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EFFECTS OF THE ALPHA S1-CASEIN GENOTYPE ON MILK YIELD AND MILK COMPOSITION OF BALKAN GOAT IN MONTENEGRO

SUMMARY

The objective of this study was to investigate genetic polymorphism of the alpha S1 casein gene and its association with milk production parameters (milk yield and milk composition) of indigenous Balkan goat breed reared in Montenegro. Two genetic variants (A and F) and three genotypes AA, AF and FF of alpha S1-casein have been identified. The analysis showed a prevalence of strong A allele (0.626) compared to the weak F allele (0.374), while the frequencies of genotypes were 0.398, 0.456 and 0.146 respectively. The effect of genotype of alpha S1 casein on the total milk yield, length of lactation and daily milk yield was not significant ($p>0.05$), while it is significantly influenced to the milk composition parameters. Goats with AA genotype of alpha S1 casein had significantly higher ($p<0.001$) average content of protein (3.54%) than AF and FF genotype (3.40% and 3.33%). The same genotypes order were for fat content and solid non-fat content (AA>AF>FF) with significant differences ($p<0.05$) of AA genotype in comparison to AF and FF.

Genetic characterization of the alpha S1-casein gene contributes to the knowledge of the genetic structure of Balkan goat breed in Montenegro. These results could be important for establishing of the concept of conservation and sustainable use of this indigenous goat breed, as well as for selection scheme and breeding program.

Key words: goat, milk, alpha S1 casein, polymorphism

INTRODUCTION

One of the most valuable components in goat milk is protein. In goat milk, as in the other domesticated ruminants, total milk protein contains two main whey proteins (α-lacto albumin and β-lacto albumin) and four casein proteins (αS1-casein, αS2–casein, β-casein, κ-casein). These four caseins are expressed and encoded by CSN1S1, CSN2, CSN1S2 and CSN3 genes, respectively (Martin

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et al., 2002; Clark and Sherbron, 2000). The casein accounts approximately 80% of the total protein, while whey proteins stand for the remaining 20%. Genetic polymorphisms of caseins are important and well known due to their effects on quantitative traits of milk and its technological properties. The casein at the locus alpha S1 is highly polymorphic and it is associated with the milk quality and composition, with some technological properties of the milk and also with the yield and quality of cheese (Martin et al., 2002; Roncada et al., 2002; Gómez et al., 2004;Feligini et al., 2005). Seven variants of αs1gene were identified initially (A, B, C, D, E, F and O), even more genetic variants and numerous possible allelic combinations (Grosclaude et al., 1987; Caravaca et al., 2011; Szatankoova et al., 2007).In the last decades several studies have reported that the remarkable genetic polymorphism at the αs1-casein locus is greatly responsible for the individual variations observed in the casein content of the goat milk.

On the base more research the next expression levels are identified: strong alleles (A, B1, B2, B3, B4, C) associated with high content of αs1-casein (3.5 g/l), intermediate alleles (E and I) associated with 1,1 g/l of casein, weak alleles (D, F and G) synthesized at a low level (0.45 g/l) and null alleles (O1, O2, O3 and N) characterized by absence of αs1-casein in milk (Marletta et al., 2007;Torres-Vaskez et al., 2008,Feligini et al., 2005). Goat milks containing these “strong” variants (“strong” milks) are characterized by higher content of total protein, milk fat, calcium and more favorable cheese properties (coagulation properties, cheese yield and quality) than “intermediate”, “weak” or “null” variants (Clark and Sherbon, 2000; Devold et al., 2011). Different protein content in goat milk affected by polymorphism of αs1-casein generates significant cheese yield differences (Moioli et al., 2007).

The effect of αs1-casein polymorphism on goat milk quality was studied in different goat populations, but the most intensively in French breeds (Manfredi et al., 1995; Delacroix et al., 1996). These studies concluded that A allele has a significant positive effect on the whole milk protein, casein, fat contents and manufacturing properties (increasing cheese making efficiency up to 15%), in comparison with F allele; E allele have an intermediary effect between the previous two. In dairy goat populations, it is often considered convenient to identify the alpha S1 genotypes of individual animals, with the idea of incorporating this information to the selection process for commercial breeds or to conservation program for autochthonous breeds (Moioli et al., 1998).

Goat production is one of the smallest livestock sectors in Montenegro (about 35,000 heads), but the sector with increasing trend in the last years. The main product of goat production is milk which is further processed into various types of cheese.

The objective of this study was to evaluate genetic polymorphism in the alpha S1 casein gene in Balkan goat breed from Montenegro by identifying genotypes and theirs association with milk production parameters (milk yield and milk composition). These information could be used in the program of conservation as well as for the selection and improvement of this breed.

MATERIAL AND METHODS

The field study was carried out at two family farms of the Balkan goat breed, reared in south part of Montenegro characterized by Mediterranean climate. This is an autochthonous goat breed of the Balkan Peninsula and the most numerous breed in goat population of Montenegro. There are several varieties of Balkan goat breed regarding the color of hair, while red variety is the most typical in Montenegro. The system of rearing was semi extensive, based mostly on the grazing in hilly and rocky areas. Supplementing nutrition by concentrate feeds provided only during kidding time and in the beginning of the lactation and sometimes during summer season if vegetation is over dried and poor.

Milk production traits

Milk yield, recorded on the base of test day control, were carried out once a month during lactation period according to ICAR rules for AT control. Milk samples for the analysis of fat, protein and solid nonfat (SNF) content were collected for each test day(monthly) from all animals. The milk samples were analyzed in authorized laboratory by using infrared spectrophotometry on MilkoScan FT120.Milk recording and milk analysis are organized for bigger number of animals, but for this research used data and analysis performed on 103 animals which had both data - milk production parameters and genotype information.

Genotyping of alpha S1 casein

For DNA extraction, PCR amplification and sequencing blood samples (5 ml) were taken from randomly chosen goats from above-mentioned two flocks. Samples were collected in EDTA tubes and stored at+4°C. A standard phenol-chloroform protocol for DNA extraction from blood was used.

The sequence of 750 bp of αS1-casein gene from exon 12 was amplified by PCR.

A typical 38cycles of PCR were carried out with the following PCR mixture: 10 µl 10xPCR buffer (500 mMKCl, 200 mMTris-HCl, pH 8.4); 8 µl of 25 mM MgCl2; 8 µl 10 mM of each dNTP, 0.5 µl of 2.5 U AmpliTaq DNA polymerase; 4 µl of 10 pmol of forward and reverse primers, 100 ng goat genomic DNA and ddH_2O up to 100 µl. The oligonucleotide sequences of the primers for amplification of sequence of the αS1-casein gene were as follows:

Forward primer: 5' - CATTCTTTACTCCTGGGAAAG - 3';

Reverse primer: 5' – AGCACTTTTGGGAACAATTTC – 3'.

The following amplification conditions were used: denaturation; 1' at 94^{o}C, annealing; 45'' at 58^{o}C, extension; 1' and 30'' at 72^{o}C. The PCR productswere electrophoresed on 1.5% agarose.

Sequencing was carried out by ABI PRISM® BigDye™ Terminator Cycle Sequencing Ready Reaction Kit and the automatic DNA-sequencer Model 377 (PE Applied Biosystems, Wellesley, MA, USA).

Statistical analysis

Statistical data processing for milking properties and alpha S1 casein genotypes was done using the general linear model (GLM) model in SPSS Statistics, version 20.

The GLM model used for calculation effect of genotype of alpha S1 casein and other factors to lactation length and total lactation milk yield had the following form:

$Y_{ijkl} = \mu + F_i + G_j + L_k + (FG)_{ij} + e_{ijkl}$,

While the model used for calculation effect of genotype alpha S1 casein and other factors to test day milk yield and milk composition had the following form

$Y_{ijkl} = \mu + F_i + G_j + L_k + C_l + (FC)_{il} + e_{ijkl}$

Y_{ijkl} - the phenotypic value of individual traits,

μ - general mean value,

F_i - fixed effect of the farms or flock, (i = 1, 2),

G_j - fixed effect of the genotype of alpha S1 casein (j = 1, ... 3)

L_k – fixed effect of number of lactation (k = 1, …4),

C_l - fixed effect of the test days (k = 1, …7),

$(FG)_{ij}$ – interaction farm x genotype

$(FC)_{il}$ - interaction herd x test day,

e_{ijkl} –other undetermined effects (random error).

The frequency of alleles and genotypes and possible deviation from Hardy-Weinberg equilibrium were calculated by use Hardy-Weinberg equilibrium calculator (Rodrigueze et al., 2009).

RESULTS AND DISCUSSION

Genotypes of alpha S1 casein

The presence of two genetic variants of alpha S1-casein (A and F) determined by the sequencing of 750 base pairs long fragment of genomic DNA (between 3' end of intron 11 and 5' end of exon 13) which showed numerous single base substitutions, deletions and insertions. Allele A (A usually include A, B and C variants) dominated in the investigated population of Balkan goat breed in Montenegro with frequency 0.626, while the obtained frequency of weak allele F was 0.374 (Table 1). These two alleles (A and F) appeared in three genetic forms, namely genotypes of alpha S1-casein (AA, AF and FF). The most common genotype was heterozygote AF (0.456), followed by the homozygote AA (0.398), while the FF genotype frequency was the smallest 0.146.

According to the results of χ^2 test, the investigated goat population was in Hardy-Weinberg equilibrium suggesting that the genotypes of alpha S1-casein was not influenced by selection.

Based on conducted results, the genetic structure of Balkan goat breeds corresponds to a high content of alpha S1 casein what is associated with domination of strong homozygote (AA) in comparison with weak heterozygote genotypes AF and FF. Polymorphisms at the locus of alpha S1-casein have been characterized in the most economically important goat breeds as well as in some local breeds from different countries (Kalamaki et al., 2014). Genotyping of αs1-casein and used as a selection criteria for dairy goat breeds (Alpina and Sannen) in some European countries has been carried out since the 1980s.

Table 1. Frequencies of genotypes and alleles and Hardy-Weinberg equilibrium test αS1-casein gene of Balkan goat breed

Genotypes of alpha S1-casein					Alleles		χ^2
Parameter	AA	AF	FF	Total	A	F	
Ho	41	47	15	**103**	129	77	$\chi^2 = 0.0657^{ns}$
He	40.39	48.22	14.39				
Frequency	**0.398**	**0.456**	**0.146**	**1.00**	0.626	0.374	P = 0.7975

Ho: observed heterozygotes, He: expected heterozygotes on the basis of Hardy-Weinberg, $\chi 2$ =chi-square value

The high prevalence of strong A allele (0.626) obtained in our analyses is similar to that found for many other unselected goats breeds such asSpanish Canaria breed, 0.60 (Jordana et al. 1996); Italian Gargancia breed, 0.61 (Martin, 1993), Balkan goat breed reared in Albania (0.47 – A and 0.46 B allele) and Damascus goat breed from Greece, 0.87(Grosclaude and Martin, 1997). High domination of strong alleles A and B obtained in seven African local goat breeds (Missohou et al., 2006) and in American dairy goats with African origin (La Mancha, Nubian and Nigerian dwarf) reported by Maga et al. (2009) .It is important to note that a frequency of weak F allele in the most of mentioned breeds was very low, even less than it is obtained for Montenegrin Balkan goat breed.

Since Montenegro is situated in South East Europe in Mediterranean area, the prevalence of strong A allele for Balkan goat breed is in accordance with several earlier studies which revealed that the genotypes with strong or medium alleles of the alpha S1-caseindominate among goat breeds from the Mediterranean region as Sarda, Maltese, Moroccan, Tunisian (Moioli et al., 2007), and within the Iberian Peninsula, in southern breeds as Murciano-granadina, Malageña or Payoya (Jordana et al., 1996; Caravaca et al., 2009). The prevalence of the A allele or of group of strong alleles (A, B and C) were also presented by Vlaic et al. (2010) for Carpatian goat breed, Caroliet al. (2007)for some African goats breeds and Kalamaki et al. (2014) for Greek-indigenous Skopelos goat.

Different from our results, on predominance of the intermediate-expressing(E) and weak-expressing (F) alleles, reported Maga et al. (2009) for America dairy goats (Alpine, Saanen, Togenburg, Oberhasli), and Soares et al. (2009) for Saanen and Alpina breeds reared in Brazil, as well as several earlier studies on dairy goat breeds in France and Italy (Grosclaude and Martin, 1997; Martin 1993).

Effect of alpha S1-casein genotype on the milk traits

According to results presented in Table 2the autochthonous Balkan goat breed reared under extensive system in Montenegro is relatively low productive with average milk yield of 162.07 kg, in lactation that lasted 215.9 days in average. In regarding of genotype of alpha S1 – casein the highest milk production (average daily milk yield and total lactation milk yield) obtained for heterozygote AF (0.735 kg/day and 166.9 kg), while the lowest milk yield obtained for goats with AA genotype of alpha S1 casein (0.720 kg /day and 158.1 kg), but differences among genotypes were not significant (p>0.05).

Table 2. The means of alpha S1-casein genotypes for milk yield and milk composition traits in the studied goats

	Genotype	**N**	**Mean**	**S.E.**	**F -value**
Lactation duration, days	AA	41	211.2	3.83	
	AF	47	216.6	3.52	
	FF	15	220.4	5.29	
	Total	103	215.9	2.96	1.394^{NS}
Lactation milk yield, kg	AA	41	158.1	6.34	
	AF	47	166.9	5.83	
	FF	15	161.1	8.76	
	Total	103	162.07	4.90	0.833^{NS}
Daily milk yield, kg	AA	243	0.720	0.017	
	AF	290	0.735	0.016	
	FF	92	0.717	0.024	
	Total	625	0.724	0.014	0.793^{NS}
Fat, %	AA	243	3.491^{a}	0.042	
	AF	290	3.365^{b}	0.039	
	FF	92	3.295^{b}	0.059	
	Total	625	3.384	0.036	7.398**
Protein, %	AA	243	3.537^{a}	0.0230	
	AF	290	3.404^{b}	0.027	
	FF	92	3.328^{b}	0.040	
	Total	625	3.423	0.023	18.646***
SNF, %	AA	243	8.532^{a}	0.0231	
	AF	290	8.442^{b}	0.029	
	FF	92	8.436^{b}	0.042	
	Total	625	8.467	0.025	5.282*

SNF – solids non fat, Different letter in the superscript means statistical significant difference between means of genotypes (p<0.05) which are in same column.
^{NS} – non significance (p>0.05), *- significance (p<0.05), **- significance (p<0.01), *** significance (p<0.001).

On the other side, milk composition parameters (content of protein, fat and SNF on the test day) have been significantly affected by genotype of alpha S1-

casein ($p<0.01$). An average protein content for the whole investigated population was 3.42%, while the goats with AA genotype of alpha S1 casein had higher content of protein than milk of AF and FF genotype by 0.13% and 0.21%, respectively; those differences were significant ($p<0.001$).

The average fat content also was significantly higher in the goats milk with AA (3.491%) than in goats with AF and FF genotype of alpha S1 casein (3.365 and 3.295%, respectively). A similar effect of genotypes was also in the view of the content of SNF - the highest value for AA genotype was significantly different in comparison with the other two.

The autochthonous Balkan breed reared under extensive system of rearing in Montenegro is relatively low productive in regarding of total milk yield. The average milk yield of the studied population of Balkan goat breed reared under extensive condition in Montenegro was 162.07 kg, in lactation that lasted 215.9 days in average is much lower than Bogdanović et al. (2010) referred for Balkan breed of goat reared under semi intensive condition in Serbia (378.4 kg in 256 days) and results of Kominakisa et al. (2000) for Greek Skopelos dairy goat (235 kg in lactation of 187 days). Our results are very similar to the results of milk production traits of Balkan breed on Kosovo and Albanian local breed (178 kg per lactation) displayed by Memisi et al. (2004) and Kume et al. (2012).In comparison with high productive dairy breeds, total milk yield and daily milk yield in Balkan goat are very inferior (Mioč et al., 2007; Klir et al., 2015, Slysius et al., 2017).These results, however, indicate that Balkan breed similarly as many autochthonous breeds, possesses a good biological variability, that could be easily used for increasing its production potential by improvement of nutrition, system of rearing and selection.

To meet the increasing interest for goat production and goats' dairy products, investigation of different milk parameters is very useful. The effect of polymorphism in the locus of alpha S1 casein on milk traits has been investigated in several countries for a long time, due to economic implications on goat milk processing into cheese.

Although a negligible negative effect of allele A and genotype AA of alpha S1 CN on milk yield was identified, in comparison to allele F, genotype of alphaS1 casein had no overall significant effect on total milk yield and daily milk yield. These results are in accordance with the results of Felícitas Vázquez et al.(2012).A slightly higher milk yield milkyield in AF and FF genotype than in AA genotype is in agreement with the results of Szatankoova et al. (2013) obtained for Check dairy goat.

A contrary to the previous finding, significant effect of the genotype of alpha S1 casein on milk composition is obtained. The close relationship of AA genotype with higher content of protein and fat is in agreement with many previous studies (Avondo et al., 2009, Szatankoova et al., 2007; Felícitas-Vaskez et al., 2012; Johansson et al., 2015). The higher protein content in goat milk which is influenced by polymorphism in alpha S1 casein gene resulting in higher cheese yield. (Feligini et al., 2005; Moioli et al.,2007; Maga et al.,2009).

Though, the genotype of alpha S1 casein can be used as the selection criterion to improve these traits of goats.

As it claimed Boettcher et al. (2010) actions aimed to genotype for proved useful genes should be among the priorities for conservation of specific animal genetic resources, especially for breeds well adapted to difficult territories and rearing conditions as it Balkan goat breed. Generally, selection of animals for strong alpha S1-caseingenotype can lead to improvements in milk composition and quality, particularly in protein content and contribute to sustainable conservation of this breed.

CONCLUSIONS

This is the first study of genetic polymorphism of the alpha S1-casein gene of indigenous Balkan goat breed in Montenegro and their effect on milk production traits. The obtained results confirmed the existence of close association between the alpha S1-casein genotype and the parameters of milk quality in the investigated indigenous Balkan breed of goat. The proving of domination of "strong" allele A in the population of Balkan breed of goats and its associated with a higher content of protein in milk. Since A allele is desirable in regards of cheese production, it is very important because most of milk of this goat breed use for cheese production.

The next step in upgrading of these studies should be to investigate the effect of the alpha S1 casein polymorphisms on the technological characteristics of goat's milk. These results of the genetic diversity of Balkan goat breed reared under semi extensive conditions are the important argument for the conservation of its genetic diversity and consolidation because this breed very well adapted to local environment as well as for improvement of sustainable use of this indigenous goat population. Also, it would be possible to include the alpha S1 casein genotype in the selection scheme, as it is regular practice in developed countries.

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A RESEARCH ON THE INFLUENCE OF POROSITY ON PERLITE SUBSTRATE AND ITS INTERACTION ON POROSITY OF TWO TYPES OF SOIL AND PEAT SUBSTRATE

SUMMARY

Perlite is a generic name for an amorphous volcanic rock that expands by a factor of 4–20 when rapidly heated to (760–1100°C). Water trapped in the structure of the material vaporises and escapes, and this causes the expansion of the material to 7–16 times its original volume. The expanded material is a brilliant white, due to the reflectivity of the trapped bubbles. Expanded perlite has several attractive physical properties for commercial applications, including, low bulk density, low thermal conductivity, high heat resistance, low sound transmission, high surface area, and chemical inertness.

The perlite supplies the ideal balance between air and water. Perlite is sterile, inert, non-toxic, non-decomposable and easy to handle with, enhanced water retention and aeration capacity. The application of substrates which improve the properties of the soils requires knowledge of their physical and chemical characteristics that are responsible for providing adequate support and a reservoir for air, water and nutrients.

Agricultural production is increasingly concerned about the study of the impact of improvers of properties, such as perlite, that affect the properties of soils as well as their impact on yield and plant quality. The goal of this paper is to observe the influence of porosity on the Perlite substrate and its interaction with the porosity of two types of soil and the peat substrate. The laboratory part comprised preparation of the substrate perlite, soils, and substrate peat for analyses and conducting quantitative laboratory analysis.

The substrate perlite, soils and substrate peat were analysed in all five of their different ratios: Perlite (Pe) 20%; 30%; 50%; 70%; 80% by volume) and 100% perlite. Fluvial soil (FS) 80%; 70%; 50%; 30%; 20% by volume) and 100% fluvial soil. Mollic Vertic Gleysol (GS) 80%; 70%; 50%; 30%; 20% by volume) and 100% mollic vertic gleysol. Peat (P) 80%; 70%; 50%; 30%; 20% by volume) and 100% Peat. In laboratory conditions the total porosity (in percentage form) was determined with the help of apparent and specific density (apparent density through applying the Koppecki method (specific density was determined

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through the Gracanin method. The pores' total content is determined indirectly on the basis of the specific mass and volume mass. The results will be displayed through statistical data processing.

Key words: perlite, soil, porosity.

INTRODUCTION

Perlite is a 100% natural siliceous volcanic glass mineral, which traps crystalline water into its mass. Perlite expands when rapidly heated in temperatures of 700°C–1100°C (Dogan and Alkan, 2004). The way of preparation of fine expanded perlite, was given by (Sodeyama et al., 1999). Causing entrapped water molecules in the rock to turn to steam and expand the particles like popcorn.

The abrupt, controlled rise of temperature forms a white mass of minuscule glass bubbles. Perlite melts and expands in an extremely porous surface and increasing its volume up to 4-20 times of its original volume (Ennis, 2011). It is very porous, has a strong capillary action and can hold 3–4 times its weight in water. (Bures et al. 1997a). This microstructure gives the material a set of favourable properties such as excellent insulation properties, low density and high porosity (Sengul et al., 2011; Kramar and Bindiganavile 2013; Polat et al., 2015). Here are a number of obvious advantages of perlite over other substrates like stability, great properties such as: ultra-lightweight, excellent water retention up to four times its weight, advances drainage and aeration, pH natural and asbestos free, chemically inert, sterile, free of weeds and permanent, serves as an insulator to reduce extreme soil temperature fluctuations, reduces concentrations of salt and also promotes the long term effect of fertilizers (Raviv, M., and Lieth, J ,H, 2008; Asher B.,T et al., 2008).

Moreover, it is commonly used in the food industry, filter product, growing of seed, regulating of the soil in agriculture, and in so many other industrial applications (Alihosseini et al., 2010). Perlite has very good physical characteristics. The physical properties of container-growing substrates, particularly air space, container capacity, and bulk density, have a significant impact on plant growth, and knowledge of these properties is essential in properly managing nursery irrigation and fertilization programs (Yeager et al., 2000). Peat is formed as a result of the partial decomposition of plants (Sphagnum, Carex) typical of poorly drained areas (peat bogs), with low nutrients and pH, under low temperatures and anaerobic conditions (Raviv et al., 2002). Other relevant properties are the high easily available water under conditions of container capacity, i.e. after the end of free drainage and the high oxygen diffusion rate. On the other hand, as negative aspect peat can be a conducive substrate for numerous soil-borne diseases and its sterilization does not solve the problem as it leaves a biological vacuum that can be easily filled by pathogenic fungi. (Abad et al., 2001).

Peat use in horticulture increased during the last decades, resulting in rising costs and generating doubts about availability of this material in the near future due to environmental constraints. In fact, peat mining has been recently questioned because it is harvested from peat lands, highly fragile wetlands ecosystems with a great ecological and archaeological value, included in the list of natural habitats with a potential degradation. (Barber et al., 1993). Peat also plays an important role in improving groundwater quality, and peat bogs also serve as a special habitat for wild plants and animals. Moreover, these ecosystems represent important carbon dioxide (CO_2) sinks (Maher et al., 2008). Peat is the most widely used growing media and substrate component in horticulture, currently accounting for 77–80 percent of the growing media used annually in Europe's horticultural industry (Gruda, 2012),

Seedlings and transplants are grown predominantly in organic substrates based on peat it is also used in horticulture as a raw material for substrates in which container plants are grown (Gruda, 2005). The term alluvial originates from the Latin word *alluvio* which means rubble. Lately, names that originate from the Latin word *fluvius* river are being used. The following such names can be frequently encountered: fluviogenic soil, fluvizem, fluvisol, fluvent etc. In our newest classification multiple terms are used: alluvial or fluviatile soils (fluvisol) which are classified as fluvisol according to WRB 2016. In regards to physical properties fluvial soils are quite heterogeneous in their mechanical composition. All varieties of soils can appear among fluvial soils from sandy to clayish soils. However, they are most often sandy loam or loamy sand. In most cases, fluvial soils have good porosity, an advantageous relation between capillary and non – capillary pores, they are well aerated, they permeate water quite well etc.

The following definition is ascribed for the soil type of Mollic Vertic Gleysol (Filipovski, 1996) hydromorphic soils which have a darkly colored mollic humus horizon with possible signs of hydromorphy. The humus horizon usually has a dark grey color to a distinctly black color, out of which the name is derived. These soils are rich in clay and the clay content is above 40% in hor. A. The physical properties (water-air regime) of these soils depend on the mechanical composition of the substrate and the mineralogical composition (especially the contents of montmorillonite), the humus contents, some processes that are significant for the physical properties (re-covering with new rubble, duality of the layers, alkalization and human influence).

Aeration as an important physical property is of great significance for non – capillary porosity which on average is around 5%. This speaks to the fact that the soil is not aerated enough when it is saturated in regard to its field capacity. The goal of this paper is to observe the influence of porosity on the perlite substrate and its interaction with the porosity of two types of soil and the peat substrate. The water and air regime of the soil/substrate depends on the porosity and its character which means supplying the plant with sufficient quantities of water and air. Knowing the total porosity, the relationship between the macropores and micropores, the stability of the porous system and the total internal surface of the pores has an immense practical significance for the soil as well as the substrates for plants growth.

MATERIAL AND METHODS

The experimental part served to determine on the influence of porosity on perlite substrate and its interaction on porosity of two types of soil and peat substrate. The experimental part was divided into two parts: field part and laboratory part. The used perlite originates from Cera Poliana, Mariovo Gradesnica, Republic of Macedonia, and was applied in expanded (commercial) form. The experimental part was divided into two parts: field part and laboratory part. The laboratory part comprised preparation of the substrate perlite, soils, and substrate peat for analyses and conducting quantitative laboratory analysis.

The substrate perlite, soils and substrate peat were analyzed in all five of their different ratios: Perlite (Pe) 20%; 30%; 50%; 70%; 80% by volume) and 100% perlite, Fluvial soil (FS) 80%; 70%; 50%; 30%; 20% by volume) and 100% fluvial soil. Mollic Vertic Gleysol (GS) 80%, 70%, 50%; 30; 20 by volume) and 100% mollic vertic gleysol. Peat (P) 80%; 70%, 50%; 30%; 20% by volume) and 100% peat. The soil samples were taken at depth of 0-30cm. In laboratory conditions, soil samples were brought to an airy dry state. Then the soil was finely milled and sifted through a sieve with 2mm openings, and an average analytical sample was prepared in which further soil analysis was carried out. In laboratory conditions the total porosity (in percentage form) was determined with the help of apparent and specific density (apparent density through applying the Koppecki method (Mitrikeski and Mitkova, 2013) (specific density was determined through the Gracanin method (Resulovic, H. et al., 1971). The pores' total content is determined indirectly on the basis of the specific mass and volume mass.

The results will be displayed through statistical data processing. The first statistical analysis of the gathered data was made with the descriptive procedure for analysis of frequencies and data dispersion depending on the factors of influences. The obtained results are represented as an average with a ± standard deviation from the arithmetic mean value. With the help of the general linear model, the multivariate procedure, the influence of independent (factor) variables were tested and their interaction on the mean values of the different groupings from the physical and chemical properties of the examined variants. For those variables, for which the F-value has displayed statistical significance, a post – hoc test was implemented i.e the Bonferoni test. With it, the differences between the specific mean values of the pairs were assessed in a multiple comparison for the factors involved in the model. The interdependence of variables incorporated in statistical regression models was examined through Pearson's correlation coefficient. The obtained results will be presented through tables, sketches, etc.

RESULTS

In Table 1 the results are displayed with the mean values of: total porosity, water and air porosity of the analyzed samples: Perlite (Pe) 20%; 30%; 50%; 70%; 80% by volume) and 100% perlite. Fluvial soil (FS) 80%; 70%; 50%; 30%; 20% by volume) and 100% fluvial soil. Mollic Vertic Gleysol (GS) 80%; 70%, 50%; 30%; 20% by volume) and 100% Mollic Vertic Gleysol. Peat (P) 80%; 70%; 50%; 30%; 20% by volume) and 100% peat. The results of the multivariate regression statistical model will be presented for the influence of the different variants, the different correlation in variants and their interaction with total porosity, water and air porosity. Additionally, the results of the post hoc analysis for the testing of the differences in mean values of dependent variables are presented, depending on the sources of variation.

The analyzed sample of Perlite (Pe) has displayed the highest percentage of total porosity out of all analyzed samples from Table 1 with a mean value of 88.09%, out of which 60.2% in mean value is air porosity and 27.9% is water porosity. The contents of total porosity of fluvial soil (FS) is has a mean value of 77.73% out of which 39.68% with a mean value is water porosity and 38.05% is air porosity. All the other analysed samples in their various ratios are displayed in Table 1.

	Formulation	**Designation**
1.	100% Perlite (commercial substrate)	(Pe)
2.	100% Peat (commercial substrate)	(P)
3.	80% Perlite + 20%Peat	Pe80/P20
4.	70%Perlite + 30% Peat	Pe70/P30
5.	50%Perlite + 50% Peat	Pe50/P50
6.	30%Perlite + 70% Peat	Pe30/P70
7.	20%Perlite + 80% Peat	Pe20/P80

	Formulation	**Designation**
1.	100% Perlite (commercial substrate)	(Pe)
2.	100% Fluvial soil (soil)	(FS)
3.	80% Perlite + 20% Soil	Pe80/FS20
4.	70%Perlite + 30% Soil	Pe70/FS30
5.	50%Perlite + 50% Soil	Pe50/FS50
6.	30%Perlite + 70% Soil	Pe30/FS70
7.	20%Perlite + 80% Soil	Pe20/FS80

	Formulation	**Designation**
1.	100% Perlite (commercial substrate)	(Pe)
2.	100% Mollic Vertic Gleysol (Soil)	(GS)
3.	80% Perlite + 20% Soil	Pe80/GS20
4.	70%Perlite + 30% Soil	Pe70/GS30
5.	50%Perlite + 50% Soil	Pe50/GS50
6.	30%Perlite + 70% Soil	Pe30/GS70
7.	20%Perlite + 80% Soil	Pe20/GS80

Table 1. Physical properties of perlite substrate and fluvial soil

	n	Air porosity %		Water porosity %		Total porosity %	
		$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Pe-Perlite	3	60.20	0.01	27.90	0.01	88.09	0.01
FS-Fluviol Soil	3	38.05	0.28	39.68	0.60	77.73	0.84
Pe80/FS20	3	55.77	0.01	29.61	0.01	85.38	0.01
Pe70/FS30	3	53.56	0.19	31.43	0.40	84.99	0.23
Pe50/FS50	3	49.13	0.03	33.79	0.03	82.92	0.02
Pe30/FS70	3	44.69	0.62	36.15	0.22	80.84	0.79
Pe20/FS80	3	42.48	1.06	37.16	0.89	79.64	1.95

An overview of the following results is displayed in Table 2 total porosity, water and air porosity of analyzed samples of Perlite (Pe), Mollic Vertic Gleysol (GS) and their mixtures in various ratios. The highest value of total porosity was recorded in the Perlite ratio with a mean value of 88.09% while the lowest value was recorded in the Mollic Vertic Gleysol with a mean value of 49.99%. The highest percentage of water porosity was noted in the mollic vertic gleysol with a mean value of 42.79% and the lowest in perlite with a mean value of 27.9%.

The highest air porosity was noted in perlite with a mean value of 60.2% while the lowest air porosity was recorded in mollic vertic gleysol with a mean value of 4.21%. All the other analyzed samples in their various ratios are displayed in Table 2

Table 2. Physical properties of perlite substrate and mollic vertic gleysol

	n	Air porosity %		Water porosity %		Total porosity %	
		$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Pe-Perlite	3	60.20	0.01	27.90	0.01	88.09	0.01
GS- Mollic vertic gleysol	3	4.21	1.41	42.79	0.01	46.99	1.41
Pe80/GS20	3	49.00	2.74	30.23	1.06	79.23	0.86
Pe70/GS30	3	43.40	1.70	32.37	1.03	75.77	0.93
Pe50/GS50	3	32.20	1.03	35.34	1.17	67.54	1.65
Pe30/GS70	3	21.00	0.09	38.23	0.93	59.23	1.33
Pe20/GS80	3	15.40	0.92	39.65	1.22	55.05	1.13

The following results are displayed in Table 3 total porosity, water and air porosity and the total retention capacity of the analyzed samples of Perlite, Peat and their mixtures in various ratios. The highest total porosity was noted in Peat (P) with 90.8% out of which 10.7% belong to air porosity and 80.1% with a mean value belong to water porosity. A somewhat lower porosity was noted in Perlite (Pe) with a mean value of 88.09% out of which 27.9% belong to water porosity and 60.2% to air porosity. All the other analyzed samples in their various ratios are displayed in Table 3.

Table 3. Physical properties of Perlite substrate and peat

	n	Air porosity %		Water porosity %		Total porosity %	
		$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Pe-Perlite	3	60.20	0.01	27.90	0,01	88.09	0.01
P-Peat	3	10.70	1.03	80.10	0.93	90.80	1.95
Pe80/P20	3	50.30	0.16	39.96	0.53	90.26	0.68
Pe70/P30	3	45.35	0.15	44.64	1.08	89.99	1.02
Pe50/P50	3	35.45	0.28	54.00	0.28	89.45	0.55
Pe30/P70	3	25.55	0.56	63.55	0.41	88.90	1.76
Pe20/P80	3	20.60	0.83	68.03	0.60	88.63	1.58

Table 4. Multivariate general linear model for the influence of variants, various ratios within the variants and their interaction on water porosity, air porosity, total porosity

Parameters	Source of variation									
	Model		Variants		Ratios		Variants x ratios		Error	
	df	F	df	F	df	F	df	F	df	Variance
[a]Air porosity %	21	5454.2***	2	1045.4***	6	1919.9***	12	1360.8***	42	0.9
[b]Water porosity %	21	7046.9***	2	6245.3***	6	836.6***	12	1163.3***	42	0.9
[c]Total porosity %	21	14120.7***	2	3608.3***	6	335.8***	12	138.3***	42	1.2

[a]R^2 = 0,919; [b]R^2 = 1; [c]R^2 = 0,1.

***statistically significant on level p<0.001;**statistically significant on level p<0.01;*statistically significant on level p<0.05

All statistical models about the influence of the variants and the different ratio of Perlite and fluvial soil, Perlite and Peat and Perlite with Mollic Vertic Gleysol soil in the respective variants, as well as the interaction of the variant and the ratio with water porosity, air porosity and total porosity have displayed a high statistical significance ($p<0.001$).

According to the results obtained out of the statistical model, displayed in Table 4 the variants displayed a significant statistically high influence of water porosity, air porosity and total porosity ($p<0.001$). The influence of the various ratios within the variants have also displayed a significant statistically high influence on water porosity, air porosity and total porosity ($p<0.001$). The interaction of the variants and the ratios have displayed a statistically high influence ($p<0.001$) on water porosity, air porosity and total porosity. The value of R^2 in all three statistical models was high. This means that a large part of the variant for water porosity, air porosity and total porosity can be explained through the variation sources involved in the model.

The testing of the differences between the mean values of air porosity depending on the variant are displayed in Table 5. A statistically significant difference between the mean values of air porosity was recorded among all variants. Throughout it, the largest statistically significant difference in the mean values of air porosity has been determined among the Perlite/Fluvial soil and Perlite/Peat variants with a value of 12.72%.

Table 5. Testing the differences of the mean values of air porosity between the variants

Air porosity %	Perlite/ Peat	Perlite/ Mollic Vertic Gleysol
Perlite/ Fluvial soil	12.72*	9.1*
Perlite/ Peat	1	-3.6*

*statistically significant on level p<0.05

Table 6. Testing the differences of the mean values of air porosity depending on the different ratio of Perlite, Fluvial soil, Peat and Mollic Vertic Gleysol in the respective variants

Air porosity %	FS,GS, P70/Pe30	FS,GS,P80/ Pe20	FS,GS, P30/Pe70	FS,GS, P20/Pe80	Perlite	FS, FG, P
FS,GS,P50/Pe50	6.49*	9.58*	-2.27*	-5.67*	-31.11*	-11.70*
FS,GS,P70/Pe30	1	3.10*	-8.75*	-12.15*	-37.60*	-18.19*
FS,GS,P80/Pe20		1	-11.85*	-15.25*	-40.70*	-21.28*
FS,GS,P30/Pe70			1	-3.40*	-28.85*	-9.43*
FS,GS,P20/Pe80				1	-25.45*	-6.03*
Perlite					1	19,41*

*statistically significant on level p<0.05

A statistically significant negative difference was noted in the mean values of air porosity between Perlite and the different ratios which points to the realization that the percentage of air porosity in Perlite is larger compared to the presence of air porosity in the different ratios of the variants in Table 6. The biggest statistically significant difference between the values of air porosity of the variants was determined between the ratio FS, GS, P80/Pe20 and FS, GS, P20/Pe80 with a value of 15.25%. Likewise, the difference in the mean values of air porosity of the soils and Peat that are used in the formation of various ratios and the ratios within the variants has displayed a statistically significant difference. However, the greatest difference in the mean values of air porosity i.e. 19.41% was noted between Perlite and the appropriate soils and Peat which in various ratios comprised the variants.

The testing of the differences between the mean values of water porosity depending on the variant is displayed in Table 7. A statistically significant difference between the mean values of water porosity was noted among all variants. Throughout it, the largest statistically significant difference in the mean values of water porosity was determined between the variants Perlite/Mollic Vertic Gleysol and Perlite/Peat with a value of 30.94%. A very noticeable fact was the statistically significant positive difference in the mean values of water porosity between Perlite and the ratio FS, GS, P80/Pe20. The largest statistically significant difference between the values of water porosity and the variants was determined between the ratio FS, GS, P80/Pe20 and FS, GS, P20/Pe80 with a value of 15.98%.

Table 7. Testing the differences of the mean values of water porosity between the variants

Water porosity %	Perlite/Peat	Perlite / Mollic Vertic Gleysol
Perlite/ fluvial soil	-20.08*	10.86*
Perlite/Peat	1	30.94*

*statistically significant on level p<0.05

Table 8. Testing the differences of the mean values of water porosity depending on the different Perlite ratio, Fluvial soil, Peat and Mollic Vertic Gleysol in the respective variants

Water porosity **%**	FS,GS ,P70/Pe30	FS,GS P80/Pe20	FS,GS P30/Pe70	FS,GS P20/Pe80	Perlite	FS,GS,P
FS,GS,P50/Pe50	-5.70*	-8.81*	5.85*	7.17*	14.82*	11.66*
FS,GS,P70/Pe30	1	-3.11*	11.55*	12.87*	20.52*	17.36*
FS,GS,P80/Pe20		1	14.66*	15.98*	23.63*	20.47*
FS,GS,P30/Pe70			1	1.32	8.97*	5.81*
FS,GS,P20/Pe80				1	7.65*	4.49*
Perlite					1	-3.16*

*statistically significant on level p<0.05

The difference in the mean values of water porosity of the soils and Peat used in the formation of the various ratios and the ratios within the variants has also indicated a statistically significant difference depicted in Table 8. However, the largest difference in the mean values of water porosity i.e. 20.47% has been noted between Perlite and the respective soils and Peat which in various ratios formed the variants. The testing of the differences between the mean values of total porosity depending on the variant is displayed in Table 9. A statistically significant difference between the mean values of total porosity was noted among all variants. Throughout it, the largest statistically significant difference in the mean values of total porosity was determined among the Perlite/ Mollic Vertic Gleysol and Perlite/Peat variants with a value of 27.63%.

Table 9. Testing the differences of the mean values of water porosity between variants

Total porosity %	Perlite/Peat	Perlite / Mollic Vertic Gleysol
Perlite/ fluvial soil	-7.63*	19.99*
Perlite/Peat	1	27.63*

*statistically significant on level p<0.05

Table 10. Testing the differences of the mean values of total porosity depending on the different ratio of Perlite, Fluvial soil, Peat and Mollic Vertic Gleysol in the respective variants

Total porosity %	FS,GS P70/Pe30	FS,GS P80/Pe20	FS,GS P30/Pe70	FS,GS P20/Pe80	Perlite	FS, GS, P
FS,GS,P50/Pe50	0.34	0.55	3.54*	1.50	-16.29*	-0.04
FS,GS,P70/Pe30	1	0.21	3.20*	1.17	-16.63*	-0.38
FS,GS,P80/Pe20		1	2.99*	0.95	-16.84*	-0.59
FS,GS,P30/Pe70			1	-2.04*	-19.83*	-3.58*
FS,GS,P20/Pe80				1	-17.79*	-1.5
Perlite					1	16.25*

*statistically significant on level p<0.05

A statistically significant negative difference was once more noticeable in the mean values of total porosity between Perlite and the different ratios which points to the realization that the total porosity in Perlite is larger compared to the total porosity in the various ratios of the variants. The largest statistically significant difference between the values of total porosity was determined between the ratio FS, GS, P30/Pe70 and FS, GS, P50/Pe50 with a value of 3.54% which is depicted in Table 10. The difference in the mean values of total porosity of the soils and Peat used in the formation of different ratios and the variants ratios has also displayed a statistically significant difference. Never the less, the largest difference in the mean values of total porosity i.e. 16.25% was noted among Perlite and the respective soils and Peat which comprised the variants in various ratios.

DISCUSSION

Porosity or void fraction is the total volume of the pores (cavities) expressed in voluminous percentages of the total soil/raw material in a natural (undistorted) state. The volume of all pores in a certain volume i.e. soil/raw material constitutes total porosity which is encompassed by water and air. In the macropores (non – capillary pores) there is air while in the micropores (capillary pores) there is water. Through irrigation the water gets into all pores but it is only retained in the capillary pores. A different ratio between capillary and non – capillary pores will induce a different water and air regime. Total porosity, capillary and non – capillary porosity differ among each other. In our research the values of total porosity, water porous capacity, air porous capacity of Perlite substrate with Fluvial soil, Mollic Vertic Gleysol and Peat substrate were analyzed. Out of the obtained results with their respective values from Table 1 and Table 2 for total porosity it can be noted that Perlite as a substrate has a very high porosity with a mean value of 88.09% out of which 60.2% belong to air porosity and 27.9% belong to water porosity with high capillary porosity. This is due to the high porosity level which facilitates the retention of oxygen and water in the pores.

The effortless availability of nutrients and water is of great significance for the healthy growth and development of plants. (De Boodt and Verdonck, 1972) and (Fonteno et al, 1981) through their research point to the fact that an ideal substrate should have a TPS or total porous space which exceeds 85%. The pores are filled with air or water depending on their dimension and the contents of the base. The substrates' total porous space is higher than the soils' porous space whose percentage amounts to a quantity which is approximately 50% of the volume. (Michiels et al., 1993) claims in his research that in principle, according to the shape and size of the particles, organic substrates should have a total porosity that amounts to around 85-95% of the volume (Michiels et al., 1993; Raviv at al., 2002) in his research points to the fact that the total porous space in the substrates for plant cultivation should amount to 60-90% in volume. A lot of studies and authors with (Eriksson, 1982) being one of them stressed the importance of the presence of air in the pores for healthy growth of plants and high yield.

The authors (Wesseling and Van Wijik, 1957; Paul and Lee, 1976) stress that there is a general consensus on the fact that the minimal volume of the air porous space for an appropriate air exchange for supporting plant growth should amount to around 10% of the volume. (Brückner U, 1997) underscores in his research that the relative balance of air and water in the pores of the soil space is of crucial significance for the growth of plants.

The analyzed soil sample of fluvial soil in Table 1 also has a high total porosity with a mean value of 77.73% which points to the fact that this type of soil falls in the category of soils which are high in porosity which is an indicator for excellently aeriated soils rich in sand and with less clay which influence high porosity. (Filipovski, 1997) has divided soil types depending on porosity in four categories: quite porous (pores that exceed 60%), porous (45-60%), slightly porous (30-45%) and quite moderately porous (pores whose percentage is below 30%).

Knowing the value of total porosity, the relation between macropores and micropores, the porous system's stability and the total internal surface of the pores has immense practical significance for the soil and the growth of plants. It is not beneficial for the plant when only non - capillary or only capillary pores are present in the soil. In the first case the soil doesn't retain water and in the second all the pores fill with water and enough air isn't available or there is weak aeration. The obtained values from the air porous space of the fluvial soil has a mean value of 38.05% while the water porous space has a mean value of 39.68% which can be explained with the fact that fluvial soil has an optimal water and air regime. (Gajic, 2006) points to the fact that optimal physical and water physical properties and their water – air regime can be obtained when the capillary and non – capillary porosity are in a mutual relation of 1:1 or 2:3. (Filipovski, 1996) claims that the most advantageous relation of porosity occurs when out of the total porosity 60% of the pores are capillary pores and 40% of the pores are non – capillary pores. All the other analyzed samples have displayed optimal water – air porosity.

Out of the analyzed properties of the Mollic Vertic Gleysol soil type and Perlite substrate with their mixtures in Table 2 we can draw the conclusion that the examined trials of total porosity of Mollic Vertic Gleysol have displayed a total porosity with a mean value of 46.99% which points to a soil which is porous. But, the Mollic Vertic Gleysol soil type falls in the category of clay soils with high porosity. Its characteristics are low presence of non – capillary pores with a presence which usually doesn't exceed 8% which makes the water – air regime of these soils disadvantageous. In our research the obtained values from the analyzed properties of water porosity had a mean value of 42.79% and a high capacity of the capillary pores while the analyzed samples of air porosity displayed quite low values with a result of 4.21%. This is due to the high percentage of clay present in that soil, the low presence of non – capillary pores, poor filtration and infiltration with the low diffusion of gasses which characterize poorly aerated soil. The authors (Steffens D, et al., 2005) point to the fact that soils which access to limited conditions for aeration in the inside of the soil's porous volume have an increase in CO_2concentration with a transient increase of pH around the root's absorption system. The author (Spirovski, 1965) achieved similarly low values of air porosity in his research with a value of 6.44%. Because of the higher content of clay, Mollic Vertic Gleysol is falls into the category of heavy soils in which non – capillary pores dominate while clay soils despite the greater content of capillary pores often have a low quantity of easily accessible water because of the high content of micropores (smaller than 3 microns). The water in these micropores isn't easily accessible for the plants. Out of the results in Table 3 it can be easily noted that out of the analyzed properties of Peat substrate the highest result for total porosity stands out with a mean value of 90.8% which defines a high total porous volume. This high percentage of porosity present in Peat is due to the high content of organic matter which can be found in Peat.

With the increase of organic matter the total porosity also increases. Never the less, the mutual water and air regime is disadvantageous because the capillary pores have a mean value of 80.1% which points to a very high content of water capacity while air capacity has a low capillarity or insufficient retention of air with a mean value of 10.7%. All the other analyzed samples in various ratios indicate a different balance between the water and air regime. With adding or mixing of Perlite and Peat, the percentage of air porosity increases. For example, the analyzed sample in a mixture with a ratio Pe20/P80 or 20% Perlite + 80% Peat displays a total porosity with a mean value of 88.63%, which points to high porosity and an advantageous water – air regime. Air capacity has a mean value of 20.6% and water porosity has a high mean value of 68.03%. All the other analyzed samples of mixtures in ratios of Pe50/P50 and Pe70/P30 display different values. By adding a mixture of 50% Perlite and 50% Peat total porosity reaches a mean value of 89.45%, air porosity reaches a mean value of 35.45% while water porosity displays a mean value of 54.0%. These states allow us to claim that the analyzed sample Pe50/P50 has a high total porosity and advantageous water – air capacity. By adding a mixture of 30% Perlite and 70% Peat the total volume of the pores (both capillary and non – capillary) reaches a value of 88.9%.

The water regime is high and filled with capillary pores with a mean value of 63.55%. Non – capillary pores have an advantageous air porosity with a mean value of 25.55%. Similar results to ours were also obtained by the authors (Jeb S, Fields et al., 2014) in their research on the hydrohpysical properties of Perlite and Peat. They reached the following results: total porosity of Peat with a value of 91.0%, air porosity with a value of 10.7%, water porosity of Perlite with a value of 66.4% and air porosity with a value of 12.2%.

CONCLUSIONS

Once more, the analyzed properties of total porosity of Perlite as a substrate have displayed a very high porosity with a mean value of 88.09% out of which 27.9% belong to water porosity which points to a presence of solid capillary porosity while the researched properties of air porosity in Perlite have displayed a very high air capacity with a mean value of 60.2%. That points to the fact that this is a substrate with a high level of superiority for appropriate retention of air whose application can act as a betterment for the increase of aeration of problematic heavy soils which will impact plants directly in their roots when the need for stable supply with oxygen exists. Through the application of Perlite in mixtures in various ratios an influence of the water and air porosity is displayed in the analyzed samples of fluvial soil, mollic vertic gleysol and Peat.

The fluvial soil type also has a high total porosity with a mean value of 77.73%. The obtained values from the air porous space of the fluvial soil type has a mean value of 38.05% while the water porous space has a mean value of 39.68%. This can be explained with the high water and air porosity because of the high quantity of sand and the lower quantity of clay. While it is a characteristic of mollic vertic gleysol soil type to have a low percentage of non – capillary pores with a value which doesn’t exceed 8% which points to a low percentage of air porosity. Water porosity has an average value of 46.99% which points to an advantageous water porosity. This soil type has a disadvantageous ratio of water and air i.e. a weak exchange between the water regime and the aeration regime. Here the positive influence of Perlite substrate on air porosity is the most visible.

There is a drastic improvement of the air porosity in soil. It can be derived out of all of this that Perlite as a substrate improves the soil’s aeration power of soil types with a poorer aeration capacity. It can be derived out of the analyzed properties of Peat substrate that this substrate stands out with the highest total porosity with a mean value of 90.8% that defines a high total porous volume. This high Porosity percentage in Peat is due to the high content of organic matter which can be found in Peat. With the increase of organic matter itself the total porosity increases. Nevertheless, the mutual water and air regime is disadvantageous because capillary pores have a mean value of 80% which points to a very high content of water capacity while air capacity has a low capillarity or insufficient air retention with a mean value of 10.7%. In all the other ratios a different balance between the water and air regime can be noted while with the mixing of Perlite and Peat, air porosity displays a higher percentage.

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PHYTOCHEMISTRY, BIOCHEMICAL AND INSECTICIDAL ACTIVITIES OF *RUTA CHALEPENSIS* ESSENTIAL OILS ON *TRIBOLIUM CONFUSUM*

SUMMARY

The confused flour beetle, *Tribolium confusum* (Coleoptera: Tenebrionidae) is a common pest insect known for attacking and infesting stored flour and grain. Biodegradable and ecologically natural products such as essential oils are emerging candidates for replacement of usually applied chemical pesticides. The essential oils of *Ruta chalepensis* flowering aerial parts were investigated for their contact toxicity and physiological aspects on *T. confusum*.

Essential oils were obtained by hydrodistillation and 56 components were identified by GC-MS. Our results clearly indicated that these compounds exhibited toxicity against *T. confusum* pupae and adults with an LC_{50} value of 0.08 and 0.055 $\mu L/cm^2$, respectively after 7 days of treatment. In repellency assay, essential oils repelled *T. confusum* adults significantly even at 0.06 $\mu L/cm^2$ concentration in an area preference test. Result also showed that maximum exposure time resulted in maximum repellency of the pest at all the concentrations. The repellent activity could be related to the abundance of the 2-undecanone (25.94%) in the oils. In other experiments, the essential oils were investigated on the activities of acetylcholinesterase (AChE) and glutathione *S*-transferases (GSTs). Biochemical analysis demonstrated that the essential oils of *R. chalepensis* induced GSTs and reduced the activity of AChE. Based on these results, essential oils of *R. chalepensis* origin could have greater potential in future in stored-product pest management.

Keywords: Essential oils, phytochemistry, toxicity, Acetylcholinesterase, glutathione S-transferases.

INTRODUCTION

Stored products of agricultural and animal origin are attacked by more than 600 species of beetle pests, 70 species of moths and about 355 species of mites causing quantitative and qualitative losses (Rajendran and Sriranjini, 2008).

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

Stored-grain insect pests have been damaging food grains in granaries and store houses and accounts for 10-40% loss worldwide (Chaubey, 2012). The confused flour beetle, *Tribolium confusum* (Coleoptera: Tenebrionidae) is a very common pest infesting many flour mills, warehouses and grocery stores. It is cosmopolitan and considered one of the major stored-product insects (Garcìa et al., 2005). In Tunisia and North Africa, Jarraya (2003) reported that this insect is amid the most important and destructive pests in mills.

Infestations of stored product insects are typically controlled by the use of synthetic insecticides and fumigants. Currently, phosphine and methyl bromide are the common product worldwide used for stored protection (Suthisut et al., 2011). These insecticides are the most effective applications for the protection of flour mills, grocery shops, warehouses, and other agricultural commodities from stored insect infestation (Park et al., 2004). However, they have a number of associated disadvantages, such as environmental pollution, development of insect resistance and potential toxicity to non target organisms. The use of methyl bromide is being restricted because of its potential to damage the ozone layer (Butler and Rodriguez, 1996; MBTOC, 1998). The Montreal Protocol of the United Nations Environment Program (UNEP 1995) recommends the phasing out of methyl bromide by 2005 in developed countries and by 2015 in developing countries (MBTOC, 1998). Insect resistance to phosphine is a global issue now and control failures have been reported in field situations in some countries (Rajendran and Sriranjini, 2008). Collins et al. (2005) and Pimentel et al. (2009) reported that phosphine is not effective against some insect populations in India, Australia and Brazil, because of resistance. Therefore, today there is a need to develop alternatives that is capable of reducing the large-scale utilization of synthetic pesticides for crop protection.

Research in recent years has been turning more towards selective biorational pesticides. Among these botanical insecticides have attracted the greatest attention and have been reviewed extensively (Abdellaoui et al., 2013). Plants have always been rich source of chemicals and drugs for human and synthesize a wide array of compounds that are generally thought to be involved in plant-insect interactions (Amason et al., 1981). Thus, organic molecules of botanical origin may offer a safe source of compounds for pest management, being environmentally friendly, and an excellent alternative to persistent synthetic insecticides (Abdellaoui et al., 2015).

Among the natural compounds produced by plants, the essential oils appear to directly or indirectly influence the patterns of growth and reproduction of associated phytophagous insects. Essential oils, secondary metabolites that plants produce for their own needs other than for nutrition, have traditionally been utilized to protect stored grain and legumes (Isman, 2000). In recent years, they received a great deal of attention as pest control agents. These complex mixtures, and their individual constituents, have been shown to possess multiple pest control properties, including toxic, repellent, ovicidal, antifeedant and antioviposition effects (Ho et al., 1996; Bakkali et al., 2008; Rajendran and

Sriranjini, 2008). Liu et al. (2013) reported that many essential oils from plants including medicinal herbs, spices and fruits have been evaluated and shown to be effective as pesticides against stored product insects. In addition, they are low toxic to human and animals, volatile and can function as fumigants, and may also be applicable to the protection of stored products (Sahaf et al., 2008).

In an effort to identify novel active natural products derived from plants as alternatives to conventional insecticides, we have studied the contact toxicity and repellency effect of *R. chalepensis***,** medicinal plant which occur in the Tunisia flora, essential oils against *T. confusum*.

MATERIAL AND METHODS

Insect rearing

T. confusum was reared in 2-L plastic containers containing wheat flour mixed with yeast (10:1 w/w). The cultures were maintained in darkness in a growth chamber set at 30±1°C and 60-70% r.h.

Plant materials

Aerial parts of *R. chalepensis* were collected at full-flowering stage during spring season (March-April) from the region of Kairouan (N: 35.67°, E: 10.09°) in the Center of Tunisia. The samples were dried in the shade at room temperature for two weeks. After drying, the samples were ground to a fine powder used for the extraction of essential oils.

Essential oil extraction and identification

The essential oils have been extracted from 100 g air-dried flowering aerial parts of *R. chalepensis* by hydrodistillation for 4 h, using a Clevenger-type apparatus. The essential oils were dried using anhydrous sodium sulphate and then stored in sterile tubes at 4°C until analyses.

Chemical components of essential oils were identified with gas chromatography-mass spectrometry instrument (GCMS-QP 2010 Plus Shimadzu, Japan). RTX-5 ms capillary column 30m x 0.25mm x 0.25μm film thickness was used. The column temperature was initially set at 50°C for 2 min, then gradually ramped at 7°C/min to 250°C and then left at 250°C for 5 min. Injection and detector temperatures were kept respectively at 250 and 280°C. The carrier gas was helium (99.995% purity), the flow through the column was 1.2 mL/min and the split ratio was set to 1:50 with injection of 1 µL of oil sample. The mass spectrometer conditions were as follow: ionization voltage 70 eV, ion source temperature 200°C and the scan range was from 50 to 550 m/z. The identification of the components separated by GC-MS was made by comparing the obtained mass spectra for each component with the values stored in NIST Mass Spectral Library (NIST 08). The percentage composition of the oils was calculated in peak areas using the normalization method.

Contact toxicity

In an effort to determine the toxicity by contact of the *R. chalepensis* essential oils and the median effective time to cause mortality in 50% of tested insects (LT_{50} values), three solutions of essential oils were prepared in acetone.

500 µL of each solution was applied to the surface of filter papers (Whatman No. 1, cut into 5 cm diameter pieces) and homogeneously dispersed, giving a range of concentration of 0.06, 0.12 and 0.25 µL/cm^2. Controls were treated with solvent alone. The solvent was allowed to evaporate for 10 min prior to the introduction of 20 *T. confusum* adults (1-6 days old) or pupae (0-1 day old) selected randomly. In the adult boxes, 0.5 g of food (artificial diet of wheat flour mixed with beer yeast: 10/1 w/w) was added. Control and treated groups were kept under the same conditions described above for mass rearing. Each treatment was replicated four times. The mortality was assessed daily via direct observation for a period of 7 days, and when no antennal movements were observed, the insects were considered dead. Probit analysis (Finney, 1971) was conducted to estimate the LC_{50} and LC_{95} values with their 95% confidence limits. Time-mortality data for each experiment were analyzed via the method developed by Finney (1971), with time as the explanatory variable to derive the estimated time for 50% mortality (LT_{50}).

Repellency bioassay

An area preference method (Zhang et al., 2011) was adopted to assess the repellent activity of *R. chalepensis* essential oils against *T. confusum* adults. Experiments were carried out in glass Petri dishes (diameter 8.5 cm and height 1.2 cm) using concentrations of 0.06, 0.12 and 0.25 µL/cm^2 prepared in acetone. Whatman filter paper was cut into two equal halves and each test solution (500 µL) was applied to filter paper half as uniform as possible using micropipette. The other half of filter paper was treated with acetone only as a control. The treated and control half disks were air-dried to evaporate solvent completely. Both treated and untreated halves were then attached with cellophane tape and placed at the bottom in Petri dish. Twenty adults of *T. confusum* (7-10 days old) were released at the centre of each filter paper disk and then Petri dishes were covered, sealed with parafilm and kept in dark. Five replicates were performed for each tested concentration. The number of insects on both treated and untreated halves was recorded after 1, 2 and 4 h of exposure. Percentage repellency (PR) was calculated according to Nerio et al. (2009) as follows: PR = [(Nc - Nt)/(Nc + Nt)] x 100, where Nc was the number of insects on the untreated area and Nt was the number of insects on the treated area.

Enzymatic assays

Larvae were homogenized in 1 mL of 0.1 M phosphate buffer (pH 7.4) using a Teflon glass tissue homogenizer. Homogenates were centrifuged (15,000 g for 20 min at 4°C) and supernatants were used for enzyme assays.

The acetylcholinesterase activity (AChE) was determined according to the method of Ellman et al. (1961) using the Jenway 6105 spectrophotometer. The reaction medium included sodium phosphate buffer (0.1 M, pH 7.2), DTNB (1.6 mM), AcSChI (156 mM) and sample (S9). Kinetics was recorded at 412 nm and the assay was carried out at 25°C. The enzymatic activity was expressed as nmol of acetylthiocholine hydrolyzed per min per mg of proteins.

The glutathione *S*-transferase (GST) activity was measured by the method of Habig et al. (1974) using 10 mg of cytosolic protein, 1 mM 1-chloro- 2,4-dinitrobenzene (CDNB) (Sigma-Aldrich, Saint Louis, MO, USA) and 4 mM glutathione (reduced form; GSH) in 100 mM sodium phosphate buffer, pH substrate 7.5. GST activity was determined by kinetic measurement at 20°C using a Jenway 6105 spectrophotometer (λ=340 nm). The results were expressed as nmol GSH-CDNB produced per min and per mg proteins. Proteins in the S9 fraction were quantified according to the Bradford (1976) method using Coomassie Blue reagent.

Data analysis

Statistical analysis was performed using Probit analysis (Fienny, 1971) to determine LC_{50} and LC_{95} values with their 95% confidence limits. Data from repellency and enzymes activities were expressed as means ± standard deviation (SD) and subjected to analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS: version 18.0). The significance between control and treated series was made by Student-Newman-Keuls (*SNK*) test at the 5% level.

RESULTS AND DISCUSSION

Chemical composition of essential oils

The essential oils yield of *R. chalepensis* flowering aerial parts was 0.87 ± 0.05% (V/W) on the basis of dry matter weight. The results of the chemical analysis are shown in Table 1. A total number of 56 components were identified by GC-MS. The main components of *R. chalepensis* essential oils were 2-Octanol acetate (30.98%), 2-Undecanone (25.94%), 2-Nonanone (16.28%) and 5-Dodecanone acetate (9.35%). Major compounds were followed by others components with lower percentages which are 2-Nonanol (2.54%) and 2-Decanone (2.42%) (Table 1).

Contact toxicity bioassay

Contact toxicity after 7 days of treatment was determined. LC_{50} and LC_{95} values, and their 95% confidence limits expressed as microliter per square centimeter are shown in Table 2. The evaluation of contact toxicity data revealed that *R. chalepensis* essential oil was toxic for both pupae and adults of *T. confusum*. Probit analysis showed that *T. confusum* pupae were more susceptible to *R. chalepensis* essential oil than adults. The corresponding LC_{50} and LC_{95} values were respectively 0.05 and 0.12 $\mu L/cm^2$ against 0.08 and 0.2 $\mu L/cm^2$ (Table 2). Our experiments also showed that the essential oil of *R. chalepensis* flowering aerial parts exhibited acute toxic effects especially when applied at the two highest concentrations (0.12 and 0.25 $\mu L/cm^2$).

Bioassay was also designed to determine median effective time to cause mortality of 50% of treated insects (LT_{50}). Considerable differences in LT_{50} values were noted with different concentrations and developmental stage. Results in Table 3 show that pupae were significantly more susceptible than adults. For pupa stage, LT_{50} values ranged from 3.75 days for the lowest dose (0.06 $\mu L/cm^2$)

to 1.04 days for the highest dose (0.25 µL/cm^2). With adults, the LT_{50} values ranged from 7.6 days to 1.16 days for the lowest and highest doses, respectively.

Table 1. Chemical constituents of the essential oils from *R. chalepensis* flowering aerial parts collected from Kairouan, Tunisia.

No.	Compounds	Retention time	%
1	2-Hexanal, (E)-	5.135	0.18
2	Butanoic acid, 2-methyl-,1-methylethyl ester	5.873	0.16
3	2-Octanone	8.301	0.35
4	2-Octanol, (R)-	8.524	0.12
5	2-Heptanol, acetate	9.508	0.43
6	2-Nonanone	10.848	16.28
7	2-Nonanol	11.039	2.54
8	Nonanal	11.110	0.19
9	1,3-Cycloheptadiene	11.995	0.69
10	2-Decanone	13.053	2.42
11	2-Octanol, acetate	14.133	30.98
12	2-Tridecanone	14.468	1.72
13	2-Undecanone	15.389	25.94
14	Acetic acid, nonyl ester	15.596	0.13
15	2-Heptanol, acetate	16.035	0.37
16	2-Dodecanone	16.706	1.84
17	5-Dodecanol acetate	18.034	9.35
18	Lauryl acetate	19.278	0.16
19	(E)-2-Decenyl acetate	19.893	0.39
20	6-Dodecanol acetate	20.300	0.78
21	4-(3,4-Methylenedioxyphenyl)-2-butanone	21.238	0.28
22	Propanedioic acid, (phenylmethyl)-, diethyl ester	23.677	0.36
23	1, 3-Benzodoxole, 5-propyl-	24.802	0.89
24	1-Adamantaneacetic acid	28.179	0.31
25	Phytol	29.077	0.12
26	Pyrrolo [3, 2-g] quinolone, 9-methoxy-2, 3, 5, 7-tetramethyl-	30.295	1.47
27-56	Other components	-	1.59
EO yields (%)			0.87 ± 0.05

Table 2. Toxicity of *T. confusum* pupae and adults treated with *R. chalepensis* essential oil in contact toxicity bioassay.

Insects	LC_{50} [a, b]	LC_{95} [a, b]	Chi square ($\chi 2$)	df	*P*-value
Adults	0.08 (0.01- 0.16)	0.20 (0.13 - 0.64)	7.52	2	0.02
Pupae	0.055 (0.02 - 0.09)	0.12 (0.1 - 0.29)	6.22	2	0.04

[a] Units LC_{50} and LC_{95}=µL/cm^2.
[b] 95% lower and upper fiducial limits are shown in parenthesis.

Table 3. LT_{50} values of *R. chalepensis* essential oil against pupae and adults of *T. confusum.*

Insects	Concentration (µL/cm^2)	LT_{50} (days)[a]	df	Chi square ($\chi 2$)
Adults	0.06	7.6 (6.82-8.81)	5	2.04
	0.12	4.34 (3.99-4.72)	5	1.25
	0.25	1.16 (0.41-1.70)	5	3.37
Pupae	0.06	3.75 (3.06-4.39)	5	3.66
	0.12	1.48 (0.84-1.95)	5	8.05
	0.25	1.04 (0.77-1.25)	5	9.68

[a] 95% lower and upper fiducial limits are shown in parenthesis.

In our observation, *R. chalepensis* essential oil was characterized by typical neurotoxic symptoms including hyperactivity, convulsion, and paralysis with mortality of treated adults. In the pupal stage, we observed other syndromes of intoxication resulting in malformations that can affect the whole body of the insect, and a change in color that becomes darker with the appearance of some necrotic areas (Fig. 1A).

Figure 1. Toxicity effects of *R. chalepensis* essential oils on *T. confusum* pupae (A) and disruption of adult emergence due to the impossibility to reject the old integuments (B).

We also noted exuviations difficulties due to the impossibility to reject the old integuments causing mortality at the beginning of the adult stage and increased percentage of abnormal insects emerging (Fig. 1B).

Repellency bioassay

The results of the repellent activity of the essential oils from *R. chalepensis* flowering aerial parts against *T. confusum* adults were presented in Table 4. The percentage repellency (PR) was determined as function of the oil concentrations and the periods of exposure. As illustrated in table 4, in the binary choice bioassays, the essential oil affects significantly the distribution of insects between the treated and untreated areas and caused acute repellent activity even at the low concentrations tested. The PR values were higher than 50% at the testing concentrations from the first hour of exposure. The lowest concentration (0.06 $\mu L/cm^2$) led to percentage repellency of 52 ± 8.36% at 1 h after exposure, but it increased to 72 ± 10.95% after 4 h. The repellent activity becomes more evident by increasing the concentration of the oil and the maximum PR, independently to the period of exposure, was observed with the highest concentration tested (0.25 $\mu L/cm^2$). Indeed, with this latter, repellency was arranged between 78 ± 14.83 and 100% for respectively 1 and 4 h of exposure (Table 4). The analysis of variance with the oil concentrations as classification criteria shows a significant difference among treatments and the *SNK*-test gives heterogeneous groups represented by different letters in Table 4. Moreover, it was found that PR values are widely correlated to the exposure times and the most effect was noted after 4 h of exposure with all concentrations applied. The ANOVA analysis indicated that for each tested concentration, significant differences ($P<0.05$) were obtained between the time periods evaluated. Therefore, the repellent activity of essential oils could be related to the testing concentrations and the exposure duration (Table 4).

Table 4. Percentage repellency (mean ± SD) of *R. chalepensis* essential oils against *T. confusum* adults after various periods of exposure.

Concentrations ($\mu L/cm^2$)	1h	2h	4h
0.06	$52 \pm 8.36^{a,A}$	$66 \pm 5.47^{a,B}$	$72 \pm 10.95^{a,B}$
0.12	$74\pm 13.41^{b,A}$	$84 \pm 11.4^{b,AB}$	$98 \pm 4.47^{b,B}$
0.25	$78 \pm 14.83^{b,A}$	$94 \pm 8.94^{b,B}$	$100 \pm 0^{b,B}$
F-value	6.25	12.58	26.14
P-value	0.014	0.001	<0.001

Within column, comparison was made between concentrations (letter in lowercase). Within rows, comparison was made between exposure times for each concentration (letter in uppercase). Means followed by same letter were not statistically different by *SNK* test at $P<0.05$.

AChE inhibition assay

R. chalepensis essential oils were screened for their AChE inhibitory effects at different concentrations. Results are summarized in Table 5. In control, the mean AChE activity remained constant during the experimental period. Values ranged from 1.039 ± 0.08 nM/min/mg proteins at the first day to 1.14 ± 0.06 nM/min/mg protein at day 3. However, in the treated series, ANOVA revealed a significant effect (P=0.000 after 72h of exposure) of the essential oils on the AChE activity which varied as function of the dose and the duration of treatment. As compared to control, the mean values recorded during the experimental period decreased by raising the concentration of the oil until reaching 40.6 and 50.88 % inhibition after 72 hours of exposure at 0.12 and 0.25 µL/cm^2, respectively. Data also indicated that the highest concentration had the greatest inhibitory activity at all the time periods evaluated and there was no significant difference between the two elevated concentrations (Table 5).

Table 5. Effect of *R. chalepensis* essential oils on the activity of acetylcholinesterase (means ± SD, nM/min/mg proteins) in surviving insects as function of the concentration and the exposure time.

Exposure time	Control	EO Concentrations (µL/cm^2)			P-value
		0.06	0.12	0.25	
24h	1.03±0.08^a (100)	0.93±0.12^a (89.5)	0.73±0.02^b (70.25)	0.68±0.05^b (65.44)	0.003
48h	1.01±0.07^a (100)	0.92±0.1^a (91.08)	0.72±0.07^b (71.28)	0.60±0.04^b (59.4)	0.001
72h	1.14±0.06^a (100)	0.64±0.13^b (56.14)	0.70±0.06^b (61.4)	0.56±0.05^b (49.12)	<0.001

For each exposure time, mean values followed by different letters are significantly different (P<0.05). Values in parentheses indicate per cent change with respect to control taken as 100%.

Glutathione *S*-transferases activity

As illustrated in table 6, the *R. chalepensis* essential oils caused significant change in the GST activity with a dose-response relationship.

Table 6. Effect of *R. chalepensis* essential oils on the activity of of glutathione *S*-transferases (means ± SD, nM/min/mg proteins) in surviving insects as function of the concentration and the exposure time.

Exposure time	Control	EO Concentrations (µL/cm^2)			P-value
		0.06	0.12	0.25	
24h	2.36±0.38^a	5.34±0.42^b	7.86±1.32^c	9.49±0.94^d	<0.001
48h	1.32±0.22^a	5.77±0.88^b	8.08±0.84^c	11.1±1.27^d	<0.001
72h	3.3±0.24^a	8.01±1.31^b	9.06±0.54^{bc}	10.53±1.21^c	<0.001

For each exposure time, mean values followed by different letters are significantly different (P<0.05).

A significance increase in the GST activity was recorded with the three tested doses starting day 1 of oils application. The enzymatic activity of the treated larvae varied significantly ($P<0.05$) among themselves and also when compared to the control. The maximum increase, independently to the exposure duration, was observed with the highest concentration tested (0.25 µL/cm^2). Indeed, the GST activity recorded in the treated group at this concentration is 11.1 ± 1.27 and 10.53 ± 1.21 nM/min/mg proteins respectively for 48 and 72 h of exposure. However, we measured in control insects 1.32 ± 0.22 and 3.3 ± 0.24 nM/min/mg proteins, respectively to the same exposure times (Table 6).

DISCUSSION

Some plant extracts and phytochemicals are known to possess insecticidal activity against various insect pests in stored-product and the essential oils extracted from aromatic plants have been widely investigated in this connection. Use of plant oils and its components as pesticide has received much attention of the scientific communities in pest management programme (Chaubey, 2012). In the previous studies, Tapondjou et al. (2005) evaluated contact toxicity of the essential oils extracted from *Eucalyptus saligna* and *Cupressus sempervirens* leaves by impregnation on filter paper discs against *T. confusum*. They claimed that these chemicals caused significant mortality with LC_{50} values of 0.48 and 0.74 µL/cm^2, respectively for *Eucalyptus* and *Cupressus* oils. Similarly, Russo et al. (2015) indicated that essential oils from *E. globulus* showed interesting insecticidal properties against *T. confusum* adults. Contact toxicity increased according to exposure time and concentration. Indeed, data showed that, at 4h exposure, essential oils applied at the lowest concentration (0.5 µL/cm^2), exhibited the toxicity of 63.33%, while a dose of 0.75 µL/cm^2 was required to obtain 100% mortality. However, after 12h of exposure, both concentrations killed all tested insects. In their study on the insecticidal activity of the essential oils of three *Chrysanthemum* species growing in Tunisia (*C. coronarium*, *C. fuscatum*, and *C. grandiflorum*) against *T. confusum*, Houas et al. (2012) reported that these chemicals caused an antifeeding effect and a high mortality (80%) of *T. confusum* larvae. The most effective essential oil was obtained from the leaves of *C. grandiflorum*.

Consulting many papers on this thematic, we can deduce that compared with the other essential oils in the literature, the essential oil of *R. chalepensis* flowering aerial parts possessed stronger contact toxicity against *T. confusum* adults, e.g. essential oils of *E. saligna* and *C. sempervirens* (LC_{95} = 1.2 and 2.42 µL/cm^2 calculated for mortality within 3 days of exposure, respectively) (Tapondjou et al., 2005).

In the present study, *R. chalepensis* essential oils have been also evaluated for their repellency. It appears that these compounds repelled *T. confusum* adults significantly even at the lowest concentration used. Similar repellent effects from other plant extracts have been reported. For example, essential oils extracted from *E. camaldulensis* (Myrtaceae) repelled *T. confusum* adults in an area preference test (Huang et al., 1997). *Evodia rutaecarpa* (Rutaceae) essential oils also had a repellent effect on *T. castaneum* adults and reduced the growth rate of larvae (Liu and Ho, 1999). Also, Tripathi et al. (1999) reported that fruit oil of *Piper retrofractum* exhibited high repellency against *T. castaneum* (52, 76 and 90%) at 0.5, 1 and 2% concentrations. In the same context, Naseem and Khan (2011), reported in their study on the repellency of the essential oils of *P. nigrum* and *E. camaldulensis* against *T. castaneum* under laboratory conditions that maximum exposure time resulted in maximum repellency of the pest at all the concentrations.

Repellency of *R. chalepensis* essential oils against *T. confusum* adults appears to be related to the presence of 2-undecanone among the major compounds. In 2007, the arthropod repellent BioUD® (7.75% 2-undecanone) was registered by the US Environmental Protection Agency for use against mosquitoes and ticks. The active ingredient in BioUD® is the 11-carbon methyl ketone, 2-undecanone that was originally isolated from the glandular trichomes on the stems and foliage of the wild tomato plant, *Lycopersicon hirsutum* Dunal *f. glabratum* (Farrar and Kennedy, 1987). Resistance of *L. hirsutum f. glabratum* to insect herbivory is due in part to the presence of 2-undecanone (Kennedy, 2003). Witting-Bissinger et al. (2008) showed that BioUD® was an efficacious repellent against *Dermacentor variabilis* (Arachnida: Ixodidae) in laboratory studies on treated human skin, cloth, and filter paper. Additional research was conducted by Witting-Bissinger et al. (2009) to evaluate the repellency of BioUD® against three tick species (Acari: Ixodidae): *Amblyomma americanum*, *D. variabilis*, and *Ixodes scapularis*, important in disease pathogen transmission and to determine quantitative differences with DEET (*N,N* diethyl-*m*-toluamide) (98.11%), the most extensively used active ingredient in commercial arthropod repellents. A two-choice bioassay between repellent-treated and untreated filter paper surfaces was used under controlled laboratory conditions. The results showed that BioUD® is at least 2-4 times more active as a repellent than DEET against the three species of ixodid ticks.

In a second series of experiments, the essential oils of *R. chalepensis* were investigated on the activities of acetylcholinesterase (AChE) and glutathione *S*-transferases (GSTs). Data showed that the compound induced GSTs and reduced the activity of AChE. The GSTs are known to play a central role in the detoxification of both endogenous and xenobiotic compounds and are involved in intracellular transport and various biosynthetic pathways (Che-Mendoza et al. 2009). They play an important role in insecticide resistance and are implicated in the metabolism of organophosphorus and organochlorine compounds (Fang, 2012). Other xenobiotics such as plant secondary metabolites induce GST activity in phytophagous insects, and similarly in predators that feed on these herbivores (Vanhaelen et al. 2004, Acheuk et al., 2018). AchE has a key role in neurotransmission by hydrolyzing the neurotransmitter acetylcholine in cholinergic synapses of the nervous system and is the target site of several neurotoxic insecticides. The essential oils of *R. chalepensis* exhibited a neurotoxic action in *T. confusum* resulting in convulsions, lack of mechanical coordination and tremors. Several previous studies reported that rapid action of essential oils against insect pests is an indicative of neurotoxic actions. Bessette et al. (2013) noted that in direct contact, essential oils may penetrate via insect's cuticle and contact the nerve endings in the invertebrate pest's trachea, and cause neurotoxic activity and more rapid death. The neurotoxic modes of action on insects are mainly related to AChE levels and several reports have demonstrated the interference of essential oils or its constituents with AChE enzyme activity in insects (Yeom et al. 2013). However, it is suspected that, in addition to AChE inhibition, the monoterpenes may act on other vulnerable sites (e.g. cytochrome P450-dependent monooxygenases) (Rajendran and Sriranjini, 2008). In fumigant toxicity tests with monoterpenes (limonene, linalool, menthol, menthone, α-pinene, *β*-pinene) against *Sitophilus oryzae* adults, Lee et al. (2001) did not find a direct correlation between insect toxicity and AChE inhibition.

As we have shown previously, *R. chalepensis* essential oils induced GSTs activity in *T. confusum*. Similarly, Vanhaelen et al. (2004) showed that Brassicacea secondary metabolites induced GST activity in *Myzus persicae* and several Lepidopteran species such as *Heliothis virescens* and *Trichoplusia ni*. By treating *Artemisia annua* extracts on *Eurygaster integriceps* adults, Zibaee and Bandani (2010) also reported that activity level of GST in 24 h post-treatment increased significantly.

CONCLUSIONS

The development of natural insecticides would help to decrease the negative impact of synthetic insecticides, such as residues, resistance, and environmental pollution. In the present study, *R. chalepensis* essential oils showed strong contact toxicity against *T. confusum*. Based on these findings, it can be suggested that essential oils of plant origin could have greater potential in future on the basis of their efficacy, economic value and use in large-scale storage.

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COMMUNITY FOREST FOR GLOBAL WARMING MITIGATION: THE TECHNIQUE FOR ESTIMATION OF BIOMASS AND ABOVE GROUND CARBON STORAGE USING REMOTE SENSING METHOD

SUMMARY

This study aims to present remote sensing methods for the analysis and comparison of different physical parameters for estimation of biomass and above ground carbon storage in the study areas of the pilot project for the chosen community forest in Maha Sarakham Province, Thailand. The implementation method was divided into four steps as follows. (1) Analysis of Landsat-8 satellite data with six physical parameters in the study (NDVI, TNDVI, GVI, IR-R, IR/R, and MSAVI2) and analyzing the results obtained to find out Fraction Cover (FC) value, (2) Field surveying by creating survey plots, measuring the circumference and the height of trees, and bringing the results to calculate above ground biomass with allometric equations (3) Creating correlation equations from the analysis results from Step 1 and Step 2, then applying the correlation equations obtained to calculate Carbon Dioxide (CO_2) storage by using remote sensing methods, and (4) Comparing optimal methods from six physical parameters. The analysis results of six physical parameters were found that GVI was the most appropriate method, the correlation equation $y = 1.8312e^{0.0168x}$, and the coefficient of determination (R^2) = 0.8107. And the CO_2 storage capacity of the community forests in the study areas in Maha Sarakham Province was equal to 106.04 tonCO_2e from the total area of 61.24 ha.

Keywords: Community Forest, Remote Sensing, Biomass, Above Ground Carbon Storage

INTRODUCTION

Environmental issues have gained widespread attentions from all regions of the world, especially the problem of global warming (Ghent et al., 2011; Jones et al., 2012; Gomasathit et al., 2015; Hawkins et al., 2017). The main cause of this problem is greenhouse gases that store some of solar heat in the earth and do not reflect into the atmosphere (Lee et al., 2009). At any rate, Intergovernmental Panel on Climate Change (IPCC) of The United Nations had concluded that, from the observation of the average global temperature increase in the mid-20th century, it is quite evident that the increase in greenhouse gas emissions is caused

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by human activities and results in the phenomenon of greenhouse gases (Goody and Yung, 1989). The solution for this problem is to reduce greenhouse gas emissions. Ones of the most acceptable methods are reforestation and forest management for conservation purposes due to verdant forests can effectively absorb CO_2, which is one of greenhouse gases (Vicharnakorn et al., 2014). Forests are directly related to the amount of CO_2 that is a major greenhouse gas. In other words, forests play a role as a source and a sink. It depends on the land use change and ecological characteristics of each forest. In the growth of trees, there is a process of photosynthesis or CO_2 absorption in the forest which changes CO_2 into biomass above ground (stem, branches and leaves) and below ground (roots) (Ogawa et al., 1965). In addition, forests also play an important role in CO_2 cycle on the earth's surface. In each year, forests will absorb CO_2 approximately at 2.6 billion tones, while deforestation or land use changes generate approximately 1.6 billion tons of CO_2 or equivalent to 5.9 billion tons of CO_2 (IPCC, 2006).

Previously, the amount of CO_2 storage in the forest areas surveyed by people took quite a long time, especially in large areas of forests (Senpaseuth et al.,2009; Liaghat and Balasundram, 2010). Therefore, remote sensing technology had been applied to help evaluate CO_2 storage (Gibert et al., 2008; Anindya and Yadavand, 2012; Robinson et al., 2013; Vagen and Winowiecki, 2013; Han et al., ;Laosuwan and Uttaruk, 2016).Currently, the study of remote sensing technology is on the progress so that the study of phenomena on the earth’s surface can be implemented easier and the data obtained are quite real-time. As a result, there are many researchers applied the information from remote sensing technology widespread due to it is suitable in many aspects, namely wide range of data surveying, spatial resolution, spectral resolution, and multi temporal resolution with appropriate costs (Jiao et al.,2016;Yu et al.,2017).At any rate, the researches related to estimation of CO_2 storage in community forests are hardly found in Thailand, especially the issue of community forests in Northeastern Thailand. For this reason, this study aims to present a technique for the analysis and comparison of different physical parameters for estimation of biomass and above ground CO_2 storage in community forests in Maha Sarakham Province by using remote sensing methods.

MATERIAL AND METHODS

Study areas and data collection

- *Study areas:* Community forests (Figure 1) in Maha Sarakham Province in Northeastern Thailand were chosen to be pilot areas in this study including (1) the community forest in Nondaeng Sub-district, Borabue District, (2) the community forest in Nong Ruea Sub-district, Wapi Pathum District, (3) the community forest in Na Kha Sub-district, Wapi Pathum District, and (4) the community forest in Khwao Rai Sub-district, Kosum Phisai District. All these community forests have a total area of 61.24 ha.

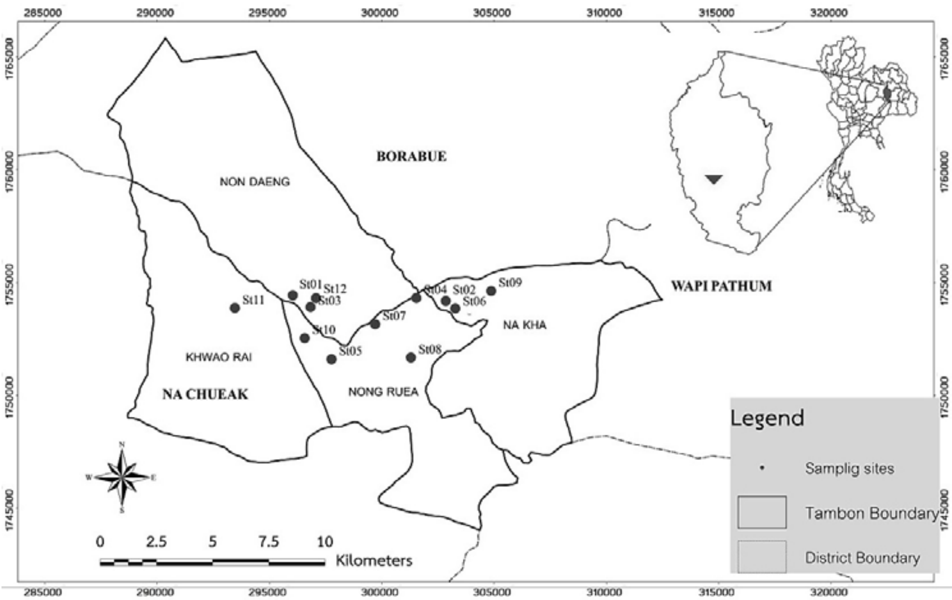

Figure 1. Community forests and 12 plots in order to be samples of the study areas

- ***Data collection:*** This study used the data from Landsat-8 satellite, path 127 and low 49 by recording the data on 29 November 2017.

Implementation methods

The implementation methods were divided into 4 main steps as follows.

Analysis of Landsat-8 satellite data

- ***Preparing data before analysis:*** Electromagnetic waves that travel from the sun to the top of the atmosphere (ToA) will depend on the distance between the earth and the sun including the angle of incidence. Some parts of electromagnetic waves will cause some phenomena, such as scattering, by air molecules, clouds and dust. And some will partially have absorbed by ozone, gases, dust and clouds. The rest will reflect objects on the earth’s surface back into the satellite data recorders. This phenomenon may result in erroneous recording of satellite data. To reduce the effect of electromagnetic waves from the mentioned phenomena, this research aims to adjust the top of atmosphere (ToA) for data accuracy in two steps: (1) Converting digital number to radiance and (2) Converting radiance to ToA reflectance (Laosuwan and Uttaruk, 2014).

- ***Satellite data analysis*:** Analyzing Landsat-8 satellite data with six physical parameters including Normalized Difference Vegetation Index (NDVI), Transformed Normalized Difference Vegetation Index (TNDVI), Green Vegetation Index (GVI) (Gandia et al., 2004), Difference Vegetation Index (DVI) or IR-R (Tucker, 1979; Agapiou et al., 2012), Simple Ratio (SR) or IR/R (Jordan, 1969), and Second Modified Soil Adjusted Vegetation Index (MSAVI2) (Qi et al., 1994) (Equation 1 to Equation 6) and bringing the results from these six physical parameters to find out FC value (Uttaruk and Laosuwan, 2016) as shown in Equation 7.

$$NDVI = \frac{NIR - RED}{NIR + RED} \qquad (1)$$

Where;
NIR = Near infrared band of Landsat 8 OLI
R = Red band of Landsat 8 OLI

$$TNDVI = ((NIR - RED)/(NIR + RED) + 0.5) \qquad (2)$$

Where;
NIR = Near infrared band of Landsat 8 OLI
R = Red band of Landsat 8 OLI

$$DVI = NIR - RED \qquad (3)$$

Where;
NIR = Near Infrared Band
RED = Red band

$$SR = NIR / RED \qquad (4)$$

Where;
NIR = Near Infrared Band
RED = Red band

$$\begin{aligned} GVI = {} & (0.2848 \times B1) - (0.2435 \times B2) - (0.5436 \times B3) + \\ & (0.7243 \times B4) + (0.0840 \times B5) - (0.1800 \times B7) \end{aligned} \qquad (5)$$

Where;
$B1$ $B2$ $B3$ $B4$ $B5$ $B7$ = band of Landsat 8 OLI

$$MSAVI2 = \frac{(2NIR + 1) - \sqrt{(2NIR + 1)^2 - 8(NIR - RED)}}{2} \qquad (6)$$

Where:
$MSAVI2$ = Vegetation Index
NIR = Near Infrared Band Reflectance
RED = Red Band Reflectance

$$FC = \frac{(VI - VI_{open})}{(VI_{canopy} - VI_{open})} \times 100 \qquad (7)$$

Where;
FC = Tree canopy fractional cover
VI = Vegetation index
VI_{open} = Vegetation index of open areas
VI_{canopy} = Vegetation index of tree canopy

Field Survey

- *Measurement of trees:* The sample plots 20 m x 20 m were arranged in the study areas in a total of 12 plots in order to be samples of the study areas of 61.24 ha. Then, the data of types and the number of large trees with diameters at Breast Height (DBH) of 4.5 cm and above, and the height of trees were measured and collected by using Clinometer. All the data collected in this study will be recorded in a record form for further analysis.

- *Above ground biomass calculation:* The field survey data were used to calculate quantitative ecological characteristics based on the height and diameter of trees and represented in the allometric equation (Ogawa et al., 1965). For the amount of above ground biomass dry dipterocarp forest was calculated in the stem, branches and leaves. The amount of biomass of above ground trees obtained was calculated to estimate the amount of CO_2 (Ogawa et al., 1965).

Creating statistical correlation equations

This step created correlation equations from the analysis results of Step 1 and Step 2. Then, the correlation equations obtained were calculated to estimate the amount of CO_2 storage in the study areas.

Comparison of appropriate implementation methods

This step compared the analysis results of the data from six physical parameters including NDVI, TNDVI, GVI, IR-R, IR/R, and MSAVI2. Then, the most appropriate method will be chosen to calculate the amount of CO_2 storage in the study areas.

RESULTS AND DISCUSSION

Result

- Analysis of Landsat-8 satellite data

Analyzing Landsat-8 satellite data with six physical parameters including NDVI, TNDVI, GVI, IR-R, IR/R, and MSAVI2 and bringing the results from these six physical parameters to find out FC value as shown in Figure 2. From the Figure 2, display that the location of the brightest area shows the area covered by the vegetation. On the other hand, gray to black areas have low vegetation cover and no vegetation cover respectively.

-Field survey results

From the sample plots of the study areas in a total of 61.24 ha and the tree measurements as mentioned above, the researchers brought the results to analyze the amount of biomass by allometric equations and converted biomass to carbon. The results of the calculation are shown in Table 1.

-Creating statistical correlation equations and data analysis

Creating statistical correlation equations between Landsat-8 satellite data and field data was to find out the correlation between FC value analyzed by six physical parameters (NDVI, TNDVI, GVI, IR-R, IR/R, and MSAVI2) and the amount of biomass analyzed from the field data. Then, the correlation equations obtained were calculated to estimate the amount of CO_2 storage. The results of creating statistical correlation equations are shown in Figure 3 and Landsat-8 satellite data analysis can be displayed as shown in Figure 4.

Table 1. Field survey results

Plot name	Area (ha)	Carbon value tons/ha
1	05.44	00.86
2	04.32	10.34
3	06.40	01.76
4	08.16	02.88
5	04.00	00.61
6	03.20	01.16
7	11.20	01.84
8	06.72	01.62
9	02.56	04.43
10	02.40	02.02
11	03.20	00.42
12	03.64	00.68
Total	61.24	28.63

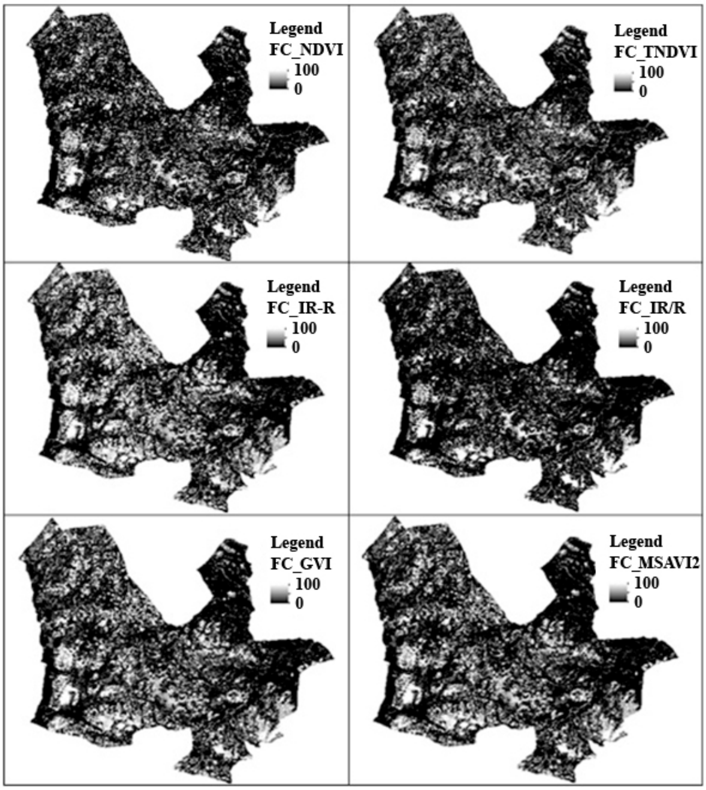

Figure 2. Analyzing Landsat-8 satellite data with six physical parameters

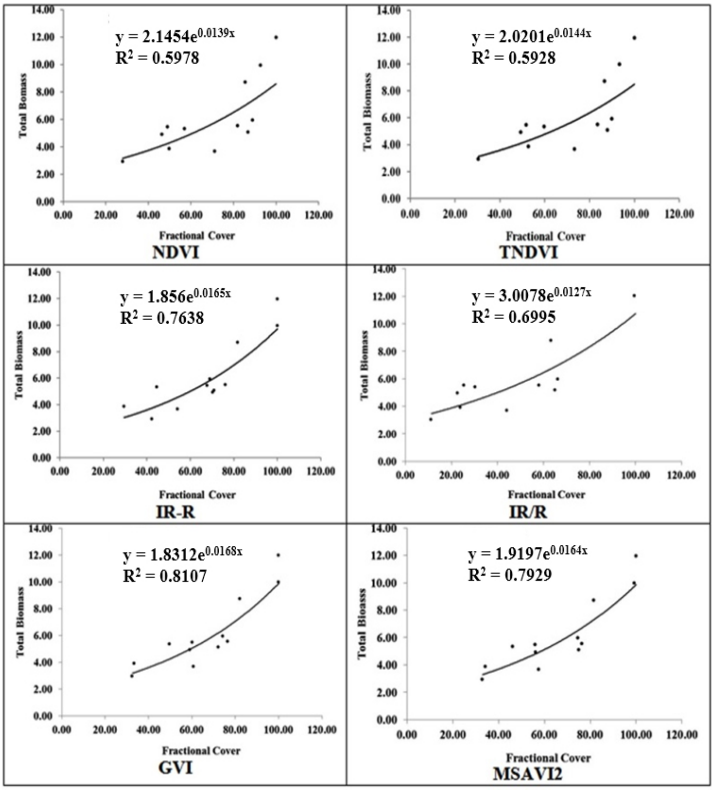

Figure 3. Relationship of the statistical data

-Comparison results of the appropriate methods

The comparison results of the six physical parameters in this study (NDVI, TNDVI, GVI, IR-R, IR/R, and MSAVI2) can be explained as follows: 1) The physical parameter NDVI obtained the correlation equation $y = 2.1454e^{0.0139x}$ and coefficient of determination (R^2) = 0.5978. As a result, the amount of biomass can be estimated at 25.43 tC/ha and the CO_2 storage in the study areas can be estimated at 93.24 tonCO_2e. 2) The physical parameter TNDVI obtained the correlation equation $y = 2.0201e^{0.0144x}$ and coefficient of determination (R^2) = 0.5928. As a result, the amount of biomass can be estimated at 25.95 tC/ha and the CO_2 storage in the study areas can be estimated at 95.15 tonCO_2e. 3) The physical parameter GVI obtained the correlation equation $y = 1.8312e^{0.0168x}$ and coefficient of determination (R^2) = 0.8107. As a result, the amount of biomass can be estimated at 28.91 tC/ha and the CO_2 storage in the study areas can be estimated at 106.04 tonCO_2e. 4) The physical parameter IR-R obtained the correlation equation $y = 1.856e^{0.0165x}$ and coefficient of determination (R^2) = 0.7638. As a result, the amount of biomass can be estimated at 28.33 tC/ha and the CO_2 storage in the study areas can be estimated at 103.87 tonCO_2e. 5) The physical parameter IR/R obtained the correlation equation $y = 3.0078e^{0.0127x}$ and coefficient of determination (R^2) = 0.6995. As a result, the amount of biomass can be estimated at 26.26 tC/ha and the CO_2 storage in the study areas can be estimated at 96.32 tonCO_2e. 6) The physical parameter MSAVI2 obtained the correlation equation $y = 1.9197e^{0.0164x}$ and coefficient of determination (R^2) = 0.7929. As a result, the amount of biomass can be estimated at 28.01 tC/ha and the CO_2 storage in the study areas can be estimated at 102.70 tonCO_2e.

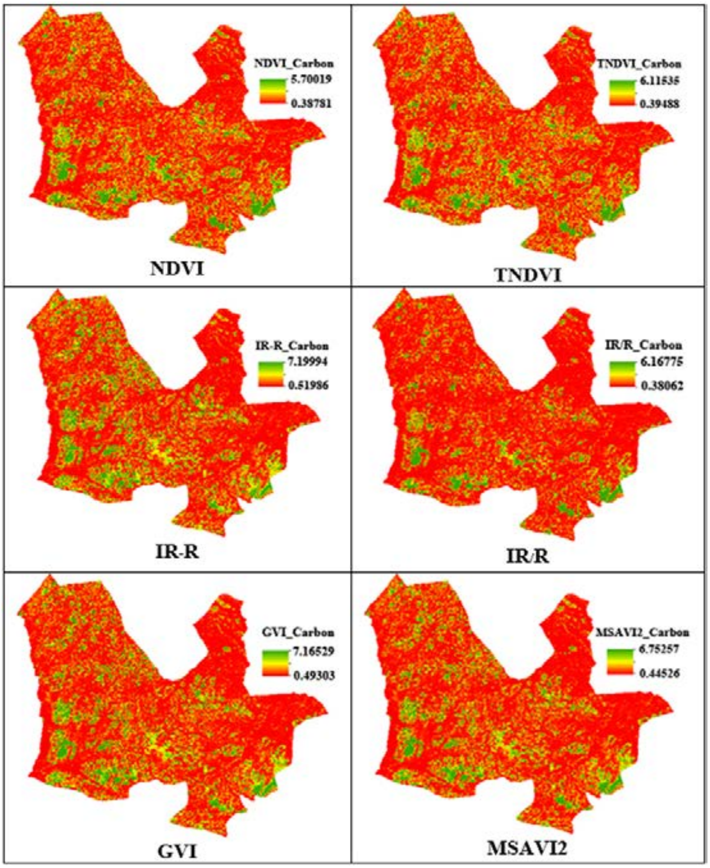

Figure 4. The spatial data after substituting value

For the analysis to find the appropriateness of the assessment of biomass and carbon storage above the community forest, considering from coefficient of determination R^2 received from the relationship between FC analyzed from the six patterns of physical parameters and the volume of biomass in the field, coefficient of determination R^2 from GVI physical parameter has the highest value and therefore it is the most appropriate method. GVI will lead to $y = 1.8312e^{0.0168x}$ with coefficient of determination $R^2 = 0.8107$. It can calculate the volume of CO_2 storage at 106.04 tonCO_2e. Regarding tCO2e/ha, it was found that the volume of CO_2 = 1.73 tCO2e/ha. As compared the result with the research of dry dipterocarp forest in Thailand by Boonsang and Arunpraparat (2011), it was discovered that the volume of CO_2 storage is 200.49 tCO2e/ha.

Meanwhile, the work of Vicharnakhon et al., (2014) found that the volume of carbon storage is 82.70 tCO2e/ha and the work of Ounkerd et al (2015) is 391.42 tCO2e/ha. The three research articles studied the large single patch forest type which differs from this study, which means the type of this community forest is small forest patch surrounding with paddy field and another land use types (small scatter forest patch) that was counted as the common divisor. For this reason, the result of the volume of carbon storage, tCO2e/ha, is quiet low. Moreover, Paired Samples Test was applied to test the statistical significance between field data and data analyzed from the 6 physical parameters which found that the statistical significance is at 95%.

CONCLUSIONS

The purpose of this study was to present the technique to analyze and compare the different physical parameters for the above ground assessment of biomass and carbon storage in a pilot study of the community forest in Maha Sarakham Province, using the data received from Landsat-8 satellite and six patterns of physical parameters including field study to create equation model of relationship before the assessment of biomass and carbon storage. According to the study of the above-ground assessment of biomass and carbon storage for the forest community in 12 studying fields, the forest community is classified as dry dipterocarp forest. As in the survey, 31 families of plants and 51 types were founds. The total number of trees is 692 and the average DBH is 8.54 cm while the average height is 7.10 m. Considering the study, the assessment of biomass and carbon storage requires no field study in all areas which will reduce the expense of field study and also reduce the time of researching while the data will be up-to-date and respond the demand of data in the urgent time.

ACKNOWLEDGEMENTS

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THE EFFECT OF CARBON SOURCE CONCENTRATION ON TOXIGENESIS AND LIPASE ACTIVITY OF *Penicillium aurantiogriseum*

SUMMARY

Penicillium aurantiogriseum (P. aurantiogriseum) is a species frequently contaminates olives and other foodstuffs including cereals and their derivatives. Because of its secondary metabolites, *P. aurantiogriseum* is used in the food and pharmaceutical industries. The aim of this work is to study the influence of carbon source concentration on the secondary metabolites production of *P. aurantiogriseum* in order to fight against this species and also to improve the production of its lipases. Lipase activity was measured by the titrimetric method with olive oil as a substrate. Mycotoxins production was determined by TLC technique. Our study revealed that *P. aurantiogriseum* secretes three mycotoxins (terrestric acid, penicillic acid, and aurantiamine) as well as two types of lipase, namely lipases I and II. The production of penicillic acid was detected in CYA, BM, and YES media, while aurantiamine was produced only in BM medium and terrestric acid in YES. Terrestric acid production increased proportionally to the sucrose concentration in YES. However, the penicillic acid production is inversely proportional to the concentration of sucrose in YES. On the other hand, a maximum of lipase activity was obtained in the CYA* medium under agitation condition.

Keywords: *P. aurantiogriseum*, carbon source, mycotoxins, lipases.

INTRODUCTION

Penicillium has long been used in food and pharmaceutical industry. However, some species are toxigenic and, thus, could be very harmful to human health. Numerous studies have reported that some mycotoxins can be secreted by a huge number of *Penicillium* species (Lu et al., 1994) including those used in food processing and pharmaceutical industry, whenever favorable physicochemical and trophic conditions are present. *P. aurantiogriseum* is frequently isolated from food (Yu et al. 2010; Khaddor et al., 2007; Maouni et al., 2002)**,** it secretes numerous mycotoxins namely terrestric acid, penicillic acid,

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and aurantiamine. *P. aurantiogriseum* is the interesting source of lipase (Li and Zong, 2010), which are typically extracellular and therefore relatively easy to recover after fermentation (Li and Zong, 2010; Lima et al., 2004). Lipases of *P. aurantiogriseum* are widely used in the agri-food industry as they play a major role in the development of characteristic flavors in ripened cheeses (Chopra et al., 1980; Salihu et al., 2012), pesticide production, and also their role in the pharmaceutical industry through the synthesis of drugs (Mase et al., 1995; Torre et al., 1996). Although several methods have been developed for the determination of lipase activity and mycotoxins production, our study was limited by the use of the titrimetric method using olive oil as a substrate. Mycotoxins production was determined by TLC technique. The aim of this work is to study the influence of carbon source concentration on the toxigenesis of *P. aurantiogriseum* and to improve lipases productivity.

MATERIAL AND METHODS

The strain

P. aurantiogriseum used in this study belongs to the collection of our EFBRT (Environmental and Food Biotechnology Research Team) which was used in previous studies (Khaddor et al., 2007), (Maouni et al., 2002; Sebti and Tantaoui-Elaraki, 1994). The strain was first cultivated on MEA (malt extract agar) at 25°C for 7 days, and the spores were suspended in 0,1 % of tween 80. The suspension density was adjusted to 10^7spores/ml.

The purification was then made on three different media MEA (Samson and Gams, 1984), CYA (czapeck yeast extract agar) (Samson, 1981) and G25N (glycerol 25% nitrate agar) (Pitt, 1973) (Table 1).

Table 1: Composition of the media used for growth and metabolites production of *P. aurantiogriseum*

Medium	Composition per 1 liter of distilled water
G25N	Concentrated Czapeck**: 10ml; K_2HPO_4: 1g; Yeast Extract: 5g; Glycerol: 250 g; Agar: 16g.
MEA	Malt extract agar: 30 g; Glucose: 20 g; Agar: 10 g.
CYA	Concentrated Czapeck**: 10ml; K_2HPO_4: 1 g; Yeast extract: 5g; Sucrose : 30g; Agar: 15 g.
YES	Yeast Extract: 20g; Sucrose: 100g.
BM	Malt Extract: 130g; Agar : 15g.
** Czapek concentrate: $NaNO_3$: 30g ; KCl : 5g ; $MgSO_4$:5g ; $FeSO_4$: 0,1g ; $ZnSO_4$: 0,1g ; $CuSO_4$: 0,05g; Distilled water: 100ml	

Determination of mycelial dry weight

The growth of *P. aurantiogriseum* colonies was estimated by determination of cell dry weight and radial growth according to Zain et al. (2009). For cell dry weight determination, the whole of culture plate agar was mixed with 100 ml of distilled water and boiled to dissolve the agar. The agar solution containing the fungal biomass was filtered through Whatman dry filter paper number 5, and the filter paper with the retained fungal cells was then dried in an oven at 80°C until a weight was reached constant dry. Radial growth was estimated by measuring the diameter of each colony with a ruler. All the experiments were performed in triplicate.

Research of mycotoxins

The experiment was done in two steps; we first used three growth media (CYA, MB, and YES) to identify mycotoxins produced by *P. aurantiogriseum* in each medium. After that, we have used only the medium that gave more than one mycotoxin and we tried to change the concentration of its basic carbon source to determine the maximum production of each mycotoxin. For the second step, the medium chosen for mycotoxins production was the YES (Yeast Extract Sucrose) (Frisvad, 1983). For that, 25 ml of YES, with different concentrations of sucrose, ranging from 0 to 300 g/l, was distributed in 100 ml Erlenmeyer flasks and inoculated with 2 ml of spore suspension (10^7 spores/ml). All cultures were incubated for 10 days at 25°C. The experiment was done three times.

The toxigenesis study was made according to the method reported by (Khaddor et al., 2007). The TLC plates used are 60 Kieselguhr F254. Mycotoxins standards used by the reference of migration forehead (Rf) were patulin (P), citrinin (C), ochratoxin A (OTA), penicillic acid (PA), and griseofulvin (Gi). Ten ml each of ethanol extract and of standard solutions (1 mg/ml) were spotted on TLC plates. Elution systems used are: toluene-ethyl acetate - formic acid (5/4/1, v/v/v) and chloroform - acetone - 2-propanol (85/15/20, v/v/v). The plates were examined in daylight and by ultraviolet 365 and 254 nm after spraying the spots by ANIS (p-anisaldehyde solution) and 8 min heating to 120°C. The ratio (Rf), color and fluorescence intensity of the extracts were compared with different reference concentrations of P, C, Gi, PA and OTA (Cunniff and Association of Official Analytical Chemists, 1995). Fluorescence intensity was expressed by a variable number of "+" signs (Hameed et al., 2012)

Lipase production

Detection of enzymatic activity in the solid medium

The determination of lipase production by *P. aurantiogriseum* was carried out according to Beisson et al. (2000). The growth medium contained the following elements: 5 g of peptone, 2 g of NaCl; 1 g of NO_3NH_4, 15 g of agar; 25 g of the olive oil and 20 ml of rhodamine B 0.5%. 10^8 spores are cultured in the medium for 4 days at 25 °C. Rhodamine B is used as an indicator of the enzymatic activity.

Determination of lipase I and II in liquid medium

In this study, we used the media of *P. aurantiogriseum* growth and toxinogenesis as media for lipase production with modification of the carbon sources that have been substituted by their values in olive oil. The purpose of this study is to improve the production of P.A lipase while maintaining the same basic culture conditions. It is for this reason that we think of just replacing the carbon sources with their value in olive oil instead of changing the whole culture medium.

The extracellular lipase was measured by free fatty acids assay in the supernatant of growth medium (Ginalska et al., 2004; Sarda et al., 1958). A volume of 100 ml, of each medium, was placed in Erlenmeyer flask (1000 ml) and added with 1 ml of 10^8/ml of spore suspension. The medium, thus seeded, was incubated for one week at 25°C. This incubation was carried out without agitation (for lipase I production) and with agitation on shaker-incubator at 120 rpm (for lipase II production). After incubation, the media were filtered to determine the lipolytic activity which depends on the number of fatty acids released by the hydrolysis of the triglycerides contained in the buffered emulsion.

A volume of 200 µl of the filtrate was incubated for 20 minutes at room temperature with 2.5 ml of 50 mM Tris buffer pH 7 and 2.5 ml of an emulsion prepared by homogenizing by ultraturax (the emulsion is made with Ultra-Turrax, with stirring at maximum speed for 2 minutes) 2 g of tributyrin in 100 ml of an aqueous solution of 3% gum arabic. After 20 minutes of incubation, 10 ml of alcohol was added to block lipolysis. The same volume of the filtrate, in this case, 200 µl, was used as a control by incubating it for 10 min at a temperature of 100 °C to inactivate the lipases secreted by *P. aurantiogriseum*. The released fatty acids were titrated with a 0.05 M NaOH solution and phenolphthalein as an indicator. One unit of lipase was defined as the number of enzymes that releases 1 µmol of fatty acid/min. The experiment was done three times.

RESULTS

Mycotoxin production

Media effect on radial growth rate and cell mass production

The effect of medium composition on growth and toxigenesis of *P. aurantiogriseum* are summarized in Table 2. The radial growth rate was significantly high in CYA medium compared to YES and BM media. The highest cell production was obtained from CYA, while the lowest was obtained from BM. A slight difference in cell production was observed between CYA and YES.

Media effect on mycotoxins production

The chromatographic analysis of the extracts obtained from different media of *P. aurantiogriseum* (YES, CYA and BM) allowed highlighting three mycotoxins: penicillic acid, terrestric acid, and aurantiamine (Table 2**).** The production of penicillic acid was detected in CYA, BM and YES media, while aurantiamine was produced only in the BM medium and terrestric acid in the YES.

Effect of sucrose concentration on mycotoxins production

Terrestric acid production was observed in the YES medium. This production increased proportionally to the sucrose concentration in YES.

However, the penicillic acid production is inversely proportional to the concentration of sucrose in YES (Figure 1 and Table 3).

Table 2: Growth and toxigenesis of *P. aurantiogriseum* on different media

Medium	Mycotoxins intensity on chromatogram			Dry weight (mg)	Colony diameter (cm)
	Penicillic acid	Terrestric acid	aurantiamine		
CYA	+	-	-	1.1	3.5
BM	+	-	+	0.5	2.5
YES	+	+	-	0.8	3.0

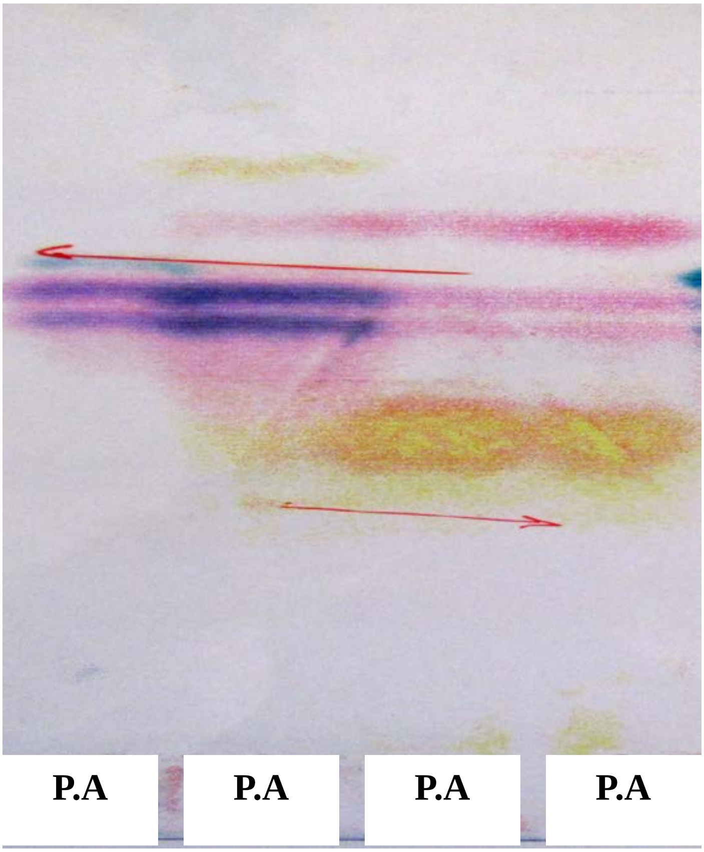

Figure 1: Chromatogram showing the mycotoxins produced by *P. aurantiogriseum* in different sucrose concentrations on YES medium.

P.A: *P. aurantiogriseum***;** **S**: the concentration of sucrose in the YES medium expressed in g/l. The arrows indicate the degree of mycotoxins production; from left to right: the increase in the production of terrestric acid (yellow spot). From right to left: increased production of penicillic acid (blue lilac spot).

Table 3: Growth and mycotoxins production at different sucrose concentrations

Sucrose concentration in YES medium (g/l)	Colony diameter (cm)	Mycelial dry weight (mg)	Mycotoxins intensity	
			Terrestric acid	Penicillic acid
0	3.8	1.3	-	++
100	3.0	0.8	+	>+++
200	2.5	0.7	>+++	>+
300	3.2	1	++	+

Lipase activity of P. aurantiogriseum

Rhodamine B assay

P. aurantiogriseum showed rapid growth after 24 h. Positive results were obtained using rhodamine B as indicators. After 24 hours of incubation, we observed a lysis zone in *P. aurantiogriseum* colonies. The enzymatic concentration is related to the diameter of the intensification zone. This diameter increases with the incubation time (Figure 2). The intense development of color can be observed around lipase production in colonies. Therefore, we propose that this technique is a simple and reliable technique for detection of lipolytic activity in *P. aurantiogriseum.*

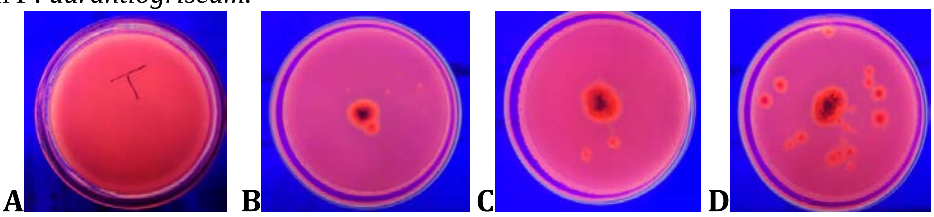

Figure 2: Detection of enzymatic activity in *P. aurantiogriseum*.

Rhodamine B was complexed with the fatty acids released during lipolysis. This complex gave an orange coloration which is read at 350 nm. **A:** control, **B:** after 24 h of incubation, **C:** after 3 days, **D:** after 4 days.

Determination of lipase activity

The lipase activity of *P. aurantiogriseum* on different media was detected after 20 min of the substrate hydrolysis reaction. The results showed a maximum lipase activity of 4.25 U/ml of fatty acid obtained in the CYA* medium. The activity was low in MEA* and YES* media with 3.04 U/ml and 1.6 U/ml respectively (Figure 3).

P. aurantiogriseum recorded an activity of 2.0125 U/ml of fatty acid in the non-agitated medium and 4.25 U/ml in the agitated medium indicating that lipase II is the most dominant in our strain (Figure 4).

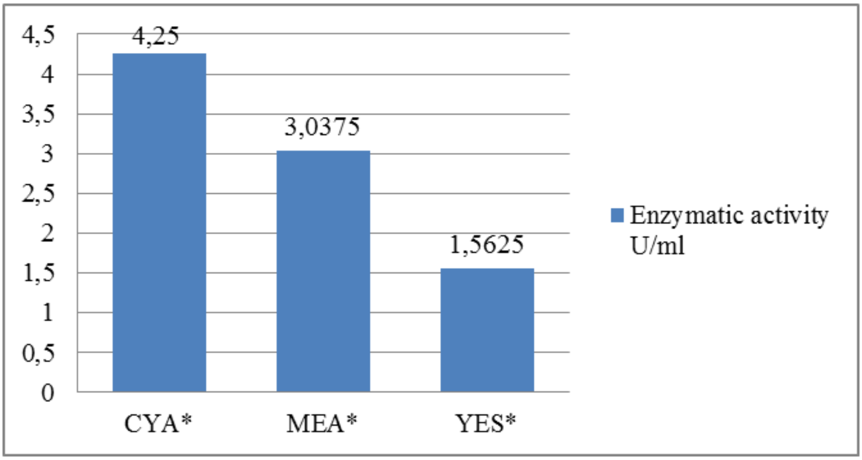

Figure 3: Enzymatic activity on different culture media.

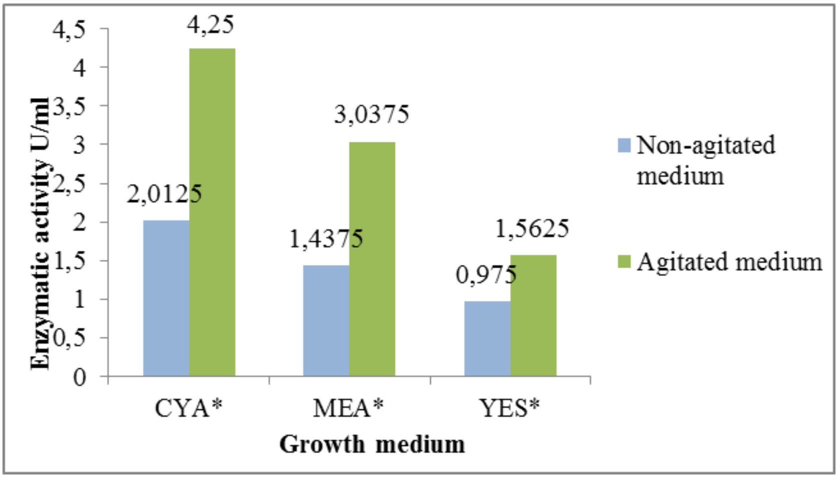

Figure 4: Determination of *P. aurantiogriseum* enzyme activity in two media states.

DISCUSSION

Mycotoxin production in P. aurantiogriseum

Effect of media on mycotoxins production

In this study, three different media were used to obtain a basal medium favorable to improve *P. aurantiogriseum* toxigenesis. The results demonstrated that mycotoxin production in *P. aurantiogriseum* was greatly influenced by the carbon source. The strain was able to grow and produce mycotoxins greatly when sucrose has been used. The YES medium was preferred to *P. aurantiogriseum* for growth and toxigenesis.

Chromatographic profile of extracts obtained from *P. aurantiogriseum* on different media (YES, CYA and BM) revealed three mycotoxins: penicillic acid, terrestric acid, and aurantiamine. These mycotoxins have been reported to be produced by this species in previous works (Colombo et al., 2003; Frisvad and Samson, 2004; Khaddor et al., 2007).

In this study, we showed a great diversity regarding mycotoxin production by *P. aurantiogriseum* on different media. Penicillic acid production was detected in CYA, BM and YES media, while aurantiamine was produced only in the BM medium and terrestric acid in the YES medium. The comparison between media (CYA, BM and YES) indicates that the variation in mycotoxin production was related to different carbon sources used in different media (Davis et al., 1969; Mills et al., 1995). *P. aurantiogriseum* growth was increased when sucrose was present in YES as a carbon source. The production of mycotoxins was observed in the BM and YES media as it was demonstrated in similar studies (Davis et al., 1969; Mills et al., 1995a; Zain et al., 2009).

Effect of sucrose concentration on mycotoxin production

As it was mentioned in the Material and Methods section, we chose the medium which the mycotoxin production was clearer and contains more than one mycotoxin. This medium was the YES on which we changed the concentration of sucrose from 0 to 300 g/l to determine the maximum production of terrestric acid and penicillic acid.

We have observed, on the one hand, the terrestric acid production in YES medium. This production increased proportionally to the sucrose concentration in the medium. On the other hand, penicillic acid production is inversely proportional to the sucrose concentration in the YES. It appears that the production of terrestric acid is induced by sucrose. Therefore, we suggest that the terrestric acid has a strong affinity towards sucrose since it is produced only in the YES medium. This is in agreement with the results of Mills et al. (1995) which showed that the terrestric acid is produced in YES medium in which sucrose is the sole carbon source. Unlike the terrestric acid, penicillic acid is produced in three media (CYA, MB and YES), so it rhymed with several types of carbon source. However, when we increased the sucrose concentration in the medium, penicillic acid production decreased and this could be due to the difficulty of assimilation of sucrose by the strain even in the presence of the invertase enzyme which appeared to be little in the intracellular medium.

P. aurantiogriseum develops on various carbon sources, including sucrose. Sucrose growth resulted in high levels of biomass, but mycotoxin production decreased as carbohydrate concentration increased. Therefore, the maximum product concentrations were not reached under conditions supporting the highest biomass level. Our results suggest some control of the carbon catabolism of mycotoxin production in *P. aurantiogriseum*. To achieve the highest volumetric title, it is not sufficient to provide conditions for a high specific output. A critical level of mycotoxin production is also needed. Sucrose at the concentration between 100 and 200 g/l provided the critical degree of production of penicillic acid and terrestric acid, respectively. We postulate that this is due to the slow sucrose hydrolysis. This created a situation of carbon limitation and releases the culture to the carbon source regulation (Aharonowitz and Demain, 1978). It has been shown in several works (Chander et al., 1981; Flipphi et al., 2009; Zain et al., 2011) that the production of antibiotics and other secondary metabolites is negatively affected when the carbon source is rapidly used for growth; however, when the carbon source is slowly introduced into the culture, the secondary metabolite is efficiently produced.

Lipase activity in P. aurantiogriseum

Our results showed a maximum lipase activity of 4.25 U/ml of the fatty acid obtained in the CYA* medium. The activity was low in the MEA* and the YES* medium with 3.04 U/ml and 1.56 U/ml respectively. These results are in agreement with those reported by Singh et al. (2012) where the enzymatic activity of *P. aurantiogriseum* in YES was very low, whereas, in a medium supplemented with another source of carbon (like maltose on MEA) or other trace elements (like microelements on CYA), the production was remarkable.

Salihu et al. (2012) have shown that the presence of olive oil in the medium induces lipase production during the growth phase. The triglycerides of olive oil are the best carbon sources for lipase production. It is possible that the consumption of fatty acids leads to the immediate induction of lipases. Cordova et al (1998) had suggested that when *P. aurantiogriseum* is grown in the presence of an inducer, the lipase that produced is derived from a primary metabolism.

In this work, the concentration of olive oil was 3 % in CYA* medium and the corresponding lipase activity was 4.25 U/ml. This is not coherent with the results obtained by Lima et al (2003) where the concentration of 2 % of olive oil gave an activity of 3 U/ml. On the other hand, in MEA* medium (2 % olive oil) and YES* (10 %), the activity was much lower compared to CYA* with values of 3.04 and 1.56 U/ml respectively. Lima (2003) explains that the inhibition of lipase synthesis at high concentrations of olive oil may be due to the difficulty of transferring oxygen to the environment. Low oxygen stores can alter fungal metabolism and consequently lipase production. However, in our study, the high concentrations of olive oil did not affect lipase activity. This could be explained that the strain can grow both in anaerobic and in aerobic conditions. Moreover, the concentration of olive oil, the sole carbon source, in the medium could be assimilated by our strain by using it in the growth, survival, and production of metabolites.

It should be noted that the production of lipase is influenced by certain factors, such as aeration. Chahinian et al**.** (2000) reported that when *Penicillium cyclopium* (nearest equivalent description of *P. aurantiogriseum* according to Raper and Thom (1949)) was cultured in a non-agitated liquid medium, it produced lipase I specific for triacylglycerols. But in the agitated medium, the strain produced the lipase II which is active only on mono- and diacylglycerols. In the present study, *P. aurantiogriseum* recorded an activity of 2.01 U/ml of fatty acid in non-agitated medium and 4.25 U/ml in the agitated medium, indicating that lipase II is the most dominant in the strain.

In our study, the non-agitated medium showed a difference in the enzymatic activity of *P. aurantiogriseum* in the three media with the values of

2.01 U/ml of fatty acids on CYA*, 1.44 U/ml on MEA* and 0.97 U/ml on YES*. These media contained an inducer of lipase production, which is olive oil, with different concentrations. This induction caused the lipase to be produced in the exponential phase of the strain as a primary metabolite. That explains the high activity in CYA* which olive oil is the sole carbon source. In the MEA* medium, which contained maltose in addition to olive oil, the strain first used maltose as a carbon source and consequently the production of lipase was delayed by giving a small amount of lipase compared to CYA*. The high concentration of olive oil in the YES* medium makes oxygen uptake very slow and the strain has no other source of carbon which influences its growth and, therefore, its lipase production.

These results have a low value compared to that obtained in agitated medium, suggesting that in the non-agitated medium, the growth and production of lipase was limited by the lack of oxygen used during respiration. The agitation created a kind of aeration in the colony to release CO2 and use the maximum of O_2 in the medium (Börjesson et al., 1990; Chander et al., 1981).

CONCLUSIONS

The mode of secondary metabolites production has long been very useful for the identification and classification of fungi. However, production of these compounds can be affected by environmental conditions. Our results showed that the growth parameters and secondary metabolites production of *P. aurantiogriseum* were affected by the nature and concentration of carbon source. Results from this study revealed that mycelium weight, colony diameter, and mycotoxins production of *P. aurantiogriseum* were significantly affected by increased sucrose concentrations. As a result, secondary metabolites are sensitive to environmental conditions and their production is limited under certain conditions, therefore the use of secondary metabolites in fungal taxonomy should be reconsidered. The current study showed that olive oil significantly increased lipase production in *P. aurantiogriseum*. This is the first time that higher lipolytic activity and shorter production times were achieved for this species.

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PHENOTYPIC VARIABILITY OF YIELD COMPONENTS OF *Triticum spelta* IN ORGANIC PRODUCTION

SUMMARY

The aim of this study was to investigate phenotypic variability of yield components for different spelt wheat genotypes (*Triticum spelta* L.). Six genotypes of winter spelt wheat (Nirvana, KG-37-8/3, KG -54-7/3, KG -54-8/1, KG -54-4/2, and KG-54-2/3) were grown during two growing seasons (2011/2012 and 2012/2013) at certified organic trial parcel in the Municipality of Čačak, Serbia. Through variance analysis, highly significant differences in mean values for both investigated yield components (number of grains per spike and grain weight per spike) were established. Higher values of coefficient of variability for grain weight per spike (*CV* = 12.8%) than grain number per spike (*CV* = 10.2%) were determined. The highest average value for number of grains per spike had genotype KG-54-7/3 (46.22). Genotype KG-54-2/3 (1.94 g) had significantly higher mass of hulled grains per spike compared to other investigated genotypes. Phenotypic analysis of variance indicated that ecological factors had higher impact on the expression of grain weight per spike, but genetic factors had higher impact on the expression of number of grains per spike.

Keywords: Wheat, spelt, variability, yield components, phenotypic variance.

INTRODUCTION

Spelt (*Triticum spelta* L.) is an old wheat species whose production greatly decreased in the 20th century but the interest for this low-input wheat has recently increased again, and spelt is recognized as one of the most appropriate cereals for organic production (Bavec and Bavec, 2011). The growing of spelt wheat has recently become very popular, particularly with regard to use in special bakery products, health and organic foods. Growing this cereal is also becoming more interesting in Serbia. Areas under spelt wheat are increasing in hilly mountains and lowland regions as well (Glamočlija et al., 2013).

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

Spelt's popularity has seen a more recent resurgence largely due to its health benefits and its distinctive taste attributes. Spelt is very highly regarded as an alternative grain due to its great nutritional value. This species is very high in protein (Kohajdova and Karičova, 2008, Moudrý et al., 2011, Siemianowska et al., 2011) and various minerals and vitamins (Piergiovanni et al., 1997, Bojňanská and Frančaková, 2002), higher in selenium content (Zhao et al., 2009), abundant in soluble fibers (Bonifacia et al., 2000) and has more favorable ratio of unsaturated to saturated fatty acids (Ruibal-Mendieta et al., 2004), while containing a low amount of fat and a moderate amount of gluten. The grain's gluten content makes it suitable for some baking. Today, more spelt-based products are available including flour, bread, crackers (Konvalina et al., 2013, Filipčev et al., 2013), and pasta (Marques et al., 2007). There are many applications of spelt in bakery industry but not in the beverage industry, which makes new approaches in the malting and brewing process for further investigation. Study of Muñoz-Insa et al. (2013) shows a new possibility to adopt the production of spelt beverages and further expands the market of alternative cereals.

Spelt is adapted to lower fertility soils and shows higher resistance to environmental influences than common wheat, giving it greater suitability for organic farming. Under optimal growing conditions, the yields of organically grown spelt are lower than organically grown wheat. Under sub-optimal growing conditions some research suggests that spelt out-performs many traditional grains (such as wheat) and is able to better utilize nutrients when grown in a low-input system, suggesting that spelt could play a greater role in organic cereal rotations (Neeson, 2011). Experience shows that yield potential in organic farming varies much more widely than in conventional agriculture. Therefore expectations for organic varieties include higher adaptability and plasticity, combined with consistently high quality.

Organic plant breeding is a new direction in plant breeding aimed at creating so called organic agricultural plant varieties that are adapted to the conditions and requirements of organic production. New varieties for organic production must have a greater resistance to abiotic and biotic stress conditions, more efficient uptake of mineral nutrients and better exploit of the existing environmental conditions. Since the problems of weeds in organic crop production are more pronounced if it's the initial criteria, it is certain that varieties incurred by this means should be selected, based on the other grounds (Berenji, 2009). One of the basic mechanisms of weed control in organic production is the competitiveness of organic species and weeds that expressed the power suppressing weeds by organic varieties. Resistance / tolerance to pests and diseases are among the most important expectations of the organic varieties (Berenji and Sikora, 2009). Organic plant breeding aims to respect natural species authenticity. Crosses between species should not be forced if fertilization and embryo growth do not succeed on the plant. Also, organic plant breeding aims to achieve a species-specific equilibrium for an optimal development of species-specific characteristics, such a taste, color, bouquet and shape (Lammerst van Bueren et al., 1999).

The aim of this paper was to study phenotypic variability for yield components in spelt genotypes which can be used as parent cultivars in organic plant breeding programs for improvement of grain yield and quality of spelt wheat.

MATERIAL AND METHODS

Six genotypes of winter spelt wheat (Nirvana, KG-37-8/3, KG -54-7/3, KG -54-8/1, KG -54-4/2, and KG-54-2/3) were grown during two growing seasons (2011/2012 and 2012/2013) at certified organic trial parcel which is located in Mršinci, in the Municipality of Čačak, Serbia (20°20' E, 43°50' N, 204 m asl). The yearly mean air temperature of the area is 11.6°C; and the average annual rainfall in the area is around 669 mm. Average temperature and precipitation for vegetative period (October-June) in the 2011/2012 were 8.7 ºC and 345.5 mm, respectively. In the 2012/2013 average temperature and precipitation were higher (10.1 ºC and 503.0 mm, respectively).

Cultivar Nirvana is the property of the Institute of Field and Vegetable Crops, Novi Sad, and other investigated genotypes are the property of the Small Grains Research Centre Kragujevac.

The field experiment was conducted in a randomized block design with three replications with plot of 5 m^2 on the soil which belongs to the loamy clay type. The sowing density was 600 seeds per square meter. Experiment was carried out by the organic technology of scientific farming production of wheat. Soybean in the first and potato in the second year were used as the preceding crops. Sowing was carried out in the first decade of November. The treatment of the crops during the growing season respected the principles of the organic farming.

For analysis of spike characteristics 60 plants in full maturity stage (20 plants per replication) were used. The primary spike was used for analysis of yield components. The number of grains per spike and dehulled grain mass per spike were determined.

The following parameters were computed: the average value ($\bar{x}$), the standard deviation (SD), the coefficient of variation (*CV*), and analysis of variance (ANOVA). The significant differences between the average values were estimated by LSD-test. The ANOVA was performed according to a random block system with two factors using the MSTAT-C program (Michigan State University, 1990). Components of variance (σ^2_g-genetic, σ^2_{gl}-interaction; σ^2_E-environment; σ^2_f-phenotypic) were calculated according to the Falconer (1981).

RESULTS AND DISCUSSION

Grain number per spike is an important yield component, which directly affects genetic yield potential. The results for number of grains per spike are presented in Table 1. According to the results, number of grains per spike depended significantly on genotype and genotype × year interaction. Variability between varieties was higher than between investigated growing seasons. In this research, average value for this trait is varied in the range of 34.01 to 46.22 grains per spike. The highest average value for number of grains per spike was observed in genotype KG-54-7/3 (46.22), and the lowest in Nirvana variety (34.01). Variability of this yield component differed between growing seasons. Higher variability was established in the first (CV=11.82%) than in the second year (CV=8.68%).

The coefficient of variation varied in the range from 5.3% (Nirvana in the second year) to 19.5% (KG-37-8/3 in the first year). Cultivar Nirvana had about 34 grains per spike on average, which concurred with previous investigations. According to Pospisil et al. (2011), cultivar Nirvana produced from 32.9 to 40.0 grains per spike in dependence to crop management and growing season. The number of grains per spike is a quantitative trait whose expression depends on a large number of genes that are strongly influenced by environmental factors that cause high variability, as confirmed by this research.

Table 1. Average values and variability for number of grains per spike

Genotype	Year						Average
	2011/2012			2012/2013			
	$\bar{x}$	SD	*CV* (%)	$\bar{x}$	SD	*CV* (%)	
Nirvana	36.55±0.51[d]	3.94	10.8	31.47±0.22[e]	1.67	5.3	34.01±0.36[C]
KG-37-8/3	37.26±0.94[cd]	7.28	19.5	40.63±0.85[bc]	6.60	16.2	38.94±0.89[B]
KG-54-7/3	45.44±0.66[a]	5.08	11.2	46.99±0.46[a]	3.53	7.5	46.22±0.56[A]
KG-54-8/1	39.51±0.62[bcd]	4.78	12.1	40.90±0.54[bc]	4.18	10.2	40.21±0.58[B]
KG-54-4/2	40.05±0.52[bcd]	4.02	10.0	41.60±0.40[b]	3.11	7.4	40.83±0.46[B]
KG-54-2/3	45.41±0.43[a]	3.32	7.3	46.67±0.33[a]	2.56	5.5	46.04±0.38[A]
Average	40.70±0.61	4.74	11.82	41.38±0.47	3.61	8.68	41.04±0.54

Mean values of genotypes in both years denoted with different small letters are significantly different according to LSD test ($P \leq 0.05$); Mean values of genotypes for both years denoted with different capital letters are significantly different according to LSD test ($P \leq 0.05$).

Analysis of variance for the number of grains per spike is shown in Table 2. The analysis of phenotypic variance established highly significant F values for varieties and significant for interaction varieties × year. Most of the total phenotypic variance belongs to genotype (73.48%), less to interaction (12.30%), and the least belonged to environmental factors (1.87%).

Average values and variability of the hulled grain weight per spike are shown in Table 3. The grain weight per spike is very variable trait because it depends on grain number and grain chemical composition. This trait is very important yield component, which directly influences harvest index and yield.

This yield component is very variable and its expression depends highly on the environmental factors. Examined varieties reacted differently to environmental changes during particular years. Average grain weight per spike in all analyzed varieties was 1.66 g, ranging from 1.525 g (KG-54-8/1) to 1.940 g (KG-54-2/3). Genotype KG-54-2/3 produced significantly higher mass of hulled grains compared to other investigated genotypes.

Table 2. Results of ANOVA and variance components for number of grains per spike

Source	*df*	Mean square	*F*	Components of variance		*LSD*	
				s^2	%	0.05	0.01
Replication	2	0.578	0.1798	-	-	-	-
Genotype	5	127.680	39.6773**	19.142	73.48	2.662	4.176
Year	1	4.101	1.2743^{ns}	0.485	1.87	1.240	1.686
Genotype × year	5	12.831	3.9873*	3.204	12.30	3.765	5.906
Error	22	3.218		3.218	12.35	-	-
Total	35	-	-	26.049	100.00	-	-

^{ns} $P > 0.05$; $*P \leq 0.05$; $**P \leq 0.01$

High variability and significant differences for grain weight per spike in different varieties were established by analysis of variance (Table 4). Phenotypic variance analysis showed highly significant differences for grain weight per spike between cultivars, experimental years and their interactions, which agree with previous studies (Jablonskytė-Raščė et al., 2013).

The highest percentage of the total phenotypic variability was assigned to year (50.00%), less to genotype (29.73%), and only 14.86% to interaction genotype × year. This suggests that environmental factors in this study played a major role in the expression of grain weight per spike, what agree with previous results (Zečević et al., 2010). In previous studies (Lacko-Bartošová et al., 2010), weight of grains per spike was also statistically significantly dependent on variety and weather conditions.

Spike properties of wheat depended highly on the genotypes, environmental conditions (Zečević et al., 2013), soil quality (Glamočlija et al., 2013), and applied cultivation technology (Bicanova et al. 2008). Bavec et al. (2011) also established significant influence of the production year and production system on yields for spelt and common wheat. They pointed out that differences among production systems in spelt are not as accentuated as in the case of common wheat, possibly due to the lower breeding modifications of spelt as compared to wheat and the somewhat unresponsive reaction to additional nitrogen fertilizer applications.

Table 3. Average values and variability of grain weight per spike

Genotype	Year						Average
	2011/2012			2012/2013			
	$\bar{x}$ (g)	SD	*CV* (%)	$\bar{x}$ (g)	SD	*CV* (%)	
Nirvana	1.497±0.02[d]	0.17	11.4	1.633±0.13[bc]	0.10	6.1	1.565±0.08[C]
KG-37-8/3	1.493±0.03[d]	0.25	16.7	1.713±0.04[bc]	0.29	16.9	1.603±0.04[C]
KG-54-7/3	1.607±0.02[cd]	0.16	10.0	1.987±0.04[a]	0.28	14.1	1.797±0.03[B]
KG-54-8/1	1.317±0.02[e]	0.14	10.6	1.733±0.04[bc]	0.34	19.6	1.525±0.03[C]
KG-54-4/2	1.313±0.03[e]	0.20	15.2	1.763±0.02[b]	0.20	11.3	1.538±0.02[C]
KG-54-2/3	1.907±0.02[ab]	0.16	8.4	1.973±0.04[a]	0.28	14.2	1.940±0.03[A]
Average	1.52±0.02	0.18	12.0	1.80±0.05	0.38	13.7	1.66±0.04

Mean values of genotypes in both years denoted with different small letters are significantly different according to LSD test ($P \leq 0.05$); Mean values of genotypes for both years denoted with different capital letters are significantly different according to LSD test ($P \leq 0.05$).

Table 4. Results of ANOVA and variance components for grain weight per spike

Source	*df*	Mean square	*F*	Components of variance		*LSD*	
				s^2	%	0.05	0.01
Replication	2	0.000	0.0407	-	-	-	-
Genotype	5	0.171	48.1048**	0.022	29.73	0.0984	0.1472
Year	1	0.697	196.3736**	0.037	50.00	0.0435	0.0591
Genotype × year	5	0.038	10.7492**	0.011	14.86	0.1327	0.2082
Error	22	0.004	-	0.004	5.41	-	-
Total	35	-	-	0.074	100.00	-	-

**$P \leq 0.01$

For organic farming, varieties with high tillering capacity where yield is based on number of ears should not be chosen. These varieties need sufficiency of nutrients at the beginning of spring vegetation. However, there is lack of nutrients in soil because of low microbial activity and low level of mineralization (Bicanova et al., 2008).

CONCLUSIONS

These investigations indicated that ecological factors had high impact on the expression of grain weight per spike and number of grains per spike of spelt wheat in organic production. Through variance analysis, highly significant differences in mean values for both investigated yield components were established. High variability for both investigated yield components was determined. The highest average value for number of grains per spike had genotype KG-54-7/3, while genotype KG-54-2/3 produced significantly higher mass of hulled grains compared to other investigated genotypes. These genotypes can be used in breeding programs for increasing yield in spelt wheat.

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IMPACT OF THE SHOOT MATURITY LEVEL ON ROOTING, ACCLIMATISATION OF GREEN AND SEMI-HARDWOOD CUTTINGS OF THE BLUEBERRY (*Vaccinium corymbosum* L.) CV. BLUECROP STIMULATED WITH INDOL BUTYRIC ACID AND NAPHTHALENE ACETIC ACID

SUMMARY

In this research we have tested the rooting abilities of green and semi-hardwood cuttings with leaves in the ennobled blueberry (*Vaccinium corymbosum* L.) Bluecrop cultivar taken at the end of August, from shoots of the year when growth was stopped and they had not reached one year. These cuttings were stimulated with IBA (Indol butyric Acid) and NAA (Naphthalene Acetic Acid) in concentrations of 1500 ppm, 3000 ppm, 4500 ppm and in the variation control without treatment, in substrate turf, turf-perlite 2:1. The results in both types of cuttings show differences of statistical significance ($p = 0.05$) and ($p = 0.01$). Bearing in mind that the greatest rooting ability is in special time periods and based on results from several years, propagation with green cuttings did not produce a high percentage of rooting based on the fact that their rooting is difficult and has reached 37.5% whilst semi-hardwood shoots taken at the same time period have reached a higher percentage (42.5%) which is definitely related to the physiological condition of the shoot.

Keywords: green cuttings; semi-hardwood cuttings; acclimatization; IBA; NAA

INTRODUCTION

There are several ways for a successful vegetative propagation of blueberry that can potentially be used to spread blueberry *V. corymbosum* L. This is achieved using hardwood cuttings, semi-hardwood cuttings and soft-wood cuttings which are the most commonly used for spreading blueberry during which the rooting percentage reaches over 50% (Mainland, 1993; Miller *et al.*, 2006). And the blueberry propagation with green cuttings is applied on blueberry cultivars with various successes in the rooting process. Even if this type of blueberry propagation with green cuttings is applied less, it still has some advantages since in this way it can provide a larger amount of seedling material from a mother plant, which enables a rapid spread of new cultivars as well as cultivars which have difficulties propagating with hardwood cuttings (Stanić *et al.*, 2004). Vegetative propagation ensures the preservation of germplasm and

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enables the creation of homogenous descendents from the genetic aspect (clones), since it enables the transfer of all the genetic potential from the donor fruit to the new fruit (Zobel and Talbert, 1991; Barbat, 2006; Soudre, 2008). However, through vegetative or clonal propagation, the plant has the advantage of capturing all genetic superiority without including any segregation gene. Factors that have an impact on the successful rooting of cuttings were reported by (Garner and Chaudri, 1976), (Hartman *et al* 1990), as: the age of mother plant, the season when the cutting was taken, the type of cutting, presence of vegetative buds, the content of water and other ingredients. Therefore, the basic function of vegetative propagation is the formation of adventitious roots and in special cases of adventitious buds as well (Hartman *et al.,* 2002). One way to support the increase of areas with the blueberry culture *V. corymbosum* L. is to graft it over a plant which has a higher tolerance for pH and is easier adjusted to environmental conditions. One possible rootstock would be *V. arboreum*, which has the ability to grow in many fields which are unsuitable for commercial blueberries. Firstly, *V. arboreum* has a thick root system with long roots, and because of this, it is resistant towards draught and is able to tolerate draught more than *V. corymbosum*. *Vaccinium corymbosum* L. has specific requirements regarding soil pH, it can tolerate a pH at around 4,0-5,5 but *V. arboreum* can tolerate soil pH between 4 and 7 (Darnell and Hiss, 2006). In general through grafting *V. corymbosum* over *V. arboreum* the aim is to raise the crown (Ballington *et al.*, 1990), have a more powerful root system and that the blueberry is more tolerant towards soil reaction starting from pH 4-7 bearing in mind that the soil pH is a limiting factor for blueberry cultivation.

Lack of nitrogen or excessive amounts may have a negative effect on the metabolism of the plant, (Huang *et al.,* 2004). High nitrogen content decreases the rooting percentage, while nitrogen shortage prevents it. Carbohydrates are important for the root formation because they are the basic blocks of building structural elements and are used as energy sources in plants (Struve, 1981). The adventitious root formation from the stem has been known from ancient times and is used for the vegetative propagation of elite plants that have been or were selected by natural populations (De Klerk, 2002). Auxin was widely used in hardwood cuttings to accelerate the adventitious root formation (Galavi *et al.*, 2013). The initiation of adventitious roots and their growth is an intensive metabolic process which is promoted by auxin and other growth regulators that lead towards the increase of enzyme activities for the ARN and protein synthesis (Hartmann and Kester, 1983; De Klerk *et al.,* 1999; Legue *et al*., 2014).

MATERIAL AND METHODS

The experiment was conducted during the vegetation period of 2016 in Llukë e Epërme village (Peja region). The experiment follows the method (G. Zec et al., 2001). The propagation material used were one-year old green and semi-hardwood shoots with 6 - 8 mm thick leaves without fruit buds taken by parent plants of the ennobled blueberry, Bluecrop cultivar at the time when shoots have stopped their vegetative growth. The shoots taken were cut at a length of 15 cm, i.e. several mm above the high bud and several mm under the lower bud. The cuttings taken before being placed in rooting bangos (boxes) filled in with substrate turf-only and turf-perlite 2:1 (substrate thickness 25 cm) are prepared, tied into tubes and their base part is dipped in 2.5 cm in a solution of IBA and NAA in various concentrations of 1500 ppm, 3000 ppm and 4500 ppm, in a duration of 5-7 seconds, whilst one row per box is not treated (controlled) at all.

At the bottom of boxes a layer of gravel was placed to ensure the drainage of excessive water. Such treated cuttings have stayed for 15 minutes (until they have well absorbed IBA and NAA), and after drying they were powdered at their base with captan powder mixed with talk (at a ratio 1:10 against rotting), than they were placed in boxes for rooting at a distance of 10 x 5 cm, at a depth of around ½ of the length of the cutting, leaving at least two buds above the substrate where they have stayed for 4 - 5 weeks.

The experiment was placed in four boxes with four repetitions each, where one repetition = 40 cuttings, 40 x 4 = 160 cutting/box. The rooting of cuttings was carried out under a misting system, which has enabled the maintenance of a certain moisture and temperature. The boxes filled with substrate for the rooting of green and semi-hardwood cuttings were placed in a glasshouse where the relative air moisture is 85-90%.

a. green cuttings; b. semi-hardwood cuttings

Figure 1. Rooted blueberry cuttings (*Vaccinum corymbosum* L.) Bluecrop cultivar

RESULTS AND DISCUSSION

Results show that semi-hardwood cuttings have reached a rooting percentage of 42.5 % compared to green cuttings where the rooting percentage reaches 37.5% taken at the same time in the same substrate (figure. 2).

This difference in the rooting percentage is definitely related to the shoot maturity level, reserve nutrients and hormone substances which help the rooting and in different stages of plant development are present in different amounts.

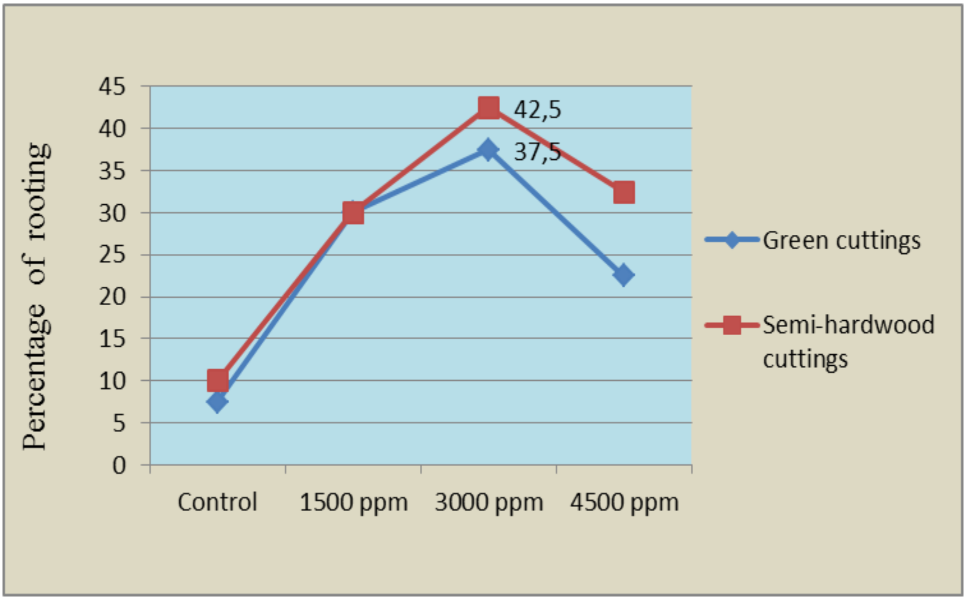

Figure 2. Averages of rooting percentage for green and semi-hardwood cuttings, Bluecrop cultivar, taken at the end of August, stimulated with IBA

Among green and semi-hardwood cuttings taken during the vegetation it was observed that the rooting ability increases from the base towards the top. This is because of auxin and other matter which is synthesized into new leaves and in the apical bud of the shoot. Moreover, the tissues of the top are less lignified, thus favouring the formation of adventitious roots. Our results are similar to the researches of other authors conducted previously, (Pokorny, F. A., and M. E. Austin. 1982), (Giroux *et al.*, 1999) (Gough, 1993; Lee et al., 2004; Yong et al., 2005). The differences in the rooting percentage among green and semi-hardwood cuttings were observed in all variants and in both types of substrate. The control variant also shows a small difference in the rooting percentage favouring a more mature shoot (more lignified).

These research results and the data of previous researchers provide a justification to recommend, respectively, favour the taking of cuttings from semi-hardwood and lignified shoots in order to increase the rooting percentage, because such shoots may live longer in a rooting substrate without their base part rotting and they are more resistant to dehydration. This dynamic of the rooting percentage (Figure 2) shows a dependency between the maturity level of the shoot and the rooting percentage of cuttings.

The analysis of variance (ANOVA) of the rooting percentage in green cuttings taken at the end of August (Table1) shows that differences were observed at the level of statistical significance (p= 0.01) in the middle of control (variant without stimulation) and variant with stimulation IBA, NAA 3000 ppm in both types of substrate. Whilst, between variants 1500 and 3000 ppm differences were observed at the level of statistical significance (p=0.05).

Table 1. The three way analysis of variance (ANOVA) on the rooting percentage of green cuttings with two leaves taken at the end of August.

Factor A - Substrate		Factor -B	Factor -C	Average	Average (A)
Turf	Turf-Perlite	Growth regulators	Concentration	(AB)	
0.50	0.75	Control	-	0.63	0.5**
0.25	0.50	Control	-	0.38	
Average AC					
0.38	0.63				
2.0	3.0	IBA	1500 ppm	2.5	2.1*
1.5	2.25	NAA	1500 ppm	1.8	
Average AC					
1.75	2.75				
2.5	3.75	IBA	3000 ppm	3.1	2.8**
2.0	3.0	NAA	3000 ppm	2.5	
Average AC					
2.25	3.4				
1.5	2.25	IBA	4500 ppm	1.8	1.7**
1.25	1.75	NAA	4500 ppm	1.5	
Average AC					
1.3	1.9				
Average C					
0.50	2.18				
Average BC				Average B	
0.5**	2.2**			1.35*	
2.8**	1.7**			2.25*	

Factors		A**	B*	C**	AB	AC	BC	ABC
LSD	1 %	0.20	0.36	0.71	0.59	1.15	1.15	2.24
	5 %	0.15	0.28	0.51	0.42	0.79	0.79	1.35

The three way ANOVA according to the test by Vukadinovic. **Significant 0.01% level. Numbers in bold show the most important attribute for variant.

The three way analysis of variance (ANOVA) on the rooting percentage of semi-hardwood cuttings (Table 2) shows that statistically verified changes were observed on both types of substrate at the level of statistical significance p=0.01 between the control variant and variants IBA, NAA 3000 ppm, as well as with variants 1500 and 4500 ppm.

Table 2. The three way analysis of variance (ANOVA) on the rooting percentage of semi-hardwood cuttings with leaves taken at the end of August

Factor A - Substrate		Factor -B Growth regulators	Factor -C Concentration	Average (AB)	Average (A)
Turf	Turf-Perlite				
0.75	1.0	Control	-	0.88	0.75**
0.50	0.75	Control	-	0.63	
Average AC					
0.63	0.88				
2.25	3.00	IBA	1500 ppm	2.62	2.25*
1.75	2.00	NAA	1500 ppm	1.88	
Average AC					
2.00	2.50				
3.00	4.25	IBA	3000 ppm	3.62	3.25**
2.50	3.25	NAA	3000 ppm	2.88	
Average AC					
2.80	3.80				
2.50	3.25	IBA	4500 ppm	2.88	2.56*
2.00	2.50	NAA	4500 ppm	2.25	
Average AC					
2.25	2.88				
Average C					
0.75	2.25				
Average BC				Average B	
0.75**	2.25*			1.50**	
3.25**	2.56*			2.90**	

Factors		A**	B**	C**	AB	AC	BC	ABC
LSD	1 %	0.15	0.38	0.66	0.61	1.07	1.07	2.08
	5 %	0.11	0.28	0.48	0.44	0.73	0.73	1.25

The three way ANOVA according to the test by Vukadinoviç. **Significant 0.01% level. Numbers in bold show the most important attribute for variant

The stimulation of cuttings with auxin has played a central role in the development process of adventitious roots, and highly significant differences seem to exist between control variants and variants stimulated with IBA and NAA. Our results are also matching with the results of other authors (Smith and Thorpe 1975, De Klerk *et al.*, 1995, De Klerk *et al.*, 1999, Luckman and Menary 2002). The stimulation of cuttings with auxin apart from inducing the propagation of roots and their development, has also had an impact on reducing the time for rooting as well as on the uniformity of rooting of cuttings in general.

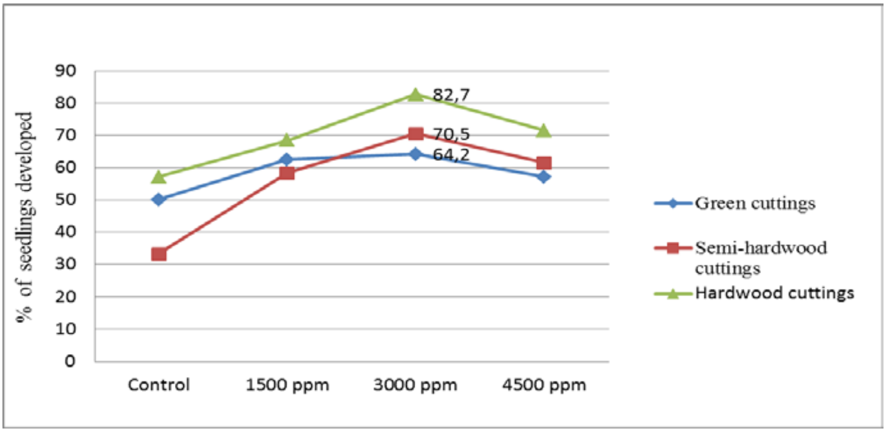

Figure 3. Average percentages of shoots developed at the end of acclimatization process, substrate turf-perlite 2:1 stimulated with IBA

Figure 3 shows that hardwood cuttings perform better during the acclimatisation process where the percentage of shoots developed at the end of the acclimatisation process reaches 82.7%. In these cuttings, the tissue is more mature and resistant towards the change of moisture and temperature which happens during the transplantation of shoots from rooting bangos in the vases. Before the removal of shoots, the amount of watering in the rooting bangos should gradually be reduced in order for the plant to be prepared for transplantation in vases, which should be placed in environments protected from the direct sunlight in order to avoid plant dehydration.

Whilst among semi-hardwood cuttings the percentage of shoots developed at the end of the acclimatisation process is 70.5% which is 12.2% lower than in hardwood cuttings. Yet, among green cuttings this percentage is even lower (only 64.2%) as a result of being more sensitive during the acclimatisation process and can very easily dehydrate, which is related to the level of shoot maturity.

CONCLUSIONS

- The geen cuttings are very sensitive and cannot endure for long in the loam, and thus their base part gets rotten. However, this propagation method has an advantage since it can provide a greater amount of seedling material which enables the rapid propagation of young cultivars and of those cultivars which have difficulties in rooting with hardwood cuttings.

- Results of the percentage of seedlings developed in hardwood and vegetative cuttings cv. ‘Bluecrop’ show that hardwood cuttings are more sustainable during the acclimatisation process and this is due to the physiological and anatomical status of the offshoot.

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SITE VARIABILITY AND DIEBACK OF ATLAS CEDAR IN THE CEDARFOREST OF THENIET EL HAD (WEST OF ALGERIA)

SUMMARY

Main goal of the paper was to investigate the wasting of the Atlas cedar (*Cedrus atlantica* (Endl.) Manetti ex Carrière) in the national park of Théniet El Had (west of Algeria).We have installed 59 temporary plots of circular shape and a surface of 10 acres each to study this effect. At the level of each plot, we have performed measurements (density, basal area, dominant circumference and dominant height), analyze of soil (pH, organic matter, humidity, rate of limestone, texture and nitrogen content) and noted some site parameters (exposure, altitude, slope and micro relief). The results show that the total basal area and density of trees are very important in the plots of the altitudinal floor 1400-1600 m, oriented toward the north and steep slope (>20°) where there is a positive relations on soils basaltic and texture of sandy-loam soils. The soil has an average depth at low varies from one canton to another. It is sandy-loam type with a pH of 5.92 to 7.8, not salty rich in organic matter, poor in nitrogen and non-limestone. The decline of the Atlas cedar is frequent through the northeastern, sunniest and driest exposures, along the altitudinal range between 1400 and 1600 m, where water losses are greater than the contributions on slopes > 20°.

Key words: Atlas cedar, decline, Theniet El Had, physic - chemical properties of soil, site factors.

INTRODUCTION

The cedar of the Atlas is a noble species, majestic and endemic in the North Africa, existing for millennia. It presents the bioecological and socio-economic value giving him a universal importance (M'Hirit, 1994). In North Africa, *Cedrus atlantica* covers an area of 145,000 ha in Morocco (Demarteau et *al.*, 2007; Zine El Abidine et al., 2013) and 16000 ha in Algeria (FAO, 2000). It's the first national park established during the colonial period in 1923 year and proclaimed once again as a national park after the independence in 1983 year. In Algeria, since the year 1999 to 2002 (the beginning of drought), the cedar forest

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dieback symptoms affecting all age classes were observed (Bentouati, 2008). This situation has only worsened from Djebels Boumerzoug and Tuggurt to Batna (national Park of Belezma) in an area of 300 ha (Meftah, 2001). As well as the study of Kherchouche et al (2013) showed that the drought is responsible for triggering dieback in this forest. In contrast, Talbi and Bouhraoua (2015) reported that the *Buprestidae* and *Scolytinae* beetles are responsible for the majority of the damage observed on Cedar. In 1984, the forest service revealed that hundreds of stems of cedar are affected by dieback in the Cedar Forest of Theniet El Had. The number of dead cedar on foot is lifted up to 2891 stems (PNTH, 2006). They were divided into small bouquets or isolated among the healthy stands. In July 1992, the cedars declining stands were 3/4 of the canton Rond-point, 1/4 of the canton Kef Siga and 1/4 of the canton Djouareb. In 2008, the work of Sarmoum (2008) was shown that among the probable causes of this phenomenon is the drought. According to the results of Naggar (2010), the local over density and the spatial arrangement inappropriate contribute to the decline of the cedar of the Atlas of Theniet El Had. Concerning the settings measurements and essentially the density and basal area of the cedar to Teniet was found that the density is slightly decreased after 30 years of growth because of the absence of the natural regeneration, dieback and illegal logging (Mairif, 2014). It is mainly due to illegal logging while the basal area is strongly reduced. In contrast, Taleb et al. (2016) has reported that there is no relationship between the rate of decline and topographic features and other edaphic factors. The hypothesis raised assumes the effect of factors site on the measured parameters and on the rate of decline of cedar. The main objective of this study is to search for possible relationships existing between the environmental parameters, soil and indexes with dieback of the Atlas cedar in the various cantons of the National park of Theniet El Had.

MATERIAL AND METHODS

The study area is located in the National park of Theniet El Had (Tissemssilt, west of Algeria) (Fig. 1).

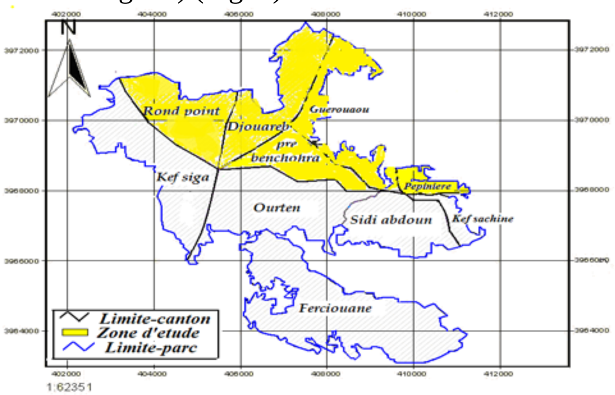

Figure 1. Delimitation of the study area (Guerouaou, Djouareb, Pre-Benchohra, Pepiniere et Rond-point) within the National park of Theniet El Had.

This park is part of the prolongation of the massif of the Ouarsenis and occupies the two slopes of Djebel El Meddad.It is located in the sub-humid bioclimatic floor in cold winter. 2,968 ha is covered by forest stands (PNTH, 2006).This study is based on the percentage of needle desiccation and branch mortality.

Four classes of cedar dieback based on the Nageleisen (1994) and Benhalima (2006) were defined in Table 1.

Table 1. Dieback classes of the Cedar

Cantons	Number of deserted trees	Classes	Lost hands	Health Categories
Pepiniere	0	C0	0 –5%	Healthy tree
	3	C1	10 – 25%	Tree weakened
	0 2 2	C2	30 –45% 50 - 60% 65 – 90%	Trees weakly depleted Tree moderately depleted Tree heavily depleted
	27	C3	>95%	Dead tree
Guerouaou	0	C0	0 –5%	Healthy tree
	10	C1	10 – 25%	Tree weakened
	0 11 10	C2	30 –45% 50 - 60% 65 – 90%	Trees weakly depleted Tree moderately depleted Tree heavily depleted
	71	C3	>95%	Dead tree
Rond-point	0	C0	0 –5%	Healthy tree
	9	C1	10 – 25%	Tree weakened
	1 1 4	C2	30 –45% 50 - 60% 65 – 90%	Trees weakly depleted Tree moderately depleted Tree heavily depleted
	49	C3	>95%	Dead tree
Djouareb	0	C0	0 –5%	Healthy tree
	1	C1	10 – 25%	Tree weakened
	0 4 0	C2	30 –45% 50 - 60% 65 – 90%	Trees weakly depleted Tree moderately depleted Tree heavily depleted
	27	C3	>95%	Dead tree
Pre-Benchohra	0	C0	0 –5%	Healthy tree
	21	C1	10 – 25%	Tree weakened
	3 13 12	C2	30 –45% 50 - 60% 65 – 90%	Trees weakly depleted Tree moderately depleted Tree heavily depleted
	109	C3	>95%	Dead tree

The dendrometric characterization of stand is defined for an irregular forest. The dendrometric data characterized with dominant circumference, height, total basal area and density. They correspond to the measures carried out on 1456 trees. In particular, 390 trees have got worsening indexes.

In addition, the topographic data collected at each plot were as following: exposure, altitude, slope of the terrain and type of microrelief. The microrelief qualitative criterion is classified among the most complex stationary factors (Masson, 2005). It is evaluated on the basis of input and loss of water. *TOPO* 1: the side losses of water are superior to inputs; *TOPO* 2: the inputs are zero or equal to the losses of water by drainage; *TOPO* 3: water flows more slowly at this level, which is a favorable situation; *TOPO* 4: the water situation is exceptionally favorable. "TOPO" meaning is the topography peculiarity. According to the homogeneity of the stations, 21 samples of the soil in the A1 horizon were collected. The determination of the total limestone was measured using the volumetric method using the Bernard calcimeter; pH (water) and pH (KCl) are measured using a pH meter with a soil / water ratio of 1:5.The soil organic matter was based on the determination of organic carbon multiplier 1.72 was used to change from carbon to total organic matter. The total soil nitrogen content was measured follow the Kjeldahl method. Particle size analysis has four different stages (Mathieu and Pieltain, 2007);

RESULTS AND DISCUSSION

The statistical characteristics of dominant circumference and height, density and total basal area are recorded in Table 2.

Table 2. Descriptive statistics of the various dendrometric variables.

Dendrometric variables	Descriptive statistics				
	Min	Max	Moy	Standard deviation	CV (%)
C dom (m)	1.23	3.45	2.04	0.53	25.78
H dom (m)	6.37	30.25	17.23	4.35	25.24
G (m²/ha)	14.7	83.6	39.87	17.51	43.92
Density (feet/ha)	100	700	175	98.64	56.50

Symbol: ***Min*** : Minimum ; ***Max*** :Maximum ; ***Moy*** : means ; ***CV*** : Coefficient of variation ; C***dom*** : Dominant circumference ; ***H dom*** : dominant height ; ***G*** : total basal area.

The distribution of the dominant circumference classes of the stands (Fig.2 A) is decreasing. The class of 1.23 to 1.78m has a relative frequency of about 36%.

Nevertheless, the largest stands (from 2 to 3 m) are more represented with a relative frequency of 64.15%. It was established that approximately 36% of the stand has dominant height (Fig. 2B), while stands with dominant height from 15.93 to 20.71 m (the most productive class) show the highest frequency (45.28%). If we refer to the study of Bentouati (2006) carried out at the Belezma cedar, the differences in height and circumference reveal much more variations of the state and of the stands age.

The distribution of the relative frequencies of the classes of the total density of the Atlas cedar can be better visualized in Figure 2C. Through this figure, 90.57% of stands have a total density less than 300 feet/ha. On the other hand, about 2% of the stands have a very high density (500-700 feet/ha).These results allow us to say that there is no great competition between individuals. It is therefore a moderately dense forest at different stages of development. Throughout Figure 2 D, the total basal area shows a decreasing pattern where about 38% of stands have basal areas less than 32 m²/ha. On the other hand, 11.32% of stands (the most fertile class) have land areas more than 66.36 m²/ha.

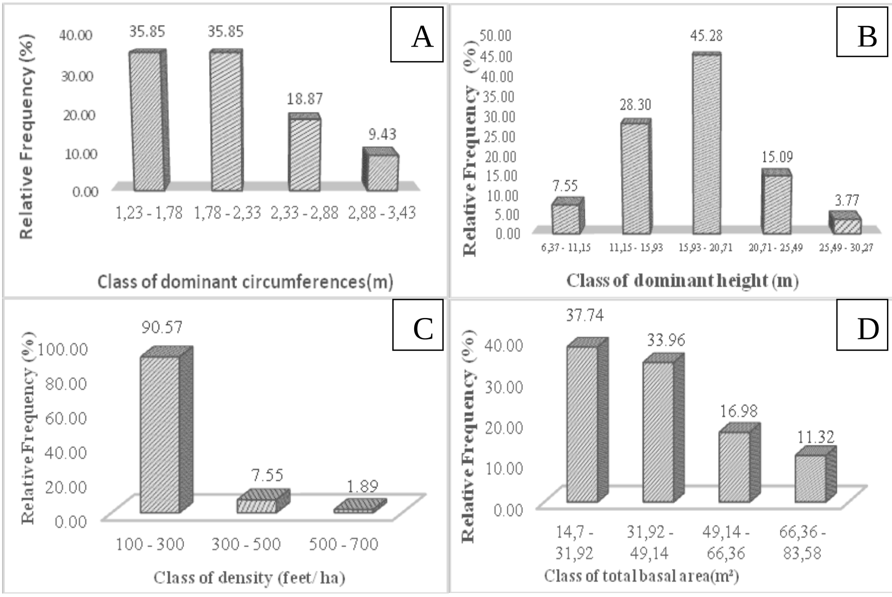

Figure 2. Distribution of dominant circumference (A), dominant heights (B), density (C) and total basal area (D) of Atlas cedar stands.

For all the plots, three variants of exposition are retained (North, East and West). Due to the diversity of the topographic sites of the study plots the topographic stratification selected reveals four types of topography observed in the field.

TOPO1 : presenting 37.29% of all observations ; *TOPO2* : presenting 20.34% of all observations ; *TOPO3* : presenting 27.12% of all observations ; *TOPO4* : presenting 15.25% of all observations.

On the basis of a staging of 200m of altitude, three altitudinal classes are retained. The slope values recorded in the study plots are from 0 to> 20°. Thus, on the basis of a difference of 10° of slope, three classes of slope are retained.

At the level of the study plots, it should be noted that the depth of the soil varies greatly from one canton to another. For example in Canton Guerouaou, it is moderately weak (30 cm) with an increase in stony load and rock outcroppings every time we climb up. These outcrops constitute a constraint for the growth of trees. On the other hand, in Pepiniere, Rond-point and Pres-Benchohra township, the coarse-grained load is moderately present and the soil is deep (more than 50 cm deep), allowing good root exploitation. The observation of the charge in coarse elements is moderately present. Two layers of texture characterize all soil samples: sandy and sandy loam. Since it is a gardened stand, where the notion of age makes no sense, the total density and the total basal area are chosen as dendrometric variables that explain the rate of this type of stand. The choice of these two variables allows us to explain the evolution of stand structure and to identify stationary fertility regardless of age. Based on total density stratification, the analysis of the variance showed no significant difference in Cedar dieback. Dewatering is very common (90.63%) in plots where density classes are less than 300 feet/ha. Indeed, the high density of the stand causes strong competition between the trees and consequently a difficulty of growth of the stems and a weakening of the trees.

If we refer to the total recorded basal area values, the 14 to 32 m²/ha class has the highest dieback rate (44.30%). This descriptive situation, which is subject to analysis of one-factor variance, does not reveal any significant difference in the Cedar dieback rate across the total basal area values recorded is shown in Table 3.

Table 3. Results of the variance analysis at the 95% significance level between cedar dieback and dendrometric variables (total basal area and density).

Dendrometric variables		**Dewatering rate (%)**	**F observed**	**F theoretical**
Density (Feet / ha)	100 - 300	90.63	0.47	3.16
	300 - 500	8.35		
	500 - 700	1.01		
Total basal area (m²)	14.7 – 31.92	44.30	0.33	2.77
	31.92 – 49.14	29.87		
	49.14 – 66.36	17.22		
	66.36 – 83.58	8.61		

The rate of decline per plot is evaluated against all cedar stems identified, whether healthy, deserted or declining. This evaluation translates into the following formulation :

$$\text{Rate of decline per plot in } (\%) = \frac{\text{Number of subjects (healthy, declining or declining)}}{\text{Total number of plot subjects}} \times 100$$

As shown in Figure 3, plot (50) is in Pre-Benschohra Township where the rate of dieback is 100% (there is no healthy subject), this plot is characterized by an east orientation, an altitude of 1555m, a slope of 24° and a *TOPO 1*. This situation, clearly evident on land, shows a singular peculiarity relative to this canton. This one, shaded and moist, reveals the most cedar deadwood. The plot (7) in the Pepiniere canton, on the contrary, remains the one where the decline is least noticeable (5.71%), and this, since the Cedar initiation until today. This plot is located in the lower elevations, on a flat terrain of 7 ° slope and an exposure of NNE. In addition to this peculiar tendency of the Pepiniere to be the one where the dieback is relatively weak, it is also the one where the cedar is almost in the pure state.

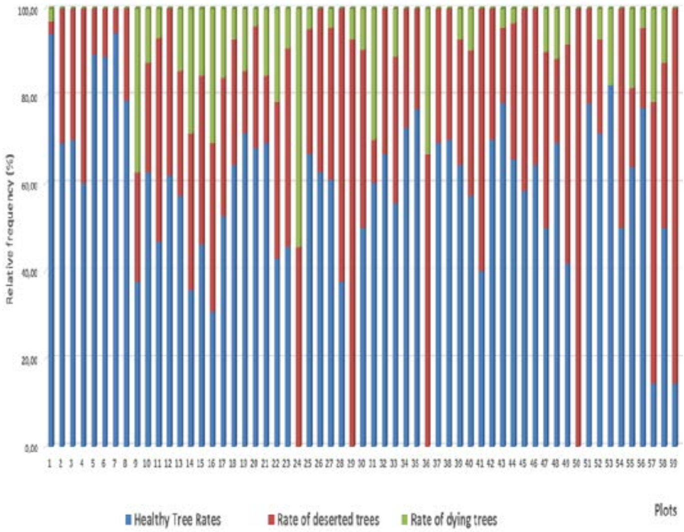

Figure 3. Rate of dieback per plot

Observations of the different exposures across the plots recorded in Table 4 show that there is a significant relationship between exposure and decline, despite it being encountered across all exposures, remains very common (52.66%).

The dieback is found to be very frequent, 75.44%, through the altitudinal stratum "1400 to 1600 m". On the contrary, it is less important at altitudes less than 1200 m. This low rate of decline seems to be related to the forest stand structure since the presence of Atlas Cedars remains low at this altitude (Zedek, 1993). We note that 57.21% of deserted trees are located on highly rugged terrain where gradient values are > 20. On the other hand, the decline is less frequent across slope classes "0 - 10°" and "10 - 20°", resulting in rates of 17.47 and 25.32% respectively.

Table 4. Analysis of variance at 95% significance level between decline rate and stationary factors

Stationary Factors	Site specifity	Dewatering rate (%)	Analysis of the variance (F obs)
Exposure	E – EE	26.84	**3.17***
	N – NE	52.66	
	NW - WW	20.1	
Altitude	1000-1200m	6.33	1.78
	1200-1400m	18.23	
	1400-1600m	75.44	
Slope	0°- 10°	17.47	0.31
	10° - 20°	25.32	
	>20°	57.21	
Microrelief	TOPO 1	34.68	1.98
	TOPO 2	15.44	
	TOPO 3	28.61	
	TOPO 4	21.27	

*: meaning at 5%.

Due to the diversity of the topographic sites of the study plots, the rate of decline is relatively high (34.68%) on "*TOPO 1*" type land, which results in water losses greater than the inputs. However, the type "*TOPO 4*", where the water situation is exceptionally favorable, has a rate of dieback of 21.27%. The results of Bonneau et al. (1969) show that the dieback is more intense in the more hydromorphic soils, that is to say in the soils where the water table is closer to the surface. Also, the topographic position affects the depth of the soil and its ability to retain water. Nevertheless, we do not record any significant difference in the rate of dieback through the topographic formations and the different altitudinal classes.

Through the texture strata, the frequency of the dieback phenomenon is low about 31% in the sand stratum (Table 5).

On the other hand, it is very common in the sandy loam stratum (69%). According to Oueld Safi (2014), the sandy texture is characterized by a low water and nutrient retention capacity.The phenomenon of dieback is very common in the classes where the humidity is low, a rate of 66.37% of the total population, whereas it is less frequent (33.63%) in the classes with very high humidity. The first limestone level stratum comprises a dieback of the order of 85%, whereas the second stratum comprises only a dieback of 15%.The phenomenon of dieback is very common (48%) in samples with high nitrogen content, whereas it is less frequent in the 11.93 to 22.06 % classes where a 21% rate of deserted trees is noted.

On the whole of the physicochemical parameters of the soil taken into account in our work, only the pH seems to have an effect on the dieback of the

Cedar of the Atlas. Indeed, the results obtained on the acidity of the soil show that the decline is less frequent (35%) in the soils where the pH is slightly acid (5.92 to 6.86), very favorable values for the vegetation, and it is very frequent (65%) in soils with low pH (7.8).

Table 5. Analysis of variance at the 95% significance level between the rate of dieback and soil factors

Edaphic Factors	Variants	Dewatering rate (%)	F observed	F theoretical
Soil texture	Sandy loam	69.03	1.67	4.38
	Sandblaster	30.97		
Humidity (%)	9.78 - 25.60	66.37	0.08	4.38
	25.60 – 41.00	33.63		
Organic material (%)	0.92 – 3.42	30.97	1.27	3.55
	3.42 – 5.92	37.17		
	5.92 – 8.42	31.86		
Limestone content (%)	0 – 0.67	84.96	0.60	4.38
	0.67 – 1.35	15.04		
pH	5.92 – 6.86	35.0	5.99*	4.38
	6.86 – 7.80	65.0		
Nitrogen content (%)	1.8 – 11.93	31.0	1.15	3.55
	11.93 – 22.06	21.0		
	22.06 – 32.19	48.0		

*: meaning at 5%.

CONCLUSION

In order to better elucidate the problem of dieback, we were able to statistically investigate the possible relationships between the rate of decline and the ecological descriptors from the one hand and the soil parameters on the other hand. The decline of the Atlas Cedar is frequent through the most sunny and dry-oriented exposures to the north-east, where about 53% of the trees in the Atlas mountains are depleted.

Along the altitudinal stretch between 1400 and 1600 m, the rate of decline is about 75.44% of the total number of Cedars inventoried.On *TOPO 1* type sites where water losses are greater than contributions, the decline is estimated at 34.68% of the total population.On slopes > 20°, we have a decline rate of 57.21%. The data obtained from this work certainly allow us to better understanding the biological, ecological factors that govern the functioning, dynamics and evolution of cedar trees. They will be used in the implementation an adequate management plan and objective and rational silvicultural interventions.

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EVALUATION OF YIELD AND SOME QUALITY CHARACTERS OF WINTER BARLEY (*Hordeum vulgare* L.) GENOTYPES USING BIPLOT ANALYSIS

SUMMARY

In this study, it was used 19 winter barley advanced lines which are selected from regional yield trials and 5 cultivars (TARM-92, Aydanhanım, Sladoran, Karatay-94 and Kalaycı-97) in order to determine grain yield and some quality traits under ecological condition of Diyarbakır. The experiment was conducted in 2004-2005 growing seasons in Diyarbakır under rainfed conditions, with randomized complete block design with 3 replications. Grain yield and different quality traits were considered: test weight (HLT), thousand kernel weight (TKW), grain protein content (PC), starch value (STR) and 2.5-2.8 mm sieve ratio (SV). According to the results; the highest grain yield obtained from G16 (5269 kg ha^{-1}), G17 (4930 kg ha^{-1}) and Karatay-94 (4868 kg ha^{-1}) genotypes, the lowest grain yield obtained from G6 (2625 kg ha^{-1}), G21 (3186 kg ha^{-1}) and G11 (3428 kg ha^{-1}) genotypes. According to biplot analysis that based on the visual correlation between traits, HLT, SV, GY and STR were involved in the same group, while TKW and PC were involved individually in separate groups genotypes located in corner of the polygon, G11 and G21 had highest values or desirable traits for protein content, G8 was the best for TKW while G7, Kalaycı-97 and Sladoran were the best for HLT and SV. The results also showed that genotypes with appropriate combination in terms of examined traits were selected for national winter wheat breeding programs.

Key words: Biplot, grain yield, quality, winter barley.

INTRODUCTION

Barley is classified as a long-day plant, which means that it will flower earlier when exposed to increasing day lengths (Casao et al, 2011). Barley (*Hordeum vulgare* L.), a member of the grass family, is one of the eight founder crops (einkorn wheat, emmer wheat, barley, lentil, pea, chick pea, bitter vetch, and flax) (Kant and Babu 2016). Barley was first domesticated in the Fertile Crescent in the Near East which spans present-day Palestina-Israel, Northern Syria, Southern Turkey, Eastern Irak and Western Iran (Harlan 1979). The total

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

area covered by barley is about 2.74 million hectares with total production of 6.7 million tons and the yield of the crop is low with national average of 2.48 tha^{-1} İn Turkey (TMO, 2016). It is the second important cereal crop of Turkey and accounts for about 25% of the total cereal production (Oralet al., 2018).

In Southeastern Anatolia region, the total area covered by barley is about 0.327 million hectares with total production of 1.035 million tons and the yield of the crop is low with regional average of 3.04 tha^{-1} (Gaputaem, 2013). In Southeastern Anatolia, barley is sown in late autumn, after the occurrence of the first rains in the arid environments. Kılıç et al, (2010) reported that barley has been cultivated for many years and has significant role in dry areas of Eastern Transitional Zone, Turkey and barley grain yield and quality are exposed to different factors varying on a large scale. The Northern part of southern region's climate is sufficiently humid (annual precipitation rate 500 mm) with mild-hard winter weather which enable winter barley to perform well. Because the interaction between environmental stress and barley genotypes has not been sufficiently investigated (Ceccarelli et al. 2010; Alemayehu et al., 2014;), this target should be involved in breeding strategies. Mohammadi et al. (2013) reported that some barley varieties require certain times expose to cold temperatures to initiate flowering, while others initiate flowering without any obligation for vernalization.

Thus, learning more about genetic variation of winter and spring types in barley breeding genotypes is useful for developing and adaptation new varieties for worked environments. The aim of this study was to evaluate the yield performances of various winter barley varieties and advanced lines for the Diyarbakır region in terms of adaptability.

MATERIAL AND METHODS

The study was carried out on the experimental area of GAP International Agricultural Research and Training Center, Turkey, (37°56' N; 40°15' E; 599 m altitude) during the growing seasons of 2004 to 2005 (Fig 1).

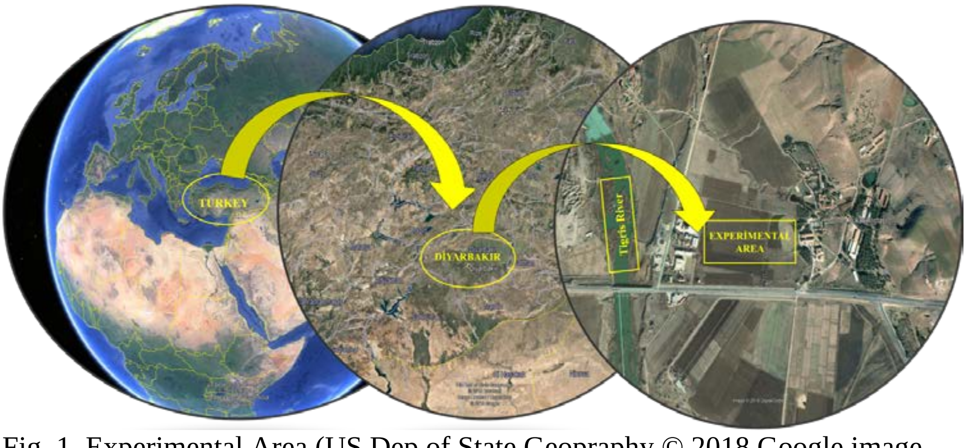

Fig. 1. Experimental Area (US Dep of State Geopraphy © 2018 Google image landset/Copernics)

The 25 two row-barley genotypes were tested under dryland conditions of Northern part of southern Anatolia (Diyarbakır) (Table 1).

Table 1. Name and origin of barley genotypes used in experiment.

Code	Name	Year of release	Origin
G1	Eskişehir-18	Advanced line	Anadolu Agricultural Research Inst
G2	Eskişehir-14	Advanced line	Anadolu Agricultural Research Inst
G3	Eskişehir-13	Advanced line	Anadolu Agricultural Research Inst
G4	Eskişehir-8	Advanced line	Anadolu Agricultural Research Inst
G5	TARM-92	1992	Central Res Inst. for Field Crops
G6	Eskişehir-2	Advanced line	Anadolu Agricultural Research Inst
G7	Edirne-11	Advanced line	Thrace Agricultural Research Inst.
G8	Edirne-10	Advanced line	Thrace Agricultural Research Inst.
G9	Edirne-9	Advanced line	Thrace Agricultural Research Inst.
G10	Aydanhanım	2002	Central Res Inst. for Field Crops
G11	Ankara-4	Advanced line	Central Res Inst. for Field Crops
G12	Ankara-13	Advanced line	Central Res Inst. for Field Crops
G13	Ankara-14	Advanced line	Central Res Inst. for Field Crops
G14	Ankara-16	Advanced line	Central Res Inst. for Field Crops
G15	Sladoran	1998	Thrace Agricultural Research Inst.
G16	Ankara-21	Advanced line	Central Res Inst. for Field Crops
G17	Ankara-59	Advanced line	Central Res Inst. for Field Crops
G18	Ankara-62	Advanced line	Central Res Inst. for Field Crops
G19	Ankara-64	Advanced line	Central Res Inst. for Field Crops
G20	Karatay-94	1994	Bahri Dagdas Intern. Agr.Res. Inst.
G21	Ankara-416	Advanced line	Anadolu Agricultural Research Inst
G22	Ankara-107	Advanced line	Anadolu Agricultural Research Inst
G23	Ankara-116	Advanced line	Anadolu Agricultural Research Inst
G24	Ankara-148	Advanced line	Anadolu Agricultural Research Inst
G25	Kalaycı 97	1997	Anadolu Agricultural Research Inst

The soil of the experimental field was silty loam with pH of 7.87, the organic matter was 1.16%, phosphorus (P_2O_5) 32 kg ha^{-1}, potassium (K_2O) 950 kg ha^{-1}, saturation 68% and lime (C_aCO_3) 10.2%. The weather conditions during the crop cycles are presented in Table 2. Sowing was done by a Wintersteiger drill on 15 November in 2004.

The experiment was conducted in a Randomized Block Design with three replications. Seeding rates were 400 seeds m^{-2}. Plot size was 7.2 m^{-2} (1.2 × 6 m). The plots were fertilized with 60 kg N ha^{-1} and 60 kg P_2O_5 ha^{-1} at the sowing and 40 kg N ha^{-1} in spring at stem elongation for drought conditions. Harvest was done using Hege 140 harvester in 6 m^{-2}. Grain yield was recorded in kg per hectare (kg ha^{-1}) after combine harvesting. Thousand grain weights (TGW) and hectoliter (HL) were determined according to the ICC standard method. The barley samples were graded by size fractionation with sieving fractions with three slotted sieves of different widths (2.8, 2.5 and 2.2 mm).

Table 2. Monthly and long term averages of the climatic data in the experimental area.

Months	Precipitation mm^{-1}		Mean air temperature ^{o}C	
	Long-term	2004-2005	Long-term	2004-2005
September	2.7	0.0	24.8	25.0
October	31.1	1.3	17.0	18.2
November	54.0	123.1	9.6	8.2
December	71.5	4.7	4.1	1.4
January	73.5	58.7	1.6	2.3
Ferbruary	67.1	46.8	3.6	3.0
March	67.9	58.4	8.1	8.4
April	70.5	36.8	13.8	14.1
May	42.1	26.5	19.3	19.6
Jun	7.0	33.1	25.9	25.8
July	0.7	0	31.0	32.0
August	0.5	0	30.3	31.8
Total	488.6	389.4		

A sample of 100 g of grain was placed onto the top sieve and shaken for 5 min (Magliano et al., 2014). Protein and starch content are analyzed by **Whole Grain Analyzer (**NIT) instrument that principles described by Maghirang et al (2006) and Dowell et al. (2006).

Analysis of variance (ANOVA) was used to determine the effects of genotype on yield and quality traits. Tukey test was performed to determine the significant differences between individual means. All statistical analyses were performed using the SAS program (SAS Institute, 1999) and GenStat 14 software. GGE biplot analyses were carried out using GGE biplot software to assess traits (Yan and Thinker, 2006, Dogan et al, 2016). In multi-traits (MT) for genotypes, biplots were constructed by plotting the first two principal components (PC1 and PC2) derived from centered quality criteria data to singular value separation. Also, with the GGE biplot analysis graphs in the study: It was aimed at revealing relation among examined traits and genotypes means by scatter plot (Fig. 2), and grouped traits and performance of each genotype at each trait (Fig. 3), Which-Won-Where based on traits and genotypes (Fig. 4), compare the desirable genotypes to ideal center on traits by comparison model (Fig. 5).

RESULTS AND DISCUSSION

Grain yield

Grain yield is ultimate component, which is not only genetically determined but also related to the growing conditions or environment (Popović et al., 2011; Mladenović et al., 2009; Đekić et al., 2012.a) Sabaghnia at al., 2013; Chamurliyski et al., 2015). The analyses of variance revealed that grain yield had significant differences between genotypes (Table 3).

Table 3. Means of Test weight, TGW, coarse grain, protein, starch and grain yield of winter barley genotypes

Deneme Adı	Test Weight kg m^{-3}	TKW g	Coarse grain >2.5 mm (%)	% Protein	% Starch	Grain yield kg ha^{-1}
G1	59.6	34.8	37.08	12.9	62.3	4258 abc
G2	53.2	33.0	25.81	15.1	60.1	3564 bcd
G3	60.1	34.5	42.35	14.7	61.2	4042 a-d
G4	60.8	37.3	52.66	13.5	62.0	4233 a-d
G5 TARM-92	60.9	33.6	41.35	15.1	62.0	4275 abc
G6	49.6	23.7	15.18	14.8	58.9	2625 d
G7	62.7	35.4	46.68	15.3	61.2	3822 a-d
G8	66.5	34.6	67.88	14.2	61.5	3758 a-d
G9	66.5	37.0	67.85	12.8	62.9	4011 a-d
G10 Aydanhanım	62.3	31.1	73.43	13.7	62.4	4672 abc
G11	59.1	36.7	38.58	16.1	60.1	3428 bcd
G12	61.5	35.4	57.55	14.3	61.8	3978 a-d
G13	62.6	36.5	47.83	15.3	62.0	4000 a-d
G14	61.8	33.9	63.61	15.2	61.5	4156 a-d
G15 Sladoran	62.3	37.1	67.27	12.8	62.2	3542 bcd
G16	64.1	37.5	60.87	13.1	63.5	5269 a
G17	65.6	36.5	66.50	13.7	62.2	4931 ab
G18	59.8	34.9	48.81	13.7	61.8	4511 abc
G19	62.0	36.4	60.01	13.5	62.4	3828 a-d
G20 Karatay-94	63.4	32.4	56.02	13.8	62.6	4858 ab
G21	62.0	36.4	60.00	13.5	62.4	3186 cd
G22	55.6	36.4	52.35	15.5	59.9	4000 a-d
G23	65.7	34.6	51.59	14.8	61.4	3947 a-d
G24	60.5	37.0	52.67	12.7	62.6	4458 abc
G25 Kalaycı 97	62.8	37.1	57.09	13.6	62.5	4258 abc
Min.value	53.2	23.7	15.18	12.7	58.9	2625
Max. value	66.5	37.3	67.88	16.1	63.5	5269
CV%						12.6

According to the results; the highest grain yield obtained from G16 (5269 kg ha^{-1}), G17 (4931 kg ha^{-1}) and Karatay-94 (4858 kg ha^{-1}) genotypes, the lowest grain yield obtained from G6 (2625 kg ha^{-1}), G21 (3186 kg ha^{-1}) and G11 (3428 kg ha^{-1}) varieties. The results are consistent with those of Kılıç et al. (2010) and

Bayram et al. (2017) while the results are lower than those of Öztürk et al. (2007); Sirat and Sezer (2017); in these, higher yields can be obtained in a warmer climate only by means of better adapted genotypes. Moreover, Akıncı and Yildirim (2009), who studied barley accessions from South East region of Turkey, reported that the decreasing of grain yield at high rainfall environment depend on lodging.

Biplot analysis

Principal component analysis was used to show the distribution of genotypes based on traits. The two dimensional PCA score plot for the 25 genotypes data, derived from multi-traits and explained 78.86% (64.57% and 14.29% by PC1 and PC2, respectively) of the total variation (Figs. 1-4). This view of the biplot explains understanding of the interrelationships among the traits (Yan, Rajcan, 2002; Segherloo et al., 2016). GT biplot analysis graphs revealed significant results that there is high relation among examined traits and genotypes means by scatter plot (Fig. 2), and grouped traits and performance of each genotype at each trait (Fig. 3), which-won-where based on traits and genotypes (Fig. 4), compare the desirable genotypes to ideal center on traits by comparison model (Fig. 5).

The relationship each genotype by each trait: Both the genotypes vectors and the traits vectors are drawn in Fig. 1, so that the specific interactions between a genotype and a trait (i.e., the performance of each genotype in each trait) can be visualized. Therefore this figure can be used (1) to rank the genotypes based on performance in any trait, and (2) to rank traits on the relative performance of any genotype. The interpretation of performance a genotype in a trait is better than average if the angle between its vector and the trait's vector is <90°; it is poorer than average if the angle is >90°; and it is near average if the angle is about 90° (Yan and Tinker, 2006). The results of traits showed that there is high variation among genotypes. According to results, there was high correlation among GY-STR, SV-HLT-TKW and while negative correlations among these Traits and PC. Moreover the effect of each trait generally was the same because the long of each trait vector was the same. On the other hand; some genotypes related with special trait; G16 for GY-STR, G17 for SV-HLT-TKW, G11and G21 for PC. The genotypes are far from center of biplot graphs, are specific genotypes (G16, G11 and G21) for specific trait. While G6 located opposite of traits, so this genotype did not related with any genotypes. Therefore, there is major contribution of trait to traits; because of they have opposite direction, so they can make up different genetic contribution (Jalata 2011). The GT biplot mainly allows the visualization of any crossover GT interaction, which is very important for the breeding program (Atnaf et al., 2017). The GT (genotype-trait) biplot provides an excellent tool for visualizing genotype × trait data (Adjabi et al., 2014).

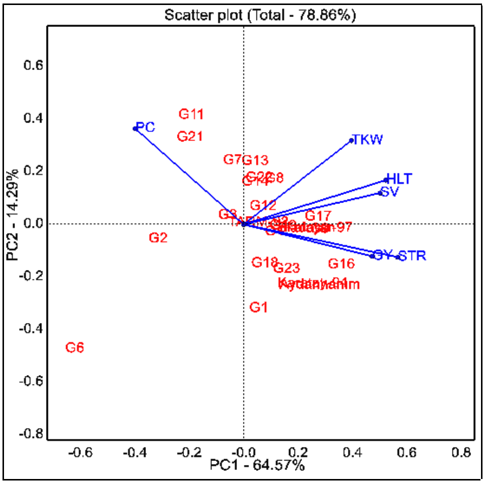

Figure 2. Relation of traits and genotypes based GT Biplot

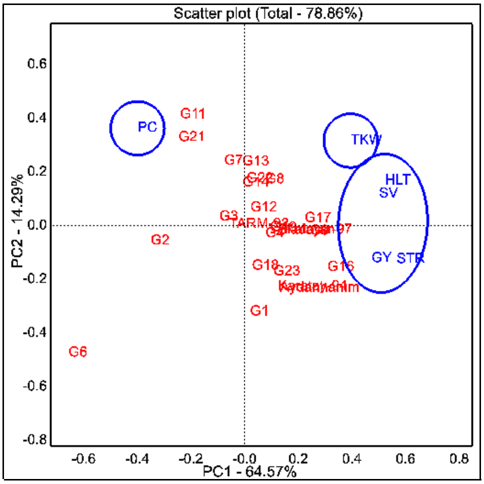

Figure 3.Group of traits based on genotypes by GT Biplot.

Group of traits based on genotypes by GT Biplot: The GGE biplot visualize the correlation amongs traits and grouping them also to visualize the interaction patterns between genotypes and traits (Yan and Tinker 2006). The partitioning of GT interaction divided into three groups (Fig. 3). The traits (GY, STR, SV and HLT) took place in first group; TKW in second group; and PC in third group. The majority of genotypes showed general adaptability for traits. On the other hand, there was correlation among traits which took places in same group. These genotypes took places near group of trait can be select for this group traits (G2 for PC) or some of them is poorest genotypes (G6) for all of traits because they were farthest from the origin of the biplot. GT biplots were found to be effective to reveal important relationships among genotypes and traits of winter barley for ease multi-variety selection (Yan and Kang 2003). The Genotype by Trait (GT) biplot can be used to compare cultivars on the basis of multiple traits and to identify cultivars that are particularly good in certain traits and therefore can be candidates for parents in plant breeding program (Dolatabad et al., 2010). Mega traits "which-won-where" pattern to identify the best genotypes in each environment: Discriminating the target environment into meaningful mega-traits and deploying different genotypes for different mega-traits is the only way to utilize positive GT and avoid negative GT and the sole purpose for genotype by environment interaction analysis (Yan and Tinker 2006; Yan and Rajcan 2002). This definition explains the following biplot based on the multi-traits trials (MT) data of barley yield illustrates two points: 1) A mega-traits may have more than one winning genotypes (sector 2), and 2) even if there exists a universal winner (G16, G17), it is still possible and beneficial, to divide the target traits into meaningful mega-traits (Fig. 4). Mainly, the six lines of biplot graph divide the biplot into six sectors. The traits located in three separate sectors; this means that the traits can be used in the selection special genotypes. On the other hand, second sector consist of all controls variety with some genotypes and related with GY, STR, SV, HLT, and G16 represented of vertex this sector. While TKW took places in third sector and did not related with any genotype and PC consists of consists of took places in fifth sector and related with G11, G21. The result of this study showed that G16, G11 is suitable to recommend to high potential for special traits. Kendal and Sayar (2016) reported that there is a strong correlations between traits, which located in same sector. Mohammadi et al. (2011), the large variation due to traits indicated strong influence of trait and existence of mega-traits among trial conducting traits; this suggests the usefulness of GT biplot technique for identifying mega- traits among barley genotypes. According to Yan and Rajcan (2002), multiple trait data illustrated that GT biplots graphically displayed the interrelationships among traits.

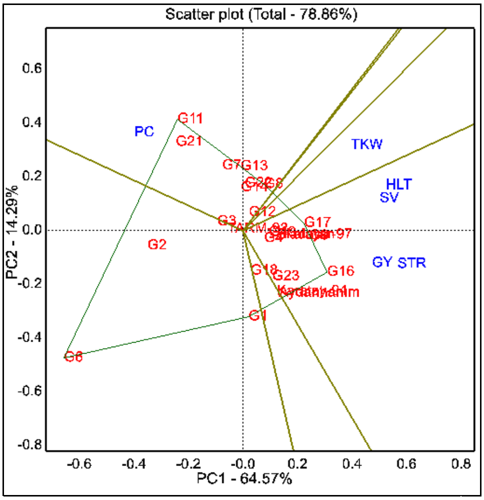

Figure 4.Which-Won-Where based on traits and genotypes by GT Biplot.

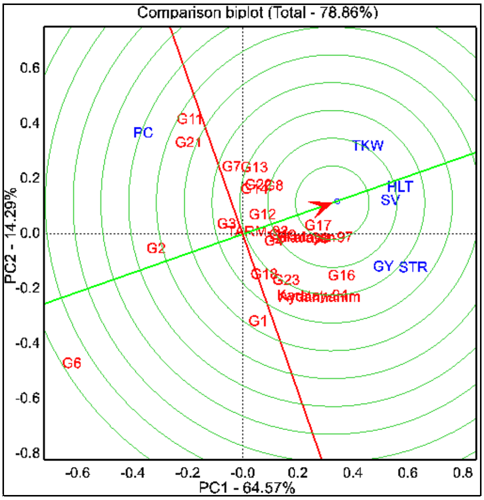

Figure 5.Comparison genotypes based on traits by GT Biplot.

Comparison of genotypes based on traits by ideal genotype: The genotype has both high traits mean and high stability is called an ideal genotype (Fig. 5). The center of the concentric circles is a point on the AEA ("absolutely ideal") in the positive direction and has a vector length equal to the longest vectors of the traits on the positive side of AEA (highest mean performance). So, genotypes located closer to the ideal circle are meaning that it is ideal genotype than others (Yan and Tinker, 2006). In the study, G17 located in center of AEA (absolutely stable), but; G16 took place of near center of AEA. On the other hand; G1, G2 and G6, G11 and G21 were undesirable for all traits except PC, because they took places under mean of trait s values. So, G17 is ideal than other genotypes. Consequently, G17 can be recommended for release in terms of all traits. Ngozi (2011), genotype evaluation and selection of parents for traits are facilitated by GT biplot. The genotype with both high mean performance and high stability for all of the traits was called an ideal genotype (Akinwale et al., 2014). Therefore the center of the concentric circles (i.e., ideal genotype) was the

AEA in the positive direction. Genotypes located closer to the ideal genotype were more desirable than others.

CONCLUSIONS

In the study, the GT Biplot results indicated that yield performance and quality performances of barley genotypes were highly influenced by growing season conditions (rainfal). The genotype G17, demonstrated best performance among genotypes tested growing seasons, while G21 and G11 had good result for PC, while G16 for STV, GY, SV, and HLW. Therefore, G17 was desirable in terms of majority of traits; while the specific genotypes were appropriate for specific traits (G 11 for PC, G16 for GY). As a result indicated that G17 and G16 are suitable to recommend for release, while G21 and G11 valuable source for PC to use in barley breeding program. The result showed that GT Biplot analysis permitted a meaningful and useful summary of GT interaction data and assisted in examining the natural relationships and variations in genotype performance on traits.

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DETERMINATION OF WATER RETENTION CHARACTERISTICS OF PERLITE AND PEAT

SUMMARY

The results of the water retention curves between (pF -2 and -4.2) matric potential for two substrates perlite and peat, are presented in this paper. Perlite is an inorganic, expanded aluminosilicate of volcanic origin. Peat is an organic substrate. For assessing this parameter, the method of bar extractors and Porous plate extractors have been explored. The method is applied on 7 different regimes of pressure (0.1; 0.33; 1; 3; 6.25; 11; 15 bars) in samples composed of perlite and peat present at different volume ratios of 80% Perlite + 20% Peat, 70%Perlite + 30% Peat, 50% Perlite + 50% Peat, 30% Perlite + 70% Peat, 20%Perlite + 80% Peat. The retention capacity of the perlite, at all applied different point of tension, is: 67.88% for 0.1 bar, 58.35%, for 0.33 bar, 47.70% for 1 bar, 39.78%; for 3 bars, 34.84 for 6.25 bars, 30.10% for 11 bars and 26.65% for 15 bars and for the peat are: for 0.1 bar = 89.16%, for 0.33 bar = 74.84%, for 1 bar = 57.94%; for 3 bars = 45.15%; for 6.25 bars = 39.57; for 11 bars = 33.89%; for 15 bars = 23.17%. The peat substrate shows higher retention at all points of tension of 0.1; 0.33; 1; 3; 6.25; 11, with the exception of 15 bars, when the retention is lower than the substrate perlite.

The reason for the higher water retention at peat than at perlite, is the result of the high content of the humus in the peat. Of all the analyzed samples, it can be seen that all curves show a favorable water retention capacity, which is due to the fact that the peat and the perlite as substrates have high porosity. The aim of this paper is to examine the impact of the water retention capacity of both substrates and their mixtures. Also to see the ability which substrate retains a greater amount of water that will be easily accessible to the plants for their proper growth and development.

Key words: perlite, peat, water retention.

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INTRODUCTION

Substrates are formulated from various inorganic and organic components to provide suitable physical and chemical properties as required by the specific crop and growing conditions (Bunt, 1988). An important physical property of substrates is air-filled pore space. Individual components of mixed substrates are often chosen considering their properties so that they complement each other and the resultant medium possesses most of the desirable attributes for good plant growth and production. Soil and organic components used in substrate mixes, like peat or compost, often lack coarse particles necessary for adequate aeration and hold moisture relatively tightly around the particles, predominantly by adsorptive forces (Bilderback & Jones, 2001).

Perlite is an inorganic, expanded aluminosilicate of volcanic origin (Nelson, 2003). Perlite is a very lightweight soil amendment perlite is used throughout the world as a component of soil-less growing mixes where it provides aeration and optimum moisture retention for superior plant growth. Expanded perlite has several attractive physical properties for commercial applications including low bulk density, low thermal conductivity, high heat resistance, low sound transmission, high surface area, and chemical inertness.

The use of perlite reportedly dates back to the 1800s and modern exploitation of this resource in the United States began in the 1940s (Austin & Barker, 1998; Ennis, 2011; Allen, 1992; Weber, 1963). Expanded perlite is commonly used for herbicides, insecticides, and fertilizer as a carrier (Tekin, 2004), Moreover, it is commonly used in the food industry, filter product, growing of seed, regulating of the soil in agriculture, and in so many other industrial applications (Alihosseini et al., 2010). Perlite has very good physical characteristics. The physical properties of container-growing substrates, particularly air space, container capacity, and bulk density, have a significant impact on plant growth, and knowledge of these properties is essential in properly managing nursery irrigation and fertilization programs (Yeager et al., 2000). As such, physical properties of container-growing substrates and individual substrate components have been investigated and reported in numerous research studies in past years (Bilderback et al., 1982; Bilderback & Lorscheider et al., 1995) and continue to be emphasized in more recent studies (Abad et al., 2005; Bilderback et al., 2005; Blythe et al., 2005; Cole et al., 2005) perlite is one of the best media for growing plants, it is possible to grow most plants in perlite alone and is just as successful as traditional peat mixes. However there are no nutrients in perlite. Perlite on the other hand has been widely used in soil-less cultures. Perlite, is rather inert (low buffering and cation exchange capacities. In general, it has a closed cellular structure, with the majority of water being retained superficially and released slowly at a relatively low tension, providing excellent drainage of the medium and aeration of rhizosphere. Therefore, it requires frequent irrigation to prevent a fast developing water stress (Maloupa et al., 1992).


Figure 1. a) Substrate peat b) Substrate perlite

Peat is an organic substrate. In 1960 the peat as a substrate began to be used in gardening for growing vegetables (Puustjarvi, 1973). There are different types of peat that differ in their degree of decay (Handreck and Black, 2005; Handreck, 1992). Depending on what the peat is formed of, there are different types of peat, from different plant species, created at different climatic conditions, and all of these conditions affect the different characteristics of the peat (Raviv et al ., 2002). Peat is a very porous substrate with excellent water capacity, and therefore is used together with other substrates. Advantages of peat as a substrate have been studied by many authors. Its long-time success is certainly due to the physical properties (slow degradation rate, low bulk density, high porosity, high water holding capacity and the chemical characteristics (relatively high cation exchange capacity, CEC) that makes peat particularly suitable as growing media for a large number of vegetables and ornamentals (Bohlin et al., 2004). Peat is formed as a result of the partial decomposition of plants (Sphagnum, Carex) typical of poorly drained areas (peat bogs), with low nutrients and pH, under low temperatures and anaerobic conditions (Raviv et al., 2002).

Other relevant properties are the high easily available water under conditions of container capacity, i.e. after the end of free drainage and the high oxygen diffusion rate. On the other hand, as negative aspect peat can be a conducive substrate for numerous soil-borne diseases and its sterilization does not solve the problem as it leaves a biological vacuum that can be easily filled by pathogenic fungi (Abad et al., 2001). Peat use in horticulture increased during the last decades, resulting in rising costs and generating doubts about availability of this material in the near future due to environmental constraints. In fact, peat mining has been recently questioned because it is harvested from peat lands, highly fragile wetlands ecosystems with a great ecological and archaeological value, included in the list of natural habitats with a potential degradation. (Barber et al., 1993). Peat also plays an important role in improving groundwater quality, and peat bogs also serve as a special habitat for wild plants and animals. Moreover, these ecosystems represent important carbon dioxide (CO_2) sinks. (Maher et al., 2008). Peat is the most widely used growing media and substrate component in horticulture, currently accounting for 77–80 percent of the growing media used annually in Europe’s horticultural industry (Gruda, 2012a). Seedlings and transplants are grown predominantly in organic substrates based on peat it is also used in horticulture as a raw material for substrates in which container plants are grown (Gruda, 2005). Peat has long been used as a component of standardized growing media; however, research in the1960s showed that it could be used as a growing medium in its own right both for container plants and for vegetable and cut flower production (Puustjarvi, 1973). Peat substrates offer numerous advantages and their nutrient content and pH are easy to control because both are initially low. The purpose of this paper is to see which substrate retains a greater amount of water, and that water to be easily accessible to plants for their proper growth and development. Also to see the impact of the water retention capacity of both substrates and their mixtures. Retention curves have great practical and theoretical significance, because they show data about water properties in substrate. These curves give the opportunity to determinate when and what amount of water the plant needs. In this way we can see the relations among the water, substrate and plants.

MATERIAL AND METHODS

The experimental part served to determine the retention of moisture of substrates perlite and peat at different pressures. The used perlite originates from Cera Poliana, Mariovo Gradesnica, Republic of Macedonia, and was appled in expanded (commercial) form.

Peat was used in a commercial form. The peat and perlite were analysed in all five of their different ratios: (P: Perlite 20%, 30%, 50%, 70%, 80% by volume) and 100% perlite, (Pe: Peat 80%, 70%, 50%, 30%, 20% by volume) and 100% peat, with the ultimate goal to determine the ability to retain water in the substrate. In laboratory conditions, perlite moisture and peat was determined at higher pressures with application of a pressure limiter with Bar extractor for determination of moisture retention at 0.1 bar (pF - 2); 0.33 bar (pF - 2.54); 1 bar (pF - 3); To determine perlite and peat moisture retention in higher pressures, the Richard Porous plate extractor method was applied, 2.00 bar (pF - 3.3); 6.25 bar (pF - 3.90); 11 bar (pF - 4.04) and 15 bar (pF - 4.2), described by (Belić et al., 2014).

Figure 2. Preparing soil and placing samples on Bar extractor and Porous plate extractor

Figure 3. Substrate and mix- ratio

RESULTS AND DISCUSSION

Keeping water in the peat or perlite is marked as retention. The characteristics of moisture retention include the relations between the matrix potential and the moisture content and can be represented by a retention curve. It shows the moisture content at different tensions. Water retention is the result of two forces: adhesion (attraction of water molecules by the particles) and cohesion (attraction of water molecules to each other). Atheism is much stronger than cohesion. The force with which the water is retained in the substrates, that is, the force it needs to squeeze out of the substrates is denoted as capillary potential and is closely related to the water content. To obtain a clearer representation of the intensity of moisture retention, especially for peat and perlite, the peat along with perlite, the mean humidity values in mass percent tabular and graphic with pF values are displayed, the height of the water column in cm (1 bar = 1063 cm / cm^2).

All examined samples of perlite and peat and their respective ratios were placed on 7 different pressure modes (0,1; 0.33; 1; 3; 6.25; 11; 15 bar) using Bar extractor and Porous plate extractor, and the obtained results for moisture retention in weight percent are presented in Table 1 and Table 2.

	Formulation	**Designation**
1.	100% Perlite (commercial substrate)	(Pe)
2.	100% Peat (commercial substrate)	(P)
3.	80% Perlite + 20% Peat	Pe80/P20
4.	70%Perlite + 30% Peat	Pe70/P30
5.	50%Perlite + 50% Peat	Pe50/P50
6.	30%Perlite + 70% Peat	Pe30/P70
7.	20%Perlite + 80% Peat	Pe20/P80

Table 1. Moisture retention in weight percent % at different tension in substrate perlite and peat at 0.1 bar; 0.33 bar; and 1 bar.

Substrate and mix- ratio	n	**0.1 bar**		**0.33 bars**		**1 bar**	
		$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
100% Perlite	3	67.85	1.88	58.35	1.59	47.70	1.57
100% Peat	3	89.16	0.83	74.84	1.17	57.94	1.03
Pe80/P20	3	72.11	1.07	61.65	1.01	49.75	1.49
Pe70/P30	3	74.25	1.39	63.30	1.80	50.77	0.77
Pe50/P50	3	78.51	0.81	66.63	0.64	52.73	1.12
Pe30/P70	3	82.76	0.64	69.88	0.96	54.87	1.06
Pe20/P80	3	84.89	0.94	71.54	1.34	55.89	1.08

Table 2. Moisture retention in weight % at different tension in substrate perlite and peat at 3 bars; 6.25 bars; 11 bars and 15 bars.

Substrate and mix-ratio	n	3 bars		6.25 bars		11 bars		15 bars	
		$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
100% Perlite	3	39.78	2.58	34.84	2.66	30.10	2.40	26.65	2.75
100% Peat	3	45.15	1.07	39.57	1.18	33.89	1.07	23.17	1.45
Pe80/P20	3	40.85	1.21	35.78	1.15	30.86	1.03	25.94	1.12
Pe70/P30	3	41.40	1.20	36.25	1.40	31.24	1.26	25.60	1.21
Pe50/P50	3	42.46	0.55	37.21	0.17	31.96	0.52	23.86	0.20
Pe30/P70	3	43.54	0.18	38.15	0.72	32.75	0.51	23.16	0.17
Pe20/P80	3	44.07	0.33	38.62	0.81	33.13	0.40	23.83	0.57

To understand more clearly the intensity of moisture retention in peat with perlite, the mean moisture values in weight percent are shown. The experimental data presented in Table 1 and 2 show that the peat substrate has the largest retention capacity in all variants and at all points of pressure tension such as: at pressure of 0.1 bar with an average value of 89,16% at pressure of 0.33 bar with an average value of 74,84 %, at pressure of 1 bar – 57,94%; 3 bars – 45,15%; 6.25 bars - 39.57; at pressure of 11 bar - 33.89%; except at pressure of 15 bars - average value of 23.17%.

The retention capacity of the perlite is lower than the peat in at all applied pressures of different tension: for 0.1 bar = 67.88%, for 0.33 bar = 58.35%, for 1 bar = 47.70%; for 3 bars = 39.78%; for 6.25 bars = 34.84; for 11 bars = 30.10%; for 15 bars = 26.65%. In other analysed ratios, where the peat was represented by 20%, 30% and 50% 70%, 80% in the analysed sample, the perlite retention capacity is increased dramatically. The retention pressure of other ratios such as Pe70/P30 and Pe50/P50, Pe30/P70, P80/Pe20, is presented in Table 1 and Table 2. From the presented data, it is obvious that the addition of a larger percentage of peat to perlite, always resulted in increase of the sample retention pressure.

Figure 1, 2, 3, 4, 5, 6, and Figure 7 represent the retention curves of the analysed samples of substrate perlite and the peat respectively. The ability of the substrate to retain and maintain moisture is crucial for improving the efficiency of water use for growing crops in closed (greenhouses) and open-field conditions. According to (Richards 1955), retention curves have great practical and theoretical importance, because they show all important data about water properties and management in soil and substrates. Moisture retention curves (MRCs) in soilless substrates were first described by (Bunt, 1961). However, the suction range is generally conducted at lower tensions (0 to 30 kPa) than in mineral soils, because soilless mixes are more porous and normally have larger diameter pores, enabling water to drain at lower tensions. Moisture retention curves provide data about substrate capacity for available moisture, with the upper limit of field water capacity and the lower limit of the coefficient of the set.

For estimation of substrate moisture, using capillary potential quantified, pF-(soil moisture tension), values were determined, whereby the water force in the substrate was expressed through the height of the water column in cm (1 bar = 1063 cm/cm^2) (Vućić, 1987).

Filipovski (1996), also explains that retention of moisture in various tensions is closely related to the content of humus, clay, dust and mineral clay composition. According to (Kutilek and Novak, 1998) the hydrological characteristics such as water retention and the rate of water movement, depend, to a large degree on the total porosity and pore-size distribution of the material. The moisture content depends on the percentage of pores in the perlite itself, higher porosity- higher moisture content.

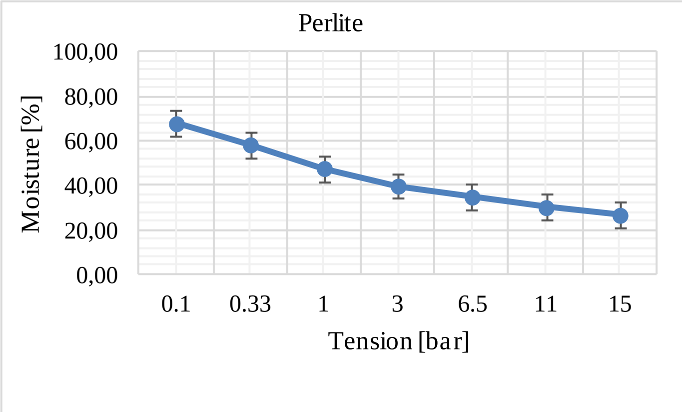

Figure 1. Moisture retention curve of substrate perlite.

Figure 1 shows the results of the retention curve of the substrate perlite. The analysis shows higher water retention at points of tension of 0.1, 0.33, 1 up to 3 bars with a percentage of moisture of 67.88, 58.35, 47.70 and 39.78 vol.%, respectively. At higher pressures of 6.25, 11, and 15 bars the percentage of moisture drops from 34.84, 30.10 to 26.65 vol.%, respectively. The curve of retention has slight slope, where the percentage of moisture gradually decreases at higher tensions.

Examined the physical properties of the perlite and tested the moisture retention by methods from the manual by (Fonteno & Harden, 2010) with Volumetric Pressure Plate Extractors with (-Kpa), which yielded similar results with ours, the percentage of moisture in the perlite substrate was 66% per 0.1 bar, 43% per 1 bar and 31% of moisture per 10 bars. The water retention curve of perlite shows moderate hysteresis (Bures et al., 1997b; Wever et al., 1997) reported that the saturation of perlite was very rapid, independent of its initial moisture.

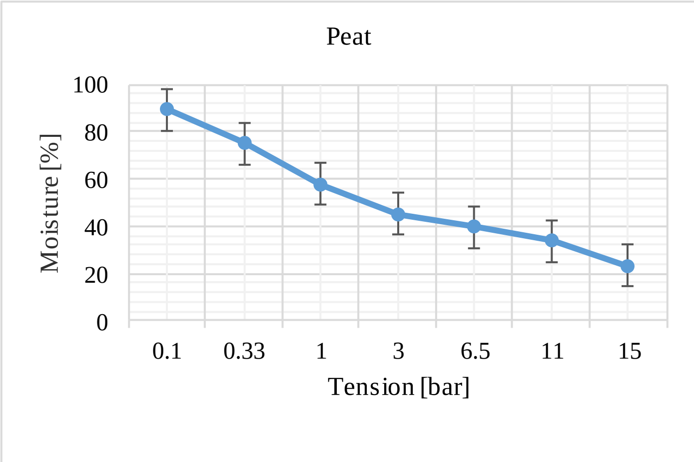

Figure 2. Moisture retention curve of substrate peat.

Figure 1 shows the results of the retention curve of the substrate perlite. The analysis shows higher water retention at points of tension of 0.1, 0.33, 1 up to 3 bars with a percentage of moisture of 67.88, 58.35, 47.70 and 39.78 vol.%, respectively. At higher pressures of 6.25, 11, and 15 bars the percentage of moisture drops from 34.84, 30.10 to 26.65 vol.%, respectively. The curve of retention has slight slope, where the percentage of moisture gradually decreases at higher tensions.

Figure 2 shows the results of the retention curve of the substrate peat. From the results it can be seen that the peat shows higher retention of water than perlite. The curve has sharp slope starting from 0.1, 0.33, 1, 3, 6.25 and 11 bars, except with a mild drop in a lower retention of moisture at 15 bars. The reason for the higher retention of the substrate peat than the perlite is due to the high content of organic matter in the peat. Comparing the two substrates, it can be ascertained that the peat substrate has higher moisture retention from the perlite at all points of tension except at 15 bars. The moisture in the perlite that is stored at 15 bars, which is higher than the peat, has an approximate value of the point or humidity wilting range.This means that under conditions wilting range, the substrate perlite retains higher percentage of moisture than the peat. Similar results as ours, received authors (Fields et al., 2004), where the percentage of moisture in the substrate peat, at a point of tension of 0.1 to 3 bars is around 40-90 vol.%. At the point of tension of 3 to 15 bars, the percentage of moisture ranges from 50-27 vol.%, while the substrate perlite at a point of tension of 0.1 to 3 bars has moisture percentage of 67-40 vol.%, and at tension of 3 up to 15 bars the retention curve of moisture ranges from 40-30 vol.%. According to the author (Raviv et al., 2002), the total pore space in the plant growth substrates should range from 60-90% by volume.

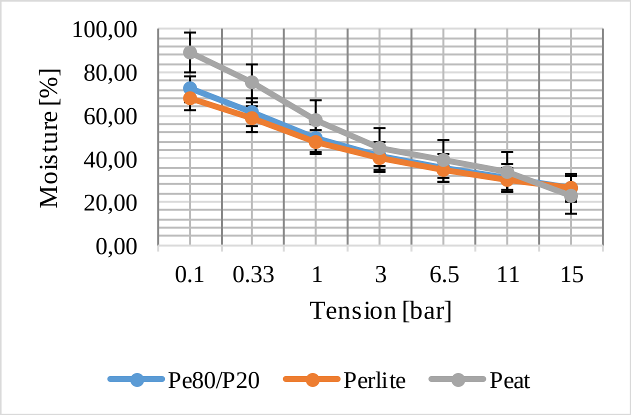

Figure 3. Moisture retention curve of substrates peat, perlite and mix ratio Pe80/P20.

Figures 3, 4, 5, 6, and 7 represent the moisture retention curves of the analyzed samples of substrates perlite and peat and their mixtures of different volume ratios Pe80 / P20; Pe70 / P30; Pe 50 / P50; Pe30 / P70; Pe20 / P80. The pF curves of moisture retention of substrate perlite and substrate peat is the same as in Figures 1 and 2, while for other mixtures of perlite and peat there are some differences between the retention curves. In the analyzed sample of the mixture with ratio Pe80 / P20 retention curve shows moderate decline in the retention of moisture, starting from 0.1 bar to 15 bars with a moisture content of around (71 to 25 vol.%).

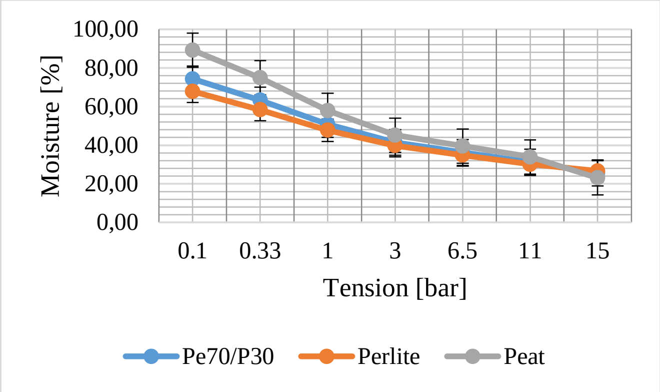

Figure 4. Moisture retention curve in substrates peat, perlite and mix ratio Pe70/P30

The curve of retention on Pe50/P50 shows a slightly more noticeable decline in values between 0.1 and 3 bars when percentage of moisture decreases from (78.51 to 42.46 vol.%), and then up to 15 bars tension it decreases moderately to 23.86 vol%.

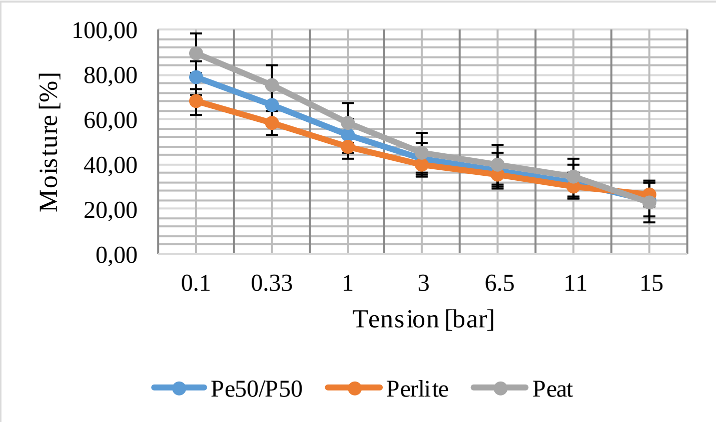

Figure 5 Moisture retention curve in substrates peat, perlite and mix ratio Pe50/P50.

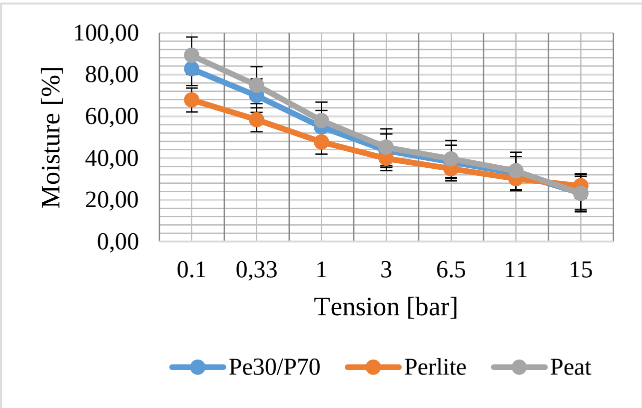

Figure 6. Moisture retention curve in substrates peat, perlite and mix ratio Pe30/P70.

The retention curve of the analyzed sample with volume ratio Pe30/P70, for tension from 0.1 to 3 bars shows higher moisture percentage from 82 to 53 vol. %. Then the retention curve gradually drops in a horizontal fall, when the percentage of moisture slightly decreases from 38 to 23 vol.%.

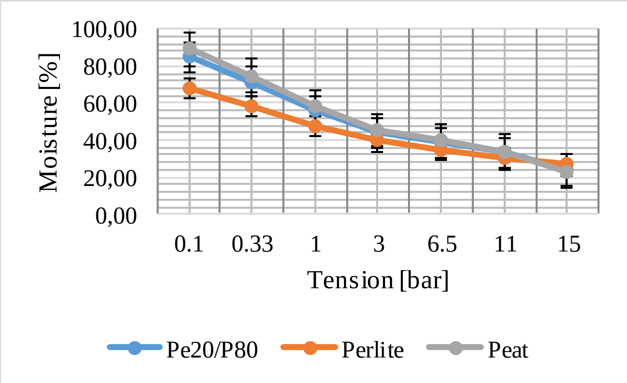

Figure 7. Moisture retention curve in substrates peat, perlite and ratio Pe20/P80.

Table 3. Correlation coefficients between the tension points of 0.33, 1, 3, 6.25, 11, 15 bars and humus content.

Correlation coefficients	**0,1 bar**	**0,33 bars**	**1 bar**	**3 bars**	**6.25 bars**	**11 bars**	**15 bars**	**Organic mater**
0,1 bars	1	0.996**	0.959**	0.912**	0.879**	0.772**	-0.664**	0.645**
0,33 bars		1	0.953**	0.926**	0.894**	0.781**	-0.649**	0.640**
1 bar			1	0.830**	0.811**	0.828**	-0.720**	0,627**
3 bars				1	0.975**	0.611**	-0.401**	0,566**
6.25 bars					1	0.570**	-0.326**	0,537**
11 bars						1	-0.750**	0,514**
15 bars							1	-0,510*
Organic mater								1

Based on the correlation analysis for the investigated properties in different ratios of the perlite substrate and the peat, it can be noted that there is a positive significant correlation in almost all of the retention constants, the highest is r = 0.996. High positive significance correlation (r = 0.645) exists between organic matter and all points of tension except at 15 bar (r = - 0.510).

CONCLUSIONS

Based on the data from laboratory investigations for moisture retention in the substrate perlite and the peat substrate and their mixtures with ratios Pe80 / P20, Pe70/P30, Pe50/P50, Pe30 / P70, Pe20 / P80 ratio, the following can be concluded: Most of the moisture at all the analyzed samples is run out at lower pressures of 0.1 to 3 bar around 85-40 vol.%. A smaller percentage of moisture is run out to higher pressures of 6.25 to 15 bar (40-20%). However, for the plants, the most important is the physiologically available moisture that in all the analyzed samples ranges somewhere around 71-34 vol.%. The total amount of moisture from 0.33 bar to 15 bar ranges from 31.7 % in perlite, 51.72% at peat and in the mixtures of substrate perlite and the peat substrate with Pe20/P80 ratio the total available moisture is approximately 47.72%.

Comparing the retention curves of the perlite and the peat, they are relatively close to each other. There are certain differences at the peat, where the peat unlike the perlite shows a higher moisture retention capacity, from 0.33 bars to 11 bars and the slightly decreasing of water retention occurs at higher pressure of 15 bars. Higher retention curves in the peat substrate are due to the higher percentage of organic matter (humus). Also, the perlite substrate shows the optimum water retention capacity, which has slightly higher value at tension of 15 bars compared to that of peat. The perlite retains more water at 15 bars, because that moisture in the substrate perlite is retained by very large retentive forces. This is particulary important because the moisture content is retained in the substrate under conditions when the plant is at wilting range at 15 bars. pF curves of moisture retention provide data on the capacity of the available moisture, which gives us the opportunity to draw conclusions when and what amount of water the plant needs. That is the best way to understand the relationship between water, substrate and plants. For each water content, its holding strength in the substrate can be determined.

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EFFECT OF POLYAMINES APPLICATION ON GERMINATION AND PHYSIOLOGICAL CHARACTERISTICS OF BORAGE (*Borago officinalis* L.)

SUMMARY

The aim of study was determine the advantages of re-drying after seed priming by polyamines. As biologically active compounds, polyamines (PAs) have been considered as modulator of plant growth and development, they play a significant role in plant response to environmental stress. The effects of polyamines priming on seed germination, emergence and seedling growth of borage plants was investigated by a laboratory experiment in factorial layout with complete randomized design (CRD) conducted in three replications. The seeds were classified into five sub-samples one of which was kept as control (unprimed) while the rest of them were primed with polyamines. Seeds pre-treatments included: control (unprimed), water pretreatment for 4 and 8 h, spermidine at 5 and 5.5 mM for 4 and 8 h, spermine at 2.5 and 3 mM for 4 and 8 h, putrescine at 2.5 and 3 mM for 4 and 8 h. Sseed treatments with polyamine led to earlier and enhanced germination. Improved seedling length, seedling fresh and dry weight as well as vigor index were found in polyamine-treated seeds. Moreover, the majority of priming treatments enhanced seedling emergence percentage, emergence energy and coefficient of uniformity of emergence (CUE) as compared with control samples. Non-primed seeds (control samples) significantly showed the least α-amylase and β-amylase activity (0.293 and 4.923 $U.mg^{-1}$ Protein, respectively) and shortest of plant height. 4-hour seed treatment by 3mM putrescine and 8-hour treatment with 3mM spermine were recognized as the most effective treatments in most of the studied traits.

Key words: Pre-treatment, Polyamines (PAs), Germination, Vigor index, Amylase enzymes, Emergence.

INTRODUCTION

As one of the precious medicinal plants, Borage is capable of wide cultivation in the semi-arid regions. However, its seedling establishes in the field with difficulty. High plant crops yield is achievable by high seedlings establishment which enables the plant to can cope with the environment and produce high rate of crops (Kamithi et al., 2016). Fast and uniform germination

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

as well as seeds emergence and vigorous seedlings are important factors in its establishment and therefore increase of the yield quality and quantity (Cantliffe, 2003). Seed germination performance has been improved by several techniques; these techniques are applicable for environmental stresses and/or aged seeds.

One of the simple and low-cost techniques to break dormancy, expand germination and stand establishment is seed priming which controls seeds hydration followed by their redrying (Afzal et al., 2009). Primed seeds usually exhibit developed germination percent (Nathawat et al., 2007); such an enhanced performance can be attributed to numerous physiological, biochemical and molecular modifications (Taylor et al., 1998; Powell et al., 2000; Afzal et al., 2008). Haigh and Barlow (1987) reported faster imbibition, enhanced extensibility of radicle cell walls and weakened endosperm as a result of priming which could shorten the lag phase of tomato before its radicle emergence. Application of plant growth regulators during priming and other pre-sowing treatments can improve their seed germination performance (Farooq et al., 2007). Seed priming can be conducted in various media including tap water (hydropriming), aerated low water potential solutions like polyethylene glycol or a salt solution (KNO_3, KCl, K_3PO_4, KH_2PO_4, $MgSO_4$, $CaCl_2$ and NaCl) (osmopriming), solid matrix (matripriming), plant growth regulators and polyamines (hormonal priming) (Chiu et al. 2002; Basra et al. 2006; Farooq et al. 2006a, b, c, d, 2007).

As biologically active compounds, polyamines (PAs) can modulate plant growth and development; they play a crucial role in plant responses to environmental stress. Putrescine (Put – diamine), spermidine (Spd – triamine) and spermine (Spm – tetramine) are widely used PAs in higher plants and exist in free, soluble conjugated, and insoluble bound forms (Gill and Tuteja, 2010). Increasing evidences have revealed the role of PAs in regulating plants' responses to different environmental stresses such as drought or osmotic stress, salinity, heat and chilling through direct binding to membrane phospholipids, direct scavenge of free radicals, osmotic adjustment, maintaining a cation-anion balance and binding to the antioxidant enzymes which can enhance their function (Alcazar, 2010; Puyang et al., 2015). Furthermore, PAs may be involved in accumulation of seed protein storage and its maturation (Santanen and Simola 1999) which can in turn promote seed germination (Sińska and Lewandoska 1991, Zeid and Shedeed 2006). PAs are proven to activate protein synthesis in early germination stages (Takahashi and Kakehi, 2010). Seed priming by PAs solutions could improve germination and stress resistance of seedlings under abiotic stress. PAs biosynthesis dramatically elevates under stresses; its function has been recognized as a protective response as it scavenges free radicals (Bagni and Pistocchi, 1991; Kuznetsov et al. 2002). Xu et al. (2011) expressed that Put priming treatment will enhance germination percentage and chilling tolerance of tobacco seedlings. In a similar study by Farooq et al., effective improvement of germination and early seedling growth of sunflower seed priming by Spd (Farooq et al., 2007) and rice (Farooq et al., 2008) were observed. Use of putrescine on

Syngonium plants resulted in significant enhancement of leaves fresh and dry weights and leaf area (El-Quensi et al., 2010).

This study was conducted on the basis of this hypothesis that PA-treatment of the seeds that are would enhance their germination and hence, the borage seeds performance would be improved due to subsequent seedling emergence. T the best of our knowledge, the majority of the previous studies has been devoted to improvement of germination and emergence though seed priming by PAs. In this content, no study has addressed the possibility of borage seed invigoration by polyamines treatment of seeds. In this regard, the aim of this study was to examine seed PA-treatment potential for improving vigor in borage. Moreover, the present study wants to evaluate the possible benefits (if any) of seed priming by PAs for borage by studying the responses at seed germination (vigor) and early growth stages.

MATERIAL AND METHODS

The impact of polyamines priming on seed germination, emergence and seedling growth of borage plants under the saline condition was assessed through a laboratory experiment in University of Maragheh, Iran. For this purpose, a complete randomized design (CRD)-based factorial experiment with three replications was performed. Borage seeds were provided from Pakan Bazr Co., Isfahan, Iran. These seeds were divided into 5 sub-samples. One of them was considered as control (unprimed) and the other four experienced polyamines priming as follows:

1: Control (unprimed)
2: Priming with water for 4 (h_1) and 8 (h_2) hours
3: Priming with spermidine at 5 (sd_1) and 5.5 (sd_2) mM for 4 and 8 hours
4: Priming with spermine at 2.5 (sm_1) and 3 (sm_2) mM for 4 and 8 hours
5: Priming with putrescine at 2.5 (p_1) and 3 (p_2) mM for 4 and 8 hours

After priming, the seeds were washed with tap water and dried for ~ 2 h at room temperature (20-25 $^{\circ}$C). Two experiments were performed at the laboratory. The first experiment involved seeds placement in petri dishes (40 seeds per petri dish) between layers of moistened Whatman paper at 25 $^{\circ}$C in germinator. While in the second experiment, the seeds were cultured in pots (25 seeds per pot). Petri dishes and pots were drenched with water. Germination observation was daily performed as mentioned in association of official seed analysts (AOSA) method (AOSA, 1990).

The field environment was simulated by pot planting, so, the results of this step could be compared with those of petri dishes. In this regard, the pre-treated seeds (P2, P3, P4 and P5) and control samples (P1) were quantitatively and qualitatively tested. Physiological measurements were conducted on petri dish seeds at day 7 of germination; while the other batch was sampled at day 10 of germination to determine the seedling growth. This study determined various traits including final germination (FGP) and emergence (FGE) percentage, germination rate, energy of germination (GE) and emergence (EE), mean germination time (MGT), seedling fresh weight (SeFW) and dry weight (SeDW), seedling length (SeL), vigor index (VI), plant height and coefficient of uniformity of emergence (CUE). For evaluation of physiological traits, amylase enzymes activities were assessed.

The traits were measured by following methods:

Final Germination Percentage (FGP):

FGP = (the number of germinated seed up to the day)/(the total number of seeds) ×100

Mean germination time (MGT) was calculated by equation developed by Ellis and Roberts (1981):

$$\mathrm{MGT} = \frac{\sum \mathrm{Dn}}{\sum \mathrm{n}}$$

In which, n shows the number of seeds germinated on day D, and D denotes the day number from the initiation of germination.

The germination energy measurement was conducted on 4th day of planting. This parameter is defined as the percentage of germinated seeds 4 days after planting compared to the total number of studied seeds (Farooq et al., 2005).

Seeds germinated Vigor Index (VI):

VI = [seedling length (cm) × germination percentage] / 100.

The coefficient of uniformity of emergence (CUE) was calculated by formulae proposed by Bewley and Black (1994):

CUE = $\sum n \,/\, \sum[(\bar{t} - t)^2 . n]$

Here, t represents the time in days, starting from day 0, the day of sowing and n denotes the number of seeds which completed the emergence on day t; while $\bar{t}$ is MET.

Amylase enzymes activities were evaluated by the method developed by Tarrago and Nicolas (1976) and Kishorekumar et al. (2007). In a typical experiment, the seeds (0.1 g) were ground with distilled water (8 mL) at 4 °C. Then the extract was obtained by 25-min centrifugation at 20,000 *g* at 4 °C. The supernatant was then applied to assess the activities of α-amylase and β-amylase. 3 mL of supernatant was mixed with 3 mL of $CaCl_2$ (3 mM) followed by 5-min incubation at 70 °C. The reaction mixture (consisting 0.1 mM citrate buffer, 2% soluble starch solution, 0.7 mL hot enzyme extract) underwent 6-min incubation at 30 °C followed by 5-min heating at 50 °C. Spectrophotometric measurements at 540 nm were employed to assess α-amylase activity. β-amylase activity was evaluated after α-amylase inactivation at pH value of 3.4. The reaction solution included 0.1 mM citrate buffer, 2% soluble starch, 0.7 mL EDTA treated enzyme extract; 2 mL of the mentioned mixture was incubated at 30 °C for 5 min after adding starch. Activity of β-amylase was then measured by the method similar to that of α-amylase.

Suitable analysis of variance was performed by SPSS and MSTATC software. Means of each trait were compared as mentioned in Duncan multiple range test at P value of 0.05. Excel software was also employed for plotting the figures.

RESULTS AND DISCUSSION

According to the obtained results, seed germination was significantly enhanced upon PAs application (Figure 1a). Seeds soaking in Put at 2.5 and 3 mM for 4h resulted in better and higher than germinations as compared with other treated seeds (Figure 1a). Mean germination time (MGT day) declined by prolonging the soaking duration (Figure 1b). Incorporation of PAs into priming media resulted in MGT decrease as compared with control samples. In PAs treated samples, the minimum MGT was observed in seeds primed with Spm and Spd (Figure 1b).As suggested by Figure 1c, seed priming with PAs resulted in increased energy of germination. Seeds primed in Put for 4h, 3mM Spm for 4h and Spd for 8h exhibited the highest energy of germination; while the control sample showed lowest value of the mentioned trait.

Seed germination is a complex physiological process which can be modulated by phytohormones or physiological activators like abscisic acid (Finkelstein et al., 2000), nitric oxide (Beligni and Lamattina, 2000) or polyamines (Zapata et al., 2004). Vigorous seedlings responded to seed priming by polyamines and evidently showed enhanced resistance against the adverse effects of environmental stresses (Li et al., 2014). Moreover, polyamine priming resulted in significant improvement of germination and early seedling growth in borage. Our study also revealed that polyamine pretreatment can stimulate borage germination. Earlier and more uniform germination were detected in PA-pretreated seeds. Enhanced germination percentage due to priming could be attributed to breakdown of reserve food material, elevated cell division and embryonic axis expansion (Basra et al., 2006).

The earlier and more synchronized germination could be also due to enhanced metabolic activities of the treated samples (yang et al., 2016). Previous studies have also reported the positive impacts on seed germination through Spd priming (Rebecca et al., 2010; Sedagahat and Rahemi, 2011). Recently, it has been shown that Spd soaking can significantly improve seed germination in corn, while exogenous application of cyclohexylamine (CHA; an inhibitor of Spd biosynthesis) resulted in significant inhibition of seed germination and declined seed vigour (Huang et al., 2017).

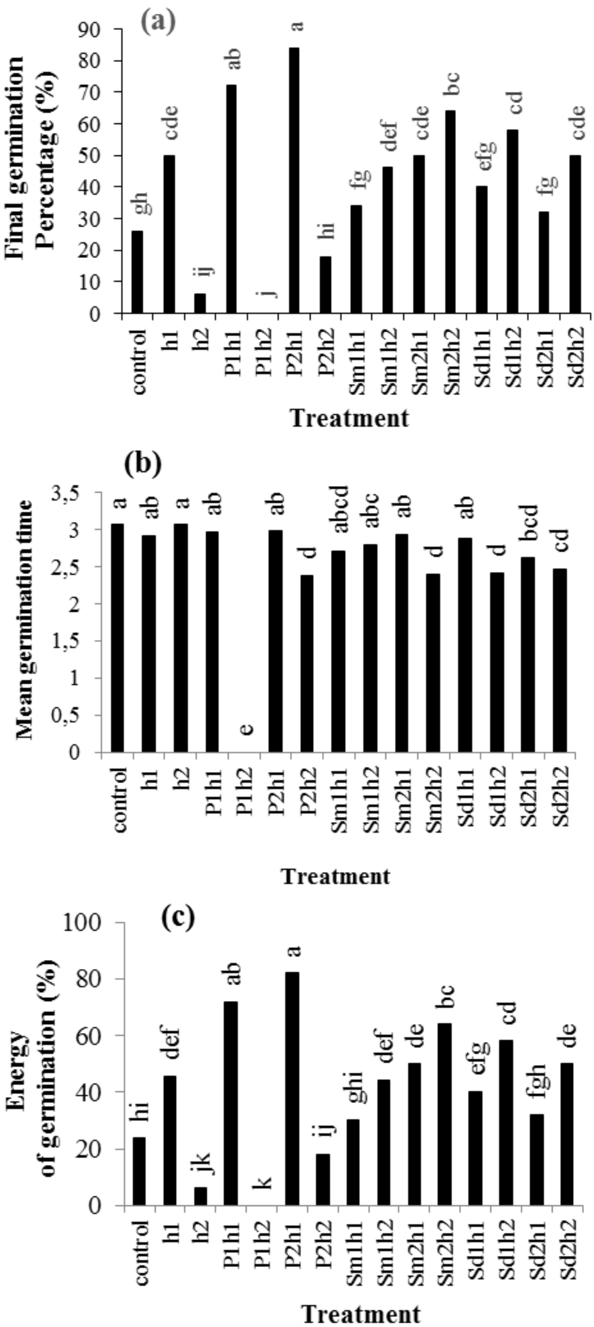

Figure 1- Effect of PA- priming strategies on (a) final germination percentage (b) mean germination time (c) and energy of germination of the seeds

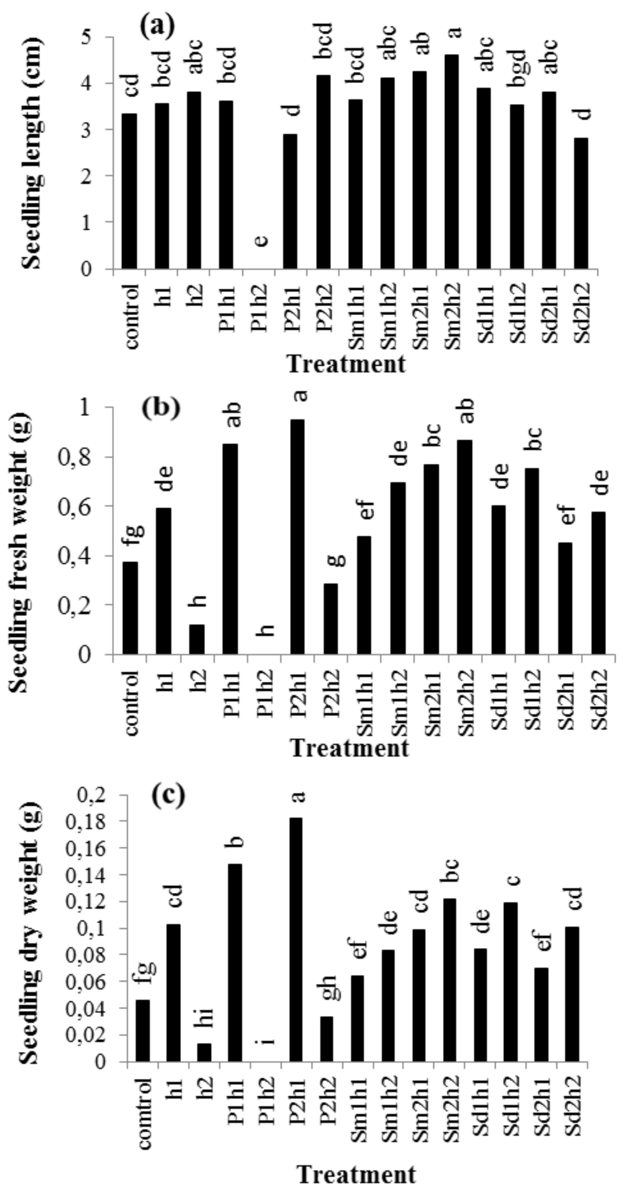

Figure 2- Effect of PAs priming strategies on (a) seedling length (b) seedling fresh (c) and dry weight of the seeds

In comparison with control, seeds pre-soaking in polyamine solutions drastically improved the seedling growth. Seedling length was also dramatically influenced by priming treatments. Priming with 3mM of Spm for 4 and 8 h resulted in the highest seedling length (Figure 2a). Furthermore, polyamine seed treatments led to significant enhancment of seedling fresh and dry weights. Among various tested treatments, the Put-treated seeds exhibited more vigorous seedlings. Substantially higher seedling fresh and dry weights were observed in sampled treated by Put (3mM) for 4 h (Figure 2b,c). While statistical minimums of seedling fresh and dry weights were measured in samples pretreated with water for 8 h and Put (3mM) for 8 h (Figure 2b, and c).

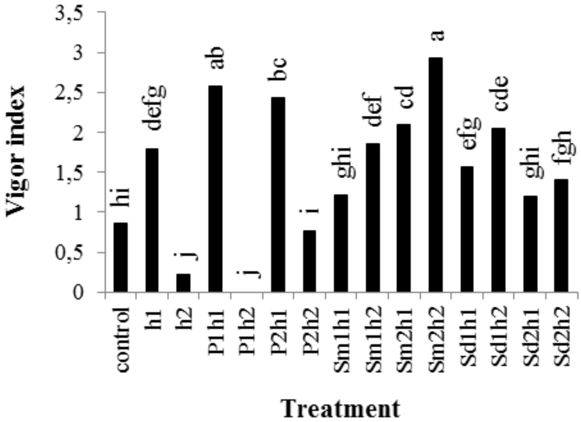

Figure 3- Effect of seed priming with polyamine on vigor index.

Similarly, vigor index measurments showed high values in polyamines-treated samples (Figure 3). Almost all of the polyamine-treated samples revealed similar results in terms of vigor index improvement with little variation among concentration levels. The maximum vigor index was recorded in samples treated with Spm (3 mM) for 8 h (Figure 3). The results also indicated that seed priming by polyamines will increase seed vigor as suggested by seedling fresh and dry weights comparison with control samples. The enhanced growth could be assigned to earlier germination and emergence (Hammad et al., 2012). Such earlier synchronized and faster emergence could be the consequence of the improved DNA, RNA and protein syntheses during priming, which will lead to augmented seedling growth (Huang and Villanueva, 1992; Farooq et al., 2011). This shows the polyamines significance for plant development justifying the performance of polyamines in this study. According to the obtained results, all the PA treatments led to the seed germination and seedling growth stimulation as compared with control; among which, Put showed more effectiveness. The findings of this study are consistent with those obtained by Farooq et al. (2008) indicating that Put at lower concentrations will result in effective improvement of germination performance as compared with other Pas. Our results are however in contrast with the findings reported by Farooq et al. (2007) and Farooq et al. (2011). Exogenous Spd enhanced seed germination in numerous plant systems, as well. For example, based on the studies conducted with white clover indicated that Spd-primed seeds not only led to enhanced germination percentages and shortened mean germination, but also improved the seed vigor shown by longer root length and higher seedling fresh and dry weights in comparison with control (Li et al., 2014).

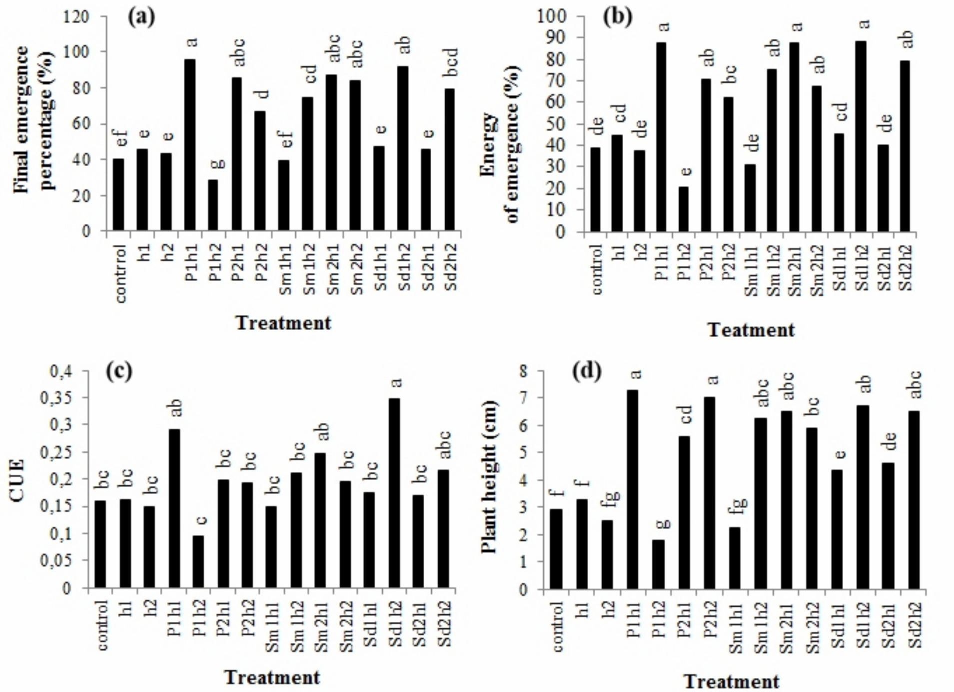

Figure 4- Effect of polyamine treatment on (a) Emergence percentage (b) Energy of emergence (c) CUE (d) Plat height of samples

Most of the priming treatments resulted in improved seedling emergence percentage, energy of emergency and coefficient of uniformity of emergence (CUE) (Figure 4a,b,c). Among the mentioned priming methods, the maximum and minimum percentage of emergence were measured in seeds treated with Put (2.5 mM) for 4 h and 8h, respectively. The maximum energy of emergence was observed in seeds primed by Spd (5mM) for 8h which showed a 2.5-fold increase relative to control (Figure 4a, and b). Pre-sowing of the seeds with polyamines led to enhanced coefficient of uniformity of emergence (CUE) where the maximum value was recorded for samples undergoint Spd (5mM) treatment for 8h (Figure 4c). Seed soaking in PAs also improved the plant height (Figure 4d). Apparently, improvement of seedling emergence and growth through polyamines priming can be assigned to stimulation of germination metabolism (Figure 1) and increased seed vigor (Figure 3). Seed germination is the most important period of seedling establishment (Hubbard et al. 2012; Shi et al. 2014). Polyamine was involved in seed germination of plants. PA content increased during the first 15 days of Ocotea catharinensis seed germination it was then decreased and stabilized between 30 and 60 day of germination (Dias et al. 2009). Exogenous polyamines showed enhancing effects on seed germination of the hot pepper (Khan et al. 2012). A signifcant relationship was observed between PAs and hormones in plant growth regulation. So, enhanced polyamines may rise the plant hormones and enzymes (Pieruzzi et al., 2011; yang et al., 2016). Moreover, Increased EE (energy of emergence), FEP (Final emergence percentage), CUE (coefficient of uniformity of emergence) and plant height seemed to be attributed to efficient mobilization and use of seed reserves and improved genetic repair, i.e., earlier and faster DNA, RNA and proteins syntheses (Srivastava 2002). These changes could be due to earlier initiation of germination suggested by lower E_{50} values (Basra et al. 2006). Boothe et al. (2010) showed that accumulation of the various seed storage compounds plays an important role because: (i) these reserves support the early growth of seedlings after germination, thus affecting the seedling vigor; and (ii) they are widely applied as food and feed. A sturdy relationship between seed metabolites and CUE (coefficient of uniformity of emergence) and MET (mean emergence time) supported this assumption that faster starch metabolism may take part in uniform and early emergence and vigorous seedling growth.

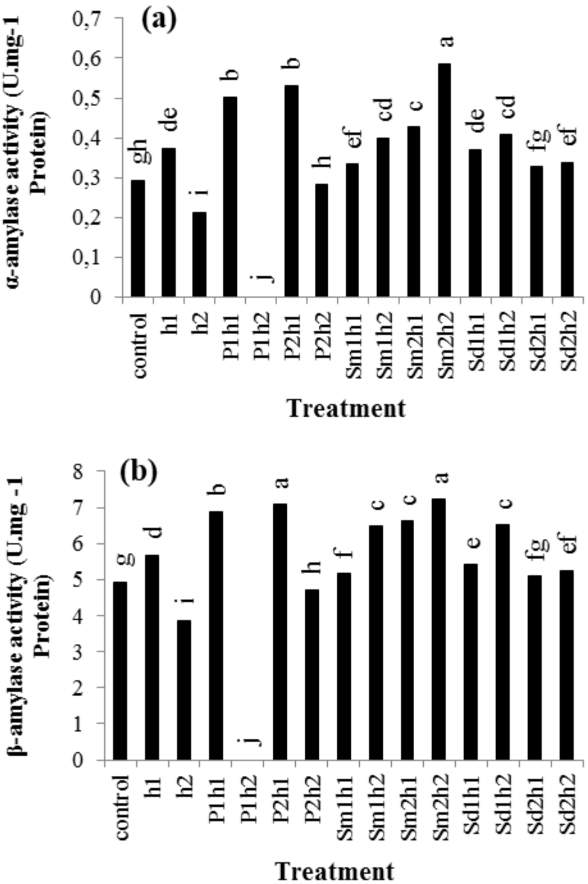

Figure 5- Effect of polyamine treatment on (a)α-amylase and(b) β-amylase activities

Maximum (0.587 U.mg $^{-1}$ Protein) and minimum (0.293 U.mg $^{-1}$ Protein) values of alpha-amylase activity were observed when borage seeds were primed with spermine (3mM) for 8 h and Put(2.5 mM) for 8 h (Figure 5a). Accordingly, polyamine seed treatments resulted in significant improvement of β-amylase activity. Maximum β-amylase activity was detected for seeds soaked in 3 mM Spm solution for 8 h (Figure 5). The polyamines-induced improvement of seed germination, seedling growth, vigor index and seed emergence was assigned to germination metabolism stimulation as a result of increased α-amylase and β-amylase activities. Priming may significantly affect the enzyme activities necessary in fast seed germination (Varier et al., 2010). Amylases are essential enzymes with prominent role in hydrolyzing the seeds starch reserve, which will provide sugar for developing embryo (Andoh and Kobata, 2002; Kamithi et al., 2016). Li et al. (2014) stated that seed priming with Spd could enhance starch metabolism possibly because of elevated α- and β-amylase activities. Farooq et al. (2011) reported a strong correlation between amylase and soluble sugars supporting this hypothesis that fast starch metabolism could be helpful in the early emergence of seeds and vigorous seedling growth observed by Spd priming. Starch metabolites such as glucose play crucial role in seed germination since they act as osmolytes in cellular turgor maintenance and energy sources. That’s why PAs plays a significant role in accelerating starch metabolism due to enhanced α-amylase and β-amylase activity during seed germination. Seed germination potential is determined by cellular metabolism during germination (Bewley and Black 1994; Holdsworth et al. 2008a; Rajjou and Debeaujon 2008); thus the success of the new plant establishment. Deeper understanding of these mechanisms will result in development of molecular and biochemical markers, which can be employed as quality markers of seed sector in high-vigor seed lots markets (Catusse et al. 2011; yang et al. 2016).

CONCLUSIONS

Based on the results of this study, seed priming by polyamine techniques can significantly enhance the emergence uniformity coefficient through improving the seeds vigor; although its effectiveness significantly varied for different polyamines and their concentrations. Seed priming treatments exhibited higher beneficial impacts on stimulating amylase enzymes activities and improvement of the seedling emergence and growth. It can be concluded that seed priming by PAs could be regarded as a promising approach to improve the seed germination especially under adverse conditions. Furthermore, for practical uses, investigation of the polyamines effects on different crops under biotic and abiotic conditions sounds necessary. This interesting topic could be considered for future studies.

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VARIABILITY OF YIELD AND YIELD COMPONENTS OF SELECTED GENOTYPES OF ITALIAN RYEGRASS

SUMMARY

Italian ryegrass (*Lolium multiflorim* L.) is a short-term grass species that is of great importance for the production of quality fodder on arable land. It is grown in pure sowing or in short-term grass-leguminous mixtures. For the sowing of grasslands in Bosnia and Herzegovina, the significant seed quantities of the Italian ryegrass are used. The aim of this paper is to examine the yield variability and yield components of the progeny of the own selection of Italian ryegrass "BL Vubo" in relation to the four varieties of the Italian ryegrass that are most often used in our area. The research was carried out in the testing field and in the laboratories of the Agricultural Institute of the Republic of Srpska in Banja Luka during 2014 and 2015. These tests include the local selection "BL Vubo" and 4 foreign and local diploid and tetraploid varieties of the Italian ryegrass: Tur, Danergo, Draga and Tetraflorum. During the study, the following properties were monitored: number of generative stems per plant, spike length, number of spikelets per spike and seed yield per plant.

During the two-year study, the highest average number of generative stems per plant was found in the Draga variety (183,6) and the lowest in the Tur variety (158,2). The average length of the Italian ryegrass in the tested genotypes varied from 23,4 cm (Draga) to 29,5 cm (Tetraflorum). The highest average number of spikelets/spike was found in the Tur variety (27,1). When it comes to yield of seeds/plants, the variety Draga (72,4 g) and the local selection "BL Vubo" (68,8 g) were distinguished. The variety had a highly significant influence on all tested properties ($p<0,01$). For the spike length, it is determined that year had highly significant influence ($p<0,01$), while for the rest of the examined properties, the effect of the year was significant ($p<0,05$). The existence of an interaction genotype x year has not been determined for any property.

Key words: Italian ryegrass, genotype, spike length, spikelets, seed yield.

INTRODUCTION

Italian ryegrass is one of the most common cultivated short-term grass species. It is a plant species of moderate climates, which best grows on fertile soil

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in conditions of sufficient amount of precipitation or with the application of irrigation. It is very important to note that the Italian rye grass starts with vegetation early in the spring, and can grow until late autumn. In the Italian ryegrass we distinguish short-term (one-year) and two-year forms. The length of life of the plants of the Italian ryegrass almost no exceeds longer than two years. On suitable land for cultivation it grows very quickly and is able to push other grass species. The low tolerance of the Italian ryegrass to the cold prevents its spread in colder areas (Redfearn et al., 2002). Tolerance of the Italian ryegrass to the cold varies depending on the genotype. Experimental studies of diploid and tetraploid varieties of the Italian ryegrass showed that the diploid varieties had greater tolerance to cold than tetraploid (Park et al., 1987). Tetraploid varieties are interesting because they give higher yield of green mass, but yields of seeds are lower in relation to diploid. It is noticed that tetraploid ryegrasses has larger seeds than the corresponding values recorded for diploid strains (Simić et al., 2010)

In Bosnia and Herzegovina, there are no recognized domestic varieties of Italian ryegrass. The needs for the seed of this plant species are met by importing and own production of seeds of domesticated varieties of Italian ryegrass. At the annual level, the need for Italian ryegrass seeds in Bosnia and Herzegovina varies considerably, ranging from 18 tonnes to over 50 tonnes.

In the Agricultural Institute of the Republic of Srpska, Banja Luka, the program of creating its own varieties of Italian ryegrass was launched. After many years of work using the method of individual selection and polycross from selected domestic populations of Italian ryegrass from the mountainous region of the Republic of Srpska, a variety was created under the name "BL Vubo" and was introduced to the Commission for the recognition of varieties in Serbia.

The aim of this paper was to examine the variability of yield and seed yield components of the progeny of their own selection of Italian ryegrass "BL Vubo" in relation to the four varieties of the Italian ryegrass that are most often used in our area.

MATERIAL AND METHODS

The research was carried out in the experimental field and in the laboratories of the Agricultural Institute of the Republic of Srpska in Banja Luka during 2014 and 2015. These tests include the domestic selection "BL Vubo" and 4 foreign and domesticated diploid and tetraploid varieties of the Italian ryegrass: Tur, Danergo, Draga and Tetraflorum. During the examination, each genotype was represented with 52 plants (clone) in the experimental field. Individual plants were sown at the beginning of September 2013 with three seeds each. Each genotype was sown in 4 rows. It was 13 plants per row. The distance between plants in row and between rows was 50 cm. During the examination, harrowing and intertillage was performed. Plants were top dressed early in the spring with 81 kg/ha of N twice during both years of testing. During the study, the following properties were monitored: number of generative stems per plant, spike length, number of spikelets per spike and seed yield per plant. After harvest, the crop is top dressed and mowed until the end of the vegetation.

The obtained data were processed by statistical calculation of the mean value and the corresponding measures of variability. The results obtained during these trials were analysed by two factor analysis of variance (ANOVA), and the significance of the difference in mean values was determined by the LSD test.

RESULTS AND DISCUSSION

Soil properties

In the experimental field of the Agricultural Institute of the Republic of Srpska, Banja Luka dominated type of soil is valley-brown soil on the alluvial substrate of Vrbas. The soil by mechanical composition belongs to a clay-loam group. The structure of this type of soil in the surface layer is crumbly. The results of the chemical analyses of the arable layer of soil on which the experiment is placed are shown in Table 1.

Table 1. The results of the chemical analyses of the arable layer of soil

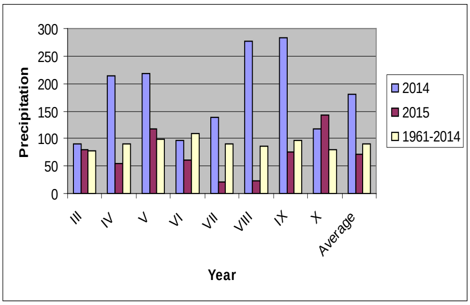
Depth (cm)	Humus %	pH in H_2O	pH in KCl	P_2O_5 mg/100g of soil	K_2O mg/100g of soil
0-30	2,0	7,1	6,4	16,1	22,9

In terms of pH, the soil reaction is neutral and, according to the content of humus, it belongs to the land with low humus content (2,0%). The availability of easily accessible phosphorus is good (16,1 mg / 100g). According to the results of chemical analysis of the arable layer, the availability of easily accessible potassium is good (Tab.1).

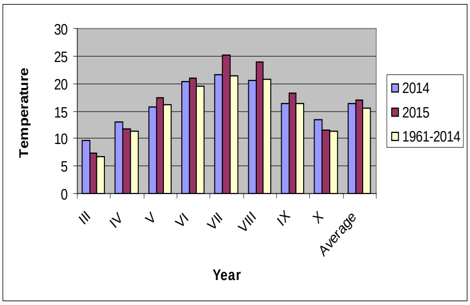
Based on the results of chemical analysis of the arable layer of soil, it can be concluded that the land is suitable for cultivating the Italian rye grass.

Meteorological conditions

For analysing the weather conditions during the performance of the experiment, data of the Hydro meteorological Station in Banja Luka were used (Graphs 1 and 2).



Graph1. Temperature



Graph 2. Precipitation

The average multi-year temperature during the vegetation period (III-X) was 15,4°C. Total amount of precipitation in the vegetation period (III-X) for the period 1961-2014 was 729,0 l/m^2.

The average temperatures for both years of testing during the vegetation period were higher than the multi-year average (1961-2014). In the first year of testing the amount of precipitation during the vegetation period, compared to the multi-year average, was increased for 707,0 l/m^2. During the vegetation period 2015 it fell 572,2 l/m^2, which is 153,8 l/m^2 of precipitation less compared to the multi-year average.

Number of generative stems per plant

The average number of generative stems/plant is shown by the year of testing in Table 2.

Table 2. Average number of generative stems per plant at the investigated genotypes of the Italian ryegrass

No	Genotype (A)		Year (B)		$\bar{X}$ A
			2014	2015	
			$\bar{X} \pm S_x$	$\bar{X} \pm S_x$	
1.	Tur		162,8±20,9	153,5±19,6	158,2
2.	Danergo		184,3±12,2	177,5±14,0	180,9
3.	Draga		190,8±14,0	176,3±17,2	183,6
4.	Tetraflorum		168,5±14,2	161,8±12,6	165,2
5.	BL-Vubo		182,3±14,5	172,0±19,6	177,2
$\bar{X}$ B			177,7	168,2	
Basic factors			A	B	AB
Variance analysis– $F_{calculated}$			5,187**	4,953*	0,112
LSD		0,05	13,8	8,8	19,6
		0,01	18,7	11,8	26,4

After two years of testing, the largest average number of stems was found in the Italian ryegrass of the Draga variety (183,6 stems/plant). In all genotypes, a larger number of generative stems were determined in the first year of testing. The analysis of the variance of the average number of generative stems per plant in the investigated years show highly significant differences between the investigated genotypes. The differences between the years are significant. The variance analysis does not show the existence of an interaction effect of the genotype x year. During the two-year study, the lowest average number of generative stems/plant had a Tur genotype (153,5 stems/ plant), and the largest number had genotype Draga (190,8 stems/plant). The number of generative stems per plant depending on the nitrogen fertilization intensity varies from 156 to 208 (Chastain, 2000).

Average spike length

The average length of the spikes of studied Italian ryegrass genotypes, expressed in centimetres, are given in Table 3.

Table 3. Average spike length (cm) of studied Italian ryegrass genotypes

No	Genotype (A)		Year (B) 2014 $\bar{X} \pm S_x$	Year (B) 2015 $\bar{X} \pm S_x$	$\bar{X}$ A (cm)
1.	Tur		28,8±4,2	26,3±4,0	27,6
2.	Danergo		29,3±3,3	27,8±4,7	28,6
3.	Draga		24,0±3,6	22,8±4,3	23,4
4.	Tetraflorum		30,1±5,0	28,9±4,3	29,5
5.	BL-Vubo		28,9±3,0	26,4±5,3	27,7
$\bar{X}$ B (cm)			28,2	26,4	
Basic factors			A	B	AB
Variance analysis– $F_{calculated}$			11,323**	7,817**	0,200
LSD		0,05	2,0	1,3	2,9
		0,01	2,7	1,7	3,9

The lowest average spike length during the test is found in the Draga variety in 2015 (22,8 cm), and the largest in the Tetraflorum variety in 2014 (30,1 cm). The average length of the spike in variety Florida 80 was 21 cm, variety Kowinearly 24 cm and variety Hwasan 101 was 34 cm (Choi et al., 2011). During a four-year trial (2003-2006), the average length of spikes in the Tetraflorum variety of ryegrass, grown in conditions of different inter row spacing, was from 24,1 cm to 35,0 cm (Simić et al., 2009). The average length of the spikes of investigated Italian ryegrass genotypes shows the existence of a highly significant difference between the investigated genotypes as well as between the years studied. No interaction of the genotype x year has been noticed during these studies.

The average number of spikelets per spike

The results of the average number of spikelets per spike of examined genotypes of the Italian ryegrass are given in Table 4. During the two-year trials, the number of spikelets per spike was high among the tested genotypes of the Italian ryegrass, and ranged from 23,1 (Draga) to 27,1 (Tur). The smallest number of spikelets was found in the Draga variety in the second year of the testing, and the highest in the Tur variety in the first year of testing (2014). Hides et al. (1993) found an average of 24 spikelets per spike in diploid italian ryegrass.The average number of spikelets per spike during the study of several varieties of the Italian ryegrass, in the period 2003-2006 at the Suwon site in South Korea, ranged from 20 in diploid varieties up to 24 in tetraploid varieties (Choi et al., 2011). In the case of Tetraflorum variety of Italian ryegrass, during the multy-year trial conducted in the Sabac area in Serbia, the average number of spikelets per spike was 21,6-27,2 (Simić et al., 2009). Kovačević et al (2012) state that tetraploid variety of italian ryegrass "Mir" has 24,9-25,2 spikelets per spike.

Table 4. The average number of spikelets per spike in examined genotypes of the Italian ryegrass

No	Genotype (A)		Year (B)		$\bar{X}$ *A*
			2014	2015	
			$\bar{X} \pm S_x$	$\bar{X} \pm S_x$	
1.	Tur		27,8±3,7	26,3±4,0	27,1
2.	Danergo		26,8±4,7	25,1±3,8	26,0
3.	Draga		23,3±3,2	22,8±3,5	23,1
4.	Tetraflorum		25,8±4,6	24,1±3,0	25,0
5.	BL-Vubo		27,5±3,0	25,8±3,3	26,7
$\bar{X}$ *B*			26,2	62,1	
Basic factors			A	B	AB
Variance analysis– F $_{calculated}$			4,372**	4,453*	0,123
LSD		0,05	2,2	1,4	3,2
		0,01	3,0	1,9	4,3

An analysis of variance of the number of spikelets per spike showed a statistically highly significant difference between the investigated genotypes. The impact of the year on the number of spikelets per spike was significant.

Average seed yield per plant

The results of a two-year study of average seed yield per plant, expressed in grams, are shown in Table 5.

Table 5. Average seed yield per plant (g) in the study of the Italian ryegrass genotypes

No	Genotype (A)		Year (B)		$\bar{X}$ A (g)
			2014	2015	
			$\bar{X} \pm S_x$	$\bar{X} \pm S_x$	
1.	Tur		48,8±9,8	37,8±8,1	43,3
2.	Danergo		59,7±10,0	52,3±9,0	56,0
3.	Draga		77,4±19,3	67,3±13,8	72,4
4.	Tetraflorum		52,5±10,0	48,0±9,3	50,3
5.	BL-Vubo		74,0±10,1	63,5±12,4	68,8
$\bar{X}$ B (g)			62,5	53,8	
Basic factors			A	B	AB
Variance analysis– F $_{calculated}$			10,582**	6,691*	0,130
LSD		0,05	10,9	6,9	15,5
		0,01	14,8	9,4	20,9

The seed yield in the tested genotypes of the Italian ryegrass varied considerably during the year and between the years of testing. e. This is because seed loss can occur throughout the following processes: pollination, fertiliation, seed set and development, harvesting and seed cleaning (Elgersma, 1985). In the first year (2014), higher yield of seeds was achieved in all tested genotypes. During these examinations, the highest average seed yield per plant was achieved with the Draga variety (72,4 g / plant). During both years of testing, the lowest yield of seed/plant was achieved with Tur variety (Tab.5).

The analysis of variance of seed yield/plant shows the existence of statistically significant differences between the investigated genotypes. Also during the studied time, the effect of the year on seed yield of the investigated genotypes was significant. The variance analysis did not determine the existence of interaction of the genotype x year.

CONCLUSIONS

Based on the results of testing 5 genotypes of the Italian ryegrass in the agroecological conditions of the Banja Luka region, the following conclusions can be made:

– Meteorological conditions for the growing of the Italian ryegrass were more favourable in the first year of testing.
–During the investigation of yield and seed yield components, the new domestic variety of the Italian ryegrass "BL-Vubo", statistically, was not falling behind the most cultivated varieties in our country in any tested parameter.
–According to the number of generative stems/plant, the genotypes Draga (183,6) and Danergo (180,9) were noticed.
–During these tests, the highest average length of spike (29,5 cm) was measured in the Tetraflorum variety of Italian ryegrass.
–The highest number of spikelets per spike were determined for genotypes Tur and "BL Vubo".
–When it comes to the yield of the seed/plant, during the studied time, the genotypes Draga (72,4 g) and "BL Vubo" (68,8 g) were noticed..

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INFLUENCE OF ARBUSCULAR MYCORRHIZAL FUNGI IN THE ESTABLISHMENT OF PRE-BROKEN SUGAR CANE

SUMMARY

The objective of this work was to evaluate the influence of arbuscular mycorrhizal fungi on the initial development of pre-sprouted seedlings in three sugarcane varieties (CTC 9004 M, IACSP 955094 and IACSP 962042). The experiment was conducted in a greenhouse in the experimental field and in the agricultural microbiology laboratory of the Goianesia Evangelical Faculty located in the city of Goianésia, Goiás. The experimental design was a 3x2 factorial scheme with 5 replications in which the first factor was constituted by three varieties of sugarcane and the second factor by treatments: sterile soil with inoculation of arbuscular mycorrhizal fungi (AMF) and sterile soil without inoculation. AMF were inoculated directly into the roots of the seedlings. For the determination of growth and development were made count of number of tiller, stem diameter, dry mass of roots, shoot height, mycorrhizal colonization, spore density and identification of associated genera. The data received statistical treatment through the Assistat program. There was statistical difference only in the variety factor, in the variables plant height, spore density and mycorrhizal colonization rate. The genera Claroideglomus, Diversipora and Glomus were identified in all three varieties.

Key words: FMA, *Saccharum spp.*, mycorrhiza.

INTRODUCTION

Brazil is today the largest producer of sugarcane, which makes the country the world leader in raw material production and ethanol production (UNICA, 2017). The Agronomic Institute of Campinas (IAC) launched in 2009 a technique that intends to change the concept of sugarcane plantation in Brazil. The system of pre-budded seedlings (PBS) of cane, as it is known, foresees the rapid production of seedlings, associated with high planting pattern, vigor and uniformity of planting (Landell et al., 2013).

Unlike the conventional system that uses whole stems for planting, the PBS takes to the field only the pre-budded seedlings originated from the cutting

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of the stem, known as wheels, which have an average of 3 cm in length, where the buds are located. The technology has already been adopted by small producers and associations of Goias and Central region of Sao Paulo (Souza & Junior, 2013). However, it takes approximately 60 days for the entire process to be completed and the seedlings are perfectly rooted and ready for planting in the field.

The PBS system that uses individualized sugarcane grinding wheels is a rapid multiplication technology that allows the reduction of the volume of vegetal material used in the planting, better control of vigor and high phytosanitary standard, promote sugarcane of excellent clonal standard. The fact of substantially reducing the volume of plant material needed for planting automatically results in a higher gain for the mills, since this material can be used for its production of alcohol or sugar, which will depend on the company's objective (Landell et al., 2013; Xavier et al., 2014).

Unlike traditional planting, seeding pre-sprouted seedlings can be done with adapted machinery which has made the process faster. It is estimated that there is a reduction of 18 to 20 tons (t) of plant material per hectare when the planting is performed with the PBS system, which represents considerable financial gain in the sugar and alcohol industry (Souza & Junior, 2013). Although it is a simple technique, studies on this technique are still lacking, especially those related to the use of plant growth promoting microorganisms, which can be easily applied in encapsulated gems, as predicted in this work. Therefore, the present proposal may contribute significantly to the understanding of the sprouting process and establishment of the seedling in assessing the influence of arbuscular mycorrhizal fungi without its establishment.

According Landell et al. (2012) with the advent of mechanical planting, the faults became more frequent and, in order not to result in significant losses in productivity, the volume of seedlings used became very high, being used up to more than 20 t/ha. If a tonne of sugarcane contains 8,000 to 20,000 yolks, it is concluded that the number of yolks per meter is between 24 and 60 yolks, which is therefore an overspending of stalks that could be destined to industry.

Mycorrhizal fungi perform a mutualistic symbiotic association with most cultivated and native plants, benefit the development of the plant due to the greater absorption of water and nutrients, especially phosphorus that has low mobility in the soil (George et al., 1995; Elbon & Whalen, 2014). These microorganisms occupy an important ecological niche in the ecosystems and bring benefits to the associated plant that can increase the rate of seedlings establishments in field conditions (Mohan et al., 2014; Soka & Ritchie, 2015).

When it comes to mycorrhization in sugar cane, it can be noticed the shortage of work, this happens because the culture presents a long and large cycle, which makes it difficult to evaluate it in controlled environments, there are no conclusive results about this mycorrhizal interaction (Reis et al., 1999; Silveira & Freitas, 2007).

This work aimed to evaluate the influence of arbuscular mycorrhizal fungi on the early development of pre-sprouted seedlings in three varieties of sugarcane.

MATERIAL AND METHODS

The experimental design was the 3x2 factorial scheme with five replications, the first factor was constituted by three sugarcane varieties (CTC 9004M, IAC SP 95-5094 and IAC SP 96-2042) and the second factor by treatments: soil sterile with spore inoculation of mycorrhizal fungi and sterile soil without inoculation of spores. The experiment was carried out in the greenhouse and agricultural microbiology laboratory of the Evangelical Faculty of Goianesia.

The sugarcane varieties (*Saccharum officinarum* L.) were chosen according to regional utilization. The buds were from the Jalles Machado mill and were planted in polystyrene trays with commercial Bioplant® substrate and kept in a greenhouse for 30 days.

After 30 days of growth the seedlings were transplanted into 290 cm3 tubes with Bioplant®. Then the seedlings were grown in a greenhouse. After this, they were transferred to 5-liter pots with sterile soil according to the proposed design and with formulated NPK fertilizer (04-30-10). It was conducted inside the greenhouse and irrigation occurred due to the need of the plant.

The spores of arbuscular mycorrhizal fungi (AMF) were extracted from 500 cm3 soil of native cerrado area with characteristics of ciliary forest closed by the wet sieving technique (Gerdemann & Nicolson, 1963), followed by centrifugation in water and 50% sucrose solution. The spores were separated according to their phenotypic characteristics as color, size and shape, composing the different morphotypes under stereoscopic binocular loupe.

For the determination of growth and development the following biometric analyzes were made of plant height, stalk diameter, root dry mass weight, number of tillers, mycorrhizal colonization rate, spore density and identification of associated genera.

In order to determine the percentage of colonization, the roots were clarified and stained with 0.05% Trypan Blue in lactoglycerol (Phillips & Hayman, 1970) and the evaluation of the colonization was made under a stereoscopic microscope, following the technique of intersection of the quadrants (Giovannetti & Mosse, 1980).

For the identification of the AMF genera from the morphological characteristics, the spores were separated according to their morphotypes and assembled on slides with pure polyvinyl lactoglycerol (PVLG) and PVLG mixed with Melzer (1:1 v/v). To support the work of identification, we used original articles of the species description and descriptions of species provided on the website of the "International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi” (Invam, 2018).

RESULTS AND DISCUSSION

The F test shows the significance of the differences of means, in which statistical differences were verified only in the variety factor, in the variables plant height, spore density and mycorrhizal colonization rate (Table 1). No statistical difference was observed between the varieties in relation to root dry weight, stalk diameter and number of tillers. There was no statistical difference regarding inoculation.

Table 1. F test of sugarcane varieties without and with inoculation of arbuscular mycorrhizal fungi

Source of Variation	Root Dough	Height	Diameter	Thorns	Density of Spores	Mycorrhizal Colonization
Variety	0,7811 ns	4.3168 *	1.5444 ns	1.7528 ns	9.5967 **	5.6986 **
Inoculation	0,0117 ns	1.2756 ns	1.2000 ns	0.5506 ns	0.6928 ns	0.4139 ns
Var. x Inoc.	0,5037 ns	0.3289 ns	0.4111 ns	0.7191 ns	1.4135 ns	1.4175 ns

* significant at the 5% probability level (.01 =< p < .05); ns - not significant (p >= .05)

Tristão et al. (2016), when evaluating initial parameters of sugarcane under influence of mycorrhizal fungi, did not observe statistical differences in stem diameter values. In the same study, the authors found a positive influence of the AMF on the variety IAC 91-1099 on the variables of root dry mass and number of tillers.

Andreola et al. (1985) evaluated the influence of six species of arbuscular mycorrhizal fungi on the growth and development of three varieties of sugarcane, they found benefits in the development of the varieties, and showed that their efficiency differs according to fungus and variety. Studies have shown that mycorrhizal fungi increase the uptake of nutrients, especially nutrients with low soil mobility, such as phosphorus, copper and zinc, so it is more appropriate to choose varieties that are more efficient in the use of nutrients and which have easier interactions with mycorrhizal fungi arbuscular (Tellechea, 2007). Silva et al. (2016) did not verify differences in the diameter of the colon in embaúba seedlings under the inoculation of arbuscular mycorrhizal fungi.

There was difference in relation to plant heights of the three varieties, and the variety IAC SP 96-2042 was statically inferior to the varieties IAC SP 95-5094 and CTC 9004M (Figure 1).

Tristão et al. (2016), when evaluating plant height under the influence of mycorrhizal fungi, verified differences among the varieties, and demonstrated that mycorrhizal fungi can develop symbiosis with some varieties more easily than with others. The authors verified higher average values in the varieties IAC SP 95-5094 and IAC 91-1099 and lower average values in IAC SP 97-4039 and IAC SP 95-5000. It is possible to observe that the variety IAC SP 95-5094 has an ease of interaction with arbuscular mycorrhizal fungi. The height of the plant is a very determinant index for the growth and development in the sugar cane, because it is a factor that will determine the final productivity.

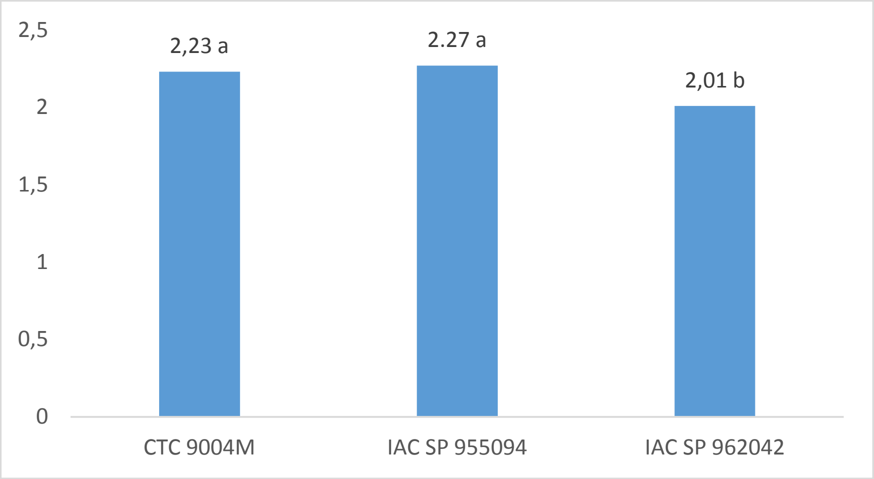

Figure 1. Plant height of three varieties of sugarcane.
CV = 9.67 %

The symbiosis between mycorrhizal seedlings and fungi has positive effects such as increases in CO assimilation rates, transpiration rates and a higher rate of stomatal opening, as well as a higher vegetative growth rate (Schwob et al., 1998; Diniz, 2007; Oliveira et al., 2015).

There was a difference in spore density in samples of sugarcane varieties, being the CTC 9004M variety statically inferior to the varieties IAC SP 95-5094 and IAC SP 96-2042 (Figure 2).

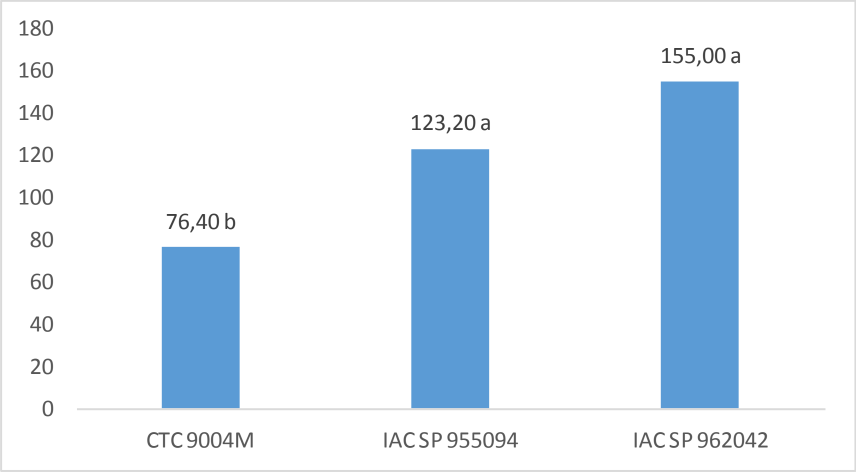

Figure 2. Density of spores in three varieties of sugarcane.
CV = 34.15 %

The papers of Ambrosano et al. (2011) and Moura et al. (2016) presented that there was no significant difference in spore density in the sugar cane crop.

In relation to the rate of mycorrhizal colonization in samples of sugarcane varieties, a significant difference was observed between the averages, in which the CTC 9004M variety was statically inferior to the varieties IAC SP 95-5094 and IAC SP 96-2042 (Figure 3).

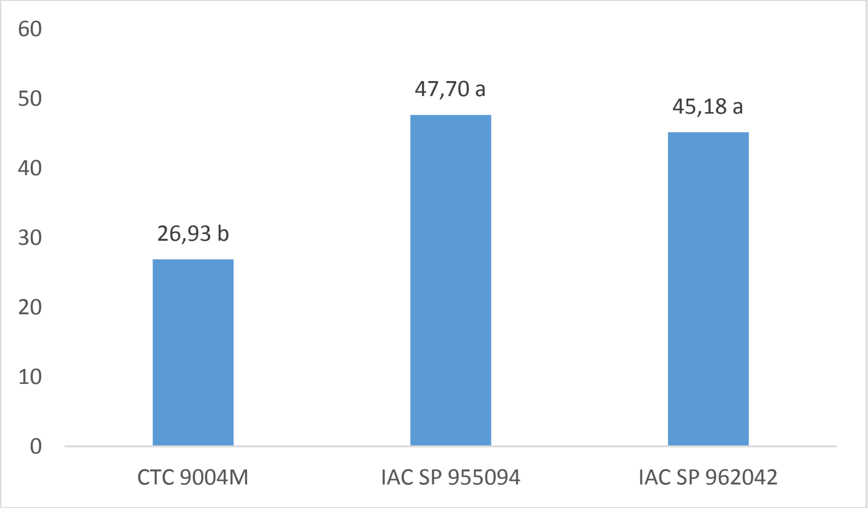

Figure 3. Mycorrhizal colonization rate in three varieties of sugarcane.
CV = 31.11 %

Authors report that fungi have an easier mycorrhizal interaction and more sporulation in certain crops and the particular varieties or cultivars, in relation to others. In the work of Silva et al. (2016), the number of AMF spores was significant in embaúba seedlings, the same result regarding sporulation was found in the acerola crop when inoculated with mycorrhizal fungi (Balota et al., 2011) and papaya culture (Machineski et al., 2011). Significant values were found in the myriad culture belonging to the family Ericaceae with the cultivars Georgia, Misty and O´neal and lower values in the cultivars of Delite and Climax (Lima, 2014), this reinforces that fungi perform mycorrhizal interaction more easily in some crops and in certain varieties.

In the analyzed samples the genera that interacted with sugarcane varieties were identified, among them the genres: Acaulospora, Claroideglomus, Diversispora, Gigaspora and Glomus (Table 2).

Three of these genera were identified and observed according to Tellechea (2007), the species that are most predominant to interact with sugarcane are of the genres: Acaulospora, Scutelospora, Glomus e Gigaspora. In the work of Miranda (2008) higher rates were identified in the genus Glomus, which is one of the most common genus in the Cerrado.

Table 2. Genus of arbuscular mycorrhizal fungi found associated with the rhizosphere of three sugarcane varieties without and with inoculation of arbuscular mycorrhizal fungi

Genres	Inoculated varieties			Uninoculated varieties		
	CTC 9004M	IAC SP 955094	IAC SP 962042	CTC 9004M	IAC SP 955094	IAC SP 962042
Acaulospora	-	+	+	-	+	+
Claroideglomus	+	+	+	-	+	+
Diversispora	+	+	+	+	+	+
Glomus	+	+	+	+	+	+
Gigaspora	-	-	+	-	-	-

According Fernandes et al. (2010), the genus Glomus was the genus for which a greater number of spores were observed under the pigeon pea, in carrots and beans, Glomus and Gigaspora were the largest numbers of spores, also.

CONCLUSIONS

The use of arbuscular mycorrhizal fungi in the sprout phase of sugarcane promoted higher results at the height of the varieties in IAC SP 955094 and CTC 9004M in relation to the variety IAC SP 962042.

Mycorrhizal colonization and spore density showed significant values in the varieties IAC SP 955094 and IAC SP 962042.

The genres Claroideglomus, Diversispora and Glomus were identified in all three varieties. The variety IAC SP 962042 presented five genera of mycorrhizal fungi.

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RELATIONSHIPS AMONG WATER USE EFFICIENCY AND THE PHYSIO-AGRONOMIC TRAITS IN DURUM WHEAT (*TRITICUM DURUM* DESF.) CULTIVARS ASSESSED UNDER RAINFED CONDITIONS OF THE EASTERN HIGH PLATEAUS OF ALGERIA.

SUMMARY

Genetic advances in grain yield under rainfed conditions haves been low, slowed by genotype x environment interaction arising from unpredictable rainfall in drought prone areas. A good understanding of factors regulating yield provides the opportunity to identify and select for physiological and agronomic traits that increase both water use efficiency and grain yield under rainfed conditions. The results of this investigation exhibited large variation for physiological and agronomic traits among varieties and cropping seasons. Modern varieties had high harvest index, grain yield, and leaf chlorophyll content, low leaf relative water content, and were shorter than varieties derived from land races. Total dry matter and specific leaf area differences, among groups of varieties, were not significant. Water use efficiency for total dry matter showed no significant correlations with the measured physiological and agronomic traits, while water use efficiency for grain yield was significantly correlated with harvest index, plant height and to a lesser extent with leaf chlorophyll content. Path analysis, based on phenotypic correlations, showed the consistent direct and indirect effects of harvest index and to a lesser extent those of plant height. Selecting for plant height and harvest index could improve both water use efficiency and grain yield under drought prone environments.

Key words: *Triticum durum*, water use efficiency, harvest index, grain yield, path analysis, rainfed.

INTRODUCTION

Durum wheat cultivation, in Algeria, is practiced in a fallow-wheat rotation, relying on stored water during the fallow period, in addition to the cropping season's rainfall**.** Annual precipitations, inherently low in amount, varied quantitatively and qualitatively, mainly on the high plateaus area, where nearly 70% are receipted during the cold winter months. Under such growing conditions, the occurrence of intermittent drought stress limits grain yield and

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renders water scarcity as the most penalizing production factor (Chennafi *et al.*, 2006). The high plateaus area belongs to a vast geographical region where agriculture has been forecast to be at greater risk due to an increase in the frequency and severity of drought episodes (Sahnoune *et al.*, 2013). Selection of drought tolerant cultivars is sought to minimize the effects of water scarcity and to sustain crop production. The release of improved cultivars requiring lower amounts of water per unit yield and characterized by high yield potential is essential for more sustainable agricultural practices, particularly in rainfed, drought prone areas. Water conserving breeding strategy could combine high yield, high WUE and good drought resistance traits in one variety (Zhang *et al.*, 2004). Water use efficiency (WUE) is seen as an important determinant of yield under stress and as a component of crop drought resistance (Ehdaie, 1995 ; Kirda *et al.*, 1999). This trait remains among the most appropriate strategies to cope with drought stress under rainfed conditions. Several studies have shown that selection based on this trait improved grain yield potential (Rebetzke *et al.*, 2002, Franks *et al.*, 2015). Zhang *et al. (*2005) reported that grain yield improved by 50%, resulting in significant WUE increases. Studies are needed to focus on plant traits that are beneficial to both grain yield and WUE improvement.

Besides crop husbandry, numerous plant characteristics are reported to affect WUE and grain yield (GY). In fact GY and WUE, due to their close association with harvest index (HI), could be improved by manipulating this trait (Ehdaie and Waines, 1993; Zhang *et al.*, 2008). Siddique *et al.* (1990) reported that WUE of modern cultivars was higher than old cultivars among Australian tested wheat varieties, because of significant changes in plant stature and crop cycle duration, leading to improved HI and stress escaping. Slafer and Araus (1998) reported that the improved crop performance may be achieved by improvements in water use (WU), WUE and HI. Several plant traits such as chlorophyll content, osmotic adjustment, relative water content, translocation of stem stored carbohydrate, stay green, early seedling vigor, earliness, canopy temperature, carbon isotopic discrimination, coleoptile length, stem and leaf waxiness, leaf and root architecture as well as the amount of soil moisture available to the crop and its partitioning between evaporation and transpiration are reported to related to WUE and GY(Quin *et al.*, 2013; Richard *et al.*, 2015; Farjam *et al.*, 2015; Nakhforoosh *et al.*, 2016; Christy *et al.*, 2018; Rashid *et al.*, 2018; Abdolahi *et al,*2018). The present investigation aimed to analyze the association between some physio-agronomic traits and WUE in eight durum wheat (*Triticum durum* Desf.) varieties, belonging to two different eras, evaluated under semi-arid conditions during three cropping seasons.

MATERIAL AND METHODS

Plant material and experimental design

The experiment was carried out at the Field Crop Institute-Agricultural Experimental Station of Setif (ITGC-AES, 36°12' N and 05°24' E, 1080 masl, Algeria), under rainfed conditions during three growing seasons (2013/14-

2015/16). Eight durum wheat varieties were evaluated (Table 1). Waha and Gaviota durum are selections from Cimmyt–Icarda joint durum wheat breeding program. Simeto is an Italian cultivar while Megress is an ITGC-AES Setif selection. These varieties proved to be well adapted to the Setif region and are classified as early-heading genotypes (Haddad *et al.*, 2016). Mohamed Ben Bachir (MBB), $Hedba_3$, Guemgoum Rkhem, and Oued $Zenati_{368}$ are old varieties selected from land races. MBB is selected from a land race native to the Setif region. $Hedba_3$, alias Pelissier, is a drought tolerant cultivar. Guemgoum Rkhem is native from Tiaret region (Western Algeria), while Oued $Zenati_{368}$ is a selection from a population native to the Guelma region (Eastern Algeria). Varieties derived from landraces are taller and late maturing compared to recently released ones (Nouar *et al.* 2012).

Table 1.Name of varieties evaluated during the 2013/14 - 2015/16 cropping seasons at the ARS-ITGC, Setif, Algeria.

Variety name	Abv	Cross name	Origin (released year)
Waha	WAH	Plc/Ruff//Gta/3/Rolett e	Cimmyt-Icarda (1985)
Gaviota durum	GTA	Crane/4/Polonicum PI_{185309}//*T.glutin enano*/2* Tc60/3/Gll	Cimmyt-Icarda (1985)
Simeto	SMT	$Capeiti_8$/Valvona	Italy
Megress	MGS	Ofanto/Waha//MBB	ITGC- AES, Setif (2015)
Med Ben Bachir	MBB	Local variety	INRA Algeria (1950)
$Hedba_3$	H_3	Local variety	INRA Algeria (1950)
Guemgoum Rkhem	GMG	Local variety	INRA Algeria (1950)
Oued $Zenati_{368}$	OZ_{368}	Local variety	INRA Algeria (1950)

The experiment was arranged according to a randomized complete block design, with four replications. Soil site is a silt-clay soil with calcium carbonate and organic matter contents of 30.4 % and 1.4%, respectively. Sowing dates were 09/12/2013,15/2014,29/11/2015for 2013/14, 2014/15 and 2015/16 cropping seasons, respectively. Recommended cultural practices for the area were followed to raise a good crop. Monoammonium phosphate (52% P_2O_5 + 12% N) with 80 kg ha^{-1} was applied just before sowing and 80 kg ha^{-1} of urea (46%) were broadcasted at the tillering stage. Weeds were controlled chemically by application of 150 g ha^{-1} of Zoom [*Dicamba 66% Triasulfuron 4%]* and 1.2 L ha^{-1} of Traxos [*22.5 g/l de Pinoxaden, 22.5 g/l Clodinafop-propargyl, 6.5g/l de Cloquintocet-méxyl]* herbicides.

Measurements

At the heading stage, leaf relative water content (LRWC), leaf chlorophyll content (LCHC) and specific leaf area (SLA) were measured. LRWC was determined by the method of Barrs and Weartherly (1962) described by Pask *et al.*, (2012). Four leaves were sampled per plot and immediately weighed to obtain the fresh weight. Leaf samples were then placed in test tubes containing distilled water, and let to stand for four hours, under dim light at laboratory ambient temperature. Leaf samples were then reweighed to obtain the leaf turgid weight. Leaf samples were then oven dried at 80°C for 48 h for leaf dry weight determination. The LRWC was calculated according to the following formulae reported by Pask *et al.*, (2012):

$$\mathbf{LRWC} = \left[\frac{\mathbf{FW - DW}}{\mathbf{TW - DW}}\right]\mathbf{x}100$$

where FW is the sample fresh weight, TW is the sample turgid weight, and DW is the sample dry weight. SPAD chlorophyll meter (Minolta SPAD-502 meter, Tokyo, Japan) was used to estimate leaf chlorophyll content. Three readings were taken per leaf from a sample of five fully expanded flag leaves per plot. Readings were averaged to get the plot mean SPAD value. The same leaf samples were used to estimate the specific leaf area, which was measured with an image scanner software (Mesurim pro, version 3.4). Leaf dry weight (LDW) was determined after oven-drying at 80 °C for 48 hours. SLA, derived as leaf area (LA) per unit leaf dry weight (cm^2. g^{-1}), was calculated using the following formulae reported by Rashid *et al.*, (2018):

$$\mathbf{SLA} = \frac{\mathbf{LA(cm^2)}}{\mathbf{LDW(g)}}$$

At crop maturity, 2-row segments, 2 m long, were sampled per plot to estimate plant height, measured from ground level to the tip of the terminal spikelet, awns excluded; total dry matter, grain yield, and harvest index, derived as the ratio of grain yield over total dry matter yield. The amount of water evaporated and that transpired by each variety during the cropping cycle (water used =WU) was determined as the sum of the soil moisture available at seeding minus soil moisture available at harvest, plus the accumulated rainfall, from seeding to harvest. Soil available moisture (ASM, mm), at sowing and at harvest was deduced by the following formulae: ASM (mm) = [(H%-WP) x h x ρb]/100, where H% = 100(wet soil weight-dry soil weight)/dry soil weight, WP =wilting point =12%, average of the soil of the experimental site, h = soil profile depth in mm(600 mm), and ρb = bulk density = 1.23 (Chennafi et al. 2011;Belagrouz et al.,2016). Water use efficiency for total dry matter ($WUE_{TDM,}$ kg ha^{-1} mm^{-1}) and grain yield ($WUE_{GY,}$ kg ha^{-1} mm^{-1}) were derived according to Cheikh M'hamed *et al.*, (2015) as follow:

$$\mathbf{WUE_{TDM}} = \frac{\mathbf{TDM}}{\mathbf{WU}}$$

$$\mathbf{WUE_{GY}} = \frac{\mathbf{GY}}{\mathbf{WU}}$$

Where TDM= total dry matter (kg ha^{-1}) and GY=grain yield (kg ha^{-1}).

Data analysis

Collected data were subjected to a combined analysis of variance using balanced anova subroutine implemented in Cropstat software package (Cropstat, 2007). Years, replications within years, and genotype by year interaction effects were considered as random and genotype effect was considered as fixed. Year main effect was tested against the replication hierarchized within years, while the genotype main effect was tested against the interaction which was tested against the residual. Mean comparisons were performed using the Fisher's protected least significant difference test at 5% probability level. Relationships among the measured traits were computed using Pearson's simple correlation test implemented in Past software (Hammer *et al.*, 2001). Path coefficient analysis was performed to divide the correlation coefficient between WUE and the physio-agronomic traits (r_{iy}) into direct (p_{iy}) and indirect effects ($r_{ij}\ p_{jy}$) according to the following equation reported by Garcıa del Moral *et al.*, (2003):

$$\mathbf{r}_{iy} = \mathbf{P}_{iy} + \mathbf{r}_{ij}.\mathbf{P}_{jy}$$

RESULTS AND DISCUSSION

1.Physiological characteristics

The combined analysis of variance indicated significant year main effect for leaf chlorophyll content and leaf relative water content, but not for specific leaf area. Genotype main effect was significant only for leaf chlorophyll content, while the genotype x year interaction was significant for the three measured physiological traits (Table 2). The significant interaction indicated that ranking order of the varieties changed between years suggesting that differences existed for the same trait between varieties within year and varied significantly also for the same variety among years.

Table 2. Combined analysis of variance mean squares of the measured traits.

Traits	Year (Y)	Rep/year	Variety (V)	V x Y	Residual
			Physiological traits		
LCHC	3280.00^{**}	24.0	150.25^{**}	25.87^{*}	10.80
LRWC	2346.00^{**}	56.7	78.80^{ns}	85.10^{**}	29.80
SLA	10.90^{ns}	5.7	24.60^{ns}	24.30^{**}	2.40
			Agronomic traits		
PHT	4632.89^{**}	17.10	1980.10^{**}	346.30^{**}	17.40
TDM	25930.32^{**}	68.50	364.10^{ns}	232.10^{**}	24.10
GY	3173.52^{**}	3.40	247.26^{**}	27.21^{**}	5.60
HI	1225.98^{**}	14.70	947.82^{**}	30.09^{**}	8.20
			Water use efficiency		
WUE_{TDM}	3397.67^{**}	8.10	36.30^{ns}	22.70^{**}	2.80
WUE_{GY}	436.32^{**}	0.40	28.40^{**}	3.60^{**}	0.61

*, ** = Significant effect at the 5 and 1% probability level, respectively; LCHC= Leaf chlorophyll content, LRWC= Leaf relative water content, SLA = Specific leaf area, PHT = Plant height, TDM= Total dry matter, GY= Grain yield, HI = Harvest index, WUE_{TDM}= Water use efficiency for total dry matter, WUEGY= Water use efficiency for grain yield.

Leaf chlorophyll content values, averaged over varieties, varied from 25.6 to 45.8 spad units indicating that 2013/14 cropping season was less favorable to the expression of high chlorophyll content compared to the 2014/15 cropping season. Averaged over cropping seasons, chlorophyll index values ranged from 32.7 spad units, measured in GMG, to 42.3 Spad units, measured in MGS. This indicated that MGS possesses higher potential for chlorophyll content expression than GMG. Per cropping season, GMG, in 2013/14, (20.3 spad units), MBB, in 2014/15 (43.0 spad units), and H3, in 2015/16, (31.6 spad units), expressed the lowest leaf chlorophyll content. SMT, in 2013/14, (30.3 spad units), and MGS, in 2014/15 and 215/16, (49.8 and 47.1 spad units), exhibited the highest chlorophyll content mean values (Table 3).

Table 3. Mean values of the three measured physiological traits, averaged over years (variety main effect), averaged over varieties (year main effect), variety mean value per cropping season and the least significant difference at 5% probability level.

	LCHC				LRWC				SLA			
	Cropping seasons			Variety effect	Cropping seasons			Variety effect	Cropping seasons			Variety effect
Varieties	2014	2015	2016		2014	2015	2016		2014	2015	2016	
GMG	20.3	45.5	32.4	32.7	74.6	90.1	78.8	81.1	10.8	12.5	8.9	10.7
OZ3	25.4	45.8	31.9	34.4	77.7	93.4	88.5	86.5	9.5	15.4	8.6	11.2
H3	23.3	45.2	31.6	33.3	76.3	88.7	91.3	85.4	9	5.3	10.2	8.2
MBB	24.5	43.0	32.1	33.2	78.6	85.4	86.3	83.4	9.8	10.7	6.9	9.1
SMT	30.3	47.3	43.1	40.2	74.3	83.8	88.2	82.1	9.9	5.4	8	7.8
WAH	26.3	45.5	39.8	37.2	69.3	90.4	90.1	83.3	9.6	7.8	7.6	8.3
GTA	25.1	44.7	35.7	35.1	68.7	97.2	90.8	85.6	9.2	3.5	8.6	7.1
MGS	29.9	49.8	47.1	42.3	69.3	89.6	77.7	78.9	9.2	11.1	8.9	9.7
Lsd5%		*4.6*		*4.5*		*7.7*		*8.1*		*2.2*		*4.3*
Year effect	25.6	45.8	36.7		73.6	89.8	86.5		9.6	9	8.5	
Lsd5%		*2.5*				*4.3*				*1.4*		

LCHC= Leaf chlorophyll content, LRWC= Leaf relative water content, SLA = Specific leaf area. GMG =Guemgoum Rkhem, OZ_{368}= Oued Zenati 368, H_3= Hedba3, MBB= Mohammed ben Bachir, SMT= Simeto, WAH= Waha, GTA= Gaviota, MGS= Megress, LSD5%= Least significant difference at the 5% probability level.

Leaf relative water content mean values, averaged over varieties, varied from 73.6 to 89.8% indicating that 2013/14 cropping season was less favorable to the expression of high leaf relative water content compared to the 2014/15 cropping season. Averaged over cropping seasons, leaf relative water content mean values ranged from 78.9%, in MGS, to 86.5%, in OZ_{368}. The range among varieties main effect was not statistically significant when compared to the value of 8.1% taken by the least significant difference at 5% probability level. Per cropping season, GTA, in 2013/14, (68.7%), SMT, in 2014/15 (83.8%), and MGS, in 2015/16, (77.7%), expressed the lowest leaf relative water content. MBB, in 2013/14, (78.6%), GTA, in 2014/15, (97.2%), and H_3, in 215/16, (91.3%), showed the highest leaf relative water content mean values.

Differences among extreme mean values were statistically significant as indicated by the significant genotype x cropping season interaction (Tables 2 and 3). Differences among cropping seasons (average over varieties) and among varieties (average over cropping seasons) main effects were not statistically significant for specific leaf area, whose mean values ranged from 8.5 to 9.6 cm² g^{-1}, among cropping seasons and from 7.1 to 11.2 cm² g^{-1} among varieties main effect. Per cropping season, GMG, in 2013/14, (10.8 cm² g^{-1}), SMT, in 2014/15, (83.8%), and MGS, in 2015/16, (77.7%), expressed the lowest leaf relative water content. Meanwhile MBB, in 2013/14, (78.6%), GTA, in 2014/15, (97.2%), and H3, 215/16, (91.3%), showed the highest leaf relative water content mean values. Differences among extreme varieties mean values were statistically significant as indicated by the significant genotype x cropping season interaction (Tables 2 and 3). These results indicated that the expression of the physiological traits was strongly affected by the environment and to a lesser extent by the genotype.

2.Agronomic performances

The combined analysis of variance indicated significant year main effect for the four measured agronomic traits. Plant height, grain yield and harvest index showed significant genotype main effect. The genotype x year interaction was significant for the four measured agronomic traits (Table 2). The 2015/16 cropping season was the most favorable environment for the expression of the potential of plant height, total dry matter and grain yield. The less favorable environment for the expression of these traits were the 2014/15 for plant height and the 2013/14 for both grain yield and total dry matter. Plant height was reduced from the favorable to less favorable environments by 23.4 cm which represents 29.3% of plant height mean value recorded under favorable environment (Table 4). Total dry matter and grain yield were reduced by 56.6 and 17.5 q ha^{-1} , respectively, which represents 59.8 and 57.4 % of the mean values recorded under favorable environment for total dry matter and grain yield (Table 4). The best mean value of harvest index (34.3 %) was expressed under the 2013/14 cropping season, which was less favorable to the expression of grain yield and total dry matter. The lowest harvest index mean value (22.9%) was recorded in 2014/15 cropping season. These results suggested that the measured respectively (Table 4).

Even though the small set of varieties assessed, the results showed the presence of variability for all the measured traits. Globally, newly released varieties were shorter, high grain yielding and allocating more dry matter to the grain than old varieties. Difference in terms of total dry matter produced was not significant. This corroborated results of Waddington et al., (1987) whom mentioned that increases in HI have accounted, in many instances, for the grain yield improvement in wheat since new high-yielding wheat varieties have higher HI than older ones. Samarrai et al. (1987) reported that HI is influenced by environment, as the results of the present study suggested.

Table 4. Mean values of the four measured agronomic traits, averaged over years (variety main effect), averaged over varieties (year main effect), variety mean value per cropping season (year) and the least significant difference at 5% probability level.

	PHT				TDM			
	Cropping seasons			Variety	Cropping seasons			Variety
Varieties	2014	2015	2016	main effect	2014	2015	2016	main effect
GMG	70.1	60.3	97.9	76.1	35.3	47.1	90.1	57.5
OZ3	72.8	59.0	96.0	75.9	42.8	57.0	106.9	68.9
H3	75.5	73.5	106.4	85.1	39.9	70.2	102.0	70.7
MBB	62.5	58.5	101.1	74.0	33.4	60.0	97.1	63.5
SMT	54.6	54.5	57.9	55.7	36.6	45.9	88.7	57.1
WAH	59.3	54.3	58.3	57.3	40.1	59.4	89.9	63.1
GTA	54.8	48.3	57.0	53.3	34.5	80.4	98.9	71.3
MGS	53.9	41.5	62.2	52.5	43.9	68.1	85.5	65.8
Lsd5%		*5.9*		*16.3*		*6.9*		*13.3*
Year main effect	62.9	56.2	79.6		38.3	61.0	94.9	
Lsd5%		*2.3*				*4.7*		
	GY				HI			
	Cropping seasons			Variety	Cropping seasons			Variety
Varieties	2014	2015	2016	main effect	2014	2015	2016	main effect
GMG	9.1	12.8	23.0	15.0	27.6	16.0	23.3	22.3
OZ3	9.4	10.3	25.2	15.0	22.2	18.1	23.6	21.3
H3	8.2	10.0	24.5	14.2	20.7	14.2	24.0	19.7
MBB	9.0	11.3	24.4	14.9	27.1	19.0	25.1	23.7
SMT	16.5	13.8	36.2	22.2	45.1	30.2	40.9	38.7
WAH	17.0	18.0	37.4	24.1	42.6	30.3	41.5	38.1
GTA	15.4	14.2	39.0	22.9	44.1	30.1	43.3	39.2
MGS	19.7	17.0	34.2	23.6	44.9	25.2	40.1	36.7
Lsd5%		*3.3*		*4.6*		*4.1*		*4.8*
Year main effect	13.0	13.4	30.5		34.3	22.9	32.7	
Lsd5%		*1.0*				*2.2*		

PHT = Plant height,(cm) TDM= Total dry matter,(q $ha^{-1)}$ GY= Grain yield, ,(q $ha^{-1)}$, HI = Harvest index, (%) WUE_{TDM}= Water use efficiency for total dry matter, (kg ha^{-1} mm^{-1}) WUEGY= Water use efficiency for grain yield (kg ha^{-1} mm^{-1}). GMG =Guemgoum Rkhem, OZ_{368}= Oued Zenati 368, H_3= Hedba3, MBB= Mohammed ben Bachir, SMT= Simeto, WAH= Waha, GTA= Gaviota, MGS= Megress, LSD5%= Least significant difference at the 5% probability level.

3.Water use efficiency

Total rainfall, accumulated from sowing to harvest, reached 251.9, 299.4 and 237.7 mm in 2013/2014, 2014/2015 and 2015/16, respectively. Compared to the long term average of 321.2 mm reported by Mekhlouf *et al.*, (2006), these figures appeared to be very low, mainly during the 2013/14 and 2015/16 cropping seasons, suggesting a strong drought stress effect during the course of the experiment. At sowing, soil relative humidity, in the 600 mm profile, reached 19.0, 18.3 and 18.6%, in 2013/14, 2014/15 and 2015/16 cropping seasons, respectively. These figures are the equivalents of 51.6, 46.7 and 48.9 mm soil moisture available to the plant. This soil moisture resulted from early autumn rain showers and from moisture stored during the fallow season. Soil relative humidity, measured at harvest, was below the wilting point and thus the available moisture left in the soil was assumed to be nil. Water available for use (evapotranspiration) by the crop during the growing cycle reached 303.6, 346.1 and 286.7 mm, in 2013/14, 2014/15 and 2015/16 cropping seasons, respectively.

Table 5. Mean values of water use efficiency for total dry matter and for grain yield, averaged over years (variety main effect), averaged over varieties (year main effect), variety mean value per cropping season (year) and the least significant difference at 5% probability level.

	WUE_{TDM}				WUE_{GY}			
	Cropping seasons			Variety	Cropping seasons			Variety
Varieties	2014	2015	2016	main effect	2014	2015	2016	main effect
GMG	11.6	13.6	31.4	18.9	3.0	3.7	8.0	4.9
OZ_{368}	11.4	23.2	34.5	23.0	5.1	4.1	13.6	7.6
H_3	13.1	20.3	35.6	23.0	2.7	2.9	8.5	4.7
MBB	11.0	17.3	33.9	20.7	3.0	3.3	8.5	4.9
SMT	14.4	19.7	29.8	21.3	6.5	4.9	11.9	7.8
WAH	14.1	16.5	37.3	22.6	3.1	3.0	8.8	5.0
GTA	12.0	13.3	30.9	18.8	5.4	4.0	12.6	7.4
MGS	13.2	17.2	31.4	20.6	5.6	5.2	13.0	7.9
Lsd5%		*2.4*		*4.2*		*1.1*		*2.2*
Year main effect	12.6	17.6	33.1		4.3	3.9	10.6	
Lsd5%		*1.6*				*0.4*		

WUE_{TDM}= Water use efficiency for total dry matter, (kg ha^{-1} mm^{-1}) WUEGY= Water use efficiency for grain yield (kg ha^{-1} mm^{-1}). GMG =Guemgoum Rkhem, OZ_{368}= Oued Zenati 368, H_3= Hedba3, MBB= Mohammed ben Bachir, SMT= Simeto, WAH= Waha, GTA= Gaviota, MGS= Megress, LSD5%= Least significant difference at the 5% probability level.

Water use efficiency data analysis indicated significant cropping season main and genotype x cropping season interaction, for both total dry matter and grain yield. Variety main effect was significant for grain yield only (Table 2). Among cropping seasons, WUE_{TDM} and WUE_{GY} varied from 12.6 (2013/14) to 33.1 kg ha^{-1} mm^{-1} (201516), and from 3.9 (2014/15) to 10.6 kg ha^{-1} mm^{-1} (2015/16), respectively. These figures were in line with those reported by Sadras *and* Angus *al.*, (2006) whom reported that average wheat grain yield per unit water use was 9.9 kg grain ha^{-1} mm^{-1} for southeastern Australia, 9.8 kg grain ha^{-1} mm^{-1} for the China Loess Plateau, 8.9 kg grain ha^{-1} mm^{-1} for the northern Great Plains of North America, 7.6 kg grain ha^{-1} mm^{-1} for the Mediterranean Basin, and 5.3 kg grain ha^{-1} mm^{-1} for the southern-central Great Plains. Averaged over cropping seasons, GTA showed lower WUE_{TDM} (18.8 kg ha^{-1} mm^{-1}) and both, H_3 (23.0 kg ha^{-1} mm^{-1}) and OZ_{368} (23.0 kg ha^{-1} mm^{-1}), had high WUE_{TDM} mean values. Low WUE_{GY} (4.7 kg ha^{-1} mm^{-1}) was noted for H_3 and high WUE_{GY} mean value (7.9 kg ha^{-1} mm^{-1}) was exhibited by MGS (Table 5). MBB, in 2013/14 (11.0 kg ha^{-1} mm^{-1}), GTA, in 2014/15 (13.3 kg ha^{-1} mm^{-1}) and SMT, in 2015/16 (29.8 kg ha^{-1} mm^{-1}) exhibited low WUE_{TDM}. High WUE_{TDM} mean values were expressed by SMT, in 2013/14 (14.4 kg ha^{-1} mm^{-1}), OZ_{368}, in 2014/15 (23.2 kg ha^{-1} mm^{-1}) and WAH, in 2015/16 (37.3 kg ha^{-1} mm^{-1}). Lower WUE_{GY} were noted in 2013/14 and 2014/15 (2.7 and 2.9 kg ha^{-1} mm^{-1}) for H_3, and for GMG (8.0 kg ha^{-1} mm^{-1}) in 2015/16; while high WUE_{GY} mean values were exhibited by SMT, MGS and OZ_{368}, in 2013/14, 2014/15 and 2015/16, respectively (Table 5). Ancient varieties tented to have lower WUE_{GY} (5.5 kg ha^{-1} mm^{-1}) than newly realized ones (7.0 kg ha^{-1} mm^{-1}), while no clear differences appeared for WUE_{TDM} (21.4 kg ha^{-1} mm^{-1}vs 20.8 kg ha^{-1} mm^{-1}, respectively). These supported results reported by Zhang *et al.*, (2016) whom carried out studies to understand the genetic gains in yield and WUE and their associated physiologic and agronomic traits for winter wheat and found that WUE increased substantially from 1.0 to 1.2 kg m^{-3} for cultivars from the early 1970s to 1.4–1.5 kg m^{-3} for recently released cultivars. Genotypic differences in WUE_{GY} were also reported by van den Boogaard *et al.* (1997), and Zhang *et al.* (2010).

4.Relationships between WUE and the physio-agronomic traits

The correlation coefficients relating WUE_{TDM} to the measured physio-agronomic traits were statistically no significant, except the correlation coefficient between LCHC and WUE_{TDM}, measured in 2015/16, which reached significance and had a negative sign (Table 6).

Table 6. Simple correlation coefficients between water use efficiency for total dry matter and grain yield and physio-agronomic traits.

		LCHC	*LRWC*	*SLA*	*PHT*	*HI*	*WUE_{TDM}*
WUE_{TDM}	2013/14	0.398	-0.171	-0.507	0.141	-0.008	
	2014/15	-0.087	0.602	-0.429	-0.184	0.134	
	2015/16	-0.772	0.598	0.247	0.565	-0.540	
WUE_{GY}	2013/14	0.792	-0.836	-0.277	-0.876	0.950	0.295
	2014/15	0.512	0.078	-0.199	-0.746	0.767	0.081
	2015/16	0.720	0.230	-0.240	-0.977	0.990	-0.435

PHT = Plant height,(cm), HI = Harvest index, (%) WUE_{TDM}= Water use efficiency for total dry matter, (kg ha^{-1} mm^{-1}) WUEGY= Water use efficiency for grain yield (kg ha^{-1} mm^{-1}), LCHC= Leaf chlorophyll content, LRWC= Leaf relative water content, SLA = Specific leaf area, $r_{5\%}$= 0.666.

These results suggested that, among the measured physio-agronomic traits, no one could be able to predict WUE_{TDM}, and to be used as selection criterion for screening purposes. Correlation coefficients of SLA and TDM with WUE_{GY} were non-significant, suggesting that these two traits were of little value for WUE_{GY} prediction. Results about SLA didn’t supported findings of van den Boogaard *et al.* (1997) whom studied wheat plant growth and water-use efficiency and found that WUE was higher for plants with higher leaf area per unit plant weight. Richards *et al.* (2002) suggested using specific leaf area as an indirect selection criterion for yield potential in wheat. Atta (2013) found that specific leaf area was negatively correlated with WUE and grain yield and suggested that selection against this trait may be effective in raising grain yield. The relationship between WUE_{GY} and LRWC was unreliable, being dependent on the environment for its expression. However PHT and HI, and to a lesser extent LCHC were reproducible and significantly correlated with WUE_{GY}. These traits appeared to be useful for WUE_{GY} improvement (Table 6). In this context, Zhang *et al.,* (2016) found no significant correlations between WUE_{GY} and LCHC, or LRWC, but significant correlations were found between WUE_{GY} and HI. Through multiple regression analysis Atta (2013) identified several key traits that contribute to improve WUE among which leaf traits, plant height, total dry matter at maturity, harvest index and grain yield which corroborated partially the results of this study.

Taking LCHC, LRWC, PHT and HI as causing traits and WUE_{GY} as caused trait, path analysis indicated that direct and indirect effects were inconsistent and varied from one environment to another (Table 7).

Table 7. Direct and indirect effects of the physio-agronomic traits on WUE_{GY}.

	LCHC	LRWC	PHT	HI	riy
	2013/14 cropping season				
LCHC	**0.450**	0.170	0.008	0.164	0.792
LRWC	-0.161	**-0.475**	-0.008	-0.196	-0.840
PHT	-0.321	-0.325	**-0.011**	-0.224	-0.881
HI	0.313	0.394	0.010	**0.236**	0.953
	2014/15 cropping season				
LCHC	**0.200**	0.002	0.196	0.152	0.512
LRWC	-0.018	**-0.023**	0.064	0.054	0.078
PHT	-0.143	0.005	**-0.275**	-0.365	-0.746
HI	0.059	-0.002	0.195	**0.514**	0.767
	2015/16 cropping season				
LCHC	**-0.080**	-0.036	0.315	0.520	0.720
LRWC	0.027	**0.108**	0.019	0.075	0.230
PHT	0.065	-0.005	**-0.389**	-0.648	-0.977
HI	-0.063	0.012	0.383	**0.658**	0.990

LCHC= Leaf chlorophyll content, LRWC= Leaf relative water content, PHT = Plant height,(cm), HI = Harvest index, (%) , WUE_{GY}= Water use efficiency for grain yield (kg ha^{-1} mm^{-1}).

Hence LCHC exhibited a large positive direct effect (0.450) in 2016/14, which lessened in the second cropping season (0.200) then vanished (-0.080) in the third one. This trait acted indirectly via HI during the three cropping seasons (0.164, 0.152, 0.520), via LRWC, in one season (0.170) and via PHT during two seasons (0.196, 0.315). The positive sign of the direct and indirect effects of LCHC suggested that higher LCHC was desirable to improve WUE_{GY}, either directly (but depending on the environment) or indirectly via HI and to lesser extend via PHT. Recently released cultivars expressed consistently high LCHC and HI compared to old ones which explain their observed high WUE_{GY} (Table 3). Similarly LRWC exhibited a large direct effect (-0.475) associated to sizeable indirect effects via LCHC (-0.161) and HI (-0.196) in one season, and both direct and indirect effects vanished during the two other seasons (Table 7). High LRWC was expressed by local varieties which had lower HI and LCHC, but the effect of this trait were inconsistent depending on the environment. PHT expressed a direct effect variable which was lower than the consistent indirect effects via HI. Taller varieties tended to have low HI and WUE_{GY}. HI expressed a consistent positive direct effect; the indirect effects, either via PHT or via LCHC and LRWC, were inconsistent (Table 7).

CONCLUSIONS

Experiment results revealed that modern cultivars are more efficient users of rain water than all others in semi-arid conditions, It is also revealed that those varieties, which use more water, produce hi harvest index value and give more grain yields. Our studies demonstrated that LCHC, LRWC, PHT and HI are more important traits linked to the WUEg in semi-arid regions. Thus, path analysis, based on phenotypic correlations between WUE_{GY} and HI, PHT, LRWC, LCHC, showed the consistent direct and indirect effects of HI and to a lesser extent those of PHT. Selecting for PHT and HI could improve both WUE_{GY} and grain yield under variable environments. The high WUE_{GY} genotypes identified in the current study can be used to develop more efficient cultivars that increase grain yield per unit of water used, in drought prone areas. However, selection for high HI, to improve GY and WUE_{GY}, will reduce plant height and biomass production under severe drought conditions. Conversely grain yield, at excessive crop height, can be reduced because of poor HI and increased lodging. It is suggested to select for tall, high-yielding plants within dwarf segregating populations.

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