajevo, BOSNIA AND HERZEGOVINA; Hakan Geren, University of Ege, Faculty of Agriculture, Department of Field Crops, Izmir, TURKEY.

Paper presented at the 9th International Scientific Agricultural Symposium "AGROSYM 2018".
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

INTRODUCTION

Mountain soils have performed vital services on the ecosystem for a long time that help to ensure food security and nutrition to 900 million mountain people around the world and benefit billions more living downstream (FAO, 2015).

The Balkans are a mountainous region. Most of the Balkans have a harsh, continental climate with hot, dry summers and cold winters. The peninsula was once covered with many forests. However, over the centuries, they have been cut down to make room for settlements, agriculture and especially, for pastures and meadows as a forage source for animal husbandry.

Agricultural land covers about 38% of Montenegro. Meadows and pasture cover 33.1% of the territory or 88.25% of agricultural land. Only 10% of the territory is below 200 m above sea level, 35% from 200 to 1 000 m, 40% from 1 000 to 1 500 m, while about 15% is above 1 500 m (Radojičić, 2002). On the basis of the influence of agro-ecological factors, primarily the climate and soil, as well as the way and level of exploitation, Montenegro is provisionally divided into five production regions, where the Northern-mountain and Polimlje-Ibar regions are more suitable than others for fodder production and animal husbandry (Dubljević, 2009). Different forms of flysch are present in the coastal area and on the southern slope of Durmitor mountain and surrounding massifs ('Durmitor flysch'). Natural grasslands, especially pasture, cover poorer land, unsuited to intensive exploitation. They are mostly on steep slopes, shallow soils and with many large stones. Meadows are on deeper soil, flatter and fertile, especially in river basins and plateaux of hilly-mountain areas.

Forty-five percent of agricultural land in Bosnia is hilly (300– 700 m), of medium quality and well suited to semi-intensive livestock production (Alibegović-Grbić, 2009). Mountain areas (> 700 m) account for a further 35% of agricultural land but high altitude, steep slopes and lower soil fertility limit the use of this land to grazing in spring and summer.

Soils in Serbia, and especially in western Serbia, are poor in available P, while K content is variable and depends on location. Grassland dry matter yield was between 2.03 to 5.70 t ha^{-1}, confirmed that pastures and meadows need to be maintained through fertilizer application (Simić *et al*., 2016). Zlatibor is a vast rolling plateau that is geographically defined by the territory between rivers Sušica and Uvac, the eastern slopes of mountain Tara and the western slopes of Murtenica. The mountain Zlatibor in western Serbia is largely built of Upper Jurassic ultramafics. Geologic bedrock at mountain Zlatibor is composed of serpentine, peridotites and serpentinized peridotites. In the northern, part of the region limestone prevails, while, in contrast to the limestone, the main rock type, away from the northern and eastern fringes of the plateau, is green serpentine and this forms the largest serpentine massif in Serbia.

Natural grasslands, meadows and pasture are the most important sources of roughage in Balkan countries, especially in hilly-mountain areas where they provide the only feed for cattle. In winter, cattle are mainly fed hay collected

from natural meadows and in summer, cattle graze the pastures and the meadows after mowing.

Mountain pastures, which make up the majority of the natural grasslands, are of great importance for production of animal feed and protection against soil erosion on steep and rocky terrain. Better pastures at altitudes of 1 000 to 1 500 m on deeper and more fertile soil are used for both mowing and grazing, but shallow and eroded sites are only grazed. At higher altitudes, there is a zone of montane pastures which are less used due to inaccessibility and distance unless they are near larger summer settlements (Dubljević, 2005; 2007). Although the mountain region can be seen as a unique area of mountain and high mountain pastures, there are substantial differences between localities. These are characterized by varied floristic composition and grass cover influenced by differences in climate, relief and soil. The best pastures are on flat, less rocky terrain with permanent mountain settlements and higher altitude summer settlements.

Beside natural conditions, human activities have had a big influence on the floristic composition and productivity of these pastures, by manuring (moving sheepfold) and more intensive exploitation. These are the main source of animal feed for both summer and winter. The land base for agriculture is thus very limited in both quantity and quality. Excessive deforestation, inappropriate conversion of grassland to arable and uncontrolled cultivation of sloping terrain are degrading the land, even in the valleys and lowland regions.

Meadows of *Agrostietum capillaris* are of secondary anthropogenic origin, as they are the result of two anthropogenic factors: reduced area under forest on the one hand, and mowing, on the other hand. The association *Agrostietum capillaris* covers a huge area in the hilly region of the Balkan Peninsula (Vučković *et al*., 2010). The association *Agrostietum capillaris* prospers on low-nutrient acidic soils of this region. On southeast part of Bosnia, western Serbia and northern part of Montenegro, this community is the dominant meadow type and it is widely distributed. In this area, it develops on quite different sites.

In large concentrations, many of the trace elements/metals may be toxic to plants and/or animals, or may affect the quality of foodstuffs for human consumption. These potentially toxic elements include As, B, Cd, Cu, F, Pb, Hg, Mo, Ni, Se and Zn. The relationship between trace elements in plants and amounts absorbed and utilized by the animal is again complex and depends on factors such as selectivity in grazing, the degree of dependence of the animal on grass as a source of trace element dietary intake, digestibility of the diet and form and 'availability' of the ingested trace elements (Thornton and Alloway, 1974).

Therefore, our research was directed to ascertain the content of heavy metals in the specific soils of the mountains in Balkan countries (Serbia, Bosnia & Herzegovina and Montenegro), covered by grasslands, in order to estimate element concentration in the produced forage, because deficiency or excess of dietary mineral elements may cause animal health concerns.

MATERIAL AND METHODS

The experiments were carried out in 2016 and 2017 in the mountainous region of Montenegro, Serbia and Bosnia & Herzegovina. Pasture areas of the study sites are mainly occupied by plant community *Agrostietum capillaris*. For the purpose of determining the concentrations of heavy metals in soil and plants, samples were collected from permanent grasslands in diverse ecological conditions from thirteen sites in three countries (Figure 1).

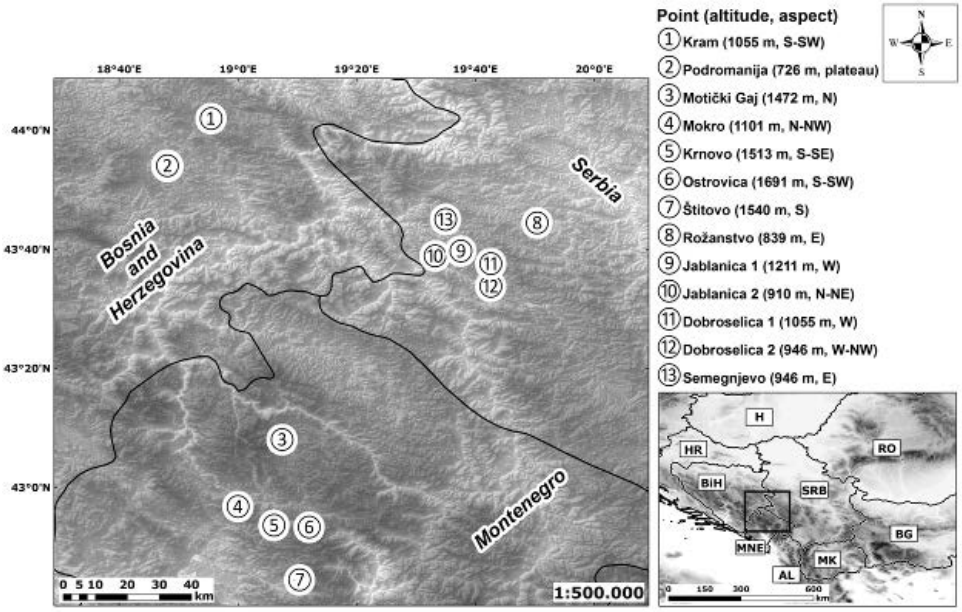


Figure 1. Study locations in three countries

Based on morphological and basic physico-chemical soil characteristics after IUSS (2014) classification, three types of pasture soils were determined: Dystric cambisol (Dystric leptosol), Eutric Cambisol (on limestone) ie. Mollic Leptosol and soils composed of serpentine, peridotites and serpentinized peridotites. Brown acid soils (Dystric cambisols), covering an area of 395 000 ha, are the second major type of soil in Montenegro, and are especially characteristic of the north-east of Montenegro. Brown eutric soils (Eutric cambisol), cover an area of 118 000 ha, and occupy the lowest land of river basins (old river terraces), ravines and karst fields. Serpentine (ophiolithic, ultramafic) rocks represent a group of siliceous rocks which are characterized by calcium deficiency, high concentrations of aluminium, iron, magnesium, nickel, cobalt and chromium, and a few plant nutrients. In contrast to other acid siliceous rocks, the pH values of the serpentine substrate vary from basic to ultrabasic (pH 5.5–8). Serpentine flora and vegetation differ from those occurring on other types of siliceous substrates or limestone. Open serpentine habitats are characterized by pronounced thermophilous character and xeric conditions. The xerothermic

character of serpentine plants is also enhanced by the specific chemical composition of the serpentine substrate (Kabaš *et al*., 2013). The study fields were mowed once in the time of inflorescences formation of the dominant grass – *Agrostis capillaris* (late June – early July). Nutritive status of the topsoil samples (0-10 cm) collected in summer in each study site was determined by followed methods: pH values in deionized water and 1M KCl (1:2.5 w v^{-1}); total organic C by dichromate redox titration method; total N by semi-micro Kjeldahl method; and available P and K by the AL-method (Pansu and Gautheyrou, 2006).

The total concentrations of heavy metals (Pb, Ni, Cd and Cr) in the samples of plant material were determined, according to procedure described by Jones and Case (1990), by the decomposition of HNO_3 at 125°C. After cooling down by 30% H_2O_2 was added and digesting was continued until the digest was clear. In order to determine the content of available microelements in the soil, dried and sieved soil was mixed continuously for 2 h in 1 M ammonium acetate and 0.01 M EDTA mixture (pH 7), according to the Standard NF X 31-120.22. The concentrations of the microelements and heavy metals were determined by atomic absorption spectrophotometry (Shimadzu AA 7000). The obtained results were processed by calculating average value and standard deviation for each sample.

RESULTS AND DISCUSSION

Environmental conditions

The basic agrochemical properties of the meadows are presented in Table 1. The soil reaction (pH in H_2O and 1M KCl) ranged from acid to very acid, while the contents of total organic C and N where high *(*Džamić *et al*., 1996).

Table 1. Chemical properties of the soil

Location	pH		Total organic C %	OM %	Total N %	AL-P_2O_5 mg 100 g^{-1}	AL-K_2O mg 100 g^{-1}
	in H_2O	in KCl					
Podromanija	5.11	3.98	3.95	6.80	0.324	23.7	34.9
Han Kram	5.33	4.42	5.89	10.2	0.408	3.22	22.7
Motički Gaj	5.82	5.11	7.27	12.5	0.146	1.9	27.7
Mokro	5.40	4.16	4.92	8.48	0.313	1.01	21.9
Krnovo	5.52	4.52	5.27	9.08	0.445	1.01	21.2
Ostrovica	5.03	4.18	9.74	16.8	0.818	3.13	33.3
Štitovo	5.26	4.16	10.5	18.1	0.844	1.37	45.5
Rožanstvo	5.98	4.88	4.92	8.49	0.323	0.21	19.8
Jablanica 1	6.56	5.55	6.28	10.8	0.465	4.57	22.3
Jablanica 2	5.80	4.22	4.15	7.15	0.298	2.38	9.89
Dobroselica 1	5.98	4.88	4.92	8.49	0.327	0.21	19.8
Dobroselica 2	6.16	4.58	5.46	9.40	0.374	5.94	31.8
Semegnjevo	6.67	5.74	9.83	17.0	0.689	9.59	61.0

Relatively large amount of rainfall and low temperatures during the year, favour the slow pace of the process of mineralization of organic substances in these mountain soil, and which result in higher amounts C and N. The amount of precipitation is relatively high in central Balkan mountainous region, more than 1000 mm.

Although the amount of precipitation has a great effect on the permanent grassland productivity, especially that during vegetation, it is frequently not well disturbed. This happens because a rainy period is frequently followed by a marked fall of temperature, which retards growth and development of grasses, and hence reduces the productivity of the biomass (Dubljević, 2007; Vučković *et al*., 2010). Analysed soil substrate had low P content (except one location in Bosnia), and well-supplied with available K (Džamić *et al*., *1996*).

Heavy metals content in plants

Mountain Zlatibor was situated in serpentine areas of Serbia. Serpentine soils are known for elevated heavy metal load. Therefore they may pose a threat that can compromise mineral content of grassland plant's organs. Serpentine soils are loaded with certain heavy metals, and are known to host and give rise to metal hyperaccumulating plants (Reeves *et al*., 1999). Heavy metal analyses were made of various plants shoots growing on soils with elevated Ni and Cr, covered mainly by *Agrostietum capillaris* communities (Table 2). According to Regulation of tolerant amount of hazardous and toxic materials in soil *(*Official gazette of RS, 1994*)*, in eight tested soil samples Ni content and in six soils samples Cr surpassed the maximum permissible concentrations (Table 2).

Table 2. Total and available heavy metal content of topsoil (0-10 cm) and herbage in permanent grasslands (mg kg^{-1} dry weight)

Available heavy metals content in topsoil				
Location	Ni	Pb	Cr	Cd
Podromanija	2.9±0.14	10.9±0.9	<0.1±0	1.3±0.08
Han Kram	1.4±0.08	6.9±0.16	<0.1±0	0.1±0.01
Motički Gaj	0.6±0.00	7.5±0.57	<0.1±0	0.4±0.01
Mokro	3.1±0.04	3.5±0.22	<0.1±0	0.1±0.0
Krnovo	1.3±0.07	5.4±0.25	<0.1±0	0.2±0.0
Ostrovica	7.9±0.35	9.9±0.02	<0.1±0	0.6±0.0
Štitovo	2.0±0.02	19.2±0.7	<0.1±0	0.7±0.01
Rožanstvo	5.06±0.14	5.8±0.02	0.43±0.0	1.14±0.01
Jablanica 1	28.7±0.85	4.6±0.0	1.69±0.23	0.06±0.0
Jablanica 2	116±1.0	2.8±0.4	4.14±0.37	0.09±0.0
Dobroselica 1	10.9±0.2	2.19±0.29	1.07±0.06	0.28±0.01
Dobroselica 2	119±7.0	2.26±0.38	3.56±0.15	0.12±0.01
Semegnjevo	319±2.9	7.19±0.03	5.3±0.63	0.17±0.02

Total heavy metals content in topsoil				
Podromanija	31.8±1.9	26.1±4.8	25.4±1.1	2.18±0.02
Han Kram	17.5±0.1	15.2±0.3	23.0±0.1	0.17±0.00
Motički Gaj	22.3±0.1	30.8±2.1	1.62±0.0	1.41±0.05
Mokro	112±2.2	28.2±1.6	92.3±2.5	<0.05±0.0
Krnovo	50.7±3.8	11.9±0.6	27.4±4.2	0.45±0.00
Ostrovica	58.9±2.8	14.7±0.1	37.7±2.6	0.73±0.01
Štitovo	33.0±1.7	24.0±0.4	29.5±1.2	1.02±0.05
Rožanstvo	543±34	36.6±2.7	140±8.3	1.48±0.05
Jablanica 1	415±4.3	21.9±0.7	113±0.4	0.20±0.0
Jablanica 2	1105±40.5	17.0±0.1	382±11.5	0.26±0.04
Dobroselica 1	260±10.3	17.0±1.1	128±5.5	1.21±0.01
Dobroselica 2	2687±7	16.0±1.0	544±11.5	0.0±0.0
Semegnjevo	2694±19	31.4±0.08	508±8.6	0.28±0.03
MPL§	**50**	**100**	**100**	**3**
Heavy metals concentrations in plants				
Podromanija	9.57±0.17	6.66±3.19	<0.1±0.0	0.31±0.0
Han Kram	7.22±0.61	11.1±3.56	<0.1±0.0	0.46±0.11
Motički Gaj	5.97±0.43	12.1±2.79	0.31±4.1	0.16±0.0
Mokro	9.53±1.15	6.17±1.92	<0.1±0.0	<0.05±0.0
Krnovo	3.79±0.29	1.26±1.18	<0.1±0.0	0.50±0.32
Ostrovica	7.08±0.22	4.62±2.09	<0.1±0.0	0.28±0.0
Štitovo	4.74±0.71	3.14±3.94	<0.1±0.0	0.69±0.0
Rožanstvo	2.85±0.46	5.70±1.76	2.21±0.15	0.42±0.03
Jablanica 1	7.70±1.25	1.62±0.43	0.73±1.03	0.13±0.02
Jablanica 2	6.56±1.06	2.45±2.0	2.02±0.05	0.12±0.05
Dobroselica 1	4.37±0.35	7.49±1.18	2.19±1.10	0.21±0.02
Dobroselica 2	7.63±0.36	9.25±1.50	3.60±0.42	0.12±0.09
Semegnjevo	12.4±0.51	2.79±1.12	1.59±0.06	0.41±0.50
NC*	**0.1-5**	**1-5**	**<0.1-1**	**<0.1-1**
TC**	**30**	**20**	**2**	**10**
MTLF***	**50**	**40**	**-**	**1**

§ MPL- maximum permissible levels of dangerous and hazardous matters according to Official Gazette of RS, 1994; *Normal concentrations in plants according to Chaney (1983); **TC - Toxic concentrations in plants according to Kabata-Pendias (2010); ***MTLF - Maximum tolerant level for fodder in plants according to NRC (2005); Official Gazette of RS (2009)

Some plant species, mainly from families *Boraginaceae, Cruciferae, Myrtaceae, Leguminosae* and *Caryophyllaceae* are accumulators of Ni. In most cases, Ni is accumulated in the roots. Almost all measured concentrations of Ni

in plants from meadows were below the critical concentration for normal plant growth except in three locations in Serbia, where locations

Jablanica 2, Dobroselica 2, and especially Semegnjevo, all situated at the mountain Zlatibor, surpassed maximum tolerant level for fodder in plants (NRC, 2005). It was effect of very high total amount of Ni registered in the soil (1105, 2687, 2694 mg kg^{-1} dry weight, respectively). It is fair to assume a risk that well adapted plants in mountain grasslands could be capable of holding increased amounts of serpentine soil-defining metals (Ni and Cr).

On the other hand, Ni content in Montenegro on three locations (table 2), was higher than maximum permissible amount in soil, but available Ni concentrations were between 0.6-7.9 mg kg^{-1}. Concentrations of Ni in plants collected from all sites were below 10 mg kg^{-1}. The elements Ni and Cr, are presumed essential for ruminants, needs for Ni is from 60-70 mg kg^{-1} (Miranda *et al.*, 2009).

The Cr concentration was <0.1 mg kg^{-1} in the soils of Bosnia and Montenegro, and, consequently, the Cr concentration in plant tissue samples was low. All locations in Serbia had much higher concentration Cr in the soil than limit concentration (>100 mg kg^{-1}). Also, concentration in plant tissue samples was close to toxic concentrations in plants according to Kabata-Pendias (2010), which could be potentially detrimental for the plant growth. Cr is an essential element for organisms as its important for normal metabolism of glucose. It is not a toxic element, and negative effects on the function of the organism halves at concentration greater than 50 mg kg^{-1} (Đorđević *et al*., 2009).

The highest amounts of Ni and Cr were found in locations Jablanica 2, Dobroselica 2, and Semegnjevo, but it seems that harmful amounts of these metals were effectively prevented from being accumulated in the stem or the photosynthetic leaf tissue. It is in a line with results Vicić *et al.* (2013) obtained in populations of *Teucrium montanum*, sampled in the study, and confirm that the mechanism employed is metal exclusion, even if the amounts of Cr and Ni were somewhat higher in the roots.

Although Pb occurs naturally in all plants, it has not been shown to play any essential role in their metabolism. The Pb concentration in forage crops is ranged from 2.1 (grasses) to 2.5 mg kg^{-1} (clovers) and Pb is considered as metal with the lowest biological accessibility and highest bioaccumulation in the roots (Kabata-Pendias, 2010). It was not confirmed by results from investigated meadows, where some the examined plant tissues accumulated Pb content > 5 mg kg^{-1}, which could be explained by the vicinity of road and traffic-related air polution. The measured Cd content in soil was low and Cd was neither readily soluble nor easily phytoavailable.

Our results suggest that Ni, Cr, Pb, and Cd content in the forage produced on the permanent grassland in the three Balkan countries can be considered safe for usage in afore-mentioned ways. However, mineral composition and soil conditions that govern metal mobility vary greatly in serpentine soils, therefore their accumulation still has a potential of reaching potentially hazardous levels.

Therefore, it is necessary to evaluate the actual heavy metal content, especially so in populations growing on metal-loaded serpentine soil.

CONCLUSIONS

Our goals were to survey selected metal content of permanent grasslands from Central Balkans. We aimed to define if the metal levels determined within their tissues are close to or exceed the thresholds that would put their nutritive properties in question. All four elements reported in this survey (Ni, Cr, Pb, Cd) represent elements, which can be toxic, if their levels surpass the usual toxicity thresholds.

We assessed the heavy metal status of extensive meadows in Serbia, Bosnia and Montenegro, in relation to the abundance of those chemical elements of the soil. Dominant species in *Agrostietum capillaris* are grasses and the level of all studied elements in the plant biomass collected from investigated meadows were within the allowed limits.

In general, it can be concluded that from the results of this study, the mineral element concentrations of analyzed herbage samples from all sites do not exceed maximal tolerance levels for fodder. Precautionary measures of metal content scanning are suggested for beneficial properties of these grasslands to remain safe and uncompromised.

ACKNOWLEDGEMENTS

The authors are grateful to the Ministry of Agriculture, Forestry and Water Economy of the Republic of Serbia, Zlatiborski Eco Agrar and Čajetina municipality for financial support.

REFERENCES

Alibegović-Grbić, S. (2009). Bosnia and Herzegovina. Country Pasture/Forage Resource Profile, FAO, p. 23.

Chaney R.L. (1983). Potential effects of waste constituents on the food chain. In: Parr, J.F., Marsh, P.B., Kla, J.M. (eds), Land Treatment of Hazardous Wastes, Noyes Data Corporation, Park Ridge, New Jersey (1983) 152-239.

Dubljević, R. (2005). Influence of fertilization on producing properties of degraded mountain grassland type of Nardetum strictae. Agroznanje, Banja Luka, B&H, 6, 4,31-38.

Dubljević R. (2007). Nitrogen fertilization influence on producing properties of meadow of type Agrostidetum vulgaris in hilly area of Polimlje. Zbornik radova, Institut za ratarstvo i povrtarstvo, Novi Sad, 44, 1, 361-367.

Dubljević R. (2009). Montenegro. Country Pasture/Forage Resource Profiles, FAO, p. 26.

FAO (2015). Understanding Mountain Soils: A contribution from mountain areas to the International Year of Soils 2015, by Romeo, R., Vita, A., Manuelli, S., Zanini, E., Freppaz, M. & Stanchi, S. Rome, Italy, p. 157.

Đorđević N., Grubić G., Maković M., Jokić Ž. (2009). Ishrana domaćih životinja. Univerzitet u Beogradu, Poljoprivredni fakultet, 1022.

Džamić, R. A., Stevanović, D., Jakovljević, M. (1996). Praktikum iz agrohemije. Poljoprivredni fakultet.

FAO (2015). Understanding Mountain Soils: A contribution from mountain areas to the International Year of Soils 2015, by Romeo, R., Vita, A., Manuelli, S., Zanini, E., Freppaz, M. & Stanchi, S. Rome, Italy

IUSS Working Group (2014). World reference base for soil resources 2014 international soil classification system for naming soils and creating legends for soil maps. FAO, Rome.

Jones J. B. Jr., Case V. W. (1990). Sampling, handling, and analyzing plant tissue samples. In: Soil testing and plant analysis, 3rd edition (Ed. R. L. Westerman), 389-427, SSSA, Madison.

Kabaš, E. N., Alegro, A. A., Kuzmanović, N. V., Jakovljević, K. M., Vukojičić, S. S., Lakušić, D. V. (2013). Stipetum novakii ass. nova–a new association of serpentine rocky grassland vegetation (Halacsyetalia sendtneri) in Serbia. Acta Botanica Croatica, 72(1), 169-184.

Kabata-Pendias A. (2010). Trace Elements in Soils and Plants, Fourth Edition, p. 548.

Mayland H. F., Shewmaker G. E. (2001). Animal health problems caused by silicon and other mineral imbalances. Journal of range management, 441-446.

Miranda M., Benedito J.L., Blanco-Penedo I., López-Lamas C., Merino A., López-Alonso M. (2009). Metal accumulation in cattle raised in a serpentine-soil area: Relationship between metal concentrations in soil, forage and animal tissues. Journal of Trace Elements in Medicine and Biology, 23, 3, 231-238.

National research council (NRC) (2005). Mineral Tolerance of Animals: Second Revised Edition, Washington, DC: The National Academies Press, 2005.

Official gazette of RS (1994). Rule book of permissible concentrations of dangerous and hazardous materials in soil and in water for irrigation and methods for analysis, 23.

Official Gazette of RS (2009). Law on Food Safety, Rule book of animal food quality, 41/09.

Pansu M., Gautheyrou J. (2006). Mineralogical separation by selective dissolution. Handbook of Soil Analysis: Mineralogical, Organic and Inorganic Methods, 167-219.

Radojičić B. (2002). Geography of Montenegro, DANU, Podgorica, 191-254.

Reeves, R., Baker, A., Borhidi, A., Berazain R. (1999). Nickel hyperaccumulation in the serpentine flora of Cuba. Ann. Bot., 83: 29-38.

Simić A., Dželetović Ž., Vučković S., Ćupina B., Mandić V., Krstić Đ. (2014). Trace elements concentrations in herbaceous plants from ash deposit of thermal power station. In: Soil 2014 - Planning and land use and landfills in terms of sustainable development and new remediation technologies (Proceedings of Integrated meeting, May 12-13, 2014., Zrenjanin, Serbia), 167-174.

Simić A., Dželetović Ž., Vučković S., Sokolović D., Delić D., Mandić V., Anđelković B. (2015). Upotrebna vrednost i akumulacija teških metala u krmnim travama odgajenim na pepelištu termoelektrane. Hemijska industrija, 69, 5, 459-467.

Simić, A., Dželetović, Ž., Vučković, S., Krga, I., Andrejić, G. (2016). Soil fertility of meadows and pastures in Western Serbia. Proceedings of 51st Croatian and 11th International Symposium on Agriculture, 15-18. 2. 2016, Opatija, Croatia: 251-255.

Thornton I., Alloway B. J. (1974). Geochemical aspects of the soil-plant-animal relationship in the development of trace element deficiency and excess. Proceedings of the Nutrition Society, 33, 3, 257-266.

Vicić, D., Polavder, S., Stojiljković, M., Jurišić, B., Bojat, N. C. (2013). Content and allocation of nickel, chromium, cobalt, copper and zinc in Teucrium montanum L.: From serpentine habitats in Serbia. Acta Agriculturae Serbica, 18(36), 101-110.

Vučković S., Simić A., Ćupina B., Krstić Đ., Duronić G. (2010). Effect of mineral fertilization on yield of Agrostidetum vulgaris – type meadows in mountainous grasslands in Serbia. XII International Symposium on Forage Crops of Republic of Serbia, Biotechnology in Animal Husbandry, 26 (spec. issue), 389-394.

DOI: 10.17707/AgricultForest.65.1.02

Tony Kevork SAJYAN*,
Nidal SHABAN, Jad RIZKALLAH, Youssef Najib SASSINE [1]

PERFORMANCE OF SALT-STRESSED TOMATO CROP AS AFFECTED BY NANO-$CACO_3$, GLYCINE BETAINE, MKP FERTILIZER AND ASPIRIN APPLICATION

SUMMARY

Salinity problem is a major abiotic stress affecting tomato growth. In Lebanon, the problem is rising in coastal zone and Northern (Baalback-Hermel belt) areas. The current work aimed to study the effect of Monopotassium-phosphate (MKP), Lithovit® (LITHO) (nano-$CaCO_3$), Glycine betaine (GB) and Aspirin (ASP) applied each in three concentrations (Low, Med and High) on tomato (*Solanum lycopersicum* L.) subjected to five salinity levels (EC=2,4,6,8 and 10 dS/m). Control treatments were those subjected to the five salinity levels with no products application. Results showed that increased salt stress reduced fresh weight of aboveground parts and roots while MKP-High improved fresh weight of aboveground parts at EC8 (by 44.6g) and EC10 (32.7g) and ASP-Med improved fresh weight of roots by 18g at EC10 compared to control. Root mass fraction was enhanced by Aspirin applied with all concentrations at EC2 and EC4 and by Lithovit at EC8. Dry matter accumulation in the aboveground parts was only improved by MKP at EC4, 6 and 10 and by Lithovit at EC6 and 8. Leaf area was reduced by 142.4g and cell electrolyte leakage was increased by 17% with increasing salinity. Lithovit enhanced leaf area with Lithovit-Med and total chlorophyll content with all concentrations at all ECs. Finally at EC4 total soluble solids increased following the application of Lithovit, MKP, ASP and GB with the highest concentrations, while Titratable acidity was increased only with GB-low. In conclusion, products' effects varied with EC level and applied dose.

Keywords: tomato, fertilizer, osmo-regulator, salt-tolerance.

INTRODUCTION

Salinity is one of the common factors causing significant reduction in crop yields and affecting plant growth (Hassan *et al*., 2015). It causes disturbance of water balance, closure of leaf stomata and inhibition of cell division (Zhang *et al*., 2016). It also reduces the production of leaf photo-assimilates due to stomatal

[1]Tony Kevork Sajyan*(corresponding author: tony.sajyan@hotmail.com), Nidal Shaban, University of Forestry, 10 Kliment Ohridski blvd, BG1797 Sofia, BULGARIA; Jad Rizkallah, Lebanese University, Faculty of Agricultural Engineering and Veterinary Medicine, Dep. Food Technology, Beirut, LEBANON; Youssef Najib Sassine, Lebanese University, Faculty of Agricultural Engineering and Veterinary Medicine,Dep. Horticulture, Beirut, LEBANON
Paper presented at the 9th International Scientific Agricultural Symposium "AGROSYM 2018".
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

closure and the accumulation of Na^+ and Cl^- in leaves (Romero-Aranda and Syvertsen, 1996). It was reported earlier that salinity negatively affects plant growth parameters like plant height, leaf area and fresh weight as well as chemical contents such as N, P, and K (Tantawy *et al*., 2013). On the contrary, high salinity positively influenced tomato fruit quality (Boamah *et al*., 2011) by increasing sugars content and acidity (Cuartero and Fernàndez-Muñoz, 1999). This is due to the inhibition and prevention of water uptake and transport improving the concentration of soluble solids (Sakamoto *et al*., 1999; Li *et al*., 2001). Del amor *et al*. (2001) found a correlation between the improvement in fruit acidity and the accumulation of Na^+ and Cl^- due to salinity. Attempts are constantly done to find new methods to alleviate the negative impacts of salinity on plants especially that this problem is raisin in many regions of the world and also in Lebanon (Darwish *et al*., 2002).

Recently, nano-fertilizers showed a potential use as a pioneer in solving problems (Froggett, 2009). LITHOVIT® or nano-$CaCO_3$ is a CO_2 foliar fertilizer (Bilal, 2010) increasing CO_2 concentration and stimulating light saturated photosynthesis in C3 plants (Ainsworth and Rogers, 2007). There are a few reviews about the effect of nano-particles on plants (Tantawy *et al.,* 2014) and minor reports on its efficiency under salt stress. On the other hand, the use of fertilizers rich in phosphorus and potassium was noted as beneficial in mitigating salinity effect on crops (Afzal *et al*., 2015) due to their contribution in ion homeostasis and osmotic balance (Perkins-Veazie and Robert, 2003). Acetylsalicylic acid or Aspirin which was previously stated to increase leaf water potential, membrane stability and soluble compounds (Agamy *et al*., 2013) could enhance tomato tolerance to salinity. Finally, the positive role of glycinebetaine (GB) against salinity which was reported on various crops, while on tomato salt-stressed plants its role is still leading to confusion due to contradictory reports upon this subject. GB being an osmolyte accumulated naturally in plants in stressful conditions, but not in tomato. It has a role in protecting photosynthetic apparatus from abiotic stress (Chaum and Kirdmanee, 2010) and in maintaining osmotic balance (McCue and Hanson, 1992).

Therefore, the current study aimed to find the optimal solution to improve physiological responses of tomato plant to salinity together with the preservation of ameliorative effect of this abiotic stress on fruit quality. This was done through the application of LITHOVIT®, MKP, Aspirin and GB in various concentrations on salt-stressed tomato plants.

MATERIAL AND METHODS

Treatments

Tomato seedlings (determinate Var. Sila) of 3-4 leaves were transplanted in pots containing washed sandy clay soil during May. The date of transplantation was referred as initiation date for all practices. After transplantation, irrigation with sweet water was carried out till 14 DAT. LITHOVIT® (LITHO), Monopotassium-phosphate (MKP) (0-52-34), Aspirin

(ASP) and Glycinebetaine (GB) products were applied in 3 different concentrations: Low, Medium and High with respectively 0.5 g/L; 0.75 g/L and 1 g/L for LITHO, 2 g/L, 3 g/L and 3.5 g/L for MKP, 4.5 g/L, 6 g/L and 7.5 g/L for GB and 50 mg/L, 75 mg/L and 100 mg/L for ASP. Each treatment was applied 3 times starting at 15 DAT with an interval of 15 days between consecutive applications. LITHO and ASP were applied by foliar spray, MKP through fertigation and GB by both methods.

All products were dissolved in distilled water except for ASP (tablets of 100mg) that was mixed at high temperature with ethanol. Salinity was induced by saline irrigation which started at 19 DAT using different solution's ECs according to the corresponding treatment: 2, 4, 6, 8 and 10 dS/m. Saline irrigation was done continuously with a frequency of 3 days and a dose of 1 L per plant. Control consisted of tomato plants irrigated by all ECs, however not treated by the various products.

Physiological indicators

Six plants of each treatment were selected for measuring their fresh (aboveground and root parts) and dry weights. Fresh weight was measured first and dry weight was then assessed after oven-drying at 100^{o} C until constant weight. Consequently dry matter content was measured based on fresh and dry weights of plants parts. Root mass fraction and were measured based on dry weights of plant parts following the method of Poorter *et al*. (2012). Three tomato plants were selected from each treatment for measuring leaf area on their total number of leaves.

Cell electrolyte leakage was measured as described by Mumtaz Khan *et al*. (2013). Chlorophyll content test was performed as follows: 0.1 g of calcium carbonate was added to 1g of fresh leaves. The mix was macerated in 50 mL of acetone (80 %). The liquid phase was then transferred into small beakers and the remaining solution was macerated once more in acetone (80%) until full discoloration of leaves. The solution was subjected to centrifugation at 3000 rpm for 5 minutes. The absorbance was red on a spectrophotometer at the wavelengths: 663 nm and 645 nm. Finally, total chlorophyll was determined in µg/g (mg/L) according to Porra (2002).

Fruit quality

Total Soluble Solids (TSS) content was evaluated by Euromex RF (360) refractometer (Tigchelaar, 1986). Titratable acidity (TTA) in fruits was measured by titration of tomato juice (6g of tomato juice in 50 mL of distilled water) with 0.1M NaOH to pH=8.1 (Rangana, 1979).

Statistical analysis

Data was subjected to analysis of variance which consisted on means ±SE compared by Fisher's least-significant differences test (LSD) using STATISTICA 10 program.

RESULTS AND DISCUSSION

Physiological parameters

In general, from the probabilities associated with Fisher statistics for the different effects (Table 1), it was found that the separate effects of both EC and Treatments (product application) were statistically (P_{value}<0.05) significant on all parameters except for the non-interactive effect of EC on fresh weight of aboveground parts and dry matter of roots. Finally, the combined (interactive) effects of EC x Treatment was not statistically (P_{value}>0.05) significant on all parameters.

Table 1: ANOVA null hypothesis rejection probability for the effects of the experimental factors and their interactions on the different measurements averages

	F.W.A.P (g)	F.W.R (g)	D.M.A.P (%)	D.M.R (%)	RMF ($g.g^{-1}$)
EC	0.070	0.000	0.031	0.196	0.000
Treatment	0.000	0.000	0.000	0.000	0.000
EC*Treatment	0.177	0.091	0.734	0.089	0.317

F.W.A.P: Fresh Weight of Aboveground parts; F.W.R: Fresh Weight of Roots; D.M.A.P: Dry Matter of Aboveground parts; D.M.R: Dry Matter of Roots.

Increasing in salinity level (from EC2 to EC10) has significantly reduced fresh weight of aboveground parts (Figure 1a) by 41 g (77 g at EC2 compared to 36 g at EC10 in control plants). However, the application of MPK-High improved this parameter compared to control at all EC levels; with a significant difference at EC4 (by 63.2 g) and EC8 (44.6 g) and a slight difference at EC2 (16 g), EC6 (18.2 g) and EC10 (32.7 g). In addition, at EC4, MKP application (MKP-Low, MKP-Med and MKP-High) has enhanced fresh weight of roots (Figure 1b) by 38 % while at EC10, Asp-Med application has significantly enhanced it by 69 %, and at EC6, MKP-Low and ASP-High application has slightly increased this trait compared to control (respectively 26.1 g and 26.7 g compared to 16.3 g).

Root mass fraction (Figure 1c) was slightly improved by Asp-Med at EC2 and EC4 (0.43 $g.g^{-1}$ and 0.37 $g.g^{-1}$ compared to 0.25 $g.g^{-1}$ and 0.22 $g.g^{-1}$ in control at EC2 and EC4 respectively) and by Lithovit® at EC8 with all the applied concentrations (0.44 $g.g^{-1}$, 0.38 $g.g^{-1}$ and 0.46 $g.g^{-1}$ respectively at Litho Low, Med and High compared to 0.35 $g.g^{-1}$ in control). Concerning dry matter accumulation in plants (Figure 1d), there was no significant difference in the percentage of dry matter accumulated in aboveground plant parts when comparing between the various treatments and control at all EC levels with the exception of MKP-Med at EC8 (16.9 % compared to 9 % in control). On the contrary, dry matter in roots was affected variously by different treatments; it increased significantly compared to control at EC2 and EC6 with MKP-Med (by 15.3 % and 15.7 % respectively), at EC4 and EC10 with MKP-High (by 32.3 % and 11% respectively) and at EC8 with Litho-Low (by 19.9 %).

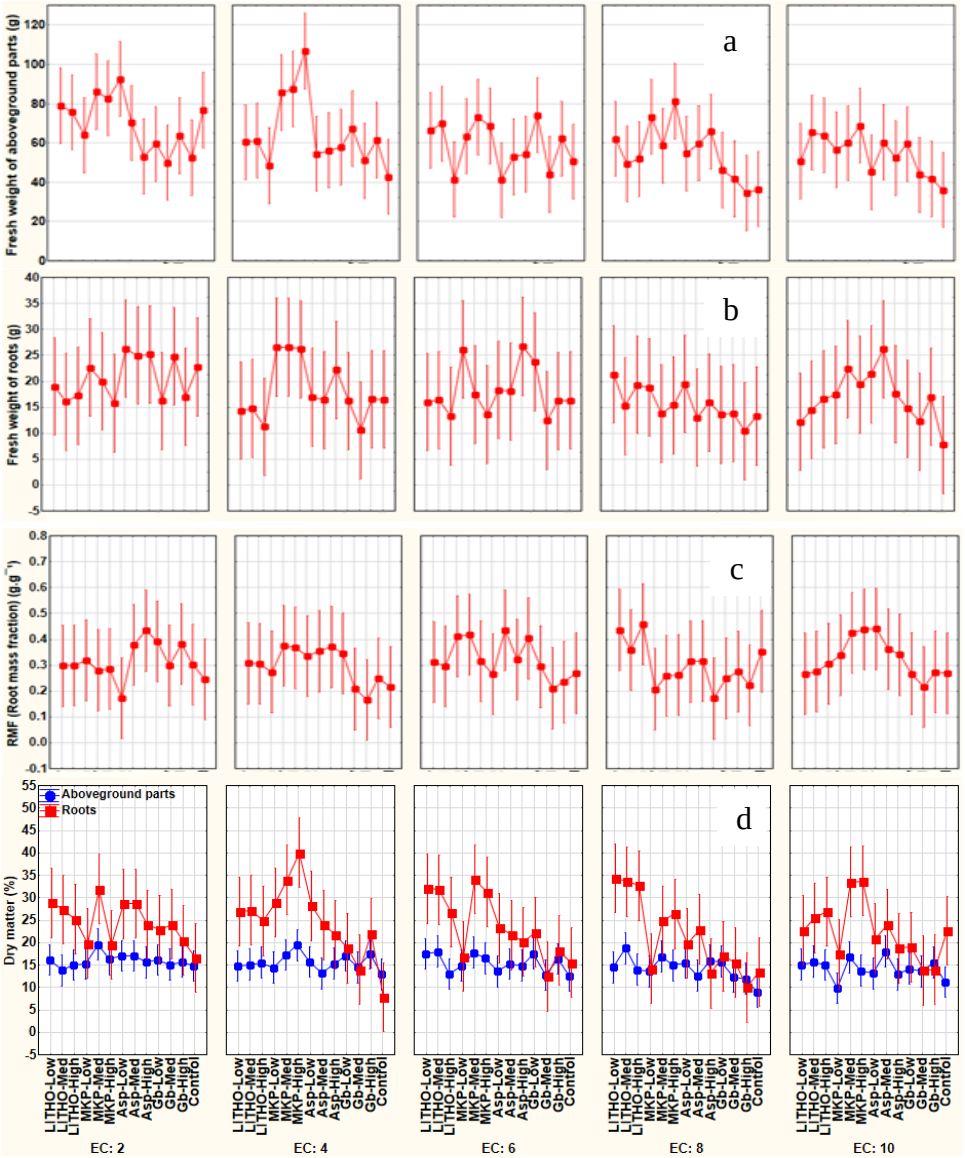


Figure 1: Averages (middle markers) and the 95% limits of confidence (±2 x standard Error: SE) (vertical bars) of various tested parameters

Leaf area was reduced by 142.4 g and cell electrolyte leakage was increased by 17% with increasing salinity level from 2 to 10 dS/m. However, Lithovit-Med enhanced leaf area at EC2 (by 50%), EC4 (46%), EC6 (44%), EC8 (65%) and EC10 (68%) compared to control. Lithovit was also beneficial with the 3 applied doses on total chlorophyll content at all ECs with the best

improvement obtained with Litho-High compared to control (by 29%, 51%, 41%, 39% and 26% respectively at EC2, 4, 6, 8 and 10).

Fruit quality

Total soluble solids increased at EC4 following the application of Lithovit, MKP, ASP and GB with high concentrations by 10%, 6%, 5% and 16% and Titratable acidity was increased by 15% following GB low application compared to control.

The reduction in fresh weight of plant parts (aboveground parts and roots) caused by salinity could be attributed to its inhibitory effect on cell expansion and division as well as stomatal closure (Flowers, 2004) which mitigates the ion flux to the shoot (Hasegawa *et al.*, 2000). According to Läuchli and Epstein (1990), under salinity stress, the reduction in shoot growth is related to leaf area decline and stunted shoots resulting in an inhibition in photosynthetic activity, reduction in energy production and protein synthesis other physiological changes (Cramer and Nowak, 1992). In fact, ion imbalances caused by salinity prevent K^+ and Ca^{2+} uptake thus reducing root cell growth and root tips expansion (Larcher, 1980). The inhibition in tomato growth has been also reported as one of the most reliable indicators under salt-stress (Cruz *et al.*, 1990); significant reductions in fresh weight of tomato shoots were observed earlier (Bolarin *et al.*, 1993).

Therefore, the beneficial effect of monopotassium phosphate application was due to the presence of both potassium and phosphorus elements. In fact, improving the potassium nutritional status and phosphorus content might have minimized the oxidative cell damage. This was possible by reducing both ROS (reactive oxygen species) and NADPH oxidase formation (Shin and Schachtman, 2004) that were previously stimulated by increasing salt-stress. On other solanaceous crops several studies stated the positive effect of K in mitigating salinity (Kaya and Higgs, 2003; Rubio *et al.*, 2009, Sajyan *et al.*, 2018). This was translated in the current study by an improvement in fresh weight of plant parts and in dry matter of roots especially at EC4. In addition, LITHOVIT® application improved dry matter percentage, chlorophyll content and leaf area in roots compared to control especially at EC8,. Actually, LITHOVIT® is rich in Ca in a micronized form ($CaCO_3$), CO_2 and Mg (Bilal, 2010) which counteracted the negative impacts of salinity especially on leaf area and total chlorophyll content. Its application improved the atmospheric CO_2 (del Amor, 2013) and Mg an essential element for chlorophyll formation (Bilal, 2010) which could explain the improvement in photosynthetic activity. Furthermore, improvement of root mass fraction by Aspirin application at EC2, 4 and 10 and total soluble solids at EC4 could be related to the product role in maintaining cellular membrane function by preventing lethal stress load (Sun *et al.*, 1994) and by enhancing the activity of antioxidant enzymes (He *et al.*, 2002). Finally, Glycine betaine was the least effective among all products and did not improved salt-tolerance of tomato crop which confirmed the findings of Heuer (2003) who has attributed the non-effect of GB to its inhibitory effect on ion accumulation in plant cells. It

seemed that the applied concentrations (4.5, 6 and 7.5 g/L) were too high and glycine betaine should be applied in lower rates.

CONCLUSIONS

Under salinity stress, LITHOVIT® and MKP were more beneficial more than Aspirin and GB products. It seemed that improving ion uptake (K, P, Ca, Mg and others) have better reduced the salinity-caused effects compared to the use of an osmoprotectant (GB) or aspirin (acetyl salicylic acid).

REFERENCES

Afzal I., Hussain B., Basra S.M.A., Ullah S.H, Shakeel Q. and Kamran M. (2015). Foliar application of potassium improves fruit quality and yield of tomato plants. Acta Sci Pol Hortorum Cultus. 14(1): 3-13.

Agamy R.A., Hafez E.E. and Taha T.H. (2013). Acquired Resistant Motivated by Salicylic Acid applications on salt stressed tomato (Lycopersicon esculentum Mill.). American-Eurasian J Agric & Environ Sci. 13: 50-57.

Ainsworth E.A. and Rogers A. (2007). The response of photosynthesis and stomatal conductance to rising [CO2]: Mechanisms and environmental interactions. Plant, Cell and Environment. 30: 258–270.

Bilal B.A. (2010). Lithovit®: An innovative fertilizer. The 3rd e- Conference on Agricultural Biosciences (IeCAB 2010), 1st- 15th June 2010. http://www.slideserve.com/madison/lithovitan-innovative-fertilizer.

Boamah P.O., Sam-Amoah L.K. and Onumah J. (2011). Effect of salinity level of irrigation water on the yield of tomato. ARPN Journal of Agricultural and Biological Science. 6(8): 49-53.

Bolarin M.C., Perez-Alfocea F., Cano E.A., Estan M.T. and Caro M. (1993). Growth, fruit yield, and ion concentration in tomato epotypes after pre- and post-emergence salt treatments. J. Am. Soc. Hort. Sci. 118: 655-660.

Chaum S. and Kirdmanee C. (2010). Effect of glycinebetaine on proline, water use and photosynthetic efficiencies and growth of rice seedlings under salt stress. Turk. J. Agric. For. 34: 517-527

Cramer G.R. and Nowak R.S. (1992). Supplemental manganese improves the relative growth, net assimilation and photosynthetic rates of salt-stressed barley. Physiol. Plant. 84: 600-605.

Cruz V., Cuartero J., Bolarin MC. and Romero M. (1990) Evaluation of characters for ascertaining salt stress responses in Lycopersicon species. J. Am. Soc. Hortic. Sci. 115: 1000-1003.

Cuartero J. and Fernández-Muñoz R. (1999). Tomato and salinity. Sci Hort. 78, 83-125.

Darwish T., Atallah T., El-Khatib M. and Hajhasan S. (2002). Impact of irrigation and fertilization on NO3 leaching and soil-ground water contamination in Lebanon. Transactions 17th World Congress of Soil Science. Bangkok, Thailand: 13-21 August 2002: 406.1- 406.11.

del Amor F.M., Martinez V. and Cerda, A. (2001). Salt Tolerance of Tomato Plants as Affected by Stage of Plant Development. HortScience. 36(7): 1260-1263.

del Amor F.M. (2013). Variation in the leaf 13o C elevated CO2 concentration. Journal of Plant Physiology. 170: 283-290.

Flowers T.J. (2004). Improving salt tolerance. J. Exp. Bot. 55: 307-329.

Froggett S. (2009). Nanotechnology and agricultural trade. OECD Conference on the Potential Environmental Benefits of Nanotechnology: Fostering Safe Innovation-Led Growth.

Hasegawa P.M., Bressan R.A., Zhu J.-K. and Bohnert H.J. (2000). "Plant cellular and molecular responses to high salinity," Annual Review of Plant Biology. 51: 463–499.

Hassan M.A., Martínez M.F., Ramos F.J.S., Vicente O. and Boscaiu M. (2015). Effects of salt and water stress on plant growth and on accumulation of osmolytes and antioxidant compounds in cherry tomato. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 43:1-11.

He Y.L, Liu Y.L, Chen Q, Bian A.H. (2002). Thermo tolerance related to antioxidation induced by salicylic acid and heat hardening in tall fescue seedlings. J. Plant Physiol Mol Biol. 28: 89-95.

Heuer B. (2003). Influence of exogenous application of proline and glycinebetaine on growth of salt-stressed tomato plants. Plant Sci. 165: 693 699.

Kaya C. and Higgs D. (2003). Supplementary potassium nitrate improves salt tolerance in bell pepper plants. J Plant Nutr. 26: 1367-1382.

Läuchli A. and Epstein E. (1990). Plant responses to saline and sodic conditions. In K.K. Tanji (ed). Agricultural salinity assessment and management. ASCE manuals and reports on engineering practice. 71: 113–137.

Larcher W. (1980). Physiological plant ecology. In 2nd totally rev. edition ed. Berlin and New York: Springer-Verlag. pp. 303.

Li Y.L., Stanghellini C. and Challa H. (2001). Effect of electrical conductivity and transpiration on production of greenhouse tomato (Lycopersicon esculentum L.). Sci. Hortic. 88: 11-29.

McCue K.F. and Hanson A.D. (1992). Salt-inducible betaine aldehyde dehydrogenase from sugarbeet: cDNA cloning and expression. Plant Mol Biol. 18: 1-11.

Mumtaz Khan M., Masoudi S.M.A., Al-Said F. and Khan I. (2013). Salinity Effects on Growth, electrolyte leakage, chlorophyll content and lipid peroxidation in cucumber (Cucumis sativus L.). 2nd International Conference on Food and Agricultural Sciences IPCBEE vol.55. IACSIT Press, Singapore.

Perkins-Veazie P. and Roberts W. (2003). Can potassium application affect the mineral and antioxidant content of horticultural crops? Amer. Soc. Agron., Proc. Symposium on Fertilizing Crops for Functional Foods, 2/1–2/6.

Poorter H., Niklas K.J., Reich P.B., Oleksyn J., Poot P. and Mommer L. (2012). Biomass allocation to leaves stems and roots: meta-analyses of interspecific variation and environmental control. Tansley review. New Phytol. 193: 30 50.

Porra R.J. (2002). The chequered history of the development and use of simultaneous equations for the accurate determination of chlorophylls a and b. Photosynth. Res. 73: 149-156.

Rangana (1979) Manual Analysis of Fruits and Vegetables Product. Tata McGraw Hill Co. Ltd., New Delhi.

Romero-Aranda R. and Syvertsen J.R. (1996). The influence of foliar-applied urea nitrogen and saline solutions on net gas exchange of citrus leaves. J. Am. Soc. Hort. Sci. 121: 501-506.

Rubio J.S., Garcia-Sanchez F., Rubio F. and Martinez V. (2009). Yield, blossom-end rot incidence, and fruit quality in pepper plants under moderate salinity are affected by K+ and Ca2+ fertilization. Sci Hortic-Amsterdam. 119: 79–87

Sajyan T.K., Shaban N., Rizkallah J. and Sassine Y.N. (2018). Effects of Monopotassium-phosphate, Nano-calcium fertilizer, Acetyl salicylic acid and Glycinebetaine application on growth and production of tomato (Solanum lycopersicum) crop under salt stress. Agronomy Research. 16(3): 872-883.

Sakamoto Y., Watanabe S., Nakashima T. and Okano K. (1999). Effects of salinity at two ripening stages on the fruit quality of single-truss tomato grown in hydroponics. J. Hort. Sci. Biotechnol. 74: 690-693.

Shin R. and Schachtman D.P. (2004). Hydrogen peroxide mediates plant root cell response to nutrient deprivation. Proc Natl Acad Sci. 101: 8827–8832

Sun W.Q., Irving T.C. and Leopold A.C. (1994). The role of sugar, vitrification and membrane phase transition in seed desiccation tolerance. Physiol. Plant. 90: 621-628.

Tantawy A.S., Salama Y.A.M., Abdel-Mawgoud A.M.R. and Zaki M.F. (2013). Interaction of Fe and salinity on growth and production of tomato plants. World Applied Sciences Journal. 27 (5): 597-609.

Tantawy A.S., Salama Y.A.M., Abdel-Mawgoud, M.R. and Ghoname A.A. (2014). Comparison of Chelated Calcium with Nano Calcium on Alleviation of Salinity Negative Effects on Tomato Plants. Middle East Journal of Agriculture Research. 3(4), 912-916.

Tigchelaar E.C. (1986). Tomato breeding. In: M. J. Bassett (ed.), Breeding Vegetable Crops, Avi publishing company, Inc., Westport, Connecticut, USA. 135-171.

Zhang P., Senge M. and Dai Y. (2016). Effects of salinity stress on growth, yield, fruit quality and water use efficiency of tomato under hydroponics system. Reviews in Agricultural Science, 4: 46-55.

DOI: 10.17707/AgricultForest.65.1.03

Raisa IVANOVA*, Svetlana SMEREA [1]

SAFFLOWER YIELD RESPONSE TO IRRIGATION AND GAMMA IRRADIATION

SUMMARY

Safflower (*Carthamus tinctorius* L.) is drought resistant plant, but the adequate irrigation is important for the increasing of safflower yield. The effects of drip irrigation associated with pre-sowing gamma irradiation of seeds on plant growth and yield were investigated. The seeds were irradiated by gamma rays (^{60}Co) at various doses (50, 100, 150Gy). The safflower plants were grown under drip irrigation and rainfed conditions of the Republic of Moldova in season of 2017. Modifications of bio-morphological (height, number of secondary branch, developed and undeveloped inflorescence) and yield attributing (number of seeds per head and per plant, weight of seeds per plant and 1000-seed weight) characters of safflower were studied. The results showed that the drip irrigation influenced positive and statistically significant ($p \leq 0.001$) on all studied characters of safflower. The plant grown under irrigation had the number of developed inflorescences, the number of seeds and the weight of seeds per plant, respectively 1.83, 1.81 and 2.50 times more than rainfed plants; as well as undeveloped inflorescence less 2.47 times. The contribution of pre-sowing irradiation was not so pronounced. The impact of factors (irradiation, irrigation) and their interaction for the improvement of bio-morphological and yield attributing characters were determined. The significant impact of growing condition on the number of seeds per plant at $p \leq 0.01$, weight of seeds per plant and 1000-seeds weight at $p \leq 0.01$ was established. The gamma radiation had the contribution to changes in 1000-seeds weight but the contribution of growing condition on this character was 15.7 times stronger.

Keywords: safflower, seed yield, pre-sowing seed irradiation, rainfed, irrigation.

INTRODUCTION

Safflower (*Carthamus tinctorius* L.) is characterized as drought tolerant plant which could be cultivated under arid and semi-arid conditions (Beyyavas et al., 2011). According to pedoclimatical conditions the Republic of Moldova is not considered as arid or semi-arid area, but during last three-five years it was observed the rising of mean summer temperatures by 2.2…3.3ºC and amount decreasing of precipitation by 100…200 mm. Previous studies (Ivanova, 2016*;*

[1]Raisa Ivanova*(corresponding author: raisa.a.ivanova@gmail.com), Svetlana Smerea, Institute of Genetics, Physiology and Plant Protection, Republic of MOLDOVA

Paper presented at the 9th International Scientific Agricultural Symposium "AGROSYM 2018".
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

Ivanova et al., 2017) showed that the safflower is a new crop for Republic of Moldova as it easily adapted to these conditions. However, the safflower plants depend on an adequate water supply for optimum growth, development and for improvement of seed and oil yield. Numerous studies have examined the effects of irrigation on plant height, number of secondary branches, number of head, head diameter, number of seed per head, 100-seed or 1000-seed weight, seed and oil yields of safflower as well as the relationships between seed yield and some morphological traits (Ozturk et al., 2008; Istanbulluoglu et al., 2009; Khalili et al., 2013; Feyzollahzadeh et al., 2014; Shahrokhnia & Sepaskhah, 2017).

The bio-morphological and yield attributing characters of safflower plants could also be improved by pre-sowing treatment of seed with natural bioregulators (Ivanova et al., 2017) and gamma irradiation (Patil et al., 2001; Parameshwarappa & Meghannavar, 2001). Only several publications focus on the impact of combined abiotic factors such as irrigated or non-irrigated conditions with the pre-sowing treatment of seeds by different doses of γ-radiation on biological potentials of safflower (Mozaffari et al., 2009; Kaya et al., 2009).

This study was initiated to evaluate the effects of drip irrigation associated with pre-sowing gamma irradiation of seeds on growth and seed yield of safflower plant, cultivated in the Republic of Moldova.

MATERIAL AND METHODS

Plant materials were obtained on the experimental fields of the Institute of Genetics, Physiology and Plant Protection in Chisinau area of Republic of Moldova (lat. 47°01', long. 28°75', alt. 85 m above sea level), in the season of 2017. Safflower seeds were treated with three doses of γ-radiation (50, 100, 150Gy) using gamma RXM-V-20 system, the radiation source - ^{60}Co. Irradiated seeds were sown in rainfed and drip irrigation plots in first decade of April. The row spacing of plantation was 50cm and intra-row spacing - 15cm. Each row consisted of 50 seeds, in triplicate. The plants grown from intact (untreated with gamma radiation) seeds served as control.

T**he bio-morphological and yield attributing characters of safflower plants** were studied according to Ahmadzadeh (2013), namely plant height (cm), number of secondary branches, number of inflorescences per plant, number of seeds per plant, number of seeds per head, 1000-seed weight (g), seed yield (g).

The statistical analysis were done using software package Statgraphics Plus 2.1. The ANOVA test was applied for variance analysis of bio-morphological and yield attributing characters, Student test in assessment of statistically significant differences between plots (Raudonius, 2017).

RESULTS AND DISCUSSION

Bio-morphological characters of safflower plants grown in irrigation and rainfed conditions are given in Figure 1.

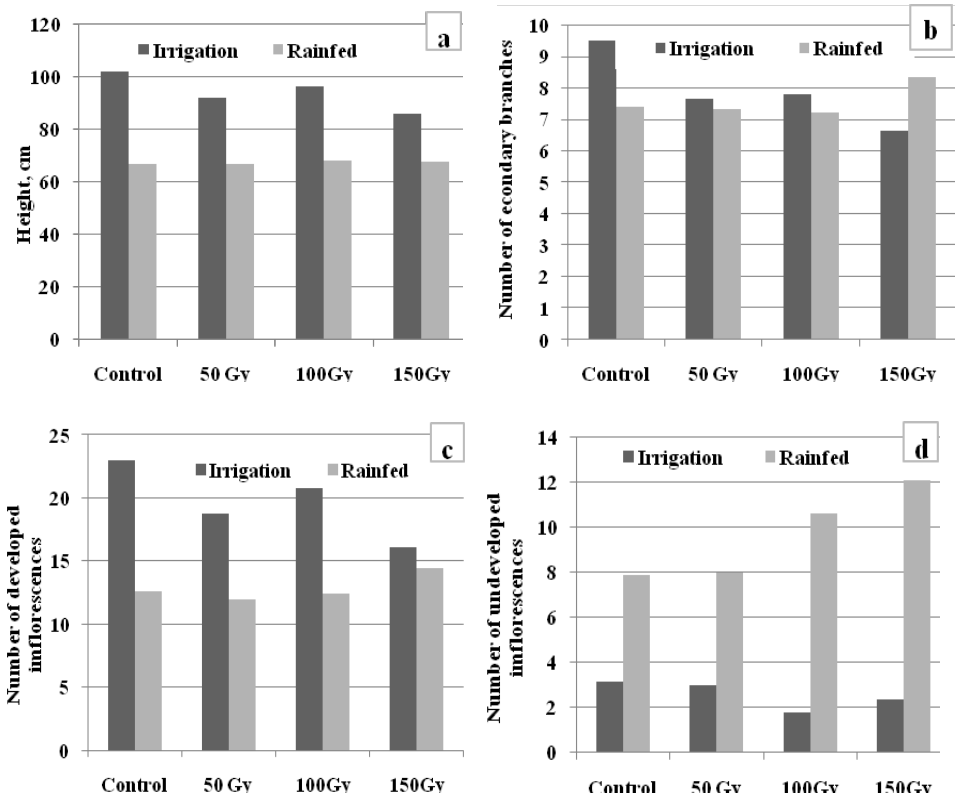


Figure 1. Bio-morphological characters: height (a), number of secondary branches (b), developed (c) and undeveloped (d) inflorescences of safflower grown in different conditions

It was observed that the intact seeds that were not exposed to pre-sowing treatment by gamma radiation under different growth conditions (control) give the plants of dissimilar characters.

Under irrigation conditions (Fig. 1a), in the control plot the plant height varied from 64.0 to 130.0cm (101.80±2.54cm), however under rainfed condition it was 41.0 - 95.0cm (66.86±1.47cm). Similar differences of height between irrigated and non-irrigated plants equal to 8.23 - 33.85cm were evaluated on various genotypes of safflower during two growing seasons (Ozturk et al., 2008).

Additionally, the irrigated safflower were more ramified, with the mean of two secondary branches per plant (Fig.1b); and had 2.0 times more developed inflorescences (Fig. 1c) and 2.5 times less undeveloped inflorescences than the rainfed plants (Fig. 1d). Thus irrigated safflower plants are characterized by better bio-morphological values than plants growing under rainfed conditions.

Pre-sowing irradiation of seeds and subsequent cultivation under irrigation condition led to decreasing in bio-morphological values of safflower plant (Fig.1). A statistically significant difference in comparison with control was

observed on plant height at 50Gy ($p \leq 0.05$) and 150Gy ($p \leq 0.001$) as well as on number of secondary branches at 150Gy ($p \leq 0.01$). Gamma irradiation did not affect the height of rainfed plants. Moreover doses of 50 and 100Gy did not influence on number of secondary branches and developed inflorescences of rainfed safflower. Dose effects of 150Gy were detected as an increase in the number of secondary branches and developed inflorescences as well as, in number of undeveloped inflorescences (Fig. 1).

When compare the factors impact in following mode: radiation and growing conditions such as with or without irrigation, it was detected that the condition had the statistically significant ($p \leq 0.001$) influence on height of safflower, number of developed and undeveloped inflorescences per plant (Tab. 1).

The interaction between radiation and growing condition factors had major contributions at $p \leq 0.01$ to changes in number of secondary branches per plant. Thus, it could be suggested that the bio-morphological characters of safflower plants depends on cultivation conditions more than on gamma radiation doses applied to seeds in pre-sowing time.

The study of growing conditions influence on yield attributing characters of safflower indicated that the seed number per head of control plants was practical equal for irrigated (23.50±1.50) and non-irrigated (21.62±1.16) safflower (Fig.2a). No significant differences of seed number per head between irrigated and non-irrigated plants were also reported (Ozturk et al., 2008). It was shown that this character depends significantly at $p \leq 0.01$ on genotype of safflower and growing season

However, the number of seeds obtained from one control plant cultivated under drip irrigation (536.60±60.22) and rainfed (295.41±29.05) condition varied significantly (Fig. 2b). In addition, the weight of seeds harvested from one irrigated plant was 2.5 times greater than the seeds weight from rainfed plant (Fig. 2c). The seeds of irrigated plants were 1.5 times heavier compared to the seeds of non irrigated safflower; 1000-seed weight was equal 36.67±1.45g for irrigated plants and 25.47±0.97g for plants from rainfed condition (Fig. 2d). The minimum and maximum values of this character in our experiments varied from 10.70 to 60.30g, and our data is in excellent agreement with the results presented by other scientists (Beyyavas et al., 2011; Ahmadzadeh, 2013).

The character 1000-seed weight significant depends on genotype of safflower and interaction between genotype and growing season. Ozturk et al. (2008) reported that seven studied genotypes had higher 1000-seed weight in non-irrigated and three genotypes – in irrigated condition.

According to obtained results, the safflower plants grown under irrigation conditions had better yield attributing characters. Previous studies also reported that the irrigation during safflower grown cycle increased in seeds yield (Lovelli et al., 2007: Istanbulluoglu et al., 2009) and conditions of water stress in safflower reduced its productivity (Eslam, 2011; Singh et al., 2016). In Brazil the seed yield of safflower under irrigation regime was 1,552.1 kg/ha and the mean

value of seed yield under water deficit followed by rehydration regime - 1,144.9 kg/ha (Bortolheiro & Silva, 2017). It was observed (Feyzollahzadeh et al., 2014) that weight of one safflower seed within the various irrigation regimes was significantly different at ($p \leq 0.01$) and modified into the range of 0.040 - 0.055 g. The most direct effect on seed yield was obtained from 1000-seed weight of safflower under irrigation.

Table 1.Impact of radiation and conditions of growth on bio-morphological characters

Factor	**Sum of squares**	**Df**	**Mean square**	**F-ratio**	**P-value**	**Contribution of factor, %**
Height of plant, cm						
Radiation (R)	1764.79	3	588.26	4.71**	0.0033	2.51
Condition (C)	24304.70	1	24304.70	194.50***	0.0000	34.66
Interactions R-C	1938.17	3	646.06	5.17**	0.0018	2.76
Total	70121.90	233				
Secondary branches						
Radiation (R)	42.83	3	14.28	1.83	0.1422	ns
Condition (C)	3.99	1	3.99	0.51	0.4749	ns
Interactions R-C	92.66	3	30.89	3.96**	0.0088	4.86
Total	1904.65	233				
Developed inflorescence per plant						
Radiation (R)	265.75	3	88.58	1.08	0.3573	ns
Condition (C)	1601.19	1	1601.19	19.57***	0.0000	7.29
Interactions R-C	489.06	3	163.02	1.99	0.1160	ns
Total	21964.30	233				
Undeveloped inflorescence per plant						
Radiation (R)	87.52	3	29.17	1.01	0.3883	ns
Condition (C)	1694.14	1	1694.14	58.76***	0.0000	19.28
Interactions R-C	208.40	3	69.47	2.41	0.0679	ns
Total	8789.12	233				

Note: **; *** - denotes the statistically significant difference at p≤0.01; p≤0.001

Pre-sowing irradiation of seeds resulted in some fluctuation of yield attributing characters of plants (Fig. 2). Concerning to irrigated plants the number of seeds per head (Fig. 2a) decreased significantly ($p \leq 0.05$) from 23.50±1.50 (control) to 16.88±1.46 (150Gy). Number and weight of seeds harvested from one plant were also 1.58-1.74 times reduced (Fig. 2b, c), control plant produced a mean of 536.60±60.22 seeds, and safflower from seeds irradiated by 150Gy dose

- 308.0±76.13 seeds ($p \leq 0.1$). On the contrary, the weight of 1000 seeds from irrigated plants augmented depending on the rising of radiation doses (Fig. 2 d).

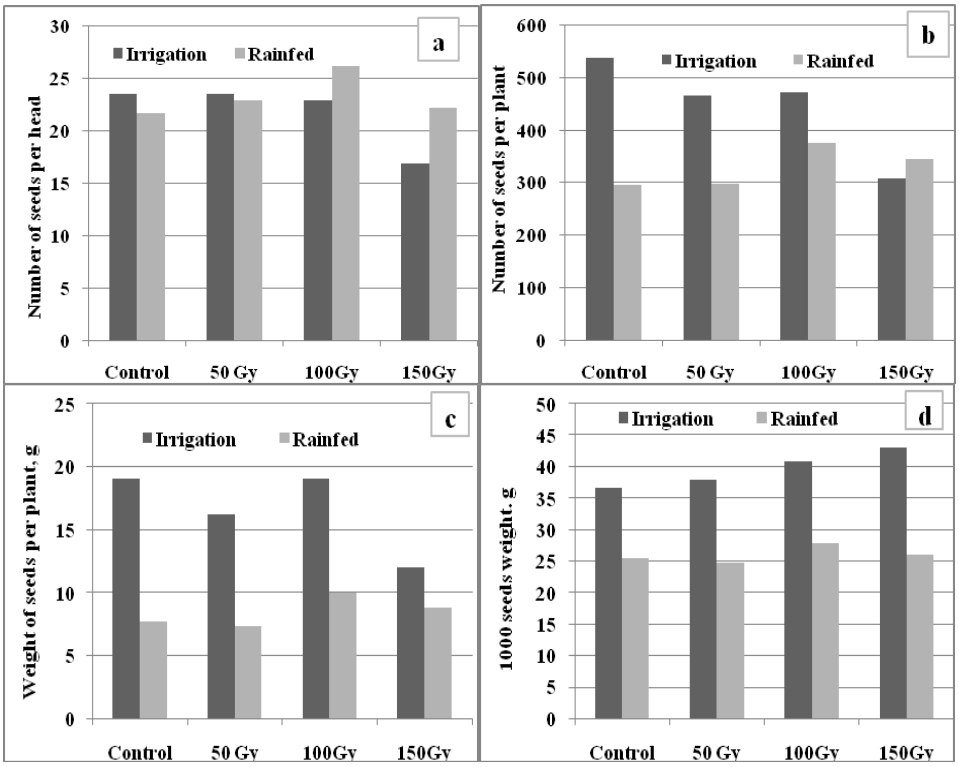


Figure 2. Yield attributing characters: number of seeds per head (a) and per plant (b), weight of seeds per plant (c) and 1000 seeds weight (d) of safflower grown in different conditions

More advantageous influence of gamma radiation was observed on yield attributing characters of safflower grown in rainfed conditions. All studied characters of these plants tended to increase in comparison with the control. The safflower germinated from seeds irradiated by 100Gy and cultivated in rainfed condition possessed the higher values of yield attributed characters (Fig. 2a-d), namely: 26.08±2.10 seeds per head; 374.61±60.63 seeds per plant; 9.94±1.68 g of seeds weight per plant and 27.77±1.42 of 1000-seeds weight. Thus, it was shown that the values of safflower yield attributing characters modified under both factors: the growing condition and pre-sowing gamma irradiation.

The contribution of these factors was evaluated by statistical analysis of the data (Tab. 2). The significant impact of growing condition was observed on the number of seeds per plant at $p \leq 0.01$, weight of seeds per plant and 1000-seeds weight at $p \leq 0.01$. The factor of radiation had the contribution to changes in

1000-seeds weight but the contribution of cultivation condition on this character was 15.7 times stronger.

Neither growing conditions nor irradiation significantly influence on the number of seeds per head (Tab. 2). Thus it could be concluded that the seed yield of safflower predicted by number of heads or number of developed inflorescences per plant. Khalilli et al. (2013) reported that under irrigation the most direct effect of characters on seed yield of safflower was from head diameter and 100-seed weight, and under rainfed condition – from number of head per plant. The results of path analysis reported by Bahmankar et al. (2014) strongly suggested that 1000-seed weight; heads per plants and main head diameter contain positive direct effect on seed yield.

Table 2.Impact of radiation and growing conditions on safflower seed yield

Factor	Sum of squares	Df	Mean square	F-ratio	P-value	Contribution of factor, %
Number of seeds per plant						
Radiation (R)	220720.00	3	73573.20	1.08	0.3580	ns
Condition (C)	470923.00	1	470923.00	6.92**	0.0091	2.78
Interactions R-C	491921.00	3	163974.00	2.41*	0.0679	2.91
Total	1.69288E7	230				
Weight of seeds per plant						
Radiation (R)	310.05	3	103.35	1.67	0.1743	ns
Condition (C)	2262.07	1	2262.07	36.55***	0.0000	12.43
Interactions R-C	416.19	3	138.73	2.24*	0.0843	2.29
Total	18198.70	230				
Weight of 1000 seeds						
Radiation (R)	401.718	3	133.91	2.47*	0.0629	1.92
Condition (C)	6316.02	1	6316.02	116.40***	0.0000	30.20
Interactions R-C	209.20	3	69.73	1.29	0.2803	ns
Total	20915.30	230				
Number of seeds per head						
Radiation (R)	410.77	3	136.93	2.00	0.1149	ns
Condition (C)	75.53	1	75.53	1.10	0.2947	ns
Interactions R-C	360.50	3	120.17	1.76	0.1566	ns
Total	16108.80	230				

Note: *; **; ***- denotes the statistically significant difference at $p \leq 0.1$; $p \leq 0.01$; $p \leq 0.001$ respectively

The strong direct proportional dependences between the developed inflorescences and number of seeds per plant were observed in this study (Tab. 3). The high correlation coefficients (>0.75) of these dependences were detected in all experimental plots of safflower plants. The safflower yield was significantly correlated with number of heads (R^2=0,916) and number of secondary branches (R^2=0,639) per plant (Tabrizi, 2001). The correlation between the number of secondary branches and developed inflorescences of different safflower cultivars also was determined (Beyyavas et al. 2011). Concerning to direct selection of safflower characters for improvement of the seed yield the author opinions differ. Ahmadzadeh et al. (2012) suggested that the improvement of the seed yield will immensely be efficient via 100-seed weight based selection in both conditions. Moreover direct selection could be made for plant height under irrigated conditions and number of seeds per head under non-irrigated conditions. Khalili et al. (2013) concluded that under rainfed conditions selection could be made for number of head per plant which confirm the results of the present study.

Table3. Correlation coefficient between developed inflorescences and seeds per plant

Variant	Control	50Gy	100Gy	150Gy
Irrigation	0.8660	0.8949	0.9552	0.9733
Rainfed	0.9252	0.8781	0.9440	0.7596

CONCLUSIONS

It was observed that the bio-morphological and yield attributing characters of safflower plant could be improved under adequate drip irrigation. The response of safflower growth and yield to pre-sowing treatment of seeds by gamma radiation with doses 50.100.150Gy was various (negative or positive) and depended on subsequent condition of cultivation. The cultivation conditions contributed more for plant growth and seed yield of safflower than the pre-sowing gamma irradiation. The number of head per plant is the most suitable selection character for improving seeds yield in safflower.

ACKNOWLEDGEMENT

The authors are thankful to the Science and Technology Centre in Ukraine for the financial support via the project STCU #6097.

REFERENCES

Ahmadzadeh A. (2013). Genetic diversity and classification of spring safflower (Carthamus tinctorius) cultivars using morphological characters. Advances in Bioresearch. 14 (4), 125-131.

Ahmadzadeh A.R., Alizadeh B., Aghdam Shahryar H., Narimani-Rad M. (2012). Path analysis of the relationships between grain yield and some morphological characters in spring safflower (Carthamus tinctorius) under normal irrigation and drought stress condition. Journal of Medicinal Plant Research. 6 (7), 1268-1271.

Bahmankar M., Nabati D.A., Dehdari M. (2014). Correlation, multiple regression and path analysis for some yield-related traits in safflower. Journal of Biodiversity and Environmental Sciences. 4 (2), 111-118. http://www.innspub.net.

Beyyavas V., Haliloglu H., Copur O., Yilmaz A. (2011). Determination of seed yield and yield components of some safflower (Carthamus tinctorius L.) cultivars, lines and populations under the semi-arid conditions. African Journal of Biotechnology. 10 (4), 527-534. DOI: 10.5897/AJB09.1395.

Bortolheiro F.P.A.P., Silva M.A. (2017). Physiological response and productivity of safflower lines under water deficit and rehydration. Anais da Academia Brasileira de Ciências ((Annals of the Brazilian Academy of Sciences), 89 (4), 3051-3066.

Eslam B.P. (2011). Evaluation of physiological indices for improving water deficit tolerance in spring safflower. J Agric Sci Technol. 13, 327-338.

Feyzollahzadeh M., Motlagh A.M., Nikbakhtt A.M. (2014). Effect of irrigation and nutrient on physical properties of safflower seeds. Int. Agrophys. 28, 7-14. DOI: 10.2478/intag-2013-0022.

Istanbulluoglu A., Gocmen E., Gezer E., Pasa C., Konukcu F. (2009). Effects of water stress at different development stages on yield and water productivity of winter and summer safflower (Carthamus tinctorius L.). Agric. Water Manage. 96, 1429-1434.

Ivanova R. (2016). Theoretical and practical aspects of the introduction of safflower (Carthamus tinctorius L.) in the Republic of Moldova. Studii şi comunicari. Stiintele Naturii. Muzeul Olteniei Craiova, Romania. 32 (2), 48-51.

Ivanova R., Mascenco N., Bejinari M. (2017). Influence of row spacing and bioregulators application on safflower yield. Scientific Papers. Series A. Agronomy, Bucharest, Romania. 60, 281-284

Kaya M.D., BayramİN S., KayaçetİN F., Katar D., Şenay A. (2009). Determination of proper gamma radiation (60Co) dose to induce variation in safflower. Ziraat Fakültesi Dergisi - Süleyman Demirel Üniversitesi. 4 (2), 28-33.

Khalili M., Aboughadareh A.P., Naghavi M.R., Rad H.N. (2013). Path analysis of the relationships between seed yield and some of morphological traits in safflower (Carthamus tinctorius L.) under normal irrigation and rainfed conditions. Technical Journal of Engineering and Applied Sciences. 15 (3), 1692-1696. www.tjeas.com

Lovelli S., Perniola M., Ferrara A., Di Tommaso T. (2007). Yield response factor to water (Ky) and water use efficiency of Carthamus tinctorius L. and Solanum melongena L. Agric. Water Manage. 92, 73–80.

Mozaffari K., Asadi A. A., Rahimi M. (2009).Investigation of yield and earliness traits in safflower mutants in irrigated and drought stress condition Journal of Nuclear Science and Technology. 46, 65-69

Ozturk E., Ozer H., Polat T. (2008). Growth and yield of safflower genotypes grown under irrigated and non-irrigated conditions in a highland environment. Plant Soil Environ. 54 (10), 453-460.

Patil S.A., Ravikumar R.L., Prabhu T.G., Parameshwarappa K.G. (2001). Gamma radiation induced polygenic variation homozygous and heterozygous genotypes of safflower. Reports of 5th International safflower conference, Williston, North Dakota and Sidney, USA. 99-101.

Raudonius S. (2017). Application of statistics in plant and crop research: important issues. Zemdirbyste-Agriculture. 104 (4), 377-382. DOI: 10.13080/z-a.2017.104.048.

Shahrokhnia M.H., Sepaskhah A.R. (2017). Safflower model for simulation of growth and yield under various irrigation strategies, planting methods and nitrogen

fertilization. International Journal of Plant Production. 11 (1), 167-192. http://ijpp.gau.ac.ir/article_3316_40d3bda947310b9e5439b20247b26ebe.pdf

Singh S., Angadi S.V., Grover K., Begna S., Auld D. (2016). Drought response and yield formation of spring safflower under different water regimes in the semiarid Southern High Plains. Agric Water Manage. 163, 354-362.

Tabrizi A.H.O. (2001). Correlation between traits and path analysis for grain and oil yield in spring safflower. Reports of Vth International safflower conferences, July 23-27, 2001. Williston, North Dakota and Sidney, Montana, USA. 95-98.

Parameshwarappa K.G., Meghannavar R.D. (2001). Combining hybridization and irradiation for enhancing genetic variability in early segregating generations of safflower crosses. Reports of 5th International safflower conference, Williston, North Dakota and Sidney, Montana, USA. 83-89

DOI: 10.17707/AgricultForest.65.1.04

Oksana KLYACHENKO, Larysa PRYSIAZHNIUK [1]

EVALUATION OF GENETIC DISTANCES CORRELATIONS AMONG SUGAR BEET GENOTYPES (*BETA VULGARIS* L.)

SUMMARY

A search and a combination of different statistical methods for determining the similarity and difference between genotypes is a hot topic for breeding. In the formation of adaptive potential and appropriate protective mechanisms for the implementation of the adaptive potential of sugar beet, terpene compounds and saponins are involved.

To determine polymorphism based on DNA of sugar beet materials under investigation, RAPD analysis was performed. A cluster analysis of the hybrids affinity was carried out in terms of the qualitative biochemical state of the system and DNA polymorphism. As a result, it was found that hybrids that demonstrated high activity of the secondary metabolism and, consequently, showed different ability to form a high adaptive potential in the conditions of long-term cultivation *in vitro*, formed separate clusters. With the aid of RAPD markers, the authors also determined two clusters that were formed on the basis of genotype affinity. In order to determine the correlations between genetic distances obtained by RAPD markers and terpene compounds, the Mantel test (linear correlation for Pearson) was performed. The values of coefficients $r = 0.517$ were obtained at $\alpha = 0.05$. However, according to the data interpretation, the calculated value of p (0.088) was higher than the significance level of $\alpha = 0.05$, which indicates the absence of correlations between the matrices under investigation. Thus, the revealed genotypic features of the secondary metabolism and genetic distances are additional characteristics for evaluation of the ecological plasticity of sugar beet plants, which is important in the breeding process.

Keywords: terpene compounds, saponins, DNA markers, Mantel test.

INTRODUCTION

One promising way to increase sugar beet (*Beta vulgaris* L.) productivity is to create new hybrids featuring the best response to environmental changes that can be adjusted by cultivation technology and genetical resistance to uncontrollable environmental factors at certain ontogenetic stages (Roik, 2010; Srivastava *et al*., 2017; Pazuki *et al.*, 2018).

[1]Oksana Klyachenko, National University of Life and Environmental Sciences of Ukraine, UKRAINE; Larysa Prysiazhniuk (corresponding author: prysiazhniuk_l@ukr.net), Ukrainian Institute for Plant Variety Examination, UKRAINE

Paper presented at the 9th International Scientific Agricultural Symposium "AGROSYM 2018".
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

Increasing environmental problems and anthropogenic influences leads to narrowing borders of tolerance and reducing crops resistance to abiotic and biotic stress factors (Strausbaugh *et al.*, 2010; Kito *et al.*, 2017). Therefore, in recent years, the development of crop production is focused on creating agro-ecosystem capable of fast responding to stressful effects and subsequent self-regulation (Bray, 2002; Zhuchenko, 2008; Pazuki *et al.*, 2017).

A complex nature of the issue of plant resistance to stress factors, in particular, drought-tolerance and salt-resistance, requires new approaches to the solution (Chirkova, 2002; Kito *et al.*, 2017). Today, cell selection is a promising and highly efficient method that makes it possible to create new starting material resistant to adverse conditions. Selection in vitro is performed in regard to the signs that can be detected at the cellular level, in particular an increase in the expression of certain genes that control the metabolic pathways providing tolerance to stress factors (Serheeva *et al.*, 2001; Tsilke, 2002; Dubrovna and Morhun, 2009; Golovko and Tabalenkova, 2014). A cell reacts to all deviations from its normal state with a change in a number of physiological and biochemical characteristics. This fact allows in vitro selection aimed at stability based on these characteristics (Dolgih, 1993; Gubanova *et al.*, 2000; Kalashnikova, 2003; Sidorov, 2004; Shearman *et al.*, 2005; Dubrovna and Morhun, 2009; Chugunkova, 2009; Dubrovna *et al.*, 2012).

When culturing in vitro, plants remain in conditions of partially heterotrophic nutrition. This causes changes in metabolic processes and plant adaptation to specific conditions of the environment. The ability of plants in vitro to synthesize terpene compounds and saponins is of great importance. These compounds perform important structural, constitutional, regulatory, and protective functions. Their production depends on the formation of assimilation organs and the activity of the photosynthetic apparatus. Along with the development of DNA technologies, new methods of direct genome analysis and the related search for molecular markers appear, in particular, RAPD analysis that allows differentiation, identification, and monitoring of various accessions independently of the stage of plant development and environmental conditions. The method is highly informative, technological, reproducible, and has a high potential of polymorphism (Havkin, 2003; Capistrano-Gossmann *et al.*, 2017; Ibrahim *et al.*, 2017; Wang *et al.*, 2018).

The issue of determining the relationship between DNA markers and the physiological state of plants is promising for the creation of new sugar beet lines based on the effect of heterosis. Research on the correlation of genetic distances of physiological parameters and DNA markers is an important field for the development of breeding in order to find and combine different statistical methods to determine the affinity and differences between genotypes that are involved in the breeding process and increase the efficiency of breeding.

The purpose of the research was to estimate the correlations in terms of the genetic distances of polymorphism of sugar beet genotypes based on RAPD analysis and metabolic profile of the biochemical status of the leaves.

MATERIAL AND METHODS

Five Ukrainian sugar beet genotypes were studied: variety Yaltushkivskyi monogerm 64, diploid hybrids Uladovo-Verkhniatskyi MS 37, Ukrainian MS 70, Ivanivskyi MS 33, and triploid hybrid Oleksandriia. The studies have been carried out for 2015–2017 in The Problem Laboratory Of Phytovirology and Biotechnology of National University of Life and Environmental Sciences of Ukraine (Kyiv, Ukraine).

DNA isolation and PCR

The sampling within each genotype numbered 30 accessions. DNA was isolated from 100 mg of green leaves of 5-day sprouts using CTAB and dissolved in TE buffer (Velikov, 2013; Roik *et al.*, 2007). The authors used four RAPD markers (GEN 2-80-7: GCAGGTCGCG; GEN 1-80-5: ACCCCAGCCG; GEN 1-70-9/2: TGCAGCACCG; GEN 4-70-2: GGACCGACTG) (Smulders *et al.*, 2010; Lučić *et al.*, 2011; Klyachenko and Prysiazhniuk, 2016). The size of the alleles obtained by electrophoretic spectra was analysed in pairs of nucleotides using computer program TotalLab v.2.01. Genetic distances, the frequency of detected alleles, and the index of polymorphism of the locus (PIC) were determined using the allele presence/absence matrix (Roik *et al*., 2007; Fedulova, 2014).

Identification of triterpene glycosides

The identification of triterpene glycosides was carried out according to the thin-layer chromatography method on 100 mm by 150 mm plates using sorbent Silica gel G60 (Merck, Germany) (Ladyigina *et al.*, 1983). The chromatograms were exposed 7–10 min at a temperature of 110 °C until the characteristic bands appeared. Digital data processing was performed using the Image-Pro Premier 9.0 (Trial version) program and chromatographic analysis using Sorbflil TLC.

Statistical analysis of data

The genetic distances between the genotypes under study were determined using a computer program STATISTICA 12 (Trial version) based on cluster analysis. The grouping of genotypes in terms of RAPD analysis and the metabolic profile of the biochemical status of the leaves was carried out by the unweighted average method with the calculation of Euclidian distances (Fortin *et al*., 2002; Drozdov, 2010; Everitt *et al*., 2011; Dong *et al.*, 2014).

Correlation by genetic distances of sugar beet genotype polymorphism based on RAPD analysis and metabolic profile of the biochemical status of leaves was calculated by Mantel test using the computer program XLSTAT 2018 (Trial version) (Legendre *et al*., 2010; Diniz-Filho *et al.*, 2013).

RESULTS AND DISCUSSION

In vitro cell selection aimed at increased complex tolerance to drought and salinity usually consists of two main steps: the very selection of resistant cell lines in the appropriate selective media and plant regeneration. Complex cell selection for drought- and salt-tolerant lines was carried out using selective agents PEG 6000 (20 %) and Na2SO4 (2.0 %). The stability of the signs of

tolerance of the resulting lines to the complex of stress factors was determined through the alternation of transplantations to nutrient media containing / without stress factors. Obtaining of the drought- and salt-tolerant lines was performed through a long-term (up to seven passages) in vitro culturing of both callus and produced regenerated plants. The highest regeneration capacity was demonstrated by the callus lines of variety Yaltushkivskyi monogerm 64 (77 %), triploid hybrid Alexandria (60 %), and diploid hybrid Ukrainian MS 70 (50 %).

As a result of RAPD analysis, 14 alleles with a frequency varied from 0.2 to 0.8 were detected. The average PIC value was 0.45. According to the obtained data, the vast majority of the genotypes under investigation had an allele measuring 50 bp (at a frequency of 0.8). Noteworthy, that the allele of 176 bp (at a frequency of 0.2) was identified only in Yaltushkivskyi monogerm 64.

The analysis of the metabolic profile of the biochemical status of the leaves allowed to find out the peculiarities of the genotypes under study in respect of synthesis of terpene compounds and saponins. Using the method of photodensitometric analysis of chromatogram, the authors determined the ratio of individual compounds to the corresponding indices of electrophoretic mobility (Rf) and their occurrence in the examined sugar beet samples (Klyachenko and Likhanov, 2017).

In order to analyse the polymorphism based on the ratio of terpene compounds to saponins and RAPD analysis, a cluster analysis was performed, and genetic distances between genotypes were calculated. Shown in Table 1 are the genetic distances between sugar beet genotypes in terms of the ratio of terpene compounds to saponins.

Table 1. Euclidean distances between sugar beet genotypes based on the metabolic profile of triterpene compounds and saponins.

Variable	Uladovo-Verkhniatskyi MS-37	Ukrainian MS-70	Oleksandriia	Yaltushkivskyi monogerm 64
Ivanivskyi MS-33	2.83	2.65	2.45	2.65
Uladovo-Verkhniatskyi MS-37	-	1.73	2.00	1.73
Ukrainian MS-70	-	-	1,00	2.00
Oleksandriia	-	-	-	1.73

Given that objects with zero digital expression of genetic distances are considered to be identical, according to cluster analysis results, the least value of genetic distance (1.00) was found between triploid hybrid Oleksandriia and diploid hybrid Ukrainian MS 70. Diploid hybrids Uladovo-Verkhniatskyi MS 37 and Ukrainian MS 70, Yaltushkivskyi monogerm 64 and triploid hybrid Oleksandriia had a fairly close distance (1.73). The diploid hybrids Uladovo-Verkhniatskyi MS 37 and Ivanivskyi MS 33 appeared to be the most remote (2.83) by the metabolic profile of triterpene compounds and saponins.

In the conditions of long-term cultivation in vitro, hybrids Oleksandriia and Ivanivskyi MS 33 demonstrated high activity of secondary metabolism and, accordingly, a potentially high adaptive potential. Hybrids Uladovo-Verkhniatskyi MS 37 and Ukrainian MS 70, as well as variety Yaltushkivskyi monogerm 64, demonstrated the least activity in the synthesis of triterpene compounds and saponins. Thus, according to the results of the biochemical test of the metabolic profiles of terpenes and triterpene saponins in vitro, genotypic features that serve as additional signs for assessing the ecological plasticity of sugar beet plants were identified, which is very important for breeding work.

According to the distribution of the RAPD analysis results, genetic distances between genotypes ranged between 2.45 and 3.46 (Table 2).

Table 2. Euclidean distances between sugar beet genotypes based on RAPD analysis

Variable	Uladovo-Verkhniatskyi MS-37	Ukrainian MS-70	Ivanivskyi MS-33	Oleksandriia
Yaltushkivskyi monogerm 64	3.46	2.83	2.65	2.83
Uladovo-Verkhniatskyi MS-37	-	2.83	2.65	2.83
Ukrainian MS-70	-	-	2.65	2.45
Ivanivskyi MS-33	-	-	-	2.65

Triploid hybrid Oleksandriia and diploid hybrid Ukrainian MS 70 were found to be the most affine, which also coincided with the results of cluster analysis of the profile of triterpene compounds and saponins. According to the obtained distribution, most genotypes were at the same distance from each other. However, it should be noted that according to the results of RAPD analysis, the most remote genotypes proved to be Yaltushkivskyi monogerm 64 and diploid hybrid Uladovo-Verkhniatskyi MS 37 (3.46). Consequently, based on RAPD analysis, the molecular genetic polymorphism of sugar beet genotypes was found, specific alleles were identified, and their genetic affinity was determined.

The research identified specific and non-specific qualitative and quantitative changes in the regenerants of different sugar beet genotypes in the conditions of long-term cultivation in vitro on the basis of the content of triterpene compounds and saponins, which production varies in different genotypes. Owing to RAPD analysis, which allows involving methods of DNA analysis for the analysis of the varietal and hybrid material of sugar beet, it was possible to determine the genetic affinity of the sugar beet genotypes under study.

Noticeable, that cluster analysis on the basis of individual indicators shows a different distribution of genotypes and, accordingly, different degree of their affinity. However, effective breeding work requires involving modern methods of integrated genotype assessment in order to determine the most effective schemes.

The Mantel test (linear correlation by Pearson) was used to determine correlations between genetic distances obtained with the aid of RAPD analysis and the metabolic profile of triterpene compounds and saponins. As a result, p-value and r (AB) values at $\alpha = 0.05$ were determined, which, according to the test interpretation, allowed accepting one of the hypotheses: presence (Ha) or absence (H0) of correlation.

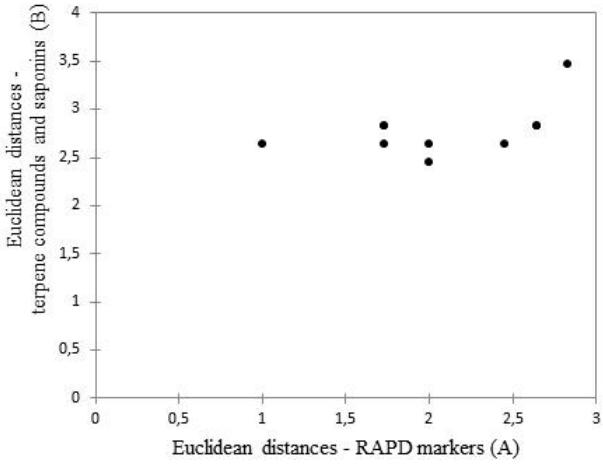


Fig. 4. Interactions between genetic distances of sugar beet genotypes by four RAPD markers and the metabolic profile of triterpene compounds and saponins.

It is known that the H0 hypothesis of the absence of correlation can be assumed given that $p > \alpha$. In this research, the calculated value of p (0.088) was higher than the significance level $\alpha = 0.05$, therefore the zero hypothesis H0 should be accepted and the alternative Ha hypothesis of the existence of correlation should be rejected (Burstin *et al.*, 1997; Diniz-Filho *et al.*, 2013). Shown in Fig. 5 are the correlation coefficient and normality of data distribution by matrixes of genetic distances.

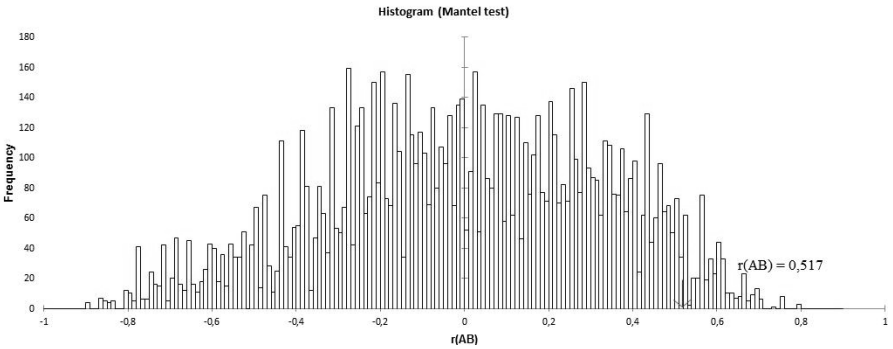


Fig. 5. Normality of Mantel test results distribution for sugar beet genotypes on the basis of genetic distances.

As a result of the analysis, it was found that there was no correlation between the genetic distances of five sugar beet genotypes by four RAPD markers and the metabolic profile of triterpene compounds and saponins. The shape of a diagram showing the normality of the distribution also indicated this fact. Consequently, it was found that a system consisting of four RAPD markers is effective for evaluation of genetic relationships of different sugar beet genotypes and analysis of their metabolic profile that reflects genotype ability to synthesize secondary metabolites, which is an indicator of adaptive potential.

CONCLUSIONS

As a result of the research, the molecular-genetic polymorphism of five sugar beet genotypes was investigated by four RAPD markers and the metabolic profile of triterpenes and saponins at long-term cultivation in vitro.

Based on cluster analysis and in accordance with genetic distances, it was found that triploid hybrid Oleksandriia and diploid hybrid Ukrainian MS 70 appeared to be the most affine ones, whereas the most remote genotypes were found to be variety Yaltushkivskyi monogerm 64 and diploid hybrid Uladovo-Verkhniatskyi MS 37. The results of this research are recommended to use in planning crossing schemes in breeding work.

According to the metabolic profile of triterpene compounds and saponins, diploids Uladovo-Verkhniatskyi MS 37 and Ivanivskyi MS 33 are the most remote hybrids, whereas the smallest distance is observed between the triploid hybrid Oleksandriia and the diploid hybrid Ukrainian MS 70.

It was proved that prolonged in vitro vegetation of sugar beet caused adaptive changes in metabolic processes, specifically in the synthesis of secondary metabolites. The changes in the metabolic processes are involved in the development of adaptive potential and appropriate protective mechanisms of sugar beet plants.

Based on the Mantel test results, there is no correlation between genetic distances on the basis of RAPD analysis and the metabolic profile of triterpene compounds and saponins. Therefore, in breeding work aimed at obtaining plants with complex resistance to drought and salinity, when selecting start material, it is necessary to consider not only genetic distances between genotypes but also their distribution in terms of adaptive potential.

REFERENCES

Bray, E. A. (2002). Classification of genes differentially expressed during water-deficit stress in Arabidopsis thaliana: an analysis using microarray and differential expression data. Annals of botany, 89(7), 803-811.

Burstin J., Charcosset A. 1997. Relationship between phenotypic and marker distances: theoretical and experimental investigations. Heredity, 79 (5): 477.

Capistrano-Gossmann, G. G., Ries, D., Holtgräwe, D., Minoche, A., Kraft, T., Frerichmann, S. L. M., ... & Varrelmann, M. (2017). Crop wild relative populations of Beta vulgaris allow direct mapping of agronomically important genes. Nature communications, 8, 15708. https://doi:10.1038/ncomms15708

Chirkova, T. V. (2002). Physiological basis of plant resistance. Publishing St. Petersburg University, St. Petersburg, Russian Federation. (in Russian).

Chugunkova, T. V. (2009). Use of cell selection to create resistant forms beets. Physiology and biochemistry of cultivated plants, 41(6), 509-515. (in Ukrainian).

Diniz-Filho, J. A. F., Soares, T. N., Lima, J. S., Dobrovolski, R., Landeiro, V. L., Telles, M. P. D. C., ... & Bini, L. M. (2013). Mantel test in population genetics. Genetics and molecular biology, 36(4): 475-485. http://dx.doi.org/10.1590/S1415-47572013000400002

Dolgih, O. I. (1993). Principles of screening cells in vitro for the purpose of obtaining forms of plants resistant to abiotic stresses. 2nd Russian Symposium «New methods of plant biotechnology», May, 18-20 1993 г., Pushchino, Russian Federation. (in Russian).

Dong D., Fu X., Yuan F., Chen P., Zhu S., Li B., ... Zhu D. 2014. Genetic diversity and population structure of vegetable soybean (Glycine max (L.) Merr.) in China as revealed by SSR markers. Genetic Resources and Crop Evolution, 61 (1): 173-183. http://doi:10.1007/s10722-013-0024-y

Drozdov, V. I. (2010). Instructions for using the Statistica 6.0. (in Russian).

Dubrovna, O. V., & Morhun, B. V. (2009). Wheat Cell Selection for Resistance to Stressful Environmental Factors. Physiology and biochemistry of cultivated plants. (in Ukrainian).

Dubrovna, O. V., Chuhunkova, T. V., Bavol, A. V., & Lialko, I. I. (2012). Biotechnological bases for the creation of plants resistant to stress. Logos, Kyiv, Ukraine. (in Ukrainian).

Everitt, B.S., Landau, S., Leese, M., &Stahl, D. Cluster. (2011). Analysis 5th Edition.

Fedulova, T. P. (2014). DNA markers for identification of sortotypes in the gene pool of beet root. Sugar beet, (5), 14.

Fortin, M. J., Dale, M. R., & Ver Hoef, J. M. (2002). Spatial analysis in ecology. Wiley StatsRef: Statistics Reference Online. http://onlinelibrary.wiley.com/doi/10.1002/9781118445112.stat07766.pub2/full.

Golovko, T., & Tabalenkova, G. (2014). Pigments and productivity of the crop plants. Photosynthetic pigments: chemical structure, biological function and ecology.

Gubanova, N. Ya., Dubrovnaya, O. V., & Chugunkova, T. V. (2000). Selection and comparative analysis of salt-resistant callus cultures of fodder beet obtained from explants of different ploidy. Physiology and biochemistry of cultivated plants, 32(5), 362-369. (in Russian).

Havkin, E. E. (2003). Molecular selection of plants: DNA technology for creating new varieties of crops. Agricultural Biology, (3), 26-41.

Ibrahim, H. M., El-Leel, O. F., & Emam, K. A. (2017). Molecular Profiling for Geneti c Variability in Petroselinum crispum Based on ISSR and RAPD Markers. Middle East J, 6(1), 67-75.

Kalashnikova, E. V. (2003). Cell selection for resistance to fungal diseases (Cand. Agric. Sci. Diss). Moscow, Russian Federation. (in Russian).

Kito, K., Tsutsumi, K., Rai, V., Theerawitaya, C., Cha-um, S., Yamada-Kato, N., ... & Takabe, T. (2017). Isolation and functional characterization of 3-phosphoglycerate dehydrogenase involved in salt responses in sugar beet. Protoplasma, 254(6), 2305-2313. https://doi.org/10.1007/s00709-017-1127-7

Kito, K., Yamane, K., Yamamori, T., Matsuhira, H., Tanaka, Y., & Takabe, T. (2018). Isolation, functional characterization and stress responses of raffinose synthase genes in sugar beet. Journal of Plant Biochemistry and Biotechnology, 27(1), 36-45. https://doi.org/10.1007/s13562-017-0413-y

Klyachenko, O. L., & Likhanov, A. F. (2017). Content of Triterpene saponins and phenolics compounds in leaves of in vitro Beta vulgaris L. genotypes. Ukrainian Journal of Ecology, 7(4), 564-568. https://doi:10.15421/2017_161

Klyachenko, O. L., & Prysiazhniuk, L. M. (2016). Differentiation and identification of different genotypes of sugar beet (Beta vulgaris L.) using DNA markers. Scientific reports of NULES of Ukraine, (4 (61)).

Ladyigina, E. Ya., Safronich, L. N., & Otryashenkova, V. E. (1983). Chemical analysis of medicinal plants. Vyisshaya shkola. Moscow. Russian Federation. (in Russian).

Legendre, P., & Fortin, M. J. (2010). Comparison of the Mantel test and alternative approaches for detecting complex multivariate relationships in the spatial analysis of genetic data. Molecular ecology resources, 10(5): 831-844. https://doi:10.1111/j.1755-0998.2010.02866.x

Lučić, A., Isajev, V., Rakonjac, L., Ristić, D., Kostadinović, M., Babić, V., & Nikolić, A. (2011). Genetic divergence of Scots pine (Pinus sylvestris L.) populations in Serbia revealed by RAPD. Archives of biological sciences, 63(2), 371-380. http://doi:10.2298/ABS1102371L

Pazuki, A., Aflaki, F., Gürel, E., Ergül, A., & Gürel, S. (2017). A robust method for haploid sugar beet in vitro proliferation and hyperhydricity reduction. Folia Horticulturae, 29(2), 241-250. https://doi:10.1515/fhort-2017-0022

Pazuki, A., Aflaki, F., Gürel, S., Ergül, A., & Gürel, E. (2018). Production of doubled haploids in sugar beet (Beta vulgaris): an efficient method by a multivariate experiment. Plant Cell, Tissue and Organ Culture (PCTOC), 132(1), 85-97. https://doi:10.1007/s11240-017-1313-5

Roik, M. V. (2007). Determination of molecular genetic polymorphism of the genus BETA L. with polymerase chain reaction. K.: TOV «PolihrafKonsaltynh».

Royk, N. V. (2010). Combinative ability of sugar beet pollinators of different genetic structure by elements of productivity. Encyclopedia of the genus Beta: Biology, genetics and selection of beets, 525-541. (in Russian).

Serheeva, L. E. (2001). Changes in cell culture due to stress. Logos, Kiev, Ukraine (in Russian).

Shearman, V. J., Sylvester-Bradley, R., Scott, R. K., & Foulkes, M. J. (2005). Physiological processes associated with wheat yield progress in the UK. Crop Science, 45(1), 175-185.

Sidorov, V. A. (2004). Biotechnology of plants. Cell selection of plants. Naukova Dumka, Kiev, Ukraine. (in Russian).

Smulders, M. J., Esselink, G. D., Everaert, I., De Riek, J., & Vosman, B. (2010). Characterisation of sugar beet (Beta vulgaris L. ssp. vulgaris) varieties using microsatellite markers. BMC genetics, 11(1), 41. https://doi.org/10.1186/1471-2156-11-41

Srivastava, S., Pathak, A. D., Kumar, R., & Joshi, B. B. (2017). Genetic diversity of sugar beet genotypes evaluated by microsatellite DNA markers. Journal of Environmental Biology, 38(5), 777. http://doi.org/10.22438/jeb/38/5/MS-141

Strausbaugh, C. A., Eujayl, I. A., & Foote, P. (2010). Sugarbeet cultivar evaluation for bacterial root rot. Journal of Sugarbeet Research, 47, 51-64.

Tsilke, R. A. (2002). Some aspects of genetic engineering in plants. Improving the efficiency of plant selection and seed production of agriculture crops: of scientific articles, 17-23. (in Russian).

Velikov, V. A. (2013). Molecular biology. A Practical Guide. Saratov:«Saratovskiy istochnik ».–2013.–84 c.

Wang, M., Xu, Y., Wu, Z., Wang, H., & Zhang, H. (2018). High-Density Genetic Map Construction in Sugar Beet (Beta vulgaris L.) by High-Throughput Technology. Sugar Tech, 20(2), 212-219. https://doi.org/10.1007/s12355-017-0550-6

Zhuchenko, A. A. (2008). Adaptive plant growing (ecological and genetic basis): theory and practice. Agrorus, Moscow, Russian Federation. (in Russian).

DOI: 10.17707/AgricultForest.65.1.05

Yusuf YANAR*, Dürdane YANAR,
Büşra DEMİR, Yasin Bedrettin KARAN [1]

EFFECTS OF LOCAL ENTOMOPATHOGENIC *BEAUVERIA BASSIANA* ISOLATES AGAINST *SITOPHILUS GRANARIUS* (COLEOPTERA)

SUMMARY

The granary weevil *Sitophilus granarius* L. is one of the most damaging grains pest in many parts of the World and Turkey. Chemical insecticides have been widely employed for the control of stored grain pests. This has caused such problems as insecticide resistance along with contamination of foodstuffs with chemical residues. Thus, there is a growing interest in using pathogenic control agents as an alternative.

This study aimed to evaluate the efficacy of entomopathogenic fungus *Beauveria bassiana* isolates (F-52, F-53, and F-56) on adults of granary beetle *S. granarius*. Five different doses, including 1×10^3, $1x10^5$, 1×10^7, $1x10^8$, and 1×10^9 conidia/ml for insect dipping, had been used. The experiment was laid out in a completely randomised block design with five replications and replicated two times.

Mortalities were recorded on the 1st, 3rd, 5th, and seventh days of incubation. The highest mortality rate of 70% was observed at the end of the five-day incubation period with isolate F-53. Mortality increased with increase in the incubation period, and the highest mortality was observed after seven days of incubation period.

Although the results indicated that isolate F-53 was effective against *S. granarius* and resulted in a high mortality 98% at the end of seventh day incubation period at 1×10^9 conidia/ml and followed by isolates F-52 and F-56 with 94% mortality. LC50 values confirmed that *S. granarius* was more susceptible to the isolate F-52 than the other two isolates F-53 and F-56 where the LC50's were $1x10^5$, $2x10^5$, and $5x10^5$ conidia/ml respectively. Mycosis was observed in all the treatments except the control.

Our study indicates that all the isolates could be used as potential biological control agents. Further studies are ongoing for determination of the efficacy of this isolate under storage conditions.

Keywords: Biological control, entomopathogen, *Beauveria bassiana*, granary weevil

[1]Yusuf Yanar*(corresponding author: yusuf.yanar@gop.edu.tr), Dürdane Yanar, Büşra Demir, Yasin Bedrettin Karan, Gaziosmanpaşa University, Faculty of Agriculture, Department of Plant Protection, Tokat, TURKEY

Paper presented at the 9th International Scientific Agricultural Symposium "AGROSYM 2018".
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

INTRODUCTION

Stored grains and their by-products are infested by insect pests causing approximately 10-25% losses worldwide. Losses caused by insects include not only the direct feeding damage resulting in loss of weight but they also severely reduce nutrients, lowering percentage of seeds germination, reducing grade and lowering their marketing value due to the accumulation of waste, webbing and insect cadavers (Hill, 1990). Most of these are coleopterans (Vinuela et al. 1993). Among them, the granary weevil *Sitophilus granarius* L. (Coleoptera: Curculionidae) and is known for its economic importance. The damages consist of the reduction in weight, quality, commercial value and seed viability (Hill, 1990). Residual insecticides have been employed to control insect pests of stored grains, but alternative control strategies are desirable because of the loss of insecticides due to pest resistance and consumer desire for pesticide-free grain (Arthur, 1996).

The most significant impetus for the growth of biopesticides comes from the growing awareness by farmers of the value of integrated pest management as a more environmentally sound, economical, safer and a selective approach to crop protection (Menn, 1996). Therefore, it is necessary to find out safer alternative control strategies such as the use of microbial control agents against stored–product insect pests. Using fungi and selected insecticides can potentially reduce the use of chemical insecticides and subsequently their residues and side effects in agriculture. *Beauveria bassiana* and *Metarhizium anisopliae* are naturally occurring entomopathogenic fungi with a broad host range (Sheeba et al. 2001, Wakil and Ghazanfar, 2010, Sewify et al. 2014).

The entomopathogenic fungus *B. bassiana* bears a considerable potential for the control of the different stored product pests. Cherry et al. (2005) suggested that *B. bassiana* is a potential microbial control agent against some stored product pests. Nowadays, several *B. bassiana* formulations (Boverosil ®, Mycotrol® ES, Mycotrol® 22WP, Naturalis® SC) are commercially available and are registered for use in storage facilities. Entomopathogenic fungi within stored food products can be employed to treat empty stores to control residual pests before the new harvest is brought in or may be applied as the direct admixture of conidia to grain, either as preventative or curative treatments of bulk grain. The present work aimed to evaluate the efficacy of the local entomopathogenic *B. bassiana* isolates against the granary weevil *S. granarius.*

MATERIAL AND METHODS

Test Insect Rearing: Tested stored product insect species *Sitophilus granarius* L. were obtained from laboratory cultures reared for several generations at the Department of Plant Protection, Agricultural Faculty, Gaziosmanpasa University, Tokat/Turkey. The insects were reared on whole wheat grains. Insect cultures were maintained in glass jars (2 litres) covered with a muslin cloth. All insects were reared under laboratory conditions of 24±2 °C and 75±2% R.H.

Fungal Isolates: The entomopathogenic fungi, *B. bassiana*, isolates F-52, F-53, and F-56 were initially isolated from the meadow soil at Kelkit Valley. The fungi were grown on Potato dextrose agar (PDA) medium. The media was autoclaved at 121 °C for 15 minutes and poured into Petri dishes (9 cm diameter x 1.5 cm). The fungal isolates kept at 25 ±2 °C and 85± 5 % RH. The fungal isolate was sub-cultured every 14 – 30 days and kept at 4 °C.

Bioassay: The isolates F-52, F-53, and F-56 were grown on Potato Dextrose Agar (PDA) medium at 27 ^{o}C for four weeks to get sporulation. The conidia were harvested under sterile conditions by flooding the plate with 10 ml of sterile distilled water containing 0,02% tween 80 then scraping the colony with a sterile glass hockey stick. The spore suspension was filtered through four layer sterile cheesecloths to remove mycelia. The concentrations of conidial suspensions were determined, using a Neubauer hemocytometer. The conidial suspensions were stored at 4 ^{o}C for up to 1 week until used in the assays. The viability of conidia was determined by spread-plating 0.1 ml of the suspension on the PDA plates. A sterile microscope coverslip was placed on each plate. Plates were incubated at 27 ^{o}C and examined after 24 h. The percentage of germination was determined by counting 100 spores for each plate, and over 95% of the spores germinated. For the dose-mortality test, *Sitophilus granarius* healthy adults were randomly selected and used for bioassays. Conidial suspensions of fungal isolates were prepared as described above, and a series of dilutions was prepared as 1×10^{3}, 1×10^{5}, 1×10^{7}, 1×10^{8}, and 1×10^{9} conidia/ml. Ten adults were dipped in conidia suspension for 5 seconds than transferred into the glass wails containing ten wheat grains as food. The experiment was laid out in a completely randomised block design with five replications and replicated two times. The control insects treated with sterile distilled water containing 0,02% tween 80. Mortalities were recorded on the 1st, 3rd, 5th, and seventh days of incubation. Dead insects were transferred in humid sterile 90 mm glass petri dishes for 14 days to determine the mycosis rates. The percentage mortality and LC50 were estimated.

Statistical Analysis

The data were analysed by analysis of variance (ANOVA) and the means compared by Tukey's multiple comparison test. All statistical analyses were carried out using the SPSS Release 16 packet program. The lethal concentration (LC50) was calculated using probit analysis. (Finney 1978).

RESULTS AND DISCUSSION

Several studies documented the high potential of entomopathogenic fungi for the control of insect pests in stored grains and their byproducts (Moore et al., 2000, Cox et al. 2003 and 2004, Cherry et al. 2005). The insecticidal efficacy of *B. bassiana* is highly influenced by several factors such as characteristics of host insects, and physiology of pathogen fungi (enzymes and toxins) (Fargues et al., 1996, Cox et al., 2004). So, the testing of different isolates of *B. bassiana* against different storage pests is needed. In the present study it was found that three *B.*

bassiana isolates tested (F-52, F-53, and F-56) were pathogenic in *S. granarius* adults.

The percentages of mortality varied from 76% to 84% at $1x10^8$ conidia/ml concentration 7 day after inoculation (Table 1, 2, and 3). The control group had 8% mortality.

Table 1. Mortality of *Sitophilus granarius* exposed to different doses (1×10^3, 1×10^5, 1×10^7, 1×10^8, and 1×10^9 conidia/ml) of *Beauveria bassiana* isolates F-52 and controls over seven days after treatment.

	Mortality±SEM* (%)			
Doses(conidia/ml)	**1 DAT****	**3 DAT**	**5 DAT**	**7 DAT**
$1x10^3$	4,00±5,47a***	16,00±5,47b	32,00±4,47b	44,00±5,47b
$1x10^5$	2,00±4,47a	24,00±5,47bc	36,00±5,47b	48,00±4,47b
$1x10^7$	2,00±4,47a	32,00±4,47cd	38,00±4,47bc	66,00±5,47c
$1x10^8$	4,00±5,47a	34,00±5,47cd	46,00±5,47c	76,00±5,47c
$1x10^9$	4,00±5,47a	36,00±5,47d	56,00±5,47d	94,00±5,47d
Control	0,00±0,00a	2,00±4,47a	2,00±4,47a	8,00±4,47a

* SEM: Standard error of the mean;
** DAT: Days after treatment;
*** Means in a column followed by the same letter are not statistical significantly different ($P < 0.05$).

In all the experiments, mortality increased with increase in conidia concentrations and incubation periods while the highest mortality was observed after seven days of incubation at $1x10^9$ conidia/ml concentrations. The results indicated that isolate F-53 was effective against *S. granarius* and resulted in a high mortality 98% at the end of the 7^{th} day incubation period at 1×10^9 conidia/ml.

Followed by isolates F-52 and F-56 with 94% mortality (table 1,2 and 3). Mycosis was observed in all the treatments except the control. The present study agrees with the results of previous studies where higher doses produce the highest percentage of mortality (Athanassiou and Steenberg 2007, Khashaveh et al., 2011; Magda and Mohamed, 2015).

Table 2. Mortality of *Sitophilus granarius* exposed to different doses (1×10^3, 1×10^5, 1×10^7, 1×10^8, and 1×10^9 conidia/ml) of *Beauveria bassiana* isolates F-53 and controls over seven days after treatment.

	Mortality±SEM* (%)			
Doses(conidia/ml)	**1 DAT****	**3 DAT**	**5 DAT**	**7 DAT**
$1x10^3$	0,00±0,00a***	18,00±4,47b	28,00±4,47b	38,00±4,47b
$1x10^5$	0,00±0,00a	22,00±8,36b	36,00±5,47bc	46,00±5,47b
$1x10^7$	6,00±8,94a	34,00±5,47c	44,00±5,47c	66,00±5,47c
$1x10^8$	0,00±0,00a	38,00±4,47c	56,00±5,47d	82,00±4,47d
$1x10^9$	8,00±10,95a	40,00±0,00c	70,00±7,07e	98,00±4,47e
Control	2,00±4,47a	2,00±4,47a	2,00±4,47a	8,00±4,47a

* SEM: Standard error of the mean; ** DAT: Days after treatment;
*** Means in a column followed by the same letter are not statistical significantly different ($P < 0.05$).

Table 3. Mortality of *Sitophilus granarius* exposed to different doses (1×10^3, 1×10^5, 1×10^7, 1×10^8, and 1×10^9 conidia/ml) of *Beauveria bassiana* isolates F-56 and controls over seven days after treatment.

	Mortality±SEM* (%)			
Doses(conidia/ml)	**1 DAT****	**3 DAT**	**5 DAT**	**7 DAT**
$1x10^3$	6,00±8,94a***	10,00±7,07ab	20,00±7,07b	34,00±5,47b
$1x10^5$	2,00±4,47a	16,00±5,47bc	30,00±7,07bc	44,00±5,47b
$1x10^7$	2,00±4,47a	24,00±5,47cd	36,00±5,47cd	56,00±5,47c
$1x10^8$	2,00±4,47a	28,00±4,47de	44,00±5,47d	84,00±5,47d
$1x10^9$	0,00±0,00a	36,00±5,47e	56,00±5,47e	94,00±5,47d
Control	2,00±4,47a	2,00±4,47a	2,00±4,47a	8,00±4,47a

* SEM: Standard error of the mean; ** DAT: Days after treatment;
*** Means in a column followed by the same letter are not statistical significantly different ($P < 0.05$).

Probit analysis was carried out to determine LC50. The parameters of the probit analysis and LC50 are given in Table 4. LC50 values confirmed that *S. granarius* was more susceptible to the isolate F-52 than the other two isolates F-53 and F-56 where the LC50's were $1x10^5$, $2x10^5$, and $5x10^5$ conidia/ml respectively (Table 4).

Table 4. Lethal concentrations (LC_{50}) values of the adult of *Sitophilus granarius* treated *Beauveria bassiana* isolates F-52, F-53, and F-56.

Isolates	Slope±SE	LC_{50} (95% fiducial limit)	χ^2
F-52	0,26±0,015	$1x10^5$ ($2.8x10^4$ – $4x10^5$)	91,5
F-53	0,34±0,016	$2x10^5$ ($6,0x10^4$ – $5x10^5$)	105,5
F-56	0,34±0,016	$5x10^5$ ($1,0x10^5$ – $1x10^6$)	121,9

[*] Slope value (±standart deviation) of dose-mortality respose of *Sitophilus granarius* to *Beauveria basiana* isolates F-52, F-53, and F-56.
[**]Pearson $\chi 2$ value (α=0.05)

CONCLUSIONS

It can be concluded that the isolates F-52 was more efficient against *S. granarius* than the isolates F-53 and F-56 and proved that F-52 had higher virulence than the others, All the three *B. bassiana* isolates have the potential for practical and economically feasible control of *S. granarius.*

REFERENCES

Arthur, F. H. (1996). Grain Protectants: Current Status and Prospects for the Future. J. Stored Prod. Res., 32(4): 293302.

Athanassiou C. G., Steenberg T. (2007). Insecticidal Effect of *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales) in Combination with Three Diatomaceous Earth Formulations against *Sitophilus granarius* (L.) (Coleoptera: Curculionidae). *Biol. Control*, 40: 411–416.

Cherry A. J., Abalo P., Hell K. (2005). A Laboratory Assessment of the Potential of Different Strains of the Entomopathogenic Fungi *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) to Control *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) in Stored Cowpea. J. Stored Prod. Res., 41(3): 295–309.

Cox P. D., Wakefield M. E., Price N. R., Wildey K. B., Moore D., Aquino de Muro M. Bell B. A. (2003). Entomopathogenic Fungi for the Control of Invertebrate Pests in Storage Structures: Advances in Stored Product Protection. Proceedings of the 8th International Working Conference on Stored Product Protect., 22-26 July 2002, York, UK. PP. 87-94.

Cox P. D., Wakefield M. E., Price N., Wildey K. B., Chambers J., Moore D., Aquino de Muro M. Bell B. A. (2004). The Potential Use of Insect Specific Fungi to Control Grain Storage Pests in Empty Grain Stores. HGCA Project Report No. 341, 49 pp.

Fargues J., Goettel M. S., Smites N., Quedraogo A., Vidal C., Lacey L. A., Lomer C. J., Rougier M. (1996). Variability in susceptibility to simulated sunlight of conidia among isolates of entomopathogenic Hyphomycetes. Mycopathologia, 135: 171-181.

Finney D. J. (1978). Probit Analysis, Cambridge University Press, Cambridge.
Hill D. S. (1990). Pests of Stored Products and Their Control. Belhaven Press, London. 8-55.
Khashaveh A., Ghosta Y., Safaralizadeh M. H., Ziaee M. (2011). The Use of Entomopathogenic Fungus, *Beauveria bassiana* (Bals.) Vuill. in Assays with Storage Grain Beetles. J. Agr. Sci. Tech. 13: 35-43.
Magda S., Mohamed A. (2015). Toxicity of the fungus Beauveria bassiana and three oils extract against Sitophilus granaries under laboratory and store condition, American Journal of Innovative Research and Applied Sciences.1(7):251-256.
Menn J. J. (1996). Biopesticides: Has Their Time Come. J. Environ. Sci. Health. 31: 383-389.
Moore D., Lord J .C., Smith S. M. (2000). Pathogens. In: Subramanyam Bh, Hagstrum DW(Eds). Alternatives to Pesticides in Stored-Product IPM. Kluwer Academic Publishers, Dordrecht, pp. 193-227.
Sheeba G., Sundaram S., Raja N., Janarthanan S., Ignacimuthu S. (2001). Efficacy of *Beauveria bassiana* for control of the rice weevil *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). Applied Entomology Zoology 2001; 36(1):117-120.
Sewify G. H., El Shabrawy H. A., Eweis M. E., Magda Naroz H. (2014). Efficacy of Entomopathogenic Fungi, *Beauveria bassiana* and *Metarhizium anisopliae* for controlling certain stored Product Insects Egypt, Journal of Biological Pest Control. 2014; 24(1):191-196.
Wakil W., Ghazanfar M. U. (2010). Entomopathogenic fungus as a biological control agent against Rhyzopertha dominica F. (Coleoptera: Bostrychidae) on stored wheat. Arch. Phytopathol. Plant Prot. 43: 1236 – 1242.
Vinuela E., Adan A., Del Estal P., Marco V., Budia F. (1993). Plagas de los Productos
Almacenados. Hojas Divulgadoras. Ministerio de Agricultura Pesca y Alimentacio´ n, Madrid, Espana.

DOI: 10.17707/AgricultForest.65.1.06

Ivana GLIŠIĆ, Dragan MILATOVIĆ, Radosav CEROVIĆ, Sanja RADIČEVIĆ, Milena ĐORĐEVIĆ, Nebojša MILOŠEVIĆ* [1]

UNUSUAL GROWTH OF POLLEN TUBES IN THE OVARY OF PLUM GENOTYPES DEVELOPED AT FRUIT RESEARCH INSTITUTE ČAČAK (SERBIA)

SUMMARY

Unusual growth of pollen tubes in different pollination variants (self-, cross- and open pollination) was observed in the ovary of six promising plum (*Prunus domestica* L.) genotypes developed at Fruit Research Institute (FRI), Čačak (hybrids 38/62/70, IV/63/81, 32/21/87, 34/41/87 and 22/17/87 and cultivar 'Nada') in Serbia. The cross-pollination was performed using the pollen of plum cultivar 'Čačanska Lepotica'. The occurrence of unusual pollen tubes growth in the ovary was studied over the three-year period (2009–2011) using fluorescent microscopy method. The appearance of unusual growth of pollen tubes in the ovary of all studied genotypes during the whole examination period was recorded in the region of the obturator and the micropyle, with or without further growth of the pollen tubes to the nucellus.

Several different types of unusual growth of pollen tubes were observed, i.e. the growth of pollen tubes in opposite direction in relation to the nucellus, the curling up of pollen tubes, the branching of pollen tubes, as well as combinations of some of the above mentioned types of specific pollen tubes growth. Average values of all analyzed genotypes showed that the highest frequency of the ovaries with unusual growth of pollen tubes was determined in the open pollination variant (7.89%), followed by the cross-pollination variant (6.27%), while the smallest frequency was found in the self-pollination variant (3.12%).

Keywords: *Prunus domestica* L., promising genotypes, pollination variant, pollen tubes growth, ovary, unusual growth

INTRODUCTION

The efficiency of the pollen tubes growth in the style and in the ovary and the final outcome of pollination are influenced by a large number of factors. Thus, in this respect, Neumüller (2010) favours the influence of the female genotype, Stott et al. (1973) emphasize the effect of the pollination variant.

[1]Ivana Glišić*(corresponding author: iglisic@institut-cacak.org), Sanja Radičević, Milena Đorđević, Nebojša Milošević, Fruit Research Institute, Čačak, Kralja Petra I, 9, 32000 Čačak, Republic of SERBIA; Dragan Milatović, University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Belgrade, Republic of SERBIA; Radosav Cerović, Innovation Centre, Faculty of Technology and Metallurgy, Karnegijeva 4, 11120 Belgrade, Republic of SERBIA

Paper presented at the 9th International Scientific Agricultural Symposium "AGROSYM 2018".
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

Cerović and Mićić (1996) and Hedhly et al. (2005) point out the significant influence of interaction between polleniser genotype and environmental factors, while Radičević et al. (2016) emphasize the impact of polleniser, its requirement to temperatures during flowering, the influence of pollinated cultivar on pollen performance, and the response of male–female relations to air temperature.

The final phases of the pollen tubes growth occur in the tissue of the ovary. When the pollen tube enters the locule, they grow further along the placenta, through the obturator and through the micropyle into the nucellus, and then through one of the synergids in the embryo sac, penetrates the ovule and achieves fertilisation. Opposite the aforementioned in some cases, pollen tubes lose direction and do not achieve fertilisation (Herrero, 2000). The appearance of the so-called specific growth of pollen tubes in the ovary, deviating from the normal or usual was observed and described in some species of the *Prunus* genus, (Cerović, 1996; Hedhly et al., 2009; Đorđević et al., 2010; Radičević et al., 2016). This kind of pollen tubes growth has been described as chaotic, specific growth or growth abnormalities (Herrero, 2000). This phenomenon was firstly described as some kind of incompatibility in the ovary (Sage et al., 1994). Later, the occurrence of unusual growth of pollen tubes in the ovary was also observed in cases of pollination of compatible genotypes (Herrero, 2000). Today, after it has been proven that several structures all along the pollen tube pathway are involved in regulation of pollen tube growth (Yadegari and Drewsb, 2004; Palanivelu and Tsukamoto, 2011), the occurrence of unusual growth is explained by three main reasons. One relates to developmental changes in the female tissues and how they affect pollen tube growth (Herrero, 2001). The second refers to mutations, which disrupt pollen tubes guidance and cause pollen tubes to migrate aberrantly (Hülskamp et al., 1995; Ray et al., 1997) while the third is concerned with potential molecules involved in this signalling (Johnson and Preuss, 2002).

The aim of this study was to determine the appearance of the unusual pollen tubes growth in the ovary of the plum hybrids 38/62/70, IV/63/81, 32/21/87, 34/41/87 and 22/17/87 and plum cultivar ‘Nada’ in different pollination variants as well as their possible effect on achieved fertilisation.

MATERIAL AND METHODS

Plant material and experimental design

The research was carried out over a three-year period (2009–2011) at the experimental plum orchard at the Ljubić facility of the Fruit Research Institute, Čačak. The orchard was set up in spring 2002, using standard one-year old plantings of hybrids 38/62/70, IV/63/81, 32/21/87, 34/41/87 and 22/17/87 and cultivar ‘Nada’. Myrobalan (*Prunus cerasifera* Ehrh.) seedling was used as a rootstock. The planting was performed at 6 × 5 m planting distance (333 trees ha^{-1}), using the random block system with five trees in three replications. The cultivation system is the Central Leader, and the standard cultural practices were performed in the orchard. The appearance of unusual growth of pollen tubes in

the ovary of abovementioned plum genotypes was conducted in three pollination variants: self-, cross- and open pollination. The cross-pollination was performed using the pollen of plum cultivar 'Čačanska Lepotica'.

Pollination procedure and pistil sampling

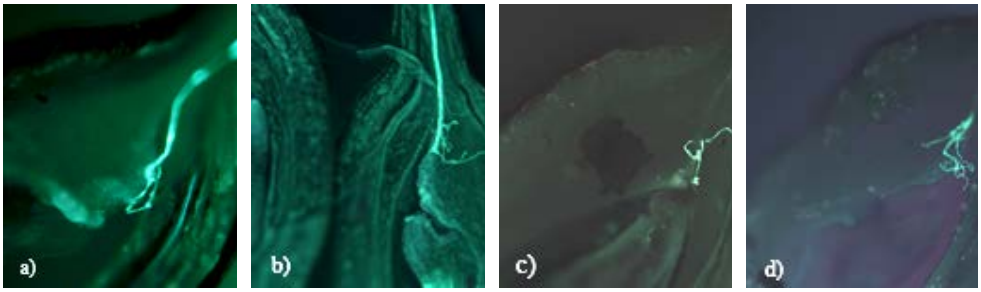
Three years old and younger branches were chosen on the trees under field conditions and flowers at the late balloon stage were emasculated, while other flowers were removed. Emasculated flowers were isolated in parchment bags. At the beginning of full flowering, when stigma receptivity was visible, pistils of each genotype were hand-pollinated by their own pollen (self- pollination) or pollen of cultivar 'Čačanska Lepotica' (cross-pollination). Pollinated pistils were isolated again by parchment bags during three weeks after pollination. For the open pollination variant branches with flowers at the beginning of full flowering stage were also chosen but without emasculation and isolation. A total of 30 pistils of each genotype per each pollination variant were fixed in FPA fixative (formaldehyde, propionic acid, 70% ethanol in a volume ratio of 5:5:90) in three terms: 72 h, 144 h and 240 h after pollination. The aniline blue staining was used (Kho and Baër, 1968). Further methodology involved: separation of the style from the ovary; separation of the ovary along the suture; longitudinal and tangential sections of the ovule for better observation of pollen tube penetration into the micropyle and nucellus of the ovule (Cerović and Ružić, 1992). Observation of ovaries was done under UV light on Olympus BX61 microscope (Tokyo, Japan). Multiple Image Analysis software (Münster, Germany) was used to obtain an image of the entire ovary. The number of pistils (%) with the occurrence of unusual growth of pollen tubes in each pollination variant represents the sum values for all three fixing terms. The average values for examination period were presented in the paper. The occurrence of unusual growth of pollen tubes was analyzed with respect to whether or not pollen tubes penetrated into the nucellus.

RESULTS AND DISCUSSION

The presence of pollen tubes whose growth towards the nucellus deviated from the normal was observed in the regions of the obturator or/and mycropyle of all studied plum genotypes and in all variants of pollination. Several different types of unusual growth of pollen tubes were observed, i.e. the growth of pollen tubes in opposite direction in relation to the nucellus, the curling up and branching of pollen tubes, as well as combinations of some of above mentioned types of specific growth of pollen tubes (Fig. 1 a-e). In some cases, further growth of pollen tubes towards the nucelleus was not observed (Fig. 1-a, 1-b and 1-d), resulting in the absence of fertilization, although the pollen tubes were present in the ovary.

On the other hand, the occurrence of unusual pollen tubes growth with further growth of pollen tubes and their penetration into the nucellus was observed more often (Fig. 1-c and 1-e). Similar types of unusual growth of pollen

tubes were observed by Đorđević et al. (2010) in plum, as well as by Radičević (2013) in sweet cherry.



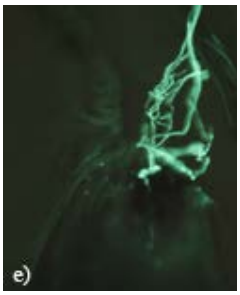


Figure 1. Unusual growth of pollen tubes in the ovary of the studied plum genotypes. a) the growth of pollen tubes in opposite direction in relation to the nucellus; b) a bundle in the obturator without pollen tubes penetration into the nucellus; c) a bundle in the obturator with pollen tubes penetration into the nucellus; d) a bundle in the micropyle without pollen tubes penetration into the nucellus; e) a bundle in the micropyle with pollen tubes penetration into the nucellus.

Table 1. Unusual growth of pollen tubes in the ovary of the studied plum genotypes in the variant of self-pollination.

Genotyp	**Obturator**		**Micropyle**	
	***PTDPN (%)**	****PTPN (%)**	***PTDPN (%)**	****PTPN (%)**
Hybrid 38/62/70	3.76	2.46	0.00	3.34
Hybrid IV/63/81	0.00	0.00	0.95	4.92
Hybrid 32/21/87	0.00	0.00	0.51	0.00
Nada	1.55	0.00	0.00	0.00
Hybrid 34/41/87	0.00	0.70	3.32	2.95
Hybrid 22/17/87	0.00	0.60	6.79	5.60
Average	0.44	0.31	0.96	1.41

*PTDPN – Ovaries where pollen tubes did not penetrate into the nucellus
**PTPN – Ovaries where pollen tubes penetrated into the nucellus

The average values for all analyzed genotypes (Tables 1-3) point that the highest frequency of the ovaries with unusual growth of pollen tubes was determined in the open pollination variant (7.89%), followed by the cross-

pollination variant (6.27%), while the smallest frequency was found in the self-pollination variant (3.12%). These results are in agreement with the results of Radović et al. (2017), but in contrast with the results of Đorđević et al. (2010) who have found that the occurrence of the unusual pollen tubes growth in the ovary was more frequent in self-pollination variant compared to open-pollination variant.

Table 2. Unusual growth of pollen tubes in the ovary of the studied plum genotypes in the variant of open-pollination.

Genotyp	**Obturator**		**Micropyle**	
	***PTDPN (%)**	****PTPN (%)**	***PTDPN (%)**	****PTPN (%)**
Hybrid 38/62/70	2.42	1.61	1.82	5.72
Hybrid IV/63/81	0.00	0.85	2.94	3.57
Hybrid 32/21/87	0.70	1.17	0.66	1.56
Nada	0.00	0.00	1.41	4.74
Hybrid 34/41/87	0.00	1.90	2.13	3.01
Hybrid 22/17/87	0.40	0.36	4.89	5.48
Average	0.59	0.98	2.31	4.01

*PTDPN – Ovaries where pollen tubes did not penetrate into the nucellus
**PTPN – Ovaries where pollen tubes penetrated into the nucellus

On average, all pollination variants were characterized by the largest number of pollen tubes with unusual growth in the region of the mycropile, where there was penetration of pollen tubes into the nucellus. This rule was typical for all of the studied plum genotypes in the variant of open-pollination, but not for two other pollination variants.

For example, the largest number of pollen tubes with unusual growth in the region of obturator without pollen tubes penetration into the nucellus was observed in the ovaries of hybrid 38/62/70 (3.76) and cultivar Nada (1.55) in the variant of self-pollination as well as in the ovaries of hybrid 38/62/70 (5.89) in the variant of cross-pollination (Tables 1 and 3). Also, hybrids 34/41/87 and 22/17/87 in the variant of self-pollination (3.32; 6.79, respectively) and hybrid 32/21/87 in the variant of cross-pollination (5.63) had the largest number of ovaries with unusual pollen tubes growth in the micropyle region, where there was no further growth of pollen tubes (Tables 1 and 3).

The appearance of unusual pollen tubes growth in ovary without their further growth towards the nucellus of studied plum genotypes, lead to the absence of fertilization in a certain percentage of ovules. This phenomenon was

most prevalent in hybrid 22/17/87 in variants of self- and open-pollination and in hybrids 38/62/70 and 32/21/87 in the variant of cross-pollination.

Table 3. Unusual growth of pollen tubes in the ovary of the studied plum genotypes in the variant of cross-pollination.

Genotyp	Obturator		Micropyle	
	*PTDPN (%)	**PTPN (%)	*PTDPN (%)	**PTPN (%)
Hybrid 38/62/70	5.89	0.00	0.86	0.00
Hybrid IV/63/81	0.37	0.97	1.87	3.79
Hybrid 32/21/87	0.00	1.22	5.63	0.00
Nada	0.00	0.00	0.00	4.85
Hybrid 34/41/87	0.00	0.00	0.00	2.39
Hybrid 22/17/87	3.90	0.00	3.50	2.20
Average	1.69	0.39	1.98	2.21

*PTDPN – Ovaries where pollen tubes did not penetrate into the nucellus
**PTPN – Ovaries where pollen tubes penetrated into the nucellus

Results of our study showed that some pollen tubes followed a route and penetrated nucellus, while others lost their orientation and grew chaotic. The first place where unusual growth of pollen tubes was observed was obturator while the second place was micropyla. The obturator is placental protuberance and represents some kind of bridge between the stylar transmitting tissue and the ovule entrance. This region of ovary is first critical territory or place of the first stop of pollen tubes. Pollen tubes growth is not possible until this structure enters a secretory phase. In some species like kiwi fruit, a secretion phase of obturator starts from flower opening (González et al., 1996). In contrast, secretory phase of obturator cells of peach starts 12 days after anthesis (Arbeloa and Herrero, 1987). In the ovary of sour cherry, the occurrence of specific pollen tube growth was first observed in the region of the obturator, with starch grain found in its cells (Cerović, 1994).

The newest results obtained in apple emphasize that the arrival of pollen tubes at the obturator was followed by secretion of proteins, saccharides and glycoproteins as well as that following pollen tube passage, these glycoproteins were exhausted (Lossada and Herrero, 2017). The embryo sac is required for directing pollen tubes during late stages of its growth (Johnson and Preuss, 2002). Dramatic defect in direction of pollen tube growth in the region of micropyla was obtained when the synergid cells were ablated (Higashiyama et al., 2001).

CONCLUSIONS

The occurrence of unusual growth of pollen tubes in the ovary, in the region of the obturator and the micropyle, with or without further growth of the pollen tubes to the nucellus, was observed in all of the studied genotypes and in all pollination variants.

The highest frequency of the ovaries with unusual growth of pollen tubes was determined in the open pollination variant, followed by the cross-pollination variant, while the smallest frequency was found in the self-pollination variant. All pollination variants were characterized by the largest number of pollen tubes with unusual growth in the region of the mycropile, where there was penetration of pollen tubes into the nucellus.

The phenomenon of the unusual growth of pollen tubes in the ovary without further growth of the pollen tubes to the nucellus of some of examined plum cultivars resulted in the absence of fertilization, although the pollen tubes were present in the ovary.

ACKNOWLEDGEMENTS

This paper is supported by the grant of Ministry of Education, Science and Technological Development of the Republic of Serbia, Project No 31064.

REFERENCES

Arbeloa A., Herrero M. (1987). The significance of the obturator in the control of pollen tube entry into the ovary in peach (*Prunus persica*), Annals of Botany, 60 (1), 681–685.

Cerović, R. (1994). Histocitološki aspekti dinamike oplodnje kod višnje (*Prunus cerasus* L.), PhD Thesis, University of Belgrade, Faculty of Agriculture, Belgrade.

Cerović R. (1996). Unusual behavior of growing pollen tubes in the ovary of sour cherry, Acta Horticulturae, 423, 171–176.

Cerović R., Ružić Đ. (1992). Senescence of ovules at different temperatures and their effect on the behavior of pollen tubes in sour cherry, Scientia Horticulturae, 51, 321–327.

Cerović R., Mićić N. (1996). Pollination and fertilization of pome and stone fruit, Journal of Pomology, 30 (113–114), 73–98.

Đorđević M., Cerović R., Nikolić D., Radičević S. (2010). Unusual behaviour of growing pollen tubes in the ovary of plum culture (*Prunus domestica* L.), Archives of Biological Sciences, 62 (1), 137–142.

González M.V., Coque M., Herrero M. (1996). Pollen-pistil interaction in kiwifruit (*Actinidia deliciosa*; Actinidiaceae), American Journal of Botany, 83,148–154.

Hedhly A., Hormaza J.I., Herrero M. (2005). Influence of genotype-temperature interaction on pollen performance, Journal of Evolutionary Biology, 18 (6), 1494–1502.

Hedhly A., Hormaza J.I., Herrero M. (2009). Flower emasculation accelerates ovule degeneration and reduces fruit set in sweet cherry, Scientia Horticulturae, 119, 455–457.

Herrero M. (2000). Changes in the ovary related to pollen tube guidance, Annals of Botany, 85 (1), 79–85.

Herrero M. (2001). Ovary signals for directional pollen tube growth, Sexual Plant Reproduction, 14, 3–7.

Higashiyama T., Yabe S., Sasaki N., Nishimura Y., Miyagishima S., Kuroiwa H., Kuroiwa T. (2001). Pollen tube attraction by the synergid cell, Science, 293, 1480–1483.

Hülskamp, M., Schneitz, K., Pruitt, R. (1995). Genetic evidence for a long-range activity that directs pollen tube guidance in *Arabidopsis*, Plant Cell 7, 57–64.

Johnson M. A., Preuss D. (2002). Plotting a course: multiple signals Guide Pollen Tubes to Their Targets, Developmental Cell, 2, 273–281.

Kho Y.O., Baër J. (1968). Observing pollen tubes by means of fluorescence, Euphytica 17, 298–302.

Lossada J.M., Herrero M. (2017). Pollen tube access to the ovule is mediated by glycoprotein secretion on the obturator of apple (*Malus* × *domestica*, Borkh), Annals of Botany, 119 (6), 989–1000.

Neumüller M. (2010). Fundamental and applied aspects of plum (*Prunus domestica* L.) breeding. In: Flachowsky H., Hanke V.M. (Eds), 'Methods in temperate fruit breeding', Fruit, vegetable and cereal science and biotechnology, Global Science Books, Kagawa, Japan, 5 (1), 139–154.

Palanivelu, R., Tsukamoto, T. (2011). Pathfinding in angiosperm reproduction: pollen tube guidance by pistils ensures successful double fertilization, WIREs Developmental Biology, 1 (1), 96–113.

Radičević S. (2013). Fertilization biology and pomological properties of newly introduced sweet cherry (*Prunus avium* L.) cultivars, PhD Thesis, University of Belgrade, Faculty of Agriculture, Belgrade.

Radičević S., Cerović R., Nikolić D., Đorđević M. (2016). The effect of genotype and temperature on pollen tube growth and fertilization in sweet cherry (*Prunus avium* L.), Euphytica, 209, 121–136.

Radović A., Nikolić D., Cerović R., Milatović D., Đorđević B., Zec G. (2017). Unusual growth of pollen tubes in the ovary of quince (*Cydonia oblonga* mill.), Acta Scientiarum Polonorum Hortorum Cultus, 16 (2), 133–138.

Ray, S.M., Park, S.S., and Ray, A. (1997). Pollen tube guidance by the female gametophyte, Development 124, 2489–2498.

Sage T.L., Bertin R.I., Williams E.G. (1994). Ovarian and other late-action self-incompatibility systems, In: Williams E.G., Clarke A.E., and. Knox R.B. (Eds.), Genetic control of self-incompatibility and reproductive development in flowering plants, Kluwer Academic Publishers, Dordrecht, Netherlands, pp. 116–140.

Stott K.G., Jefferies C.J., Jago C. (1973). Pollination and fruit set in plum, Report of Long Ashton Research Station for 1972, University of Bristol, pp. 23-26.

Yadegari R., Drewsb N.G. (2004). Female gametophyte development, The Plant Cell, 16, 133–141.

DOI: 10.17707/AgricultForest.65.1.07

Fazilah MUSA*,
Nor Asyirah LILE, Diana Demiyah MOHD HAMDAN[1]

AGROFORESTRY PRACTICES CONTRIBUTION TOWARDS SOCIOECONOMICS: A CASE STUDY OF TAWAU COMMUNITIES IN MALAYSIA

SUMMARY

Agroforestry is a key indicator in terms of socioeconomic level towards developing countries especially to rural communities for sustainable development. Generally, agroforestry practices are valued environmentally, economically and socially. However, a key problem within recent literatures in relation to agroforestry practices is lack of awareness and knowledge among local community in rural areas. The aim of this study was to identify the contribution of agroforestry practices towards socioeconomics of communities in Merotai Besar, Tawau, Sabah, Malaysia. The data collection was conducted by questionnaire, which was randomly distributed to 250 respondents from five (5) villages namely Merotai Besar, Simpang Tiga, Kijang, Langsat and Iban. Majority of respondents strongly agreed that agroforestry practices could provide food resources for the wellbeing of rural communities. More than half of the communities in Merotai Besar area practiced agrisilvicultural system. A small number of residences in the study area also practiced agrosilvopastoral and silvopastoral systems. In spite of the fact that 93.2% of respondents were practicing agroforestry, they lack awareness that they were practicing agroforestry. This was due to poor dissemination of agroforestry information. This paper suggests that policy makers should encourage stakeholders to provide training and skills development centre to enhance the community's knowledge. Furthermore, it is necessary to encourage active community-based management practices within respective villages for sustainable economic development and to ensure prosperity for all. In conclusion, agroforestry practices can expand the socioeconomics level to reduce poverty of rural communities in Tawau area.

Keywords: Socioeconomic, agroforestry practices, rural communities, Borneo

INTRODUCTION

Agroforestry is one of the sectors that have contributed to the socioeconomics' of Malaysia communities especially in rural areas. Agroforestry

[1]Fazilah Musa*(corresponding author: fazilah.musa@ums.edu.my), Nor Asyirah Lile, Diana Demiyah Mohd Hamdan, Faculty of Science and Natural Resources, University Malaysia Sabah, MALAYSIA

Paper presented at the 9th International Scientific Agricultural Symposium "AGROSYM 2018".
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

have the potential to become an effective tool for land uses' sustainable management. It also plays a crucial part in ensuring sustainable development by balancing and managing natural resources (Azmy *et al*., 2013). Generally agroforestry have a positive impact in terms of ecological, economics and social to most communities. For some communities agroforestry was the main source to generate income, provided safe and healthy food and ensured job opportunity (Keat, 2018). Although agroforestry practices provide greater social impacts, it is more labour intensive and requires local knowledge of agriculture and forestry (Hashim *et al*., 2012). Tree planting is one of the strategies applied in agroforestry to alleviate farmer's quality of life by fully utilization of available resources (Nair, 1993).

Agroforestry is defined as systems of land use on the same plot that integrates diverse outputs production with key component inclusive of combination of trees with crop or animals (Nair, 1991). Agroforestry practices in Malaysia involve four major systems of agroforestry, namely agrosilvicultural, silvopastoral, silvofisheries and agrosilvopastoral (Mohd. Nazip *et al*., 2000). In Malaysia majority practiced agrosilvicultural systems. However, research on agroforestry in Malaysia is limited (Keat, 2018). Communities in rural areas that are practising agroforestry were unaware that they are actually involved in agroforestry sector were reported in recent studies (Mohd. Nazip *et al*., 2000; Aminuddin *et al*., 2008; Azmy *et al*., 2013). This was due to lack of knowledge and understanding on agroforestry practices among local community in rural areas. Agroforestry practices are largely monopolized by rural community who do not have high levels of education resulting difficulties to identify the constraints in relation practicing it. Furthermore, without better understanding and knowledge, rural community will use excessive land without knowing the negative effects that will be faced in the future.

Smallholders are one of the potential users of agroforestry but scientific data on smallholders' agroforestry practices is still lacking in Malaysia especially on the East Coast area (Aminuddin *et al*., 2008). Even though local community lack awareness on agroforestry practices, indigenous knowledge passed down to the next generation is crucial for conservation of environmental and biodiversity for livelihood sustainability (Ab. Halim *et al*., 2012). Therefore, these studies were conducted to identify Merotai Besar communities' knowledge on agroforestry practices and whether this sector contributed on the communities' socioeconomics.

MATERIAL AND METHODS

This study was conducted in Merotai Besar, Tawau, Sabah, Malaysia (4° 25' 8" N; 117° 46' 32" E), which is 24 km away from Tawau city. Five villages in Merotai Besar have been selected for this study area: Merotai Besar, Simpang Tiga, Kijang, Langsat and Iban.

The selection of villages included in this study were based on that local communities were practicing agroforestry systems was done after discussion with

Mr. Harris Chashuri as the Head of Villages (Ketua Kampung). In addition, people living in these areas carry out agricultural activities as food resources for household consumption and livelihood. Other than that, these villages had been listed as the best in agricultural activity and have received social media coverage (Harris Chashuri, personal communication, 2017). From these five selected villages, 670 households had been identified as practising agroforestry systems from respective head of villages. A total of 250 respondents were successfully interviewed between July and August 2017. A survey written in Malay Language was also distributed to respondents which sociodemographic and socioeconomics of the household were successfully recorded. The questionnaire was designed to assess agroforestry knowledge and socioeconomic contribution of local communities practising agroforestry systems in Merotai Besar, Tawau.

RESULTS AND DISCUSSION

Agroforestry Practitioners Sociodemographic

Men are more involved than women in agroforestry activities (Keat, 2018). The majority of the respondents were male (63.2%) and 36.8% were female (Table 1). Most of the respondent age was 31-54 years. Half of the number of respondents only attended primary school and a significant number did not receive any formal education. Merotai Besar communities consist of multi diverse group ethnics and majority practices Islam (Mohd Hamdan *et al.*, 2017). Based on this result, the respondents were working in agriculture and business sector that were related to agroforestry activities. Majority of the respondents earn below the poverty line. Agroforestry is a sector that can decrease poverty rate of community living in rural areas (Ahmed Azhar *et al.*, 2008).

Agroforestry Practices Contribution

Agroforestry practices had improved the wellbeing of rural communities in terms of environment, economic and social in Merotai Besar (Figure 1). The majority of agroforestry practitioners in Merotai Besar strongly agreed that agroforestry practices had provided them food resources such as vegetables, fruits and meat. The agroforestry practices support sustainable development in providing food resources to local communities and conjointly ensuring food security (Fanish & Priya, 2013). Simultaneously, agroforestry practices contributed to the development of their residential areas (Figure 1). Furthermore, agroforestry had created job opportunities for local communities living in Merotai Besar, which is located far away from the closest city, Tawau city. By venturing into agroforestry related activities, local communities in Merotai Besar were able to generate more income. Job opportunities can reduce the poverty rate of the local communities in line with the government's efforts to eradicate poverty in rural communities (Nik Hashim, 1996). Significantly, agroforestry practices give more positive impact compare to negative impact to rural communities.

Table 1. Sociodemographic and socioeconomic of agroforestry practitioners living in Merotai Besar, East Malaysia.

Variables	Frequency	Percentage (%)
Gender		
Male	158	63.2
Female	92	36.8
Ethnic		
Tidung	89	35.6
Bugis	56	22.4
Javanese	64	25.6
Iban	23	9.2
Others	18	7.2
Religion		
Islam	220	88
Christian	26	10.4
Hinduism	1	0.4
Buddhist	3	1.2
Age		
≤18 years	1	4
19-30 years	30	12
31-54 years	164	65.6
≥55 years	55	22
Education Status		
No Formal Education	39	15.6
Primary School	124	49.6
Secondary School	83	33.2
University/College	4	1.6
Marital Status		
Single	27	10.8
Married	199	79.6
Divorced/Widowed	24	9.6
Monthly Income		
≤ RM400	53	21.2
RM401 - RM800	121	48.4
RM801 - RM1, 200	60	24
≥ RM1, 201	16	6.4
Job		
Public Sector	4	1.6
Private Sector	49	19.6
Farmer	99	39.6
Fishermen	5	2
Business	90	36
Others	3	1.2
Side Job		
Farmer	137	54.4
Business	112	44.8
Others	1	0.4

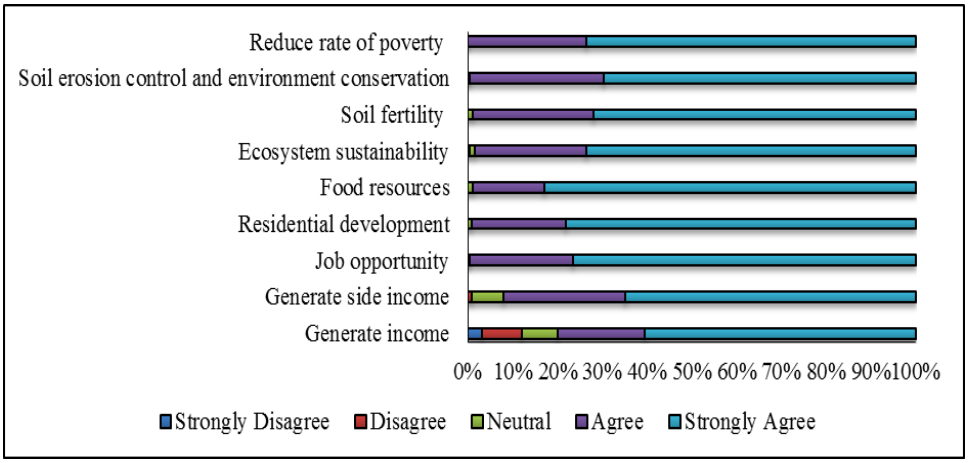


Figure 1. Agroforestry practitioners' insight on the contribution of agroforestry practices towards their socioeconomics development.

Agroforestry Awareness

Local communities practising agroforestry in Sandakan, which is also located in the same state, Sabah with Merotai Besar were found lacking in understanding of agroforestry systems concepts (Aminuddin *et al.*, 2008). This is because they did not have any formal agroforestry education. Surprisingly, a survey in agroforestry education conducted among professional staffs (top level officers, managerial officers, field officers) in agro-based agencies resulted in more than half of these professionals never had agroforestry education (Azmy *et al.*, 2013). Professionals working in agro-based agencies had perceived the low numbers of staff that had taken agroforestry education because it was not strongly emphasized at tertiary level education in Malaysia, as job opportunities were low.

Not surprisingly, almost all agroforestry practitioners in Merotai Besar lack awareness that they are practicing agroforestry activities. Only 17 respondents understood agroforestry practices (Figure 2a). Most of the respondents have never heard the word *"agroforestry"* which clearly indicates that respondents' knowledge of agroforestry is very limited. The main reason might be they had less exposure of agroforestry knowledge due to their education level (Table 1).

Sharing and transferring of knowledge is very important for making successful awareness programs to community (Hudcova, 2014). In consideration of the education and socioeconomic background of the local communities in Merotai Besar must also be taken account of the best strategies to increase the level of agroforestry awareness (Mohd. Hamdan *et al.*, 2017). Campaign is one of the ways to improve communities understanding and enhance knowledge on agroforestry practices. It will be more difficult for the professionals to transfer the knowledge and educate the rural community due to lack of agroforestry education themselves (Azmy *et al.*, 2013). Therefore, the government should

acknowledge this issue and train agroforestry related professionals for successful dissemination of agroforestry practices among potential communities.

In Sandakan District, Sabah there is six types of agroforestry systems that were practised in smallholders' farm: agrosilvicultural, agrisilvicultural, agrosilvopastoral, aqua-agrosilvicultural, silvopastoral, and aqua-agrisilvicultural (Aminuddin *et al.*, 2008). Sabah is a centre of biodiversity hotspot reflected by major tree/crop components in agroforestry practice area makes agrosilvicultural is the main system being practised (Mohd. Nazip *et al.*, 2000). The agroforestry education is very important to sustain agroforestry practices in Malaysia due to lack of successful models, constraint to make right species selection and diversion of resources (Awang Noor *et al*., 2010). Nevertheless agroforestry systems may vary according to the location of agroforestry activities that is being practiced by the local communities in certain areas. In Merotai Besar, agrisilvicultural system is the most agroforestry systems that were practised by agroforestry practitioners followed by agrosilvopastoral system and silvopastoral system (Figure 2b).

Agrisilvicultural were more applied by the agroforestry practitioners in Merotai Besar because it can increase the source of income and more practical to manage compared to the other systems (Table 1). The application of agrisilvicultural system by local communities in this area emphasized more on the planting of woody trees together with crops without any livestock. On the other hand, the management of the agrosilvopastoral system is more difficult to sustain because livestock has the potential to damage the crops in certain areas (Gutteridge & Sheldon, 1994).

Popular combination of crops such as rubber trees and crops like vegetables were observed in Merotai Besar. The main function of the tree is to provide natural shading to the crop as well as to avoid soil erosion because of the tap root’s crops can hold the soil stronger than the fibrous root's crops (Simons, 1992). Merotai Besar local communities strongly agreed by practicing agroforestry systems has helped in preventing soil erosion and sustained soil fertility (Figure 1).

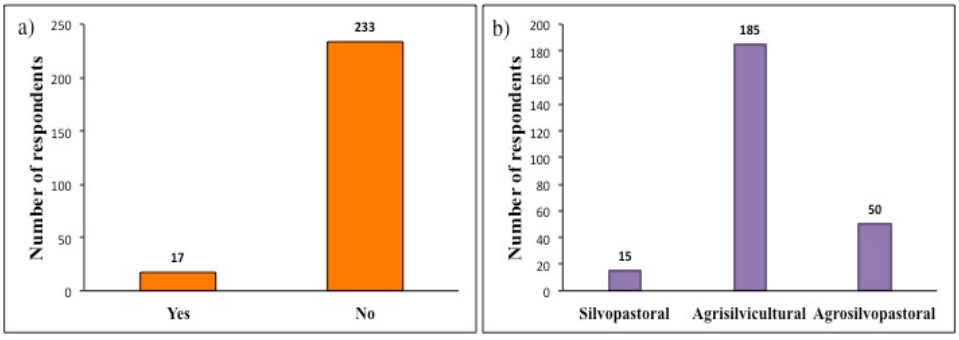


Figure 2. a) Agroforestry systems awareness of respondents; b) Agroforestry systems that is being practiced in Merotai Besar.

CONCLUSIONS

This study offers findings that can explain the linkages between knowledge at rural communities' levels and agroforestry activities, with references to agroforestry in Malaysia. Local communities in rural areas have low education that obstructed them for a better understanding of agroforestry systems as holistic even generally they are practicing the agroforestry activities. Poor dissemination of agroforestry information and lack of awareness within rural communities in relation towards agroforestry practices could influence community's socioeconomics. Therefore, the involvement of active community-based management practices among respective villages plays an important role for sustainable economic development.

Knowledge of the contribution of agroforestry practices in rural area is crucial for maintaining sustainability and future improvement of agroforestry practices.

ACKNOWLEDGEMENT

The authors wish to extend their appreciation to the Heads of Villages and all of respondents in Merotai Besar, Tawau, Sabah for the cooperation and provision of invaluable research data and information for this study.

REFERENCES

Ab Halim, A., Othman, N., Ismail, S. R., Jawan, J. A., & Ibrahim, N. N. (2012). Indigenous knowledge and biodiversity conservation in Sabah, Malaysia. International Journal of Social Science and Humanity, Vol. 2 (2), pp. 159-163.

Ahmed Azhar, J., Norman, K., Suhaimi, M., & Wan Hanisah, W. I. (2008). Agroforestry practices in Malaysia-Integrating plantations crops with timber species. Retrieved from http://www.iipm.com.my/ipicex2014/docs/posters/Ahmed%20Azhar%20et%20al.pdf

Aminuddin, M., Musa, S., & Muhammad Razali, M. (2008). Agroforestry practices in Sandakan, Sabah, Malaysia. In The International Agroforestry Conference (IAC) 2006, 1-2 August 2006. Forest Research Institute Malaysia (FRIM), Kuala Lumpur, Malaysia.

Azmy, M., Nor Izaida, I., & Awang Noor, A. G. (2013). Agroforestry education And professional level links in Peninsular Malaysia. Journal of Sustainability Science and Management, Vol. 8 (2), pp. 161-170.

Awang Noor, A. G., Azmy, M., & Rasip, A. G. (2010). Agroforestry Education Research and Development and Extension in Malaysia: The Way Forward. In International Conference on Agroforestry Education, 15-17 December 2010, Maejo University, Chiang Mai, Thailand.

Fanish, S. A., & Priya, R. S. (2013). Review of benefits of agroforestry systems. International Journal of Education and Research, Vol. 1 (1), pp. 1-12.

Gutteridge, R. C., & Sheldon, H. M. (1994). Animal Production Potential of Agroforestry System. In ACIAR proceedings, July 1994, Australian Centre for International Agriculture Research, Canberra, Australia.

Hashim, M. N., Rosdi, K., Abd Razak, O., Lok, E. H., & Ahmad Zuhaidi, Y. (2012). Generation of high income through compatible combinations of forest trees and agricultural crops under agroforestry ecosystem. In 3rd APICEX 2012, 5-7 November 2012. Kota Kinabalu, Sabah.

Hudcova, S. 2014. Tools of internal communication from knowledge transfer perspective. Journal of Competitiveness, Vol. 6 (4), pp. 50-62.

Keat, N. J., Nath, T. K., & Jose, S. (2018). Indigenous agroforestry practices by Orang Asli in Peninsular Malaysia: Management, sustainability and contribution to household economy. Indian Journal of Traditional Knowledge, Vol. 17 (3), pp. 542-549.

Mohd Hamdan, D. D., Udin, L., Tair, R., Adnan, M. H. (2017). Communication gap of heavy metals knowledge among community in Batu Payung, Tawau and its impact on food safety, security and livelihood sustainability. Jurnal Komunikasi Borneo Special Issue (Konvokesyen ke-19 UMS), Vol. 3 (1), pp. 1-25.

Mohd. Nazip, S., Suhaimi, M., Nasaruddin, R., & Jiwan, D. (2000). A preliminary survey of agroforestry practices in Malaysia. In Conference on Forestry and Forest Products Research 1997: Proceedings of the Fourth Conference, Forest Research Institute Malaysia, 2-4 October 1997. Forest Research Institute Malaysia.

Nair, P. K. R. (1991). State-of-the-art of agroforestry systems. Forest Ecology and Management, Vol. 45, pp. 5-29.

Nair, P. K. R. (1993). An Introduction to Agroforestry. Kluwer Academics Publishers in Cooperation with the International Centre for Research in Agroforestry, Dordrecht, The Netherlands.

Nik Hashim, N. M. (1996). Perancangan Pembangunan Pertanian di Malaysia. Dewan Bahasa dan Pustaka, Kuala Lumpur.

Simons, A. J. (1992). Genetic Improvement of Non-Industrial Trees. Agroforestry System, Vol. 18 (3), pp. 197-212.

DOI: 10.17707/AgricultForest.65.1.08

Zeina EL SEBAALY*, Falah ASSADI,
Youssef Najib SASSINE, Nidal SHABAN [1]

SUBSTRATE TYPES EFFECT ON NUTRITIONAL COMPOSITION OF BUTTON MUSHROOM (*AGARICUS BISPORUS*)

SUMMARY

Button mushroom (*Agaricus bisporus*) has been recognized as a fair substitute for meat and is the most intensively cultivated mushroom worldwide. The nutritional value of the mushroom is one of the main factors determining its quality. The current study investigated the variation in nutritional composition of button mushroom as affected by substrate type. Three locally composted wastes were used; chic: deep litter chicken manure, ban: banana wastes (leaves and pseudo-stems) and win: winery wastes (grape marc). Each type of compost was mixed separately with horse manure compost (hors) in different ratios (30%, 50%, 70% and 100%) on volume basis. Control substrate consisted of 100% horse manure compost.

Results showed that in the mixture hors-win: 30-70 water, fat, Mg, Fe, Cu, Zn, Na and Ca contents were reduced, while ash and K contents were increased. There was a peak of water (90%), proteins (5.2%), Fe (21.9mg/kg), Cu (18.6mg/kg), Zn (10.2mg/kg), Na (74.5mg/kg), K (2mg/kg) and Ca (65.8mg/kg) contents in mushrooms picked from hors-chic: 0-100. Moreover, growing the mushroom on composted banana wastes (hors-ban: 0-100) produced fruits with the lowest protein (2.9%), fat (0.01%), Mg (147.5mg/kg), Na (64.8mg/kg), K (1.4mg/kg) and Ca (55.8mg/kg) contents and the highest ash content (2%). It seemed that the total use of chicken manure compost allowed the production of mushrooms with the closest nutritional composition to those of the traditional compost.

Keywords: Button mushroom, nutritional value, substrate.

INTRODUCTION

Mushrooms are very popular and valuable food items in modern dietary regimes because of their nutritional value (Tshinyangu, 1996). *Agaricus bisporus* mushrooms are often considered to provide a substitute for meat with a comparable nutritional value to many vegetables. The authors assume that they both add flavor and are a valuable food which can make a valuable addition to

[1]Zeina El Sebaaly*(corresponding author: zeinasebaaly1@hotmail.com), Nidal Shaban, University of Forestry, 10 Kliment Ohridski blvd, BG1797 Sofia, BULGARIA; Falah Assadi, Youssef Najib Sassine, Lebanese University, Faculty of Agricultural Engineering and Veterinary Medicine, Dep. Horticulture, Beirut, LEBANON

Paper presented at the 9th International Scientific Agricultural Symposium "AGROSYM 2018".
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

the often unbalanced diets of people in developing countries (Chang and Miles, 2004). Protein is an important constituent of mushrooms dry matter (Chang and Buswell, 1996), carbohydrate represent the bulk of their fruiting bodies and their fat content is very low (Wani *et al.*, 2010). Given the crucial role that minerals play in the biological process and metabolism and their therapeutic use in dietary supplements, foods are often tested for nutrient minerals in relation to specific health benefits (Tomkins, 2002). *Agaricus bisporus* (button mushroom) has an especially high content of phosphorus, sodium and potassium (Bąkowski *et al.*, 1986) followed by Ca, Mg, Na, Fe and Zn (Guillamón *et al.*, 2010; Falandysz and Borovička, 2013). It is also an excellent source of several essential amino acids and vitamins (B2, niacin and folate) (Manzi *et al.*, 2001).

The *Agaricus* mushroom is a heterotrophic fungus growing naturally on a partially degraded organic substrate. It requires for its nutrition and subsequent metabolism, carbon compounds that have already been produced by green plants (Wood and Fermor, 1985). Traditionally, the mushroom is grown on composted horse manure and wheat straw (Chang and Miles, 2004). Compost is normally supplemented by adding proteins and lipids additives (Weil *et al.*, 2004) or by ingredients with high levels of vegetable proteins (Zied *et al.*, 2011). However, according to Arce-Cervantez *et al.* (2015) the development of inexpensive, readily available alternative supplements is a necessity to reduce the costs of mushroom production.

In Lebanon, *Agaricus bisporus* is highly demanded however the development of mushroom sector is highly dependent on the availability of substrates at the local level (Karam *et al.*, 2010). Due to the scarcity of horse manure, alternative substrates based partially or totally on chicken manure and winery wastes were reported by Sebaaly *et al.* (2018a) and Sebaaly *et al.* (2018b) as being efficient for producing good yields of button mushroom. However, since the efficient use of local agricultural wastes is quite evident in order to optimize not only yield but also quality of button mushroom produced. Therefore, the study investigated the nutritional composition of mushrooms produced from substrates containing local winery wastes, banana wastes and chicken manure in order to evaluate the substrate effect on the various mushroom components.

MATERIAL AND METHODS

Eleven substrates of different properties (Table 1) were formed by three types of local agricultural wastes that were used alone (100 %) or mixed with the traditional compost (based on horse manure and wheat straw) in various proportions (30 %, 50 % or 70 %) on a volume basis. The nutritional composition of *A. bisporus* mushrooms of the strain A15 was evaluated and compared among the various substrates and a control substrate consisting of traditional compost.

Ten representative samples of mushrooms harvested from each substrate type were dried in an oven at 70°C for 12 hours and made into fine powder before being analyzed for composition. Complete mushrooms (stipe and cap) were

dried. Thereafter, moisture, proteins, carbohydrates, fat, crude fibers and ash contents using standard method of analysis (AOAC, 1995).

For the determination of mineral elements (Ca, K, Mg and Na) and Fe, dried mushroom samples were digested in concentrated HNO_3. Then, elements were quantified by atomic emission spectrometry (Matila and Konko, 2001). All the elements were analyzed in triplicate.

Table 1: Compositional characteristics of growing substrates

Substrate mixture	Moisture content (%)	Organic matter (%d.w.)	Total nitrogen (%d.w.)	Carbon (%d.w.)	C:N	pH
S1	54.8	65.5	1.9	34.2	18:1	5.9
S2	63.4	69.4	1.9	36.1	19:1	6.1
S3	58.7	75.8	1.8	34.2	19:1	6.4
S4	62.1	81.7	2	42	21:1	6.5
S5	55.1	64.8	1.9	34.2	18:1	5.9
S6	58.5	61.9	2	36	18:1	5.6
S7	63.1	56.2	2.1	35.7	17:1	5.6
S8	62.7	54.7	2.3	39.1	17:1	5.1
S9	63.9	70.2	1.9	37.1	20:1	6.2
S10	65.1	73.8	1.8	40.4	22:1	6.3
S11	68.2	78.5	1.8	43.2	24:1	6.7
S12	72.4	80.9	1.6	41.6	26:1	6.8

S1: control, S2: Hors-win: 70-30, S3: Hors-win:50-50, S4: Hors-win:30-70, S5: Hors-chic:70-30, S6: Hors-chic:50-50, S7: Hors-chic:30-70, S8: Hors-chic:0-100, S9: Hors-ban:70-30, S10: Hors-ban:50-50, S11: Hors-ban:30-70, S12: Hors-ban:0-100

Data analysis

Data analysis was performed using SPSS 23. ANOVA and Duncan tests were applied with a $P_{value}<0.05$ as a significant difference between treatments.

RESULTS AND DISCUSSION

Results of ANOVA test showed that the effect of substrate type was statistically significant ($P_{value}<0.05$) on water, proteins, carbohydrates, fat, ash, magnesium, iron, sodium, potassium and calcium contents, while it was not significant on crude fibers content in mushrooms.

Compositional characteristics varied among mushrooms produced by the different compost formulations (Figure 1). In general, mushroom moisture content was the highest in chicken manure compost (hors-ban: 0-100) (90.7 %) and the lowest in the substrates hors-win: 30-70 and hors-ban: 50-50 (around 85.5 %). Mushroom moisture content in control was higher compared to treatments containing horse manure mixed with banana or winery wastes in all ratios except hors-ban: 30-70 (88.2 %) and hors-win: 50-50 (88.6 %), while it was lower compared to treatments containing horse manure mixed with chicken manure in all ratios except hors-chic: 50-50 (88.1 %).

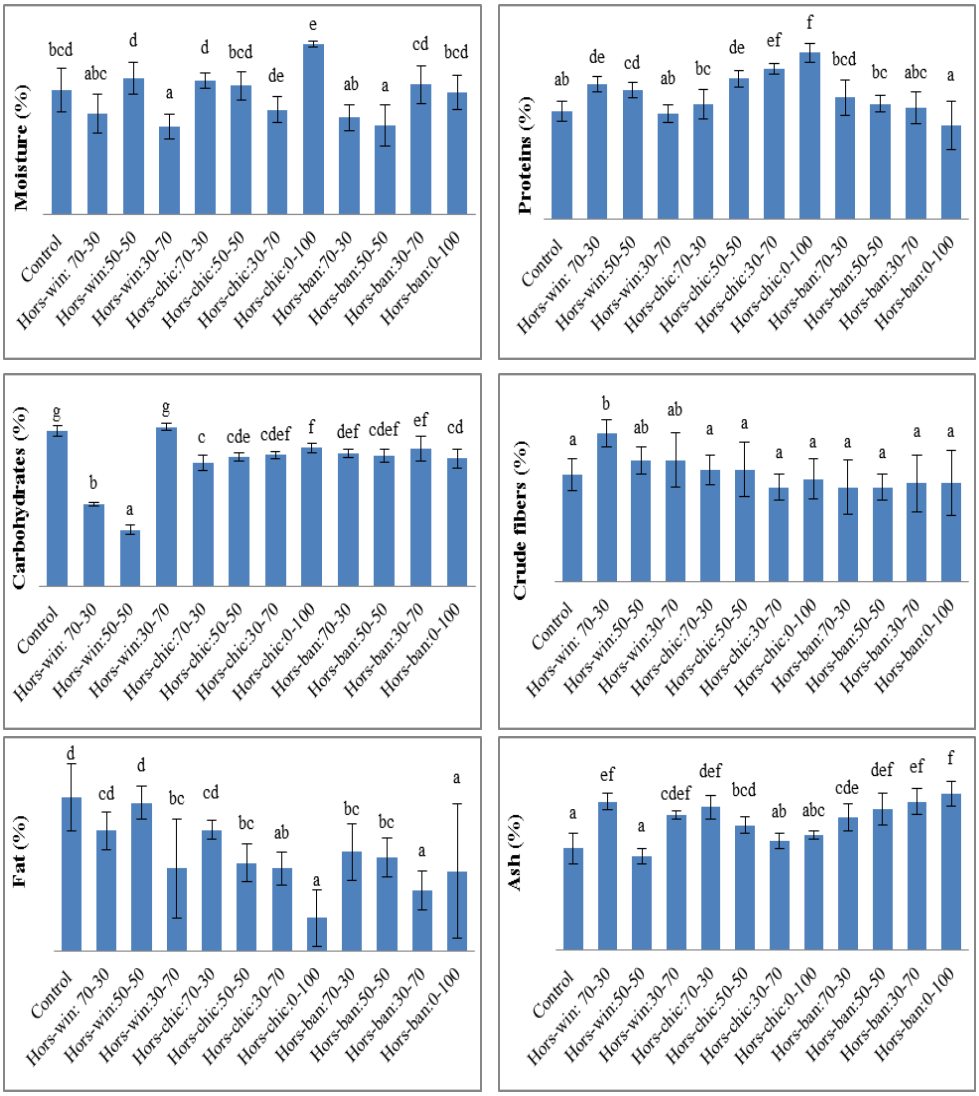


Figure 1: Effect of substrate type on moisture, proteins, carbohydrates, crude fibers, fat and ash contents of mushrooms (Means followed by the same letter are not significantly different according to Duncan's significant difference $P<0.05$).

In addition, mushroom protein content was ameliorated by the presence of chicken manure with various ratios in the substrate (3.6 %, 4.4 %, and 4.7 % in hors-chic: 70-30, hors-chic: 50-50 and hors-chic: 30-70 respectively) as well as by the presence of winery wastes in proportions up to 50% (4.2 % and 4.0 % in hors-win: 70-30 and hors-win: 50-50 respectively) compared to control (3.4 %). It was the highest in chicken manure compost (hors-chic: 0-100) with 5.2 % and the lowest in banana compost (hors-ban: 0-100) with 2.9 %. Carbohydrates content was generally reduced in all treatments compared to control except in the

treatment hors-win: 30-70 where values were comparable (8.5 % in hors-win: 30-70 compared to 8.3 % in control). Moreover, mushrooms produced in substrates containing winery wastes were the richest in crude fibers compared to all other substrates including control.

Mushroom fat content decreased in all treatments compared to control except in hors-win: 50-50 and it was the lowest in substrates based totally on chicken manure and banana wastes (0.02 % and 0.01 % in hors-chic: 0-100 and hors-ban: 0-100 respectively). Ash content was the lowest in hors-ban: 50-50 (1.2 %) and control (1.3 %). It was slightly higher in substrates containing more than 50 % chicken manure compost (1.4 % and 1.5 % in hors-chic: 30-70 and hors-chic: 0-100 respectively), while it was significantly improved in all remaining substrates.

Furthermore, in comparison with control, mushroom sodium content was gradually reduced when horse manure was mixed with increasing proportions of winery or banana wastes, while it was gradually increased when horse manure was mixed with increasing proportions of chicken manure. Magnesium content did not significantly differ among all treatments and control. In addition, mushroom iron content was the highest in substrates containing chicken manure in all proportions and calcium content was the highest in hors-chic: 50-50 and hors-chic: 30-70. Both elements were the lowest in substrates containing more than 50 % banana wastes.

Finally, compared to control, mushroom potassium content was significantly lower in substrates with more than 30 % banana wastes, significantly higher in those containing more than 50 % chicken manure, almost equal in mushrooms of hors-ban: 70-30 and slightly higher in remaining substrates (Table 2).

Table 2: Effect of substrate type on mushroom mineral composition

	Na (mg/kg)	**Mg** (mg/kg)	**K** (mg/kg)	**Ca** (mg/kg)	**Fe** (mg/kg)
S1	$72.9^{cd}\pm1.2$	$160.5^{d}\pm2.5$	$1.6^{ab}\pm0.1$	$62.5^{cd}\pm1.2$	$20.4^{de}\pm1.0$
S2	$70.2^{b}\pm1.3$	$159.7^{d}\pm1.7$	$1.7^{abc}\pm0.2$	$62.1^{cd}\pm1.5$	$19.5^{cd}\pm1.0$
S3	$69.5^{b}\pm0.6$	$157.5^{cd}\pm1.3$	$1.9^{bc}\pm0.2$	$60.2^{bc}\pm1.8$	$19.0^{bcd}\pm1.7$
S4	$65.5^{a}\pm1.4$	$150.5^{a}\pm1.5$	$1.9^{bc}\pm0.2$	$59.1^{b}\pm2.0$	$19.2^{bcd}\pm0.9$
S5	$73.3^{cd}\pm1.5$	$159.8^{d}\pm2.4$	$1.8^{abc}\pm0.4$	$62.9^{cd}\pm1.1$	$20.8^{de}\pm0.9$
S6	$73.2^{cd}\pm0.7$	$159.4^{d}\pm1.6$	$1.7^{abc}\pm0.1$	$63.3^{de}\pm1.9$	$21.4^{e}\pm0.8$
S7	$74.2^{d}\pm0.8$	$155.1^{bc}\pm2.1$	$2.3^{d}\pm0.3$	$64.7^{de}\pm0.7$	$21.8^{e}\pm1.2$
S8	$74.5^{d}\pm1.3$	$158.3^{cd}\pm1.6$	$2.0^{cd}\pm0.1$	$65.8^{e}\pm0.7$	$27.9^{e}\pm1.0$
S9	$71.2^{bc}\pm1.7$	$155.5^{bc}\pm2.4$	$1.6^{ab}\pm0.2$	$60.4^{bc}\pm2.1$	$19.5^{cd}\pm0.9$
S10	$70.1^{b}\pm2.3$	$153.1^{ab}\pm0.8$	$1.5^{a}\pm0.2$	$60.0^{bc}\pm2.9$	$18.1^{abc}\pm0.5$
S11	$65.9^{a}\pm1.0$	$160.8^{d}\pm1.5$	$1.4^{a}\pm0.2$	$57.8^{ab}\pm1.3$	$17.5^{ab}\pm0.5$
S12	$64.8^{a}\pm4.3$	$161.2^{d}\pm3.0$	$1.4^{a}\pm0.3$	$55.8^{a}\pm5.7$	$16.6^{a}\pm3.0$

S1: control, S2: Hors-win: 70-30, S3: Hors-win:50-50, S4: Hors-win:30-70, S5: Hors-chic:70-30, S6: Hors-chic:50-50, S7: Hors-chic:30-70, S8: Hors-chic:0-100, S9: Hors-ban:70-30, S10: Hors-ban:50-50, S11: Hors-ban:30-70, S12: Hors-ban:0-100

The study reflected the effect of substrate type on mushroom composition. Supplementing horse manure with banana or winery wastes with up to 50 % (on volume basis) would extend the mushroom shelf-life since in those substrates moisture content was reduced. On the contrary, the addition of chicken manure induced an average moisture content of 89.2 % which would affect negatively the mushroom shelf-life. In products with such high water content (>85%) and with no conventional cuticle as mushrooms, evaporation and consequently loss of weight usually have detrimental effect on shelf-life (Hammond and Nichols, 1975). Mushrooms secrete enzymes to digest surrounding foodstuff to get nutrients from organic matter contained in compost. In other terms, they are grown by bioconversion of agricultural wastes into edible food (Goyal *et al.*, 2006). As a result, their nutritional value largely depends on the chemical composition of the compost (Tshinyangu, 1996; Gothwal *et al*., 2012). It was observed that the lowest and highest mushroom protein contents were obtained respectively with the substrates with lowest (hors-ban: 0-100 with 1.6% N) and highest (hors-chic: 0-100 with 2.3% N) nitrogen contents. Even with the same species, values obtained values of protein content were lower than those reported by Sadiq *et al*. (2008): 11.01 %, Muszyńska *et al*. (2011): 25%, Mohiuddin *et al*. (2015): 17.7%–24.7% and Ahlavat *et al*. (2016): 29.14 % in different growing substrates, while they were in the range indicated by Chang and Miles (2004): 3.5 to 4%.

In general, the total substitution of the traditional compost or its supplementation by the various types of tested wastes has allowed the production of mushrooms with qualitative attributes highly demanded by consumers of healthy food such as low fat and carbohydrates contents and good composition in dietary fibers which play a key role in healthy properties of mushrooms (Cheung, 2009). Furthermore, mushrooms are known to be an excellent accumulator of minerals from the environment in which they grow (Atilda *et al.*, 2017). The mineral composition of the button mushroom is undoubtedly affected by the composition of growth substrate (Bąkowski *et al.*, 1986) and is differently presented by various authors (Beelman and Edwards, 1989; Mattila and Konko, 2001; Vetter, 2003). It seemed that the utilization of chicken manure as a main substrate or as a supplement for the traditional compost has increased sodium, potassium, calcium and iron contents in fruits which is convenient for consumers with calcium deficiencies or anemia but is not suitable for consumers with blood pressure problems.

CONCLUSIONS

Mushrooms obtained in substrates where local wastes were implemented were of comparable composition to those obtained from the traditional horse manure-based compost. Results varied with the type of waste as well as with the ratio of each waste type in the substrate. The safe usage of chicken manure, winery and banana wastes were earlier reported on same or other mushroom

species. Consequently, we recommend the implementation of such types of agricultural wastes in button mushroom production in Lebanon.

REFERENCES

Ahlavat O.P., Manikandan K. and Singh M. (2016). Proximate composition of different mushroom varieties and effect of UV light exposure on vitamin D content in Agaricus bisporus and Volvariella volvacea. Mushroom Research, 25(1): 1-8.

AOAC (1995). Official methods of analysis. In: Association of Analytical Chemists, 16th edn. Arlington.

Arce-Cervantes O., Saucedo-García M., Leal Lara H., Ramírez-Carrillo R.,Cruz-Sosa F. and Loera O. (2015). Alternative supplements for Agaricus bisporus production and the response on lignocellulolytic enzymes. Scientia Horticulturae 192: 375-380.

Atilda F., Owaid M.N. and Shariati M.A. (2017). The Nutritional and Medical Benefits of Agaricusbisporus : A review. Journal of Microbiology, Biotechnology and Food Science, 7(3): 281-286.

Bąkowski J., Kosson R. and Horbowicz M. (1986). Nutritional value of different species of mushrooms (Agaricus bisporus). 39(1): 111-121.

Beelman R.B. and Edwards C.G. (1989). Variability in the composition and nutritional value of the cultivated mushroom Agaricus bisporus. Mushroom News 37: 17-26.

Chang S.T. and Buswell J.A. (1996). Mushroom Nutriceuticals. World J. Microbiol. Biotechnol., 12: 473-476.

Chang S.T. and Miles P.G. (2004). MUSHROOMS. Cultivation, nutritional value, medicinal effect and environmental impact, eds., CRC. Florida, pp. 395.

Cheung P.C.K. (2009). Mushrooms as functional foods (ed P. C. K. Cheung), John Wiley & Sons, Inc., Hoboken, NJ, USA.

Falandysz J. and Borovička J. (2013). Macro and trace mineral constituents and radionuclides in mushrooms: health benefits and risk. Applied Microbiology and Biotechnology, 97: 477-501.

Gothwal R., Gupta A., Kumar A., Sharma S. and Alappat B.J. (2012). Feasibility of dairy waste water (DWW) and distillery spent wash (DSW) effluents in increasing the yield potential of Pleurotus flabellatus (PF 1832) and Pleurotus sajor-caju (PS 1610) on bagasse. 3 Biotechnology, 2, 249-257. https://doi.org/10.1007/s13205-012-0053-9.

Goyal R., Grewal R.B. and Goyal R.K. (2006). Nutritional attributes of Agaricus bisporus and Pleurotus sajor caju mushrooms. Nutrition and Health, 2006, Vol. 18, pp. 179-184.

Guillamón E., García-Lafuente A., Lozano M., D´Arrigo M., Rostagno M.A., Villares A. and Martínez J.A. (2010). Edible mushrooms: Role in the prevention of cardiovascular diseases. Fitoterapia, 81: 715-723.

Hammond J.B.W. and Nichols R. (1975). Changes in respiration and soluble carbohydrates during the post-harvest storage of mushrooms (Agaricus bisporus). J Sci Food Agric 26: 835–842.

Karam C., Sassine Y.N., Mounayar D. and Abdel-Menhem G. (2010). Prospective of local Oyster mushroom production in Lebanon. Journal of Applied Sciences Research, 6(12): 2139-2142.

Mattila P. and Konko K. (2001). Content of vitamins, mineral elements and some phenolic compounds in cultivated mushrooms. J. Agric. Food Chem, 49, 2343-2348.

Manzi I., Aguzzi A. and Pizzoferrato L. (2001). Nutritional value of mushrooms widely consumed in Italy. Food Chem., 73: 321-325.

Mohiuddin K.M., Alam M., Arefin T. and Ahmed I. (2015). Assessment of nutritional composition and heavy metal content in some edible mushroom varieties collected from different areas of Bangladesh. Asian Journal of Medical and Biological Research, 1(3): 495-501.

Muszyńska B., Sułkowska-Ziaja K. and Ekiert H. (2011). Indole compounds in fruiting bodies of some edible Basidiomycota species. Food Chemistry, 125: 1306-1308.

Sadiq S., Bhatti H.N. and Hanif M.A. (2008). Studies on chemical composition and nutritive evaluation of wild edible mushrooms. Iran Journal of Chemistry and Chemical Engineering, 27(3): 151-154.

Sebaaly Z., Hayek P., Sassine Y.N., Kfoury L., Kattar S. and Chalhoub S. (2018a). Using locally available chicken manure as a substitute to horse manure in compost formulas for growing Agaricus bisporus in Lebanon. Paper presented at: International Symposium on culinary herbs and edible fungi, Istanbul, Turkey.

Sebaaly Z., Semaan H., Rizkallah J. and Sassine Y.N. (2018b). Investigating the potential use of composted grape marc in the production of Agaricus bisporus. Paper presented at: International Symposium on culinary herbs and edible fungi, Istanbul, Turkey.

Tomkins A. (2002). Nutrition, infection and immunity: public health implications. In Calder P.C., Filed C.J., Gill H.S., editors: Nutrition and Immune function. CABI Publishing, Wallingford, UK, 375-412.

Tshinyangu K.K. (1996). Effect of grass hay substrate on nutritional value of Pleurotus ostreatus var. Columbinus. Die Nahrung, 40: 79-83.

Vetter J. (2003). Chemical composition of fresh and conserved Agaricus bisporus mushroom. European Food Research and Technology 217: 10-12.

Wani A.B., Bodha R.H. and Wani A.H. (2010). Nutritional and medicinal importance of mushrooms. Journal of medicinal plant research 4(24): 1-7.

Weil D.A., Beelman R.B. and Beyer D.M. (2004). Effect of adding a micronutrient-rich supplement to compost on yield and quality of Agaricus bisporus. Science and Cultivation of edible and medicinal fungi. Rinker & Royse (eds), Romaine, Keil. pp. 365-371.

Wood D.A. and Fermor T.R. (1985). Nutrition of Agaricus bisporus. In: Flegg P.B., Spencer D.M and Wood D.A., eds: The Biology and Technology of the cultivated mushroom. Glasshouse Crops Research Institute, Littlehampton, U.K., pp. 43-60.

Zied D.C., Savoie J.M. and Pardo-Giménez A. (2011). Soybean the main nitrogen source in cultivation substrate of edible and medicinal mushrooms, soybeans and nutrition, in: Prof. Hany El-Shemy (Ed.), ISBN: 978-953-307-536-5. InTech, Available from: http://www.Intechopen.com/books/soybean-and-nutrition/soybean-the-main-nitrogen-source-in-cultivation-substrates-of-edible-andmedicinal-mushrooms

DOI: 10.17707/AgricultForest.65.1.09

Mahboobeh KIANI-HARCHEGANI,
Seyed Hamidreza SADEGHI* and Sadegh BOOR [1]

COMPARATIVE ANALYSIS OF SOIL VARIABLES IN DIFFERENT LAND USES OF THE SHAZAND WATERSHED, IRAN

SUMMARY

Different land uses affect physical, chemical and biological properties of the soil and hence change the quality of soil. However, limited researches have been conducted in due course on the basis of high resolution field surveying. Therefore, the current study aimed to evaluate the effects of land use types on different variables of the Shazand Watershed (Iran) with a calcareous soil in central semi-arid region of Iran with an area about 1740 km^2. Towards this, five different main land uses viz. irrigated farms, rain fed farms, rangelands, orchards and outcrops dominant areas were primarily selected. Some 140 soil samples were then taken from the top 30-cm of the soil from homogeneous units representing an area about ≥ one km^2 and various soil properties such as sand, silt, clay, gravel, bulk density (BD), soil organic carbon (SOC), pH, electrical connectivity (EC), calcium carbonate ($CaCO_3$), nitrogen (N) were analyzed. The findings indicated that land use types had no significant effect (P> 0.05) on different soil variables. Nevertheless, the SOC and $CaCO_3$ in irrigated farm with respective values of 0.69 and 29.88 % were found to be more than those of other land uses. It is suggested from the results that other factors of slope, elevation and micro-climate might affect inter-variation of the study soil variables. These findings can be used for designating proper soil management strategies in the study watershed.

Keywords: Land use/Land cover change, Land degradation, Soil organic carbon, Watershed management.

INTRODUCTION

In a watershed, there are potent relationships between land use types and hydrological processes such as runoff and water quality, flooding, soil erosion, and sediment yield (Gao et al., 2014; Zhao et al., 2015; Davudirad et al., 2016). Over the past 50 years, humans have altered ecosystems more rapidly and extensively, largely to meet rapidly growing demands for resources along with economic development. The change and degradation of forests, agricultural lands, grasslands, and other land uses have a great impact on the intensity,

[1]Mahboobeh Kiani-Harchegani, Seyed Hamidreza Sadeghi *(corresponding author: sadeghi@modares.ac.ir) and Sadegh Boor, Department of Watershed Management Engineering, Faculty of Natural Resources, Tarbiat Modares University, Noor, IRAN
Paper presented at the 9th International Scientific Agricultural Symposium "AGROSYM 2018".
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

duration, and continuity of all of aforementioned phenomena. Changes in land use have a severe effect on chemical, physical and biological properties of soil and hence change the soil health and quality (Irshad et al., 2015; Jaiarree et al., 2011). The relationships among various land uses could be considered as water–soil–plant relationships. Therefore, to clarify these relationships, it is necessary to achieve adequate information about soil. Many studies (e.g., Franzluebbers and Stuedemann, 2010; Mohawesh et al., 2015; Deng et al., 2016) indicated that strong and statistically significant relationships between soil quality, land degradation and land use type. Improper agricultural practices and overgrazing reduce the soil resistance to the forces of erosion (Conant et al., 2016). Forestry activities changed the top soil surface structure (Enez et al., 2015; Watson et al., 2000).

Effects of land use changes on soil properties is inherently regional and highly dependent on the soil type (Abu –Hashim et al., 2016), climate (Berhongaray et al., 2013; Yang et al., 2016), and topography (Ayoubi et al., 2012; Dessalegn et al., 2014; Falahatkar et al., 2016; de Blécourt et al., 2017). So, there is a need to assess the effects of different land uses on soil properties in different ecological regions.

The Shazand watershed with a calcareous soil in central semi-arid region of Iran is a fertile region, so that over the past decades, most of the inhabitants were supported by farming. There are various land uses and excessive destructive human activities in the Shazand watershed whose effects on changing soil characteristics have not been minutely studied.

MATERIAL AND METHODS

Study area

The study was carried out for the Shazand watershed in Markazi Province. The study watershed is 1,740 km2 in area located between 49° 04′ 15″ to 49° 52′ 12″ E and 33° 44′42″ to 34° 12′ 13″ N (Fig. 1). From its total area, 50.15% includes highlands and hard formations, and 49.85% contains alluvial sediments and/or sub-mountain screes. A complex topography, with elevation ranges from 1,800 m to more than 3,300 m (above mean sea level), results in steep gradients of rainfall on both spatial and temporal scale. More than 90% of geologic formations belong to second and third geological era (i.e., Quaternary, Neogene and Cretaceous).

The climate is semi-arid with an average annual precipitation of 420 mm, mostly falling in winter, autumn and spring and a mean annual temperature of 12ºC. Agricultural lands located mostly in 5–15% slopes and mainly are utilized for wheat dry farming. The exchanges of land use for period of 1973 to 2014 showed that rangeland area reduced about 380 km^2. In the study period, rain fed and irrigation farm lands first increased and then reduced as well as area of orchards increased with the highest annual rate (0.265%) (Davudirad et al., 2016; Hazbavi and Sadeghi, 2017). The soils have low organic matter (about 1%) and are mainly calcareous and clay loam texture.

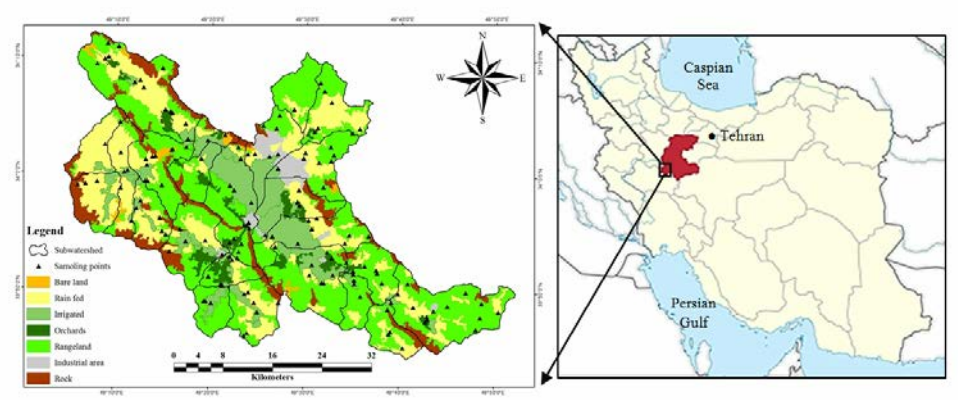


Figure 1. General view and location of the Shazand Watershed in Markazi Province, Iran

Field sampling and laboratory analyses

The investigations were carried out within the Shazand watershed in five different land uses. Some 140 soil samples were collected from different land uses. The soil samples were taken from the center of each quadrate by driving a core sampler up to 30 cm in depth. The soils were collected by an augur and kept in polythene bags so that they remained in field moist condition. After completion of collecting soil samples, the unwanted materials like stones, granules, leaves, roots etc. were discarded from the samples. The samples were then dried at room temperature, crushed, mixed thoroughly and sieved with a 2-mm sieve (Islam et al., 2016). Particle size distribution was determined by the hydrometery method (Bouyoucos, 1951). Bulk density (BD) was calculated using the cold method (Blake and Hartge, 1986). Soil pH and electrical conductivity (EC) were also measured on a 1:5 soil to water ratio suspensions with the help of by a pH/conductivity meter (Rhoades, 1996). Nitrogen (N) and Carbonate ($CaCO_3$) were determined using the Kjeldahl method (Bremner, 1996) and pressure calcimeter method, respectively (Richard and Donald, 1996). The Walkley and Black method was ultimately used to determine SOC (Nelson and Sommers, 1996).

All data were analyzed using SPSS 22.0 statistical software. One-Way ANOVA (analysis of variance) was used to detect the differences in the measured variables among land use types at significant level 5%. In addition, comparing the means among groups was investigated by different methods of Post Hoc Test (Kiani-Hrachegani et al., 2016).

RESULTS AND DISCUSSION

Table 1 shows the descriptive statistics of the properties of the soils studied. $CaCo_3$ varied from 22.29 to 29.88 % in outcrops dominant areas and irrigated land use, respectively. High amounts of calcium carbonate in different land uses indicate the soils of the Shazand watershed are calcareous. The results

of Table 1 also show that SOC varied from 0.57 to 0.69 % in different land uses, indicating low to moderate storage of organic carbon in the soil of the area. In addition, the results of particle size distribution show that orchards have the highest silt + clay contents (32.64 + 33.00%) compared to other land uses. The highest percentage of sand and gravel (39.10 and 31.64 %) were also observed at irrigated farms and outcrops dominant areas, respectively. Therefore, these findings can help to local manages designate proper soil management strategies in the study watershed (Mohawesh et al., 2015; Deng et al., 2016).

Table 1. Mean and standard divination of soil properties in different land use within the Shazand watershed, Iran

Land use	Area (%)	Sample size (n)	Fine earth			Rock fragment (%)	Bulk density (gr cm^{-3})	Organic carbon (%)	pH	Electrical connectivity (ds m^{-1})	Calcium carbonate (%)	Nitrogen (%)
			Sand (%)	Silt (%)	Clay (%)							
Irrigated	11.81	26	39.10 ±16.49	32.08 ±13.06	28.82 ±9.30	21.42 ±13.55	1.47 ±0.35	0.69 ±0.27	7.89 ±0.14	0.21 ±0.05	29.88 ±11.08	0.12 ±0.06
Rain fed	25.38	37	38.20 ±13.43	33.12 ±10.73	28.68 ±9.14	19.18 ±10.25	1.46 ±0.31	0.59 ±0.27	7.86 ±0.11	0.20 ±0.05	28.98 ±9.92	0.10 ±0.06
Rangeland	38.31	59	39.07 ±12.80	33.04 ±9.89	27.89 ±9.54	24.41 ±14.91	1.39 ±0.31	0.59 ±0.28	7.84 ±0.14	0.20 ±0.06	27.37 ±11.77	0.12 ±0.07
Orchards	7.79	10	34.36 ±11.37	32.64 ±5.48	33.00 ±7.88	23.18 ±15.72	1.49 ±0.12	0.57 ±0.34	7.87 ±0.09	0.21 ±0.05	29.80 ±9.16	0.13 ±0.09
Outcrops dominant areas	16.71	8	35.85 ±9.25	33.13 ±7.68	31.02 ±5.57	31.64 ±13.53	1.50 ±0.21	0.63 ±0.32	7.84 ±0.18	0.23 ±0.15	22.29 ±12.28	0.11 ±0.07

The results of the One-Way ANOVA to assess the effect of different land uses on soil characteristics have been presented in Table 2. The One-Way ANOVA clearly showed that land uses had no significant effect ($P > 0.05$) on different soil variables. Also, comparing the mean between groups was investigated by different methods of Post Hoc Test and various variables of soil were observed only in a group. It is probably attributed to inherent similarity in main affecting factors on soil formation and development (i.e., dominant geologic formation, topography and climate) in the study area, which different researchers have considered them (i.e., Dessalegn et al., 2014; Abu –Hashim et al., 2016; Ayoubi et al., 2018).

There was no significant difference ($P > 0.05$) among SOCs in different land uses, which agrees with Blécourt et al. (2017) who stated there was no statistically significant difference among land uses in viewpoint of SOC in Xishuangbanna Region, China. However, it disagree with the findings of Falahatkar et al. (2016) who expressed the mean values of the SOCs in the selected land uses in the Deylaman Region were significantly different ($p <$

0.05). Nevertheless, the SOC and $CaCo_3$ in irrigated farm with respective values of 0.69 and 29.88 % were found to be more than those of other land uses.

Table 2. Results of One-Way ANOVA for comparison of soil characteristics in different land uses within the Shazand watershed, Iran

variable	p-Value	Mean Squared		F-Value
		Between Groups	Within Groups	
Sand (%)	0.79	0.46	1.13	0.41
Silt (%)	0.97	12.79	110.76	0.12
Clay (%)	0.56	63.72	84.58	0.75
Gravel (%)	0.28	0.11	0.09	1.26
Bulk density (gr cm^{-3})	0.58	0.01	0.01	0.71
Organic carbon (%)	0.46	0.05	0.06	0.91
pH	0.51	0.01	0.02	0.83
Electrical connectivity (ds m^{-1})	0.89	0.004	0.01	0.27
Calcium carbonate (%)	0.56	91.89	122.55	0.75
Nitrogen (%)	0.35	0.01	0.01	1.11

CONCLUSIONS

In recent decades, land degradation processes are driven or exacerbated by human activity as change in land use. Changes in land uses are expected having a severe effect on chemical, physical and biological properties of soil and hence change the soil health and quality. In this regards, the effects of different land uses on soil properties of the Shazand Watershed (Iran) with a calcareous soil in central semi-arid region of Iran was investigated. We found non-significant difference among study variables in different land uses of the Shazand watershed, Iran. It is therefore suggested from the results that in the study of spatial and temporal scales, other factors of slope, elevation and micro-climate might affect inter-variation of the study soil variables, which need to be accurately studied in future endeavors.

ACKNOWLEDGMENT

This research has also been supported by the National Elites Foundation (Project No. 15.90186) whose valuable assistance is appreciated. The authors wish to thank the National Elites Foundation, Islamic Republic of Iran, for the Post Doctorate Fellowship awarded to the first author.

REFERENCES

Abu–Hashim M., Elsayed M., Belal A.E. (2016). Effect of land use changes and site variables on surface soil organic carbon pool at Mediterranean Region, Journal of African Earth Sciences, 114, 78-84.

Ayoubi S., Karchegani P.M., Mosaddeghi M.R., Honarjoo N. (2012). Soil aggregation and organic carbon as affected by topography and land use change in western Iran. Soil and Tillage Research 121, 18-26.

Ayoubi S., Mokhtari J., Mosaddeghi, M.R., Zeraatpisheh M. (2018). Erodibility of calcareous soils as influenced by land use and intrinsic soil properties in a semiarid region of central Iran. Environmental Monitoring and Assessment, 190(4), 192.

Berhongaray G., Alvarez R., De Paepe J., Caride C., Cantet R. (2013). Land use effects on soil carbon in the Argentine Pampas. Geoderma 192, 97-110.

Blake G.R., Hartge K.H. (1986). Bulk Density, in A. Klute, ed., Methods of Soil Analysis, Part I. Physical and Mineralogical Methods: Agronomy Monograph no. 9 (2nd ed.), pp. 363-375.

Bouyoucos G.J. (1951). A recalibration of the hydrometer method for making mechanical analysis of soils. Agronomy Journal, 43, 434-438.

Bremner J.M. (1996). Total Nitrogen, in: Sparks, D.L. (Ed.), Methods of Soil Analysis. Part 3 Chemical Methods, SSSA Book Ser. 5. 3., Soil Science Society of America, Madison, USA, pp. 1085-1122.

Conant R.T., Cerri C.E., Osborne B.B., Paustian K. (2016). Grassland management impacts on soil carbon stocks: A new synthesis. Global Environmental Change-Human and Policy Dimensions, 23, 240-251.

Davudirad A.A., Sadeghi S.H., Sadoddin A. (2016). The impact of development plans on hydrological changes in the Shazand Watershed, Iran. Land Degradation and Development, 27(4), 1236-1244.

De Blécourt M., Corre M.D., Paudel E., Harrison R.D., Brumme R., Veldkamp E. (2017). Spatial variability in soil organic carbon in a tropical montane landscape: associations between soil organic carbon and land use, soil properties, vegetation, and topography vary across plot to landscape scales. Soil, 3(3), 123.

Deng L., Wang G.L., Liu G.B., Shangguan Z.P. (2016). Effects of age and land-use changes on soil carbon and nitrogen sequestrations following cropland abandonment on the Loess Plateau, China. Ecological Engineering, 90, 105-112.

Dessalegn D., Beyene S., Ram N., Walley F., Gala T.S. (2014). Effects of topography and land use on soil characteristics along the toposequence of Ele watershed in southern Ethiopia. Catena 115, 47-54.

Enez K., Arıcak B. Sarıyıldız, T. (2015). Effects of harvesting activities on litter decomposition rates of scots pine, Trojan fir, and sweet chestnut. Šumarski List, 7-8, 361-368.

Falahatkar S., Hosseini S. M., Ayoubi S., Salmanmahiny A. (2016). Predicting soil organic carbon density using auxiliary environmental variables in northern Iran. Archives of Agronomy and Soil Science, 62(3), 375-393.

Franzluebbers A.J., Stuedemann J.A. (2010). Surface soil changes during twelve years of pasture management in the Southern Piedmont USA. Soil Science Society of America Journal, 74, 2131-2141.

Gao X., Wu P., Zhao X., Wang J., Shi Y. (2014). Effects of land use on soil moisture variations in a semi-arid catchment: implications for land and agricultural water management. Land Degradation and Development, 25, 163-172.

Hazbavi Z., Sadeghi S.H.R. (2017). Watershed health characterization using reliability–resilience–vulnerability conceptual framework based on hydrological responses. Land Degradation and Development, 28(5), 1528-1537.

Irshad M., Ali J., Eneji A.E. (2015). Chemical properties of soil and runoff water under different land uses in Abbottabad, Pakistan. Environmental Earth Sciences, 74, 3501-3506.

Islam N., Hossen S., Baten A. (2016). Soil Carbon and Nitrogen Dynamics in Agricultural Soils of Mymensingh, Bangladesh. International Journal of Agricultural and Biosystems Engineering, 1(1), 1-8

Jaiarree S., Chidt haisong A., Tangtham N., Polprasert C., Sarobol E., Tyler S. (2011). Soil organic carbon loss and turnover resulting from forest conversion to Maize fields in Eastern Thailand. Pedosphere, 21, 581-590.

Kiani Harchegani M., Sadeghi S.H.R., Asadi, H (2016). Comparative analysis of the effects of rainfall intensity and experimental plot slope on raindrop impact induced erosion (RIIE), Iranian Journal of Water and Soil Researches, 46(4), 631-640.

Mohawesh, Y., Taimeh A., Ziadat, F. (2015). Effects of land use changes and soil conservation intervention on soil properties as indicators for land degradation under a Mediterranean climate. Solid Earth, 6, 857-868.

Nelson D.W., Sommers L.E. (1996). Total carbon, organic carbon and organic matter, in: Sparks, D.L. (Ed.), Methods of Soil Analysis. Part 3. Chemical Methods, SSSA Book Ser. 5. 3., Madison, USA, Soil Science Society of America, pp. 961-1010.

Rhoades J.D. (1996). Salinity: Electrical Conductivity and Total Dissolved Solids, in: Sparks, D.L. (Ed.), Methods of Soil Analysis. Part 3. Chemical Methods, SSSA Book Ser. 5. 3., Soil Science Society of America, Madison, USA, pp. 417-436.

Richard, H.L., L.S. Donald, (1996). Carbonate and Gypsum, in: Sparks, D.L. (Ed.), Methods of Soil Analysis. Part 3 Chemical Methods, SSSA Book Ser. 5. 3., Soil Science Society of America, Madison, USA, pp. 437-474.

Watson R.T., Noble I.R., Bolin B., Ravindranath N., Verardo D.J., Dokken D.J. (2000). Land use, land-use change and forestry. A special report of the IPCC, IPCC Serial Report. Intergovernmental Panel on Climate Change, WMO, UNEP, pp. 1-23.

Yang R.M., Zhang G.L., Liu F., Lu Y.Y., Yang F., Yang F., Yang M., Zhao Y.G., Li D.C. (2016). Comparison of boosted regression tree and random forest models for mapping topsoil organic carbon concentration in an alpine ecosystem. Ecological Indicators 60, 870-878.

Zhao X., Wu P., Gao X., Persaud N. (2015). Soil quality indicators in relation to land use and topography in a small catchment on the Loess Plateau of China. Land Degradation and Development, 26, 54-61.

DOI: 10.17707/AgricultForest.65.1.10

Ljiljana KEČA,
Aleksandar MARKOVIĆ [1]

PAYMENTS FOR ECOSYSTEM SERVICES AND STAKEHOLDER'S PERSPECTIVES IN SERBIA

SUMMARY

Payments for environmental services (PES) have attracted increasing interest as a mechanism to translate non-market values of the environment, into real financial incentives for local actors to provide environmental services. The PES concept is relatively new to Serbia. However, recent laws regulating the use of natural resources have enabled some basic environmental economics mechanisms.

The current Environmental Protection Act in Serbia adopted the "user pays" principle and introduced fees for the use of natural resources and some ecological services. Other laws, such as the Water Act and the Forest Act, also regulate payments for the use of natural resources. Two ministries share the responsibility of water management in Serbia: the Ministry of the Environment (ME) and the Ministry of Agriculture, Forestry and Water Management (MAFWM). Fund for Water is not an institution, but a separate budget item in the national budget. It was established in 2010 by the Law on Waters. Six types of charges go into the Fund for Water. Financial plan for water management activities for 2011. shows that the highest financial part is directed to: management of water courses and flood control (32,93%). Some of the important characteristics of a PES schemes that are absent in existing financial mechanisms in Serbia are: explicit internalization of environmental costs; transparent pricing system; clear indications of purpose of charges; control of use of generated income; monitoring the efficiency of financed conservational measures.

Keywords: PES, stakeholders, Serbia

INTRODUCTION

Global policies have started to acknowledge the importance of ecosystem services and to incorporate them in economic systems during last decade. Payment for Ecosystem Services (PES) is becoming increasingly popular as a way to manage ecosystems using economic incentives (Kosoy and Corbera, 2010). Previous experience with incentive-based approaches suggests it is unlikely a PES approach will always be able to simultaneously improve livelihoods, increase ecosystem services, and reduce costs (Jack et al., 2008).

[1]Ljiljana Keča (corresponding author: ljiljana.keca@sfb.bg.ac.rs), Aleksandar Marković, University of Belgrade, Faculty of Forestry, Belgrade, SERBIA
Paper is presented on Green Room Sessions - International Conference, Podgorica 2018
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

Numerous international programs promote PES such as: UNEP, CBD, IUCN. These laws do not regulate the investment of collected fees (Farley and Costanza, 2010).

Provisions for the use of these funds are defined broadly, and they do not explicitly ensure the financing of conservation initiatives. Classification of ecosystem services is based on Millennium Ecosystem Assessment (Bennett et al., 2009). The PES concept is relatively new to Serbia. Different Laws define "user's pays" (Vatn, 2010) for natural resources: Law of Forests (2011a), Law on Environmental Protection (2004), Law on Waters (2010). There are 4 main group of funds connected to PES in Serbia. They have different functions:

a. Fund for environmental protection - funding for activities in the area of preservation, sustainable use, protection and advancement of the environment, as well as in the area of energy efficiency and use of renewable energy sources (2009, 2011b). b. Fund for waters - Ministry of Environmental Protection is in charge for water pollution issues and Ministry of Agriculture, Forestry and Water Protection for water use, flood prevention and technical aspects of water management. c. Fund for forests - directs funds for growth of forest coverage by reforestation; improvement of the state of coniferous plantations; conversion of coppice forests to high forests, etc. d. Charges for use of protected areas and tourism in protected areas - Article 70 of the Law on Nature Protection (2016) allow protected area managers to collect fees for different types of use of the protected areas they manage.

Water management is under the jurisdiction of the national government, which has delegated the various tasks to the Ministry of Environmental Protection, other ministries, provincial administrative bodies, agencies of local administrations, and government-held water management companies. Major administrative functions related to water management reside with Ministry of Environmental Protection, or rather the National Water Directorate attached to it. Three government-held water management companies operate in Serbia: Srbijavode (Serbia Waters), Vode Vojvodine (Waters of Vojvodina) and Beogradvode (Belgrade Waters) (2017).

There is a variety of PES schemes all around the world (350). Implementing and managing a PES scheme is demanding for all actors involved (Mahanty et al., 2013). Base is natural capital (Guerry et al., 2015), which refers to the living and nonliving components of ecosystems—other than people and what they manufacture— that contribute to the generation of goods and services of value for people. PES are direct and flexible incentive-based mechanisms, under which a user or a beneficiary of an ecosystem service makes a direct payment in cash or in kind to an individual or community whose decisions on the use of natural resources have an impact on the ecosystem service provision (Plieninger et al., 2012).

Aim of the article is to analyze financial options connected to PES in Serbia and the mail goal of the research is to discover ways of possible improvement of PES model in Serbia in the future.

MATERIAL AND METHODS

The methods applied in this paper were selected according to the nature of the problem and the purpose of research. Because of the specificity and comprehensiveness of the problem, the various research methods are applied. The research is mainly based on a review and comparative analysis (Wunder et al., 2008) of elements in the field of forestry, nature conservation and environmental protection in Serbia.

Source of data are: Serbian Fund for Environmental Protection, Fund for waters, Fund of Forests, Databases of national parks, financial plans, and Management plans.

RESULTS AND DISCUSSION

The Fund for Environmental Protection was established by the Law on Environmental Protection (2004). A separate Law on Fund for Environmental Protection was adopted in 2009. The fund aims to secure funding for activities in the area of preservation, sustainable use, protection and advancement of the environment, as well as in the area of energy efficiency and use of renewable energy sources. Different charges are connected to different legislation acts (Table 1).

According to the Fund's report, the total revenue was nearly 4.8 billion RSD (ca. 48 million EUR) in 2010, and 4.2 billion RSD (ca. 42 million EUR) in 2011. (Sekulic, 2012) structure of the revenues is presented in the Figure 1.

Table 1. Laws connected to PES and type of charges

Laws, strategy, rulebooks, acts	Type of charges
Law on Environmental Protection	
Law on Fund for Environmental Protection	
Decree on Control of Use and Trade of Wild Flora and Fauna	•charge for the use and trade with wild fauna and flora amount of the charge is 10% of the market value of used/traded natural goods.
Decree on Types of Pollution, Criteria for Calculating of Charges for Environmental Pollution and Payers, Amount and Way of Calculating and Collecting of Charges	•environmental pollution charges, calculated by the produced ton of contaminating compounds and by the type and volume of vehicles with internal combustion engines
Other	•charge for the registration in the European Eco-management and Audit Scheme (EMAS) system (cca 600€), and charges for the use of fishing areas

The activity of the Fund for Environmental Protection has some elements of a PES scheme, because it is partly based on the “user pays” principle (Ferraro, 2011) . However, it lacks some important aspects to be considered as a comprehensive mechanism that ensures financial support for conservation of ecological services (Sekulic, 2012). Ecological services are neither explicitly mentioned nor defined;

The Fund’s objectives do cover some provisioning service, but regulating and supporting services are neglected, ie. ecosystems also contribute to pollution reduction. but that is not reflected in the allocation of financial resources. Users are not well informed about what they pay for and what ecosystem services they actually use. There are no clear regulations on using the income generated, i.e. no specific conservation measures. No clear provisions on monitoring the effect of conservation measures applied are in place. Activities which are financed by the Fund for Environmental Protection are numerous: protection, preservation and improvement of the quality of air, water, soil and forests, mitigation of climate changes and ozone layer protection; rehabilitation of waste landfills, encouragement of reduction of waste creation, recycling; incentives for cleaner production; technology and products that could reduce the burden to and pollution of the environment; biodiversity and geodiversity protection and preservation; incentives for sustainable use of protected natural areas; improvement of existing and building of new infrastructure for environmental protection; incentives for use of renewable energy sources and increased energy efficiency; incentives for cleaner transport; incentives for sustainable development; development of the system of information about the environmental state, preventive and intervention measures in emergency environmental pollution, projects and programs for geological research; incentives for ecological education and raising awareness of environmental problems and sustainable development; co-financing the obligations of the Republic in relation to subsidiary measures (Sekulic, 2012).

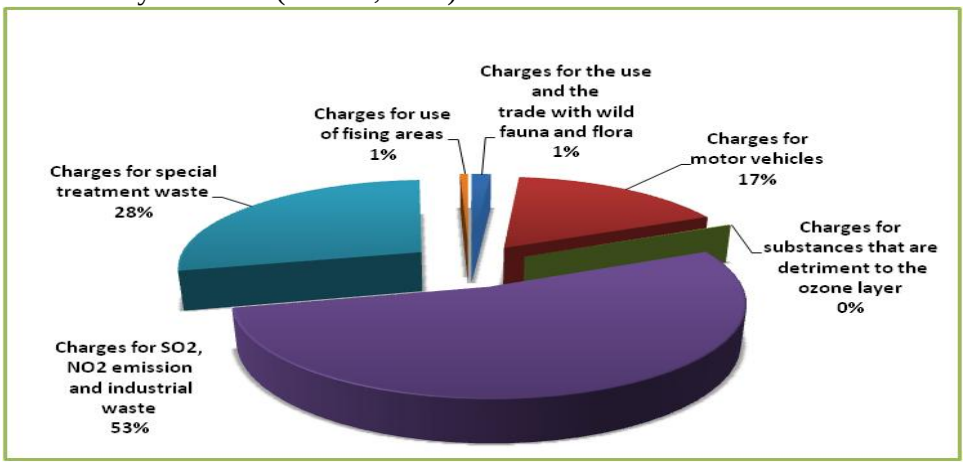


Figure 1. Structure of the revenues of the Fund of the Environmental Protection (Source: Serbian Fund for Environmental Protection)

The annual program for 2011 defines 14 priority fields, of which 5 are related to waste management, 1 to water, air and soil protection each, 1 to nature conservation, 1 to renewable energy sources, 1 to cleaner production, 2 to education and 1 to other activities defined by law. Two ministries share the responsibility of water management in Serbia: the Ministry of the Environment Protection (MEP) and the Ministry of Agriculture, Forestry and Water Management (MAFWM).

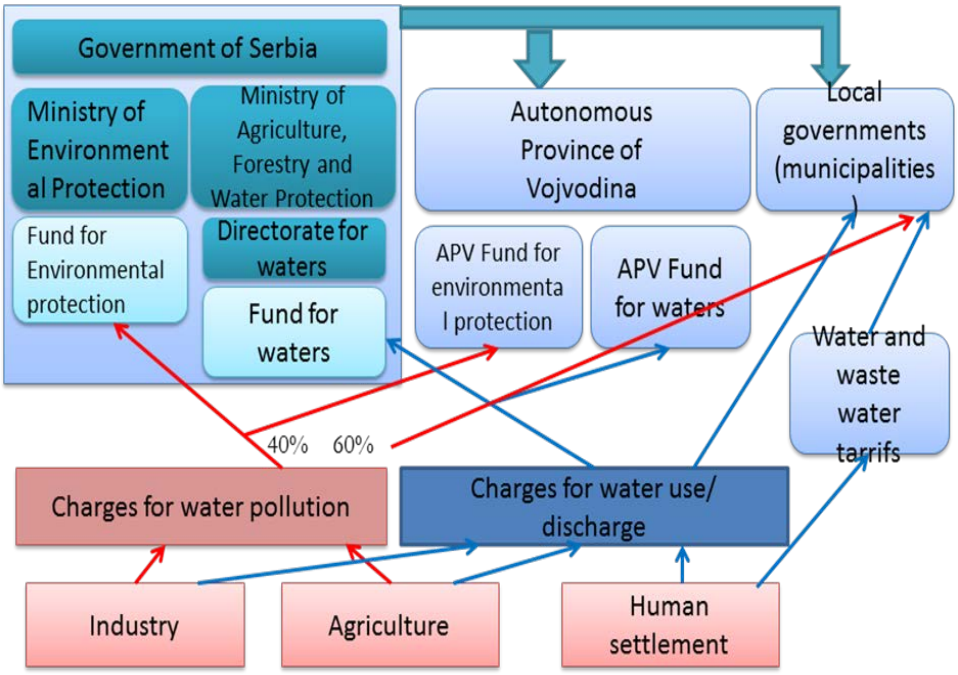


Figure 2. Structure of the revenues of the Fund of the Environmental Protection (Source: WWF, 2012)

In general, MEP is responsible for water pollution issues, while MAFWM is responsible for water use, flood prevention and for other technical aspects of water management. Six types of charges go into the Fund for Water: charge for use of water resource; charge for water discharge; charge for pollution of water; charge for drainage; charge for use of public water facilities and systems; charge for basin water management. The first three charges are fees for economic instruments related to water protection; the remaining three are water management charges. System of charges in the water management sector in Serbia is related to numerous of stakeholders (Figure 2).

Charge for use of water resources is paid for: drinking water supply (per m^3); bottling of water (per m^3); use of thermal waters (per m^3); irrigation (per m^3 or per ha); fisheries (per m^3 or per ha); energy production in hydro power plants (per kWh); other production facilities (per kW); use of river sediments (per m^3 of

extracted sediment); use of "water land" for commercial purposes (per m^2 or type of activities); locating a temporary floating object for commercial purposes (per m^2); mooring and placement of floating objects (per m^2 of an object) (Sekulic, 2012). Charge for water discharge is paid for discharging of waste waters in water courses, channels, lakes, accumulations or in canalization systems (Mays, 2010). In the context of PES, it is important to note that the fee for water discharge is higher in protected domains that have specific importance for water protection (van Ittersum and van Steenbergen, 2003). Charge for water pollution focuses more on the level and type of pollution than on volume of discharged water (2017). Charge for drainage every owner or user of land, infrastructure, road infrastructure or public land has to pay this charge, unless a drainage system for atmospheric waters is in place. An increase in the **drainage charge** will increase the pollution savings from switching technologies, but the extent will be determined by the characteristics of the crop and the technologies (Caswell et al., 1990). Charge for use of public water facilities and systems all users of public water facilities for water supply, for discharge of waste waters and for transport pay this charge. Charge for water basin management this charge is used for water course management and for flood protection (Kaštelan-Macan et al., 2007). The two charges are mutually exclusive: users who pay one are exempt from paying the other. In the context of PES schemes, it is noteworthy that managers of protected areas don't have to pay this charge. Revenues are used for water management in particular catchment areas and for drainage systems. Many such areas are natural floodplains and these revenues could be used for their restoration and management.

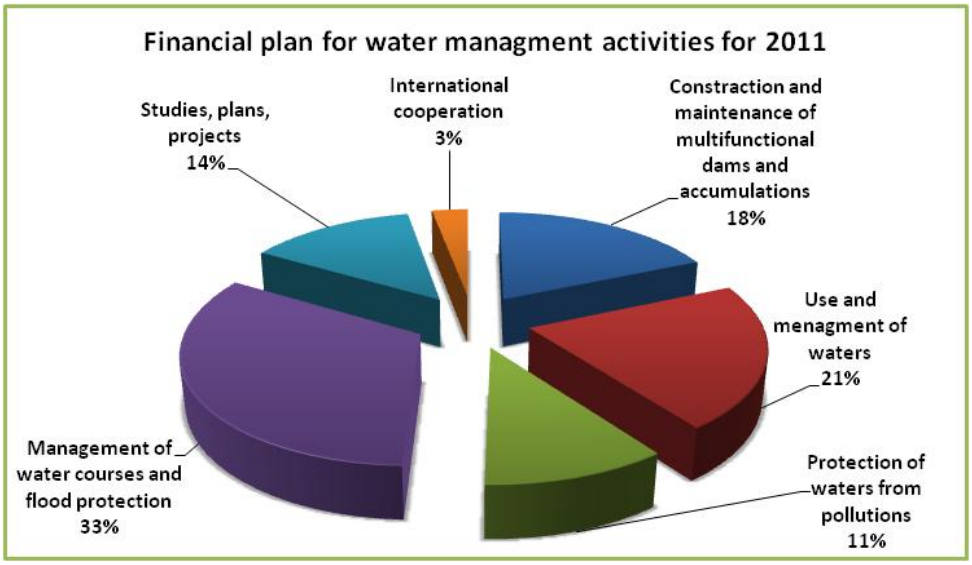


Figure 3. Financial Plan for Water management activities in 2011 (Source: WWF, 2012)

Financial plan for water management activities shows that the majority of the financial means are in the management of water courses and flood protection and use and management of waters (Figure 3).

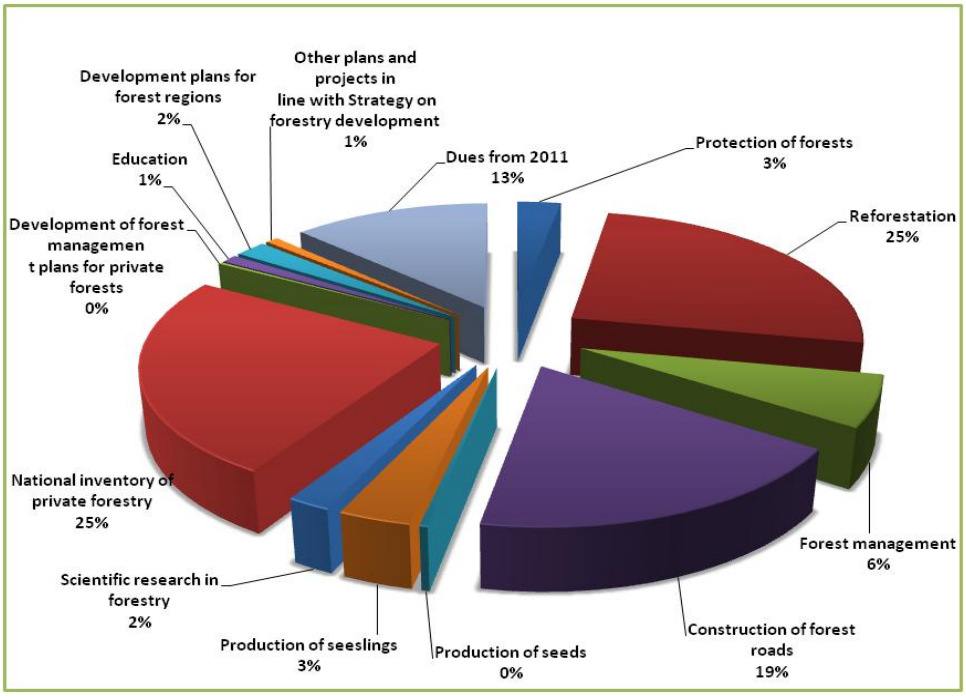


Figure 4. Financial Plan funded by the Fund of Forests in 2012 (Source: Serbian Fund of Forests, 2012)

The Fund for Forests is a separate budget item in the national budget. Fund finance the following activities: growth of forest coverage by reforestation; improvement of the state of coniferous plantations; conversion of coppice forests to high forests; production of reproductive material. The Fund's assets come from the following sources: fee for forest and forestland use; fee for protection, use and improvement of forest service's of public interest; other sources (national budget, rural development funds, donations, etc.). Every owner or user of forest pays this fee. The fee is a percentage of the total income from forest management. Forest users (public enterprises that manage public forests) pay 3% of their total annual income, while forest owners pay 5%. Any legal entity in Serbia, except for public enterprises established for forest and national park management, has to pay this fee equal to 0,025% of the entity's gross annual income (Lalic et al., 2011).

Although significant funds are allocated for reforestation, that is not necessarily a biodiversity friendly activity. Sometimes, valuable and biodiversity rich non-forest areas are being forested for economic reasons (extension of forest areas used for intensive timber use). Article 90 of the same law requires forest

users and owners to assess the forest value in their forest management plans. The law defines forest value as the value of timber, land, non-timber forest products and forest functions of public interest. This article actually provides basic steps for the development of PES schemes in forestry, and it is very important that it also recognize forest functions (services) of public interest. However, there are no by-laws regarding this issue, and the lack of experience and capacities for integrated valuing of forests has stalled progress on this front. Half of the forests in Serbia are privately owned, which is a good opportunity for the development of PES schemes.

The financial plan allocates significant sum to conservation measures, such as protection, reforestation and management, also for national inventory and forest roads construction (Figure 4).

Article 70 of the Law on Nature Protection allows protected area managers to collect fees for different types of use of the protected areas they manage. The charges are set by the Decree of Closer Criteria, Manner of Calculation and Collection of Fees, as well as by protected area management decisions.

CONCLUSIONS

There are few financial schemes related to natural resources in Serbia. Currently, none of them fully complies with the concept of PES schemes, but most of them have the potential to be adapted into functional PES schemes. Most of these schemes have been in place for many years and are traditional financial mechanisms for the use of natural resources.

Characteristics which are absent in PES schemes in Serbia are: explicit internalization of environmental costs; transparent pricing system; clear indications of purpose of charges; control of use of generated income (it should be mainly used for improvement of the state of the ecosystems); monitoring the efficiency of financed conservational measures.

Some steps necessary in order to establish a PES scheme using these funds: revenues from charges for the use of flora and fauna have to be, at least partly, allocated to financing projects/measures whose explicit objective is improving the conservation state of used species and their habitats; responsible institutions (Ministry of the Environment Protection) have to define criteria for project/measures eligibility with a focus on improving the status of used species and more efficient system of control have to be established.

In the future it would be important to establish and promote charges for the use of protected area on the national level, so that all protected managers can benefit from these opportunities; establish a transparent pricing system (in agreement with users) with reference to estimated environmental costs; account for these charges separately from the rest of the budget and use the revenues for conservational measures only, as mandated by law; avoid using the revenues from these charges to finance general management costs; focus on the conservation of habitats and species, including proactive measures, such as restoration, reintroduction, repopulation, etc.

ACKNOWLEDGMENTS

Scientific researches on the paper are financed by the COST TN 1401 CAPABAL Capacity Building in Forest Policy and Governance in Western Balkan Region.

REFERENCES

Kosoy, N., & Corbera, E. (2010). Payments for ecosystem services as commodity fetishism. Ecological economics, 69(6), 1228-1236.

Jack, B. K., Kousky, C., & Sims, K. R. (2008). Designing payments for ecosystem services: Lessons from previous experience with incentive-based mechanisms. Proceedings of the National Academy of Sciences.

Farley, J., & Costanza, R. (2010). Payments for ecosystem services: from local to global. Ecological economics, 69(11), 2060-2068.

Bennett, E. M., Peterson, G. D., & Gordon, L. J. (2009). Understanding relationships among multiple ecosystem services. Ecology letters, 12(12), 1394-1404.

Vatn, A. (2010). An institutional analysis of payments for environmental services. Ecological economics, 69(6), 1245-1252.

(2011a): Official Gazette of the Republic of Serbia no. 30/2011.

(2004): Official Gazette of the Republic of Serbia no. 135/2004

(2010): Official Gazette of the Republic of Serbia no. 30/2010.

(2011b): Official Gazette of the Republic of Serbia no. 72/2009 & 101/2011

(2016): Official Gazette of RS, no. 36/2009, 88/2010 and 91/2010 – corr. and 14/2016

Mahanty, S., Suich, H., & Tacconi, L. (2013). Access and benefits in payments for environmental services and implications for REDD+: Lessons from seven PES schemes. Land Use Policy, 31, 38-47.

Guerry, A. D., Polasky, S., Lubchenco, J., Chaplin-Kramer, R., Daily, G. C., Griffin, R., ... & Feldman, M. W. (2015). Natural capital and ecosystem services informing decisions: From promise to practice. Proceedings of the National Academy of Sciences, 112(24), 7348-7355.

(2017): Water sector in Serbia, Brussels Regional Public Services (16)

Plieninger, T., Schleyer, C., Schaich, H., Ohnesorge, B., Gerdes, H., Hernández-Morcillo, M., & Bieling, C. (2012). Mainstreaming ecosystem services through reformed European agricultural policies. Conservation Letters, 5(4), 281-288.

Wunder, S., Engel, S., & Pagiola, S. (2008). Taking stock: A comparative analysis of payments for environmental services programs in developed and developing countries. Ecological economics, 65(4), 834-852.

Sekulic G. (2012): Analysis of PES Needs and Feasibility in Serbia,WWF, (33)

Ferraro, P. J. (2011). The future of payments for environmental services. Conservation Biology, 25(6), 1134-1138.

Mays, L. W. (2010). Water resources engineering. John Wiley & Sons.

van Ittersum, M., & van Steenbergen, F. (2003). Ideas for local action in water management. Global Water Partnership.

Caswell, M., Lichtenberg, E., & Zilberman, D. (1990). The effects of pricing policies on water conservation and drainage. American journal of agricultural economics, 72(4), 883-890.

Kaštelan-Macan, M., Ahel, M., Horvat, A. J., Jabučar, D., & Jovančić, P. (2007). Water resources and waste water management in Bosnia and Herzegovina, Croatia and the State Union of Serbia and Montenegro. Water Policy, 9(3), 319-343.

Lalic, D., Popovski, K., Gecevska, V., Vasilevska, S. P., & Tesic, Z. (2011). Analysis of the opportunities and challenges for renewable energy market in the Western Balkan countries. Renewable and Sustainable Energy Reviews, 15(6), 3187-3195.

DOI: 10.17707/AgricultForest.65.1.11

Danielle de Moraes LÚCIO, Richeliel Albert Rodrigues SILVA, Kyvia Pontes Teixeira das CHAGAS, Fábio de Almeida VIEIRA [1]

BIOMETRY OF FRUITS AND SEEDS OF *Hancornia speciosa* Gomes (APOCYNACEAE) IN NATURAL POPULATIONS

SUMMARY

This study aimed verify the physical characteristics of the fruits and seeds of *Hancornia speciosa* in natural populations of the State of Rio Grande do Norte (RN), Brazil. The data were collected in two municipalities of the RN, Macaíba and Nísia Floresta. For the biometrics (n = 86 fruits and 381 seeds), fresh weight (g), length (mm) and diameter (mm) were evaluated for fruits and seeds, seed number, seed thickness (mm), yield of the pulp (%). The biometrics data were analyzed through descriptive statistics (position and dispersion measurements). Finally, the t test was applied in order to detect differences between the averages of the biometric variables of this study and of other natural populations described in the literature. The pulp contributes on average 93.56% of the total fresh fruit mass, demonstrating high yield in the studied populations. In the comparison between the populations, it was verified that the variations in the characteristics of the mangabeira fruits are probably influenced by environmental and genetic factors, which should also be correlated in future works.

Keywords: Morphometry, *Hancornia speciosa*, conservation, Brazil.

INTRODUCTION

The fruits of the mangabeira (*Hancornia speciosa* Gomes) are used in abundance mainly in the northeast region to supply the agroindustrial sector for the production of juices, sweets, ice cream and other derivatives (Ferreira et al., 2003). The fruit has good acceptance in the market, because it produces tasty fruits and with good nutritional sources (Ferreira and Marinho, 2007). In addition to this fact, the species also has a high yield in which it is possible to select fruits with higher amounts of pulp for the industry (Gonçalves et al., 2013). Capinan (2007) argues that native fruits, such as mangaba, for example, have good nutritional sources for low-income populations living in the extractive trade as an alternative source of income.

The biometry of fruits and seeds is important to characterize the intra and interpopulational variations, allowing to infer about environmental and genetic factors that determine these divergences (Silva et al., 2007; Vieira and Carvalho,

[1]Danielle De Moraes Lúcio (corresponding author: danimoraesluc@hotmail.com), Richeliel Albert Rodrigues Silva, Midwestern State University, Postgraduate Program in Forestry Sciences, Paraná, BRAZIL; Kyvia Pontes Teixeira Das Chagas, Fábio De Almeida Vieira, Federal University of Rio Grande do Norte, Postgraduate Program in Forestry Sciences, Natal, BRAZIL

Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

2009). Such information is relevant to support strategies for conservation, management of natural populations and genetic improvement.

Biometry plays an important role related to ecological aspects such as different forms of dispersion, dispersing agents, establishment of seedlings and phases of ecological succession. In addition, it contributes to the knowledge of the process of germination, viability and propagation methods (Matheus and Lopes, 2007).

The general objective of this study was to physically characterize *Hancornia speciosa* fruits and seeds in naturally occurring populations in the state of Rio Grande do Norte, Brazil.

MATERIAL AND METHODS

Area of study

The data were collected in two locations where the species *Hancornia speciosa* occurs naturally, with the purpose of capturing the variations in the species in relation to the physical characteristics of the fruits and seeds. These localities were divided and denominated in: Population Macaíba and Population Nísia Floresta. Sampling was carried out during the July 2013, a time of natural dispersion of mature fruits.

The Macaíba Population is located in the Specialized Academic Unit of Agricultural Sciences of UFRN (UAECIA), in the municipality of Macaíba, in the state of Rio Grande do Norte, approximately 26.6 km from the capital Natal. This area is covered by semideciduous seasonal forest and transition forest formation between Atlantic Forest and Caatinga biomes (EMPARN, 2013). The study population was divided into subpopulations. The subpopulation 1 is inserted in a forest fragment between the native vegetation and the banks of the UAECIA, whose coordinates are 5°53'21.30" South and 35°21'23.16" West, and elevation of 38 m (Figure 1A). The subpopulation 2 of collection in Macaíba, is about 740 m from the first collection area, in an area of native vegetation and close to pastures, between the coordinates 5°53'35.23" South and 35°21'02.18" West, and elevation of 54 m

The Nísia Floresta Population is included in the Bonfim Guaraíra Environmental Protection Area (APA), in the municipality of Nísia Floresta, state of Rio Grande do Norte. It is an area with a natural occurrence of *Hancornia speciosa* and it is possible to observe the presence of communities around the fragment. In this region, there are several ecosystems, including dunes, remnants of Atlantic Forest and other associated ones (mangrove and coastal board) (MMA, 2012), whose coordinates of the study area are 5°58'23.50" South and 35°11'51.34" West, and elevation of 32 m. The municipality of Nísia Floresta is approximately 40.1 km from the capital Natal, traveling by BR-101.

Macaíba and Nísia Floresta are municipalities that participate in the metropolitan region of Natal (capital of the RN). They present climatic classification according to Köppen, type As', of tropical climate with dry summer and rainy season. It has an annual rainfall of 1,442.8 mm, average temperature of

27.1 °C and average annual relative humidity around 76% (MME, 2005a); (MME, 2005b).

Sampling

The fruit sampling was carried out in two natural populations of the State of Rio Grande do Norte, in order to capture the variations in the species regarding the physical characteristics of the fruits and seeds. Sampling was carried out during July 2013, a time of natural dispersion of mature fruits.

At the total, 86 mature fruits of *Hancornia speciosa* were collected directly from the ground, under the matrices. The sampling used was simple random (where each element of the population has the same probability of being sampled), comprising 6 matrices. The mean size of the sampled trees was 3.06 m in height and 7.0 cm in diameter at chest height. After being collected, the fruits were sent to the Laboratory of Genetics and Forest Improvement of UFRN to begin the biometric analysis, discarding those that showed visible physical damages and rot.

The biometric characteristics were: fresh mass (g), length (mm) and diameter (mm) of fruits and seeds. The dimensions were determined with the aid of a digital caliper and the fresh mass was determined from a precision analytical balance. In order to determine the seed size, after being measured, the fruits were manually pulped. The excess pulp surrounding the seeds was withdrawn with the aid of a stylet and, subsequently, the seeds were washed in running water. In addition, the number of seeds (unity), seed thickness (mm) and yield of the pulp (YP) (%), obtained from the formula proposed by Freitas et al. (2012), where: YP = MP × 100MF. The mass of the pulp (MP) was determined by subtracting the fresh mass of the whole fruit (MF) by the fresh mass of the seeds.

Statistical analysis

The biometric data were analyzed using the statistical software BIOESTAT version 5.3 (Ayres et al., 2007). For each variable were calculated: arithmetic mean, standard deviation, standard error, coefficient of variation (*CV*), asymmetry (*S*) and kurtosis (*K*). The distribution was considered asymmetric on the left when the values of $S < 0$; and asymmetric distribution on the right when the values have $S > 0$. The reference values for the kurtosis coefficient (*K*) were $K > 3$ for sharp distribution in relation to the normal (leptokurtic) curve. For $K < 3$, the distribution was considered flatter than the normal (platicurtic) curve, according to Silva et al. (2007).

To determine if the data are statistically different from the normal distribution, the fruit and seed variables were submitted to the normality test of Lilliefors. After detecting that the data had a curve different from normal ($P < 0.05$), the use of non-parametric statistics was considered. Thus, the Spearman correlation coefficient (r_s) was calculated using the STATISTICA 7 program (Statsoft, 2004). In addition, the Student t test was applied in order to know how

much the values of the populations raised in this study are significantly similar to that of a distinct population mentioned in the literature.

The Student's t-test of this study was based on two independent samples, when only the means, variances and sample sizes of each population were available. The analyzes were performed using the BIOESTAT program version 5.3 (Ayres et al., 2007), following the following hypotheses: H_0 (null hypothesis): There are no differences between the means of the individuals of the populations sampled: $\mu_1 = \mu_2$. H_1 (alternative hypothesis): There is difference between the means of the individuals of the populations sampled: $\mu_1 \neq \mu_2$.

RESULTS AND DISCUSSION

In Table 1, the results can be observed, including position and dispersion measurements for each biometric variable that was evaluated.

Table 1. Biometric characteristics of fruits and seeds of *Hancornia speciosa*.

Biometric Features	*n*	Max	Min	Mean ± standard error	*CV* (%)	*S*	*K*
FRUITS							
Fresh mass (g)	86	56.56	2.07	13.86 ± 1.13	76.00	1.87	4.30
Lenght (mm)	86	48.10	16.80	28.32 ± 0.71	23.39	0.50	0.07
Diameter (mm)	86	45.80	16.60	26.24 ± 0.70	24.82	0.76	0.33
Number of seeds (unity)	66	32.00	1.00	5.77 ± 0.70	99.01	2.40	8.00
Yield of the pulp (%)	63	99.95	60.90	93.56 ± 0.83	7.02	-3.50	13.51
SEEDS							
Fresh mass (g)	381	1.67	0.01	0.23 ± 0.01	121.54	3.80	14.85
Length (mm)	381	16.60	4.80	10.47 ± 0.08	14.23	-0.43	1.17
Diameter (mm)	381	10.60	3.10	8.18 ± 0.06	14.12	-0.85	1.95
Thickness (mm)	381	4.50	0.30	3.01 ± 0.03	20.72	-1.23	2.97

**n*: sample size, *CV*: coefficient of variation, *S*: asymmetry, *K*: kurtosis, Max: Maximum, Min: Minimum.

There was considerable variation in the amplitude (maximum and minimum values) of the biometric characteristics of the fruits. The arithmetic mean of the fresh fruit mass was 13.86 g. Capinan (2007) presented the following average values for fresh mass of mangabeira fruits in three municipalities of the state of Bahia: 22.61 g in Conde (north coast); 10.39 g in Nova Soure (semi-arid) and 13.05 g in Ouriçangas (agreste). It is possible to affirm that, according to the biometric characteristics evaluated in the RN state region, there are similarities with the values found in the Bahia region by Capinan (2007), mainly with the fruits of the wild population. It is known that variations in fruit characteristics are also influenced by environmental factors, such as the availability of water, which is an essential factor for the production of fleshy fruits (Tabarelli et al., 2003), and consequently reflects in the total fresh mass of the fruits.

As for the fruit length, the mean was 28.32 mm. This result is close to that found by Alves et al. (2010), which evaluated five genotypes from the Piauí semi-arid region and obtained averages between 27.64 mm and 29.08 mm. In this comparison, the authors found no significant statistical difference between the genotypes. In the present work, the average fruit diameter was 26.24 mm, the parameter in which it did not present greater differences than those found by Capinan (2007): 34.49 mm; 26.87 mm and 30.74 mm in three populations in Bahia. The mean number of seeds per fruit was 5.77. Capinan (2007) obtained the following values: 14.95 for the municipality of Conde; 6.23 for the municipality of Nova Soure and 9.98 for the municipality of Ouriçangas. These differences suggest that the mean number of seeds per fruit is probably not influenced by the environment, but is determined by the biological potential of the species for seed production (Carvalho et al., 1997). In addition, no significant correlations were observed between fruit size and number of seeds, as can be observed in Table 2.

Table 2. Spearman correlation (r_s) between the biometric variables of the fruits and seeds of *Hancornia speciosa*.

Correlations	r_S
Fruits	
Fresh mass x Length of the fruit	0,600*
Fresh mass x Diameter of the fruit	0,445*
Length x Diameter of the fruit	0,396*
Mass / Diameter of the fruit x Number of Seeds	-0,006 ns /0,131 ns /0,068 ns
Fresh mass x Yield of the pulp	0,300*
Number of seeds x Yield of the pulp	-0,425*
Seeds	
Fresh mass x Seeds length	0,637*
Fresh mass x Seeds diameter	0,576*
Fresh mass x Seeds thickness	0,464*
Seeds Length x Diameter	0,716*
Seeds Length x thickness	0,261*
Seeds Diameter x thickness	0,211*
Fresh mass x Yield of the pulp	-0,620*

* = P <0.05; ns = not significant.

Zuffo et al. (2012) evaluated the biometric characteristics of the mango seeds and obtained the following means: 9.43 mm long, 3.42 mm thick and 7.35 mm diameter, values close to those found in this study. This phenotypic similarity indicates that the biometric characterization of the seeds can be applied as an important tool in the taxonomy and identification of varieties. In summary, the biometric variables analyzed do not present significant differences with other studies carried out in natural populations of the Northeast.

Capinan (2007) obtained 82.58% yield of the pulp in a sample from the municipality of Conde, on the North coast of the state of Bahia; lower than that found in this study, which was 93.56%. It can be affirmed that most of the fruit is constituted by the pulp, being thus, a species with great use for its diverse means of consumption.

The standard error for all evaluated fruit characteristics was low, demonstrating that the sample effort was expressive for the entire population (Table 1). Regarding the coefficient of variation (*CV*), the values of length, fruit diameter and yield of the pulp had little variation. On the other hand, the variables fresh mass of the fruits and the number of seeds presented high *CV*, indicating high variability. Among the biometric characteristics of the seeds, a high coefficient of variation was observed for the fresh mass when compared to the other characteristics analyzed.

The biometric characteristics of the fruits were positive with respect to the asymmetry (*S*), indicating asymmetric behavior to the right, thus, fruits with lower fresh mass, length and diameter predominated in the analyzed sample, except for the yield of the pulp value. Unlike the fruits, the seeds had an asymmetric distribution to the left and negative, evidencing the predominance of seeds with greater length, diameter and thickness in the analyzed sample, except for the value of fresh seed mass.

Regarding the kurtosis coefficient, the values of fresh fruit mass, number of seeds, yield of the pulp and fresh seed mass were $K > 3$, that is, leptokurtic, indicating that there is a large concentration of the values around the mean. As for the variables length, fruit and seed diameter and seed thickness, the distribution was more flattened than the normal, therefore, platicurtic ($K < 3$) curve, indicating dispersion of the data around the mean.

According to Spearman's correlation, only the values between the mass / length / diameter of the fruit and the number of seeds were not significant (Table 2). The correlations between the variables fresh mass x fruit length and fresh mass x diameter were positive and significant, demonstrating that larger fruits also have greater mass. In addition, it was found that fruits with greater length have larger diameter, demonstrating the circular (rounded) shape of the fruit.

There was a significant and positive correlation ($P < 0.05$) for all evaluated parameters related to the seeds. The correlation between the length and diameter of the seeds presented a higher value in relation to the other correlated parameters. Despite the lower correlation value between seed diameter and seed thickness, it was observed that the correlation was significant and positive. This suggests that larger seeds have little gain in thickness.

Positive and significant correlations were observed for the relationship between fresh fruit mass and yield of the pulp, confirming the hypothesis that fruits with higher fresh mass are directly related to fruits with higher pulp content. Although this relationship is apparently logical, it was found that fruits with higher number of seeds have a lower yield ($r_s = -0.425$). In this case, the greater fresh mass of the fruit is due to the greater number or mass of the seeds,

resulting in the lower yield of the pulp. Specifically, negative and significant correlations were observed between the number of seeds x yield of the pulp and between the fresh mass of the seeds x yield of the pulp, because in fruits with higher yield of the pulp the seeds contribute less with the total mass of the fruits (Table 2).

Biometry studies of fruits and seeds subsidize information relevant to the conservation of species. Thus, as studies for *Hancornia speciosa* are still scarce, such information may support the programs of genetic conservation of natural populations and breeding of the species.

In this sense, it is also necessary to quantify the variations between populations. For this, Student's t test allows the comparison of the means of two independent groups and it does not require the same sample size. It has the purpose of analyzing statistically the variations occurred in the two samplings. For the physical characteristics of mangabeira, for the most part, there were no significant differences between populations, both for the Macaíba (Table 3) and Nísia Floresta populations (Table 4).

There is a trend towards significant statistical differences mainly related to the municipality of Nova Xavantina (MT) in the Cerrado biome, compared to the populations of this study (Macaíba and Nísia Floresta). Ganga et al. (2010) affirm that the *speciosa* botanical variety occurs in a generalized way in the Northeast region and presently in the work, it shows a table with the localities and the respective botanical varieties sampled in the Cerrado, where *speciosa* variety is less common. The municipalities sampled in the state of Mato Grosso, the varieties are mostly: gardneri and cuyabensis. With this, this statistical divergence can be justified by the different botanical varieties among the comparative localities. These authors also affirm that there is great phenotypic variation between populations sampled and also within the botanical varieties.

Moura et al. (2008), by analyzing the genetic variability by RAPD (Random DNA Amplified DNA Polymorphism) between the *speciosa* variety and the *pubescens* and *gardinerii* varieties, found 90.70% genetic variability within the studied varieties, 4.59% between subpopulations and 4.71% among varieties within subpopulations. These variations are, according to the authors, due to the restriction of gene flow among mangabeira varieties, probably determined by the behavior of the pollinating agent, the lack of synchronism in the flowering, the population density and the location of the individuals within the subpopulation.

The comparisons between the values for fresh fruit mass, in general, were not significant. However, significant values were discarded when comparing the means between the municipalities of Macaíba (RN) and Nova Xavantina (MT), according to Table 3; and between Nísia Floresta (RN) and Conde (BA) and Nísia Floresta (RN) and Nova Xavantina (MT), according to Table 4.

Table 3. "t" test comparing the biometric variables of the fruits of *Hancornia speciosa* among the population Macaíba and other regions.

Comparisons	t
Fresh mass (g)	
Macaíba (RN) [a] *x* Conde (BA) [b]	-2,93 [ns]
Macaíba (RN) [a] *x* Nova Soure (BA) [b]	1,91 [ns]
Macaíba (RN) [a] *x* Ouriçangas (BA) [b]	0,86 [ns]
Macaíba (RN) [a] *x* Nova Xavantina (MT) [c]	-8,79*
Macaíba (RN) [a] *x* Nísia Floresta (RN) [d]	-0,11 [ns]
Macaíba (RN) [a] *x* Ipojuca (PE) [d]	0,80 [ns]
Macaíba (RN) [a] *x* Parnamirim (RN) [d]	1,01 [ns]
Macaíba (RN) [a] *x* Extremoz (RN) [d]	0,33 [ns]
Macaíba (RN) [a] *x* Rio Tinto (PE) [d]	0,18 [ns]
Lenght (mm)	
Macaíba (RN) [a] *x* Conde (BA) [b]	-5,25*
Macaíba (RN) [a] *x* Nova Soure (BA) [b]	-2,16 [ns]
Macaíba (RN) [a] *x* Ouriçangas (BA) [b]	-4,52*
Macaíba (RN) [a] *x* Nova Xavantina (MT) [c]	-11,99*
Macaíba (RN) [a] *x* Nísia Floresta (RN) [d]	-4,39 [ns]
Macaíba (RN) [a] *x* Ipojuca (PE) [d]	-2,25 [ns]
Macaíba (RN) [a] *x* Parnamirim (RN) [d]	-2,72 [ns]
Macaíba (RN) [a] *x* Extremoz (RN) [d]	-2,59 [ns]
Macaíba (RN) [a] *x* Rio Tinto (PE) [d]	7,97*
Diameter (mm)	
Macaíba (RN) [a] *x* Conde (BA) [b]	-7,56*
Macaíba (RN) [a] *x* Nova Soure (BA) [b]	0,19 [ns]
Macaíba (RN) [a] *x* Ouriçangas (BA) [b]	-2,31 [ns]
Macaíba (RN) [a] *x* Nova Xavantina (MT) [c]	-11,59*
Macaíba (RN) [a] *x* Nísia Floresta (RN) [d]	-6,19*
Macaíba (RN) [a] *x* Ipojuca (PE) [d]	0,19 [ns]
Macaíba (RN) [a] *x* Parnamirim (RN) [d]	0,62 [ns]
Macaíba (RN) [a] *x* Extremoz (RN) [d]	-0,17 [ns]
Macaíba (RN) [a] *x* Rio Tinto (PE) [d]	0,55 [ns]
Number of seeds (unity)	
Macaíba (RN) [a] *x* Conde (BA) [b]	-6,97*
Macaíba (RN) [a] *x* Nova Soure (BA) [b]	-0,01 [ns]
Macaíba (RN) [a] *x* Ouriçangas (BA) [b]	-2,95 [ns]
Macaíba (RN) [a] *x* Nova Xavantina (MT) [c]	-8,63*
Yield of the pulp (%)	
Macaíba (RN) [a] *x* Conde (BA) [b]	6,51*
Macaíba (RN) [a] *x* Nova Soure (BA) [b]	5,68*
Macaíba (RN) [a] *x* Ouriçangas (BA) [b]	3,93 [ns]
Macaíba (RN) [a] *x* Nísia Floresta (RN) [d]	2,04 [ns]
Macaíba (RN) [a] *x* Ipojuca (PE) [d]	3,60 [ns]
Macaíba (RN) [a] *x* Parnamirim (RN) [d]	1,11 [ns]
Macaíba (RN) [a] *x* Extremoz (RN) [d]	1,41 [ns]
Macaíba (RN) [a] *x* Rio Tinto (PE) [d]	2,40 [ns]

* = $P < 0.05$; ns = not significant.

Where: [a] = this study; [b] = Capinan (2007); [c] = Gonçalves et al. (2013); [d] = Araújo et al. (2003).

Table 4. "t" test comparing the biometric variables of the fruits of *Hancornia speciosa* among the population of Nísia Floresta and other regions.

Comparisons	t
Fresh mass (g)	
Nísia Floresta (RN) [a] *x* Conde (BA) [b]	-6,59*
Nísia Floresta (RN) [a] *x* Nova Soure (BA) [b]	1,97 [ns]
Nísia Floresta (RN) [a] *x* Ouriçangas (BA) [b]	0,13 [ns]
Nísia Floresta (RN) [a] *x* Nova Xavantina (MT) [c]	-13,65*
Nísia Floresta (RN) [a] *x* Nísia Floresta (RN) [d]	-1,46 [ns]
Nísia Floresta (RN) [a] *x* Ipojuca (PE) [d]	0,02 [ns]
Nísia Floresta (RN) [a] *x* Parnamirim (RN) [d]	0,38 [ns]
Nísia Floresta (RN) [a] *x* Extremoz (RN) [d]	-0,79 [ns]
Nísia Floresta (RN) [a] *x* Rio Tinto (PE) [d]	-0,95 [ns]
Lenght (mm)	
Nísia Floresta (RN) [a] *x* Conde (BA) [b]	-10,17*
Nísia Floresta (RN) [a] *x* Nova Soure (BA) [b]	-4,80*
Nísia Floresta (RN) [a] *x* Ouriçangas (BA) [b]	-7,09*
Nísia Floresta (RN) [a] *x* Nova Xavantina (MT) [c]	-18,15*
Nísia Floresta (RN) [a] *x* Nísia Floresta (RN) [d]	-9,77 [ns]
Nísia Floresta (RN) [a] *x* Ipojuca (PE) [d]	-5,73*
Nísia Floresta (RN) [a] *x* Parnamirim (RN) [d]	-6,62*
Nísia Floresta (RN) [a] *x* Extremoz (RN) [d]	-6,35*
Nísia Floresta (RN) [a] *x* Rio Tinto (PE) [d]	-6,13*
Diameter (mm)	
Nísia Floresta (RN) [a] *x* Conde (BA) [b]	-6,83*
Nísia Floresta (RN) [a] *x* Nova Soure (BA) [b]	-0,84 [ns]
Nísia Floresta (RN) [a] *x* Ouriçangas (BA) [b]	-3,88 [ns]
Nísia Floresta (RN) [a] *x* Nova Xavantina (MT) [c]	-17,07*
Nísia Floresta (RN) [a] *x* Nísia Floresta (RN) [d]	-1,58 [ns]
Nísia Floresta (RN) [a] *x* Ipojuca (PE) [d]	-1,33 [ns]
Nísia Floresta (RN) [a] *x* Parnamirim (RN) [d]	-0,59 [ns]
Nísia Floresta (RN) [a] *x* Extremoz (RN) [d]	-1,95 [ns]
Nísia Floresta (RN) [a] *x* Rio Tinto (PE) [d]	-0,72 [ns]
Number of seeds (unity)	
Nísia Floresta (RN) [a] *x* Conde (BA) [b]	-8,16*
Nísia Floresta (RN) [a] *x* Nova Soure (BA) [b]	-0,67 [ns]
Nísia Floresta (RN) [a] *x* Ouriçangas (BA) [b]	-3,85 [ns]
Nísia Floresta (RN) [a] *x* Nova Xavantina (MT) [c]	-12,74*
Yield of the pulp (%)	
Nísia Floresta (RN) [a] *x* Conde (BA) [b]	9,48*
Nísia Floresta (RN) [a] *x* Nova Soure (BA) [b]	7,65*
Nísia Floresta (RN) [a] *x* Ouriçangas (BA) [b]	5,23*
Nísia Floresta (RN) [a] *x* Nísia Floresta (RN) [d]	3,74 [ns]
Nísia Floresta (RN) [a] *x* Ipojuca (PE) [d]	5,87*
Nísia Floresta (RN) [a] *x* Parnamirim (RN) [d]	2,43 [ns]
Nísia Floresta (RN) [a] *x* Extremoz (RN) [d]	2,86 [ns]
Nísia Floresta (RN) [a] *x* Rio Tinto (PE) [d]	4,26 [ns]

* = $P < 0.05$; ns = not significant.

Where: [a] = this study; [b] = Capinan (2007); [c] = Gonçalves et al. (2013); [d] = Araújo et al. (2003).

For the variable fruit length, Conde and Ouriçangas, the municipalities pertaining to the state of Bahia, presented divergent and significant means when compared to Macaíba/RN and Nísia Floresta/RN (Table 3 and Table 4). This divergence also occurred in the comparison between the means of Nísia Floresta/RN and Nova Soure/BA (Table 4). Capinan (2007) studied the genetic variation within and among these three populations of Bahia and detected that there is greater intrapopulational genetic variation than the interpopulational variation, through RAPD marker. According to the results of the author, one should consider the main methods that evaluate genetic divergence among the populations of Bahia are fruit mass and diameter. The author also suggests studies of breeding of the species aiming at exploitation in the industrial market.

Ganga et al. (2010) found high levels of phenotypic variation for the biometric characteristics of the fruits among natural populations of mangabeira in the Cerrado. In fact, phenotypic variations among populations, such as fruit mass and yield of the pulp, are influenced by environmental factors, such as water availability, which is essential for fruit production with succulent mesocarp (Tabarelli et al., 2003). This variation can be even more significant considering the high variability in fruit size of this species. In addition, it can be determined by uncontrolled environmental conditions, such as anthropization, soil and climate.

Due to the phenotypic variability of this species, regardless of the geographic distribution, there are difficulties in the production and subsequent commercialization of the fruits, since it does not meet the income demanded by the market. This may compromise the production of species such as mangabeira in the long term, once it is important to select and multiply individuals that produce quality fruits. In this case, studies of physical and chemical characterization of fruits are necessary to direct breeding programs. Furthermore, the variability of genetic origin, which is passed on to each generation, is important for maintaining the evolutionary potential of the species and it must be conserved in the natural populations.

Finally, it is possible to affirm that most of the means for the sampled populations agree with the null hypothesis, which says that there are no differences in the pairwise comparison with the means of other populations published in the literature. However, in the same way, significant differences were found in the populations sampled, mainly because there are environmental or genetic influences in the populations, thus they agree with the alternative hypothesis.

CONCLUSIONS

The study carried out with the populations of *Hancornia speciosa* in the state of Rio Grande do Norte has reached the following conclusions:

Larger fruits present a higher amount of pulp, which contributes on average with 93.56% of the total fresh fruit mass, demonstrating high yield in the studied populations.

There is potential for conservation and genetic improvement of the species, since the coefficient of variation of fresh fruit mass was high (76%), representing considerable intrapopulation variability.

There are statistical differences between the populations of this study (Macaíba and Nísia Floresta) mainly when compared to the municipality of Nova Xavantina (MT), due to the differences between the botanical varieties found in different regions of the country.

Environmental and genetic variations are the main factors attributed to phenotypic (morphological) differences within and among mangabeira populations, which should be investigated in future work.

ACKNOWLEDGMENTS

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001

REFERENCES

Alves, T. De A.; Alves, R. E.; Moura, C. F. H.; Silveira, R. S. Da; Figueiredo, R. W. de. Características físicas de frutos da mangabeira (*Hancornia speciosa* Gomes) nativos do semi-árido piauiense. In: CONGRESSO BRASILEIRO DE FRUTICULTURA, 21., 2010, Natal. Anais... Natal: Sociedade Brasileira de Fruticultura, 2010.

Araújo, I. A. De; Ferreira, E. G.; Soares, K. T; Fontinélli, I. S. C. Características Físicas de Frutos da Mangabeira (*Hancornia speciosa* Gomes) Cultivada na Zona da Mata Paraibana. In: SIMPÓSIO BRASILEIRO SOBRE A CULTURA DA MANGABA, 1., 2003, Aracaju. Anais do Simpósio Brasileiro sobre a Cultura da Mangaba. Aracaju: Embrapa Semiárido, 2003. p. 1 - 3. CD-ROM.

Ayres, M. Et Al. BioEstat: aplicações estatísticas nas áreas de ciências biométricas. Versão 5.0. Belém: Sociedade Civil Mamirauá, MCT-CNPq, 2007.

Capinan, G. C. S. Seleção de germoplasma de mangabeira (*Hancornia speciosa*) definidos por marcadores morfológicos e moleculares. 2007. 98 f. Dissertação de mestrado - Univesidade Federal do Recôncavo da Bahia, Cruz Das Almas, 2007.

Carvalho, M. R. T. De; Bordignon, R.; Ballvé, R. M. L.; Pinto-Maglio, C. A. F.; Filho, H. P. M. Aspectos biológicos do reduzido número de sementes da tangerina 'sunki'. Bragantia, v. 56, p. 69-77, 1997.

EMPARN. Empresa de Pesquisa Agropecuária do Rio Grande do Norte. Disponível em: <www.emparn.rn.gov.br>. Acesso em: 20 ago. 2013.

Ferreira, E. G.; Marinho, S. J. O. Produção de frutos da mangabeira para consumo in natura e industrialização. Revista Tecnologia & Ciência Agropecuária, João Pessoa, v. 1, n. 1, p.9-14, set. 2007.

Ferreira, E. G.; Santos, E. S. Dos; Araújo, I. A. De. Avaliações biométricas de plantas e físico-químicas de frutos de mangabeira de pomares nativo e cultivado. In: I SIMPÓSIO BRASILEIRO SOBRE A CULTURA DA MANGABA, 2003, Aracaju. Embrapa Semi-árido. Aracaju, 2003.

Freitas, M. K. C. De; Coimbra, R. R, Aguiar, G. B.; Aguiar, C. B. N.; Chagas, D. B. Das; Ferreira, W. M.; Oliveira, R. J. Variabilidade fenotípica e caracterização morfológica de uma população natural *de Hancornia speciosa* Gomes. Bioscience Journal, Uberlândia, v. 28, n. 5, p. 833-841. out. 2012.

Ganga, R. M. D.; Ferreira, G. A.; Chaves, L. J; Naves, R. V.; Nascimento, J. L. do. Caracterização de frutos e árvores de populações naturais de *Hancornia speciosa*

Gomes do Cerrado. Revista Brasileira de Fruticultura, Jaboticabal, v. 32, n. 1, p.101-113, mar. 2010.

Gonçalves, L. G. V.; Andrade, F. R.; Marimon Junior, B. H.; Schossler, T. R.; Lenza, E.; Marimon, B. S. Biometria de frutos e sementes de mangaba (*Hancornia speciosa* Gomes) em vegetação natural na região leste de Mato Grosso, Brasil. Revista de Ciências Agrárias, [s.l], v. 36, n. 1, p.31-40, 2013.

Matheus, M. T.; Lopes, J. C. Morfologia de frutos, sementes e plântulas e germinação de sementes de *Erythrina variegata* L. Revista Brasileira de Sementes, v. 29, n. 3, p. 08-17, 2007.

MMA - MINISTÉRIO DO MEIO AMBIENTE (Nísia Floresta). Plano de Manejo da Floresta Nacional de Nísia Floresta, Rio Grande do Norte. Nísia Floresta: Ministério do Meio Ambiente, 2012. 48 p.

MME - MINISTÉRIO DE MINAS E ENERGIA (Recife). Serviço Geológico do Brasil - CPRM. Projeto cadastro de fontes de abastecimento por água subterrânea: Diagnóstico do município de Macaíba. Recife: Ministério de Minas e Energia, 2005a. 25 p.

MME - MINISTÉRIO DE MINAS E ENERGIA (Recife). Serviço Geológico do Brasil - CPRM. Projeto cadastro de fontes de abastecimento por água subterrânea: Diagnóstico do município de Nísia Floresta. Recife: Ministério de Minas e Energia, 2005b. 32 p.

Moura, N. F.; Moura, M. F.; Pereira, M. De F.; Chaves, L. J. Diversidade genética e variação fenotípica de caracteres morfológicos em mangaba (*Hancornia speciosa* Gomes). In: SIMPÓSIO NACIONAL DO CERRADO E II SIMPÓSIO INTERNACIONAL SAVANAS TROPICAIS, 9., 2008, Brasília. Anais eletrônicos... Brasília: Embrapa, 2008. p. 1 - 7. Disponível em: <http://www.cpac.embrapa.br/publicacoes/search_pbl/1?q=Variação genética>. Acesso em: 25 nov. 2013.

Silva, M. S.; Vieira, F. A.; Carvalho, D. Biometria dos frutos e divergência genética em uma população de *Geonoma schottiana* Mart. Revista Brasileira de Biociências (Impresso), v. 5, p. 582-584, 2007.

STATSOFT, INC. 2004. STATISTICA (data analysis software system). Version 7. www.statsoft.com.

Tabarelli, M.; Vicente, A.; Barbosa, D. C. A. Variation of seed dispersal spectrum of woody plants across a rainfall gradient in northeastern Brazil. Journal of Arid Environments, p. 197-210. fev. 2003.

Vieira, F. A.; Carvalho, D. de. Maturação e morfometria dos frutos de *Miconia albicans* (Swartz) Triana (Melastomataceae) em um remanescente de Floresta Estacional Semidecídua Montana em Lavras, MG. Revista Árvore, v. 33, p.1015-1023, 2009.

Zuffo, A. M; Gonçalves, L. G. V; Andrade, F. R.; Souza, T. R. S.; Marimon-Junior, B. H. Biometria de sementes de mangaba (*Hancornia speciosa* Gomes). In: IV ENAAG - ENCONTRO AMAZÔNICO DE AGRÁRIAS, 4, 2012, Belém. Biometria de sementes de mangaba (*Hancornia speciosa* Gomes). Belém: Anais do Encontro Amazônico de Agrárias, 2012.

DOI: 10.17707/AgricultForest.65.1.12

Filali LATRECHE, Mohamed BENIDIR,
Elhadi MECHENTEL, Khaled ABBAS, Sameh- SEBIHI [1]

ASSESSING AGRO-ECOLOGICAL AND ECONOMIC SUSTAINABILITY OF CEREALS-BASED CROPPING SYSTEMS IN SOUK AHRAS HIGH PLAINS (EAST ALGERIA)

SUMMARY

The food deficit that has prevailed for more than 50 years in the Algerian agricultural sector, the problem of land depletion due mainly to the regression of organic matter levels in soils generated by cereals fallow system, widely practiced in the Algerian semi-arid zones, and the evolution of the expectations of consumers, multiply to infinity the need to assess cropping systems sustainability. To reach this goal we have integrated 10 agro ecologic and economic indicators values into a global sustainability index (Sg) ranking from 0 to 1, applying continuous non-linear sustainability functions that use thresholds defining what is sustainable, unsustainable, or intermediate. Data needed to calculate the indicators was getting from face to face interview with farmers for 140 fields practicing 6 cereals-based cropping systems (cereal worked and pastured fallow, continues cereal, cereal potatoes, grain legumes or other crops) in Souk Ahras high plains region (east of Algeria).The evaluation results showed that cereal other crops and cereal potatoes systems have a better overall sustainability despite the fact that some of the indicators can be negatively impacted. Cereal worked fallow has the lowest sustainability. The high value of economic indicators; variable cost and gross income explain the first result, poor economic performance, and inadequate soil management make that cereal worked fallow has the lowest sustainability. Cereal grain legumes system is supported by the state as part of the program of resorption of fallow; but it has an intermediate durability, with high agro ecological performances, and moderate economic sustainability due to the lack of mastery of technical itinerary.

Keywords: cropping system, sustainability, assessment, indicators

INTRODUCTION

The emergence of large-scale phenomena such as food crises and environmental degradation around the world has resulted in the appearance of the

[1]Filali Latreche (corresponding author: filalila84@gmail.com), Department of Agricultural Sciences, University of Farhat Abbas Sétif1 (UFAS1). Research Laboratory Sciences and Technologies of the Living, institute of agro-veterinaries University of Souk –Ahras, ALGERIA; Mohamed Benidir, Agrosystems East Division of INRA Algeria – Setif, ALGERIA; Elhadi Mechentel, Department of Agricultural Sciences, Institute of Agro-Veterinaries Sciences, University of Souk Ahras, ALGERIA; Khaled Abbas, National Institute of Agronomic Research Algers (INRAA Alger), ALGERIA; Sameh- Sebihi, professional formation center SA, Algeria.
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

concept of sustainable development. Kane (1999), defines sustainable development as the development that meets the needs of the present without compromising the ability of future generations to meet their own needs. This new model of development is based on three main concerns: maintaining a viable economy, developing social projects and preserving the environment, resources and capabilities production (Payraudeau &Van der Werf, 2005). The millennium ecosystem assessment report, makes out the responsibility of agriculture: i) In the removal of ground and superficial water quality (nitrogen and pesticide pollution), soil (erosion and heavy metal contamination), and air (greenhouse effect); ii) In the loss of biodiversity (homogenization of habitat, mortality due to pesticides); iii) In non-renewable resources (fossil energy, nutrients); iv) In increasing disaster risk (flooding, flows mud).

In the south of the Mediterranean, the challenges of sustainable development require greater productivity per unit of available resources (input, water, energy), production methods are more adapted to the diversity of local constraints (Meynard, 2008). In Algeria, the main challenges for the agricultural sector are: increasing crop and livestock production to cope with population growth and fill the food deficit by mobilizing more resources; these are scarce and poorly exploited (Cherif et al., 2012) meeting the social expectations by increasing the contribution of this sector to employment and provide healthy products to consumers. The crucial situation of the Algerian agriculture, accentuates the need for an evaluation of existing cropping systems in order to determine the weak points and the strengths of these systems, to re-design or design new cropping systems those satisfied farmers, agronomists, decision makers and society attention's and meet the challenges of sustainable agriculture.

In the two recent decades, many indicator-based methods for evaluating the sustainability of agricultural systems at various scales have been developed (Binder et al., 2010, Bockstaller et al., 2009, Niemeijer & Groot, 2008). Recourse to indicators is useful for cropping systems assessment, designing and policies making at all scales of decision making. However, the development and application of suitable performance indicators to monitor change and sustainability have been subject to significant debate (Nortcliff, 2002& Girardin et al., 2000), generally five criteria are sought in a good indicator; it must be easy to implement, immediately understandable, sensitive to the variations of cultural practices and reflecting the reality of the field (Kristle Nathan & Sudhakara Reddy, 2010). Likewise, the choice of relevant indicators has the big importance in the evaluation process.

However, methods used for cropping systems assessments require the use of a multi criteria approach, taking into account the various components of sustainability; those methods may contain indicators that are irrelevant to the context of the region (groundwater pollution by nitrogen) or the scale of evaluation (IDEA method for farming system); and they need a parameterization and an adaptation to the local context by using a set of data which can be difficult to acquire. Differently from works that evaluate sustainability by using methods,

the aim of this paper was the assessment of agro ecological and economic sustainability of cereals-based cropping systems using a sustainability function in a semi-arid high plains region (Souk -Ahras) in the east of Algeria, to diagnose their strong and weak points and, on this basis, to encourage discussions during the design of innovative cropping systems that will afterwards be tested in fields.

MATERIAL AND METHODS

Study area and farms monitored

The study was conducted in the Souka- Ahars region (east Algeria) for two years (2015 and 2016).The region was selected because of their inner diversity based on two factors (a) the diversity of the environment, particularly according to the climatic degree of aridity and vegetation distribution. There are three semi-arid climate zones in the Souk- Ahras region: upper, central and lower. The average annual rainfall decreases from the upper to the lower zones (from 600 to 150 mm), while the inter-annual precipitation variability increases; (b) the diversity of cultivation systems practiced despite the dominance of cereal fallow system with these different types (worked , pastured or cultivated fallow).

Cropping systems management was monitored during two years (2015,2016), by using a structured questionnaire completed during face-to-face interviews with farmers. The structured schedules included seventy seven questions, designed to collect data required to calculate indicators; to describe the function of cropping system, the economic environment and to identify potentials and constraints facing the farmers. The 140 surveyed farms belong to the median zone where the climate is less intense (precipitation oscillates from 600 to 350mm. They represent 2 % of the total exploitation and cover a useful agricultural surface of 10688ha. 75% of the monitored farms combine between livestock and crop production. On the other hand 25 % practices only crop production.

Choice of the indicators

A set of ten indicators (table1) was selected after an extensive literature review described by Deytieux(2016), Bockstaller *et al*(2013), Bekhouche-Guendouz (2011), and Yakhelf *et al(2008).*The aim of the literature review and investigation of the sustainability assessment methods was to select pertinent indicators that quantify the effects of cropping systems management on agro--ecological and on economic profitability, using data that can be easily obtained (through face-to-face interviews).

Three criteria applied in the selection of indicators: (i) data availability (inputs for indicator calculation should be easily derived from farmer); (ii) synthesis (the indicator needs to summarize various aspects of the studied area, providing a good compromise between the description of the processes and their simplification into a single value); (iii) simplicity (the indicator needs to be easily calculated and interpreted. Indicators that require direct measures on soils, or crops were excluded.

Table 1. Agro-ecological, economic and soil management indicators used to evaluate cropping systems sustainability.

indicator name	**indicator acronym**	**indicator definition**	**reference**
Treatment Frequency Index	TFI	Number of registered doses of pesticides applied on a parcel for one cultural campaign.	Eckert et al (2000)
Diversity	Div	Minimum number of cultivation to cover ¾ of the area of the farm.	Turpin, et al (2010)
Rotational cropping	RC	Assessed by two factors: crop diversity and plot size.	Scholtus, & Bockstaller (2015)
Crop succession	CS	Calculation at the parcel level on the succession of the last four years.	Scholtus, & Bockstaller 2015
Soil Cover index	SC	Percentage of soil cover by crops in one year	Pervanchon, *et al* (2002)
Variable Costs	VC	Sum of the costs for gasoline, lubricants, pesticides, fertilisers, and seeds	-
Gross income	GI	Yield of harvested product multiplied by its price	-
Gross margin	GM	GI – VC	-
Economic efficiency	Ee	Ratio between gross product and total expenses multiply by 100	-
Energy consumption due to machinery	$E_{machine}$	Calculated first, at the plot scale, and then aggregated at the farm.	Donaldson, *et al* (1994)

The indicators are grouped in tree classes (Table 2). The economic index include variable costs (VC), gross income (GI), gross margin (GM) and the ratio between VC and GI; economic efficiency (Ee). Agro ecological index consists of crop succession (CS); its goal is the assessment of the cultural successions implemented in relation to the principles of integrated production to diagnose consistency of crop systems; which can influence the outcome of the other indicators, treatment frequency Index (TFI); this indicator reflects the intensity of pesticide use for one cultural campaign. Diversity (Div); it is defined as the minimum number of cultivation to cover ¾ of the area of the farm (Turpin *et al*.,2010). Rather this number is high, rather the biodiversity of the farm assumed

high. Energy consumption due to machinery ($E_{machine}$); the calculation of this indicator is done by estimating the energy for each tool passage using an adapted equation developed by Donaldson *et al* (1994). Soil management class was represented by soil cover index (SC); assessed by two factors: crop diversity and plot size; this indicator gives an appreciation for biodiversity and landscape, and rotational cropping

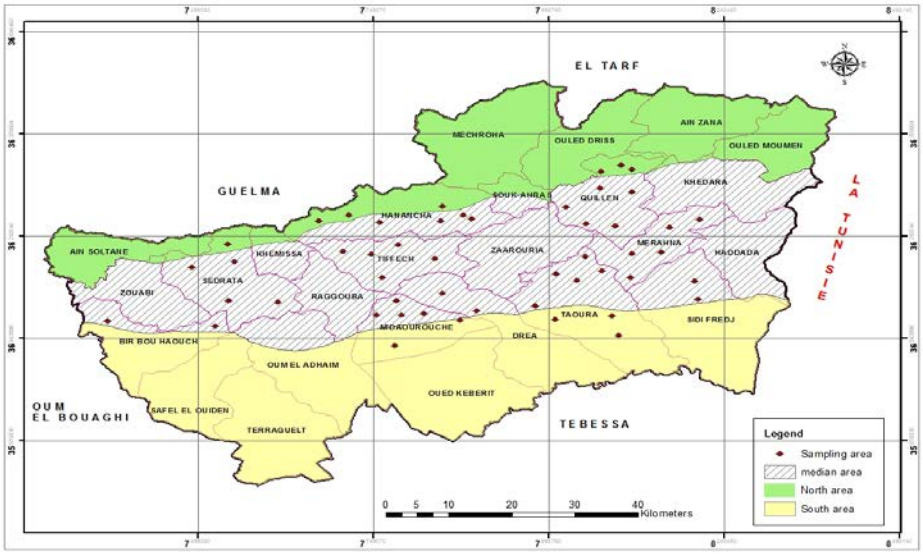


Figure 1. Location of the study area within Souk-Ahras province. 1/500000 Source: Author's own elaboration

Application of the methodology

The sustainability values of the indicators (Si), for the 6 cropping systems ware calculated according to the sustainability function described by Castoldi, And Bechini (2010). The used parameters are given in Table 2. Whenever possible the thresholds were determined by referring to the bibliography for certain indicators (variable cost and cross margin), or using statistics from the measured distribution in the fields monitored. The assignment of the K value is done in such a way that the derivative of the durability function needed to be low close to the optimum range for these indicators and higher at the extremes (a variation of the indicator close to the optimum range does not affect sustainability); for that a value of k equal to 0.5 was attribute for GI. GM. Ee. and TFI. Contrarily value of k equal to two was assigned to soil cover index to make variability of sustainability more pronounced and influenced by SC index.

Each Si calculated for the 140 fields were aggregated into tree values of Sc; these last were gathered into a unique index (Sg). The sets of weights used to aggregate indicators sustainability (Si) into sustainability class (Sc) (figure 2 (a)) then to global sustainability (Sc) (figure 2(b)), were defined by face-to-face

interviews with 26 stakeholders (farmers, agronomic, researchers, agronomists, decision-makers, and environmentalists).

Table2: Parameters used to calculate the sustainability functions for the economic and agro-ecological indicators.

Indicator	indicator acronym	unites	S_{min}	S_{optL}	S_{optU}	S_{max}	k
Economic calss							
Variable Costs	VC	DA ha^{-1}	-	-	35908.3	43630.5	1
Gross income	GI	DA ha^{-1}	45308.9	61322.2	-	-	0.5
Gross margin	GM	DA ha^{-1}	1029.6	19872.9	-	-	0.5
Economic efficiency	Ee	%	1.66	4.62	-	-	0.5
Agro ecological calass							
Crop succession	CS	0-7	1	7	-	-	1
Treatment Frequency Index	TFI	IFT point	-	-	0.73	0.99	0.5
Diversity	Div	0-4	1	2	-	-	1.0
Energy consumption due to machinery	$E_{machine}$	MJ ha^{-1}	2493.90	2688.80	-	-	1.0
Soil management calss							
Soil Cover index	SC	0-1	0.38	0.48	-	-	**2.0**
Rotational cropping	RC	0.5-10	1	7	-	-	1.0

*Smin: minimal sustainability. S_{optL}: Law optimal sustainability. S_{optU}. Upper optimal sustainability. Smax maximal sustainability. K: coefficient.

RESULTS

Indicators values

The typology of the cropping system according to crop succession makes out six groups: the head of de crop rotation is always a cereal succeeded by a worked fallow, pastured fallow, grain legumes (lentils or chickpeas), potatoes, other crops (gardening) or continues cereals. The results of the calculation of ten agro-ecological and economic indicators for 140 cropping systems are presented in table 3. The variability of the calculated indicators among succession types was moderate for the first four cropping systems, but it was very high between cereals potatoes, cereals gardening succession and the first four successions. Within succession variability was high except for the soil cover index (table 3).

Continuous cereals (Cc) was the succession with the lowest crop succession indicator (CSI=0.4) followed by cereals worked and pastured fallow (table 3), for the rotational cropping indicator, cereals grain legume (Cgl)

recorded the highest values (5.5). The treatment frequency index, which represents the intensity of pesticide use for a given crop during a farming season, raises the abuse of pesticides for the cereal-gardening system (3.8) with the use of the insecticide and the fungicide and low pesticide use for the cereal - grain legume system (0.69); this shows a benign ecological effect (reduced use of pesticides) for cereal grain legume rotation. Energy consumption due to machinery varied from 2523.1 MJ ha-1 for cereal pastured fallow Cpf to 2923.1MJ ha-1 for cereals potatoes Cp.

Table 3. Average and standard deviation (in parenthesis) of indicators calculated for 140 fields monitored over two years period in Souk-Ahras region (eastern Algeria)

	Farms number / Indicators	cereals worked Fallow (Cwf) 19	cereals pastured fallow (Cpf) 39	continues cereals (Cc) 38	cereals grain legumes (Cgl) 15	cereals potatoes (Cp) 6	cereals other crops (Coc) 23
(a)	Variable Costs (DA ha $^{-1}$)	45795.2 (53898.7)	37678.7 (12626.6)	33909.1 (13681.4)	36479.4 (14036.1)	198511.9 (235781.0)	81707.7 (179135.8)
	Cross income (DA ha $^{-1}$)	37032.4 (42505.2)	34315.1 (15538.6)	47419.3 (24513.7)	87955.1 (48720.2)	3577120.7 (5388948.4)	211229.5 (647986.2)
	Gross margin (DA ha $^{-1}$)	14101.9 (16598.1)	13010.4 (10531.6)	15401.2 (14375.9)	27880.7 (23228.4)	39719.9 (5153862.6)	55056.7 (124924.1)
	Economic efficiency (%)	4.5 (4.9)	5.4 (6.8)	3.9 (3.1)	4.1(4.7)	48.3(71.4)	11.4(16.8)
(b)	Crop succession (0-7)	0.5(0.1)	0.5 (0.0)	0.4(0.1)	2.2(0.8)	2.1(1.1)	1.7(0.6)
	Treatment Frequency Index-point	1.0(1.6)	0.77(0.7)	0.9(1.9)	0.69(0.45)	1.2(0.6)	3.3(11.1)
	Diversity (0-1)	2.0 (0.8	1.8(0.7)	1.9 (0.7)	2.53(0.83)	2.7(1.0)	2.0(0.7)
	Energy consumption due to machinery (MJ ha^{-1})	2713.08 (451.1)	2630.0 (418.7)	2676.6 (542.6)	2924.4 (514.7)	2523.1 (329.0)	2900.9 (419.7)
(c)	Soil Cover index (0-1)	0.5 (0.0)	0.5(0.0)	0.4(0.0)	0.4 (0.0)	0.4 (0.0)	0.4(0.1)
	Rotational cropping (1-7)	3.0(2.4)	3.1 (2.2)	3.4(2.2)	5.5(2.6)	3.8(1.9)	3.8(2.2)

(a)Economic indicators. (b) Agro-ecological indicators. (c) Soil management indicators

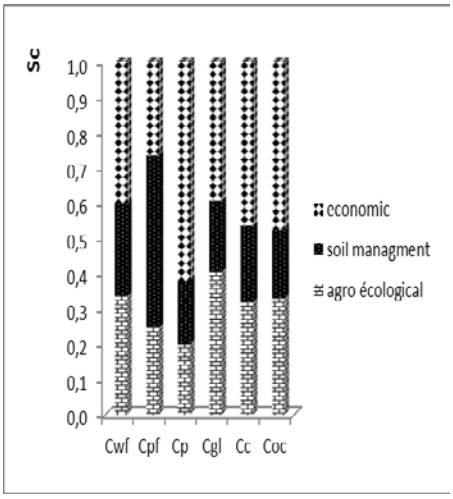
Sustainability values.

Variability within crop succession types

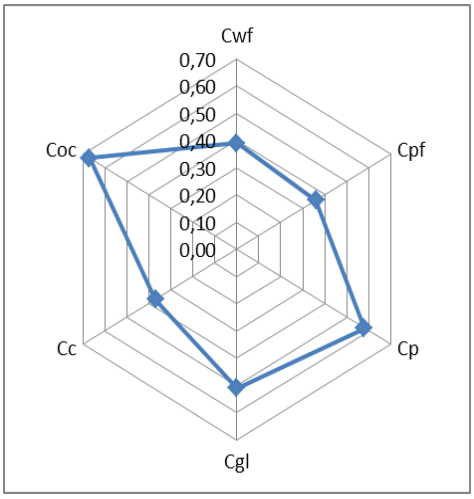
With the exception of cereal pastured fallow and cereal potatoes, the variability of sustainability within succession types was low (figure 3). This was predictable because of narrow variability in the indicator values (table 3) and the similarity of crop management.

Comparison the global sustainability according to crop succession

Despite having substantial differences in all economic indicators, cereal potatoes have the biggest value for the economic sustainability (figure2 a), and therefore it holds the highest durability (figure 2 b). For soil management (represented by crop succession and soil cover index), Cpf was the best succession type, the other successions have low sustainability values (lower than 0.34). However, for agro ecological indicators, Cgl has the biggest sustainability (0.69) with very high diversity and crop succession (figure 2a), These results can be explained by close values of indicators constituting this scale of sustainability; this scale has the worst values of durability, with values of Sc reaching zero for some fields.



a)



b)

Figure 2. a) Contribution of each indicators classes to global sustainability: Cwf: cereal worked fallow. cereal pastured fallow. Cp; cereal potatoes. Cgl; cereal grain legums Cc; continues cereal and Coc; cereal other crops) b) global sustainability for the 6 cropping system.

Weighted global sustainability index

As indicated in the methodology the global sustainability index Sg was calculated according to the sets of weights (researchers. agronomists. ecologists. decision-makers. and farmers.). The resulting rankings of the six cropping systems are reported in table 4.

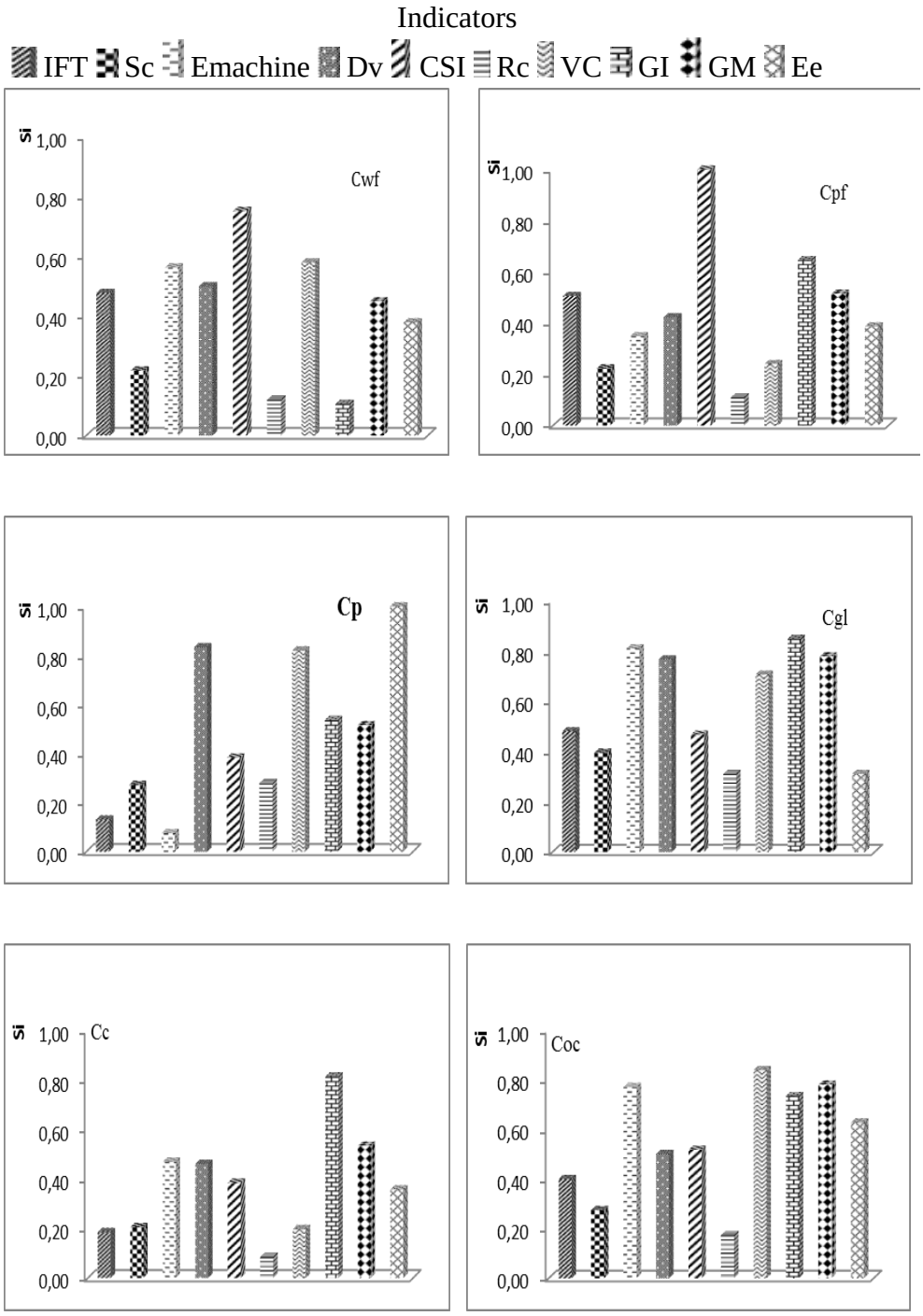


Figure 3: Contribution of the indicators to the global sustainability index (Sg) for the six cropping systems.

Table 4: Ranking of six crops successions types based on global sustainability index (Sg) calculated with six different sets of weights.

stakeholders / cropping systems	equal weights	researchers	agronomists	Ecologist	decision-makers	farmer	Average rank
cereals worked Fallow	3	3	3	2	3	1	2.5
cereal pastured fallow	5	1	5	4	4	2	3.5
continues cereals	2	2	1	1	2	3	1.8
cereals grains legumes	1	5	6	3	6	4	4.2
wheat potatoes	6	6	2	6	1	6	4.5
cereals other crops	4	5	4	5	5	5	4.7

For agronomist due to the larger weight assigned to the agro ecological and soil management indicators, cereal grains legumes was ranked in the first; likewise cereal potatoes was ranked in the first by farmers decisions makers and ecologist this ranking is due to the high weight attributed to economic indicators. Depending on the weights the cereal other crop successions were given different rankings by different stakeholder groups. Continuous cereal is the worst system with an average ranking equal to 1.8 (normally scored very poorly, due to low values for most indicators) followed by cereals worked fallow. Contrariwise, cereal rotated with other crops was ranked fifth by researchers, ecologist decision-makers and farmers, due to a better economic and agro ecological performance of other crops, while for the other stakeholders it was fourth.

The contribution of the 10 indicators to the global sustainability index Sg is reported in Figure3. It appears that variability of cropping system sustainability among crop successions is moderate due to difference existing in the mode of production which influences the value of indicator, and weights attributed by some stakeholder (table 4). For farmers for example: coefficient of variation of Sg = 17%), while for other groups it was higher (researchers 27, agronomists. 30%). According to the assigning weights the lowest average Sg (0.55) was calculated for agronomists and the highest (0.61) for researchers, decision-makers and ecologists.

DISCUSSION

Thresholds for the sustainability functions and stakeholder groups

It is clear that a large degree of uncertainty was inherent in our analysis. The choice of thresholds made sustainability functions somewhat subjective

using the median and the quartiles. The verification of the adaptability of the sustainability equation was done by re-recalculating ranks of the 6 crop successions by defining Smin. and Smax. using the 10th and 90th percentiles as a substitute of first and 3rd quartile. We always find that Si was higher (using the new thresholds) in the partially sustainable range compared to the case when the old thresholds were used; likewise Sg was always equal or higher with the new thresholds (data not shown).Continues cereal remained the worst succession for most stakeholder groups. Cereal worked fallow has an intermediate position among the six cropping systems.

From all that is said, it turns out that the choice of thresholds is a delicate and decisive phase that requires further research to carry out a task of evaluation of the sustainability of cropping systems. Statistical distribution of the indicators is a common practice for determining maximum values of indicators. For example, Reig-Martinez *et al.*(2011) recommend the use of values that are consistent with the context and performance of cropping systems taking into account the statistical distribution, and the standards of the region where the evaluation takes place. Liebig *et al.*(2001) , defined the optimum value as the highest (or lowest depending on the type of indicator) value measured in the population. The strong point of this method is the use of quartiles to set thresholds as it was the case for the agro- ecological index (except the crop succession indictor), for the economic index (except economic efficiency) and for the soil index management (except rotational cropping indicator),thus giving a more or less different distribution of Sc; which is the result of the compensating effect between the indicators constituting the three sustainability indexes (economic. agro ecological an soil management). For example, potatoes and other crop (vegetable) required high economic inputs (and therefore had low Si for VC), but giving high Si for GM. This provided a good value of Sc for cereal potatoes and cereal other crops (Figure 3),which was not too different compared to that calculated for cereals grains legumes Cgl. Among classes we can make distinction between “very good “ ,“good”, “moderate” and “bad” systems according to values obtained for each class by using quartile limitation

Comparison of crop successions

From the 6 cropping systems, cereal other crops and cereal potatoes are the leading systems, with a very high economic income due to the added value of the vegetables and potato given by price fluctuation in favour of the farmers over the past few years. The second year of cereal, which is durum wheat the technical itinerary, includes only one or two passes through the disc harrow to have finally yields higher than three to five faith to national average (Benniou & Aubry,2012) , farmers are aware of the draining effect of the potatoes and vegetables; they bring in high amounts of fertilizers and organic matter; they have low surface in the land if they do not rent land to produce the potato. The compensation effect is very clear between a high pressure of the pesticide recommended to control diseases (the seasonal potato and vegetables are susceptible to diseases especially fungal diseases for potatoes and pest for vegetables).(Rousselle & Ropert, 1996)

and the consumption of energy in the potato crop and the gross margin provided by this crop. In our study the number of farms that practice potato cultivation is low (6) because this culture is very demanding in terms of financial and water resources, the installation of an irrigation network (irrigated perimeter) and the use of local seeds can change the situation in the coming year.

In terms of abundance, cereal pastured fallow systems (39 farms) and continues cereals (38 farms) occupy the lion's share of the surveyed farms. Fallow is an integral part of the systems cereals-sheep production in the semi-arid zone, characterized by fragile soils and limited rainfall. The share of the worked fallow decreases while that of the pastured fallow increases and would represent 9% of the total forage supply (Abbas & Abdelguerfi, 2005). On the sustainability side, this system ranks fourth after Cp, Coc and Cgl, and it is characterized by low economic performance.

The continuous cereal is concentrated in the farms that rent the land or that work in association with the owners of the land this rotation is in this case imposed by the owner. Low yield which results in a low economic performance, massive recourse to pesticides (herbicide), low contribution to employment, a very low diversity, a rather low rate of soil cover in winters are the most characteristics of this system, the conjugation of those factors make this system at the least in terms of sustainability (table 3. figure 2b). Land tenure is therefore the guarantor of the continuity of this system and not their agro-ecological or economic performances.

The cereal grain legumes system occupies the third place and shows agro-ecological and economic performances (figure3). The first year, the cereal is conducted by a summary manner; with a deep tillage, P.K fertilizers, surface tillage and a nitrogen fertilization of 120 kg on average (Benniou & Aubry,2012) the second year, the legume is also conducted in a summary manner; the poor economic performance of the cereal legume system is justified by low yields of legumes, despite the high price of grain legumes (Daoudi, & Wampfler 2010). Agro-ecological effects appear in the following year related to a minimum tillage and a reduction of the nitrogen fertilization. The fallow reduction program adopted by the state which aims to replace fallow land with a grain legume in order to improve the value of agricultural land, favourite the adoption of this system by the farmers; while lack of mastery of the legume's technical itinerary, seed shortage and lack of appropriate harvesting equipment can hinder the sustainability of the system.

Although fallow has been rotated for purely agronomic purposes (Sebillotte, *et al* 1993) whose main purpose is to conserve moisture, but this role is conditioned by soil depth, precipitation volume and plowing. Worked fallow only allows water storage (more than 60 cm) if spring tillage is carried out sufficiently early (January-February); before the onset of drought and only if the soil is heavy (clay) and quite deep; in addition re-cropping is essential if rain is late to reduce the effect of weeds and creates mulch. However these conditions are not often met in the Algerian cereal zones characterized by low and irregular

rainfall and especially by shallow soils (Fenni 2013; Abbas & Abdelguerfi, 2005). This is reflected in low economic efficiency in our study, which is the result of poor performance. The excess of yield linked to the fallow cannot cover the year of fallow. If we calculate the average of the economic gains over the two years of the rotational cereals worked fallow the economic efficiency becomes 2.25, the lowest values compared to the others.

CONCLUSIONS

This paper introduced a novel method to assess and systematically compare cropping systems, this method is based on sustainability function; its application was demonstrated for 6 cereals – based cropping systems in Souk Ahras high plains (east Algeria). The highest sustainability was assigned to cereal other crops, followed by cereal potatoes system; due to good economic performance (high incomes). However both systems have low agro¬ ecological sustainability due to increased pesticide use and high energy consumption and treatment frequency; high values of these indicators affect negatively durability. The cereal grain legumes system has a good durability increased by the agro ecological effect linked to the introduction of a leguminous in the rotation. This system is very appreciated by the farmers, in addition it concord with the strategy of the State which aims is to replace fallow land with a grain legume. Cereal pastured and worked fallow have low durability with a priori for cereal pastured fallow; but the edapho climatic conditions, the socio-economic context and the vocation of the region ensure the persistence of these two systems.

ACKNOWLEDGMENTS

We would like to kindly acknowledge the contribution of all the farmers interviewed during this work for their time and their patience during the sometime tedious work of data collection over two years. We also thank the stakeholders interviewed

REFERENCES

Abbas, K. & Abdelguerfi, A. (2005). Perspectives d'avenir de la jachère pâturée dans les zones céréalières semi-arides. Fourrages. 184 : 533-546.

Bekhouche-guendouz, N., (2011). Evaluation de la Durabilité des Exploitations Bovines Laitières des Bassins de la Mitidja et d'Annaba. National Polytechnic Institute of Lorraine and National School of Agronomy (Algeria) Ph.D Thesis. pp. 310.

Benniou, R.& Aubry, C. (2012).Farm diversity and crop growing practices in semi-arid regions: A case study of the Setif high plains in Algeria. African Journal of Agricultural Research, 7:(48) 6363-6375. DOI: 10.5897/AJAR09.603.

Binder , R.C. , Feola, G. J., & Steinberger, K. (2010). Considering the normative. systemic and procedural dimensions in indicator-based sustainability assessments in agriculture, Environmental Impact Assessment Review. 30: 71-81 DOI: 10.1016/j.eiar.2009.06.002.

Bockstaller, C., M. Cariolle, M.B. Galan, L. Guichard. et al., (2013). Evaluation agri-environnementale et choix des indicateurs : acquis, enjeux et pistes. Innovations Agronomiques. 31:1-14.

Bockstaller. C., L. Guichard. O., Keichinger, P. Girardin, M. B. Galan, and G.Gaillard, (2009). Comparison of methods to assess the sustainability of agricultural systems, a review. Agronomy Sustainable Development, 29:223-235. DOI: 10.1051/agro:2008058.

Castoldi, N. & Bechini, L. (2010). Integrated sustainability assessment of cropping systems with agro-ecological and economic indicators in northern Italy. Europeen Journal of Agronomy. 32 : 59-72. DOI:10.1016/j.eja.2009.02.003. 175061/2/207.

Cherif, O., Jean-yves, M. & Arlene, A. (2012) . L'agriculture algérienne face aux défis alimentaires. Trajectoire historique et perspectives». Revue Tiers Monde. 2 :123-141 .

Daoudi, A. and B. Wampfler, (2010). Le financement informel dans l'agriculture algérienne : les principales pratiques et leurs déterminants. Cahier d'Agriculture. 19: 43-48. DOI : 10.1684/agr.2010.0414.

Deytieux, V., Munier-jolain N. & Caneill, J. (2016). Assessing the sustainability of cropping systems in single- and multi-site studies. A review of methods. Europeen. Journal of Agronomy.72: 107–126. DOI: 10.1016/j.eja.2015.10.005.

Donaldson, J.V.G., Hutcheon J.A. & Jordan, V.W.L. (1994). Evaluation of energy usage for machinery operations in the development of more environmentally benign farming systems. In Aspects of Applied Biology. 40: 87-90.

Eckert, H., breitschuh, G. and Sauerbeck, D.R. (2000). Criteria and standards for sustainable agriculture. J. Planet Nutrient . Soil Science. 163 :337 -351 .DOI :10.1002/1522-2624.

Fenni, M. (2013) .Impacts of climate change on cereal production in the setif high plains (north-east of algeria). Impacts and Solutions to Global Warming. 1:225-231 DOI :10.1007/978-1-4614-7588.

Girardin, P., Bockstaller, C. and Van der werf, H. (2000). Assessment of potential impacts of agricultural practices on the environment: the AGROECO method

Environement. Impact Assessment. Review,20: 227-239. DOI: 10.1016/S0195-9255(99)00036.

Kane, M. (1999). Sustainability Concepts: from theory to practice in Elgar.E (Ed.) Köhne. J. Gowdy. J. Henterberger. F. Straten. J.V. Sustainability in question: the search for a conceptual framework (advances in ecological economics) bookcraft(Bath)Ltd Publishers pp. 15-31.

Kristle Nathan, H. S. & Sudhakara Reddy, B. (2010). Selection Criteria for Sustainable Development Indicators. Indira Gandhi Institute of Development Research, Mumbai available at http://www.igidr.ac.in/pdf/publication/WP-2010-013.pdf.

Liebig, M.A., Varvel, G.E. & Doran,J. (2001). A simple performance-based index for assessing multiple agroecosystems functions. Agronomy. Journal 93 : 313-318. DOI: 10.2134/ agronj 2001.932313x.

Meynard, J.M. (2008). Produire autrement :réinventer les systèmes de culture in Reau. R& Doré T. Système de culture innovant et durable. (pp11-27) Educagri Publisher. France.

Niemeijer, D.& Groot, R.S. de(2008). A conceptual framework for selecting environmental indicator sets . Ecological indicators. 8:14- 25 DOI: 10.1016/j.ecolind.2006.11.012.

Nortcliff, S. (2002). Standardisation of soil quality attributes Agriculture. Ecosystems and Environment. 88:161-168 .PII: S0167-8809(01)00253-5.

Payraudeau S., Van der Werf H. M.G. (2005) . Environmental impact assessment for a farming region: a review of methods, Agriculture, Ecosystems and Environment 107: 1-19.

Pervanchon, F., Bockstaller C. & P. Girardin, (2002). Assessment of energy use in arable farming systems by means of an agro-ecological indicator: the energy indicator. In Agricultural Systems. 72: 2 149-172 .DOI:10.1016/S0308-521X(01)00073-7.

Reig-Martinez, E., Gomez-limon J.A. & Picazo-tadeo, A.J. (2011). Ranking farms with a composite indicator of sustainability. In Agriculture Economic 42: 561-575. DOI.10.1111/j.1574-0862.2011.00536.x.

Rousselle, P. & Ropert, Y. (1996). La pomme de terre production amélioration. ennemis et maladie utilisation Crosnier J.C publisher.pp.625.

Scholtus, M.T. & Bockstaller, C. (2015). Using indicators to assess the environmental impacts of wine growing activity: The INDIGO® metho. In European Journal of Agronomy. 62 :13-25 DOI: 10.1016/j.eja.2014.09.001.

Sebillotte, M., Allain, S. Dore T. & Meynard, J.M. (1993). La jachère et ses fonctions agronomiques. économiques et environnementales. Diagnostic actuel». Le Courrier de l'Environnement. 20 :11-21.

Turpin, N., Stapleton, L. Perret. E., Van der heide C.M et al., (2010). Assessment of multi-functionality and jointness of production. In: Brouwer F., Van Ittersum. M.K. (eds.). Environmental and agricultural modelling: integrated approaches for policy impact assessment. Dordrecht : Springer Publisher. pp. 11-35.

Yakhelf, H., FAR. Z., Ghozlane. F., Madani, T. & Marie, M. (2008). Evaluation de la durabilité des systèmes agropastoraux bovins dans le contexte de la zone semi-aride de Sétif (Algérie). NEW MEDIT. no. 4/2008.

DOI: 10.17707/AgricultForest.65.1.13

Mykola NAZARENKO,
Svitlana MYKOLENKO, Vadim CHERNYSKY [1]

MODERN UKRAINIAN WINTER WHEAT VARIETIES GRAIN PRODUCTIVITY AND QUALITY AT ECOLOGICAL EXAM

SUMMARY

The objectives of our investigations are to describe the variation of the main groups of modern winter wheat varieties (19 varieties, check is a national standard by grain productivity, Podolyanka) due to their interactions with environmental conditions by agronomic-value traits like as general grain productivity, components of one, protein and gluten content, developing relations between once (correlation relations), which determining wheat quality and yield in a complex. Second our purpose to estimate asset of winter wheat accessions and appear a useful diversity in comparison of modern varieties. Nineteen winter wheat genotypes have been investigated under regional conditions. Only one genotype surpassed standard in by agronomic-value traits on higher value and only one too have shown its traits in complex on standard level. Regarding to our investigations, ecological exam is necessarily to clarify true adaptability and suitability of winter wheat variety for regional conditions.

Keywords: winter wheat, variety, grain productivity, quality, ecological exam.

INTRODUCTION

With the annual production of about 757 million tons (in 2017) (USDA, 2018), bread wheat (Triticum aestivum L.) is one of the world's most important cereal crops. Winter wheat is the world's leading cereal grain and the most important food crop, occupying commanding position in Ukraine. Ukrainian agriculture takes about 48% area under cereals and contributing 38% of the total food grain production in the country (Nazarenko, 2015). Until the end of the 19th century, cultivars were mainly landraces that were well suitable to their regional ecological conditions. Since the beginning of the 20th century, as breeding methods have developed, landraces have been used as a source of variability in creating modern cultivars by classical breeding methods (Bordes et al, 2008). In the last 60 years intensive plant breeding programs led to the total replacement of landraces by modern semi-dwarf and high-yielding varieties, correlating with a

[1]Mykola Nazarenko (corresponding author: nik_nazarenko@ukr.net), Department of Plant Breeding and Seeds Production, Dnipro State Agrarian and Economic University, 49600, Dnipro, UKRAINE; Svitlana Mykolenko, Department of Agricultural Products Storage and Processing Technologies, Dnipro State Agrarian and Economic University, 49600, Dnipro, UKRAINE; Vadim Chernysky, Laboratory of mathematic data modeling and information technologies, Institute of Bioenergy Crops and Sugar Beet of NAASU, 03141, Kyiv, UKRAINE

Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

decrease in wheat genetic diversity and needs in special requirements for realization their potential higher grain productivity and protein quality (Nazarenko and Kharitonov, 2016, Nazarenko, 2017). But in spite of increasing total grain productivity tolerance to the special ecological demands of new varieties have been decreased, what, consequently, influencing on the future adaptability and special interactions with environment of winter wheat (Nazarenko and Lykholat, 2018).

In the past wheat researches was more tried to improve general grain productivity of the crop, last twenty years focused more on grain quality, but winter wheat breeders ignored special adaptability fore regional specific conditions (like as Northern Steppe of Ukraine). By conditions in terms of our investigations we mean the special combination of insufficient of water in critical growing stages which combined with high temperature and hard winter conditions. These combinations determine the properties of wheat yield and the quality of grains (Dawson et al, 2011). These agricultural-value traits in interaction actually determine the overall varieties of wheat whether good or poor for farming (Gepts, Hancock, 2006). Winter wheat yield has the most important and complex character affected directly or indirectly by gens systems present in plant (Rangare et al, 2010) as well as interaction with environment (Tester and Langridge, 2010; Serpolay et al, 2011). This has been in response to the pressure for an adequate food supply caused by constantly increasing population in Ukraine and the world as a whole (Martynov and Dobrotvorskaya, 2006; Mba et al, 2012). Therefore, ecological estimation of new wheat varieties with high yield genetic potential under regional conditions, it`s components and quality traits (Slafer and Andrade, 1993) has become a permanent purpose in the plant farming and breeding programs (Reif et al, 2005; Tuberosa and Salvi, 2006, Nazarenko and Lykholat, 2018).

Disequilibrium in influence of different nature-agricultural factors and their interactions of region determine distinguishes summarized in different genotypes grain productivity and quality (Kharytonov et all, 2017). Due to this fact we investigated varieties main agricultural-value traits under regional conditions. They determined balance of moisture, character of winter wheat growth and development, differences in seasons conditions, interaction between types of variety development (terms and specify of development stages) (Andrusevich et al, 2018).

Focused on only yield traits we have to understand that any high yield has no sense without proper quality for food and fodder demands. In mature grain, 10–15% of the dry mass is protein. Grain storage proteins (mostly gliadins and glutenins) include about 60–80% of the total protein in wheat grains and metabolic proteins, remaining part consists of the albumins and globulins (15–20%) (Dai et al, 2015). Grain storage proteins actively produce by plants during the effective filling phase of plant development (Shewry et al., 2012, Bonnot et al, 2017). Thus, the grain storage proteins of winter wheat determines its economics value.

The objectives of our investigations are to describe the phenotypic variation of the main groups of modern winter wheat varieties due to their interactions with environmental conditions by agronomic-value traits like as general grain productivity, components of one, protein and gluten content. The most target objects are developing relations between once (correlation relations), which determining wheat quality and yield in a complex. Second our purpose to estimate asset of winter wheat accessions and appear a useful diversity in comparison of modern varieties. To appreciate the interest of researches in the vast geographical representation of wheat varieties, we compared the diversity of several directions of winter wheat breeding in Ukraine from difference regions of the country with great discrepancy in natural conditions and selection purposes in breeding process. All varieties in our investigation were harvested in a location suited to growing wheat, recommended to North Steppe district as suitable for agriculture in this region. Main agronomic-value traits were determined and analyzed.

MATERIAL AND METHODS

Experiments were carried out on the experimental fields of Dnipropetrovsk State Agrarian and Economic University. The field`s geographic coordinates are: 48°30'N lat. and 35°15' E long. The experimental field is lied on 245 meters above the sea level. The air temperature during winter wheat growing season (September - July) is 8 - 11 °C, the average rainfall is about 350 - 550 mm in similar vegetation season. The field station of Dnipropetrovsk State Agrarian and Economic University use for many years (start from 60^{th} years of twenty century) as an area for intensive agricultural farming and researches (Kharytonov et.al, 2017). It is located far away from the city Dnipro (about 30 km) enough to avoid industrial or town airpollution effects.

Winter wheat seeds were procured form department of breeding and seed farming of DSAEU. The recommended intensive agronomic practice was followed. Evaluation of total grain yield per plot was calculated from 2017 to 2018 years. The trial at ecological winter wheat varieties exam was set up at a randomized block design method with three replications and with a plot size of 10 m^2 in 3 replications. The controls were national standard by productivity 'Podolyanka' and initial variety. Data on yield structure components (plant height, number of productive culms, number of grain per spike, grain weight per spike and plant, 1,000 grains weight) were taken from 50 randomly selected plants of each line representing properly morphological traits for this variety.

Wheat samples were held at room condition at 18 - 20 for several days before grinding. Each sample of 30 g weigh was separately ground on a laboratory cyclone grinder (LMT-1, PLAUN LLC, Russia).

Mathematical processing of the results was performed by the method of analysis of variance, the variability of the mean difference was evaluated by Student's t-test, cluster and correlation analyses was conducted by module ANOVA. In all cases standard tools of the program Statistica 8.0 were used.

RESULTS AND DISCUSSION

Analysis of grain productivity and its structure

Under field conditions, measurements were recorded grain yield, main components of grain productivity such as number of productive culms, number of grains, grains weight of 1000 kernels, weight of grains from one main spike, weight of grains from m^2 (table 1 – 2). Standard error (±SE) values of these varieties like as average mean and standard deviation are at tables too.

Table 1. Components of winter wheat grain productive structure

Number	Variety	Number		Weight of grains		
		Number of productive culms, pcs.	Number of grains, pcs.	Weight of 1000 grains	From 1 spike, g.	From m^2, g.
1	Voloshkova	463	32*	34,2	1,09	505
2	Novosmuglyanka	555	17	48,6*	0,82	455
3	Smuglyanka	531	17	46,8	0,79	422
4	Spivanka	440	27*	47,2*	1,27*	560
5	Podolyanka, st	580	22	42,6	1,00	580
6	Komerciyna	391	26	47,2	1,22*	476
7	Ednist	505	26	36,6	0,94	476
8	Spasivka	368	26	48,8*	1,27*	467
9	Bogdana	460	19	48,4*	0,91	420
10	Kolyadka	515	25	44,2	1,12	578
11	Lodizhinka	416	23	43,6	1,02	425
12	Gorodnicya	470	22	49,4*	0.89	417
13	Garantiya	401	26	48,0*	1,23*	495
14	Melodiya	373	25	44,6	1,12	418
15	Zluka	440	22	48,0*	1,06	468
16	Gileya	400	24	53,0*	1,27*	510
17	Mudrist	420	29*	47,8*	1,39*	582
18	Svitanok	412	26	43,4	1,12	462
19	Selevita	507	24	45,2	1,09	553
	Average	455	24	45,7	1,09	488
	Std. deviation	62	4	4,4	0,17	58

* - difference is statistically significance from check at $P_{0.05}$

The results on number of productive culms, number of grains, grains weight of 1000 kernels, weight of grains from one main spike, weight of grains from m^2 derived from varieties and compared with national standard Podolyanka (line 5 at table) are tabulated (Table 1). Next genotypes have been developed by these traits due to high its level (more than standard) – varieties Voloshkova, Spivanka, Mudrist by number of grains from main spike (first and third varieties are corresponded to Forrest-Steppe type, which adapted to most humid conditions, Spivanka is corresponded to direct Steppe type), by weight of 1000 grains varieties Novosmuglyanka, Spivanka, Spasivka, Bogdana, Gorodnicya, Garantiya, Zluka, Gileya, Mudrist (varieties Spivanka, Garantiya are Steppe ecotype, other to Forrest-Steppe), by weight of grains from main spike varieties Spivanka, Spasivka, Garantiya, Gileya, Mudrist (varieties Spivanka, Garantiya

are Steppe ecotype, other to Forrest-Steppe), weight of grains from m^2 we can find only genotypes on level of standard, but not higher.

Table 2. Winter wheat varieties grain productivity

Number	Variety	Percent of grains in total productivity	Yield, t/he (average, 2017 – 2018)	Number of cluster by grain yield
1	Voloshkova	37,9	5,05	2
2	Novosmuglyanka	27,9	4,55	2
3	Smuglyanka	29,4	4,22	3
4	Spivanka	41,5	5,60*	1
5	Podolyanka, st	42,7	5,80	1
6	Komerciyna	38,7	4,76	2
7	Ednist	42,0	4,76	2
8	Spasivka	38,9	4,67	2
9	Bogdana	38,8	4,20	3
10	Kolyadka	40,3	5,78*	1
11	Lodizhinka	32,7	4,25	3
12	Gorodnicya	36,8	4,17	3
13	Garantiya	40,1	4,95	2
14	Melodiya	40,5	4,18	3
15	Zluka	35,1	4,68	2
16	Gileya	40,3	5,10	2
17	Mudrist	38,8	5,82	1
18	Svitanok	36,0	4,62	2
19	Selevita	36,9	5,30*	1
	Average	37,7	4,81	--
	Std. deviation	4,0	0,54	--

* - difference is statistically significance from check at $P_{0.05}$

Summarized these dates next varieties have been identified as more perspective by these traits in complex Spivanka, Garantiya (Steppe ecotype, breeding special for Steppe conditions), Spasivka, Gileya, Mudrist (Forrest-Steppe ecotype). Differences of ecotypes are characterised by plant architecture and terms of several stages (date of critical stages like as evidence of spike are earlier than for other types and more suitable for higher quantity of water). We cannot see valuable forms by so key for yield characteristics as number of productive culms and grain weight from m^2. Grain productivity and percent of grains weight in a total productivity (on other way – coefficient of yield efficiency) are represented at table 2.

As we can see from the table, we could not develop genotypes with general grain productivity more than for standard Podolyanka. After cluster analyse we can subdivided all varieties on three type: 1 cluster for forms which

productivity on a level of standard with stable meaning (Spivanka, Kolyadka, Mudrist, Selevita), 2 cluster for forms with grain productivity significantly lower than Podolyanka (and cluster 1 at general), but with possibility in some years be on this level (Voloshkova, Novosmuglyanka, Komerciyna, Ednist, Spasivka, Bogdana, Garantiya, Zluka, Gileya, Svitanok), 3 cluster for forms with grain productivity significantly lower than Podolyanka (and cluster 1 at general) under any year's conditions. As we can see, this classification cannot dependent from coefficient of yield efficiency and this parameter isn't important for ecological estimation. Regarding to the cluster classification we can recommended first cluster for Northern Steppe conditions and, partly, second cluster for some years or fore farmers, which placed under river's valley conditions, more humidly. As we can see no one components of grain productivity cannot use as reliable for yield forecasting.

Grain quality and relations with traits of grain productivity. At table 3 we represent dates of the results of next parameters analyzed: grain moisture, protein content and gluten content. Standard error (± SE) values of the treated variants are shown at table 3 too.

Table 3. Parameters of winter wheat grain quality.

Number	Variety	Moisture, %	Protein content, %	Gluten, %
1	Voloshkova	17,90±0,06	13,77±0,04	26,60±0,17
2	Novosmuglyanka	18,44±0,02	13,60±0,21	25,41±0,12
3	Smuglyanka	17,10±0,03	14,40±0,05	27,33±0,17*
4	Spivanka	16,20±0,04	14,20±0,02	26,9±0,09
5	Podolyanka, st	14,90±0,04	13,73±0,03	25,20±0,08
6	Komerciyna	15,90±0,19	13,50±0,02	24,60±0,02
7	Ednist	16,60±0,05	14,30±0,01	26,40±0,05
8	Spasivka	15,90±0,01	11,70±0,04	19,34±0,08
9	Bogdana	16,70±0,03	14,13±0,02	25,30±0,12
10	Kolyadka	14,22±0,51	12,30±0,06	25,54±0,21
11	Lodizhinka	14,22±0,01	14,00±0,10	25,72±0,26
12	Gorodnicya	16,90±0,01	13,50±0,05	23,72±0,16
13	Garantiya	16,00±0,05	14,70±0,05*	27,40±0,12*
14	Melodiya	16,34±0,02	13,32±0,04	24,80±0,12
15	Zluka	15,71±0,02	13,70±0,15	25,00±0,30
16	Gileya	16,80±0,05	14,81±0,05*	27,05±0,19
17	Mudrist	16,94±0,06	15,24±0,04*	28,83±0,20*
18	Svitanok	14,80±0,03	14,70±0,05*	27,40±0,19*
19	Selevita	14,80±0,02	13,54±0,05	24,34±0,24
	Average	16,12	13,85	25,63
	Std. deviation	1,16	0,84	2,00

* - difference is statistically significance from check at $P_{0.05}$

As we can see from table 3 in spite of grain productivity by protein content as key agronomic-value trait we can identify some more perspective than standard winter wheat varieties' like as Garantiya, Gileya, Mudrist, Svitanok. Only one of these varieties was corresponded to Steppe ecotype (Garantiya), other three for Forrest-Steppe, which characterized by higher protein content than the grains of first ecotype.

Regarding gluten content varieties Smuglyanka, Garantiya, Mudrist, Svitanok can be determined due to content hicher than standard. Only in one point (variety Smuglyanka) its distinguish from protein content parameter.

In complex (by quantity and quality traits) we can recommend variety Mudrist as full suitable by all parameters for Northern Steppe subzone (for our Dnipro region), other varieties are suitable only by yield or quality parameters, but variety Spivanka is also suitable on the level of standard by agronomic-value traits complex. At table 4 correlations between main yield and quality traits have been shown.

Table 4. Correlations between difference grain productive and quality traits

Correlation between	Weight of 1000 grains	Weight from 1 spike,	Weight from m^2	Percent of grains in total productivity	Yield	Protein content	Gluten
Weight of 1000 grains	--	-0,12	-0,13	-0,36*	-0,16	-0,23	-0,34*
From 1 spike, g.	-0,12	--	0,45*	0,52*	0,44*	-0,57*	-0,05
From m^2, g.	-0,13	0,45*	--	0,49*	0,99*	-0,20	0,25
Percent of grains in total productivity	-0,36*	0,52*	0,49*	--	0,52*	-0,19	-0,03
Yield	-,016	0,44*	0,99*	0,52*	--	-0,2	0,13
Protein content, %	-0,23	-0,57*	-0,20	-0,19	-0,2	--	0,79*
Gluten, %	-0,34*	-0,05	0,25	-0,03	0,13	0,79*	--

* - true strong relation.

Enough strong reliable correlations can be observed between such traits weight of 1000 grains and percent of grains in total productivity, gluten content (forward correlation), weight from 1 spike and grain weight from m^2, percent of grains in total productivity (direct correlation), protein content (forward correlation), weight from m^2 and yield (direct correlation). Generally, quality grain traits have strong forward correlation with productive traits and strong reliable direct correlations inside these groups.

Thus, we developed that in complex (by quantity and quality traits) we can recommend variety Mudrist as full suitable by all parameters for Northern Steppe

subzone (for our Dnipro region), variety Spivanka is also suitable on the level of standard by agronomic-value traits complex..

Thereby, investigations in terms of ecological exam shows us, that general exam of winter wheat varieties isn't enough for detection suitability of winter wheat varieties for growth under regional conditions. Level of regional variability at climatic conditions is enough for significance discrepancies in genotype-environment reaction and, thus, for unsuccessful even for varieties obtaining in results of special breeding program for conditions of geographic zone (Steppe of Ukraine) and according to general variety model for this zone.

CONCLUSIONS

Due to results of our investigations our subzone has very specify requirements for winter wheat genotype grows and development. Only one genotype surpassed standard in by agronomic-value traits on higher value and only one too have shown its traits in complex on standard level.

Regarding to these statements, ecological exam is necessarily to clarify true adaptability and suitability of winter wheat variety for regional conditions. Sometimes even special breeding program for climatic zone is not enough for obtaining suitable forms. Moreover, under conditions of our exam variety Mudrist has a Forrest-Steppe ecotype and breeding not for these conditions at all.

Studies on winter wheat grain productive and quality traits are usually limited to a few types of climates (three zone for Ukraine) and measured number of varieties (without any record of variety type by special demands for realized of potential yield). Here the overall diversity of nineteen varieties in terms of many important indicators of wheat grain productivity and quality (content of protein and gluten) relating to growing, conditions was largely due to the diversity contributed by modern Ukrainian varieties. The wide phenotypic variability for the most of the agricultural-value traits investigated is indicative of the large diversity of the varieties and genotype-environment interactions, mutual influences of climatic conditions and genotype peculiarities.

ACKNOWLEDGEMENTS

This study was supported by the Ministry of Education and Science of Ukraine. We would like to thank vice-director of Institute of Bioenergy Crops and Sugar Beet of NAASU Dr. Victor Sinchenko for supporting of our investigations. Our special thanks are extended to the Ms. at Biological Sciences Elena Alexandrova for comments that greatly improved the manuscript.

REFERENCES

Andrusevich, K.V., Nazarenko, M.M., Lykholat, T.Yu., Grigoryuk, I.P., 2018. Effect of traditional agriculture technology on communities of soil invertebrates. Ukrainian journal of Ecology. 8 (1), 33–40.

Bordes, J., Branlard, G., Oury, F.X., Charmet, Balfourier, G. F., 2008. Agronomic characteristics, grain quality and flour rheology of 372 bread wheats in a worldwide core collection. Journal of Cereal Science, 48(3), 569-579.

Bonnot, T., Bance, E., Alvarez, D., Davanture, M., Boudet, J., Pailloux, M., Zivy, M., Ravel, C., Martre, P., 2017. Grain subproteome responses to nitrogen and sulfur supply in diploid wheat Triticum monococcum ssp. Monococcum. The Plant Journal, 91(5), 894-910.

Dai, Z., Plessis, A., Vincent, J., 2015. Transcriptional and metabolic alternations rebalance wheat grain storage protein accumulation under variable nitrogen and sulfur supply. Plant Journal. 83, 326–343.

Dawson, J. C., Riviиre, P., Berthellot, J. F., 2011. Collaborative Plant Breeding for Organic Agricultural Systems in Developed Countries. Sustainability, 3, 1206-1223.

Gepts, P., Hancock, J., 2006. The future of plant breeding. Crop Science, 46, 1630-1634.

Kharytonov, M.M., Pashova, V. T., Mitsik, O.O., Nazarenko, M.M., Bagorka, M.O., 2017. Estimation of winter wheat varieties suitability for difference growth of landscape conditions. Annals of the Faculty of Engineering Hunedoara. 15(4), 187–191.

Martynov, S.P., Dobrotvorskaya, T.V., 2006. Genealogical analysis of diversity of Russian winter wheat cultivars (Triticum aestivum L.). Genetic Resources and Crop Evolution, 53, 386-386.

Mba, C., Guimaraes, E.P., Ghosh, K. 2012. Re-orienting crop improvement for the changing climatic conditions of the 21st century. Agriculture & Food Security, 7**,** 1–17.

Nazarenko, M., 2015. Negativnyie posledstviya mutagennogo vozdeystviya [Peculiarities of negative consequences of mutagen action], Ecological Genetics, 4, 25-26. (in Russian).

Nazarenko, M., Kharytonov, M., 2016. Characterization of wheat mutagen depression after gamma-rays irradiated. In: Agriculture and Forestry, 62, 4, 267–276.

Nazarenko, M., 2017. Specific Features in the Negative Consequences of a Mutagenic Action. In: Russian Journal of Genetics: Applied Research, 7, 2, 195–196.

Nazarenko M., Lykholat Y., 2018. Influence of relief conditions on plant growth and development. In: Dnipro university bulletin. Geology. Geography, 26, 1. – P. 143 - 149.

Rangare, N.R., Krupakar, A., Kumar, A., Singh, S., 2010. Character association and component analysis in wheat (Triticum aestivum L.). Electronic Journal of Plant Breeding 1, 231-238.

Reif, J.C., Zhang, P., Dreisigacker, S., Warburton, M.L., 2005. Wheat genetic diversity trends during domestication and breeding. Theoretical and Applied Genetics, 110, 859-864.

Serpolay, E., Dawson, J.C., Chable, V., Lammerts Van Bueren, E.,Osman, A., Pino, S., Silveri, D., Goldringer, I., 2011. Phenotypic responses of wheat landraces, varietal associations and modern varieties when assessed in contrasting organic farming conditions in Western Europe. Organic Agriculture, 3, 12 -18.

Shewry, P.R., Mitchell, R.A.C., Tosi, P., 2012. An integrated study of grain development of wheat (cv. Hereward). Journal of Cereal Science. 56, 21–30.

Slafer, G.A., Andrade, F.H., 1993. Physiological attributes related to the generation of grain yield in bread wheat cultivars released at different eras. Field Crops Research, 31, 351-367.

Tester, M., Langridge, P., 2010. Breeding technologies to increase crop production in a changing world. Science, 327, 818-822.

Tuberosa, R., Salvi, S., 2006. Genomics-based approaches to improve drought tolerance of crops. Trends in Plant Science, 11, 405-412.

World Agricultural Supply and Demand Estimates. USDA, Washington, 2018. Retrieved from https://www.usda.gov/oce/commodity/wasde/latest.pdf.

DOI: 10.17707/AgricultForest.65.1.14

Fatmir FAIKU,
Liridon BUQAJ, Arben HAZIRI [1]

PHYTOCHEMICALS AND ANTIOXIDANT STUDY OF *TEUCRIUM CHAMAEDRYS* (L.) PLANT

SUMMARY

Phytochemicals and antioxidant activity were analysed to the extracts of aerial part of *Teucrium chamaedrys* (L.) plant growing wild in Peja (Western part of Kosovo) in two different altitude levels (790 m and 520 m). In extracts were analysed the total phenols and flavonoids by spectrophotometric methods. Total phenols were determined by Folin-Ciocalteu reagent and were in the range of 21.45 mg/g (in altitude of 790 m) to 237.51 mg/ g in altitude of 520 m (expressed as gallic acid equivalent, mg GAE/g).The amounts of flavonoids in the extracts of *Teucrium chamaedys* (L.) were in the range of 11.85 mg/g (in altitude of 520 m) to 84.50 mg/g in altitude of 790 m (expressed as rutin equivalent, mg RU/g). Antioxidative activity was determined in vitro by using the DPPH test. Experimental values are expressed as IC_{50} values (μg/mL), and they shows that the tested extracts have high antioxidant activities, which range in scope from 18.40 μg/mL (in altitude 790 m) to 264.22 μg/mL (in altitude 520 m).

Keywords: *Teucrium chamaedrys* (L.), phenols, flavonoids, antioxidant activity.

INTRODUCTION

The genus *Teucrium* L. (Lamiaceae) includes about 200 species and subspecies of herbs and shrubs, often aromatic, with a centre of distribution in the Mediterranean basin (Greuter et al., 1986). In the area of central and west Balkan, nine species of this genus was registered (Tutin et al., 1972). The herbs of *Teucrium chamaedrys* (L.) and *Teucrium montanum* (L.) are the most popular traditional remedies in Balkans used as cholagogue, tonic and antianemic, as well for treatment of diarrhea, leucorrhea, wounds and hemorrhoids. The infusion of aerial parts of *Teucrium scordium* was used as bitter aromatic, cholagogue with wound healing and fever reducing properties (Redžić, 2007; Jarić et al., 2007).

Antimicrobial and antioxidant activity, total phenolic and flavonoid concentrations of Teucrium species have been studied by a number of researchers in the different parts of the world (Kucuk et al., 2006; Sarker et al., 2007 Gursoy

[1]Fatmir Faiku, Arben Haziri (corresponding author: arbeni77chem@hotmail.com), Faculty of Natural Sciences, Department of Chemistry, University of Prishtina, Prishtinë,10000, KOSOVO; Liridon BUQAJ, Faculty of Life and Environmental Sciences, University of Prizren, Ukshin Hoti, Prizren, 20000, KOSOVO.

Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

et al., 2009; Nevcihan et al., 2009; Stanković et al., 2010; Kundaković et al., 2011; Stanković et al., 2012; Vlase et al., 2014).

Our research group was interested to analyse the chemical profile of different medicinal plants, which are growing wild in the region of Kosovo and Albania (Faiku et al., 2012, 2013, 2015a,b, 2016, 2017a,b, 2018; Haziri et al., 2009, 2010, 2013, 2017a,b).

The aim of this research was to determine the quantity of phenols, flavonoids and antioxidant activity of the different extracts from *Teucrium chamaedrys* (L.) growing wild in Peja (Western part of Kosovo).

MATERIAL AND METHODS

Plant material

The aerial part of *Teucrium chamaedrys* (L.), growing wild in east part of Kosovo, was collected in May 2017. The plants were dried at room temperature.

Preparation of plant organic extracts

A portion of the finely powdered material (200 g) was extracted three times with 70% methanol (methanol, 4 L) during a 24-h period. After removal of methanol under reduced pressure, the aqueous phase was successively extracted with three solvents of increasing polarity, namely ethanol, ethyl acetate and acetone. The extraction was carried out until a colorless extract was obtained. The residue was the aqueous extract. All five extracts (ethyl acetate, methanol, water, ethanol and acetone) were evaporated to dryness and then dissolved in 50% ethanol to make 10% (w/v) solutions. These solutions, either as such or in diluted state, were used in subsequent experiments.

Determination of total phenolic and flavonoid content

The amount of total phenolic contents in the extracts was determined spectrophotometrically with the Folin Ciocalteu (FC) reagent using the method of Fukumoto and Mazza (Fukumoto et al., 2000), with small modifications (Bozin et al., 2008). The reaction mixture contained 1.0% dilution of examined extracts (100 μL), freshly prepared 0.2 mol/L FC reagent (2.5 mL) and 10% sodium carbonate solution (2 mL). The mixture was incubated in the dark at room temperature for 1 hour to complete the reaction. The absorbance of the resulting solution was measured at 760 nm on a UV/VIS spectrophotometer using distilled water as the blank. The concentration of total phenolic contents was expressed in mg gallic acid equivalents (GAE) per g dried extract (d.e.), using a standard curve of gallic acid (0.1-2.0 μg/mL). All measurements were replicated five times.

Total flavonoid content in the extracts was determined spectrophotometrically according to Zhishen et al. 1999, using a method based on the formation of a flavonoid-aluminium complex with an absorbance maximum at 430 nm. The examined extracts (1mL) were mixed with 2% $AlCl_3 \times 6H_2O$ (0.5 mL). After incubation at room temperature for 30 min, the absorbance of the reaction mixtures was measured. The blank sample was a 1:1 mixture of the examined extracts and distilled water. Flavonoid content was expressed in μg

rutin equivalent (RE) per g dried extract by using a standard curve of rutin (concentration range 0.5–6.0 μg/mL). All measurements were replicated five times.

Antioxidant activity- DPPH assay

The DPPH assay was performed as described previously (Blois, 1958; Zhou et al., 2004), following the transformation of the DPPH radical to its reduced, neutral form (DPPH-H). The samples of all extracts of *Teucrium chamaedrys* (L.) (from 2.50 to 50.00 μg/mL) were mixed with 90 μM DPPH• solution (1 mL) and made up with 95% methanol to a final volume of 4 mL. The absorbance of the resulting solutions was recorded spectrophotometrically at 515 nm after 1 h at room temperature, against the blank (with the same chemicals, except for the sample). The same procedure was repeated with rutin and *tert*-butyl-4- hydroxyanisole (BHA) as a positive control. For each sample five replicates were recorded.

RESULTS AND DISCUSSION

The concentration of phenolics in the examined plant extracts using the Folin-Ciocalteu's reagent is expressed in terms of gallic acid equivalent. Results of the amount of total phenolic contents in *Teucrium chamaedrys* (L.) extracts are given in Table 1.

Table1. Total phenol contents in the plant extract expressed in terms of gallic acid equivalent (mg GAE)

Altitude (m)	Ethanol	Methanol	Ethyl acetate	Acetone	Water
520	237.51±085[1]	164.12±0.18	24.85±0.21	138.20±0.15	163.15±0.19
790	233.40±0.95	161.50±0.25	21.45±0.15	125.40±0.20	160.52±0.24

[1]Each value in the table vas obtained by calculating the average of five analysis ± standard deviation

The amount of total phenolics in *Teucrium chamaedrys* (L.) extracts ranged from 21.45 mg GAE/g (ethyl acetate extract to 790 m altitude) to 237.51 mg GAE/g (ethanol extract to 520 m altitude). The highest concentration of phenol was measured in ethanol, methanol and aqueous extract. Somewhat smaller concentration was found in acetone extract, while ethyl acetate extract had considerably smaller concentration of phenolics.

The content of phenolics in the plant extracts of the species *Teucrium chamaedrys* (L.) depends on the type of extract, i.e. the polarity of solvent used in extraction. The extracts obtained using more polar solvents like ethanol; methanol and water have higher concentration of phenolics (Table 1). High dissolubility of phenolics in polar solvents provides high concentration of these compounds in the extracts obtained using polar solvents for the extraction (Zhou et al., 2004).

The concentration of flavonoids in various plant extracts of the species *Teucrium chamaedrys* (L.) was determined using spectrophotometric method

with aluminum chloride. The content of flavonoids was expressed in terms of rutin equivalent (mg RU/g). The amounts of flavonoids in the tested extracts are shown in Table 2.

Table 2. Flavonoid contents in the plant extract expressed in terms of rutin equivalent (mg RU/g)

Altitude (m)	Ethanol	Methanol	Ethyl acetate	Acetone	Water
520	45.55±0.20[1]	56.0±0.62	82.27±0.75	69.52±0.55	11.85±0.65
790	41.45±0.30	58.50±0.55	84.50±0.74	72.10±0.62	12.15±0.45

[1]Each value in the table vas obtained by calculating the average of five analysis ± standard deviation

The concentration of flavonoids in plant extracts from *Teucrium chamaedrys* (L.) ranged from from 11.85 mg RU/g (water extract to 520 m altitude) to 84.50 mg RU/g (ethyl acetate extract to 790 m altitude). The concentration of flavonoids in acetone extract was 72.10 mg RU/g, which was very similar to the value of ethyl acetate extract concentration. The lowest flavonoid concentration was found in ethanol and aqueous extract. The concentration of flavonoids in plant extracts depends on the polarity of solvents used in the extract preparation (Min et al., 2005). Based on the obtained values for the concentration of flavonoids in the examined extracts of *Teucrium chamaedrys* (L.), it was found that the highest concentration of these compounds was in the extracts obtained using solvents of moderate polarity. The amount of total phenolic and total flavonoids in *Teucrium chamaedrys* (L.) extracts were given in Figure 1.

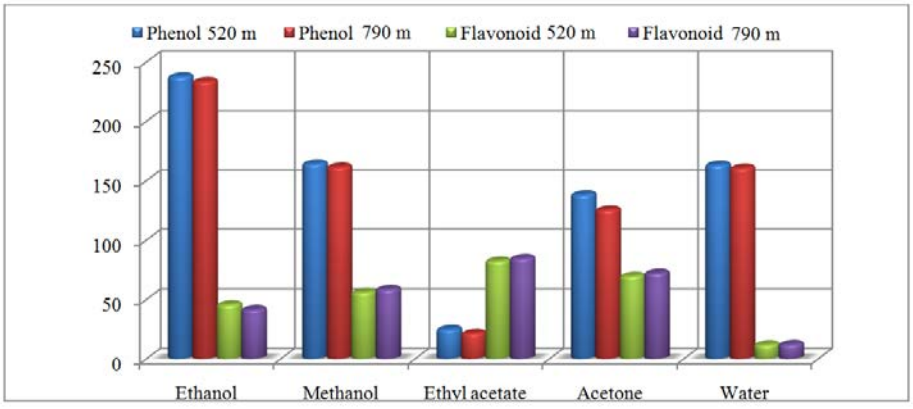


Figure 1. The amount of total phenolic contents and content of total flavonoids in *Teucrium chamaedrys* (L.) extracts

The antioxidant activity of *Teucrium chamaedrys* (L.) extracts has been evaluated in a series of *in vitro* tests. The DPPH radical is one of the most commonly used substrates for fast evaluation of antioxidant activity because of

its stability (in radical form) and the simplicity of the assay. In the DPPH assay, the ability of the investigated extracts to act as donors of hydrogen atoms or electrons in transformation of DPPH into its reduced form DPPH-H was investigated (Table 3). Parallel to examination of the antioxidant activity of plant extracts, the values for three standard compounds were obtained and compared to the values of the antioxidant activity. The standard substances were 3-tert-Butyl-4-hydroxyanisole (BHA) and rutin. Summary display of level for antioxidant activity of tested extracts is shown in Table 3.

Table 3. IC_{50} values (μg/mL) of the neutralization of DPPH radical with *Teucrium chamaedrys* (L.) extracts

Altitude (m)	Ethanol	Methanol	Ethyl acetate	Acetone	Water	BHA	Rutin
520	21.55±0.84[1]	24.55±0.85	264.22±0.98	29.95±1.1	26.25±0.65	5.25±0.78	9.27±0.77
790	18.40±0.70	25.60±0.70	259.55±0.80	27.41±0.90	22.41±0.70	5.25±0.78	9.27±0.7

[1]Each value in the table vas obtained by calculating the average of five analysis ± standard deviation

All of the assessed extracts of *Teucrium chamaedrys* (L.) were able to reduce the stable, purple-colored radical DPPH to the yellow-colored DPPH-H form with IC_{50} (50% of reduction) values as follows: 21.55 μg/mL in altitude 520 m and 18.40 μg/mL in altitude 790 m for ethanol, 24.55 μg/mL in altitude 520 m and 25.60 μg/mL in altitude 790 m for methanol, 264.22 μg/mL in altitude 520 m and 259.55 μg/mL in altitude 790 m for ethyl acetate, 29.95 μg/mL in altitude 520 m and 27.41 μg/mL in altitude 790 m for acetone, and 26.25 μg/mL in altitude 520 m and 22.41 μg/mL in altitude 790 m for water extract.

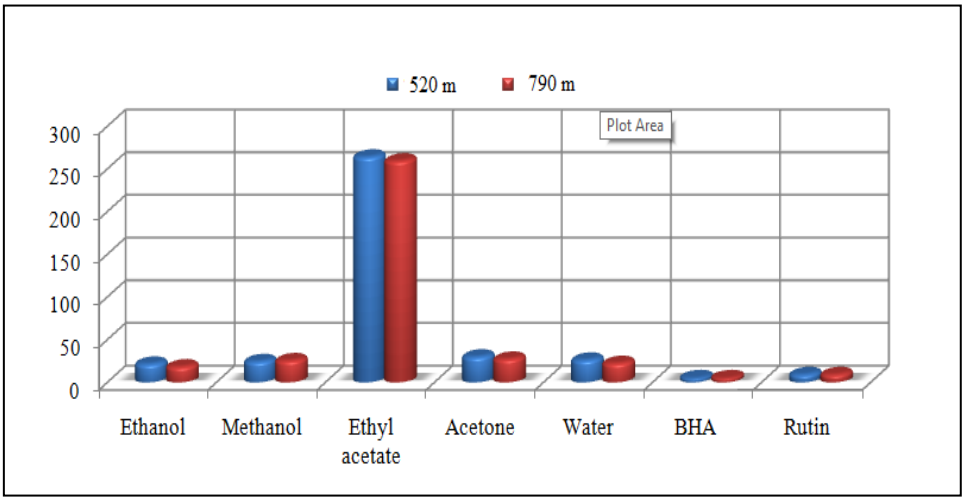


Figure 2. The IC_{50} values (μg/mL) of the neutralization of DPPH radical with *Teucrium chamaedrys* (L.) extracts

The obtained values for antioxidant activity examined with DPPH radical are in the range of 264.22 to 18.40 μg/ml. The largest capacity to neutralize DPPH radicals was found for ethanol extract, which neutralized 50% of free radicals at the concentration of 18.40 μg/ml. A similar activity was found for water, methanol and acetone extracts whose IC_{50} values were 22.41, 24.55 and 27.41μg/ml respectively. The minutest capacity to inhibit DPPH radicals was determined for ethyl acetate extract. In comparison to IC_{50} values of BHA and rutin, ethanol, methanol, water and acetone extracts from *Teucrium chamaedrys* (L.) manifested the strongest capacity for neutralization of DPPH radicals. The IC_{50} values (μg/mL) of the neutralization of DPPH radical with *Teucrium chamaedrys* (L.) extracts were given in Figure 2.

The extracts that perform the highest antioxidant activity (Table 3) have the highest concentration of phenolics (Table 1). Phenols are very important plant constituents because of their scavenging ability on free radicals due to their hydroxyl groups. Therefore, the phenolic content of plants may contribute directly to their antioxidant action (Tosun et al., 2009).

Flavonoids are class of secondary metabolites with significant antioxidant and chelating properties. Antioxidant activity of flavonoids depends on the structure and substitution pattern of hydroxyl groups (Sharififar et al., 2009). Ethyl acetate extract has a very high concentration of flavonoids as well, but it manifests less intense antioxidant activity, while aqueous extract has smaller content but manifests the antioxidant activity similar to methanol and acetone extracts. This difference is a result of different dissolubility of flavonoids in the solvents of different polarities.

CONCLUSIONS

The aim of this research was to determine the concentration of phenols, flavonoids and *in vitro* antioxidant activity of the different extracts from *Teucrium chamaedrys* (L.) growing wild in Peja (Western part of Kosovo). To conduct this type of research, we took two samples of the plant *Teucrium chamaedrys* (L.) at different altitudes 520 m and 790 m. The amount of total phenols in organic extracts of *Teucrium chamaedrys* (L.) was in the region of 21.45 mg GAE/g (ethyl acetate extract to 790m altitude) to 237.51 mg GAE/g (ethanol extract to 520 m altitude). Also, great amount of these compounds was found in the water extract (160.15 mg GAE/ g 790 m to 163.52 mg GAE/ g de 520 m). The amount of total flavonoids in organic extracts of *Teucrium chamaedrys* (L.) was in the region of 41.45 mg RU/g (ethanol extract to 790 m altitude) to 84.50 mg RU/g (ethyl acetate extract to 790 m altitude). Also, the amount of these compounds was found in the water extract (11.85 mg RU/ g 590 m to 12.15 mg RU/ g de 790 m).

Investigation of five different plant extracts from *Teucrium chamaedrys* (L.) showed high concentration of total phenols, especially flavonoids. Investigation of antioxidant activity of extracts and comparison with the activity of reference substances revealed intense antioxidant activity of plant extracts,

which was related to high concentration of phenolics in the examined extracts. In addition to its intensive use in the treatment of digestion disorders, gout and various infectious diseases, wall germander (*Teucrium chamaedrys* (L.) is a potential source of natural antioxidant substances of high value.

REFERENCES

Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, 18, 1199-1200.

Bozin, B., Mimica-Dukic, N., Samojlik, I., Anackov, G. & Igic, R. (2008). Phenolic as antioxidants in garlic (*Allium sativum* L., Alliacea). *Food Chem*., 111, 925-929.

Faiku, F. & Haziri, A. (2013). Total lipids, proteins, minerals and essential oils of *Taraxacum officinale* (L.) growing wild in Kosovo. *Int. J. Pharm. Phytopharmacol. Res*., 3, 254-257.

Faiku, F. & Haziri, A. (2015a), Total lipid, proteins, minerals, essential oils and antioxidant activity of organic extracts of *Mentha longifolia* (L.) growing wild in Kosovo. *Eur. Chem. Bull*., 4, 432-435.

Faiku, F., Haziri, A., Gashi, F., Troni, N. & Faiku, F. (2015b). Total lipids, proteins, minerals and essential oils of *Pinus nigra* Arnold and *Pinus sylvestris* growing wild in Kosovo. *Eur. Chem. Bull*., 4, 331-334.

Faiku, F., Haziri, A., Domozeti, B. & Mehmeti, A. (2012). Total lipids, proteins, minerals and essential oils of *Tussilago farfara* (L.) from south part of Kosova. *Eur., J. Exp. Biol*., 2, 1273-1277.

Faiku, F., Haziri, A., Mehmeti, A. & Reçica, B. (2017a). Antioxidant activity of the organic extracts of *Origanum vulgare* (L.) growing wild in Kosovo, *Fresen. Environ. Bull.*, 26, 1440- 1446.

Faiku, F., Haziri, A., Mehmeti, I., Muriqi, A. & Faiku, B. (2017b). Antibacterial Activity of Different Extracts of *Daucus Carota* (L.) Growing Wild in Kosovo, *Res. J. Pharm. Biol. Chem. Sci.*, 8, 987-991.

Faiku, F., Haziri, A., Mehmeti, I., Bajrami, D. & Haziri, I. (2016). Evaluation of antibacterial activity of different solvent extracts of *Cichorium intybus* (L.) grow in wild in east part of Kosovo. *J. Anim. Plant Sci.*, 26, 1486-1491.

Faiku, F., Haziri, A., Mehmeti, I., Mehmeti, A. & Hoti, Gj. (2018). *In vitro* antibacterial activity of different solvent extracts of *Achillea millefolium* (L.) growing wild in Kosovo. *Fresen. Environ. Bull*., 25, 3878- 3885.

Fukumoto, L. & Mazza, G. (2000). Assessing antioxidant and prooxidant activities of phenolic compounds. *J. Agr. Food Chem*., 48, 3597-3604.

Greuter, W., Burdet, H. M. & Long, G. (1986). Med-checklist: volume 3. Genève, Jardin Botanique, 102 pp.

Gursoy, N. & Tepe, B (2009). Determination of the antimicrobial and antioxidative properties and total phenolics of two ‘‘endemic’’ *Lamiaceae* species from Turkey: *Ballota rotundifolia* L. and *Teucrium chamaedrys* C. *Plant Foods Hum. Nutr*., 64, 135–140.

Haziri, A., Aliaga, N., Ismaili, M., Govori, S., Leci, O., Faiku, F., Arapi, V. & Haziri, I. (2010). Secondary metabolites in essential of *Achillea millefolium* (L.) growing wild in east part of Kosovo, *Amer. J. Biochem. Biotechnol*., 6, 32-34.

Haziri, A., Faiku, F., Berisha, R., Mehmeti, I., Govori, S. & Haziri, I. (2017a). Evaluation of Antibacterial Activity of Different Solvent Extracts of *Teucrium chamaedys* (L.) Growing Wild in Kosovo. *Chemistry*, 26, 431-441.

Haziri, A., Faiku, F., Rudhani, I., Mehmeti, I. & Motori, D. (2017b). Antibacterial Activity of Different Extracts of *Centaurea cyanus* (L.) Growing Wild in Kosovo, *Orient. J. Chem*., 33, 1636-1641.

Haziri, A., Faiku, F., Mehmeti, A., Govori, S., Abazi, S., Daci, M., Haziri, I., Bytyqi-Damoni, A. & Mele, A. (2013). Antimicrobial properties of the essential oil of *Juniperus Communis* (L.) growing wild in east part of Kosovo. *Amer. J. Pharmacol. Toxicol*., 8, 128-133.

Haziri, A., Govori, S., Ismaili, M., Faiku, F. & Haziri, I. (2009). Essential oil of *Tanacetum parthenium* (L.) from east part of Kosova. *Am. J. Biochem. Biotechnol*., 5. 226-228.

Jarić, S., Popović, Z., Mačukanović-Jocić, M., Djurdjević, L., Mijatović, M., Karadžić, B., Mitrović, M. & Pavlović, P. (2007). An ethnobotanical study on the usage of wild medicinal herbs from Kopaonik Mountain (Central Serbia). *J. Ethnopharmacol*., 111, 160-175.

Kucuk, M., Gulec, C., Yasar, A., Ucuncu, O., Nuran, Y., Coskuncelebi, K., Terzioglu, S. & Nurettin, Y. (2006). Chemical composition and antimicrobial activities of the essential oils of *Teucrium chamaedrys* subsp. *chamaedrys*, *T. orientale*var. *puberulens*, and *T. chamaedrys* subsp. *lydium*. *Pharm. Biol.* 44, 592 – 599.

Kundaković, T., Milenković, M., Topić, A., Stanojković, T., Juranić, Z. & Lakušić, B. (2011). Cytotoxicity and antimicrobial activity of *Teucrium scordium* L. (Lamiaceae) extracts. *Afr. J. Microbiol. Res*., 5, 2692-2696.

Min, G. A. & Chun-Zhao L., (2005), Comparison of Techniques for the Extraction of Flavonoids from Cultured Cells of *Saussurea medusa* Maxim. *Word J. Microb. Biot*., 21,1461-1463.

Nevcihan, G. & Bektas, T. (2009). Determination of the antimicrobial and antioxidative properties and total phenolics of two "endemic" Lamiaceae species from Turkey: Ballota rotundifolia L. and Teucrium chamaedrys C. Koch. *Plant Foods Hum. Nutr*., 64, 135–140.

Redžić, S. S. (2007), The ecological aspect of ethno botany and ethno pharmacology of population in Bosnia and Herzegovina. *Coll. Antropol*., 31, 869-890.

Sanchez-Moreno, C., Larrauri, A. & Saura-Calixto, F. A. (1998). Procedure to measure their anti-radical efficiency of polyphenols. *J. Agr. Food Chem*., 76, 270-276.

Sarker, S. D., Nahar, L. & Kumarasamy, Y. (2007). Microstate plate-based antibacterial assay incorporating reassuring as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. *Methods*, 42, 321-324.

Sharififar, F., Nudeh-Dehghn, G. & Mirtajaldini, M. (2009). Major flavonoids with antioxidant activity from Teucrium polium L. *Food Chem*., 112, 885-888.

Stanković, M., Stefanović, O., Čomić, L., Topuzović, M., Radojević, I. & Solujić, S, (2012). Antimicrobial activity, total phenolic content and flavonoid concentrations of Teucrium species. *Rocznik*, 7, 664-671.

Stanković, M., Topuzović, M., Marković, A., Pavlović, D., Solujić, S., Nićiforović, N. & Mihailović, V. (2010). Antioxidant Activity, Phenol and Flavonoid Contents of Different *Teucrium Chamaedrys L.* Exstracts, *Biotechnology & Biotechnological Equipment*, 24, 82- 89.

Tosun, M., Ercisli, S., Sengul, M., Ozer, H. & Polat, T. (2009). Antioxidant properties and total phenolic content of eight Salvia species from Turkey. *Biol. Res*., 42, 175-181.

Tutin, G. & Wood, D. (1972). Flora European; volume 3. Cambridge: Cambridge University Press, 57 pp.

Vlase, L., Benedec, D., Hanganu, D., Damian, G., Csillaq, I., Sevastre, B., Mot, A. C., Silaghi-Dumitrescu, R. & Tilea, I. (2014). Evaluation of antioxidant and antimicrobial activities and phenolic profile for *Hyssopus officinalis, Ocimum basilicum* and *Teucrium chamaedrys*. *Molecules*, 19, 5490-5507.

Zhishen, J., Mencheng, T. & Jianming, W. (1999). The determination of flavonoids content in mulberry and their scavenging effects on superoxide radicals. *Food Chem*., 6, 555- 559.

Zhou, K. & Yu, L. (2004), Effects of Extraction Solvent on Wheat Bran Antioxidant Activity Estimation. *LWT- Food Sci. Technol. Res*., 37, 717-721.

DOI: 10.17707/AgricultForest.65.1.15

Melisa LJUŠA, Hamid ČUSTOVIĆ* [1]

AGRICULTURAL LAND USE AND LAND LOSSES IN BOSNIA AND HERZEGOVINA IN THE PERIOD 1961-2018

SUMMARY

In Bosnia and Herzegovina (BiH) there are no available data on permanent losses of agricultural land and it is evident that all the research and professional and scientific literature still uses the information of annual land loss in the amount of 3,000 ha which, according to the available references, has been in use since 1977 (Resulović, 1977-2010).

The subject of this scientific research is to investigate changes in the use of agricultural land in BiH, sectoral policies and factors affecting these changes as well as the quality of official data on land and land use change. Analyzed as part of this research were three sets of data: statistical data (1961-1991), cadastral records (1973-1991) and CORINE data on land cover changes (2000-2018).

Researches have shown that trends in changes in total agricultural land by all categories of use were not linear and were considerably affected by the socio-economic development and sectoral policies. This study has shown that total agricultural land, according to statistical records from 1961-1991, was reduced by 101,222 ha (3,374 ha/yr). Within the category of arable land, the biggest permanent losses were recorded in cropland and gardens, amounting to 207,823 ha or 6,927 ha/yr, while in some other categories within the arable land an increase was recorded. According to cadastral records for the period 1973-1991, the total decrease of agricultural land amounts to 58,186 ha (3,232 ha/yr). According to CORINE data, the reduction of agricultural land in the period 2000-2018 is 14,152 ha or 786 ha/yr.

Keywords: agricultural land, land use, sectoral policies, cadastral records, statistical data, CORINE

INTRODUCTION

B&H has gone through several stages of socio-economic development and each one of them has left its mark on the management and use of agricultural land (from uncontrolled development of village to the II World War, the agrarian reforms, vigorous industrial development until the 1980s, economic stagnation in the eighties, wartime period in the nineties, the post-war period of reconstruction and transition, and finally the period of EU accession policies).

[1] Melisa LJUŠA*(corresponding author: melisa.ljusa@gmail.com), Hamid ČUSTOVIĆ, University of Sarajevo, Faculty of Agricultural and Food Sciences, Zmaja od Bosne 8, 71000 Sarajevo, BOSNIA AND HERZEGOVINA.

Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

Following World War II the foundations of industrial development of B&H were laid. Federal plans assigned B&H the role of producer of raw materials and energy (Bilandžić, 2003). B&H made a distinct economic growth and became, according to the European standards in the 80s of the last century, an industrialized developing country. Rapid development of industry had considerable consequences that are reflected in permanent and temporary loss of the highest quality of agricultural land in B&H, demographic change as well as deagrarization of rural area. Agricultural land had a limited value in terms of economic development of B&H as evidenced by the share of agriculture in gross domestic product of the overall economy of B&H that was reduced from 26.66% in 1960 to 14.04% in 1990. Industrial development put agriculture on the back burner. In the eighties, plans were prepared to develop agriculture and introduce planning and protection of land. However, these plans were never fully implemented, firstly because of the economic crisis and secondly because of the collapse of the former Yugoslavia.

For B&H, the new historical era began in 1992 when it became an independent country. The war in B&H and the post-war reconstruction have resulted in major changes to the land that are reflected in the permanent loss of land, change of its use as well as in the land market (Čustović et al., 2013). One of the main causes of these changes is the migration (internal and international) of around 2.2 million people, particularly from rural to urban areas. These negative trends are still present.

Existing sources of data on land are often old, uncoordinated and irrelevant. Official data on changes in agricultural land is not available and the researches and professional and scientific literature are obviously still using data on the linear loss of agricultural land of 3,000 hectares annually which, according to the available literature, has been in use since 1977 (Resulović, 1977-2010).

The aim of this paper is to use available data to analyze trends and characteristics of the way of use of agricultural land in B&H as well as the position and sustainable management of agricultural land within the socio-economic development of the country from the standpoint of impact of sectoral policies, social, political and economic factors in the period from 1961 to date.

MATERIAL AND METHODS

The two main sources of data on land until 1991 in B&H were the register (cadaster) of the Republic Geodetic Administration of B&H and the Republic Institute of Statistics. This study analyzes statistical data on agricultural areas by category of use (1961-1991) and data on arable land by the method of use (1964-1991). When it comes to cadastral data, the analysis encompassed data for the period 1973-1983 and the year 1991, since there is no integrated data for B&H for the period 1983-1991.

To analyze changes to agricultural land (>5 ha) in the period 2000-2018, the CORINE (COoRdination of INformation on the Environment) data were used. CORINE is considered to be unique and currently relevant database of

changes to land in B&H (Taletović et al., 2010). The basic principles applied to the interpretation of satellite images were the identification of changes with the following characteristics: to be larger than 5 hectares and wider than 100 m; to have occurred in the period 2000-2006, 2006-2012 and 2012-2018; and to be visible on the satellite image regardless of their position. To determine classes of land cover, the standard CORINE nomenclature grouped in three levels was used.

In addition to statistical data on the land, we also used statistical indicators of socio-economic development and data of the population census.

RESULTS AND DISCUSSION

Socio-economic development and agricultural land use and management before 1992

Although agricultural land accounts for nearly 50% of the total territory, B&H is not rich in high quality agricultural land (Čustović & Bajramović, 2008). The first three soil bonity category account for only 14.03%, and combined with the soil of IV category their participation amounts to 30.87% (Čustović, 2005).

Analysis of statistical data (1961-1991) indicate that the total agricultural land area decreased by 101,222 ha or 3,374.06 ha/year. The analysis shows that the reduction in agricultural areas has no linear character and ranges from a reduction of 148 ha, as recorded in 1977, to a reduction of as much as 51,000 ha as recorded in 1982. Within arable land, cropland and gardens were reduced by 207,823 ha and this category of use had a continuing decreasing trend in the observed period. In all the categories (arable land and gardens, orchards, vineyards, meadows, pastures) there were notable oscillations in areas by years, whether it be on the reduction or increase of the area (Chart 1). Additionally, in some years, changes in individual categories were not observed. Within arable land, the total increase in the area of orchards in the observed period is 27,279.00 ha, vineyards 479 ha, while meadows were increased by 59,605 ha. Meadows area was mostly increased at the expense of cropland, as cereal crops growing was in decline.

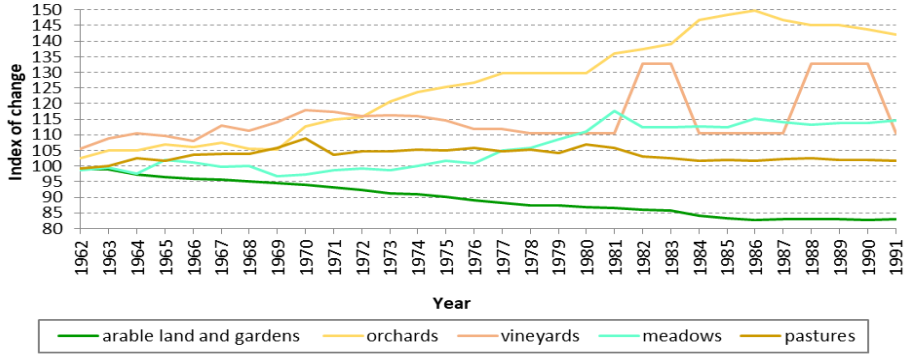


Chart 1. Index of change in agricultural areas by land use categories 1961=100

According to cadastral records, in the period 1973-1991, there is a noticeable reduction in total agricultural land area of 58,186.11 ha or 3,232.56 ha/year; out of that, 96.55% of total change was recorded in privately owned land. Reduction of agricultural land also has no linear character and by individual years it ranges from 2,318.99 ha (1977) to 13,739.32 ha (1983). In the period 1973-1991, cropland and garden areas were reduced by 62,931 ha. Cadastral records show that infertile land areas increased by 30,238 ha in the period 1973-1991.

This research has identified considerable differences between the cadastral data and statistics. For instance, according to the cadastral data, the total area of agricultural land in B&H in 1973 was bigger by 19,258.19 ha compared to the statistical ones, and in 1981 smaller by as much as 63,909.94 ha. According to the cadaster data in relation to the statistics, in 1973 the area was bigger by 40,594.17 ha in arable land and gardens, by 3,210.48 ha in orchards and by 31,819.14 ha in meadows. On the other hand, the areas under vineyards were decreased by 948.71 ha as well as pastures by 49,933.31 ha in cadastral records compared to the statistical ones.

This discrepancy in the data can be attributed to different methodology of data collection, the difference in areas between two surveys, the issue of divergent data within the very municipalities which submitted them to the Geodetic Administration and Institute of Statistics, the failure to update cadastral records contributed to by illegal construction (Ljuša, 2015) as well as the inappropriate taxation policy (Lukić et al., 1991). In the comparative data analysis, the biggest difference is observed in the category of pastures (Chart 2.).

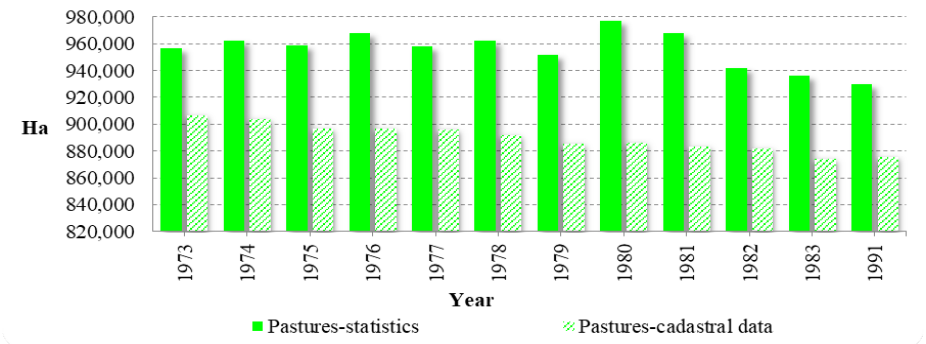


Chart 2. Comparative overview of data-pastures (1973-1991)

This can be attributed to errors in the keeping of records of agricultural, forest and barren land as well as the issue of delimitation of agricultural and forest land that has never been resolved in practice, which led to the situation where one area is recorded both as agricultural (by farmers) and forest (by foresters) at the same time (Ljuša, 2015).

Statistics show that the area of uncultivated arable land and gardens was increasing. Their surface increased from 54,283 ha in 1961 to 160,000 ha as it

was recorded in 1991. This effect was contributed by deagrarization of the area where the share of farmers in total population in 1991 compared to 1961 was reduced by 74.89%.

The favoring of industry pushed agriculture to the margins and society left agricultural land to its irrational use just because of economic relations between industry and agriculture, that is the preconception that agriculture "provides" very little (Milojevic, 1983). As Bogunović, 1983; Stefanović, 1983; Golić, 1994 reported, the bad position of land in that socio-economic development of B&H is partly a result of the discrepancy between spatial and economic planning. The problem of the implementation of the policy of physical planning is also connected with the lack of land policy and hence to the related establishment of adequate value of agricultural and forest land, the lack of a permanent spatial planning system and appropriate organization of municipal services (for the implementation and monitoring), the lack of continuous scientific and research work, systemic solution in the area of education of personnel and the like (Bublin, 2000). Negligence towards land has led to the situation where B&H has around 20,000 ha of environmental (technogenic) deposols and deserts (Resulović, 1999). The stagnation of agriculture resulted in B&H having difficulties to produce about 50% of its own food requirements (Selak, 1996).

Given the state of land resources and general negligence towards them, in the late seventies a social commitment was made to make the planning, protection and rational use of agricultural land a fundamental factor of the development of the B&H agro-industrial complex as evidenced by the five-year socio-economic plans. The need to protect agricultural land arose from the fact that the "Spatial Plan of the Socialist Republic of B&H for the period from 1981 to 2000" foresaw the requirement of around 100,000 ha of land for the construction at the expense of agricultural land.

The five-year social development plans indicate a positive turn when it comes to agricultural and land policies. Thus the "B&H Social Plan for the period from 1981 to 1985" outlined an orientation toward more rational land use as well as land development through agro hydro-melioration, land consolidation and redistribution of holdings. The problems of small holdings were recognized as a major constraint in agricultural production (average household had 3.3 ha of agricultural land of which 2 ha were more extensively used). Given the tendency of constant decrease in arable land areas or increase in the area of uncultivated arable land, specific actions were taken to cultivate those agricultural areas that were left fallow (94.000 ha in 1981) in order for them to be completely cultivated in 1985. Also, it was planned to reduce the uncultivated arable land areas (185.000 ha in 1981) down to 92,000 ha. In addition, the Social Plan provided for the land consolidation of 60,000 ha.

However, it is unknown how many of the planned activities in the area of planning and protection of land were actually realized. Only the data on implementation of land consolidation is available. It was carried out on a total area of 64,907 ha in seven municipalities, and the process has only recently been

administratively completed in some municipalities. Čustović & Ljuša (2006) reported problems in the implementation of land consolidation which, for example, were related to the lack of incentives for the development of agriculture, slow work of cadastral and geodetic offices in the municipalities, the lack of comprehensive soil maps, etc.

The analyzed data show that the implementation of five-year plans and programs did not yield desired results. In 1986, instead of 92,000 ha that were projected in the plans, the area of uncultivated arable land and gardens amounted to 160,000 ha. In the period 1986-1991, total agricultural areas were reduced by 2,000 ha, while arable land areas were reduced by 4,000 ha. Agricultural land continued to decrease although to a much lesser extent relative to the previous years of the studied period. Sowing area in period 1986-1991 decreased by 22,000 ha instead of the planned and expected increase. Uncultivated fields and gardens were increased by 30,000 ha in the same period of time. It has to be noted that deep economic crisis that struck the former Yugoslavia at that time certainly had an impact on investments in the agricultural sector.

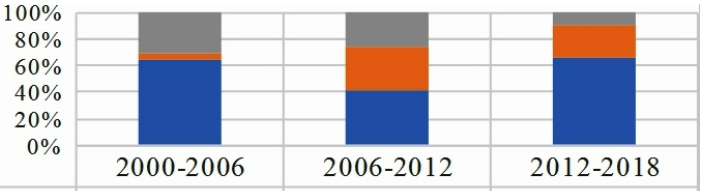
Socio-economic development and agricultural land use and management after 1992

The agricultural sector, like all other sectors, suffered enormous damage during the war, which according to a study by GTZ (2001) to $ 4.5 billion. The programs of reconstruction and restoration of international donors were more focused on supporting the local population rather than making serious investments in the revitalization of agriculture (Ivanković et al., 2006).

According to Nurković (2012), in the period 1991-2010 the number and share of urban population in the total population of B&H increased to 46.4%, while the number of residents in rural areas decreased from 75.3% to 44.2%. By comparing data from the 1991 Census and the preliminary data from the 2013 Census, a large demographic drain was identified in some areas of B&H. Many rural municipalities have experienced extraordinary changes, such as Glamoč which now has 4,038 inhabitants, which is only 32% of the total population in 1991 when it amounted 12,593, or Srebrenica municipality whose current population is only 41.56% of the pre-war one. In the area of East Bosnia, the number of inhabitants is almost halved. It is the movement of the population (over 2.2 million) that led to the occurrence of derelict and permanently lost agricultural land which, according to Čustović et al. (2013), are two basic phenomena when it comes to the way of use of agricultural land in B&H in the postwar period.

CORINE data show that in the period 2000-2018 in B&H changes occurred on a total area of 71,957 ha. The period of 1991-2000 was a period of stagnation and small activities so the changes in this period can be attributed to natural processes related mainly to succession in abandoned areas. Three characteristic changes within the agricultural areas were identified: increase, decrease and transition of one agricultural class into another (Ljuša, 2015). In the

period 2000-2018, the increase in agricultural areas amounts to 3,137 ha (eg. class 311 (broad-leaved forest) converted to 243 (land principally occupied by agriculture, with significant areas of natural vegetation class)), decrease to 14,152 ha (eg. class 211 (non-irrigated arable land) converted to 112 (discontinuous urban fabric)), while 6,235 ha transitioned from one agricultural class into another (eg. class 231 (pastures) converted to 242 (complex cultivation patterns)). Trends of change in the three observed periods (2000-2006, 2006-2012, 2012-2018) are different (Chart 3.). In the period 2000-2006, agricultural land areas increased by 554 ha, decreased by 9,327 ha, while the transition from one to another agricultural class was recorded on an area of 4,572 ha. In the period 2006-2012, agricultural land areas increased by 1,548 ha, the reduction amounted to 1,997 ha, while the transition from one into another agricultural class was recorded on an area of 1,244 ha. In the period 2012-2018, agricultural land areas increased by 1,035 ha, the reduction amounted to 2,829 ha, while the transition from one into another agricultural class was recorded on an area of 421 ha.



	2000-2006	2006-2012	2012-2018
Transition from one to another agricultural class	4.571	1.243	421
Increase of agricultural areas	554	1.548	1.035
Decrease of agricultural areas	9.326	1.997	2.829

Chart 3. Comparative overview of changes in agricultural areas (2000-2018)

In the context of conversion of agricultural areas in artificial surfaces, the largest areas (6,099.4 ha) were turned into discontinuous urban areas, where 98.3% of these changes occurred in the period 2000-2006. Agricultural areas were also converted into industrial and business areas, 340.59 ha (2000-2006), 146.61 ha (2006-2012) and 78.6 ha (2012-2018) respectively. However total conversion of agricultural areas in artificial surfaces is 7,450 ha which is 414 ha/year.

Mines and mineral resources exploitation sites occupy a considerable area covering 8,072.18 ha. In the period 2000-2006, these areas were increased by 653.72 ha, in the period 2006-2012 by 718.11 ha and in period 2012-2018 by 808.6 ha.

What stands out as a particular problem, in addition to the use of agricultural land for non-agricultural purposes, is the emergence of derelict land and transition of land in the succession of forest vegetation. According to the

CORINE data (2000-2018), 2,590 ha of agricultural land, mostly pastures (853.79 ha), were converted into the succession of forest vegetation.

However, CORINE has limitations when it comes to the identification of derelict land due to the adopted methodology (mapping of changes >5 ha) and small scale of 1:100.000. For this reason many of the permanently lost areas <5ha could not be included in the CORINE database. For example, in the municipalities of Kalesija and Gradacac, in the period 2008-2012 (M 1:10000) succession was identified in an area of 374.18 ha, and 355.76 ha respectively (Čustović et al., 2013a; Čustović et al., 2013b).

Statistical data indicate that the lack of a clear vision of development of agrarian and land policies in the country, lack of investments and incentives, fragmentation of the holdings as well as numerous other problems along with large deagrarization resulted in having over 50% of arable land uncultivated. The share of agricultural in the total population decreased to just 20.4% which means that every fifth or sixth person is engaged in agriculture.

Just like in the previous period before 1991, spatial planning nowadays is not adequately coordinated with the plans of social and economic development. There is a very pronounced problem of illegal construction, its sanctioning and the general lack of monitoring such processes. The pressure of development on the best quality agricultural land is best illustrated by the example of Kalesija municipality where the biggest expansion of the built-up areas and settlements occurred within the legally protected rating categories of land of IVb (86 ha or 47% of total change) and III rating category of high-quality land (49 ha or 27% of total change) in period 2008-2012 (Ljuša et al., 2016). Also significant occurrence of succession of agricultural areas amounting to 374.18 ha were identified in Kalesija in the period 2008-2012.

The cause of the poor state of land management/use can be sought in the absence of land market or its very weak position which is particularly contributed to by the lack of land registers that were hidden or destroyed during the war, or the inability to prove ownership, which opens the door to opportunities for illegal acquisition of wealth and corruption (Čustović et al., 2013). Lack of soil classes maps and updated information about the land and its quality creates room for speculation and manipulation of this resource in the context of conversion into construction land.

Land consolidation has not been enforced. It is evident that B&H is significantly behind schedule in terms of implementing the reforms. The lack of consensus on the long-term socio-economic development, agriculture and rural policies, information systems, database of land resources, basic strategic documents etc., contributes to the fact that the agricultural sector is considered the least regulated.

CONCLUSIONS

Through the analysis of three sets of data on agricultural land, the study has shown that the average loss of agricultural land is not linear (3,000 ha/year)

and that there are huge fluctuations in the change of use in all its categories. Studies have shown that there is a strong correlation between land use and socio-economic development policy which in all sectors, including agriculture, was based on the planned economic policy. Land policy was implemented within this framework with more or less success. With the current problems of deagrarization, ineffective agrarian and land policies and incentives, it is very unlikely to expect the area of uncultivated fields to reduce; on the contrary, it is going to grow and partly become affected by the processes of succession and degradation, especially in marginalized areas and fragmented holdings. Without making a decisive shift in policies and having clearly defined objectives aimed at protecting the agricultural areas and putting them in use, it is hard to expect any significant changes in the sector. Land consolidation whose significance has not been recognized could be the solution to many problems in the agricultural sector and rural planning in B&H.

REFERENCES

Begić K. (2000). Ekonomska politika: Uvod u ekonomsku politiku: opća pitanja ekonomske politike. Sarajevo.

Bogunović S. (1983). Osnovni preduslov za planiranje skladnijeg i racionalnijeg ukupnog društvenog razvoja. Radovi, broj 9, Institut za arhitekturu, urbanizam i prostorno planiranje Arhitektonskog fakulteta u Sarajevu. Sarajevo.

Bilandžić D. (2003). Zlatno doba Bosne i Hercegovine. Naučni skup: Bosna i Hercegovina prije i nakon ZAVNOBiH-a, Posebna izdanja, Knjiga 37. Akademija nauka i umjetnosti Bosne i Hercegovine. Sarajevo.

Bublin M. (2000). Prostorno planiranje. Univerzitetska knjiga, Studentska stamparija Univerziteta. Sarajevo.

Čustović H. (2005). An overview of general Land and Soil Water conditions in Bosnia and Herzegovina. European Soil Bureau-Research Report No. 9., JRC.

Čustović H., Ljuša M. (2006). Participatory Land Use Development in Bosnia and Herzegovina. International Conference on Land Consolidation and Land Development. FAO. Prague.

Čustović, H., Ljuša, M., Marković, M. (2013). Land use changes and loss of soil in Bosnia and Herzegovina as consequences of the war and socio-economic transition. 2nd Scientific Conference UNCCD. Bon.

Čustović H., Ljuša M. i drugi. (2013a). Studija upotrebne vrijednosti zemljišta za područje općine Kalesija.

Čustović H., Ljuša M. i drugi. (2013b). Studija upotrebne vrijednosti zemljišta za područje općine Gradačac.

Čustović, H.; Bajramović, S. (2008). State-owned land as a potential factor of improvement of ownership structure in BiH. Works Of The Faculty Of Agriculture University Of Sarajevo: 59(1), 219-231.

Golić B. (1994). Ekonomika prostora u privrednom razvoju Bosne i Hercegovine. Bosna public. Sarajevo.

GTZ (2001). Studija o razvoju prehrambene industrije u Bosni i Hercegovini. Sarajevo.

Ivanković M., Bojnec S., Kolega A., Selak V. (2006). Ekonomska i drustvena uloga poljoprivrede u Federaciji Bosne i Hercegovine. Journal for General Social Issues. Vol.15 No.4-5 (84-85) 01/2006.

Lukić V., Begić M., Imamović J. (1991). Teorijski i prakticni komentar Zakona o premjeru i katastru nekretnina. YU ISBN 86-901233-1-8. SID „STRUKA“. Sarajevo.

Ljuša M., Čustović H., Cero M. (2016). Land capability study and map in function of land protection, spatial planning and agro-ecological zoning. In print.

Ljuša M. (2015). Trendovi i karakteristike promjena načina korištenja poljoprivrednog zemljišta u Bosni i Hercegovini. Disertacija. Univerzitet u Sarajevu.

Milojević A. (1983). Selo, poljoprivreda i poljoprivredno zemljište. Savjetovanje o temi Zemljište u Prostornom planu SR BiH, referati. Poljoprivredni fakultet Sarajevo. Sarajevo.

Nurković R. (2012). Urbanization and rural development in Bosnia and Herzegovina. UGI 2011. Regional Geographic Conference, Santiago Chile, 26 Local Development. Santiago Chile.

Resulović H. (1977). Uticaj tehnološkog progresa na proces oštećenja i potrošnje zemljišta. Poljoprivredni fakultet Sarajevo. Sarajevo.

Resulović H. (1978). Poljoprivredna zemljišta SR Bosne i Hercegovine (s aspekta njihove rasprostranjenosti, upotrebne vrijednosti i potrošnje izvan sfere poljoprivrede. Sarajevo.

Resulović H. (1983). Gubici i degradacija poljoprivrednog zemljišta u SR BiH. Savjetovanje o temi „Zemljište u Prostornom planu SR BiH“, referati. Poljoprivredni fakultet Sarajevo. Sarajevo.

Resulović H. (1993). Oštećenja zemljišta izazvana ratnim dejstvima na teritoriji Republike Bosne i Hercegovine. Studija. Zavod za agropedologiju. Sarajevo.

Resulović H. (1999). Zemljišni resursi u Bosni i Hercegovini-korištenje u funkciji održivog razvoja. Posebna izdanja, Knjiga 16. Akademija nauka i umjetnosti Bosne i Hercegovine. Sarajevo.

Resulović H., Bukalo E., Kraišnik V. (2010). Načini korištenja zemljišta-suprotnosti i mogućnosti harmonizacije u funkciji održivog razvoja. Prvi naučni simpozijum agronoma sa međunarodnim učešćem-Agrosym. Jahorina.

Selak V. (1996). Tehničko-tehnološki aspekti strategije razvoja poljoprivrede R/F BiH. Prvo savjetovanje: Strategija razvoja poljoprivrede, Bihać, 10-11 maj 1996. godine.

Stefanović V. (1983). Ocjena realnosti, primjenljivosti i racionalnosti društveno-ekonomskih planova razvoja bez prostorne komponente. Radovi, broj 9, Institut za arhitekturu, urbanizam i prostorno planiranje Arhitektonskog fakulteta u Sarajevu. Studentski servis. Sarajevo.

Taletović J., Ljuša M., Đuzo F., Vojniković S., Čustović H. (2010). Priprema baze podataka o zemljišnom pokrivaču CORINE 2006-metodološki pristup i osnovni principi, Zbornik radova XXI Međunarodna naučno-stručna konferencija poljoprivrede i prehrambene industrije, Neum, str. 637-645.

DOI: 10.17707/AgricultForest.65.1.16

Dmytro ONOPRIIENKO, Mykola KHARYTONOV [1]

THE EFFECTS OF IRRIGATION AND NITROGEN APPLICATION RATES ON YIELD AND QUALITY OF CORN IN THE STEPPE ZONE OF UKRAINE

SUMMARY

The given experimental data testifies that nitrogen fertilizers can be effectively introduced with irrigation water (fertigation). The advantages of fertigation in comparison with the traditional technology of introduction of mineral fertilizers of brushwood are shown for production of corn grain in conditions of the northern part of Ukraine's Steppe zone Nitrates migrate from the root layer and is gradually impoverished when nitrogen fertilizer introduced in the autumn. By the period of intense need for N (10-12 leaves), nitrates in the soil were less than in the phase of 5-6 leaves, 15.3%, and in the phase of milk ripeness of grain their content was 50.3%. It is advisable to add N fertilizers to irrigated water in the following proportions: 40% of the overall dose during the period of 10-12 leaves, 40% - in the phase of pinnacle ejection and 20% in the phase of milky ripeness of grain. The corn grain yield of was increased up to 10.3 t/ha when the trial of N fertilizer application include 3 times of fertigation. The protein content of the grain increased with fertigation.

It is advisable to add N fertilizers to irrigated water in the following proportions: 40% of the overall dose during the period of 10-12 leaves, 40% - in the phase of pinnacle ejection and 20% in the phase of milky ripeness of grain.

Keywords: Irrigation, fertigation, corn, grain yield, mineral fertilizers.

INTRODUCTION

Application of fertilizers through an efficient irrigation system was called fertigation (Bussi et al, 1991; Hebbar et al., 2004). The combination in one technological process of fertilization and irrigation determines the phenomenon of synergy. Two of the most effective factors in the production of maize are irrigation and fertilizers mutually reinforce each other, with an additional factor - their interaction. It is one of the effective ways to intensify irrigated land, to provide more accurate and timely crop nutrition (Ardell, 2006), water use efficiency under different cropping situation (Tiwari et al., 2002). The introduction of mineral fertilizers with irrigation water fully complies with the idea of multipurpose use of irrigation systems and sprinkler technology, increases

[1]Dmitro Onopriienko, Dnipro State Agrarian and Economic University, 49600, Dnipro, UKRAINE; Mykola Kharytonov, (corresponding author: kharytonov.m.m@dsau.dp.ua), Dnipro State Agrarian and Economic University, 49600, Dnipro, UKRAINE.

Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

the efficiency of water and fertilizers, contributes to maintain the structure of the soil, improves the environmental conditions in the cultivation of crops.

Drip fertigation with water-soluble fertilizer improved the root system by inducing new secondary roots which are succulent and actively involved in physiological responses (Fanish et al., 2013).

Fertigation promotes the production of intensely branched roots that facilitates nutrient acquisition and foraging capacity. Fertigation allows the implementation of complex mechanization and automation of technological processes, ensuring, with the observance of the recommended agrotechnology. Higher grain yields of 7.5 t/ha were recorded under drip fertigation of 100 per cent recommended dose of fertilizer (RDF) with 50 per cent P and K as water soluble fertilizer (Fanish, 2013). Considering the high cost of water soluble fertilizers, drip fertigation of 150 per cent RDF with radish as intercrop could be an alternative option to realize a reasonably good yield and income in maize containing intercropping system. Similar results were obtained by Zwart and Bastiaansen (2004), who reported values of 1.1-2.7 kg/m^3 for the water productivity of maize. These finding indicate that it is essential to employ appropriate methods for determining the amount of irrigation water and the period of applying the fertilizer through the irrigation water under the drip irrigation system.

The increase in water use efficiency in all drip irrigated treatments over surface irrigation was mainly due to considerable saving of irrigation water, greater increase in yield of crops and higher nutrient use efficiency (Fanish et al., 2011). This was in agreement with Ardell (2006) reported that application of N and P fertilizer increases crop yields, thereby increasing crop water use efficiency. In order to maximize maize yields and the productivity of the irrigation water under the drip irrigation system in the sandy soil, it is recommended to irrigate maize crops using a water amount at 1.2 of crop evapotranspiration every 3 days and applying the recommended fertilizer dose in 80% of the irrigation time (Ibrahim et al., 2016). Application of fertilizers with irrigation water radically solves the problem of uniform distribution of fertilizers in the active layer of soil to the level of uniform distribution of irrigation water (Mmolawa and Or, 2000).

The traditional technology of fertilizer spreading across the field is imperfect because it is dominated by man-made factors instead of biological ones. Fertilizers that are cultivated in soil for almost six months before their intensive use by maize, are losing a lot of nutrients due to mineralization, evaporation into the air and washing into deep layers of the soil, contaminating the environment (Harold and Reetz, 2016). The technological capabilities of existing scatterers are very low. The uneven distribution of fertilizers in the field, especially in the case of large doses, reaches 50-75%. Under these conditions, even the negative effect of fertilizers on plants and soil is observed. In addition, fertilizers are unevenly fed into farms, and those purchased during vegetation of corn are practically not used. Consequently, there was a need for new approaches

to the rational use of mineral fertilizers, which involves introducing them mainly with irrigation water, as well as locally. The retail introduction of N fertilizers along with irrigation water ensures more even assimilation during the growing season than the one-time introduction at sowing (Nilahyane et al., 2018).

Rates of fertilizer should be established depending on the biological characteristics of the crops, soil conditions, and closely aligned with the irrigation schedule. Corn (Zea mays L.) harvested for silage is the most important feed crop worldwide. In semi-arid regions, scarcity of water supply reduces the potential of sustainable corn production (Bannayan et al, 2011). Efficient irrigation systems and adequate fertilization strategies could improve nutrients and water uptake and productivity (Kim et al., 2008). These strategies must be supported with research-based information addressing key issues that may decrease yield (Mansouri- Far et al., 2010). These include the negative effects of water stress on plant growth and leaf area (Stone et al., 2001; Pandey et al., 2000).

The main aim was to investigate the effects of sprinkler irrigation and N application rates on soil nitrate-N (NO_3^--N) accumulation, corn grain yield and quality.

MATERIAL AND METHODS

The research was conducted during 3 years (from 1999-2001) growing seasons in the educational - research farm "Samara" of the Dnipro State Agrarian University. Agrochemical analyzes of soil samples were carried out follow accepted approaches (Dokuchaev Soil Institute, 1965). Groundwaters lie at a depth of more than 15 m.

Weather conditions during the growing seasons were generally favorable for growing corn in irrigation by the method of sprinkling. During the vegetation period (May-September), 1999, 128 mm of precipitation was felled, in 2000 was 216 mm, and in 2001 was 192 mm.

The seeds of the medium-sized hybrid of corn Pioneer 3978 was sown in the field experiments. The rates of mineral fertilizers were calculated for the expected grain yield of 8 and 10 t/ha. There was also an option without fertilizers. Corn cultivation technology was commonly used for this crop in the northern part of the steppe zone of Ukraine. Sprinkler irrigation was performed with unit DDA-100MA. Urea (carbamide) was dosed in irrigation water with a special hydro-fluid in accordance with the program of research for cultivation and irrigation water. The irrigation mode provided for the maintenance of soil moisture in the active layer of not less than 70-80% minimum water capacity (MWC). Irrigation rate norm was 1800-2100 m^3/ha.

Rates of N fertilizers were calculated by the balance method, taking into account the content of the main nutrients in the arable soil layer. Introducing various options of urea fertilizer with irrigation water was developed in order to study this technology efficiency in comparison with the traditional spreading method and studying the optimal parameters of fertigation.

Technological schemes for the introduction of N fertilizers are as follows:

A – no fertigation;

B – fertigation under the cultivation with a full norm;

C – fractionally fertigation: 40% of the norm of sprouts for cultivation, and with irrigation water 40% in the phase of 10-12 leaves and 20% in the phase of pinnacle ejection;

D - full N norm with irrigation water (fractionally fertigation in doses, 40% in the period after sowing to the phase of 10-12 leaves, 40% in the phase of pinnacle ejection and 20% in the phase of milky ripeness of grain).

Sowing area of the experimental fields 630 m², and accounting 150 m², repetition - fourfold. Yields data were processed by the method of dispersion analysis. Grain quality was estimated on several parameters including crude protein, starch, fat and gluten using infrared analyzer “Infrapyd 61” (Hungary).

RESULTS AND DISCUSSION

The data on $N\text{-}NO_3$ content in the topsoil layer depending on the methods of nitrogen fertilizers introducing (average for 3 years) to reach the yield of 8 t/ha, mg/kg of soil (Table 1).

Table 1. The content of $N\text{-}NO_3$ in the topsoil layer depending on the methods of introducing nitrogen fertilizers at the programming of the yield of 8 ton/ha (average for 3 years), mg/kg

Trial	Vegetation stage		
	5-6 leaves	10-12 leaves	milky ripeness of grain
B	30.8	26.1	15.3
D	20.5	25.0	18.8

It was shown that N content plays an important role in plant productivity under irrigation conditions, depending on the method and time of fertilizer application. Nitrates migrate from the root layer and is gradually impoverished when nitrogen fertilizer introduced in the autumn. By the period of intense need for N (10-12 leaves), nitrates in the soil was less than in the phase of 5-6 leaves, 15.3%, and in the phase of milk ripeness of grain their content was 50.3%.

The three years corn yields data of field experiment on urea using with irrigation water are shown in the table 2. The corn grain yield of was increased up to 10.3 t/ha when the trial of N fertilizer application include 3 times of fertigation.

There were established sometimes a deterioration in the quality of grain during irrigation together with an increase in yield (Wang et al, 2012). The increased demand of limited water sources presents a constraint in crop production with deteriorating quality as result of increased consumption (Ma et al., 2005). Zhang et al. (1996) found that fertilizers are applied at double or even triple the rates required for agricultural production.

Table 2. The yield of maize hybrid Pioneer 3978, depending on the dose and method of mineral fertilizers application, t/ha

For yield. t/ha	Trials	Year			Average	± to control	
		1st	2nd	3rd		t/ha	%
	A	5.16	5.96	5.48	5.53	-	-
8.0	B	7.86	7.75	8.01	7.87	-	-
	C	8.14	8.46	8.54	8.38	0.51	6.6
	D	8.28	8.65	8.58	8.51	0.63	8.1
	Average	8.09	8.28	8.37	8.25	-	-
10.0	B	9.28	9.34	9.46	9.6	-	-
	C	9.87	10.20	10.06	10.04	0.62	6.7
	D	10.14	10.32	10.42	10.29	0.93	10.0
	Average	9.76	9.95	9.98	9.89	-	-
HIP_{05} t/ha for trial		0.03	0.47	0.21			
HIP_{05} t/ha for rate		0.24	0.32	0.13			

Meantime our experiments showed that there was a tendency to increase the content of protein with increasing calculated rates of urea (Table3).

Table 3. The grain quality of maize hybrid Pioneer 3978, depending on the methods and timing for the introduction of urea (the average for 3 years).

Expected yield, t/ha	Trials	Content in grain,%			
		Crude protein	Fat	Starch	Cluten
	A	8.9	4.9	61.8	2.9
8.0	B	9.1	4.9	62.2	3.1
	C	9.5	4.8	64.3	2.9
	D	9.4	5.0	63.1	3.0
	Average	9.3	4.9	63.2	3.0
10.0	B	9.4	4.9	62.9	2.9
	C	9.4	5.0	63.1	3.0
	D	9.6	5.0	61.8	3.0
	Average	9.4	4.9	62.6	3.0

The method of introducing nitrogen fertilizers also has an effect on the protein content of the grain. The protein content of the grain increased with fertigation. Two rates of urea introducing with fertigation way did not significantly affect the content of starch, fat and gluten in the grain.

Many factors affect grain crops yield and NO_3-N accumulation in soil. These include crop N uptake dynamics, N fertilizer management, rainfall, irrigation, soil texture, and N transformation in the soil (Wang et al, 2012). However, N fertilizer and irrigation are two major factors influencing grain crops

yield and NO_3-N accumulation but these can be controlled by the grower (Ottman and Pope, 2000; Yin et al., 2007). In our research during repeated application of N fertilizers with irrigation water, the volatility of nitrates in the soil in this period was lower. Moreover, their content in soil was bigger in the milk grazing phase.

It is necessary to take into account that N fertilization increases crop yield when the soil N supply is low (Fredrick et al., 1995a: 1995b; Sexton et al., 1996). N fertilizer applied at rates higher than the optimum requirement for crop production may cause an increase in NO_3-N accumulation below the root zone and pose a risk of NO_3-N leaching (Zhu et al., 2003; Fang et al., 2006). It was shown also that the amount of NO_3-N, decreases with the black soil depth in the DSAEU educational - research farm "Samara" (Kharytonov et al, 2016). The greatest energy of nitrification (27.1 mg/ kg) was in the stratum 0- 30 cm. The impact of nitrogen fertilization at silking was higher at the smallest rate of N during the plant vegetative development. Enhancements in grain yield with late N side - dressing resulted from increases in grain weight. Two nitrogen fertilizer rates calculation to get grain yield 8 and 10 ton/ha in our research was based for N content in soil in medium level. Obviously that 20% of N in the phase of milky ripeness enhance grain yield as well.

CONCLUSIONS

It is advisable to add N fertilizers to irrigated water in the following proportions: 40% of the overall dose during the period of 10-12 leaves, 40% - in the phase of pinnacle ejection and 20% in the phase of milky ripeness of grain.
The results of research indicate that combination of irrigation with the introduction of mineral fertilizers (fertigation) is an effective way of saving energy and material resources, increasing the yield and quality of corn grain yield, and protecting the soil from degradation.

ACKNOWLEDGEMENTS

This study was supported by the Ukrainian Ministry of Agrarian Policy.

REFERENCES

Ardell D.H. (2006). Water use efficiency under different cropping situation. Annals of Agricultural Research, 27(5):115-118.

Bannayan M., Sanjani S., Alizadeh A., Lotfabadi S.S., Mohamadian A. Association between climate indices, aridity index, and rain fed crop yield in northeast of Iran. Field Crops Res. 2011, 118, 105–114.

Bussi C., Huguet J.G., Defrance H. Fertilization Scheduling in Peach Orchard under Trickle Irrigation, *Journal of Horticulture Science*, Vol. 66, No. 4, 1991, p. 487-493.

Dokuchaev Soil Institute. Agrochemical methods of soil investigation. Ed. by USSR Academy of Science. Science Publishers, Moscow. 4th Ed. 1965. 436 p. (in Russian).

Harold F., Reetz Jr. (2016). Fertilizers and their Efficient Use International Fertilizer Industry Association (IFA) Paris, France, 2016. 116 p.

Hebbar S.S., Ramachandrappa B.K., Nanjappa H.V. and Prabhakar M. (2004). Studies on NPK drip fertigation in field grown tomato (Lycopersicon esculentum Mill.). European Journal of Agronomy, 21: 117-127.

Fanish A.S.R., Purushothaman M., Pachamuthu A. (2013). Root Characters of Maize as Influenced by Drip Fertigation Levels. American Journal of Plant Sciences, 4, 340-348, http://dx.doi.org/10.4236/ajps.2013.42045).

Fanish A.S. (2013). Water productivity and profitability of maize under drip fertigation in intensive maize based intercropping system. International Journal of Agricultural Sciences Vol. 3 (5), p. 538-543.

Fanish A.S., Muthukrishnan P., Manoharan S. (2011). Drip fertigation in maize (Zea Mays) based intercropping system. Indian J. Agric. Res., 45 (3): 233 – 238.

Ibrahim M.M., El-Baroudy A.A., Taha A.M. Irrigation and fertigation scheduling under drip irrigation for maize crop Int. Agrophys., 2016, 30, 47-55 doi: 10.1515/intag-2015-0071).

Kharytonov,M., M., Pashova, T., V., Bagorka,O.,M., Kozechko, V. (2016): Nutrition regimes of eroded lands in the northern steppe zone of Ukraine. Agriculture and Forestry, 62 (3): 175-185. DOI:10.17707/AgricultForest.62.3.15.

Kim K., Clay D.E., Carlson C.G., Clay S.A., Trooien T. Do synergistic relationships between nitrogen and water influence the ability of corn to use nitrogen derived from fertilizer and soil? Agron. J. 2008, 100, 551–556.

Ma J.Z., Wang X.S., Edmunds W.M. (2005) The characteristics of groundwater resources and their changes under the impacts of human activity in arid North-West China - a case study of the Shiyang river Basin. J Arid Environ 61:277-295

Mansouri-Far C.; Sanavy S.A.M.M., Saberali S.F. Maize yield response to deficit irrigation during low-sensitive growth stages and nitrogen rate under semi-arid climatic conditions. Agric. Water Manag. 2010, 97, 12–22.

Mmolawa K., Or D. Root Zone Solute Dynamics under Drip Irrigation: A Review," Plant and Soil, Vol. 222, No. 1-2, 2000, p. 163-190. doi:10.1023/A:1004756832038.

Nilahyane A., Islam M.A., Abdel O. Mesbah O.A., Garcia A.G. Effect of Irrigation and Nitrogen Fertilization Strategies on Silage Corn Grown in Semi-Arid Conditions. Agronomy 2018, 8, 208; doi:10.3390/agronomy8100208.

Ottman M.J., Pope N.V. (2000) Nitrogen fertilizer movement in the soil as influenced by nitrogen rate and timing in irrigated wheat. Soil Sci Soc Am J 64:1883-1892.

Pandey, R.K.; Maranville, J.W.; Admou, A. Deficit irrigation and nitrogen effects on maize in a Sahelian environment. I. Grain yield and yield components. Agric. Water Manag. 2000, 46, 1–13.

Sexton B.T., Moncrief J.F., Rosen C.J., Gupta S.C., Cheng H.H. (1996) Optimizing nitrogen and irrigation inputs for corn based on nitrate leaching and yield on a coarse-textured soil. J Environ Qual 25:982-992.

Stone P.J., Wilson, D.R., Reid J.B.,Gillespie, R.N. Water deficit effects on sweet corn. I. Water use, radiation use efficiency, growth, and yield. Aust. J. Agric. Res. 2001, 52, 103–113.

Tiwari. K.N. Singh, Ajan and P.K.Mal. (2002). Effect of drip irrigation on yield of cabbage (Brassica oleraceae L. var. capitata) under mulch and non mulch conditions. Agricultural Water Management, 58: 19-28. il.)Eur.J.Agron.21.:117-127.

Yin F., Fu B., Mao R. (2007) Effects of nitrogen fertilizer application rates on nitrate nitrogen distribution in saline soil in the Hai River Basin, China. J Soil Sediment 7 (3):136-142.

Zhang W.L., Tian Z.X., Zhang N., Li X.Q. (1996) Nitrate pollution of groundwater in Northern China. Agr Ecosyst Environ 59:223-231.Zhu JG, Liu G, Han Y, Zhang YL, Xing GX (2003) Nitrate distribution and denitrification in the saturated zone of paddy field under rice / wheat rotation. Chemosphere 50: 725-732.

Zwart, S.J. and Bastiaanssen, W.G.M. (2004) Review of Measured Crop Water Productivity Values for Irrigated Wheat, Rice, Cotton and Maize. Agricultural Water Management, 69, 115-133. http://dx.doi.org/10.1016/j.agwat.2004.04.007.

Wang Q., Li F., Zhang E., Li G. Vance M. (2012)The effects of irrigation and nitrogen application rates on yield of spring wheat (longfu-920), and water use efficiency and nitrate nitrogen accumulation in soil. Australian Journal of Crop Sciences 6(4):662- 672

DOI: 10.17707/AgricultForest.65.1.17

Fatima DAHLIA,
Cesar BENITO, Mohamed BOUSSAID [1]

GENETIC DIVERSITY OF FRUITS IN WILD JUJUBE (*Ziziphus lotus* L. DESF.) NATURAL POPULATIONS FROM ALGERIA

SUMMARY

Fruits from natural populations of wild jujube (*Ziziphus lotus* L. Desf.) collected from Algeria were evaluated through morphological and molecular markers. The analysis of variance revealed very highly significant differences for all the morphological characters studied ($P < 0.001$) with coefficients of variation ranging from 7.57% to 31.84% indicating a strong variation between populations. The analysis of genetic diversity between *Ziziphus lotus* populations using 35 ISSR primers showed a moderate polymorphism (61.31%), a polymorphism information content of 0.35, a resolving power of 2.51 and a marker index of 0.79. Cluster analysis using SM model, classify the nine populations in two main clusters. This classification seems independent to climate change.

Keywords: *Ziziphus lotus*, genetic diversity, fruits, morphological markers, ISSR markers.

INTRODUCTION

Spontaneous plant resources are a source of primary interest for humans and their needs (Bouallala et *al*. 2014). Algeria has important species of trees and shrubs that the majority are undeveloped. Their fruits are an important part of human food consumption because of their richness in nutrients. However, there are some fruits that are little consumed because of their scarcity and ignorance of their nutritional quality. Among them, we chose to conduct a study on wild jujube fruit (*Ziziphus lotus* L. Desf.).

This perennial shrub (Maraghni et *al*. 2010) is of interest in rehabilitating degraded areas (Azam-Ali et *al*. 2006). It protects soil against erosion (Laamouri et *al*. 2008). This species show a high tolerance to drought and salinity (Liu and Zhao 2009; Sudhersan and Ashkanani 2009). It provides important habitat for animals and plants (Seigne 1985; Oliet et *al*. 2012; Nebih Hadj-Sadok et *al*. 2014). It is an important source of a many nutrients, phyto-chemical components and natural bio-actives substances (Tirado and Pugnaire 2005; Wojdyło et *al*. 2016). It is rich in minerals, carbohydrates, proteins and fatty acids (Abdeddaim

[1]Fatima Dahlia (Corresponding author: fdahlia84@gmail.com), Mohamed Boussaid, Laboratory of plant physiology applied to soilless crops, Ibn Khaldoun University, BP 78 Zaâroura, Tiaret, 14000, ALGERIA; Cesar Benito, Laboratory of Molecular Genetics, Department of Genetics, Faculty of Biology, Complutense University of Madrid, SPAIN.

Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

et *al*. 2014; Hammia et *al*. 2015). *Ziziphus lotus* is, therefore, a genetic resource with universal value (Rais et *al*. 2017).

Called in Arabic « Sedra », *Ziziphus lotus* is a shrub with gray and spiny stems, deciduous leaves and small yellow flowers (Maraghni et *al*. 2011). The edible fruit called « nbag » is a dark red drupe (c. 1–1.5 cm in diameter) at maturity (Azam-Ali, 2006; Wang et *al*. 2016). The pulp is soft and pleasant; it surrounds a bone nucleus, called stone, rather small, rounded and binocular (Couverciiel, 1839). It usually contains two seeds (c. 6×5 mm) (Nasri-Ayachi and Nabli 2009).

The natural range of the majority of jujube trees is between 20° and 30° latitude (Laamouri et *al*. 2008). Wild jujube occurs throughout the Mediterranean region (Regehr and El Brahli 1995; Azam-Ali et *al*. 2006) with low penetration into the northern Sahara: Morocco, Algeria, Tunisia, Libya and Egypt. It then reappeared in Yemen, on the island of Socotra, in the Middle East: Palestine, Syria, Turkey (Quezel and Santa 1963; Ghedira, 2013; Bakhtaoui et *al*. 2014) and south to the Arabian Peninsula (Duling et *al*. 1998). It is very abundant in Iran, China and South Korea (Adeli and Samavati 2015). In Algeria, the wild jujube is very common throughout the country except in the Algerian-Constantine tell (Quezel and Santa 1963).

Environmental variations, cultivar, cultivation methods and storage conditions influence strongly the fruit quality (Guo et *al*. 2016). The important geographical and climatic distribution of wild jujube is an indicator of the presence of a large genetic diversity for this species (Singh et *al*. 2007). Exploring biodiversity and estimating genetic diversity is important for evaluating, improving, conserving and using the genetic resources (Ai et *al*. 2014; Zhang et *al*. 2015; Wang et *al*. 2016). In order to recognize resistant genotypes with good fruit properties, identification of local cultivars becomes a priority (Ghazaeian 2015; Martins et *al*. 2015; Tatari et *al*. 2016). Thereby, several ways are used to measure genetic diversity, including morphological characteristics that determine strongly the agronomic value and facilitate the classification of plants. Morphological evaluation of genetic diversity is direct, inexpensive and easy to use (Zhang et *al*. 2015). Techniques such as allozyme or DNA analysis are also use to assess directly the genetic diversity at the molecular level (Mondini et *al*., 2009). Inter Simple Sequence Repeat (ISSR) technique provides a powerful tool for the investigation of genetic variation within species. Recent ISSR studies of natural populations have demonstrated the hyper-variable nature of these markers and their potential use for population-level studies (Ge et *al*. 2005).

However, there is little information available regarding the diversity of wild jujube across several environments in the world (González-Robles et *al*. 2016; Boussaid et *al*. 2018). For this purpose, a comparative study of several populations of wild jujube can give an overview of the morphological and molecular variations existing in this species. The aims of this study were to

identify genetic diversity of fruit of nine populations of wild jujube existed in Algeria and classify them using morphological and molecular characters.

MATERIAL AND METHODS

Sampling and site characterization

Fruits of *Ziziphus lotus* were harvested at maturity in August, September and October 2016. They were collected from nine stations in different regions of Algeria (table 1). Each station represents a population and each population was represented by 10 trees spaced of more than 10 m.

The natural distribution area of harvested shrubs extends from 2°12 W to 4°18 E longitude and from 32°1 N to 36°6 N latitude (table 1). This large area is characterized by climatic and soil variations covering the main bio-climatic stage of Algeria.

The sites of Ain Defla and Medea from the sub-humid stage have a moderate climate, with abundant and regular precipitations (773,62 and 807,23 mm) and mild winter (table 1). The semi-arid stage that is under the effect of a continental climate is characterized by moderate rainfall (Chlef with 404.85 mm and Tiaret with 334.4 mm), a cold winter and a hot summer.

Table 1: Description of the selected sites of the nine natural populations of *Ziziphus lotus* in Algeria.

Populations	Ain Defla	Bechar	Chlef	El Bayadh	Ghardaia	Laghouat	M'Sila	Medea	Tiaret
Position									
Locations	Ouled Chikh	Mougheul	Ouled Ziad	Bougtoub	Metlili	Oued Nogued	Maarif	Boghar	Tidda
Long	2°1'E	2°12'W	1°6'E	0°7'E	3°33'E	2°59'E	4°18'E	2°42'E	1°14'E
Lat (N)	36°6'	32°1'	36°6'	33°59'	32°18	33°49'	35°22'	35°57'	35°40'
Alt (m)	516	1023	106	1038	526	772	410	853	705
Climate									
Tm	19.1	22.5	20.5	20.2	23.2	20.1	21.7	16.5	15.7
TR	10.2	13.8	12.6	13.4	12.2	13.3	13	8.8	14.5
Dt	28.1	37.8	31	35.3	33.9	37.1	36.3	28.4	35.8
P	807.23	107.95	404.85	218.65	17.51	198.2	172.21	773.62	334.03
Cliamte	Subhumid	Saharian	Semiarid	Arid	Saharian	Saharian	Arid	Subhumid	Semiarid
Soil									
$CaCo_3$ %	3.5	7.5	24	19.1	4.11	8.8	24.97	0	2.6
OM %	1.51	0.49	1.2	1	0.75	0.78	2.01	1.3	0.92
pH	8	7.85	7.9	7.8	8.4	7.97	8.61	6.5	7.88
Clay %	31	0.96	27	9.8	0.4	9.4	9.76	34.4	12.3
Silt %	50.6	16.62	46.8	43.6	8.6	16.73	13.68	39.9	38.8
Sand %	18.4	82.42	26.2	46.6	91	73.87	76.56	25.7	48.9
Soils	Loamy clay	Sandy-loam	Clay-loam	Sandy-loam	Sandy	Sandy-loam	Sandy-loam	Clay-loam	Loam

TM : annual mean temperature (°C) ; TR : Temperature range (°C) ; Dt : difference in temperatures between the hottest and the coolest months (°C) ; P : annual precipitation (mm);; OM : organic matter ; (M-m) : temperature differences between the hottest month and the coldest month

In the arid zone, provenances of Msila, Laghoat and El Bayadh are influenced by the local arid steppe climate characterized by little rainfall throughout the year (172.21 to 218.65 mm). The sites of Ghardaia and Bechar from the Saharean stage are characterized by very rare rainfall (17.5 to 107.95 mm), a mild winter, a very hot summer and an arid climate.

Similarly, the soils of the different sites are very variable with a dominance of sandy soils in the arid and Saharan regions. Soils of sub-humid and semi-arid sites vary between clay-loamy and sandy-loamy (table 1). pH of the different soils is neutral to slightly alkaline (6.5 to 8.61). The levels of organic matter range from 0.48 to 2.01% and the $CaCO_3$ levels vary from 0 to 24.97%.

Morphological traits

Observations were performed on the mature fruit ready for harvesting. Immature fruits that have not attained their full sizes and that are predominantly green and quite hard in texture was not considered in this study. The colors of fruits and stones were determined using the Royal Horticultural Society color chart. The number of seeds, shapes of fruits and stones and srones appearences were also recoreded according to jujube descriptor. Determination of lengths and widths of fruits, pulps, stone and seeds were carried by using a digital caliper. A precision balance was used to measure the weight of fruits, pulps, stone and seeds.

Chromosome number

Chromosome number counting was performed according to the method of (Jahier et *al*. 1992). The mitosis was stopped by keeping roots in ice distilled water for 24 hours. Plant material was immersed in the Shift reagent for 90 minutes. The colored meristematic zone was isolated and placed between Glass slides and cover-slip in a few drops of Carmen's red reagent. The observation was made on a photonic microscope equipped with a digital camera connected to a microcomputer.

Molecular analysis

DNA extraction

A total of 45 plants (Five plants from each population) were used to extract DNA which was extracted using the NZY Plant/Fungi gDNA Isolation kit (Prates, 2014). Up to 20 mg of fresh leaves were homogenized and used to extract DNA following the protocol. The genomic DNA was stored at -20 °C.

PCR amplification

Thirty-five ISSR's markers were used as shown in (Table 4). Standard PCRs buffer were performed using NZYTaq 2× Green Master Mix. Each PCR reaction contained 10 µL of NZYTaq 2× Green Master Mix, 1µL from each primer, 30 ng DNA template and nuclease (3 µL of 10 ng/µL) and 6µL of free water to obtain a volume of 20 µl. Cycling procedure was run using PTC-100 thermocycler (MJ Research, Inc.) as follows: Initial denaturation at 94°C for five minutes followed by 45 cycles of denaturation at 94°C for 30 seconds, annealing temperature of 52°C for 45 seconds and extension at 72°C for two minutes and final extension at 72°C for six minutes. The amplification products were

analyzed by electrophoresis in 1,2% agarose in TAE buffer stained by 6 µL of ethidium bromide and photographed under UV.

Data analysis

The partition of the variance was estimated between populations by the statistical analysis of the variance (ANOVA) using the Type III of SPSS for Windows version 16.0 for the calculation of the sums squares. Homogeneous groups are separated by *Tukey* test using the same software. The agglomerative hierarchical clustering procedure was based on Ward's method.

Data from ISSR markers analysis was scored for presence (1) and absence (0) of bands. Unclear bands were not counted. Dividing the number of polymorphic bands over the total number of bands gives an estimation of the polymorphism percentage.

The evaluation of the discriminatory power of the ISSR markers was done by means of three parameters: (i) Polymorphic information content (PIC) which is the probability in detecting polymorphism by a primer or primer combination between two randomly drawn genotypes, it was calculated using the formula $\mathbf{PIC = 1 - \Sigma p_i^2}$, where p_i is the frequency of the i^{th} allele (Sehgal et *al*. 2009; Lamare and Rao 2015); (ii) Resolving power (Rp) which is the ability of each primer to detect level of variation between individuals, it was calculated as $\mathbf{Rp = \Sigma I_b}$ where I_b (band informativeness) takes the values of: $\mathbf{1 - [2|0.5 - \mathit{p}|]}$, where p is the proportion of individuals containing the band (Prevost and Wilkinson 1999; Lamare and Rao 2015), and (iii) marker index (MI) in order to characterize the capacity of each primer to reveal or detect polymorphic loci among the genotypes, as a product of two functions - the polymorphic information content and effective multiplex ratio (EMR) (Milbourne et *al*. 1997; Varshney et *al*. 2007; Lamare and Rao 2015) : **MI = PIC × EMR**.

A dendrogram was constructed based on the simple matching coefficient (SM) and the UPGMA (unweighted pair-group method with arithmetic averages) cluster method with the program NTSYSpc (Numerical Taxonomy and Multivariate Analysis System version 2.1). A pair-wise difference matrix between genotypes was determined using SM coefficient to measure the resulting phenotic groups and the original matrix was bootstrapped 1000 times by employing Winboot to group the genotypes into discrete clusters.

RESULTS

Morphological variability

Analysis of variance show a very high inter-population variation justified by a very highly significant difference ($P < 0.001$) between the populations for all morphological traits (table 2). Furthermore, there were no significant differences ($P > 0.05$) between individuals in the same population (intra-population variation).

The fruits of wild jujube were characterized by a variation of the fruit color. Four colors were recorded for all populations (table 2): yellowish (0.11%), brown (52.44%), light brown (17.55%) and dark brown (29.88%). An intra-

population variability characterized by the heterogeneity in the distribution of the coloration was noticed. The populations of Laghouat, Ain Defla and El Bayadh were homogeneous and characterized by a single fruit color. Whereas the other populations presented the heterogeneity for this trait because they were characterized by several fruit colors within the same population.

An inter-population variation for the shape of fruit was also observed: the elongated shape (6,77%) and the round shape (93,23%). Two populations which were heterogeneous and characterized by the presence of the two shapes of fruit at varying percentages (table 2). These populations are Bechar and M'sila

Table 2. Qualitative traits characterization of fruit in Algerian natural populations of *Ziziphus lotus*

Populations	Ain Defla	Bechar	Chlef	El Bayed	Ghardaia	Laghouat	Médéa	M'Sila	Tiaret
	Fruit colors								
Yellowish	0	0	0	0	2	0	0	0	0
Brown	100	42	96	0	10	100	56	6	61
Light brown	0	3	1	0	1	0	28	94	31
Dark brown	0	53	3	100	87	0	16	0	8
	Fruit shape								
Oval	0	3	0	0	0	0	0	49	0
Round	100	97	100	100	100	100	100	51	100
	Stone appearance								
Smooth	9	63	7	9	0	80	46	37	14
Rough	91	37	93	91	100	20	54	63	86
	Stone shape								
Elongated	23	91	79	81	87	55	79	99	80
Round	77	9	21	19	13	45	21	1	20
	Stone color								
Light brown	87	65	100	62	65	72	3	63	92
brown	13	35	0	38	35	28	97	37	8
	Seed number								
One seed	74	72	47	59	69	59	76	41	56
Two seeds	26	28	53	41	31	41	24	59	44

There were an inter-population variation for the coloration of the stone. Two colors were observed: brown (32.33%) and light brown (67.67%). Only the population of Chlef was homogeneous and characterized by the presence of a single coloration of stone (light brown). In the other populations, the presence of the two colors of the stone was observed at various percentages (table 2).

The wild jujube showed an inter-population variation for the trait shape of the stone. Two forms were observed: the elongated shape (74.89%) and the round shape (25.11%). All populations were heterogeneous and presented the two stone shapes at various percentages (table 2).

Two stone's appearance were recorded smooth (29,44%) and rough (70,56%). Only the population of Ghardaia was homogeneous and characterized by rough appearance. In the other populations, the presence of the two appearancs of stone was recorded at various percentages (table 2).

Most wild jujube fruits had one seed per stone (61.44%). Only 38.56% of the fruit had two seeds per stone. All populations were heterogeneous (table 2) and presented various percentages of seed number per stone.

Regarding fruit characterization (Table 3), it's shown that the population of Chlef has the highest values for fruit length (12.979 ± 0.717 mm), wight (13.503 ± 0.822 mm) and weigh (0.871 ± 0.134 g). While the population of Ghardaia is chharacterised by the lowest values for these traits (11.166 ± 0.915 mm; 11.223 ± 0.884 mm and 0.464 ± 0.107 g respectively). These two populations have grown in very different environments, which suggests that this character can be influenced by the environment. Fruit length, wight and weigh of the other populations varied between these two extremes.

The pulp length and width vary between 2.776 ± 0.571 mm to 3.767 ± 0.767 mm and between 3.874 ± 0.879 mm to 5.134 ± 0.694 mm respectively. The population of Medea has shown the highest pulp length and the population of Chlef has the highest pulp width. These traits seem to be independent of the environmental effect because the values showed no relationship with environmental variation. On the other hand, the pulp weight was very related to the variations of the environment. The populations of Chlef, Medea and Ain Defla growing in favorable areas had the highest values of pulp weights (0.477 ± 0.1g; 0.381 ± 0.074g and 0.377 ± 0.091g). Whereas, the populations of Ghardaia, Laghouat and El Bayedh, growing in arid regions, had the lowest pulp weights (Table 3).

For stones, lengths varied from 8.225 ± 0.847mm (population of Ghardaia) to 10.062 ± 0.641 mm (population of Chlef). The variations of this trait between populations seem to be independent of changes in the environment. Stones also exhibited wide variability for the trait width. The values (table 3) of the population of Ain Defla (humid climate) were the highest (8.838 ± 0.913 mm). The populations of Ghardaia and Laghouat (arid climate) had the lowest values (7,156±0,687 mm and 7,188±0,747 mm respectively). Unlike the preceding trait, the width of stones seems to be correlated with environmental variations because wetland populations have the highest values. The weight

stones varies from 0.225 ± 0.057 g for the population of Ghardaia to 0.394 ± 0.064 for the population of Chlef. It is clear that this trait is positively correlated with fruit and pulp weights.

Table 3: Inter-population variability of fruit characteristics of natural *Zizyphus lotus* in Algeria.

Pop.	AinDefla	Bechar	Chlef	ElBayedh	Ghardaia	Laghouat	Medea	Msila	Tiaret	CV %
Fruit characteristics										
FL (mm)	12.09^{c}	12.13^{c}	12.97^{d}	11.63^{d}	11.16^{a}	11.19^{a}	12.59^{d}	12.67^{d}	11.87^{bc}	7.572
FW (mm)	12.71^{d}	11.81^{bc}	13.50^{d}	11.75^{bc}	11.22^{a}	11.56^{b}	12.54^{de}	11.87^{d}	12.07^{cd}	7.922
Fwe (g)	0.71^{e}	0.61^{cd}	0.87^{f}	0.58^{bc}	0.46^{a}	0.53^{b}	0.65^{de}	0.64^{d}	0.62^{cd}	20.586
Pulp characteristics										
PuL(mm)	3.59^{d}	3.28^{c}	2.91^{ab}	3.10^{bc}	2.94^{ab}	2.81^{a}	3.76^{d}	2.91^{ab}	2.77^{a}	20.732
PuW(mm)	3.87^{e}	4.47^{de}	5.13^{f}	4.52^{bc}	4.06^{a}	4.37^{b}	4.86^{e}	4.29^{cde}	4.23^{cd}	18.858
PuWe (g)	0.37^{e}	0.36^{de}	0.47^{f}	0.31^{bc}	0.23^{a}	0.29^{b}	0.38^{e}	0.35^{cde}	0.33^{cd}	27.036
Stone characteristics										
SL (mm)	8.49^{ab}	8.84^{cd}	10.06^{e}	8.52^{abc}	8.22^{a}	8.38^{a}	8.82^{bcd}	9.75^{e}	9.10^{d}	8.521
SW (mm)	8.83^{e}	7.33^{ab}	8.36^{d}	7.23^{a}	7.15^{a}	7.18^{a}	7.68^{c}	7.58^{bc}	7.83^{c}	9.653
SWe (g)	0.33^{e}	0.24^{abc}	0.39^{f}	0.26^{bc}	0.22^{a}	0.23^{ab}	0.27^{cd}	0.29^{d}	0.29^{d}	22.165
Seed characteristics										
SeL(mm)	5.38^{a}	5.75^{b}	5.88^{b}	5.50^{a}	5.43^{a}	5.36^{a}	5.51^{a}	5.95^{b}	5.83^{b}	8.126
SeW(mm)	5.18^{c}	4.95^{ab}	4.98^{abc}	5.03^{bc}	4.82^{a}	4.78^{a}	5.51^{d}	4.94^{ab}	4.85^{ab}	9.077
Chromosoms number										
Chr Num	24	36	24	36	36	24	24	36	24	

a,b,c indicate significant differences among homogeneous groups according to *Tykey* test at p value <0.05. ; **FL** : Fruit length, **FW** : Fruit width, **FWe** : Fruit weight, **PuL** : Pulp length, **PuW** : Pulp width, **PuWe** : Pulp weight, **SL** : Stone length, **SW** : Stone width, **SWe** : Stone weight, **SeL** : Seed length, **SeW** : Seed width, **SeWe** : Seed weight, **CV%** : coefficient of variation; **Chr Num :** Chromosoms number.

The seeds length character exhibits a high inter-population variation (table 3). The longest seeds were recorded in the populations of M'sila (5.953 ± 0.477 mm), Chlef (5.888 ± 0,437 mm), Tiaret (5.835 ± 0.504 mm) and Bechar (5.752 ± 0.576 mm). Seed widths varied from 4.788 ± 0.478 mm for the population of Laghouat to 5.516 ± 0.373 mm for the population of Medea. The environment does not seem to have effects on these two traits, but the variation in seed weight is strongly influenced by environmental variation. This trait showed low values in the arid zones (0.025 ± 0.0079 g for the population of Laghouat and 0.030 ± 0.0057 for the population of Bechar) compared with the values recorded in the

populations that pushed in sub-humid environments (0.0340 ± 0.0035) g for the population of Ain Defla.

Ward's method classified the nine natural populations of Ziziphus lotus L. by measuring the distance between the morphological characteristics to give rise to two closed clusters (figure 1).

The first cluster is represented by four populations: Bechar and El Bayadh which represent a subgroup and are very close. In this first cluster, we also find that the populations of Laghouat and Ghardaia. The commonality between these four populations is that they grow in arid environments that are characterized by high daily temperatures, high thermal amplitudes and low rainfall.

In the second cluster, there is a subgroup strongly linking the populations of M'sila and Tiaret which are populations of the semi-arid zones. The second cluster also includes the population of sub-humid areas: Medea, Ain Defla and Chlef.

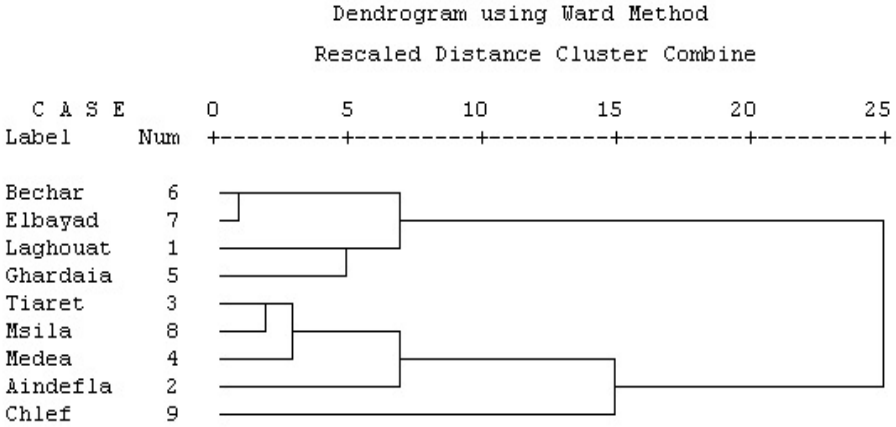


Fig 1. Dendrogram of grouping the nine natural populations of *Ziziphus lotus* L. produced using Ward method.

Chromosoms number

The number of chromosomes (Table 3) shows that wild jujube populations that grow in subhumid and semi-arid environments are diploid and have a chromosome number of 2n = 2x = 24 chromosomes. Whereas the populations of arid zones (Bechar, El Bayadh, Ghardaia and M'sila) are triploid and have a number of chromosomes equal to 2n = 3x = 36 chromosomes.

Molecular analysis

The total number of bands produced by the ISSR primers (TBN), the number of polymorphic bands (PB), the percentages of polymorphic bands (% P), the polymorphic information content (PIC), the resolving power (RP) and the marker index of the ISSR primers are shown in Table 4.

The thirty-five ISSR primers tested produced a total of 274 amplified bands with an average of 7.82 bands per primer (MR). For these primers, the number of polymorphic bands was 168 (59.14%) with an average of 4.8 polymorphic band per primer (EMR). The number of bands produced by each primer varied from 4 for the primers 847, 854, 864 and 869 to 14 for primers 812 and 876. While the number of polymorphic bands produced by each primer has varied between 2 for the primers 814, 817, 846, 849, 864 and 897 to 14 for the primer 876. The percentage of polymorphism for each primer increased from 25 % for the primer 817 to 100 % for the primers 813, 876, 892 and 900 with an average of 59.14 %. The polymorphic information content (PIC) of ISSR primers among the populations of wild jujube varied between 0.08 for the primer 814 and 0.50 for the primer 855 with an average of 0.35.

The resolving power (RP) has traded its minimum at the level of the primers 817 and 864 with a value of 0.44 and its maximum at the level of the primer 900 with a value of 8.67. The average of the RP is 2.51. At its hole, the marker index fluctuated between 0.15 for the primer 814 and 1.56 for the primer 813 with an average of 0.79.

The ISSR markers from the 35 selected primers were used to construct a dendrogram using UPGMA cluster analysis and the simple matching coefficient (SM). The degree of similarity among wild jujube populations, from different locations, varied between 31.1 % (between the populations of Laghout and Medea) and 95.7 % (between the populations of M'sila and Tairet). Cluster analysis was done to group the populations into dendrogram (figure 2). This dendrogram has two clusters. The first cluster groups the populations from Ain Defla and Bechar with a similarity of 39.1 %. The second cluster groups the populations from the other regions. This second cluster can be subdivided into two sub-clusters, the first grouping six populations two by two.

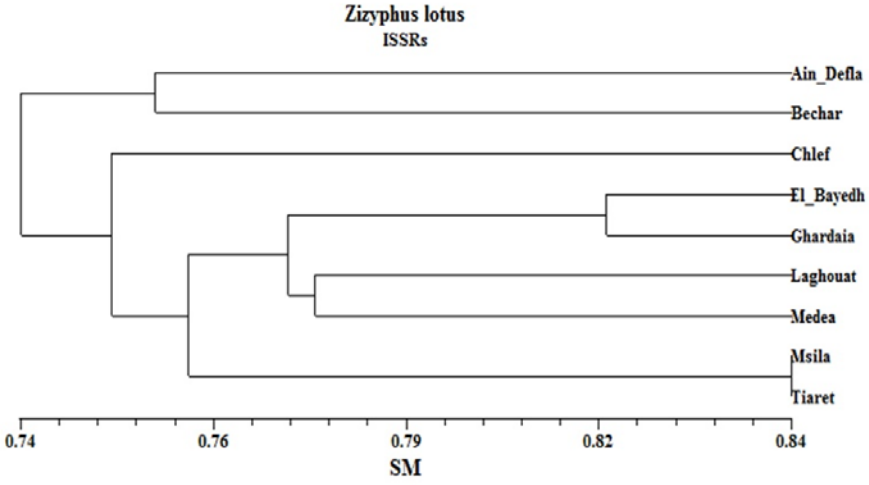


Fig 2 Dendrogram with the genetic relationship among 9 populations of *Z. lotus* growing in various regions in Algeria (SM coefficient and UPGMA method)

Table 4: Details of amplified bands generated by 35 ISSR primers in 9 populations of *Z. lotus*

Primer	Sequences 5' - 3'	TBN	PB	%P	PIC	RP	MI
807	$(AG)_8T$	8	3	37,50	0,44	1,56	0,55
808	$(AG)_8C$	8	6	75,00	0,35	3,56	1,10
810	$(GA)_8T$	12	9	75,00	0,45	3,78	0,89
811	$(GA)_8C$	10	3	30,00	0,21	2,00	0,31
812	$(GA)_8A$	14	10	71,43	0,36	4,89	1,23
813	$(CT)_8T$	6	6	100,00	0,49	2,00	1,56
814	$(CT)_8A$	7	2	28,57	0,08	1,11	0,15
815	$(CT)_8G$	5	3	60,00	0,45	1,56	0,90
817	$(CA)_8A$	8	2	25,00	0,40	0,44	0,45
823	$(TC)_8C$	8	5	62,50	0,27	2,00	0,87
834	$(AG)_8YT$	6	3	50,00	0,22	1,78	0,58
835	$(AG)_8YC$	9	4	44,44	0,48	2,44	0,63
842	$(GA)_8YG$	9	6	66,67	0,31	3,11	0,96
843	$(CT)_8RA$	10	5	50,00	0,32	3,11	0,86
845	$(CT)_8RG$	7	5	71,43	0,40	3,11	1,02
846	$(CA)_8RT$	6	2	33,33	0,12	1,33	0,25
847	$(CA)_8RC$	4	3	75,00	0,17	1,33	0,76
849	$(GT)_8YA$	8	5	62,50	0,37	3,33	0,87
854	$(TC)_8RG$	4	2	50,00	0,23	0,89	0,63
855	$(AC)_8YT$	8	4	50,00	0,50	2,44	0,69
856	$(AC)_8YA$	10	7	70,00	0,12	4,22	0,52
859	$(TG)_8RC$	6	4	66,67	0,46	2,22	0,76
864	$(ATG)_6$	4	2	50,00	0,40	0,44	0,90
866	$(CTC)_6$	8	7	87,50	0,49	2,22	1,48
868	$(GAA)_6$	6	4	66,67	0,27	2,00	0,62
869	$(GTT)_6$	4	3	75,00	0,42	2,00	1,04
876	$(GATA)_2(GACA)_2$	14	14	100,00	0,23	8,22	1,17
881	$G_3(TG_4)_2TG$	5	-	-	-	-	-
887	$DVD(TC)_7$	8	3	37,50	0,35	1,33	0,58
888	$BDB(CA)_7$	8	4	50,00	0,32	1,56	0,77
890	$VHV(GT)_7$	8	6	75,00	0,44	2,22	0,98
891	$HVHTC(TG)_6$	10	4	40,00	0,46	1,56	0,65
892	$TAGATCTGATATCTGA_2T_2C_3$	7	7	100,00	0,32	4,00	1,44
897	$C_2GACTCGAGN_6A(TG)_2G$	6	2	33,33	0,12	1,33	0,25
900	$ACT_2C_4ACAG_2T_2A_2(CA)_2$	13	13	100,00	0,23	8,67	1,17
Total		**274**	**168**				
Average		**7,82**^{MR}	**4,8**$^{EM}_{R}$	**59,14**	**0,35**	**2,51**	**0,79**

N =(A,G,C,T); R =(A,G); V =(A,G,C) ; Y =(C,T); B =(C,G,T) ; D =(A,G,T); H =(A,C,T)

The populations from Tiaret and M'sila appear to be the most similar at 95.7 %. The populations from El Bayadh and Ghardaia are grouped together with a similarity of 69.9 % and the populations from Laghouat and Medea have a similarity of 31.1 % between them. The population that is coming from Chlef represents the second sub-cluster and it seems different from the other six populations because it has a low degree of similarity with them (17.9%).

DISCUSSION

Conservation and utilization of the native plant resources is essential for long-term sustainability of biodiversity. Wild native resources are adapted to specific and diverse environmental conditions. These adaptive features can be introduced into modern cultivars either through conventional breeding or advanced molecular genetic techniques (Riaz et *al*. 2011). Estimation of genetic diversity is important for conserving, evaluating and using genetic resources. It's useful for studying the diversity of different germplasm as possible sources of genes. That can improve the performance of cultivars, and for determining the uniqueness and distinctness of the phenotypic and genetic constitution of genotypes (Geleta et *al*. 2006).

Analysis of the morphological diversity of several natural populations of *Ziziphus lotus* belonging to various environmental conditions is of a great importance. It is the foundation of plant evolution and cultivar formation in the natural wide range of distribution of this species (Boussaid et *al*. 2018).

The analysis of morphological characters shows a very large divergence between the fruits, stones and seeds of *Ziziphus lotus* natural populations collected from several sites in Algeria. Populations from Chlef, Ain Defla and Medea are considered as the best populations morphologically. They are characterized by the higher lengths, widths and weights of the different parts of the fruit. While, the populations coming from Ghardaïa and Laghouat present the lowest values. The coefficients of variation ranged from 7.57 % for fruit length to 27.036% for pulp weight. High coefficients of variation (more than 30%) for *Ziziphus jujuba* ecotypes were recorded by Tatari et *al*. (2016) for number of thorns, annual thorn length, fruit weight, shape and width, as well as stone weight and size, indicating a large variation in these traits. Our results are also similar to those recorded by Saran et *al*. (2005) and Liu et *al*. (2009) who found high coefficients of variation for several traits, particularly for fruit weight, in *Ziziphus mauritiana* and *Ziziphus jujuba* genotypes respectively.

Fruit weight is considered as appropriate for the classification of jujube ecotypes (Tatari et *al*. 2016). The studies on the Iranian ecotypes of *Ziziphus spina-christi* showed a large-scale diversity among the genotypes that show considerable morphological variation which may affect these traits (Bina et *al*. 2012; Baghazadeh-Daryaii et *al*. 2017). Tatari et *al*. (2016) confirm that climatic conditions have a large effect on the morphological characters of the *Ziziphus jujuba* ecotypes. The plants of *Ziziphus spina-christi* grown at various altitudinal zonations in Saudi Arabia showed a response to climatic conditions by using

altitudinal gradient (Moustafa et *al*. 2016). The evaluation of the morphological variability of 65 genotypes from Ziziphus genus (*Z. nummularia, Z. spina-christi* and *Z. oxyphylla*) in Iran, revealed a wide range of variation. They notified also that fruit weight among different species of jujube varied. This variation may depend on the cultivar and ecological conditions (Gao et *al*. 2011). Also, height differentiation among genotypes is caused by factors such as breeding system, isolation of population, seed and pollen dispersal distance (Norouzia et *al*. 2017). Exhibiting significant variability could be attributed to fact that the genotypes grow over a wide range of rainfall, temperature and soil type (Divakara and Das 2011).

Genetic diversity of plants based on morphological traits is difficult to measure in natural populations because these traits are influenced by environmental factors to a large degree (Riaz et *al*. 2011). To neutralize the environmental effect on the phenotypic expression among provenances for the purpose of breeding, this genetic resource should be compared under similar ecological conditions (Boussaid et *al*. 2018).

Molecular markers are widely used to assess genetic diversity and to study the relationships among genotypes and populations of many species. To study the genetic diversity of the genus Ziziphus, the ISSR, RADP, SSR, SRAP markers have been widely used on *Ziziphus spina-christi* (Alansi et *al*, 2016; Moustafa et *al*. 2016), *Ziziphus mauritiana* (Singh et *al*. 2007; Singh et *al*. 2014), *Ziziphus jujuba* (Li et *al*. 2010; Ma et *al*. 2011; Liu et *al*. 2014; Zhang et *al*. 2015;) and *Ziziphus lotus* (González-Robles et *al*. 2016). Our work is the first to study the molecular diversity between natural populations of *Ziziphus lotus* L. Desf through several environments in Algeria and the second in the world.

In order to resolve some of the inconveniences associated with RAPD (low reproducibility), the high AFLP cost, and the need to know the flanking sequences in order to developed primers for SSR polymorphism, ISSR were developed (Gomes et *al*. 2012). Also, the use of ISSR in this study is based on the fact that they are easy to employ and highly reproducible (Boussaid et *al*. 2010). Several studies have shown that the ISSR markers seem to be convenient for genetic diversity of Triticale (Tonk et *al*. 2014), *Canarium album* (Mei et *al*. 2017), *Cajanus cajan* (Hemalatha and Shanmugasundaram 2010) genotypes compared with RADP technique.

Our thirty-five ISSR primers yielded 274 bands, of which 168 were polymorphic. The percentage of polymorphism was 61.31 %. Our results are close to those found for other species: 61.54 % for *Ziziphus spina-christi* (Moustafa et *al*. 2016), 57.3% for *Dioscorea hispida* (Nudin et *al*. 2017) and 53.80 % for Triticale (Tonk et *al*. 2014). However, these results remain low compared with those found by Singh et *al*. (2007) for *Ziziphus mauritiana* (89.96 %), Alansi et *al*. (2016) for *Ziziphus spina-christi* (93.4%) and Boussaid et *al*. (2010) for *Stipa tenacissima* (87.79%).

The triploid populations of *Ziziphus lotus* showed the highest values of total bands number with 206 bands for the population of Bechar, 201 bands for

the populations of M'sila and El Bayadh and 191 bands for the population of Ghadaia. These populations had also the highest values of polymorphic bands (100, 95, 95 and 85 bands respectively) and polymorphism percent (48.54% for the population of Bechar and 47.26% for the population of El Bayadh and M'sila).

The averages obtained for polymorphism information content (PIC), resolving power (Rp) and the marker index (MI) are 0.35, 2.51 and 0.79 respectively. These averages are close to those found by Tonk et *al*. (2014) in triticale (PIC = 0.33 and Rp = 3.15), by Lamare and Rao (2015) in *Musa acuminata colla* (PIC = 0.35, Rp = 2.34 and MI = 0) , by Rashidi et *al*., (2013) *in Medicago sativa* (PIC = 0.26 and MI = 1.88) and by Singh et *al*. (2007) in *Ziziphus mauritiana* (PIC = 0.42 and Rp = 3.68).

Ward's dendrogram for morphological traits and the clustering based on SM model from ISSR data have classified wild jujube populations differently. The methods of morphology are insufficient to distinguish cultivars and the results are often liable to be influenced by the environment. It has been proved that ber genotypes earlier reported to be similar based on morphology, are genetically different (Singh et *al*. 2014).

The clustering of *Ziziphus lotus* populations according to the SM method seems independent of climatic variations. We found that the populations from M'sila and Tiaret are the most similar (95.79 %). These two populations have close latitudes (35°22 N and 35°57 N respectively). Similarly, populations from arid regions (El Bayadh and Ghardaïa) have a similarity of 69.90 % and are triploids (2n = 3x = 36 chromosomes). Populations from Laghouat and Medea, which have close longitudes (2°59E and 2°42E respectively) and which grew at the altitudes of 772 m and 853 m respectively, are classified together (31.10 %). While, the population from Ain Defla (sub-humid zone) and the population from Bechar (arid zone), which have a similarity of 39.10 %, seem independent from climatic and geographical conditions.

On their part, Rashidi et *al*. (2013) concluded that grouping of genotypes based on cluster analysis and principal coordinate analysis indicate that genetic variations of *Medicago sativa* do not agreement with the geographical distribution of genotypes. *Ziziphus spina-christi* showed a response to a suite of climatic conditions by using altitudinal gradients within circumscribed various localities in Saudi Arabia (Moustafa et *al*. 2016). A large diversity within populations was recorded in *Ziziphus spina-christi* which can be explained by the out crossing pollinisation phenomenon and a low value of genetic diversity among populations. Studies also shows that genetic variations occurred a long elevation gradient because topographical heterogeneity of plant habitat causes substantial changes in the improvement. And at different altitudes strong isolation of populations may occur due to drastic differences in phenology between higher and lower altitude and mountain barriers which restrict the gene flow between populations causing complex and varied genetic variation (Lamare and Rao 2015).

CONCLUSIONS

Wild jujube (*Ziziphus lotus* L. Desf.), despite being a frequent species in Algeria, occupying various regions and ecosystems and with various interest, remains devalued because there are very few studies on this species. Through this study, we have evaluated the genetic diversity of *Ziziphus lotus* fruits from several localities covering all bio-climatic stages of Algeria, by using morphological and molecular markers. The analysis of the morphological results revealed a strong divergence between the different populations. They also showed that the environment had a considerable effect in the development of the phenotypes of the majority of the characters. ISSR data showed moderate genetic diversity between wild jujube populations. To valorize this study, the number of populations studied must be expanded, study the genetic diversity within populations and test other molecular markers that can give more effective results.

ACKNOWLEDGEMENTS

The laboratory of molecular genetic has been of great help for the realization of this work. I am grateful to all the professors and assistants of the Department of Genetics at the Complutense University of Madrid, Spain. I thank infinitely Dr Tomas Naranjo from the laboratory of cytogenetics (Complutense University of Madrid, Spain). As I thank Barouagui S., Bakiri N., Krimat M., Tabti L., Mahieddine S., for the help provided to collect the fruits of wild jujube from several zones.

REFERENCES

Abdeddaim, M. Lombarkia, O. Bacha, A. Fahloul, D. Abdeddaim, D. Farhat, R. Saadoudi, M. Noui, Y. & Lekbir, A. (2014): Biochemical characterization and nutritional properties of *Ziziphus lotus* L. fruits in Aures region, Northeastern of Algeria. Annals: *Food Science and Technology*, 15: 75 - 81.

Ai, B. Kang, M. & Huang, H. (2014): Assessment of Genetic Diversity in Seed Plants Based on a Uniform π Criterion. *Molecules*, 19 : 20113-20127. https://doi: 10.3390/molecules191220113

Alansi, S. Tarroum, M. Al-Qurainy, F. Khan, S. & Nadeem, M. (2016): Use of ISSR markers to assess the genetic diversity in wild medicinal *Ziziphus spina-christi* (L.) Willd. collected from different regions of Saudi Arabia. *Biotechnology & Biotechnological Equipment*, 30(5): 942-947.

Azam-Ali, S. Bonkoungou, E. Bowe, C. DeKock, C. Godara, A. & Williams, J.T. (2006): Fruits for the Future 2: Ber and other jujubes. Ed. Southampton Centre for Underutilised Crops, U.K., 302 p.

Baghazadeh-Daryaii, L. Sharifi-Sirchi, G.R. & Samsampoor, D. (2017): Morphological, phytochemical and genetic diversity of *Ziziphus spina-christi* (L) Desf. in South and Southeastern of Iran. *Journal of Applied Research on Medicinal and Aromatic Plants*, https://doi.org/10.1016/j.jarmap.2017.06.006.

Bakhtaoui, F. Lakmichi, H. Megraud, F. Chait, A. & Gadhi, C.E.A. (2014) : Gastro-protective, Anti-Helicobacter pylori and, Antioxidant Properties of Moroccan *Ziziphus lotus* L. *Journal of Applied Pharmaceutical Science*, 4 (10): 81-87. https://doi.org/10.7324/JAPS.2014.40115.

Bina, F. Zamani, Z. & Nazari, V. (2012) : Morph-based Genetic variation in Christ's thorn (*Ziziphus spina-christi* (L) Wild.). *Iranian Journal of Rangelands Forest Plant Breeding and Genetic Research*, 2 (38): 274–288.

Bouallala, M. Bradai, L. & Abid, M. (2014): Diversité et utilisation des plantes spontanées du sahara septentrional algérien dans la pharmacopée saharienne: Cas de la région du Souf. *Revue ElWahat pour les reherches et les études*, 7(2) : 18-26. https://www.researchgate.net/publication/281526143

Boussaid, M. Benito, C. Kaid-Harche, M. Naranjo, T. & Zedek M. (2010): Genetic Variation in Natural Populations of *Stipa tenacissima* from Algeria. *Biochem Genet*. https://doi10.1007/s10528-010-9367-7.

Boussaid, M. Taïbi, K. Ait Abderrahim, L. & Ennajah, A. (2018): Genetic diversity of *Ziziphus lotus* natural populations from Algeria based on fruit morphological markers. *Arid land research and management*: 1 – 15: https://doi.org/10.1080/15324982.2018.1424742.

Couverciiel, (1839) : Traité Des Fruits, Tant Indigènes Qu'exotiques, Dictionnaire Carpologique. Ed. Imprimerie Et Librairie De Bouchard-Hl'zard, Paris, France, : 223-224

Divakara, & Das, R. (2011): Variability and divergence in *Pongamia pinnata* for further use in tree improvement. *Journal of Forestry Research*, 22(2): 193−200. https://doi10.1007/s11676-011-0149-9

Duling, D.W. Ghazanfar, S.A. & Prendergast, H.D.V. (1998): A New Species of Ziziphus Mill. (Rhamnaceae) from Oman. *Kew Bulletin*, 53(3): 733-739. https://doi.org/ 10.2307/4110494.

Gao, Q.H. Wu, P.T. Liu, J.R. Wu, C.S. Parry, J.W. & Wang, M. (2011): Physico chemical properties and antioxidant capacity of different jujube (*Ziziphus jujuba* Mill.) cultivars grown in loess plateau of China. *Sci. Hortic*., 130: 67–72. https://doi:10.1016/j.scienta.2011.06.005.

Ge, X.J. Yu, Y. Yuan, Y.M. Huang, H.W. & Yan, C. (2005) : Genetic Diversity and Geographic Differentiation in Endangered Ammopiptanthus (Leguminosae) Populations in Desert Regions of Northwest China as Revealed by ISSR Analysis. *Annals of Botany*, 95: 843–851. https://doi:10.1093/aob/mci089

Gelata, M. Labuschgne, M.T. & Viljoen, C.D. (2006): Genetic diversity analysis in sorghum germplasm as estimated by AFLP, SSR and morpho-agronomical markers. *Biodiversity and Conservation*, 15: 3251–3265. https://doi.org/10.1007/s10531-005-0313-7.

Ghazaeian, M. (2015): Genetic diversity of Jujube (*Ziziphus jujuba* Mill.) germplasm based on vegetative and fruits physicochemical characteristics from Golestan province of Iran. *Comunicata Scientiae*, 6(1): 10-16.

Ghedira, K. (2013): *Ziziphus lotus* (L.) Desf. (Rhamnaceae) : jujubier sauvage. *Phytothérapie*, 11 : 149-153. https://doi.org/10.1007/s10298-013-0776-8.

Gomes, S. Martins-Lopes, P. & Guedes-Pinto, H. (2012) : Chapter 2 : Olive Tree Genetic Resources Characterization Through Molecular Markers. In : Genetic diversity in plants. Eds. Çalişkan M. Croatia : 15 – 28. https://doi.org/10.1007/ 978-953-51-0185-7.

González-Robles, A. Manzaneda, A.J. Bastida, J.M. Harvey, N. Jaime, R. Salido, T. Martínez, L.M. Fernández, A. Alcántara, J.M. & Rey, P.J. (2016) : Development and characterization of microsatellite primers in the endangered Mediterranean shrub *Ziziphus lotus* (Rhamnaceae). *Applications in Plant Sciences*, 4(12): 4 p. https://doi.org/10.3732/apps.1600092.

Guo, Y. Ni, Y. Kokot, S. (2016) : Evaluation of chemical components and properties of the jujube fruit using near infrared spectroscopy and chemometrics. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 153: 79–86. https://doi.org/10.1016/j.saa.2015.08.006.

Hammia, M.K. Jdey, A. Abdelly, C. Majdoub, H. Ksouri, R. (2015): Optimization of ultrasound-assisted extraction of antioxidant compounds from Tunisian *Ziziphus lotus* fruits using response surface methodology. *Food Chemistry*, 184 : 80–89. https://doi.org/10.1016/j.foodchem.2015.03.047.

Hemalatha, T. & Shanmugasundaram, P. (2010): Efficiency of DNA Marker Systems in Discriminating *Cajanus cajan* (L.) Millsp. and its Wild Relatives. *Indian J. Plant Genet. Resour*., 23(1): 93-99.

Jahier, J. Chevre, A.M. Delourme, R. Eber, F. Tanguy, A.M. (1992): Téchniques de cytogénétique végétale. Ed. Institut Technique de la Recherche Agronomique (INRA), Paris. 184 p.

Laamouri, A. Ammari, Y. Albouchi, A. Sghaier, T. Mguis, K. & Akrimi, N. (2008) : Etude comparative de la croissance et du développement du système racinaire de trois espèces de jujubier en Tunisie. *Geo-Eco-Trop.*, 32: 37- 46.

Lamare, A. & Rao, S.R. (2015): Efficacy of RAPD, ISSR and DAMD markers in assessment of genetic variability and population structure of wild *Musa acuminata* colla. *Physiol Mol Biol Plants*, 21(3): 349–358. https://doi.org/10.1007/s12298-015-0295-1.

Li, L. Peng, J.Y. & Bai, R.X. (2009): Analysis of the Genetic Relationships in Chinese Ziziphus with SRAP Markers. *Agricultural Sciences in China*, 9(9): 1278-1284. https://doi.org/10.1016/S1671-2927(09)60217-0.

Liu, J. Liu, H. Ma, L. Wang, S. Gao, J. Li, Y. Wu, R. & Panga, X. (2014): A Chinese jujube (*Ziziphus jujuba* Mill.) fruit-expressed sequence tag (EST) library: Annotation and EST-SSR characterization. *Scientia Horticulturae*, 165: 99–105. https://doi.org/10.1016/j.scienta.2013.10.033.

Liu, M.J. Zhao, Z.H. (2009): Germplasm resources and production of jujube in China. *Acta Hort*. (ISHS), 840: 25-32. https://doi.org/10.17660/ActaHortic.2009.840.1.

Liu, P. Liu, M.J. Zhao, Z.H. Liu, X.Y. Yang, L. & Wu, Y.L. (2009): Agronomic diversity of Sour Jujube (*Ziziphus acidojujuba*) in China. Acta Horticultura, 840 :203-208. https://doi.org/10.17660/ActaHortic.2009.840.4

Ma, Q.H. Wang, G.X. & Liang, L.S. (2011): Development and characterization of SSR markers in Chinese jujube (*Ziziphus jujuba* Mill.) and its related species. *Scientia Horticulturae*, 129: 597–602. https://doi.org/10.1016/j.scienta.2011.04.032.

Maraghni, M. Gorai, M. & Neffati, M. (2010): Seed germination at different temperatures and water stress levels, and seedling emergence from different depths of *Ziziphus lotus*. *South African Journal of Botany*, 76: 453–459. https://doi.org/10.1016/j.sajb.2010.02.092.

Maraghni, M. Gorai, M. & Neffati, M. (2011) : The Influence of Water-Deficit Stress on Growth, Water Relations and Solute Accumulation in Wild Jujube (*Ziziphus lotus*). *Journal of Ornamental and Horticultural Plants*, 1(2): 63-72.

Martins, S. Ribeiro DeCarvalho, C. & Carnide, V. (2015): Assessing phenotypic diversity of *Cucurbita portuguese* germplasm. *Agriculture & Forestry*, 61(1): 27-33. https://doi.org/10.17707/AgricultForest.61.1.03

Mei, Z. Zhang, X. Liu, X. Imani, S. & Fu, J. (2017): Genetic analysis of *Canarium album* in different areas of China by improved RAPD and ISSR. *C. R. Biologies*, xxx: 8p. https://doi.org/10.1016/j.crvi.2017.09.006.

Milbourne, D. Meyer, R. Bradshaw, J.E. Baird, E. Bonar, N. Provan, J. Powell, W. & Waugh, R. (1997): Comparison of PCR-based marker systems for the analysis of genetic relationships in cultivated potato. *Mol. Breeding*, 3(2): 127-136. 10.1023/A: https://doi.org/1009649521009.

Mondini, L. Noorani, A. Pagnotta, M.A. (2009): Assessing Plant Genetic Diversity by Molecular Tools. *Diversity*, 1: 19-35. https://doi.org/10.3390/d1010019.

Moustafa, M.F. Hesham, A. Quraishi, M.S. & Alrumman, S.A. (2016): Variations in genetic and chemical constituents of *Ziziphus spina-christi* L. populations grown at various altitudinal zonation up to 2227 m height. *Journal of Genetic Engineering and Biotechnology*, 14: 349–362. https://doi.org/10.1016/j.jgeb.2016.09.001.

Nasri-Ayachi, M.B. & Nabli, M.A. (2009) : Floral Biology Study Of *Ziziphus Lotus* L. *Acta Hort.* (*ISHS*) 840, , 337-342. https://doi.org/10.17660/ActaHortic.2009.840.46

Nebih Hadj-Sadok, D. Hadroug, S. & Taoussi, F. (2014): Activité nématicide *in vitro* des extraits aqueux des plantes médicinales « *Artemisia campestris*, *Ziziphus lotus*, *Datura stramonium* et *Urginea maritima* » sur des larves de Meloidogyne. AFPP – Dixième conférence internationale sur les ravageurs en agriculture, Montpellier – 22 et 23 octobre 2014, 7 p.

Norouzia, E, Erfani-Moghadama, J, Fazelib, A, & Khadivic, A (2017): Morphological variability within and among three species of Ziziphus genus using multivariate analysis. *Scientia Horticulturae*, 222: 180–186. https://doi.org/10.1016/j.scienta.2017.05.016.

Nudin, N.F.H. Ali, A.M. Ngah, N. Mazlan, N.Z. Mat, N. AbdGhani, M.N. Alias, N. Zakaria, A. & Jahan, M.S. (2017): ISSR marker-assisted genetic diversity analysis of *Dioscorea hispida* and selection of the best variety for sustainable production. *C. R. Biologies*, xxx: 8p. https://doi.org/10.1016/j.crvi.2017.08.003.

Oliet, J.A. Artero, F. Cuadros, S. Pue´rtolas, J. Luna, L. & Grau, J.M. (2012): Deep planting with shelters improves performance of different stocktype sizes under arid Mediterranean conditions. *New Forests*, 43: 925–939. https://doi.org/10.1007/s11056-012-9345-5.

Prates, J. (2014): NZY Plant/Fungi gDNA Isolation kit. Nzytech genes & enzymes. 6 p.

Prevost, A. & Wilkinson, M.J. (1999): A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. *Theor App Genet.*, 98:107–112. https://doi.org/10.1007/s001220051046.

Quezel, P. & Santa, S. (1963): Nouvelle flore de l'Algérie et des régions désertiques méridionales. Tome II. Éditions du CNRS (Centre National de la Recherche Scientifique), Paris 7, 1170 p.

Rais, C. Lazraq, A. Houhou, M. Elhanafi, L. Fennane, A. Ghadraoui, L. Mansouri, I. Louahlia, S. (2017) : Morphometrics and morphological comparative study of three natural populations of *Ziziphus Lotus*. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 8(1): 1558 – 1564. https://www.researchgate.net/publication/323946561.

Rashidi, M. Farshadfar, M. Safari, H. & Shirvani, H. (2013): Utility of ISSR molecule marker in examine of genetic diversity 17 genotypes of perennial alfalfa (*Medicago sativa*). *Journal of Novel Applied Sciences*, 2(2S) : 969-973.

Regehr, D.L. & El Brahli, A. (1995): Wild Jujube (*Ziziphus lotus*) Control in Morocco. *Weed Technology*, 9(2): 326-330. https://doi.org/10.1017/S0890037X00023423

Riaz, A. Hameed, H. Khan, A.I. Younis, A. & Awan, F.S. (2011): Assessment of biodiversity based on morphological characteristics and RAPD markers among genotypes of wild rose species. African Journal of Biotechnology, 10(59): 12520-12526. https://doi.org/10.5897/AJB11.866.

Saran, P.L. (2005): Studies on genetic divergence in ber (*Ziziphus mauritiana* lamk.) Germplasm. Department of horticulture College of agriculture ccs haryana agricultural university, 178 p. (PhD thesis)

Sehgal, D. Rajpal, V.R. Raina, S.N. Sasanuma, T. & Sasakuma, T. (2009): Assaying polymorphism at DNA level for genetic diversity diagnostics of the safflower (*Carthamus tinctorius* L.) world germplasmresources. *Genetica,* 135: 457–470. https://doi.org/ 10.1007/s10709-008-9292-4.

Seigne, A. (1985) : La forêt circum méditerranéenne et ses problèmes : Techniques Agricoles et Productions Méditerranéennes. Eds. G.P. Maisonneuve et Larose, Paris, pp : 137-141.

Singh, A.K. Devanshi, Sharma, P. Singh, R. Singh, B. Koundal, K.R. & Singh, N.K. (2007): Assessment of Genetic Diversity in *Ziziphus mauritiana* Using Inter-Simple Sequence Repeat Markers. *J. Plant Biochemistry & Biotechnology*, 16(1): 35-40. https://doi.org/10.1007/BF03321926.

Singh, S.K. Meghwal, P.R. Pathak, R. & Gautam, R. (2014): Molecular Markers Assisted Identification of Intraspecific Hybrids in *Ziziphus mauritiana*. *Proc. Natl. Acad. Sci., India, Sect. B Biol. Sci.*, 84(3): 603–611. https://doi.org/10.1007/s40011-014-0336-5.

Sudhersan, C. & Ashkanani, J.H. (2009): Introduction, evaluation and propagation of Ziziphus In Kuwait. *Acta Hort.* (ISHS) 840, 47-54. https://doi.org/10.17660/ActaHortic.2009.840.4

Tatari, M. Ghasemi, A. & Mousavi, A. (2016): Genetic diversity in Jujube germplasm (*Ziziphus jujuba* Mill.) based on morphological and pomological traits in Isfahan province, Iran. *Crop Breeding Journal*, 4, 5 and 6 : 79-85. https://doi.org/ 10.22092/CBJ.2016.107110.

Tirado, R. & Pugnaire, F.I. (2005) : Community structure and positive interactions in constraining environments. *OIKOS*, 111 (3): 437- 444. https://doi.org/10.1111/j.1600-0706.2005.14094.x.

Tonk, F.A. Tosun, M. Ilker, E. Istipliler, D. & Tatar, O. (2014): Evaluation and comparison of issr and rapd markers for assessment of genetic diversity in triticale genotypes. *Bulgarian Journal of Agricultural Science*, 20(6): 1413-1420.

Varshney, R.K. Chabane, K. Hendre, P.S. Aggarwal, R.K. Graner, A. (2007): Comparative assessment of EST-SSR, EST-SNP and AFLP markers for evaluation of genetic diversity and conservation of genetic resources using wild, cultivated and elite barleys. *Plant Sci.*, 173(6): 638–649. https://doi.org/10.1016/j.plantsci.2007.08.010.

Wang, B. Huang, Q. Venkitasamy, C. Chai, H. Gao, H. Cheng, N. Cao, W. Lu, X. & Pan, Z. (2016): Changes in phenolic compounds and their antioxidant capacities in jujube (*Ziziphus jujuba* Miller) during three edible maturity stages. LWT - *Food Science and Technology*, 66: 56 – 62. https://doi.org/10.1016/j.lwt.2015.10.005.

Wojdyło, A. Carbonell-Barrachina, A.A. Legua, P. & Hernández, F. (2016): Phenolic composition, ascorbic acid content, and antioxidant capacity of Spanish jujube (*Ziziphus jujube* Mill.) fruits. *Food Chemistry*, 201: 307–314. https://doi.org/10.1016/j.foodchem.2016.01.090.

Zhang, Z. Gao, J. Kong, D. Wang, A. Tang, S. Li, Y. & Pang, X. (2015): Assessing genetic diversity in *Ziziphus jujuba* 'Jinsixiaozao' using morphological and microsatellite (SSR) markers. *Biochemical Systematics and Ecology*, 61: 196 – 202. https://doi.org/10.1016/j.bse.2015.06.021

DOI: 10.17707/AgricultForest.65.1.18

Tatjana ŽDRALIĆ,
Miroslav LALOVIĆ, Jelena NIKITOVIĆ [1]

THE CORRELATIVE RELATIONSHIP OF THE MASS EGSS AND TRAITS OF EGGS QUALITY OF LIGHT LINE HYBRIDS

SUMMARY

Although the production traits are still considered to be the main indicators for calculating the production index, producers must increasingly take into account the quality of eggs in order to meet consumer demand. The quality of eggs is becoming an important factor, largely depending on the placement of products on the market, and therefore the economic success of production. This means that special attention should be paid to the quality of eggs (egg height, length and width of the egg, egg shape index, cleanliness and color of the shell, color of the yolk).

Therefore, the work placed emphasis mutual correlation connections and dependencies between ground balls and qualities with light line hybrid Isa Brown, during three age periods of laying hens.

By monitoring these indicators and determining the correlation connection between these characteristics make it possible to better assessment of expression of genetic potential of the respective hybrids on a private farm near Sarajevo, Bosnia and Herzegovina.

Keywords: phenotipic correlation, mass of egg, traits of egg quality, Isa Brown.

INTRODUCTION

The quality of eggs is becoming an important factor, largely depending on the placement of products on the market, and therefore the economic success of production.

According to Schwaegele (2001), the quality of eggs is a set of all the features that are significant for the use of eggs as a foodstuff. One should not forget that the last link in the consumer egg production chain is the consumer, and the fact that the success of the production depends on the attitude of the consumer towards a product, then the definition of a quality egg is that the eggs that consumers prefer are the best (Pavlovski, 1988). Although production traits still consider basic indicators for the calculation of production index, but

[1]Tatjana Ždralić, (corresponding author: tatjana.zdralic@gmail.com), Miroslav Lalović, University of East Sarajevo, Faculty of Agriculture, East Sarajevo, BOSNIA AND HERZEGOVINA; Jelena Nikitović, University of Banja Luka, Faculty of Agriculture, Institute for genetics resources, Banja Luka, BOSNIA AND HERZEGOVINA

Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

producers must increasingly take into account the quality of the eggs, to meet the demands of consumers.

This means that special attention should be paid to the quality of eggs (egg height, length and width of the egg, egg shape index, cleanliness and color of the shell, color of the yolk).

Campo et al. (2007), Zita et al. (2009) found that the quality of the eggs in addition to genotype affects the age of hens. The authors conclude that regardless of genetic origin, the mass of eggs increases with the age of laying hens.

Contrary to previous authors Zemková et al. (2007) states that the age of the laying hens does not statistically influence the mass of the eggs.

Van den Brand et al. (2004) point out that the age of the laying hens affects the reduction in egg shape index.

Tolimir et al. (2008), on the basis of the results of the experiment in which they examined the influence of genotype and age on egg quality traits such as egg mass, index of shape and cleanliness of the shell, found that the genotype did not have any influence on any of the investigated traits. On the other hand, statistical significance was confirmed by the influence of age on the egg index alone. The values for the egg-shaped index were reduced with age (it was 82,10% at 31 weeks, from 44 weeks 82,23% and from 68 weeks 76,70%).

The aim of this paper was to determinate and test the phenotypic correlation between eggs mass and traits of egg quality (egg lenght, width of the egg, egg shape index, cleanliness egg, colour of the shell, colour of the yolk) in three production stages - Ages of Laying Hens (ALH_{20}, ALH_{28}, ALH_{48}), with a light line hybrid Isa Brown.

By monitoring these indicators and determining the correlation connection between these traits give the better possibility to evaluate expression of genetic potential light line hybrid Isa Brown on a private farm near Sarajevo, Bosnia and Herzegovina.

MATERIAL AND METHODS

Laying light line hybrid Isa Brown hybrid bred in accordance with current technological recommendations (https://www.isa-poultry.com) and in the 18th week of age were moved to a private farm around Sarajevo, Bosnia and Herzegovina.

Were reared in cages holding. The hens were fed a standard diet supplied to feed hens. Testing the quality of eggs was carried out in the laboratories of Faculty of Agriculture in East Sarajevo. In this study, 30 eggs, selected by random sample method, were individually measured from three age periods (ALH $_{28}$), $ALH_{2\,8}$ and ALH

In this study, are defined by the following indicators of the quality of eggs light line hybrid Isa Brown, such as egg weight (g), length of eggs (mm), width of the eggs (mm), egg shape index (%), cleanliness of shell (points), the color of the shell (points) and the color of the yolk (point).

The weight of eggs was measured on a technical scale with an accuracy of 0.01g. Length and width of the eggs were measured by simple accessories with an accuracy of 1mm (caliper). Shape index egg is mathematically calculated as the width in% length. The rating scales the color intensity is based on the subjective evaluation, described by points from 1 to 5 with the darkest color eggs received 5 points, a brightest point 1 are received. Cleanliness shell is visually assessed by points from 1 to 5, from very dirty to clean completely. Rating yolk color intensity using the fan 15 of the brightest shades of yellow to dark orange (Roche Color Fan).

Based on the results determined the phenotypic correlation between egg mass and the basic characteristics of the properties quality of the eggs.

Phenotypic correlation coefficient is calculated using the formula by Hadživuković (1991). The strength of the correlation of phenotypic correlation is discussed on the basis of Roemer-Orphalove classification.

Coefficient of phenotypic correlation is calculated according to the form:

$$\mathbf{r_{xy}} = \frac{\sum xy - \frac{\left(\sum x\right)\left(\sum y\right)}{n}}{\sqrt{\left[\sum x^2 - \frac{\left(\sum x\right)^2}{n}\right]\left[\sum y^2 - \frac{\left(\sum y\right)^2}{n}\right]}}.$$

Examination of the statistical significance of the obtained coefficient of correlation was done by t-test through the form:

$$\mathbf{t = r_{xy}\, x}\ \frac{\sqrt{1-r^2}}{\sqrt{n-2}}.$$

RESULTS AND DISCUSSION

The above points to the fact that between the weight of the eggs and certain qualities of quality, they precisely condition one another. In this section, we will demonstrate and discuss phenotypic correlated between mass and egg quality traits. The table below shows the calculated correlation coefficients and the level of significance between egg quality traits and mass of eggs in three age periods (ALH_{20}, ALH_{28}, ALH_{48}).

Table 1 presents the correlation coefficients between the mass of eggs and egg quality traits, as well as the level of significance coefficients.

From the data of Table 1 we see that positive correlation coefficients between the mass and the length of eggs are statistically significant at the level $P < 0.05$ and $P < 0.01$

In young laying hens at the beginning of use, to calculate the correlation coefficient was negative (r_p = - 0,023) and statistically significant at $P < 0.01$.

Table 1. Correlation coefficients and the level of significance between the mass of eggs and egg quality traits

Indicators	ALH	r_p	$t_{exp.}$
Egg lenght	ALH_{20}	-0,023**	2,801
	ALH_{28}	0,405**	9,603
	ALH_{48}	0,795**	12,350
Width of the egg	ALH_{20}	0,638**	9,871
	ALH_{28}	0,631**	10,297
	ALH_{48}	0,670**	11,325
Egg shape index	ALH_{20}	0,087ns	1,088
	ALH_{28}	0,113ns	1,445
	ALH_{48}	-0,012ns	0,176
Cleanliness egg	ALH_{20}	0,047ns	0,678
	ALH_{28}	-0,093ns	1,209
	ALH_{48}	-0.142ns	1,583
Colour of the shell	ALH_{20}	0,017ns	0,190
	ALH_{28}	-0,116ns	1,483
	ALH_{48}	-0,089ns	1,161
Colour of the yolk	ALH_{20}	0,657**	11,279
	ALH_{28}	-0,032ns	0,289
	ALH_{48}	-0,016ns	0,168

ns - P>0,05; * - P<0,05; ** - P<0,01; *** - P<0,001

Statistically significant ($P < 0.01$) correlation coefficients were determined between the mass of eggs and egg width, the strength of the connection medium to strong. The relationship between the mass of eggs and egg shape index was very weak in strength and not statistically confirmed ($P > 0.05$) for all three age groups of laying hens. Calculate the coefficients in young laying hens were positive, and in the elderly negative.

Between the weight of the egg and the cleanliness of the shell in young layer hens (ALH_{2O}) the correlation coefficient was positive ($r_p = 0,047$). With the age of the hens, the calculated correlation coefficients get a negative sign, the connections become slightly stronger, but are still very weak on the scale. In all three periods showed no statistically significant differences. Regarding the direction and strength of the connection, almost the same situation is found between the mass of eggs and the color of the shell.

In addition, Table 1 shows a positive correlation coefficient between egg yolk and yolk color at the first sampling (ALH_{20}), which was $r_p = 0,657$ (strong correlation) and was statistically significant at $P < 0.01$. For maximum egg production and mid-production cycle to calculate coefficients were negative, the strength of the weak and insignificant ($P > 0.05$).

Similar results, the correlation coefficients between the weight of the eggs and the quality characteristics were determined by Mitrovic et al. (2010), while

Đekić et al. (2008) determined a statistically significant correlation coefficient ($P < 0.05$) between egg mass and egg shape index ($r_p = 0,772$).

According to Rajičić et al. (2008), Shi et al. (2009), the importance of weight (weight) of eggs is also reflected in the fact that it significantly influences other quality properties of eggs, such as the index of egg shape, purity of the shell and color of the shell. This fact was confirmed by calculating the correlation coefficient between the weight of eggs and the abovementioned properties.

CONCLUSIONS

Based on the results of the above mentioned indicators we can conclude the following:

Between the weight of the eggs and the length, that is, the width of the eggs, statistically significant correlation coefficients were found during all three test periods. ($P < 0.01$). The same is established and the correlation relative masses of eggs and yolk color in the first period of production (ALH_{20}). On the other traits of egg quality, with different age hens, egg weight had no statistically significant effect ($P > 0.05$).

Viewed as a whole, it can be concluded that the analyzed commercially flock light line hybrid Isa Brown, grown at the poultry farm private households (B&H, RS), in most of the traits quality eggs achieved satisfactory results.

REFERENCES

Campo JL, gil MG, Dávila SG (2007): Differences among white-, tinted-, and brown-egg laying hens for incidence of eggs laid on the floor and for oviposition time. Arch Geflügelkd, 71: 105-109.

Đekić V., Milivojević J., Staletić M. 2008. Influence of hens genotype and age on tje shape index of eggs. 43rd Croatian and 3rd International Symposium on Agriculture, Opatija, Croatia (842-844).

Hadživuković S. 1991. Statistical methods with application in agricultural and biological research, p. 461, Novi Sad.

https://www.isa-poultry.com

Mitrovic S., Pandurevic T., Milic V., Djekic V., Djermanovic V. (2010): Weight and egg quality correlation relationship on different age laying hens. International Journal of Food, Agriculture & Environment - JFAE. Publisher: WFL, ISSN:1459-0263, vol. 8, Issue 3&4, p. 580-583.

Pavlovski Z. 1988. Uticaj načina proizvodnje i uslova tržišta na konzumni kvalitet jaja. Peradarstvo, 11-12, 128-133.

Rajičić V., Mitrović S., Tolimir N., Perić L. 2008. Uticaj genotipa i uzrasta nosilja na kvalitet ljuske jajeta. Savremena poljoprivreda, vol. 57, 1-2 (2008), strana 201-206, Novi Sad.

Schwaegele F. 2001. Egg quality assurance system under aspect of the EU requirementsand demand of the consumer, IX European Symposium on the Quality of eggs and eggs products.

Shi S.R., Wang K.H., Yang H.M. 2009. Egg weight affects some quality traits of chicken eggs. Journal of Food, Agriculture & Enviroment, Vol. 7(2):432-434.

Tolimir N., Perić L., Đukić-Stojčić M., Milošević N. 2008. Uticaj hibrida i uzrasta kokoši nosilja na kvalitet konzumnih jaja. Biotechnology in Animal Husbandry 24 (spec. Issue), p 245-252, Belgrade-Zemun.

Van den Brand H., Parmentier H. K., Kemp B. 2004. Effect of housing system and age of laying hens on egg characteristics“, Br. Poul Sci. 45: 745-752.

Zemková L', Simeonovová J, Lichovníková M, Somerlíková K. 2007. The effects of housing systems and age of hens on the weight and cholesterol concentration of the egg. Czech J. Anim. Sci., 52: 110-115.

Zita, L., Tůmová E., Stolc L. 2009. Effects of genotype, age nd their interaction on equlity in brown-egg laying hens. Acta Vet. Brno, 78: 85-91

DOI: 10.17707/AgricultForest.65.1.19

Mara Luana ENGEL, Angela IKEDA, Paulo César FLÔRES JR, Giovana Bomfim de ALCANTARA, Antonio Rioyei HIGA [1]

INFLUENCE OF STEM CUTTING POSITION, LEAVES CONDITION AND SIZE OF MINICUTTINGS IN ROOTING OF BLACK WATTLE

SUMMARY

Because of the commercial interest in forest resources provided by black wattle (*Acacia mearnsii* De Wildman), mainly for tannin extraction, the researches about clonal propagation techniques have been developed for this species. The objective of our study is to determine the type and size of minicuttings for the best adventitious rooting in three commercial clones of black wattle. The minicuttings were collected from a mini-clonal hedge located in the nursery at TANAGRO S.A. Company, located in Montenegro, Brazil. There were tested four sizes of minicuttings (5, 8, 11, 14 and 17 cm); two types depending on the stem cutting position where they were collected (apical or basal) and presence or absence of leaves with entire or half reduced area. The experiments were installed in randomized blocks with four blocks and six minicuttings per parcel. The evaluation of minicuttings rooting was done after 45 days. We conclude that also basal and apical minicuttings with entire or half reduced leaves exhibit higher percentage of rooting, as well as 14 cm minicuttings. Besides that, rooting response also varies according to the clone genotype.

Keywords: *Acacia mearnsii*, apical minicutting, basal minicutting, leaves in minicutting, minicutting size

INTRODUCTION

Considering the rising interest in using clones to develop forest projects, a great technological advance has been observed in processes of cloning and selection of superior genotypes. The use of vegetative propagation of forest species associated to improvement programs intents to accelerate growth and generate homogeneous and wood of quality (Valeri et al., 2012).

In Brazil, the species *Acacia mearnsii* De Wildeman, known as black wattle, is economically important in forest sector mainly for tannin extraction. Thus, vegetative propagation is an alternative to obtain genetically superior individuals. It is common that some species display limitations in seedling

[1]Mara Luana Engel, Angela Ikeda (corresponding author: aikeda@ufpr.br), Paulo César Flôres Jr, Giovana Bomfim De Alcantara, Antonio Rioyei Higa, Department of Forest Science, Federal University of Parana, Av. Pref. Lothario Meissner, 900, Jardim Botanico, 80210-170, Curitiba/PR, BRAZIL

Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

production via cutting mostly because to the complexity of rooting process of cuttings under natural conditions (Hartmann et al., 2011).

The quality, the survival and the percentage of rooting in a minicutting which will became a seedling in field, depend on several factors. In a mini-clonal hedge, the stem cutting position of which the minicutting will be prepared is one of this factors. According to Hartmann et al. (2011), woody basal minicuttings are more lignified and thus, more resistant to handling and transport. However, herbaceous apical juvenile minicuttings perform higher rooting capacity, with better percentage and speed of roots development (Wendling et al., 2002).

The maintenance of leaves in stem minicuttings is another factor that can benefit the rooting by providing carbohydrates and hormones, such as auxins. This, ensures better physiological conditions in rooting process (Xavier et al., 2013). Leaves maintenance is related to water adjustment and to the beginning of roots development, once the metabolites synthesized in the matrix plant could be transported to the rooting region in the minicuttings (Oliveira et al., 2001).

Another factor associated to the rooting success is the size of the minicutting. Small minicuttings may not have enough quantity of reserves required for adventitious rooting. Long minicuttings may be more susceptive to dehydration, once they demand more water to supply all the tissue exposed to the environment (Lima et al., 2006).

Therefore, in order to improve vegetative propagation techniques of black wattle, the objective was to determine the ideal size to induce rooting in minicuttings; if the presence, absence or leaves size influences in the rooting process and which stem cutting position is more effective to produce seedlings of black wattle commercial clones.

MATERIAL AND METHODS

Rooting experiments were installed in the forest nursery at TANAGRO S.A. Company, located in Montenegro city, state of Rio Grande do Sul, Brazil, 29º41'19" South latitude and 51º27'40" West longitude (Instituto Brasileiro de Geografia e Estatística [IBGE], 2014). Minicuttings were collected from outdoor mini-clonal hedges in summer time. The mini-clonal hedges were established with sand in a semi-hydroponic system and flood-irrigated by an automated system in which just the roots were in contact with nutritional solution (Bolle-Jones, 1954).

Mini-clonal hedges were composed by three commercial clones of black wattle nominated as A, B and C. From each one, they were collected juvenile branches and minicuttings were prepared with straight cut at the top and bevel cut at the base in adequate amount for an experiment considering type and leaves condition, and another experiment considering minicuttings size. Both experiments were installed in randomized blocks with four blocks and six minicuttings per parcel.

Evaluation for minicutting type considered: the three clones; two types of minicuttings according to stem cutting position (apical or basal) and three leaves

condition (leafless, one pair of leaves with half reduced area, one pair of leaves entire area), totaling 432 minicuttings. In the experiment to evaluate the effect of size, they were considered the three clones and five sizes: 5, 8, 11, 14 and 17 centimeters long, totaling 360 minicuttings.

Minicuttings were treated with indole butyric acid (IBA) 4000 mg L^{-1} and placed in tubes containing substrate prepared with rice husks and vermiculite medium grain size (1:1) with application of 3 Kg/m^3 of slow releasing fertilizer Osmocote® (NPK 15-03-12). Tubes containing the minicuttings were disposed in greenhouse with temperature (25 to 30 °C) and humidity (90 to 95%) controlled for 45 days.

After this period, they were evaluated: percentage of rooting (R%); number of roots per minicutting (NR) and the length of the three largest roots per minicutting (LR). Variances homogeneity was verified by Bartlett test, the homogeneous variances were evaluated by F test and the non-homogenous by Friedman test. Quantitative factors were submitted to regression analyses and treatments averages compared by Scott-Knott test. Statistical analyses were performed in Assistat 7.7 software (Silva & Azevedo, 2016).

RESULTS AND DISCUSSION

Considering the minicuttings type according to stem cutting position, apical or basal, there was no difference in rooting percentage for any clone. In clone A, leaves condition was significant for rooting percentage (R%), number of roots per minicutting (NR) and the length of the three largest roots per minicutting (LR). In clone B, it was observe interaction between the factors type and leaves condition just for NR. In clone C, leaves condition was significant to R% and LR.

In clone A, minicuttings with one pair of leaves with half reduced area presented the best results for rooting (75.0%), followed by minicuttings with one pair of leaves entire (45.8%) and leafless only 4.2%. In clone C, leafless minicuttings were the same (4.2%), minicuttings with one pair of leaves with half reduced area (66.7%) and with entire leaves (72.9%) of rooting. In clone B, it was not observed rooting differences according to leaves condition (Table 1).

Regarding the number of roots per minicutting, there was no difference to any clone considering apical or basal minicuttings. In clone A, it was observed difference in roots number according to leaves condition, minicuttings with one pair of leaves with half reduced area presented in average 22.4 roots/minicutting, with leaves entire (13.9) and leafless (0.9) (Table 2).

Length of the three largest roots per minicutting varied according to leaves condition in clones A and C. Leafless minicuttings presented the lowest mean of length, 1.35 cm to clone A and 0.92 cm to clone C. The highest roots length were observed in minicutting with one pair of leaves with half reduced area, 8.12 cm in average for the three clones, followed by minicutting with one pair of leaves entire area (6.90 cm) (Table 3).

Table 1 – Rooting percentage of three clones of black wattle considering two types of minicuttings according to stem cutting position (apical or basal) and three leaves conditions

		Leaves condition			
	Minicutting type	Leafless	One pair of leaves with half reduced area	One pair of leaves entire area	Mean
Clone A	Apical	4.17	66.67	54.17	41.67 [a]
	Basal	4.17	83.33	37.50	41.67 [a]
	Means	**4.17 C**[1]	**75.00 A**	**45.83 B**	**41.67**
Clone B	Apical	50.00	50.00	50.00	50.00 [a]
	Basal	25.00	62.50	70.83	52.78 [a]
	Means	**37.50 A**	**56.25 A**	**60.42 A**	**51.38**
Clone C	Apical	4.17	54.17	70.83	43.06 [a]
	Basal	4.17	79.17	75.00	52.78 [a]
	Mean	**4.7 B**	**66.67 A**	**72.92 A**	**47.92**

[1]Mean followed by the same lowercase letter in column and capital letter in line do not differ statistically by Scott-Knott test at 5% and 1%

Table 2 – Number of roots per minicutting of three clones of black wattle considering two types of minicuttings according to stem cutting position (apical or basal) and three leaves conditions

		Leaves condition			
	Minicutting type	Leafless	One pair of leaves with half reduced area	One pair of leaves entire area	Mean
Clone A	Apical	0.5	20.9	15.3	12.24 [a]
	Basal	1.3	23.9	12.5	12.54 [a]
	Means	**0.87 C**[1]	**22.39 A**	**13.90 B**	**12.39**
Clone B	Apical	5.49	11.52	7.70	8.24 [a]
	Basal	4.17	9.31	6.93	6.80 [a]
	Means	**4.83 A**	**10.41 A**	**7.31 A**	**7.52**
Clone C	Apical	4.50	6.01	9.96	6.82 [a]
	Basal	1.25	8.66	6.94	5.62 [a]
	Mean	**2.87 A**	**7.33 A**	**8.45 A**	**6.22**

[1]Mean followed by the same lowercase letter in column and capital letter in line do not differ statistically by Scott-Knott test at 5% and 1%

Table 3 – Length of the three largest roots per minicutting (cm) of three clones of black wattle considering two types of minicuttings according to stem cutting position (apical or basal) and three leaves conditions

		Leaves condition			
	Minicutting type	Leafless	One pair of leaves with half reduced area	One pair of leaves entire area	Mean
Clone A	Apical	1.6	6.9	7.2	5.23 [a]
	Basal	1.1	8.9	6.3	5.42 [a]
	Means	**1.35 B**[1]	**7.87 A**	**6.75 A**	**5.32**
Clone B	Apical	4.46	7.84	4.96	5.75 [a]
	Basal	3.94	7.32	6.57	5.95 [a]
	Means	**4.20 A**	**7.58 A**	**5.77 A**	**5.85**
Clone C	Apical	0.92	8.03	8.09	5.68 [a]
	Basal	0.92	9.77	8.26	6.31 [a]
	Mean	**0.92 B**	**8.90 A**	**8.17 A**	**5.99**

[1]Mean followed by the same lowercase letter in column and capital letter in line do not differ statistically by Scott-Knott test at 5% and 1%

According to Hartmann et al. (2011) maintaining leaves half reduced or entire area is an important condition to produce auxin, rooting co-factors and photosynthesis products, which are translocated and benefit the rhizogenic process. This can be applied to the black wattle minicuttings considering that singular genetic materials have different rooting response according to the presence of complete or partial leaves. In our study, clone B was the genotype that showed the best disposition to adventitious rooting, once achieved 51.4% even in leafless minicutting (37.5%) (Table 1).

In vegetative propagation of forest species, apical cuttings are typically superior for rooting, because the presence of leaves and auxin offers better rhizogenic potential (Ferreira et al., 2012). In the other hand, Câmara et al. (2017) observed in mulberry that intermediate and basal cuttings present significant increase in aerial part better than apical cuttings. Besides that, IBA application in rising doses until 2000 mg L^{-1} were related to decrease of sprout and rooting.

Black wattle did not present significant difference to rooting according to the stem cutting position, because mean of rooting for apical minicuttings was around 45% and basal 49%. The found rooting percentage can be influenced by IBA application interacting with endogenous auxin which is produced in the tissues and can act in rhizogenesis process independent to genetic constitution of each clone.

According to Santana et al. (2010) the reduction of foliar area helps to minimize pathogens occurrence, increase irrigation efficiency, prevent drought triggered by transpiration and reduce cutting bending caused by water deposition over leaves. Also, adventitious rooting in leafless minicuttings is not satisfactory to ensure survival and quality of seedling in the field. The condition of root system considering number and length of roots associated to leaves condition, should contribute to satisfactory absorption of nutrients to provide seedling growth.

In the experiment for different sizes of minicuttings, the results for rooting; number of roots per minicutting and length of the three largest roots per minicutting were significant. Minicuttings 14 cm long presented higher percentage of rooting and 5 cm long was the least efficient size (Table 4).

Table 4 – Rooting percentage of minicutting of three clones of black wattle considering four sizes

Treatments	**Rooting percentage**
Clone A	50.00 A
Clone B	56.25 A
Clone C	50.00 A
Friedman X^2	0.13^{ns}
DF	12
5 cm	5.56 D
8 cm	36.11 C
11 cm	72.22 B
14 cm	94.44 A
Friedman X^2	12**
DF	12

[1]Mean followed by the same letter in column do not differ statistically by Friedman test at 5% and 1%; DF = Degrees of Freedom

Number of roots per minicutting is also influenced by size, once minicuttings 14 cm long presented 10.41 roots/minicutting, whereas 5 and 8 cm long presented lower results (1.50 and 6.51, respectively). Minicuttings 5 cm long resulted in lower length of roots (2.29 cm) whereas 14 cm long reached 7.98 in average (Table 5).

Dias et al. (2015) and Souza (2012) found that cuttings 10 cm long of *Anadenanthera macrocarpa* (Benth.) and *Eucalyptus grandis* x *E. urophylla*, respectively, presented higher percentage of roots released when the cuttings are been removed from the greenhouse. As said by the work with *A. macrocarpa*, smaller cuttings presented larger mass and longer sprouts in aerial part. However, longer cuttings resulted in larger fresh mass and superior length of roots.

Table 5 – Number and length of the three largest roots per minicutting of three clones of black wattle considering four sizes

Variables	Clone	Minicutting size				
		5 cm	8 cm	11 cm	14 cm	Mean
Number of roots per minicutting	A	1.5	7.1	10.3	13.9	8.20 [a]
	B	1.5	5.8	7.1	10.3	6.16 [ab]
	C	1.5	6.6	5.8	7.1	5.23 [b]
	Mean	**1.50 C**	**6.51 B**	**7.71 AB**	**10.41 A**	**6.53**
Average length of roots per minicutting (%)	A	2.0	4.6	6.4	6.9	4.99 [a]
	B	4.5	6.6	6.8	8.5	6.60 [a]
	C	0.4	4.8	5.3	8.5	4.76 [a]
	Mean	**2.29 B**	**5.35 A**	**6.18 A**	**7.98 A**	**5.45**

[1]Mean followed by the same lowercase letter in column and capital letter in line do not differ statistically by Scott-Knott test at 5% and 1%

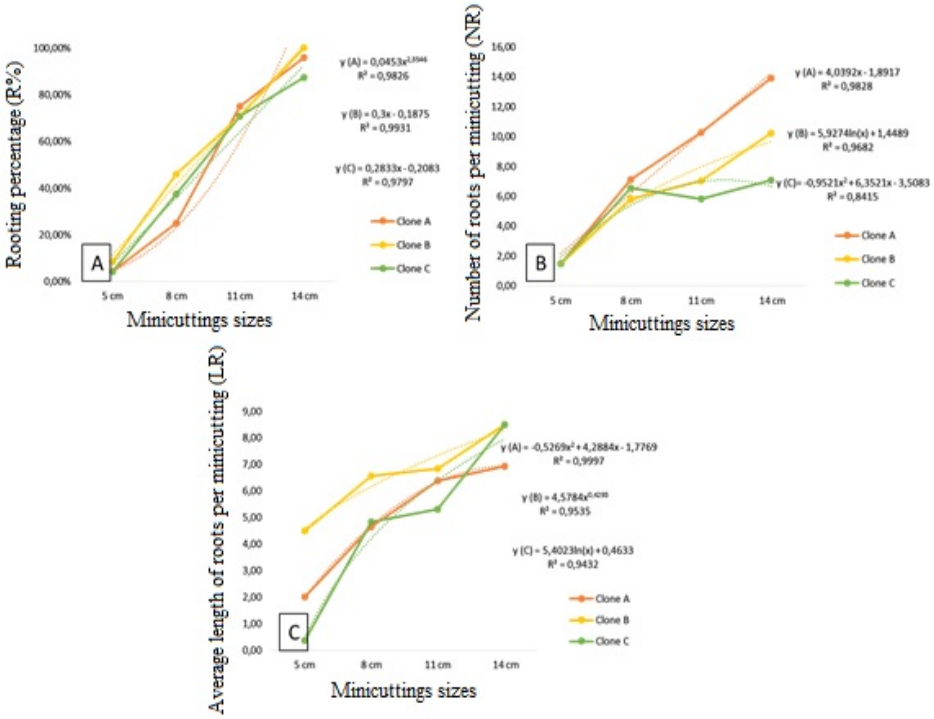


Figure 1 – Rooting of three clones of black wattle considering four minicutting sizes. A. rooting percentage; B. number of roots per minicutting and C. average of length of the three largest roots per minicutting

According to Santana et al. (2013) in hybrids of *E. urophylla*, the cutting size influenced directly the length of roots, once cuttings of 8 and 10 cm long reached 9.90 and 10.87 cm long and lower results were found with cuttings of 4 and 6 cm, similar what we found for black wattle. It should be owing to a better translocation of hormones and co-factors to form root system in longer minicuttings, which contributes to form a root system in a seedling better prepared to survival in field.

In regression analyses (Figure 1) we observed the clones related to minicutting size, which longer minicuttings size respond better to rooting. We found that there is an expressive increment in rooting results according to minicutting size increasing. All determinant model coefficients (R^2) are above 80% what is considered high and indicates that the model is highly adjusted to the equation of rooting estimative.

This behavior was observed to all the variables (R%, NR and LR) and for the three clones. The difference among the responses for rooting is low, but in general, clone B had the best performance in adventitious rooting. It indicates that in black wattle, root system development is influenced by genetic constitution.

CONCLUSIONS

Minicutting size influences in rooting response in black wattle and 14 cm long is the most efficient size. Leaves maintenance, entire or half reduced area promotes higher rooting taxes. The stem cutting position does not influence in rooting results to any clone tested. Also, clone B present higher rooting percentage, which indicate that genetic constitution provides different responses in vegetative propagation via minicutting in black wattle.

ACKNOWLEDGEMENTS

This study was supported by CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and TANAGRO S. A. Company.

REFERENCES

Bolle-Jones, E. W. (1954) Effect of nutrient deficiencies on growth, chlorophyll, rubber and mineral contents of Tjirandji seedlings. Journal of the Rubber Research Institute of Malaya, 14, 209-230.

Câmara, F. M. de M., Pereira, G. A., Mendonça, V., Pereira, E. C., Silva, F. S. de O., Oliveira, L. M. de, Cardoso, R. (2017) Types of cuttings and concentrations of indol-butyric acid (IBA) in propagation of mulberry (*Morus nigra*) Revista de la Facultad de Agronomía 116(2), 187-191. http://revista.agro.unlp.edu.ar/index.php/revagro/article/view/402/1585

Dias, P. C., Xavier, A., Oliveira, L. S., Correia, A. C. G., Barbosa, G. A. (2015) Type of mini-cutting and substrate on vegetative propagation of angico-vermelho (*Anadenanthera macrocarpa* (Benth.) Brenan). Ciência Florestal, 25(4), 909-919. http://dx.doi.org/10.5902/1980509820593

Ferreira, D. A., Barroso, D. G., Silva, M. P. S., Souza, J. S., Freitas, T. A. S., Carneiro, J. G. A. (2012) Influence of the minicutting position, in the quality of Australian cedar cutting cuttings and their initial growth. Ciência Florestal, 22(4) 715-723. http://dx.doi.org/10.5902/198050987553

Hartmann, H. T., Kester, D. E, Davies Jr, F. T., Geneve, R. L. (2011) *Plant propagation:* Principles and practices. São Paulo: Prentice-Hall.

Instituto Brasileiro de Geografia e Estatística (2014) Resolução da Presidência do IBGE de n° 5 (R.PR-5/02). https://www.ibge.gov.br

Lima, R. L. S., Siqueira, D. L., Weber, O. B., Cazzeta, J. O. (2006) Size and part of the branch to be used as Barbados cherry cuttings Revista Brasileira de Fruticultura, 28(1) 83-86. http://dx.doi.org/10.1590/S0100-29452006000100024

Oliveira, M. C., Ribeiro, J. F., Rios, M. N. S., Rezende, M. E. (2001) Enraizamento de estacas para produção de mudas de espécies nativas de matas de galeria. Brasília: Embrapa. https://www.infoteca.cnptia.embrapa.br/handle/doc/566480

Santana, R. C., Dutra, T. R., Carvalho-Neto, J. P., Nogueira, G. S., Grazziotti, P. H., Barros-Filho, N. F. (2010) Influence of leaf area reduction on clonal production of *Eucalyptus* seedling. Cerne, 16(3), 251-257. http://dx.doi.org/10.1590/S0104-77602010000300001

Santana, R. C., Fernandes, F. J. O., Titon, M., Xaxier, A., Souza, P. F., Barros-Filho, N. F. (2013) Effect of minicutting length and lief area reduction on growth and nutritional status of Eucalypt propagules. ISRN Forestry. http://dx.doi.org/10.1155/2013/905730

Silva, F. A. Z., Azevedo, C. A. V. (2016) The Assistat Software version 7.7 and its use in the analysis of experimental data. African Journal Agricultural Researh, 11(39) 3733-3740. https://doi.org/10.5897/AJAR2016.11522

Souza, C. C. (2012) Padrões de miniestaca e densidade de minicepas na propagação clonal de *Eucalyptus grandis* x *E. urophylla*. Viçosa: UFV. http://www.locus.ufv.br/handle/123456789/3104

Valeri, S. V., Sá, A. F. L., Martins, A. B. G., Barbosa, J. C. (2012) Rooting cutting of *Caesalpinia echinata* Lam. In hydroponic system. *Ciência Florestal*, 22(2), 241-250. http://dx.doi.org/10.5902/198050985731

Wendling, I., Ferrari, M. P., Grossi, F. (2002) Curso intensivo de viveiros e produção de mudas. Colombo: Embrapa Florestas. https://www.infoteca.cnptia.embrapa.br/handle/doc/306458

Xavier, A., Wendling, I., Silva, R.L. (2013) Silvicultura clonal: Princípios e técnicas. Viçosa: UFV.

DOI: 10.17707/AgricultForest.65.1.20

Volodymyr HUDZENKO, Oleksandr DEMYDOV,
Hanna VOLOSHCHUK, Mykola SARDAK, Vitalii ISHCHENKO [1]

GENOTYPE BY ENVIRONMENT INTERACTION AND YIELD STABILITY OF BARLEY BREEDING LINES IN MULTI-ENVIRONMENT TRIALS

SUMMARY

In modern conditions it is necessary to create varieties of spring barley with combination of high grain yield and stability. For this purpose, multi-environment trials of nine advanced breeding lines and the standard variety Vzirets were carried out in three different ecological zones in Ukraine: Central Forest-Steppe, Polissia and Northern Steppe. High yield variability of spring barley breeding lines has been established. The ANOVA revealed reliable contributions from all three source of the variation: genotype, environment and genotype–environment interaction. The part of influence for environment was the highest – 90.42 %. A set of statistical indices (regression coefficient (b_i), deviation mean squares (S^2d_i), variety superiority measure (P_i), nonparametric stability indices ($S_i^{(1)}$ and $S_i^{(2)}$), homeostaticity (Hom_i), breeding value (Sc_i)) and GGE biplot were used to interpret the multi-environment trial data. The applied statistical indices in different ways characterized the investigated barley breeding lines. Some indices estimated the stability only, without considering yield level (S^2d_i, $S_i^{(2)}$). Other indices were related with the mean yield (P_i), with the maximum (b_i) or minimum ($S_i^{(1)}$, Hom_i, Sc_i) its value. The combination of statistical indices and graphic model was effective for comprehensive evaluation of the genotype-environment data from multi-environment trials. This approach allows identify the best of the best breeding lines at the final stage of breeding work. The breeding lines Deficiens 5005, Nutans 4855, Nutans 4941 and Nutans 4890 have been submitted to the State Strain Testing of Ukraine as new varieties MIP Visnyk, MIP Ekspert, MIP Myroslav, MIP Vdiachnyi, respectively.

Key words: *Hordeum vulgare* L., GGE biplot, stability, statistical indices, yield

INTRODUCTION

Plant breeding has been making significant contribution to improve productivity of the main crops. Significant increase barley (*Hordeum vulgare* L.)

[1]Volodymyr Hudzenko (corresponding author: barley0482@gmail.com), Oleksandr Demydov, Hanna Voloschuk, The V.M. Remeslo Myronivka Institute of Wheat of the National Academy of Agrarian Sciences of Ukraine, Tsentralne village, Myronivka district, Kyiv region, UKRAINE; Mykola Sardak, Nosivka Plant Breeding and Experimental Station of the V. M. Remeslo MIW of NAAS, Doslidne village, Nosivka district, Chernihiv region, UKRAINE; Vitalii Ischenko, Institute of Agriculture of Steppe of NAAS, Sozonivka village, Kirovohrad district, Kirovohrad region, UKRAINE

Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

production as a result of breeding improvement was noted in Austria (Grausgruber et al., 2002), Norway (Lillemo et al., 2009), Czech Republic (Psota et al., 2009), Germany (Laidig et al., 2017). However, in conditions of climate change, there are needed not only high-yielding, but also environmentally stable varieties (Macholdt, Honermeier, 2016). One of the major problems in plant breeding for improve and stabilize yield is genotype-environment interaction (Hill, 1975). The genotype-environment interaction is part of the phenotypic variation that occurs as a result of non-compliance between genetics and environmental effects. Environments differ in the amount and quality of inputs that they convey to plants: water, nutrients, radiation, etc. This leads to the fact that the selection of genotypes under certain conditions may not provide the advantage of these genotypes in other conditions (Malosetti et al., 2013). According to mentioned, at the final stage of selection, breeding lines should be comprehensively evaluated. To assess the genotype-by-environment interaction are effective multi-environment trials (Sabaghnia et al., 2013). To interpret the experimental data of multi-environment trials, it is necessary to use the most appropriate statistical models (Eeuwijk et al., 2016). To evaluate barley yield stability a number of parametric and non-parametric stability indices have been used (Dimova et al., 2012; Verma, 2017). However, comprehensive genotype-environment data analysis must cover not only genotype evaluation, but two other important aspects: mega-environment analysis and test-environment evaluation (Yan and Tinker, 2006). For this purpose the more and more researchers have used GGE (genotype plus genotype by environment interaction) biplot (Vaezi et al., 2017; Kiliç et al., 2018; Khanzadeh et al. 2018; Solonechnyi et al., 2018). The advantages of GGE biplot are the ability to visualize the distribution of genotypes and environments in coordinates of the principal components, as well as to combine a genotype and genotype–environment interaction in mega-environment analysis (Yan, Tinker, 2006; Yan et al., 2007).

The present study aimed to evaluate of the genotype by environment interaction and identify spring barley breeding lines that combine yield performance and stability in the multi-environment trials.

MATERIALS AND METHODS

Nine spring barley advanced breeding lines and the standard variety Vzirets were tested. The breeding lines have been selected by complex of traits in competitive strain tasting at The V. M. Remeslo Myronivka Institute of Wheat of NAAS in 2015. In 2016–2018, the study was carried out through three environmental zones. 1) The V. M. Remeslo Myronivka Institute of Wheat of NAAS (Central Forest-Steppe, Latitude – 49°64', Longitude – 31°08', Altitude – 153 m). Soils are deep, little humus, slightly leached black soil. Humus content 3.8 %, alkaline hydrolyzed nitrogen – 5.9 mg/100 g, P_2O_5 – 22.1 mg/100 g, K_2O – 9.6 mg/100 g, pH = 5.8. 2) Nosivka Plant Breeding and Experimental Station of the V. M. Remeslo MIW of NAAS (Polissia, Latitude – 50°93', Longitude – 31°69', Altitude – 126 m).

Soils are modal, little humus, leached black soil. Humus content 2.6 %, nitrogen – 8.5 mg/100 g, P_2O_5 – 12.2 mg/100 g, K_2O – 7.5 mg/100 g, pH = 4.6. 3) Institute of Agriculture of Steppe of NAAS (Nothern Steppe, Latitude – 48°56', Longitude – 32°32', Altitude – 171 m). Soils are deep, middle humus, clay loamy black soil. Humus content 4.6 %, nitrogen – 12.0 mg/100 g, P_2O_5 – 11.6 mg/100 g, K_2O – 11.8 mg/100 g, pH = 5.4. Meteorological conditions of research significantly differed between ecological zones and years (Table 1).

The trial was laid out with complete randomized blocks in a three replications in each ecological zone.

Table 1. Meteorological conditions during the spring barley growing season

Year	Code	Monthly air temperature °C				Monthly precipitation mm			
		April	May	June	July	April	May	June	July
The V.M. Remeslo Myronivka Institute of Wheat of NAAS									
2015	M15	9.3	16.3	19.4	21.5	34.0	55.0	101.0	99.0
2016	M16	12.4	15.2	20.1	22.2	55.4	91.7	68.6	19.1
2017	M17	10.4	15.4	20.6	21.0	42.7	23.6	20.1	101.8
2018	M18	13.3	18.4	20.2	20.9	21.1	33.3	95.0	74.8
Long-term		8.8	15.0	18.0	19.7	42.1	51.2	85.2	86.5
Nosivka Plant Breeding and Experimental Station of the V. M. Remeslo MIW of NAAS									
2016	N16	11.7	15.3	20.0	21.8	58.4	122.9	36.5	51.3
2017	N17	9.5	13.9	18.6	19.1	35.4	44.3	33.0	109.3
2018	N18	11.4	17.5	19.2	20.3	2.0	31.0	64.0	81.0
Long-term		7.9	15.0	18.4	20.2	35.6	45.1	64.5	73.0
Institute of Agriculture of Steppe of NAAS									
2016	K16	13.9	17.3	22.2	24.3	52.3	153.2	107.5	15.5
2017	K17	10.9	17.6	23.1	23.2	23.5	10.7	22.2	66.0
2018	K18	15.0	20.8	22.9	23.7	10.0	25.5	29.2	141.0
Long-term		8.9	15.3	18.6	20.0	36.0	45.0	66.0	72.0

The widespread statistical indices were determined: regression coefficient b_i and deviation mean squares S^2d_i (Eberhart S.A., Russel W.A., 1966), variety superiority measure P_i (Lin, Binns, 1988), nonparametric stability indices $S_i^{(1)}$ and $S_i^{(2)}$ (Huehn, 1990). Also, there were determined the two less common indices: homeostaticity index (Hom_i) and breeding value (Sc_i) (Khangil'din, Litvinenko, 1981): $Hom_i = x^2/\sigma$, $Sc_i = x * x_{lim} / x_{opt}$, where x is the mean yield of genotype, σ is the standard deviation, x_{lim} is the minimum yield of genotype, x_{opt} is the maximum yield of genotype. AMMI and GGE biplot analysis was performed using non-commercial software *GEA-R.* Software review is provided in the publication (Frutos et al., 2014).

RESULTS AND DISCUSSION

Grain yield

The level of spring barley breeding lines yield significantly varied, depending on the ecological location and meteorological conditions of the year (Table 2). On average across environments, the breeding lines Nutans 4941, Deficiens 5005, Nutans 4855, Nutans 4983 reliably exceeded the standard variety Vzirets. The breeding lines Nutans 4890 and Pallidum 5023 exceeded the standard unreliably. The mean yield of the lines Nutans 4867, Nutans 4693, Nutans 5006 was unreliably lower than in the standard variety Vzirets.

Table 2. Grain yield of spring barley breeding lines in the multi-environment trial, t ha^{-1}

Standard, breeding lines	Grain yield in the environments										Mean
	M15	M16	M17	M18	N16	N17	N18	K16	K17	K18	
Vzirets	7.02	6.88	4.66	2.90	6.01	6.61	4.53	5.73	4.53	3.25	5.21
Nutans 4983	7.32	6.32	4.56	3.47	6.70	7.06	6.12	5.67	4.73	3.48	5.54
Nutans 4890	7.35	7.59	5.27	3.69	6.01	6.38	5.21	5.21	4.32	3.34	5.44
Deficiens 5005	7.63	7.88	5.20	3.24	6.73	6.55	6.55	5.66	4.20	2.83	5.65
Nutans 5006	7.67	5.39	4.56	2.52	5.20	7.03	6.30	4.28	4.22	2.80	5.00
Nutans 4941	7.74	7.78	5.42	3.95	7.07	7.01	5.65	5.16	4.56	3.09	5.74
Nutans 4693	7.12	6.74	4.75	2.74	6.28	6.01	5.35	4.99	4.26	3.25	5.15
Nutans 4855	7.57	7.41	5.37	3.22	6.80	7.15	6.19	5.69	4.12	2.80	5.63
Nutans 4867	7.34	6.52	4.92	2.65	5.65	6.23	5.72	4.86	4.36	3.63	5.19
Pallidum 5023	8.35	6.34	4.25	2.35	5.85	6.87	5.66	5.59	3.71	3.18	5.22
LSD_{05}	0.23	0.31	0.33	0.24	0.45	0.22	0.40	0.19	0.41	0.15	0.29

Analysis of variance showed a significant advantage of the contribution of environmental conditions in the total variation – 90.42 %. The genotype–environment interaction contribution was 7.03 %, the genotype – 2.55 % (Table 3).

Table 3. Analysis of variance of the grain yield in spring barley breeding lines

Source	Sum of squares	Degree of freedom	Mean square	Percentage relative to the sum of squares
Genotype (G)	630.31	9	70.03**	90.42
Environment (E)	17.74	9	1.97**	2.55
G x E	49.00	81	0.60**	7.03
Residuals	6,54	200	0.03	0

** – significant at 1 % level of a probability

Despite the low numerical values of the last two, they were reliable. Such results, in our opinion, sufficiently indicate strong variation in yield depending on the contrasting environmental conditions and conditions of the year of the research. Low numerical values of genotype variation are also due to the fact that the best breeding lines were involved in the trial. At that time, such research conditions made it possible to comprehensively evaluate the breeding lines and to select the best ones among them.

Stability analysis

Numerical values of stability are presented in Table 4. According to the regression coefficient b_i, the breeding lines Nutans 5006 (G5) and Nutans 4941 (G6) were approximated to the optimal reaction to change of environmental conditions (b_i = 1.0). The breeding line Nutans 4890 (G3) responded to the changing conditions the least of all, whereas the response of the breeding line Pallidum 5023 (G10) was the most. The breeding lines Nutans 4693 (G7), Nutans 4855 (G8), and Nutans 4867 (G9) were stable in variance S^2d_i. According to the variety superiority measure P_i, the breeding lines Nutans 4941 (G6), Deficiency 5005 (G4), and Nutans 4855 (G8) should be distinguished. The lines Nutans 4890 (G3) and Nutans 4693 (G7) were stable according to non-parametric parameters $Si^{(1)}$ and $Si^{(2)}$, respectively. The breeding lines Nutans 4983 (G2) and Nutans 4890 (G3) were the best according to homeostaticity index Hom_i and breeding value Sc_i.

Table 4. Characteristics of spring barley breeding lines by parametric and non-parametric stability indices

Standard, breeding lines	Code	Stability indices						
		b_i	S^2d_i	P_i	$S_i^{(1)}$	$S_i^{(2)}$	Hom_i	Sc_i
Vzirets	G1	0.92	0.20	0.51	0.47	7.81	18.53	2.15
Nutans 4983	G2	0.90	0.13	0.24	0.47	9.44	21.77	2.63
Nutans 4890	G3	0.89	0.16	0.28	0.38	6.33	20.71	2.39
Deficiens 5005	G4	1.12	0.15	0.12	0.56	6.67	18.17	2.03
Nutans 5006	G5	1.02	0.48	0.81	0.71	10.89	14.85	1.64
Nutans 4941	G6	1.03	0.17	0.09	0.49	7.11	20.36	2.28
Nutans 4693	G7	0.94	0.04	0.45	0.47	3.14	18.32	1.98
Nutans 4855	G8	1.12	0.07	0.13	0.51	8.22	18.28	2.08
Nutans 4867	G9	0.90	0.08	0.46	0.53	7.11	19.18	1.87
Pallidum 5023	G10	1.16	0.21	0.50	0.64	8.23	14.86	1.47

For a more detailed clarification of relationship between yield level and stability indices, correlation analysis was carried out (Table 5). Functional negative correlation was observed between mean yield and P_i. The $S_i^{(2)}$ and b_i not correlated with mean yield. The weak negative correlation was noted between

mean yield and S^2d_i, mean yield and $S_i^{(1)}$. The moderate positive relationship of Hom_i and Sc_i with mean yield was observed. Maximum yield had positively correlation with b_i only. The relationship between minimum yield and Hom_i, minimum yield and Sc_i was close to the functional. Between the individual indices, it should be noted the functional relationship between Hom_i and Sc_i. A strong negative relation was noted between $S_i^{(1)}$ and Hom_i, $S_i^{(1)}$ and Sc_i. Mean yield more correlated with the minimum yield level than with the maximum one.

Table 5. Correlation between yield and stability indices

Yield, indices	Mean	Max	Min	b_i	S^2d_i	P_i	$S_i^{(1)}$	$S_i^{(2)}$	Hom_i
Max	0.22								
Min	0.57	-0.37							
b_i	0.26	0.79	-0.56						
S^2d_i	-0.42	0.30	-0.30	0.16					
P_i	-0.96	-0.12	-0.56	-0.18	0.64				
$S_i^{(1)}$	-0.43	0.53	-0.77	0.59	0.69	0.56			
$S_i^{(2)}$	-0.07	0.27	-0.06	0.19	0.69	0.30	0.56		
Hom_i	0.59	-0.47	0.91	-0.61	-0.58	-0.65	-0.86	-0.27	
Sc_i	0.61	-0.47	0.98	-0.55	-0.40	-0.62	-0.81	-0.11	0.94

Based on data in Tables 4 and 5, it can be observed that statistical indices characterized the genotypes in different ways. Some parameters estimate the stability only, without considering yield level. Other indices related with the mean yield, with the maximum or minimum its limits.

GGE biplot analysis

The first two principal components of the GGE biplot explained 70.29 % of genotype–environment interaction. Figure 1 shows the representativeness and discriminating ability of environments. The line that intersects the center of the biplot is the average environment axis (AEA). The average environment represented by the small circle at the end of the arrow. The dashed lines indicate the vectors of individual test environments. The length of the vector characterizes discriminating ability of an environment. The angle between the vector of environment and the AEA showes its representativeness. A test environment that has a smaller angle with AEA is more representative than other test environments. The environment of M16 was characterized by the greatest differentiating ability. The environment N16 was the most representative, whereas M15 and K18 were the least representative. The environments M15 and K18 were the most distant from each other. The environments M17, M18, and K16, as well as N17 and N18 were similar among themselves.

GGE biplot "which-won-where" polygon view is effective tool to visualize the interaction patterns between genotypes and environments (Fig. 2). The polygon is formed by connecting the genotypes that are farthest away from the center of biplot, such that all other genotypes are contained within the polygon. A set of perpendicular to each side of the polygon lines divide the biplot into several sectors. In the sectors at the vertex of the polygon locates genotypes that have an advantage in a particular environment or in a set of environments (mega-environment). In this case, the first mega-environment is formed by the environments M16, M17, M18, N16, and K16. The two breeding lines Nutans 4941 (G6) and Deficiens 5005 (G4) had advantage in it. The second mega-environment was formed by the environments M15, N17, N18. The breeding line Nutans 4983 (G2) had higher yield in it. The breeding line Nutans 4855 (G8) was located on the straight line that connected the two above-mentioned mega-environments. The breeding line Nutans 4890 (G3) was distinguished in the environment K17. In the environment K18 the standard variety Vzirets (G1) was the best. Thus, it can be seen that the first two mega-environments are formed by different ecological environments. This confirms that not only ecological, but also meteorological conditions of the years of the research significantly influenced on the yield performance of the genotypes.

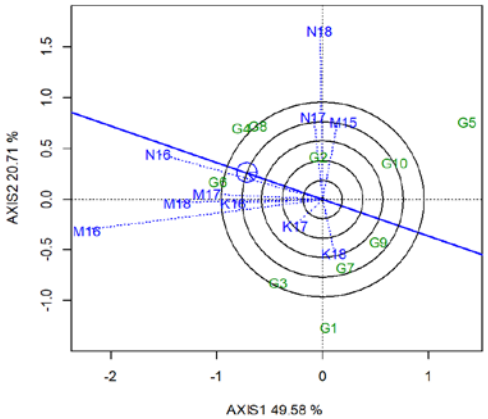


Figure 1. GGE biplot of discriminating ability and representativeness of test environments

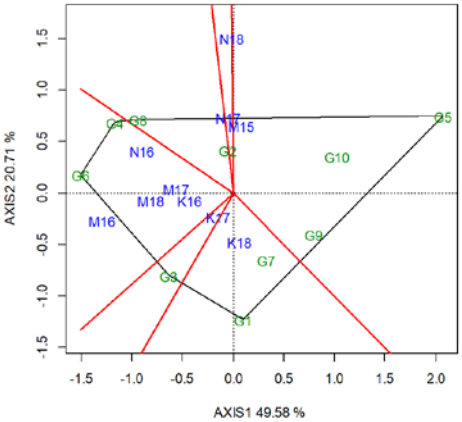


Figure 2. GGE biplot "which-won-where" polygon view for breeding lines and test environments

Figure 3 shows the average environment coordination of breeding lines in terms of mean yield and stability. The axis intersecting the center of the GGE biplot horizontally is the average environment abscissa (or AEA) for the environments. In the direction marked with arrow distinguished by a circle on the abscissa, the cultivars are ranked by mean yield. In the vertical plane, the average ordinate intersects the center of the GGE biplot. The intersection point represents the grand mean yield across environments. The displacement of genotypes along the ordinate axis from the abscissa (indicated by dash line) characterizes their variability with respect to the expected mean performance. The maximum yield

was noted in the breeding line Nutans 4941 (G6), the minimum yield was in Nutans 5006 (G5). The breeding line Nutans 5006 (G5) was also the most variable. The breeding lines Deficiens 5005 (G4), Nutans 4855 (G8) and Nutans 4867 (G9) were stable. However, the breeding line Nutans 4867 (G9) had lower yield than the mean yield in trial and standard variety Vzirets (G1).

Figure 4 shows ranking the breeding lines relative to a hypothetical “ideal genotype” which conventionally is represented as the center of centric circles. By the combination of yield and stability, the line Deficiencs 5005 (G4) was significantly distinguished. The breeding line Nutans 4855 (G8) was close to an “ideal genotype” also. The breeding line Nutans 4941 (G6) was slightly shifted away from the center as a result of higher variability. The breeding lines Nutants 4983 (G2) and Nutans 4890 (G3) were inferior to the three mentioned, but exceeded the rest of the breeding lines and the standard variety Vzirets (G1).

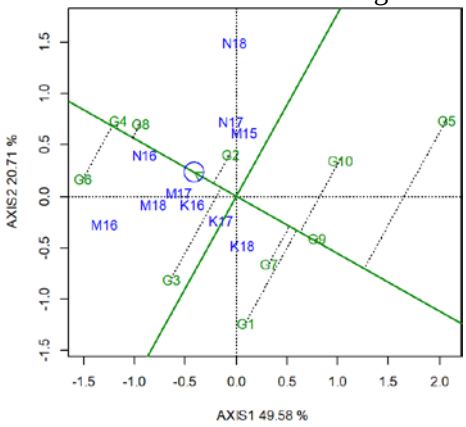


Figure 3. GGE biplot average nvironment coordination view of spring barley breeding lines for mean yield against stability

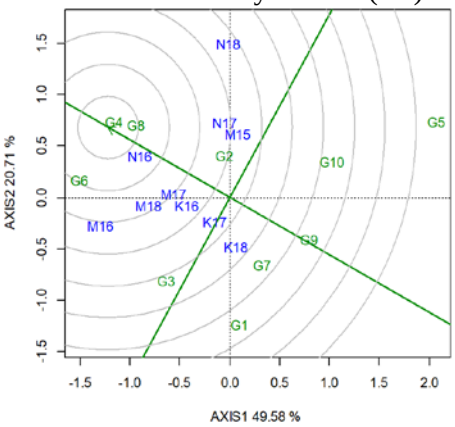


Figure 4. GGE biplot ranking spring barley breeding lines relative to an “ideal genotype”

Resulted from the research, the breeding lines Deficiens 5005, Nutans 4855, Nutans 4941 and Nutans 4890 have been submitted to the State Strain Testing of Ukraine as new varieties MIP Visnyk, MIP Ekspert, MIP Myroslav and MIP Vdiachnyi, respectively.

CONCLUSIONS

The high variability in yield of spring barley breeding lines has been established. It is caused by both different ecological conditions and meteorological conditions of the years of the research. The ANOVA revealed significant contributions from all three sources of variation: genotype, environment and genotype-environment interaction. The effect of environmental conditions was the highest – 90.42 %.

The applied statistical indices in different ways characterized the investigated spring barley breeding lines. Some indices estimated the stability

only, without considering yield level (S^2d_i, $S_i^{(2)}$). Other indices were related (positive or negative) with the mean yield (P_i), with the maximum (b_i) or minimum ($S_i^{(1)}$, Hom_i, Sc_i) its limits. The GGE biplot model has provided in-depth visual mega-environment analysis of multi-environment trial data.

The combination of statistical indices and graphic model was effective for comprehensive evaluation of the genotype-environment data from multi-environment trials. This approach allows identify the best of the best breeding lines at the final stage of breeding work.

The breeding lines Deficiens 5005, Nutans 4855, Nutans 4941 and Nutans 4890 have been submitted to the State Strain Testing of Ukraine as new varieties MIP Visnyk, MIP Ekspert, MIP Myroslav and MIP Vdiachnyi, respectively.

REFERENCES

Dimova D., Krasteva L., Panayotov N., Svetleva D., Dimitrova M., Georgieva T. 2012. Evaluation of the yield and the yield stability of perspective lines of barley. Agroznanje, 13(1): 55–60. https://doi.org/10.7251/AGREN1201055D

Eberhart S.A., Russel W.A. Stability parameters for comparing varieties. 1966. Crop Sci., 6(1): 36–40. https://doi.org/10.2135/cropsci1966.0011183X000600010011x

Frutos E., Galindo M.P., Leiva V. 2014. An interactive biplot implementation in R for modeling genotype-by-environment interaction. Stoch. Environ. Res. Risk Assess., 28(7): 1629–1641. https://doi.org/10.1007/s00477-013-0821-z

Grausgruber H., Bointer H., Tumpold R., Ruckenbauer P., Fishbeck G. 2002. Genetic improvement of agronomic and qualitative traits of spring barley. Plant Breed., 121(5): 411–416. https://doi.org/10.1046/j.1439-0523.2002.756385.x

Hill J. 1975. Genotype-environment interaction – a challenge for plant breeding. J. Agricul. Sci., 85(3): 477–493. https://doi.org/10.1017/S0021859600062365

Huehn M. 1990. Nonparametric measures of phenotypic stability. Part 1: Theory. Euphytica, 47 (3): 189–194. https://doi.org/10.1007/BF00024241

Khangildin, V.V., Litvinenko, N.A. 1981. Homeostaticity and adaptability of winter wheat varieties. Scient. Tech. Bull. PBGI, 39: 8–14.

Khanzadeh H., Vaezi B., Mohammadi R., Mehraban A., Hosseinpor T., Shahbazi K. 2018. Grain yield stability of barley genotypes in uniform regional yield trails in warm and semi warm dry land area. Ind. J. Agric. Res., 52(1): 16–21. https://doi.org/10.18805/IJARe.A-290

Kiliç H., Kendal E., Aktas H. 2018. Evaluation of yield and some quality characters of winter barley (Hordeum vulgare L.) genotypes using biplot analysis. Agriculture & Forestry, https://doi.org/10.17707/AgricultForest.64.3.09

Laidig F., Piepho H. P., Rentel D., Drobek, T., Meyer U. 2017. Breeding progress, genotypic and environmental variation and correlation of quality traits in malting barley in German official variety trials between 1983 and 2015. Theor. Appl. Genet., 130(11): 2411–2429. https://doi.org/10.1007/s00122-017-2967-4

Lillemo M., Reitan L., Bjørnstad A. 2009. Increasing impact of plant breeding on barley yields in central Norway from 1946 to 2008. Plant Breed., 129(5): 484–490. https://doi.org/10.1111/j.1439-0523.2009.01710.x

Lin C.S., Binns M.R. 1988. A superiority measure of cultivar performance for cultivar x location data. Can. J. Plant Sci., 68(1): 193–198. https://doi.org/10.4141/cjps88-018

Macholdt J., Honermeier B. 2016. Impact of climate change on cultivar choice: adaptation strategies of farmers and advisors in German cereal production. Agronomy, 6: 40. https://doi.org/10.3390/agronomy6030040.

Malosetti M., Ribaut J.-M., van Eeuwijk F.A. 2013. The statistical analysis of multi-environment data: modeling genotype-by-environment interaction and its genetic basis. Front. Physiol., 4: 44. https://doi.org/10.3389/fphys.2013.00044

Psota V., Hartmann J., Sejkorova S., Louckova T., Vejrazka K. 2009. 50 Years of progress in quality of malting barley grown in the Czech Republic. J. Instit. Brew., 115(4), 279–291. https://doi,org/10.1002/j.2050-0416.2009.tb00382.x

Sabaghnia N., Mohammadi M., Karimizadeh R. 2013. Yield stability of performance in multi-environment trials of barley (Hordeum vulgare L.) genotypes. Acta Univ. Agric. Silvic. Mendelianae Brun., 61(3): 787–793. http://dx.doi.org/10.11118/actaun201361030787

Solonechnyi P., Kozachenko M., Vasko N., Gudzenko V., Ishenko V., Kozelets G., Usova N., Logvinenko Y., Vinyukov A. 2018. AMMI and GGE biplot analysis of yield performance of spring barley (Hordeum vulgare L.) varieties in multi environment trials. Agriculture & Forestry, 64(1): 121–132. https://doi.org/10.17707/AgricultForest.64.1.15

Vaezi B., Pour-Aboughadareh A., Mohammadi R., Armion M., Mehraban A., Hossein-Pour T., Dorii M. 2017. GGE biplot and AMMI analysis of barley yield performance in Iran. Cereal Res. Commun., 45(3): 500–511. https://doi.org/10.1556/0806.45.2017.019

Verma A., Singh J., Kumar V., Kharab A. S., Singh G. P. 2017. Non parametric analysis in multi environmental trials of feed barley genotypes. Int. J. Curr. Microbiol. App. Sci., 6(6): 1201–1210. https://doi.org/10.20546/ijcmas.2017.606.139

van Eeuwijk F.A., Bustos-Korts D.V., Malosetti M. 2016. What should students in plant breeding know about the statistical aspects of genotype × environment interactions? Crop Sci., 56(5): 2119–2140. https://doi.org/10.2135/cropsci2015.06.0375

Yan W., Tinker N.A. 2006. Biplot analysis of multi-environment trial data: principles and applications. Can. J. Plant Sci., 86(3): 623–645. https://doi.org/10.4141/P05-169

Yan W., Kang M.S., Ma B., Woods S., Cornelius P.L. 2007. GGE biplot vs. AMMI analysis of genotype-by-environment data. Crop Sci., 47(2): 643–653. https://doi.org/10.2135/cropsci2006.06.0374

DOI: 10.17707/AgricultForest.65.1.21

Željko LAKIĆ,
Dejan SOKOLOVIĆ, Igor ĐURĐIĆ [1]

EXAMINATION OF EARLINESS OF AUTOCHTHONOUS POPULATIONS OF PERENNIAL RYEGRASS

SUMMARY

Monitoring of changes in phenological phases of autochthonous populations of perennial ryegrass was conducted on the experimental field of the Agricultural Institute of Republic of Srpska in Banja Luka (Bosnia and Herzegovina) during 2007, 2008 and 2009. Seven autochthonous populations collected in Bosnia and Herzegovina (BiH) and one variety of perennial ryegrass were used for this research. This research included the following populations of perennial ryegrass: Dragočaj, Kosjerovo, Laminci, TAS, Kupres, Maglajani, Manjača and variety Maya.

The aim of this research was to select populations of perennial ryegrass in the conditions of opened pollination on the basis of phenological observations. These genotypes should be selected on the basis of earliness, length of vegetation and other characteristics important for understanding the initial genetic materials included in the breeding program of forage crops.

During the research, the following changes were observed in phenological phases with the perennial ryegrass: growth of generative shoots 10 cm above the ground, the length of the vegetative phase from April 1, the period from heading to start of blossoming, duration of blossoming, and the length of the growing season. Phenological phases were observed individually by recording the date of their beginning and/or ending, and duration of the phases was expressed in number of days.

On the basis of the duration of particular phenological phases, the differences were observed among autochthonous populations of perennial ryegrass in duration of vegetation period until appearance of spiking. The earliest ear formation was determined in population Laminci with the average duration of vegetative period of 29 days. Population Manjača had the longest period until the ear formation, which lasted for 44.9 days. During these tests of the duration of the vegetation period, three groups were distinguished. The first group, characterized with a shorter vegetation period, included populations: Laminci (82.5 days), Maglajani (84 days) and Kupres (84.3 days). The second group with

[1]Željko Lakić, Faculty of Agriculture, University of Banja Luka, BOSNIA AND HERZEGOVINA; Dejan Sokolović, Institute for fodder crops, Kruševac, SERBIA; Igor Đurđić (corresponding author: i_djurdjic@yahoo.com), Faculty of Agriculture, University of East Sarajevo, BOSNIA AND HERZEGOVINA

Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

the vegetation [2]period from 87 - 89 days included populations: TAS, Kosjerovo and the variety Maya. The latest maturing group included populations: Dragočaj and Manjača with the average duration of vegetation period of 95 days.

Keywords: perennial ryegrass, populations, phenological phases, earliness, vegetation period.

INTRODUCTION

Phenological phases represent maturity stages or segments of plant development, and occur as a result of massive physiological and biochemical processes happening during plant growth. Investigation of phenology and maturity at perennial ryegrass is very important in the begenning of breeding, since in that way we can determine approximate date of beginning of tillering and flowering and duration of these phases.

Perennial ryegrass (*Lolium perenne* L.) is one of the most important and commonly grown perennial fodder grasses in northern temperate climate of the world. It is especially widespread in northwest Europe, New Zealand, Australia, South Africa and South America (Yamada, 2013). One of the main prerequisite in breeding is characterisation and evaluation of the starting germplasm. According to Sokolovic et al. (2011) autochthonous populations and local ecotypes very often represented starting material in breeding programs because of their adaptation to local agro-ecological conditions. However, breeders usually do not have enough data about variability of the most important traits of autochthonous populations.

Date of heading of perennial ryegrass is very important trait affecting biomass production (Surault et al., 2009) as well as forage quality and digestibility. It has been determined that early flowering in perennial ryegrass is connected with increasing of dry matter and seed yield. Genotypes of perennial ryegrass may differ in time of heading significantly and based to this property many varieties, differing in maturity up to a month, have been created (Jung et al., 1996; Grogan and Gilliland, 2011), which allows significant mixtures and grassland managing flexibility.

Time of heading of European populations ranged in interval of 30 to 70 days after April 1st (Charmet and Balfourier, 1994), and in certain climate conditions it can be even longer. Autochthonous material of perennial ryegrass collected from, to a certain extent, narrow area (Serbia) may also have high amount of variability for heading date (Sokolovic et al., 2006).

In collected populations of perennial ryegrass there is a great genetic variability in relation to adaptability towards different agro-ecological conditions and it is reflected in the duration of vegetation, capability and speed of regeneration, earliness or late maturing, and other important properties. Lakic et al. (2015) have estimated interaction genotype x environment for the most important trait of eight autochthonous populations of perennial ryegrass by using

AMMI method. For all tested properties, they determined additive (genotype, environment) and non-additive (interaction of genotype x environment) variations. On the bases of results, the genotypes with the best stability for all tested traits were selected.

The goal of this research was to analyse phenology of perennial ryegrass autochthonous populations and to define material that differ in earliness, vegetation duration and other traits important for forage breeding programs.

MATERIAL AND METHODS

Monitoring and evaluation of phenological phases of autochthonous populations of perennial ryegrass were conducted at the experimental field of Agricultural Institute of Republic of Srpska in Banja Luka during three-year period (2007-2009). The trial was established on the valley-brown soil (clay-loam soil in mechanical composition) on alluvial substratum of river Vrbas. Soil had neutral chemical reaction (pH H_2O-7.03) and contained 3.25% of humus, 15.52 mg/100g of phosphorous and 35.56 mg/100g of potassium.

This field research included 7 autochthonous populations of perennial ryegrass collected in BiH (Dragočaj, Kosjerovo, Laminci, TAS, Kupres, Maglajani, Manjača) and cultivar Maya as a standard (Poland, 1987). Populations were sowed on the field in the autumn 2006, as spaced plants (70 x 70 cm) with 50 plants (five rows, 10 plants per row) representing each population.

In this trial five phenological traits of perennial ryegrass were observed (Tab. 1) and expressed as number of days from April 1st . Phenological phases were evaluated individually on each plant and expressed as population average.

Table 1. Evaluated traits in collection of perennial ryegrass populations

Traits	Abbreviation
A moment when generative shoots reached 10 cm in height	GRGS
Duration of vegetative phase	VEGF,
Interval heading – beginning of flowering	IHF
Duration of flowering	FLOW
Duration of vegetation period	VEG

Obtained results have been processed with ANOVA and statistical significance of differences was determined with LSD-test. Components of variance, genetic (σ^2_g), phenotypic (σ^2_f), variance of interaction (σ^2_g /σ^2_y) and heritability in broad sense ($h^2=\sigma^2_g/\sigma^2_f$) were calculated, as well as . coeficient of correlation (r) between examined phenological phases. Hierarchical cluster analysis of populations based on all traits was done by Ward method and Euclidean distances.

Meteorological conditions

The data used for temperature and precipitation were obtained from Hydro meteorological station Banja Luka (Table 2).

Winter period in all three years of examination was warmer in relation to many-year average. Average year air temperature for the period 1961-2004 was 10.9 °C. In comparison to many-year data, average air temperatures in 2007 and 2008 were higher for 1.9 °C and in 2009 for 1.8 °C.

Table 2. Average temperature (°C) and precipitation (l/m^2) for Banja Luka in 2007, 2008 and 2009

Year	Temperature/ precipitation	Month												Average per year/ Total per year
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
2007	Temperature (°C)	6.2	7.1	9.2	13.8	18..3	22.7	24.0	22.5	14.8	9.9	4.3	0.6	12.8
	Precipitation (l/m^2)	65.6	71.3	95.6	4.5	95.0	81.0	38.2	60.9	154.5	146.2	127.6	99.3	1,039.7
2008	Temperature (°C)	2.3	5.4	7.9	12.6	17.6	21.5	22.4	21.9	15.6	13.6	8.0	4.4	12.8
	Precipitation (l/m^2)	39.2	12.3	157.7	102.9	70.9	79.6	85.2	24.3	106.7	69.3	77.7	66.0	891.8
2009	Temperature (°C)	-0.7	2.7	7.6	14.2	18.9	20.0	23.3	22.8	18.6	11.4	8.7	4.6	12.7
	Precipitation (l/m^2)	72.7	51.1	71.0	40.0	48.7	152.9	43.4	138.2	32.9	72.5	82.2	180.4	986.0
1961-2004	Temperature (°C)	-0.2	2.0	6.5	10.9	15.9	19.4	20.9	20.6	16.1	11.1	6.1	1.2	10.9
	Precipitation (l/m^2)	71.3	62.9	77.5	90.9	95.4	111.6	94.5	82.8	94.3	80.5	98.2	89.3	1,047.0

In the period of global warming, the precipitation in Banja Luka region has been the most common limiting factor of successful agricultural production including production of fodder crops in arable land and grassland. Based on precipitation data (Table 2), it can be noticed that total sum per year of precipitation during all three years of examination (2007-2009) was smaller than many years average. Quantity of precipitation during vegetation period (April-October) in 2007-2009 was significantly lower than many-year precipitation average. During examination in vegetation period of perennial ryegrass the lowest sum of precipitation was recorded in 2007 (434.1 l/m^2).

RESULTS

Highly significant differences among autochthonous populations and variety Maya for all phenological traits were determined (Tables 3 and 4). This creates the possibility of an effective choice of progenies for their inclusion in the breeding germplasm and the creation of local varieties, suitable for different ways of forage production (pure sowing or mixtures) and utilization (for grazing, mowing/ grazing or grazing/mowing), or for the establishment of grasslands for special purposes (for sports and recreation grounds, for the protection and preservation of the environment, etc).

The three-year average values of individual phenological phases in the examined autochthonous populations of perennial ryegrass and cultivar Maya are shown in Table 3. and Figure 1-5.

With a three year monitoring of growth of generative shoots, (GRGS) at the examined populations of perennial ryegrass a statistically significant difference between the populations was found, but also in relation to the variety of Maya. The GRGS was the lowest in the population Laminci (8.4 days), at the latest at Manjaca population (23.3 days). Interval of variation of this trait in population collection was 14,9 days, what was more than average value of populations (14.5).

Table 3. Phenological traits of perennial ryegrass population and cultivar (average values)

Populations	GRGS	VEGF	IHF	FLOW	VEG
Dragočaj	19.3	40.8	9.8	15.4	95.4
Kosjerovo	14.7	33.2	11.7	17.4	89.3
Laminci	8.4	29.0	12.2	19.7	82.5
TAS	11.8	30.7	11.9	19.4	87.2
Kupres	14.2	32.9	11.3	19.8	84.3
Maglajani	9.8	33.6	10.8	20.7	84.0
Manjača	23.3	44.9	7.7	16.1	95.9
$\overline{X}$ *of populations*	14.5	35.0	10.8	18.3	88.4
Variety Maya	17.6	36.3	10.5	19.1	89.1
LSD 0,05	1.4	1.4	1.0	1.2	1.7
0,01	1.8	1.8	1.3	1.5	2.2

*Source: authors' elaboration based on original data

Vegetative phase finishes with the beginning of perennial ryegrass tillering and cutting of forage and it is one of the most important traits because it affects time of utilization and therefore the quality of produced biomass. The average VEGF in population collection was 35.0 days, and the differences between individual populations and cultivar Maya were statistically significant. This phase had the longest duration for populations Manjača and Dragočaj what refers them in late maturity genotypes. Population Dragočaj showed most stabile VEGF in all three trial years (Fig.2).

According to the results of three-year research, the average number of days from heading to starting of flowering (IHF) was 10.8 days, and the differences between tested populations were statistically highly significant (Table 3, Fig. 3). In population Manjača, this phase was the shortest (7.7 days). In contrast, the maximum IHF was found in population Laminci (12.2 days).

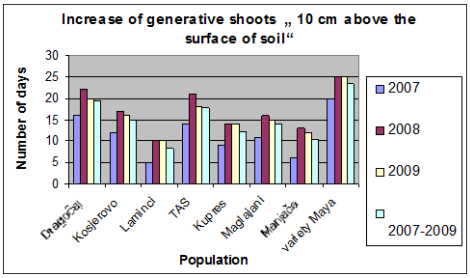


Figure 1. Increase of generative shoots „10 cm above

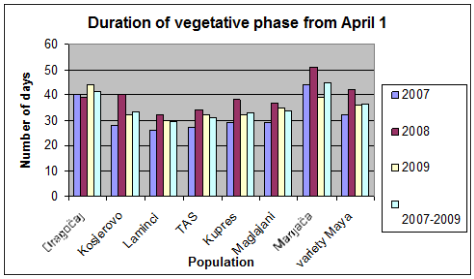


Figure 2. Duration of the surface of soil“vegetative phase from April 1

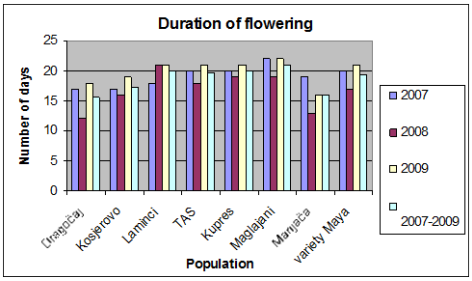


Figure 3. Interval of heading start of flowering

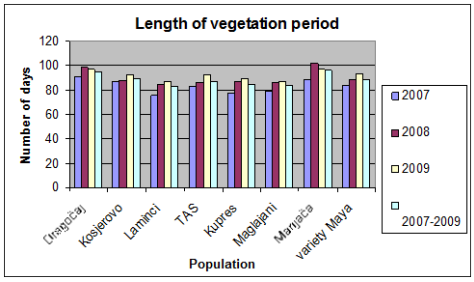


Figure 4. Duration of flowering

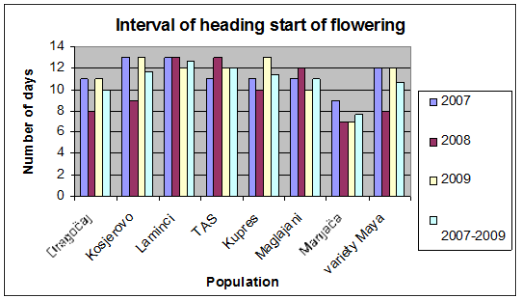


Figure 5. Length of vegetation period

The longest period of flowering (FLOW) was registered in the third, and the shortest one in the second year of study (Fig. 4). During the three-year research phenological phases of flowering lasted the longest in the population Maglajani (20.7 days) and shortest in the population Dragočaj (15.4 days). In relation to the variety Maya, four populations had longer FLOW (TAS, Laminci, Kupres and Maglajani).

The shortest average vegetation period (VEG) was found in populations Laminci (82.5 days) and Maglajani (84 days). In contrast, the longest VEG was determined in populations Manjača (95,9 days) and Dragočaj (95,4 days). Populations Dragočaj and Manjača had 7 days longer average VEG than cultivar Maya. The greatest variation in the duration of this phase observed by years, were registered on populations Kupres and Manjača (13 days), and the least in population Kosjerovo (6 days).

Table 4. ANOVA and indicators of variability of observed properties

Properties / Indicators of variability	GRGS	VEGF	IHF	FLOW	VEG
MS genotype	299.90**	340.08**	25.49**	43.35**	307.07**
MS year	291.89**	414.07**	18.82*	75.78**	716.07**
MS interaction	1.60ns	33,75**	7.95**	8.86**	26.16**
Genetic variance σ^2_g	299.45	330.44	23.22	40.82	299.60
Phenotype variance σ^2_f	303.05	349.84	28.94	47.89	317.02
Heritability h^2 (%)	98.81	94.45	80.25	85.24	94.50

Based on the results of ANOVA, it was found that the influence of genotype and year to all phenological traits was highly significant (Table 4). Presence of highly significant interaction (genotype x year) was found for all phenological traits except for the growth of generative shoots 10 cm above the ground.

The share of genetic into phenotypic variance indicates that the variability of the duration of the explored phenological phases in this collection of populations is based dominantly on the plant genotypes. Heritability in a broad sense is high for all traits and it indicates also a large proportion of the genetic in total phenotype variability of researched properties.

For better success of breeding work it is necessary to understand the genetic origin, structure of genetic and phenotype variance, heritability, and the interdependence of functionally related properties. Therefore, correlations that suggest possible directions of changes of different properties of plant material under the influence of applied method of selection are of particular importance. All coefficient of correlations were highly statistically significant except correlation between VEG and IHF, which was only significant on p > 0,95 (Tab. 5).

Table 5. Coefficient of correlation of researched traits of perennial ryegrass populations

Properties	VEGF	IHF	FLOW	VEG
GRGS	0.8553**	-0.7154**	-0.6546**	0.8630**
VEGF		-0.7198**	-0.5834**	0.7224**
IHF			0.6120**	-0.4895*
FLOW				-0.5525**

* p > 0,95 ** p > 0,99

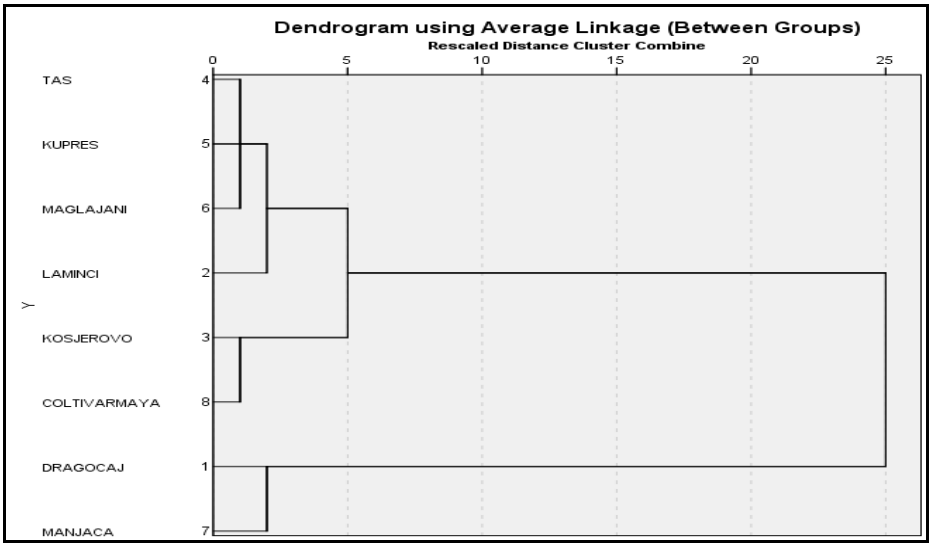


Figure 6. Cluster diagram of the studied populations of perennial ryegrass

On dendogram which was formed as a result of hierarchical cluster analysis (Fig. 6) two groups of populations can be observed. The first group is made up of two sub-groups. In the first subgroup are the following populations: TAS, Kupres, Maglajani and Laminci. The characteristic for this group is the longest period of flowering and the shortest length of vegetation. The second subgroup consists of the population Kosjerovo and variety Maya, and it is characterized by an intermediate maturity. The second group consists of the population Dragočaj and Manjača which are characterized by the latest maturity and the longest duration of the vegetation, short periods from heading to flowering and flowering per se.

DISCUSSION

By observing the date of appearance of generative shoots 10 cm above ground it was noticed that later emergence counting from April 1st resulted in late heading stage. Whyte et al. (1975) stated that the varieties of perennial ryegrass mutually differed in earliness, and late maturing, and there were early, middle early, and medium late varieties, which is in accordance with the results of this research. Earliness is a very important property in the case of perennial ryegrass, which is characterized by the time of heading (Hemphreys, 1989).

During the three-year studies, the duration of the vegetative phase of the examined perennial ryegrass populations was 35 days. The average duration of the vegetative phase in the examined perennial ryegrass populations ranged from 29 to 45 days, so these results are consistent with the results quoted by Sokolovic et al. (2001), in which the vegetative phase of perennial ryegrass lasted from 39.8 to 65.2 days. According to Charmet and Balfourier (1994), the time of heading of European population is quite long, and lasts from 30 to 70 days. Surault et al.

(2009) find that the date of heading of perennial ryegrass was very important for biomass production. These authors conducted the micro experiment in France examining 9 varieties of perennial ryegrass in pure sowing and 10 mixtures with different perennial ryegrass varieties, to determine an impact of date of heading to biomass yield. According to these authors, later maturity varieties achieved lower biomass yield as compared to the early and medium late varieties. Mixtures with two or three different varieties of perennial ryegrass with different time of heading had intermediate biomass yield and did not have higher yields than in pure sowing. Rossignol et al. (2014) found that the tested populations of three grass species depending on the current ripeness of population and phenological peak differed significantly in the amount of vegetative matter.

Based on the three-year results of research, the average length of the interval from the beginning of heading to the beginning of blossoming was 10.8 days. In the studied populations of perennial ryegrass interval of heading-start of blossoming in population Manjača was the shortes lasting for 8 days and in the population Laminci it was the longest lasting for 13 days, which is in compliance with the results of Sokolovic et al. (2001), which stated that the heading-start of blossoming period lasted from 8.6 to 19.5 days.

Blossoming of perennial ryegrass genotypes lasted 18.3 days on average, and blossoming interval was 16-21 days, indicating significant differences between populations. Phase of blossoming was the shortest in 2008, 17 days, or in other words blossoming of researched populations lasted from 12 to 21 days. In the 2009, the blossoming lasted 20 days on average, i.e. from 16 to 21 days. Martiniello (1998) pointed out that early blossoming of perennial ryegrass was an important property indicating the ability to increase seed yield. Namely, the populations of perennial ryegrass, which had previously blossomed, and whose interval of blossoming lasted longer could achieve a higher seed yields compared to the population in which the interval of blossoming was shorter. The perennial ryegrass varieties blossoming later have a higher proportion of green mass and smaller stems in area of grazing in relation to varieties that have intermediate blossoming (Stewart and Hayes, 2011). Blossoming time can significantly affect the dry weight of the leaves (Ansquer et al., 2009). During the research of a large number of indigenous populations of perennial ryegrass Lakic et al. (2013) determined the existence of a highly significant positive genetic correlation between the start of blossoming and spike length as well as the start of blossoming and mass of 1000 seeds.

During the three-year research, the average length of vegetative period of perennial ryegrass genotypes lasted 87 days. Length of vegetation period in 2007 lasted from 76 to 91 days, or 83 days on average, in the 2008 it was 90 days on average, or from 85 to 102 days, and in the 2009 it was from 87 to 97 days, or 92 days on average.

High values of heritability determined for some phenologic phases pointed to a great participation of genetic in a total phenotype variability of examined traits. Moderate heritability in jointing ($h^2 = 0,72$) revealed a large environmental

impact. In contrast, high heritability (h^2 = 0,86) in heading, flowering and the critical phase implied a strong genetic effect (Ullmann et al., 2016).

CONCLUSIONS

One of the important phenological characteristics of perennial grasses, including ryegrass, is the emergence of the reproductive organs, i.e. emergence of beginning of ears in the stem at a height of 10 cm above ground. On the basis of this trait the maturing of grasses can be determined, as well as the optimum time for short-term utilization of grassland by grazing and no adverse impact on the further growth and development of plants, i.e. taking advantage by mowing. By observing this phase, it was noted that the populations in which there was a later appearance of generative shoots in the stem and the later start of heading, had a shorter blossoming period.

ACKNOWLEDGEMENT

This work was supported by the Program of preservation of plant genetic resources of the entity of Republic of Srpska (BiH), carried out by Institute for genetic resources of University of Banja Luka.

REFERENCES

Ansquer P, Al Haj Khaled R,Cruz P, Theau JP, Therond O, Duru M (2009) Characterizing and predicting plant phenology in species-rich grasslands. Grass and Forage Science 64: 57–70.

Charmet G, Balfourier F (1994) Influence of ecological factors on population differentiation in perennial ryegrass (*Lolium perenne* L.). Genetic Resources and Crop Evolution 41: 175-184.

Grogan D, Gilliland TJ (2011) A rewiev of perennial raygrass variety evaluation in Ireland. Irish J Agric Food Res 50: 65-81.

Lakic Ž, Sokolovic D, Babic S, Vojin S, Ikanovic J, Veljovic T, Balalic I (2013) Genetic variabilyty of seed yield and seed components of autochthonous Lolium perenne L. populations. Genetika 45: 553-556.

Lakic Ž, Balalic I, Vojin S (2015) Interpretation of Genotype × Environment interaction in Perennial ryegrass (*Lolium perenne* L.). Genetika 47: 509-522.

Martiniello P (1998) Influence of agronomic factors on the relationship between forage production and seed yield in perennial forage grasses and legumes in a Mediterranean environment. Agronomie 18: 591-601.

Rossignol N, Andueza D, Carrère P, Cruz P, Duru M, Fiorelli JL, Michaud A, Plantureux

Sokolovic D, Tomic Z, Šurlan-Momirovic G (2001) Genetička vrijabilnost autohtonih populacija engleskog ljulja (*Lolium perenne* L.). Journal of Scientific Agricultural Research 62: 27-34.

Sokolovic D, Radovic J, Lugic Z, Tomic Z, Babic S (2006) Genetic variability of perennail ryegrass (*Lolium perenne* L.) autochthonous populations collected in Serbia for see yield and seed yield components. Proceedings of EUCARPIA fodder crops and amenity grasses section and Medicago spp. Group joint Meeting, Perugia, Italy, pp. 253-256.

Sokolovic D, Lugic Z, Radovic J, Zivanovic T, Babic S, Simic A, Miletic R (2011) Evaluation of morphological traits, dry matter yield and quality of *Lolium perenne* L. autochthonous populations from Serbia through multivariate analysis.Genetika 43:129 -140.

Stewart A, Hayes R (2011) Ryegrass breeding - balancing trait priorities. Irish Journal of Agricultural and Food Research 50: 31-46.

Surault F, Julier B, Veron R, Huyghe Ch (2009) Forageproduction of grasslands composed by one, tvo or three varieties of perennial ryegrass. XVIIIth Meeting of the Eucarpia Fodder Crops and Amenity Grasses Section, Book of Apstracts, La Rochelle, France, p. 62.

Ullmann I, Herrmann A, Hasler M, Cai D (2016) Variability in the critical phase of stem elongation of perennial ryegrass genotypes: a source for breeding progress? Jurnal of Agricultural Science 154: 1002-1014.

Whyte RO, Moir TRG, Cooper JP (1975) Grasses in Agriculture. FAO Agricultural Studies, Food and Agriculture Organisation of the United Nations, Italy, p. 417.

Yamada T (2013) Perennial ryegrass. In: Genetics, genomics and breeding of forage crops. (Eds. H Cai, T Jamada, C Cole). CRC Press, Boca Raton, Florida, USA, pp:11-35.

DOI: 10.17707/AgricultForest.65.1.22

Katarína HOZÁKOVÁ*, Jozef BUJKO,
Klára VAVRIŠÍNOVÁ, Július ŽITNÝ [1]

EVALUATION OF THE FACTORS AFFECTING THE GROWTH INTENSITY OF CHAROLAIS CALVES

SUMMARY

The aim of study was to evaluate the growth indicators in total of 1,986 purebred Charolais calves in relation to the sex, year of birth, birth season of the calves and also dam´s parity. At the same time, the object of work was to evaluate cows' longevity. We found higher values of live weight in bulls ($P>0.05$), the occurrence of twins showed lower birth weight ($P<0.001$) and weaning weight ($P>0.05$). The spring season of calving had an influence on lighter offspring in birth weight ($P<0.001$) as well as in pre-weaning period ($P<0.001$); the heaviest calves were born in the winter calving season. Calves born to primiparous cows were markedly lighter at birth ($P>0.05$), at the age of 120 days ($P>0.05$), and also at the weaning ($P<0.05$) than those born to older cows. The linear model characterized by the coefficient of determination for all fixed effects was $R^2 = 0.714862\%$ for birth weight (BLW), $R^2 = 0.375164\%$ for weight at 120 days (LW120) and $R^2 = 0.473550\%$ for weaning weight (LW210). On the basis of the results, we can observe the increased growth performance of calves over the years, pointing to the adaptation of Charolais to conditions in Slovakia.

Keywords: calves, cattle, coefficient of determination, Charolais, traits of growth.

INTRODUCTION

Breeding of suckling cows in Slovakia has an increasing tradition despite of the unfavourable economic situation. Increasing interest in cattle breeding results from trends of the society, especially in the consumption of quality raw materials. At the present, 11 specialized purebred cattle breeds are kept in Slovakia. The most numerous are Charolais and Limousine.

In meat production, the most important parameter is an excellent growth potential of calves represented by the live weight. In Slovakia, the live weight is determined at 120, 210 and 365 days of age. The most economically important is a live weight gained at 210 days, i.e. the weaning weight. Calves growth ability, such as the weaning weight is affected by many genetic as well as non-genetic

[1]Katarína Hozáková *(corresponding author: xsupekovak@uniag.sk), Klára Vavrišínová, Department of Animal Husbandry, Slovak Agricultural University in Nitra, SLOVAK REPUBLIC; Jozef Bujko, Július Žitný, Department of Genetic and Breeding Biology, Slovak Agricultural University in Nitra, SLOVAK REPUBLIC

Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

factors (Toušová et al., 2014). The aim of many studies was to evaluate the influence of factors such as: the breed (Bene et al., 2007), the sex of calves including occurrence of twins, the birth season (Krupa et al., 2005; Toušová et al., 2014), the dam´s age at calving (Krupa et al., 2005; Szabó et al., 2006) as well as the sire (Özlütürk et al., 2006).

Modern selecting programs tend to increase the body weight and body size at attaining maturity. The positive relationship between growth traits and live weight at maturity favourably affects the weight gains and therefore amount of muscular tissue. Just selecting cattle breeds aimed to enhance not only the quantity, but also the quality of meat with the respect to consumer preferences (Albertí et al., 2008).

The meat attributes including objectiveness and measurability of carcass. The interest of the meat industry is tended to accelerate the growth of calves and to achieve the slaughter weight earlier. Excellent maternal properties of milking cows are used in crossbreeding with beef cattle for rearing calves to greater weaning weights (Coleman et al., 2016).

According to Eriksson et al. (2004) there is a direct genetic continuity between the dam´s age at calving and the calves' birth weight. Likewise, the degree of carcass fatness has a negative effect on the birth weight and the calving difficulty.

The calving ability and calves vitality are functional traits of the cow's lifespan. In recent years, the selection criteria include increasing the longevity of milking cows such as beef cows (Różańska-Zawieja et al., 2014).

The aim of this work was to evaluate the indicators of the growth of purebred Charolais calves kept in different farming conditions in Slovakia. At the same time, the paper examines factors affecting selected utility parameters of Charolais breed. In the next part, the study examines the longevity of cows as well as numbers of reared calves.

MATERIAL AND METHODS

The material of nine herds of Charolais breed was provided from the database of Breeding Services of the Slovak republic (B.S. SR, S.E., 2017). A total of 1,986 calves (bulls = 793; heifers = 1,116; twins = 77) born between 2010 and 2016 were observed during evaluation.

In the monitored herds the mating system included seasonal mating and artificial insemination as well, 95 calves were born after embryo transfer. Subsequent calving season was distributed within individual herd throughout the year. During the entire grazing period from May to October the calves remained on the pasture with the cows. All animals were pasturing during the entire period and at the end of the grazing period were subsequently housed together in the winter blocks. The animals – bulls, sukler cows and monitored calves were during the winter period fed a feed ration based on hay, straw, alfalfa silage and maize silage in *ad libitum* amount.

In accordance with the Methodology of Performance Recording for Beef Cattle used to control the growth ability of suckler cows (Darnadiová and Debrecéni, 2009) the study evaluates the birth weight (BW), weight at 120 days (W120) and 210 days (W210). For the evaluation of the cows' longevity, the lifespan and the age at culling were the analyzed characteristics. Furthermore, the number of calves reared per a cow was calculated.

The basic statistical and variability characteristics (least square means, standard deviations) were evaluated using the Statistical Analysis System (SAS) version 9.3 (TS1M2) Enterprise Guide 5.1. (SAS INSTITUTE Inc., 2011). The mixed procedure was used for the final analysis. The value of statistical significance (P), the value of the F-test and the coefficient of determination (R^2) of the above mentioned growth parameters were evaluated with its relation to: the sex of the calves (bulls, heifers, twins), calving season, year of birth, and dam´s parity. For the impact of the verification the mixed model equation was used:

$$Y_{ijklm} = \mu + SEX_i + BS_j + YB_k + LR_l + e_{ijkl}$$

where:
Y_{ijklm} – depended variable (BLW, LW120, LW210)
μ – mean value of depended variable
SEX_i – fixed effect of i^{th} sex of calf
BS_j – fixed effect of j^{th} calving season
YB_k – fixed effect of k^{th} year of birth
LR_l – fixed effect of l^{th} class of dam´s parity
e_{ijkl} – random error

During the evaluation the calves were into four groups according to the calving season: winter calves (A) born from December to February, spring calves (B) born from March to May, summer calves (C) born from June to August, autumn calves (D) born from September to November divided. According to the sex calves were into bulls and heifers divided. The occurrence of twins was also recorded and therefore the calves were divided into male twins, female twins and mixed twins. There were seven levels of the year of birth (2010 - 2016) and six levels of dam´s parity (1^{st} lactation, 2^{nd}-3^{rd} lactation, 4^{th}-6^{th} lactation, 7^{th}-9^{th} lactation, 10^{th} lactation, and subsequent). Statistical evaluations of the differences between traits were tested at the levels of statistical significance: $^{+}$ $P<0.05$, $^{++}$ $P<0.01$, $^{+++}$ $P<0.001$ or $^{-}$ $P>0.05$.

RESULTS AND DISCUSSION

The linear model is described in Table 1. The coefficient of determination of observed traits with all fixed effects ranged from $R^2 = 0.010231\%$ with relation to the W210 to $R^2 = 0.604172\%$ in relationship to the BW. The most important factor was the effect of herd ($R^2 = 0.283454\%$ for W120 to 0.604172% for BW), the effect of sex of calves ($R^2 = 0.118623\%$ for BW), the effect of the year of birth ($R^2 = 0.063582\%$ for W210 to 0.079556% for W120). The statistical

significance of the all mentioned factors were P<0.001. According to various authors, the effect of the herd-year-season is the most significant factor with higher variability (Krupa et al., 2005; Stádník et al., 2008). Our results are similar to those of Krupa et al. (2005), who showed the highest influence of the effect for BW (R^2 = 0.867%).

Table 1. The influence of the factors affecting BW, W120 and W210 in selected herds of Charolais breed

Sources of variance	DF[a]	(BW)			(W120)			(W210)		
		MS[b]	v[c]	R-Square[d]	MS[b]	v[c]	R-Square[d]	MS[b]	v[c]	R-Square[d]
Herd	9	4291.64	10.04	0.604172^{+++}	326858.21	46.07	0.283454^{+++}	1270755.66	49.99	0.393660^{+++}
Sex of calves	7	1083.37	14.97	0.118623^{+++}	75356.56	52.99	0.050828^{+++}	146464.78	63.03	0.035290^{+++}
Calving season	3	1099.40	15.51	0.051591^{-}	60159.28	53.86	0.017390^{+++}	422857.57	62.69	0.043665^{+++}
Year of birth	6	262.93	15.74	0.024676^{+++}	137606.99	52.17	0.079556^{+++}	307867.36	62.08	0.063582^{+++}
Parity	11	108.10	15.71	0.019782^{+}	11644.25	51.95	0.013641^{+}	25853.95	64.38	0.010231^{-}

[a]grades of freedom, [b]mean squares, [c]coefficient of variation, [d] coefficient of determination (R^2);
$^{+}$ P<0.05, $^{++}$ P<0.01, $^{+++}$ P<0.001 or $^{-}$ P>0.05

The values given in Table 2 represent the effect of the herd, sex, birth year, calving season as well as dams´ parity. A statistical significance for all growth traits was detected of the level P<0.001 in relation to the herd, sex, birth year; and the level P<0.05 in relation to the parity. Our findings are similar to Szabó et al. (2006) who found the significant effect (P<0.05) of sire, herd, age of dam´s calving, birth year, season and sex of calves on the weaning weight. The growth ability of beef breeds is one of the most important parameters influencing beef production. Charolais calves show relatively high birth weight (Mujibi and Crews, 2009), statistically similar to Limousine and Blonde d´ Aquitaine calves (Szabolcs et al., 2013). According to Eriksson et al. (2004) high birth weight is associated with the gestation length and better vitality of calves. Higher growth abilities of calves are due to higher birth weight (Koch et al., 2004). On the other hand, the authors Özlütürk et al. (2004) report for Charolais higher final weight and heavier carcasses at a lower initial weight. The significance of the parity was at the level P>0.05 only for W210.

Table 2. The statistical evaluations of BW, W120 and W210 in selected herds of Charolais breed

Traits	Herd	Sex of calves	Calving season	Year of birth	Parity
BW (kg)	355.12^{+++}	38.03^{+++}	35.94^{+++}	8.35^{+++}	3.48^{+++}
W120 (kg)	86.85^{+++}	15.13^{+++}	11.69^{+++}	28.51^{+++}	2.39^{++}
W210 (kg)	142.54^{+++}	10.34^{+++}	30.17^{+++}	22.40^{+++}	1.78^{-}

+ $P<0.05$, ++ $P<0.01$, +++ $P<0.001$ or - $P>0.05$

Pursuant to the effect of the sex of calves (Table 3), bull calves were heavier than heifers in all of the monitored growth parameters (BW + 2.15 kg, W120 + 26.45, W210+ 37.28 kg), unfortunately the significance was at the level $P>0.05$. These differences are according to various authors important for the survival of the calves at the weaning period and for the growth rate before weaning as well, which is higher for Charolais and Simmental calves (Szabó et al., 2006). The majority of works recording growth abilities of the calves, including Krupa et al. (2005), Stádník et al. (2008) report the highest birth weight for bulls, followed by male twins and heifers. This does not correspond with our findings; male twins were the lightest (27.27 kg). Moreover we found different results of weaning weight which is the highest for bulls and also similar for male twins and female twins (183.90 and 185.79 kg, respectively).

In relation to the occurrence of twins (Table 3), differences of live weight were higher at birth (8.61 kg) and at weaning as well (30.78 kg), when differences were significant. These results are in agreement with Stádník et al. (2008). Despite the lower birth weight and lower weaning weight, in accordance with Davis et al. (1989) twins produce more weight per cow on sale. Birth of twins in the herds with meat production is a desirable attribute and may be important for systematic dam breeding (Stádník et al., 2008).

Relating to the effect of the dams´parity on birth weight given in table 4, we could summarize that the birth weight increase with the parity, however results were non-significant ($P>0.05$). The largest numbers of calves were born to cows on the second and third lactation (39.11%), while the lowest numbers were born to the oldest cows (0.52%). The offspring from oldest cows reached in average 5.12 kg heavier BW than calves born to primiparous cows. It is necessary to select sires and their usage for mating of heifers to avoid dystocia and improve the birth weight (Özlütürk et al., 2006). The highest average values of pre-weaning period were determined in offspring of cows with tenth and subsequent parity, the differences varied from 2.81 kg to 37.66 kg. These results point to the lower milking ability of heifers and dams with second parity as well as to their own continuing development. The highest values of W210 reached offspring of the oldest cows ($P>0.05$). These results are similar to those of Szabó et al. (2006), Stádník et al. (2008).

Table 3. Basic characteristics of BW, W120 and W210 in relation to the sex of the calves and occurrence of singles and twins

Effects		n[a]	BW (kg)		W120 (kg)		W210 (kg)	
			LSM[b]	SD[c]	LSM[b]	SD[c]	LSM[b]	SD[c]
Sex	Bull	723	37.01^{-}	5.76	150.60^{-}	66.75	208.01^{-}	121.57
	Heifer	1,091	34.86^{+}	5.03	124.15^{++}	70.91	170.73^{++}	119.08
	Male twins	30	27.27^{+++}	5.91	127.83^{-}	48.68	183.90^{+}	89.91
	Female twins	14	28.86^{+++}	4.56	119.14^{-}	43.98	185.79^{-}	86.88
	Mixed twins	33	30.27^{+++}	6.19	148.67^{-}	34.14	194.87^{+}	106.97
Occurrence of singles and twins	Singles	1,909	37.77^{++}	5.02	129.39^{-}	84.36	220.63^{++}	115.74
	Twins	77	29.16^{+++}	5.71	136.08^{+}	40.23	189.85^{-}	97.68

[a] number of observations, [b] least squares means, [c] standard deviation; $^{+}$ P<0.05, $^{++}$ P<0.01, $^{+++}$ P<0.001 or $^{-}$ P>0.05

Table 4. Basic characteristics of BW, W120 and W210 in relation to the dams´parity

Parity	n[a]	BW (kg)		W120 (kg)		W210 (kg)	
		LSM[b]	SD[c]	LSM[b]	SD[c]	LSM[b]	SD[c]
1th	462	34.69^{-}	5.45	124.41^{-}	75.26	172.20^{-}	124.30
2nd-3rd lactation	747	35.28^{-}	5.89	135.73^{-}	69.59	190.16^{-}	120.35
4th-6th lactation	537	35.91^{-}	5.87	138.54^{-}	66.41	194.34^{-}	116.09
7th-9th lactation	140	36.81^{-}	5.02	146.65^{-}	61.59	181.44^{-}	123.41
10th + lactation	14	37.43^{-}	3.55	162.07^{-}	21.01	167.79^{-}	134.63

[a] number of observations, [b] least squares means, [c] standard deviation; - P>0.05

Table 5 describes the effect of the calving year and season on the live weight of calves. A statistical significance of the level P<0.001 was recorded in relation to the calving season for all live weights except W120 (P>0.05). Differences of birth live weight in relation to the calving season were non-significant (3.97 kg). Higher and significant differences were determined at W120 and W210 (21.79 kg vs. 61.72 kg) in calves born in different season.

Table 5. Basic characteristics of BW, W120 and W210 in relation to the calving season and year of birth

Effects		n[a]	BW (kg)		W120 (kg)		W210 (kg)	
			LSM[b]	SD[c]	LSM[b]	SD[c]	LSM[b]	SD[c]
Season of birth	Winter (A)	503	35.71^{+++}	6.61	120.27^{-}	78.02	204.79^{+++}	120.09
	Spring (B)	971	36.51^{+++}	4.99	142.06^{++}	72.58	203.17^{+++}	117.79
	Summer (C)	244	35.58^{+++}	5.01	133.86^{-}	61.25	143.07^{-}	120.79
	Autumn (D)	268	32.54	5.62	124.54	64.77	148.78	115.17
Year of birth	2010	3	28.33^{++}	3.21	100.67^{-}	87.32	199.33^{-}	40.10
	2011	176	35.75^{+}	5.33	148.78^{+}	65.12	245.63^{-}	82.28
	2012	352	34.02^{+++}	5.53	106.81^{+++}	76.19	174.36^{+++}	119.58
	2013	380	35.74^{+}	5.60	108.88^{+++}	83.48	206.25^{+}	99.31
	2014	340	36.01^{-}	5.22	143.72^{++}	67.26	154.46^{+++}	142.87
	2015	472	35.88^{+}	5.74	142.68^{++}	69.12	166.24^{+++}	128.12
	2016	263	36.89	6.17	162.75	35.66	230.07	99.78

[a] number of observations, [b] least squares means, [c] standard deviation; $^{+}$ P<0.05, $^{++}$ P<0.01, $^{+++}$ P<0.001 or $^{-}$ P>0.05

The calves born in winter season were lighter in pre-weaning period than calves born in other seasons. Keeping calves on pasture with their mothers increase beef production effectiveness and give a precondition to a more intensive growth rate with cheaper feeding sources (Crosson, 2015). The majority of calving was recorded in winter (25.3%) and spring (48.89%) season, respectively. The offspring born in the winter season reached the significantly highest weaning weight (204.79 kg), while the lightest calves were born in the

summer (143.07 kg). Our results are inconsistent with findings of Kovács et al. (1994) where the heaviest calves were born in autumn and Szabó et al. (2006) for summer calves. Over the study period, the highest BW and W120 were recorded in 2016 ($P<0.01$; $P>0.05$), which is an opposite of the worst year, i.e. 2010 (+8.56 kg and 62.08 kg, respectively). Only the weaning weight was highest in year 2011 (245.63 kg), however results were non-significant. Our results demonstrate the importance of management during different weather and pasture conditions throughout the years.

Table 6 represents the indicators of dams' longevity and number of calves born in the monitored herds. We evaluated an average lifespan of 8.39 years, while the highest value was 17.49 years. The longevity is an important economic trait in beef herds and it is in close relationship with a reproductive performance, while we can expect a high rate of productivity, in average 8-12 years. The most of calves (3.26) were born at the age of 8.39 years. In comparison with Różańska-Zawieja et al. (2014) we found a longer lifespan of Charolais cows, for about 3.69 years.

Table 6. Basic characteristics of the longevity of Charolais dams and average number of dams' offspring

Effect	n[a]	LSM[b]	SD[c]	v[d]	MIN[e]	MAX[f]
Longevity of cows	979	8.39	2.87	34.137	2.39	17.49
Number of calves	1 910	3.26	2.13	66.15	1	12

[a]number of observations, [b]least squares means, [c]standard deviation, [d]coefficient of variation,[e] minimum, [f] maximum

CONCLUSIONS

In the context of this study, it can be concluded that the growth ability of monitored beef calves kept in different conditions in Slovakia, was significantly influenced by the sex, year of birth, birth season as well as by the age of dams. In the evaluated file of animals we found the average birth weight 35.935 kg, W120 137.38 kg and W210 189.37 kg. The highest differences in BW were found in relation to the dams' parity (5.12 kg). The highest differences in W120 were found in the lactation rank and sex of calves (37.66 resp. 31.46 kg) and W210 at the birth season factor (61.72 kg).

The influence of the birth year on live weights of monitored animals signifies an adaptation of Charolais breed to the conditions of the Slovak regions and despite of achieved growth results in the winter birth season, it is important to enforce mating and calving in agreement with the concrete farming conditions. During the evaluation of the lifespan we determined unsuitable results for beef breeds for which a long performance and a high number of offspring is required.

ACKNOWLEDGEMENTS

This study was supported by the Scientific Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic and the Slovak Academy of Sciences (VEGA) (Projects No. 1/0724/16 and No. 1/0742/17).

Authors would like thanks the Breeding Services of the Slovak Republic (B.S. SR, S.E.) for providing data for processing.

REFERENCES

Albertí, P., Panea, B., Sañudo, C., Olleta, J.L., Ripoll, G., Ertbjerg, P., et al. (2008). Live weight, body size and carcass characteristics of young bulls of fifteen European breeds. Livestock Science, 114(1), 19-30. DOI: 10.1016/j.livsci.2007.04.010

Bene, S., Nagy, B., Nagy, L., Kiss, B., Polgár, J. P. & Szabó, P. (2007). Comparison of body measurements of beef cows of different breeds. Archiv fur Tierzucht, Dummerstorf, 50(4), 363-373. DOI: 10.5194/aab-50-363-2007

Coleman, L.W., Hickson, R.E., Schreus, N.M., Martin, N.P., Kenyon, P.R., Lopez-Villalobos, N., et al. (2016). Carcass characteristics and meat quality of Hereford sired steers born to beef-cross-dairy and Angus breeding cows. Meat Science, 121, 403-408. DOI: 10.1016/j.meatsci.2016.07.011

Crosson, P. (2015). How to economically achieve a 365-day calving interval for suckler cows. Retrieved from http://www.agriland.ie/farming-news/how-to-economically-achieve-a-365-day-calving-interval-for-suckler-cows-teagasc/

Darnadiová, O. & Debrecéni, O. (2009). Charolais beef breed adaptation to breeding conditions in Slovakia. Adaptácia plemena charolais na chovateľské podmienky Slovenska. Acta fytotechnica et zootechnica 3, Slovaca Universitas Agriculturae Nitriae, 81-84. ISSN: 1336-9245

Davis, M.E., Harvey, W.R., Bishop, M.D. & Gearheart, W.W. (1989). Use of embryo transfer to induce twinning in beef cattle: embryo survival rate, gestation length, birth weight and weaning weight of calves. Journal of Animal Science, 67(2), 301-310. DOI: 10.2527/jas1989.672301x.

Eriksson, S., Näsholm, A., Johansson, K. & Philipsson, J. (2004). Genetic relationship between calving and carcass traits for Charolais and Hereford cattle in Sweden. Journal of Animal Science, 82(8), 2269-2276. DOI:10.2527/2004.8282269x

Koch, R. M., Cundiff, L. V., Gregory, K. E. & Van Vleck, L. D. (2004). Genetic response to selection for weaning weight or yearling weight or yearling weight and muscle score in Hereford cattle: efficiency of gain, growth, and carcass characteristics. Journal of Animal Science, 82(3), 668-682. DOI:10.2527/2004.823668x

Kovács, A., Szűcs, E., Bori, T., Nagynaska, E. & Vőlgyi Csík, J. (1994). A születési hónap ésa zivarhatása a limousine borjak választási, valaminté veskoriteljes ítményére. ÁllattenyésztésésTakarmányozás, 43(3), 209–211. ISSN 0230-1614

Krupa, E., Oravcová, M., Polák, P., Huba, J. & Krupová, Z. (2005). Factors affecting growth traits of beef cattle breeds raised in Slovakia. Czech Journal of Animal Science, 50(1), 14-21. DOI: 10.17221/3990-CJAS

Mujibi, F. D. N. & Crews, D.H. (2009). Genetic parameters for calving ease, gestation length, and birth weight in charolais cattle. Journal of Animal Science, 87(9), 2759-2766. DOI:10.2527/jas.2008-1141

Özlütürk, A., Tüzemen, N., Yanar, M., Esenbuga, N. & Dursun, E. (2004). Fattening performance, carcass traits and meat quality characteristics of calves sired by Charolais, Simmental and Eastern Anatolian Red sires mated to Eastern Anatolian Red dams. Meat Science, 67(3), 463-470. DOI: 10.1016/j.meatsci.2003.11.022

Özlütürk, A., Yanar, M., Tüzemen, N. & Kopuzlu, S. (2006). Calving and preweaning growth performance traits of calves sired by Charolais, Simmental and Eastern Anatolian Red Bulls. Turkish Journal of Veterinary and Animal science, 30(2), 257-263. ISSN: 1303-6181. http://dergipark.gov.tr/tbtkveterinary/issue/12539/151205

Różańska-Zawieja, J., Nienartowicz-Zdrojewska, A., Smorag, T. & Sobek, Z. (2014). Longevity of use and reasons for beef cattle culling in Poland. Medycyna Weterynaryjna, 70(8), 491-496. www.medycynawet.edu.pl

SAS Institute Inc. (2011) Base SAS® 9.3 Procedures Guide. Cary, NC: SAS Institute Inc., Carry. NC. USA.

Stádník, L., Louda, F., Bolečková, J., Benešová, L. & Matějů, R. (2008). Effect of charolais dam´s mating method and parity on growth ability of their progeny. Scientia agriculturae bohemica, 39(4), 304-309. www.sab.czu.cz/en/r-8044-archive/r-8064-older-issues/r-11670-04-2008#i-8e3e36041282159455001dfa35e02aa8

Szabó, F., Nagy, L., Dákay, I., Márton, D., Török, M. & Bene, S.Z. (2006). Effects of breed age of dam birth year, birth season and sex on weaning weight of beef calves. Livestock Science, 103(1-2), 181-185. DOI: 10.1016/j.livsci.2005.12.005

Szabolcs, B., Ferencz, S. & Polgar, P. (2013). Some effects on calves´ birth weight and calving difficulty of cows. 1. The results of beef cattle in Hungry. Magyar Allatorvosok Lapja, 135(5), 267-277 www.researchgate.net/publication/286020886_Some_effects_on_calves%27_birth_weight_and_calving_difficulty_of_cows_1_The_results_of_beef_cattle_in_Hungry

Toušová, R., Ducháček, J., Stádník, L., Ptáček, M. & Beran, J. (2014). The effect of selected factors on the growth ability of charolais cattle. ActaUniversitatisAgriculturaeetSilviculturaeMendelianaeBrunensis, 62(1), 255-260. DOI: 10.11118/actaun201462010255

B.S. SR, S.E.: The Breeding Service of the Slovak Republic S.E. (2017). Results of breeding herd growth traits of Charolais breed in Slovak Republic at control years 2010 to 2016.

DOI: 10.17707/AgricultForest.65.1.23

Nikola RAKAŠČAN, Gordana DRAŽIĆ, Ljubiša ŽIVANOVIĆ, Jela IKANOVIĆ, Zoran JOVOVIĆ, Miloš LONČAR, Radmila BOJOVIĆ and Vera POPOVIĆ [1]

EFFECT OF GENOTYPES AND LOCATIONS ON WHEAT YIELD COMPONENTS

SUMMARY

Due to the dominant role in world nutrition, wheat was given the character of a strategic product. Its participation in human nutrition is gradually decreasing in developed countries where changes in the nutrition structure have prioritized animal proteins. However, in underdeveloped countries where the phenomenon of hunger is present in a severe form, the main tendency is to provide a sufficient amount of it for the needs of the population's diet.

The paper examines the influence of genotype/variety and site on winter wheat yield components: length of ears, number of spikelets in the spike, number of grains in the spike and mass of grains per spike, during 2016/17. The tested factors exhibited a different impact and a strong intensity of influence on the parameters covered by the research. The cultivation site had a statistically significant influence on the length of the ear (spike), the number of spikelets in the spike and the number of grains in the spike. Within the wheat yield components, the factor of the genotype / variety had higher influence on the length of the spike. The genotype G2 had a longer spike (8.62 cm), the number of spikelets in the spike (18.30), the number of grains in the spike (42.58), and the grain weight per spike (1.57).

Keywords: wheat, variety, locality, yield components, correlations

INTRODUCTION

Wheat (*Triticum sp*. L.) is one of the oldest and most important cultivated plants because today, wheat bread is used by more than 70% of the Earth'spopulation. Likely, neither human population could develop without wheat norwheatcould survive without the presence of a man. Wheat had and will play the most important role in the nutrition of the population for a long time. Its participation in human nutrition is gradually decreasing in developed countries, where changes in the structure of nutrition made animal proteins prioritized.

[1] Nikola Rakašćan, Gordana Dražić, University of Singidunum, Belgrade, SERBIA; Ljubiša Živanović, Jela Ikanović (corresponding author: jela@agrif.bg.ac.rs, bravera@eunet.rs), Radmila Bojović, University of Belgrade, Faculty of Agriculture, Nemanjina 6, Zemun Belgrade, SERBIA; Zoran Jovović, University of Montenegro, Biotechnical faculty, Podgorica, MONTENEGRO; Miloš Lončar, University of Belgrade, Faculty of Organisational Sciences, Belgrade, SERBIA; Vera Popović, Institute of Field and Vegetable Crops, Maksima Gorkog 30, Novi Sad, SERBIA;
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

However, in underdeveloped countries where the phenomenon of hunger is present in a severe form, the main tendency is to provide a sufficient amount that will provide fulfill of the needs in population's nutrition. The great influence of wheat in human nutrition influenced the development and improvement of the accompanying industry. It is known that the milling and bakery industry has developed very well and a number of other branches of the food industry that have wheat as their basis. Today, a large number of products derived from wheat are known. Further development of its processing technology will contribute to expanding the list of these products (Lakić et al., 2018). According to FAO (2017), all types of wheat in the world are cultivated in 220.107.550 ha, and, in the Republic of Serbia about 588.820 ha. In addition to the main product, grain, a significant quantities of by-products stay in the field, in warehouses and in industrial production and processing.

The crisis to feed the ever-growing population is compounded by the counteracting issues of spatial allocation of land for accommodation vs. agriculture. This issue of food insecurity is further amplified by degrading soil fertility conditions, reduced crop productivity and unpredictable climate change, which are expected to worsen in the near future. Although several policies addressing food security have been initiated (Carraro et al., 2015; FAO, 2015), one of the most challenging propositions is to achieve higher crop productivity under stressful environmental conditions. Agriculture as an occupation depends on the ability to cultivate crops suitable for a particular climate in a defined region. Prolonged exposure to high temperatures in rainfed areas of the world, may lead to drought stress (Abhinandan et al., 2018). The characterization of a novel wheat expansin gene TaEXPB23 has also opened new avenues for the role of jasmonate-mediated abiotic stress tolerance. The expression of TaEXPB23 was induced under drought stress and following exogenous MeJA application (Han et al., 2012).

The economic importance of wheat has its international regional basis. Due to the dominant role in world nutrition, wheat was given the character of a strategic product. Many countries do not product wheat enough for fulfilling their needs and are forced to constantly import it. In such a situation there is dependence of these countries on major producers that dictate the conditions in the entire world wheat trade (Glamočlija et al., 2015).

Wheat obviously took a very important place in international trade. Today, it is the basic food for over 70% of the country's population. Wheat bread with a nutritive, vitamin and energy value of 8,500-9,400 joules is more nutritious than the bread of other types of grain, as it contains 77-78% of carbohydrates, 16-17% of total proteins, 1.2-1.5% of oil, 0.5-0.8% of mineral salts (most salts of Ca, P, Fe) is rich in vitamins B (B1-thiamine, B2-riboflavin and PP (nicotinamide). Besides flour, significant by-products of wheat grains milling are wheat germs rich in easily digestible proteins and high-quality edible oil, and are used to prepare baby food and certain treats. The bran that is also obtained by the milling of wheat grains are important in the nutrition of domestic animals because they

have a high nutritional and energy value with proteins, carbohydrates, oil, mineral salts and 9% cellulose (Popovic, 2010; Ugrenović et al., 2015; 2018; Živanović et al., 2017a; 2017b; Stevanović et al., 2018). Germs has highest nutritional value with 30-40% of the total protein and 10-12% of the oil, and the smallest has a kernel wrapper, with a high percentage of cellulose and mineral salts. The aim of this work is to examine the impact of different agro-ecological conditions and cultivation sites on the productivity of winter wheat varieties of different lengths of the vegetation period.

MATERIAL AND METHODS

Examination of the impact of different agro-ecological conditions and production potential of winter wheat cultivars of different lengths of the vegetation period was carried out during 2016/17. at two sites, namely: Leskovac (experimental plot PSSS Leskovac, village Pečenjevce) and Pančevo (experimental plot of PSS Institute Tamiš, Pančevo). On the land of type alluvium (Leskovac) (L1); and carbonate chernozem (Pančevo), were performed field demo - tests, (L2). In this study, the following two varieties of wheat were examined: Sothys, early variety with axis (G1) and Sosthenes, medium-late cultivar without axles (G2). The tested varieties originate from the French seed company Caussadesemences. Applied agro-technics at tests depended on the preconditions, agroecological conditions of the cultivation area and given mechanization. The pre-crop for wheat at the locality - Leskovac was maize, while in Pančevo, the pre-crop was soybean. After harvesting of pre-crop, fertilization was carried out with mineral nutrient NPK (15:15:15) in the amount of 300 kg ha^{-1}, and then plowing in Leskovac, or disking with a heavy disks in Pančevo. Pre-sowing preparation included disking and seedbed tillering in Leskovac, and just harrowing in Pančevo. The sowing was done according to the sowing plan, in the density according to the manufacturer's recommendation (400 - 500 grains per m2), or the distributor of the variety. In Leskovac, sowing was done on November 21, 2016, and in Pančevo on October 29, 2016. Just after sowing rolling with a smooth roller was performed. Nutrient and foliar fertilization was carried out differently, depending on the site. In Leskovac, CAN (27% N) in the amount of 250 kg ha^{-1} was applied during thestoolingphase and, in the blade phase, phosphate fertilizer Asfer universal, in the dose of 3 kg ha^{-1}, while in Pančevo for feeding was used AN (32% N) in the amount of 250 kg ha^{-1} (in the stooling phase), and CAN in the amount of 130 kg ha^{-1} at the beginning of the blade phase.

As part of the care measures, protection from weeds, from lodged (falling down), as well as the causative agent of pest diseases, were carried out, depending on the need. In Leskovac, only one treatment against weeds and disease causers was carried out with a combination of Metmark WG 0,01% and Excort 0,5 l ha^{-1}. In Pančevo, two protective treatments were carried out. Quelex 0.6 l ha^{-1} + Trend 0.2 l ha^{-1} was used for the first treatment in Pančevo. In the second treatment, the combination Duett ultra 0.6 l ha^{-1} and Fastac 10 EC 0.15 l

ha^{-1} was used in Leskovac, while Ceres 0.6 l ha^{-1} and Decis 0.2 l ha^{-1}were used in Pančevo. Within the wheat yield components, the influence of variety and site on the length of the spike, the number of spikelets in the spike, the number of grains in the spike and the mass of grains per spike were studied.

Wheat harvest was done mechanically (by harvester) in full maturity, in the first decade of July (in Pančevo), or in the middle of the second decade of July (in Leskovac). Just before harvest, samples were taken and the necessary measurements of the basic components of the yield were made. The obtained results were processed statistically using the statistical package STATISTICA 12 and the results are shown in the table.

Meteorological conditions

For the successful wheat cultivation and other herbaceous plants, meteorological conditions, especially thermal and humidity conditions, should be optimal or as close as possible to the optimum. Growth and development of cultivated plant species and their yield and quality depends largely of these. For the realization of the above program data on weather conditions obtained from meteorological stations in the experimental fields in Leskovac and Pančevo were used. Mean monthly air temperature and precipitation amounts per months of the wheat growing period in the year of examination, by location, are shown in Table 1.

Table 1. Average monthly air temperature (^{0}C) and precipitation (mm), 2016/2017

Locality/ Mounts	X	XI	XII	I	II	III	IV	V	VI	Average
	Average month temperature of air (°C)									
Leskovac	11.9	6.8	-0.7	-5.8	9.2	10.3	11.3	16.7	21.9	9.1
Pančevo	10.0	6.1	-0.8	-4.9	3.3	9.5	11.4	17.4	22.7	8.3
Summ of month precipitations (mm)										Summ
Leskovac	82.0	131.0	12.0	44.0	49.0	39.0	69.0	82.0	19.0	527.0
Pančevo	85.4	83.2	6.6	22.6	20.2	32.6	45.8	57.3	27.0	380.7

The average air temperature for the wheat vegetation period in the year of testing ranged from 8.3°C in Pančevo to 9.1°C in Leskovac. On the other hand, a smaller amount of precipitation during the wheat growing period was registered at the locality of Pančevo (380.7 mm) and higher in Leskovac (527.0 mm).

RESULTS AND DISCUSSION

In this study, the influence of variety / genotype, different lengths of vegetation period, and locality on wheat yield components were examined: length of spike, number of spikelets in the spike, number of grains per spike and grain mass per spike, Table 2-4.

The evaluation of the significance of the results obtained shows that there are statistically very significant differences between the genotype / variety and grain yield per class (F_{exp} = 6.384 **), Table 3.

Table 2. Descriptive statistics for tested parameters

Parameter	Valid N	Mean	Median	Minimum	Maximum	Std. Dev.	Std. Error
Length of spike	12	8.007	8.045	7.13	8.830	0.668	0.193
Number of spikelet at spike	12	17.342	17.550	15.200	19.200	1.073	0.309
Number of grains per spike	12	40.392	38.650	37.400	49.200	3.926	1.133
Mass of grains per spike	12	1.477	1.415	1.280	1.860	0.165	0.0476

Table 3. Anova for tested parameters

Effect	SS	Degr. of Freed.	MS	F (df 8)	p
Length of spike					
Intercept	**769.2805**	**1**	**769.2805**	**29176.25**	**0.000000**
Genotype	**4.5141**	**1**	**4.5141**	**171.21**	**0.000001**
Locality	**0.1587**	**1**	**0.1587**	**6.02**	**0.039728**
G x L	0.0243	1	0.0243	0.92	0.365163
Error	0.2109	8	0.0264		
Number of spikelet at spike					
Intercept	**3608.801**	**1**	**3608.801**	**11190.08**	**0.000000**
Genotype	**5.741**	**1**	**5.741**	**17.80**	**0.002919**
Locality	**3.741**	**1**	**3.741**	**11.60**	**0.009281**
G x L	0.608	1	0.608	1.88	0.207156
Error	2.580	8	0.322		
Number of grains per spike					
Intercept	**19577.84**	**1**	**19577.84**	**9457.894**	**0.000000**
Genotype	**57.64**	**1**	**57.64**	**27.846**	**0.000749**
Locality	**43.70**	**1**	**43.70**	**21.112**	**0.001768**
G x L	**51.67**	**1**	**51.67**	**24.960**	**0001058**
Error	16.56	8	2.07		
Mass of grains per spike					
Intercept	**26.16653**	**1**	**26.16653**	**1718.656**	**0.000000**
Genotype	**0.09720**	**1**	**0.09720**	**6.384****	**0.035437**
Locality	0.02613	1	0.02613	1.716	0.226512
G x L	0.05333	1	0.05333	3.503	0.098160
Error	0.12180	8	0.01523		

Statistically significant differences were found between the length of spike and the tested factors (genotype and locality), F_{exp} = 171.21** and F_{exp} = 6.02**), and significant between the number of spikelets in the spike and the tested factors (genotype and locality), F_{exp} = 17.80** and F_{exp} = 11.60*, Table 3.

Statistically very significant differences were found between the number of grains by spike and the tested factors (genotype, site and interaction G x L), F_{exp} = 27.846***, F_{exp} = 21.112*** and F_{exp} = 24.960*), Table 3.

The results show that, on average, for examined factors, the length of the spike was 8.01cm (Tables 3 and 4). On average, for locality, smaller spikes (7.39 cm) had plants of genotype G1, compared to G2 (8.62 cm).

On average, for varieties, the locality in Leskovac measured a greater length of spike by 0.23 and 2.92% compared to the locality in Pancevo.

Table 4. Influence of genotype and locality on parameters of yield of wheat (cm)

Genotype	**Locality**		**Average**	**IV**	**Std. Dev.**	**Std. Err.**
	Leskovac	**Pančevo**				
Length of spike, LS						
G1	7.46	7.32	7.39	0.14	0.21	0.09
G2	8.78	8.46	8.62	0.32	0.19	0.07
Average	8.12	7.89	8.01	0.23	0.67	0.19
Number of spikelets per spike, NoSS						
G1	15.87	17.43	16.65	1.53	0.98	0.40
G2	17.70	18.37	18.30	0.67	0.65	0.26
Average	16.78	17.90	17.34	1.12	1.07	0.30
Number of grain per spike, NoGS						
G1	38.03	38.37	38.20	0.34	0.70	0.28
G2	46.57	38.60	42.58	7.97	4.67	1.91
Average	42.30	38.48	40.39	3.82	3.92	1.13
Mass of grain per spike, MGS						
G1	1.37	1.41	1.39	0.04	0.06	0.02
G2	1.68	1.45	1.57	1.23	0.19	0.08
Average	1.52	1.43	1.47	0.09	0.16	0.05

Parameter	LS			NoSS			NoGS			MGS		
LSD	G	Y	GxY	G	Y	GxY	G	Y	GxY	G	Y	GxY
0.5	0.22	0.22	0.31	0.76	0.75	1.07	1.92	1.91	2.71	0.17	0.16	0.23
0.1	0.32	0.31	0.44	1.10	0.09	1.56	2.79	2.78	3.94	0.24	0.23	0.34

The average number of spikelets in the spike, for the tested factors, was 17.34 (tables 3-4). The genotype G1 had a lower number of spikelets in the spike (16.65) compared to the genotype G2 (18.30). On the average for the genotypes examined, a greater number of spikelet were found for 1.12 in relation to the

locality in Leskovac at the site in Pančevo. The interaction of the genotype x year had a statistically significant effect on the number of spikelets in the spike.

Locality, genotype and interaction of genotype x locality had a statistically significant effect on the number of grains in the spike. The number of grains in the spike was on average for the tested factors, amounting to 40.39 (tables 3 and 6). On average for genotypes, a smaller number of grains in the spike (38.20) had the genotype G1 compared to the genotype G2 (42.58). The average values of the tested parameter for genotypes were higher at the locality in Leskovac by 3.82 compared to the locality in Pančevo.

The genotype and interaction of the genotype x locality had a statistically significant effect on the grain mass in the spike, while the locality had no statistically significant influence on the tested parameter. The weight of the grains per spike, on average, for the tested factors, was 1.59 g (Table 2-4). On average, for locality, the genotype G1 had a lower weight of grain per spike (1.39 g) compared to the genotype G1 (1.57 g). The average values for genotypes were higher at the locality in Leskovac (1.52 g) by 0.09 g compared to the locality in Pančevo (1.43 g).

The strong correlation was observed between the tested parameters, Table 5. The yield of grains per spike was in very strong correlation with the number of grains per spike (r = 0.73**), in a strong positive correlation with the spike length (r = 0.59*) and in positive relationship with the number of spikelets in the spike (r = 0.37).

Table 5. Correlations between tested parameters

Parameters	Length of spike	Number of spikelets at spike	Number of grains per spike	Mass of grains per spike
Length of spike	1.00	0.63*	0.71**	0.59*
Number of spikelets at spike	0.63*	1.00	0.30^{ns}	0.37^{ns}
Number of grains per spike	0.71**	0.30^{ns}	1.00	0.73**
Mass of grains per spike	0.59*	0.37^{ns}	0.73**	1.00

The length of the spike was in a very strong positive correlation with the number of grains per spike (r = 0.71 **) and in a strong positive relationship with the number of spikelets in the spike (r = 0.63 *), Table 5.

The results of the research are in unison with the previous research (Popović, 2010; Ikanović et al., 2014; Janković et al., 2016; Terzić et al., 2016; 2017; Djekić et al., 2017; Đekić et al., 2017; 2018; Živanović et al., 2017a; 2017b; Ugrenović et al., 2015; 2018; Stevanović et al., 2018; Maksimović et al., 2018).

The use of biomass for bioenergy creates new business opportunities in agriculture sector. Bioenergy production can significantly contribute to the development of rural areas and encourage creating new supply chains for biomass feedstock.

The creation of new non-food markets for biomass could provide alternative income sources for farmers (EC 2012) Agricultural residues, may act as important source of renewable energy. However, despite progress in these segments of crop production, the greatest potential for the use of biomass as fuel is seen in field (Čurović et al. 2016; Ikanović et al., 2019; Popović et al., 2018; 2019).

CONCLUSIONS

Based on our test results on the influence of the variety of different lengths of the vegetation period on the components of the winter wheat yield, which were carried out in different agro-ecological conditions, the following conclusions can be drawn:

- Meteorological conditions during the winter wheat vegetation period, especially the amount of precipitation, differed considerably in the cultivation sites, which also affected significant differences in the values of the tested parameters.

- By theanalysis of variance statistically significant differences between genotypes / cultivars and cultivation sites in the parametersincluded in the surveywere revealed. However, when it comes to certain features, this influence was of different character and intensity of action.

- Within the wheat yield components, the factor of the genotype / variety had higher influence on length of the spike. The genotype G2 had a longer spike (8.62 cm), the number of spikelets in the spike (18.30), the number of grains in the spike (42.58), and the grain weight per spike (1.57).

- The cultivation site had a statistically significant influence on the length of spike, the number of spikelets in the spike and the number of grains in the spike. The Leskovac locality recorded higher values for the spike length (8.12 cm), the number of grains in the spike (42.30) and the weight of the grains per spike (1.52), while at the locality of Pančevo there was a higher value for the parameter - number of spikelets in the spike (17.90).

ACKNOWLEDGMENTS

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, and was created as a result of the projects: TR 31078 and TR 31025 and bilateral projects (Montenegro and Serbia; 2019-2020): “Alternative cereals and oil crops as a source of healthcare food and an important raw material for the production of biofuel“ and FAO project: “Redesigning the exploitation of small grains genetic resources towards increased sustainability of grain-value chain and improved farmers’ livelihoods in Serbia and Bulgaria – GRAINEFIT; 2019-2021“.

REFERENCES

Abhinandan, K., Skori, L., Stanic, M., Hickerson N.M.N., Jamshed, M., Samuel M., (2018): Abiotic Stress Signaling in Wheat - An Inclusive Overview of Hormonal Interactions During Abiotic Stress Responses in Wheat. Front Plant Sci. 9: 734., doi: 10.3389 / fpls.2018.00734

Božović, D., Živanović, T., Popović, V., Tatić, M., Gospavić, Z., Miloradović, Z., Stanković, G., Đokić, M. (2018): Assessment stability of maize lines yield by GGE-biplot analysis. Genetika. 50, 3: 755-770. https://doi.org/10.2298/GENSR1803755B

Carraro C., Edenhofer O., Flachsland C., Kolstad C., Stavins R., Stowe R. (2015).Climate Change. The IPCC at a crossroads: opportunities for reform. *Science*, 350, 6256, 34-35 DOI: 10.1126/science.aac4419.

Curovic, M., Pavicevic, K., Djokic, M., Drobnjak, D. (2016): Analysis of the energy potential of agricultural biomass residues in Montenegro. Agriculture and Forestry, 62 (3): 277-284.

Djekić, V., Popović, V., Branković, S., Terzić, D., Đurić, N. (2017): Grain yield and yield components of winter barley, Agriculture and Forestry, 63 (1): 179-185. DOI: 10.17707 / AgricultForest.63.1.21

Đekić, V., Popović, V., Jelić, M., Terzić, D., Branković, S. (2017): Effect of different doses of nitrogen fertilization on yield of winter wheat. Proceedings of the Institute PKB Agroekonomik. 23, 1-2, 105-112.

Đekić V., Milivojević J., Popović V., Jovović Z., Branković S., Terzić D., Ugrenović V. (2018a). Effects of fertilization on production traits of winter wheat. Green Room Sessions, International Eco Eco Conference, Podgorica, University of Montenegro, November 1-4, 2018, 25-31.

Đekić, V., Milivojević, J., Popović, V., Jovović, Z., Branković, S., Terzić, D., Ugrenović, V. (2018b): Effects of fertilization on production traits of winter wheat. Green Room Sessions, International Eco Eco Conference, Podgorica, 25-31.

Glamočlija, Đ., Janković, S., Popović, M. V., Kuzevski, J., Filipović, V., Ugrenović, V. (2015): Alternatively crop plants in conventional and organic growing systems. Monograph. Belgrade, ISBN 978-86-81689-32-5; 1-355.

Han Y. Y., Li A. X., Li F., Zhao M. R., Wang W. (2012). Characterization of a wheat (*Triticum aestivum* L.) expansin gene, TaEXPB23, involved in the abiotic stress response and phytohormone regulation. *Plant Physiol. Biochem.* 54 49–58. 10.1016/j.plaphy.2012.02.007

FAO (2015). The State of Food Insecurity in the World: Meeting the 2015 International Hunger Targets: Taking Stock of Uneven Progress. *Quebec, QC: FAO.* Adv Nutr. 2015 Sep; 6(5): 623–624. doi: 10.3945/an.115.009936

Ikanović J., Popović V., Jankovic S., ŽivanovićLj., Rakić S., Dončić D. (2014): Khorasan wheat population researching (*Triticum turgidum*, sp. Turanicum (McKEY) in the minimum tillage conditions. Genetika, Belgrade, 46 (1), 105-115.

Ikanović, J., Popović, V., Janković, S., Živanović, Lj., Kolarić Lj., Lončar, M., Kulić,G., Dražić, N. (2019): Secondary products cereals as energy products. Journal of Institute PKB Agroeconomic, Belgrade, 24, 1-2, 20-27.

Jankovic S., Popovic V., Ikanovic J., Rakic S., Kuzevski J., Gavrilovic M. (2016): Productivity traits of rye (*Secale cereale*), Khorasan wheat (*Triticum turgidum*, ssp. Taranicum MCKEY) and quinoa (*Chenopodium quinoa* Willd) grown on degraded soil. Romanian Agricultural Research, 33, www.incda-fundulea.ro,DII 2067-5720 RAR 2016-148.

Lakić Ž., Glamočlija Đ., Kondić, D., Popović M. V., Pavlović S. (2015): Krmne biljke i žita u funkciji zaštite zemljišta od degradacije. Monografija. Republika Srpska. 1-405

Maksimović L., Popović V., Stevanović P. (2018). Water and irrigation requirements of field crops grown in central Vojvodina, Serbia. Agriculture and Forestry, Podgorica, 64, 1, 133-144. DOI: 10.17707 / AgricultForest.64.1.16

Popović V. (2010): Agro-technical and agro-ecological influence on the production of wheat, maize and soybean seeds. Doctoral dissertation, University of Belgrade, Faculty of Agriculture, Zemun, 1-145.

Popović, V., Mikić, S., Jovović, Z., Čurović, M., Ignjatov, M., Rajičić, V., Ikanović, J., Maksimović, L. (2018): Effects of foliar nutrition on production biomass of broomcorn millet (*Panicum miliaceum* L.). Green Room Sessions 2018 International GEA (Geo Eco-Eco Agro) Conference, Podgorica, 60-67.

Popović, V., Mikić, S., Vučković, S., Janković, S., Živanović, Lj., Kolarić, Lj., Rajičić, V., Ikanović, J. (2019): Millet - *Panicum miliaceum* L. as health-safe food and raw material for the production of biofuels. Journal of Institute PKB Agroeconomic, Belgrade, 24, 1-2, 30-37.

Stevanović P., Popović V., Jovović Z., Ugrenović V., Rajičić V., Popović S., Filipović V. (2018): Quality of wheat seed in relation to fragment and feeding locality. Proceedings of the Institute PKB Agroekonomik, 24, 1-2, 65-74.

Terzić D., Radović J., Marković J., Popović V., Milenković J., Vasić T., Filipović V. (2016): New technical-technological process of production voluminous animal feed by combining maize and soybean grown in double cropping. A new technical solution applied at the national level. Belgrade. Serbia. 1-40.

Terzić D., Popović V., Radović J., Marković J., Milenković J., Vasić T., Filipović V. (2017): The influence of combining crops and the method of sowing on the energy and protein value maize and soybean in the second sowing. Journal of Institute PKB Agroeconomic, Belgrade, 23, 1-2, 20-27.

Ugrenović V., Filipović V., Popović V., Glamočlija Đ. (2015). Hull index: Indicator of hulled wheat productivity and quality. Selekcija i semenarstvo, 21, 2, 31-37.

Ugrenović V., Bodroža Solarov M., Pezo L., Đisalov J., Popović V., Marić B., Filipović V. (2018): Analysis of spelt variability (*Triticum spelta* L.) grown in different conditions of Serbia by organic conditions. Genetika, Belgrade. 50, 2, 635-646.

Živanović Lj, Savić J., Ikanović J., Kolarić Lj., Popović V., Novaković M. (2017a). The influence of genotype on wheat, soybean, corn and sunflower grain yield. Proceedings of the Institute PKB Agroekonomik. Belgrade. 23, 1-2, 39-49.

Živanović Lj., Popović V., Ikanović J., Kolarić Lj. (2017b). The influence of the quantity and form of nitrogen on productivity of winter wheat. XXII Simposium on Biotechnology with International participation. Čačak, 183-188.

DOI: 10.17707/AgricultForest.65.1.24

Biljana PETANOVSKA-ILIEVSKA, Mirjana S. JANKULOVSKA*, Lenche VELKOSKA-MARKOVSKA, Jasmina SPASOVSKA [1]

DETERMINATION OF BISPHENOL A IN BEVERAGES BY RP-HPLC

SUMMARY

Bisphenol A (BPA) is a monomer widely used in the production of polycarbonate, *epoxy resins, diacrylates* and phenolic resins. A small quantity of BPA can migrate into the food and thus it can be potential hazard for human health and environment. Therefore, quantitative determination of BPA is of a great importance. A fast, simple, precise and economic RP-HPLC method with UV-DAD detection for quantitative determination of BPA in beverages was developed. Three different analytical columns were tested: Hypersil ODS (250 mm x 4.6 mm; 5 μm), LiChrospher 60 RP-Select B (125 mm x 4 mm; 5 μm) and Purospher® STAR RP-18 endcapped (30 mm x 4 mm; 3 μm). Analyzed beverages were packed in plastic bottles and small glass bottles closed with a cops coated with epoxy resin on inside. For quantitative determination of BPA following experimental conditions were established: mobile phase consisted of acetonitrile/water 50/50 (*v*/*v*), flow rate of 1 mL/min, column temperature of 25 ^{o}C, injection volume of 5 μL and UV detection at 200 nm. The method was developed in an isocratic manner and with a reversed phase column. Prior the analyses the samples were filtrated through syringe filters Spartan – T with pore size 0.45 μm. The following parameters were determined: retention time, linearity, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy, selectivity and sensitivity. The RP-HPLC method with UV-DAD detection can be successfully used for quantitative determination of BPA in non-alcoholic beverages without pre-treatment. The BPA was not detected in the analyzed beverages.

Keywords: Bisphenol A, RP-HPLC, UV-DAD detection, non-alcoholic beverages.

INTRODUCTION

Food packaging allows a lot of benefits mainly related to food quality and safety such as: extension of shelf life, protection of deterioration caused by microorganisms, light, oxygen, pests that can cause disease and so on. On the other hand, packaging can be source of chemical contaminants into food and beverages which have negative influence of food safety (Barens *et al*., 2007).

[1]Biljana Petanovska - Ilievska, Mirjana S. Jankulovska *(corresponding author: jankulovska_m@yahoo.com), Lenche Velkoska-Markovska, Jasmina Spasovska, Ss. Cyril and Methodius University, Faculty of Agricultural Sciences and Food, Skopje, NORTH MACEDONIA
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

Chemical migration is the transition of certain chemical compounds from food packaging materials into food under certain conditions such as: the composition of the packaging material, the nature and degree of the contact, the food nature, the contact temperature, and duration of the contact. Among other plastic materials, polycarbonates (PC) are widely used in the manufacture of containers for packaging of milk, water, bottles closed with cops and so on, while epoxy resins are used as an interior protective layer for food and beverage cans. Bisphenol A (BPA) is a chemical monomer used as a main component in the production of polycarbonate and epoxy-phenolic resins and because of that it is a potential migrant from packaging into food (Almeida *et al*., 2018). The migration of BPA traces from both polycarbonate-based packaging and epoxy resins into food depends mainly on liquid composition and pH (Brede *et al*., 2010; Nerin *et al*., 2016). Hence, BPA is consumed each day with food *i.e.* humans are exposed to BPA. Literature data showed that BPA possess estrogenic activity *i.e*. disturbs the function of endocrine system which causes cancer, heart diseases, diabetes and hypomethylation of genes promoters (Almeida *et al*., 2018).

Migration of chemical compounds European Union is regulated with EU Directives. European Commission with Regulation (EU) 2018/213 strengthens the restriction of Bisphenol A (BPA) in certain food contact materials and articles (Commission Regulation (EU) No 10/2011, plastic materials and articles intended to come into contact with food, 2011). This regulation included a new restriction of BPA in food contact varnished and coated materials and articles. In addition, Annex I of Regulation (EU) No 10/2011 is amended by lowering migration limit of BPA in plastic food contact materials and articles. It also included a new prohibition of BPA in polycarbonate drinking cups or bottles intended for infant and young children. According to this annex specific migration limit for BPA in plastic food contact materials and articles is lowered from 0.6 mg/kg to 0.05 mg/kg (Commission Regulation (EU) 2018/213, the use of bisphenol A in varnishes and coatings intended to come into contact with food, 2018).

In the literature there are known a lot of methods for determination of BPA in food, beverages and samples of biological materials (Ballesteros-Gómez *et al*., 2009). Due to the complexity of the matrices and the low concentrations of BPA there is constant need for development and improvement of analytical techniques for precise and accurate determination of BPA. One of the most used methods is chromatographic method, *i.e*. liquid and gas chromatography [Xu-Liang, 2012; Aristiawan *et al*., 2015). Using the chromatographic methods BPA can be determined in food for infants and different food samples such as fruits, vegetables, fishes, vines, beverages, as well as drinking water (Tanigawa *et al*., 2011; Yoshida *et al*., 2010; Cao *et al*., 2010; Taskeen and Naeem, 2010; Braunrath *et al*., 2005). Each method for determination of BPA in food and beverages includes sample clean-up by liquid-liquid extraction or solid-phase extraction followed by chromatographic determination with different detectors (Struckhofova and Marki, 2006; Sadeghi *et al*., 2016; Rykowska *et al*., 2004). To

assess potential human health risks caused by BPA exposure, it is therefore essential to start from accurate data on BPA levels in foodstuffs, at very low concentrations (Ballesteros *et al*., 2009).

Therefore, the aim of our investigation was developed and validation of reverse phase - high performance liquid chromatography (RP-HPLC) method for quantitative determination of BPA in beverages packed in plastic bottles and glass bottles closed with metallic caps coated from the inside with epoxy resin. The investigation was made in order to confirm if there is a migration from the packaging material into beverages, and if the migration quantities exceed the quantities prescribed by the EU Directive (Commission Regulation (EU) No 10/2011, plastic materials and articles intended to come into contact with food, 2011; Commission Regulation (EU) 2018/213, the use of bisphenol A in varnishes and coatings intended to come into contact with food, 2018).

MATERIAL AND METHODS

Reagents and instrumentation

The analytical standard of bisphenol A (BPA) with purity 99 % was produced by Sigma Aldrich (Germany). The acetonitrile for mobile phase was produced by Sigma Aldrich (Germany) was with HPLC grade. The ultra-pure water purified by Water purification System TKA Smart 2 Pure 12 UV/UF was used. Liquid chromatography analysis were performed on an Agilent 1260 Infinity Rapid Resolution Liquid Chromatography (RRLC) system equipped with: vacuum degasser (G1322A), binary pump (G1312B), auto sampler (G1329B), a thermostatted column compartment (G1316A), UV/Vis diode array detector (G1316B) and ChemStation software. Following analytical columns were tested: Hypersil ODS (250 mm x 4.6 mm; 5 μm) – Sigma Aldrich(Germany), LiChrospher 60 RP-Select B (125 mm x 4 mm; 5 μm), Merck (Germany) and Purospher® STAR RP-18 endcapped (30 mm x 4 mm; 3 μm), Merck (Germany). The mass was measured using analytical balance with 0.1 mg accuracy by Mettler (Zürich, Switzerland). Ultrasonic bath "Elma" (D-7700 SINGEN/Htw., Germany) was applied for a better dissolution of a stock solution.

Standard and working solutions of BPA

A mass of 0.037 g of the analytical standard of BPA was measured and transferred into a volumetric flask of 10 mL which was filled up to the mark with acetonitrile. The solution was degassed by ultrasonic bath for 15 minutes and then stored at 4 ^{o}C. In order to prepare working solution 1 mL of a standard solution was transferred into volumetric flask of 10 mL and filled up with acetonitrile/water 50/50 (*v*/*v*). Linearity of the method was tested with five working solutions prepared in beverage (clear apple juice) with addition of known amount of BPA as follows: 300 ng/mL (solution 1), 420 ng/mL, (solution 2), 600 ng/mL (solution 3), 720 ng/mL (solution 4), and 900 ng/mL (solution 5). Each of these solutions was injected into HPLC system in triplicate with volume of 5 μL. The method of standard addition was used in order to evaluate the

accuracy of the method for quantitative determination of BPA. For that purpose, a known amount of BPA: 600, 720 and 900 ng/mL was added in clear apple juice. An apple juice sample in which BPA was not added was used as a blank. A 5 μL of all samples were injected into the HPLC system in triplicate.

Sample preparation

Six clear apple juices produced by different manufactures анд frequently consumed in Macedonia were analyzed. Three of them mainly consumed by infants were packed in glass bottles closed with metallic caps coated from the inside with epoxy resin. The apple juice beverages consumed by adults were packed in glass bottle (one sample beverage) and in plastic polycarbonate packaging material (two sample beverages). The samples for analyses were marked as A, B, C, D, E and F. Prior to HPLC analyses all samples were filtrated through syringe filters Spartan – T with pore size 0.45 μm. A volume of 5 μL of each sample was injected into the HPLC system in triplicate.

RESULTS AND DISCUSSION

UV spectrum of BPA

Bisphenol A (BPA) is frequently used name for chemical compound with IUPAC name 4,4'-(propane-2,2-diyl)diphenol. It is an organic compound which belongs to the phenolic group of compounds. At aqueous solution at pH below 7, BPA exist in undissociated form. A molecule of BPA is stable in solid phase, but in the air, water and soil BPA undergoes aerobic degradation. UV spectrum of BPA recorded in mobile phase of acetonitrile/water 50/50 (*v*/*v*) is presented in the Fig. 1.

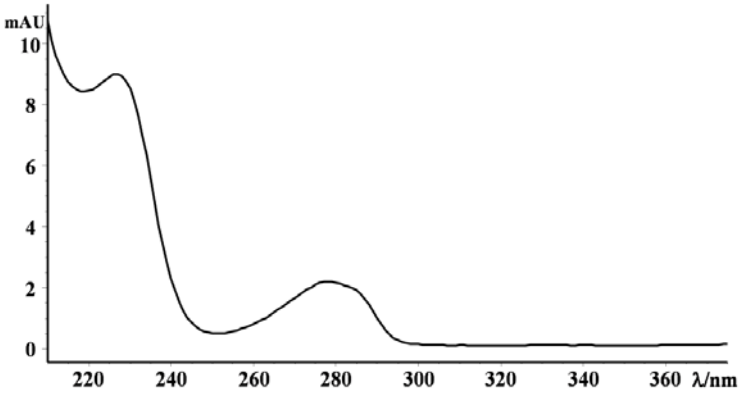


Figure 1: UV spectrum of BPA, acetonitrile/water 50/50 (*v*/*v*)

In the obtained UV spectrum of BPA were noticed two absorption bands with different intensity. First one is more intensive and it is placed at 227 nm, while the absorption maximum of the second less intensive band is located at 275 nm. However, at a wavelength of 200 nm the intensity is even greater and for that reason the chromatographic process of quantitative determination of BPA was

followed at 200 nm. The UV spectrum of a pure analytical standard of BPA was used for identification of BPA in the samples.

Matrix effect

Chromatographic analyses were performed in order to investigate matrix effect which can influence the accuracy of the applied method for quantitative determination of BPA (Niessen *et al*., 2006; Matuszewski, 2006; Silvestro *et al.*, 2013). Hence, it is very important to evaluate the influence of the co-eluting peaks onto the chromatographic response of BPA. For that purpose chromatographic analyses were performed on a standard solution of BPA and beverage in which 600 ng/mL of BPA was added. The calculation of the matrix factor (MF) can be performed as ratio between the response of the analyte present in matrix and response of pure analytical standard. If MF = 1 the matrix has no influence on the response of the investigated compound, if MF < 1 the response of the investigated compound is decreasing, if MF > 1 the response of the investigated compound is increasing.

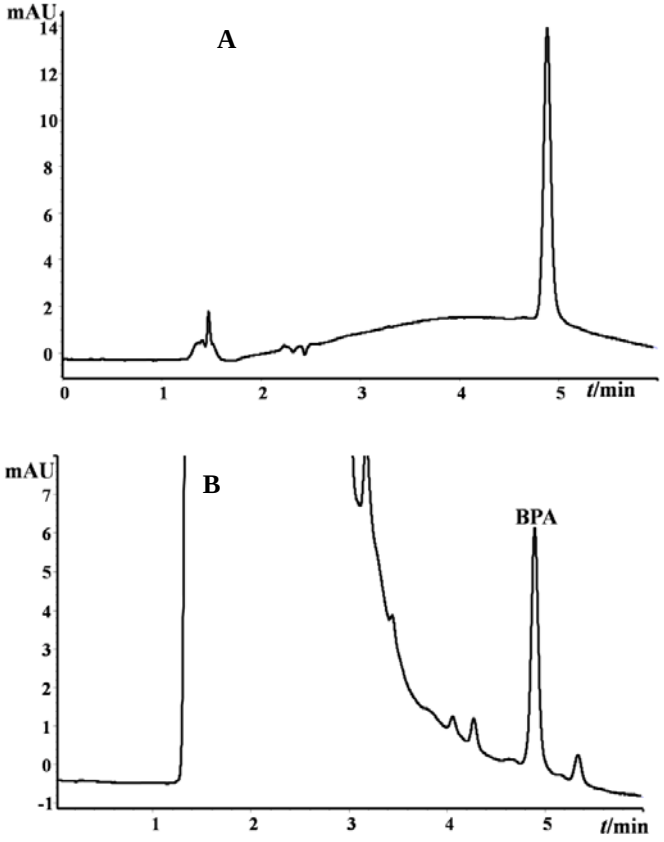


Figure 2: Chromatogram of BPA, Hypersil ODS (250 mm x 4.6 mm, 5 µm) column, mobile phase acetonitrile/water 55/45 (*v*/*v*), flow rate 1 mL/min, UV detection at 200 nm, temperature 25 ^{o}C analytical standard of BPA (A) and beverage (600 ng/mL BPA)) (B)

The matrix effect was tested with three different analytical columns: Hypersil ODS (250 mm x 4.6 mm; 5 μm), LiChrospher 60 RP-Select B (125 mm x 4 mm; 5 μm) and Purospher® STAR RP-18 endcapped (30 mm x 4 mm; 3 μm). The obtained chromatograms recorded at 200 nm with the mobile phase acetonitrile/water (55/45, *v*/*v*) are presented in the Figs. 2-4.

From Fig. 2 it can be seen that the retention time of BPA was 4.88 min. The MF factor value for peak height was 0.438, while for peak area was 0.486, which suggested that the response of BPA obtained on a Hypersil ODS (250 mm x 4.6 mm; 5 μm) column with a mobile phase consisted of acetonitrile/water (55/45, *v*/*v*) was decreasing as a result of the influence of the matrix (Silvestro *et al*., 2013; Meyer, 2010).

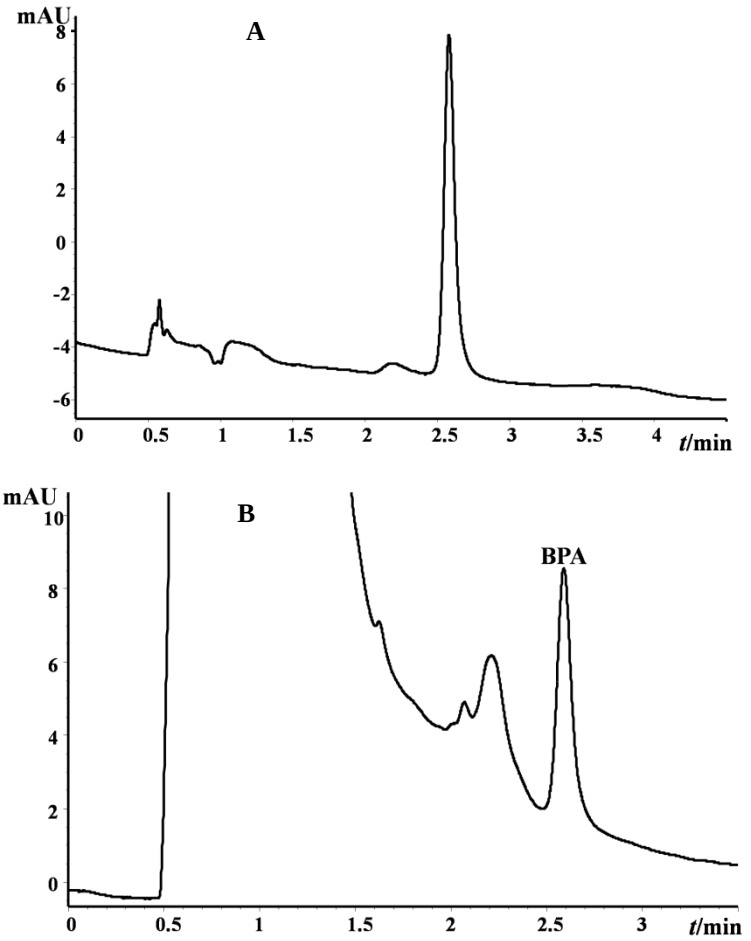


Figure 3: Chromatogram of BPA, LiChrospher 60 RP-Select B (125 mm x 4 mm, 5 μm) column, mobile phase acetonitrile/water 50/50 (*v*/*v*), flow rate 1 mL/min, UV detection at 200 nm, temperature 25 ^{o}C, analytical standard of BPA (A), beverage (600 ng/mL BPA) (B)

When LiChrospher 60 RP-Select B (125 mm x 4 mm; 5 μm) column with the mobile phase of acetonitrile/water (55/45, *v*/*v*) was used the retention time of BPA was 2.59 min, while the MF factor was 1.674 (peak height) and 1.645 (peak area). The obtained result suggested that in this case the response of BPA was increasing as a result of the influence of the co-eluting peaks (Silvestro *et al*., 2013; Meyer, 2010).

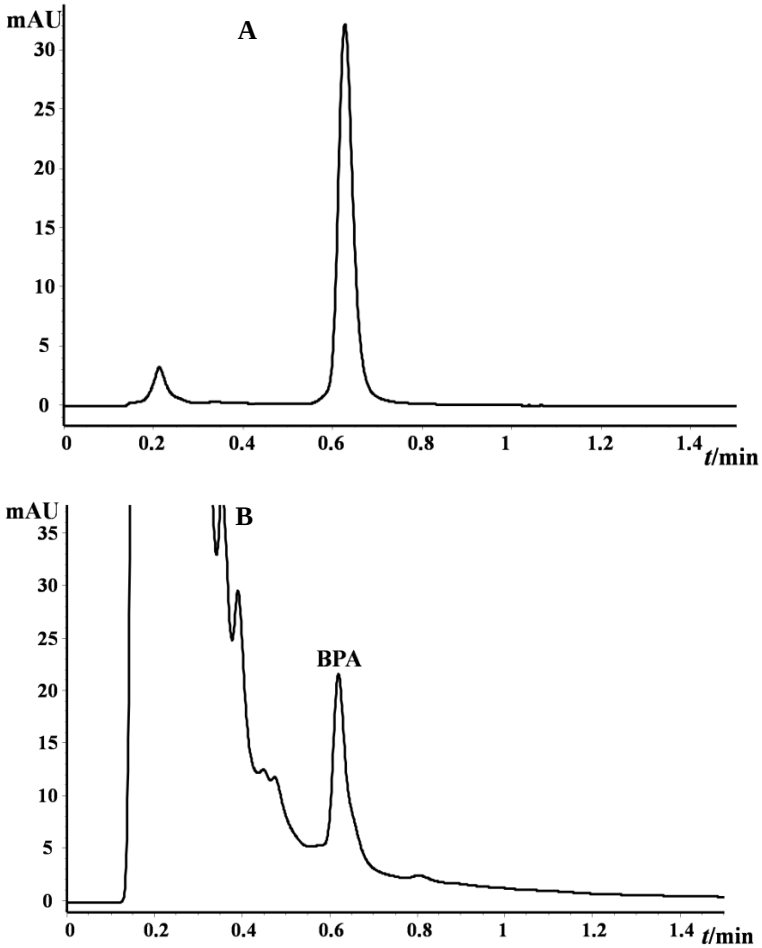


Figure 4: Chromatogram of BPA, Purospher® STAR RP-18 endcapped (30 mm x 4 mm, 3 μm) column, mobile phase acetonitrile/water 50/50 (*v*/*v*), flow rate 1 mL/min, UV detection at 200 nm, temperature 25 °C, analytical standard of BPA (A), beverage (600 ng/mL BPA) (B)

The retention time of BPA was 0.63 min, while the MF factor value was 0.832 (peak height) and 0.936 (peak area) when Purospher® STAR RP-18 endcapped (30 mm x 4 mm; 3 μm) column and acetonitrile/water (55/45, *v*/*v*) as a mobile phase was used. This suggested that in this case the influence of the co-

eluting peaks on the response of BPA was insignificant (Matuszewski, 2006; Silvestro *et al*., 2013). Taking into consideration the obtained results with tested columns the best separation was achieved with Purospher® STAR RP-18 endcapped (30 mm x 4 mm; 3 μm) with retention time of 0.63 min, dead time of 0.215 and the retention factor (*k*') of 1.916.

Method validation

In order to validate the developed method the selectivity, linearity, precision, sensitivity and accuracy were tested. Selectivity of the method for determination of BPA was tested through comparison of the UV spectrum of pure analytical standard and the UV spectrum of BPA added in the analyzed beverage (Fig. 5). The obtained value for match factor was higher than 990 confirmed that the UV spectra originated from the same compound.

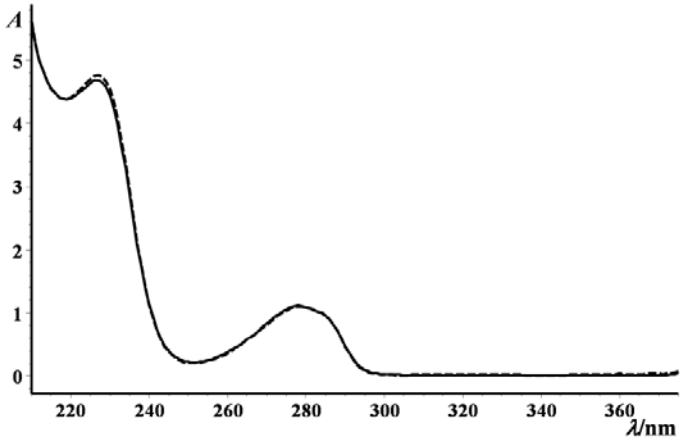


Figure 5: Overlapped UV spectra of analytical standard of BPA and beverage sample with addition of 600 ng/mL of BPA recorded at 200 nm

The linearity of the method was determined by construction of calibration curve which represented the dependence of the mass of BPA and the obtained response as peak height or peak area (Meyer, 2010). The linear dependence between the peak area and peak height on concentation of BPA was tested in the concentration range from 300 ng/mL to 900 ng/mL. The regression equations of BPA are given in Table 1. The coefficient of determination (R^2) value was 0.99 when peak area was used as dependent variable while when peak height was used as dependent variable its value was 0.98. According to literature data these values are satisfactory (Meyer, 2010).

Precision of the developed method was tested through intraday repeteability of the peak area and peak height of eight succesive injections (5 μL) of beverage in which a concentration of 600 ng/mL of BPA was added. The standard deviation (SD) and relative standard deviastion (RSD %) data were calculated using the obtained data for retention time, peak area and peak height (Table 2).

Table 1. Regression equations and R^2 values, 200 nm

Dependent variable	Regression equation	R^2
Peak area [mAU]	y = 15.545x – 3.7293	0.99
Peak height [mAU]	y = 7.2727x – 2.9171	0.98

Table 2. Chromatographic data (peak area, peak height and retention time), SD and RSD [%] of BPA (600 ng/mL), 200 nm

Parameter	1	2	3	4	5	6	7	8	$\bar{x}$	SD	RSD [%]
Retention time [min]	0.629	0.627	0.626	0.626	0.627	0.627	0.628	0.626	0.627	0.001	0.170
Peak area [mAU]	37.97	39.80	41.48	41.35	40.86	41.55	40.85	41.35	40.65	1.223	3.012
Peak height [mAU]	17.24	17.53	17.74	17.74	17.79	17.79	17.73	17.73	17.66	0.191	1.080

*mean value of three subsequent injection

According to manual for verification of the methods of AOAC (manual for the Peer Verified Methods) for compounds with mass of 0.01 pg/kg acceptable value of RSD is up to 30 % (AOAC Peer-verified methods program manual on policies and procedures, AOAC International, 1998). Hence, the obtained SD and RSD values for BPA were in the acceptable range.

Limit of detection (LOD) and limit of quantification (LOQ) were calculated in order to determine the sensitivity of the RP-HPLC method. Their determination is significant for the samples containing very low concentration of compound of interest. As it is well known LOD can be determined as the lowest amount of analyte that can be detected above baseline noise (signal to noise ratio 3:1), while LOQ can be determined as the lowest amount of analyte which can be quantified above the baseline noise (signal to noise ratio 10:1). In order to determine the LOD, 100 μL from the stock solution of BPA were transferred into volumetric flask of 10 mL which than was filled up to the mark with acetonitrile/water in volume ratio 50:50 (*v*/*v*). From this solution a series of dilution were made and injected into chromatographic system. The smallest mass of BPA when it retained its spectral characteristics was 600 pg/mL. The chromatographic peak obtained at this manner was more than three times higher compared to the noise of the base line. With further dilution BPA lost its spectral characteristics. Thus, the determined value of LOQ was 2 μg/mL.

In order to test the accuracy of the method for quantitative determination of BPA the method of standard addition was applied. The following concentrations: 600, 720 and 900 ng/mL of BPA was spiked into the beverage. Then 5 μL of each sample was injected into the chromatographic system in triplicate. Using the peak area data the analytical recovery was calculated (Table 3).

Table 3. Recovery data of the developed method, $n = 3$

Mass of BPA in the sample [ng]	Mass of BPA added in the sample [ng]	Total mass of BPA [ng]	Recovery [%]	RSD [%]
0	3.0	2.797	93.24	0.105
0	3.6	3.470	96.40	0.105
0	4.5	4.643	103.18	0.128

As it can be seen from the Table 3 the obtained recovery values ranged from 93.24 to 103.28 %. According to the analytical method criteria for the determination of bisphenol A in various matrixes the acceptable recovery is from 80 to 120 % with tolerance of 20 % (Barbalas and Garland, 1991).

Determination of BPA in samples

The developed method was used for determination of BPA in different samples of beverages frequently consumed and randomly selected from the Macedonian market. The samples were marked as A, B, C, D, E and F. Chromatograms of samples A and B are presented in Fig. 6.

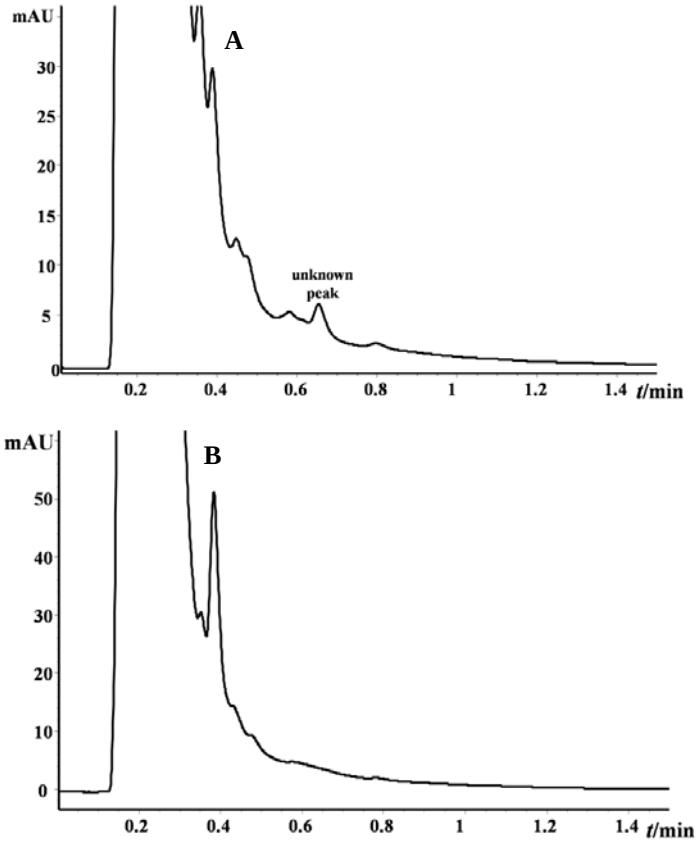


Figure 6: Chromatograms of beverages sample A (a) and sample B (b), Purospher® STAR RP-18 endcapped, 5 μL injected volume, mobile phase acetonitrile/water 50/50 (*v*/*v*), flow rate 1 mL/min, UV detection at 200 nm, temperature of 25 ^{o}C

In the chromatograms of the samples B, C, D and E there was not noticed peak with the retention time near the retention time of BPA, while in chromatograms of samples A and F a peak with retention time of 0.565 was noticed. In order to confirm if this chromatographic peak originates from BPA its UV spectrum was compared with the UV spectrum of pure analytical standard of BPA. From the compared spectra it is obvious that this peak do not have the same spectral characteristics as BPA *i.e*. the match factor was 899.561 (Fig. 7).

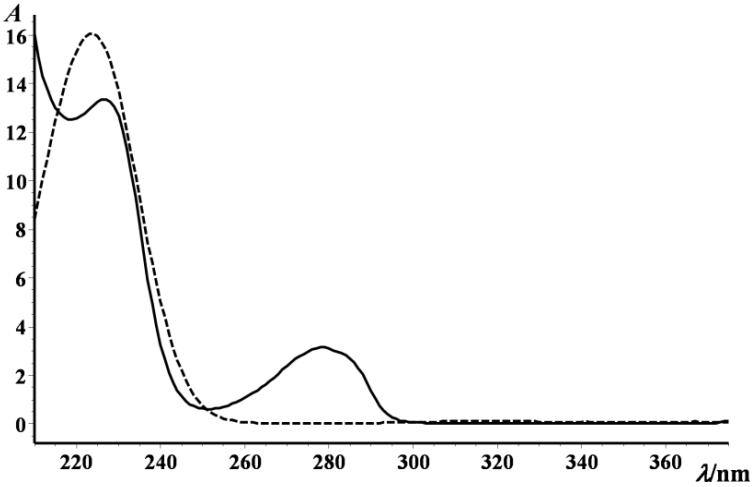


Figure 7: Overlapped UV spectra of pure analytical standard of BPA and unknown compound with retention time of 0.654 min of sample A, recorded at 200 nm

CONCLUSIONS

A method of RP-HPLC with UV-DAD detection was applied for qualitative and quantitative determination of BPA in beverages. The best results were obtained using Purospher® STAR RP-18 endcapped (30 mm x 4 mm; 3 μm) analytical column, mobile phase of acetonitrile/water 50/50 (*v*/*v*), flow rate of 1 mL/min, injection volume of 5 μL, UV detection at 200 nm and temperature of 25°C. Under these experimental conditions the retention time of BPA was 0.627 min, dead time of the column was 0.215 min and the retention factor was 1.916. Validation of the method was tested by following parameters: linearity, sensitivity, selectivity, precision and accuracy. Linearity of the method was tested over the concentration region from 300 to 900 ng/mL. Under used chromatographic conditions BPA was not detected in the analyzed samples of beverages.

REFERENCES

AOAC Peer-verified methods program manual on policies and procedures, AOAC International, 1998.

Almeida S, Raposo, A, Almeida-Gonzalez, M, Carrascosa, C. 2018. Bisphenol A: Food Exposure and Impact on Human Health, Compr. Rev. Food SCI F, 17; 1503-1517.

Aristiawan Y, Aryana N, Ramadhaningtyas D. P, Styarini D. 2015. Analytical Method Development for Bisphenol a in Tuna by Using High Performance Liquid Chromatography-UV, Procedia Chem., 16; 202-208.

Ballesteros-Gómez A, Rubio S, Pérez-Bendito D. 2009. Analytical methods for the determination of bisphenol A in food, J. Chromatogr. A., 1216(3); 449-469.

Barens, K. A.; Sinclar, C. R.; Watson, D. H. 2007. Chemical migration and food contact materials, Woodhead Publishing Limited, Cambrage, England, CRC Press, Boca Raton Boston, New York, Washington DC.

Barbalas M. P, Garland W. A. 1991. A Computer Program for the Deconvolution of Mass Spectral Peak Abundance Data from Experiments Experiments Using Stable Isotopes, J. Pharm. Sci., 80; 922-927.

Ballesteros-Gómez A, Rubio S, Pérez-Bendito D. 2009. Analytical methods for the determination of bisphenol A in food, Journal of Chromatography A, 1216(3); 449-69.

Brede C, Fjeldal P, Skjevrak I, Herikstad H. 2003. Increased migration levels of bisphenol A from polycarbonate baby bottles after dishwashing, boiling and brushing, Food Addit Contam., 20; 684-689.

Braunrath R, Podlipna D, Padlesak S, Cichna-Markl M. 2005. Determination of bisphenol A in canned foods by immunoaffinity chromatography, HPLC, and fluorescence detection, J. Agric. Food Chem., 53; 8911-8917.

Cao X. L, Corriveau J, Popovic S. 2010. Bisphenol a in canned food products from Canadian markets, J. Food Prot., 73(6); 10851089.

Commission Regulation (EU) No 10/2011, plastic materials and articles intended to come into contact with food, 2011.

Commission Regulation (EU) 2018/213, the use of bisphenol A in varnishes and coatings intended to come into contact with food, 2018.

Matuszewski B. K. 2006. Standard line slopes as a measure of a relative matrix effect in quantitative HPLC-MS Bioanalysis, J. Chromatogr. B, 830; 293-300.

Meyer, V., Practical High-Performance Liquid Chromatography, Fifth Edition, John Wiley & Sons, Ltd., 2010.

Niessen W. M. A, Manini P, Andreoli R. 2006. Matrix effects in quantitative pesticide analysis using liquid chromatography-mass spectrometry, Mass Spectrom. Rev., 25; 881-889.

Nerín C, Aznar M, Carrizo D. 2016. Food contamination during food process, Trends Food Sci. Technol., 48; 63-68.

Rykowska I, Szymanski A, Waslak W. 2004. Method Based on Solid Phase Extraction, LC and GC for Analysis of Bisphenol A in Drinking Water, Chem. Pap., 58(6); 382-385.

Silvestro, L.; Tarcomnicu, I.; Rizea Savu, S. Tandem Mass Spectrometry - Molecular Characterization, Open access peer-reviewed chapter, INTECH, pp. 1-38, 2013.

Struckhofova Z. B, Marki M. C. 2006. Determination of bisphenol A in wine by sol-gel immunoaffinity chromatography, HPLC and fluorescence detection, Food Addit. Contam., 23(11); 1227-1235.

Sadeghi M, Nematifar Z, Fattahi N, Pirsaheb M, Shamsipur M. 2016. Determination of Bisphenol A in Food and Environmental Samples Using Combined Solid-Phase Extraction–Dispersive Liquid–Liquid Microextraction with Solidification of Floating Organic Drop Followed by HPLC, Food Anal. Methods, 9; 1814-1824.

Tanigawa, T.; Watabe, Y.; Kubo, T.; Hosoya, K. 2011. Determination of bisphenol a with effective pretreatment medium using automated column-switching HPLC with fluorescence detection J. Sep. Sci., 34, 2840.

Taskeen A, Naeem I. 2010. Analysis of Bisphenol A in Canned Food: A Mini Review, Asian J. Chem., 22(5); 4133-4135.

Xu-Liang, C. J. Liq. Chromatogr. R T, 2012, 35(19), 2795.

Yoshida T, Horie M, Hoshino Y, Nakazawa H, Horie M, Nakazawa H. 2010. Determination of bisphenol A in canned vegetables and fruit by high performance liquid chromatography, Food Addit. Contam., 18(1); 69-75.

INSTRUCTIONS TO AUTHORS

The "Agriculture and Forestry" journal publishes original scientific papers, review papers, short communications on agriculture, veterinary medicine, forestry, biology and other natural sciences. It is the endeavour of the journal to give place to papers of high scientific quality and international interest, authored by all the scientists from the South Eastern European Region, as well as other international scientist in order to stimulate contacts and exchange of knowledge fostering scientific productivity.

Manuscripts, submitted via electronic journal web system should be prepared in Microsoft Word ***(Times New Roman font, 11 pt)*** and submitted in format 17 x 24 cm ***(File / Page setup / Paper / Width = 17 cm; Height = 24 cm)***, with single line spacing ***(Format / Paragraph / Line spacing = Single)***, 2 cm margins all around ***(File / Page setup / Margins / Top = 2 cm; Bottom = 2 cm; Left = 2 cm; Right = 2 cm)***, that is approximately 44 lines per page in this format. All technical details are available in section AUTHORS / Check-list for Authors.

Manuscripts are published in English. Papers that have been published elsewhere, in whole or extracts (excerpts) of their important findings, will not be accepted. A manuscript should not exceed 10 pages. Exceptions can be made if content and quality of the paper justify it (at the discretion of the Editor).

Full research papers should include the following sections:

- Author/s name/s, Title with **DOI number**

The author's name should be placed above the title and with the author's appellation and affiliation in a footnote at the bottom of the front page. Author(s) affiliation should indicate name and address of institution, including the e-mail address of the corresponding author. Title should provide a concise but also an informative synthesis of the study (recommended not more than 100 characters including spaces). Short title (not more than 70 characters) should also be provided in order to be included in the header of the Manuscript. Ensure that the title contains the most important words that relate to the topic.

- Abstract

The summary, in English language, should provide basic data on the problem that was treated and the results obtained. It should be brief, preferably one paragraph only, up to 250 words, but sufficient to inform the reader of the character of the work, its results and its conclusions. Include the keywords and phrases you repeated in your abstract.

- Key words

Keywords should provide 4-6 words or compound words, suitable for an information retrieval system. Choose the appropriate keywords and phrases for your article. Think of a phrase of 2-4 words that a researcher might search on to find your article. Repeat your keywords and phrases 3-4 times throughout the abstract in a natural, contextual way.

Main text of the manuscript includes the following sections:

- INTRODUCTION

The introduction should answer the questions what was studied, why was it an important question, what was known about it before and how the study will advance our knowledge.

- MATERIAL AND METHODS

Material and methods explain how the study was carried: the organism(s) studied; description of the study site, including the significant physical and biological features, and the precise location (latitude and longitude, map, etc); the

experimental or sampling design; the protocol for collecting data; how the data were analyzed. In this section also should be provided a clear description of instruments and equipment, machines, devices, chemicals, diagnostic kits, plants/animals studied, technology of growing/housing, sampling sites, software used etc.

- RESULTS followed by **DISCUSSION**

Results and Discussion may be combined into a single section (if appropriate) or it can be a separate section.

The results objectively present key results, without interpretation, in an orderly and logical sequence using both text and illustrative materials (tables and figures).

The discussion interpret results in light of what was already known about the subject of the investigation, and explain new understanding of the problem after taking results into consideration.

The International System of Units (SI) should be used.

- CONCLUSIONS

The conclusion should present a clear and concise review of experiments and results obtained, with possible reference to the enclosures.

- ACKNOWLEDGMENTS

If received significant help in designing, or carrying out the work, or received materials from someone who did a favour by supplying them, their assistance must be acknowledged. Acknowledgments are always brief and never flowery.

- REFERENCES (LITERATURE)

References should cover all papers cited in the text. The in-text citation format should be as follows: for one author (Karaman, 2011), for two authors (Erjavec and Volk, 2011) and for more than two authors (Rednak *et al*., 2007). Use semicolon (Rednak *et al*., 2012; Erjavec and Volk, 2011) to separate multiple citations. Multiple citations should be ordered chronologically. The literature section gives an alphabetical listing (by first author's last name) of the references. More details you can find in the Annex to the INSTRUCTIONS TO AUTHORS / Bibliographic style on the web page of the Journal: www.agricultforest.ac.me.

Short communication should include the following sections: Title, Abstract, Key words, Main text, Acknowledgments, References, Tables and Figures with captions.

SUPPLY OF ARTWORK, PHOTOS: Diagrams and graphs should be provided as finished black and white line artwork or colour images. Electronic graphics included in your manuscript should be either inserted in the word document or as .gif or .jpg formats. Please check with the editor if you wish to submit any other type of graphic for conversion suitability. Photos should be supplied un-screened in original form or in electronic form. All illustration (diagrams, graphs, tables, photos) must be fully captioned. When there are a number of illustrations, the author should endeavour to reduce the amount of text to accommodate the illustrations in the limited space available for any article.

THE REVIEW PROCESS: Submitted manuscripts are reviewed anonymously by 2 referees (duble blind review): one from the journal Editorial board, one must be an international reviewer, working out of the University of Montenegro. All tracking of manuscripts and reviewers is done by the Editor. All attempts will be made to ensure submissions will be reviewed within three months after the submission. Manuscripts will be returned to the coresponding authors when each review is completed.

Examples available on **www.agricultforest.ac.me**.