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İ Ç İ N D E K İ L E R / C O N T E N T S

Collembola Communities in Different Compost Types as Bioindicator of Substrate Quality

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Collembolans are a good indicator of the degree of mineralization and humification of the soil. Their ecological characteristics, habitat and feeding type can help the analysis of composting processes and determining the quality of the resulting substrate. A particular interest is the potential antagonistic effect of compost on soil plant euedaphic life forms pathogens and phytophagous arthropods.The aim of this study was to establish the quality differences between the four types of mature compost by studying the structure of Collembola communities in them. The investigations were carried out with two substrates composed of forest wastes and two substrates composed of agricultural wastes. The difference between the compost types was the origin and size of the substrate particles. The results were obtained by field and laboratory studies. In the studied composts, the identified species were hemiedaphic, euedaphic and atmobiont. Hemiedaphic life forms dominated in the compost of agricultural wastes. The have the highest density into the compost of forest wastes. With regard to food sources the collembolans established species were divided into three ecological functional groups: herbivores, predators and detritivores. The groups of predators and herbivores were the smallest, and the most numerous were the detritivores. The detritivores population was established in high population density in the compost of forest wastes. The studies allow evaluating the found differences in the collembolans species composition and their number in the studied compost types. The results could be used as an estimate of the composting process and the quality of the compost.

Keywords: Collemlola, agricultural and wastes, compost

Introduction

Artificial fertilization and intensive soil tillage have a negative impact on the soil in the long term. Therefore, measures to revitalize soils are becoming increasingly important. The soil is home to an enormous number and variety of organisms. In the maintenance of a healthy soil with sustainable soil fertility, they take over a number of important tasks. An alternative to it is an intake of low-pollution compost in the soil, the safest and cheapest. The prominent role of soil fauna in the process of humus formation has been proved, (Coleman & Wall 2015, Bagyaraj et al. 2016, Chertov et al. 2017).

The soil mesofauna is also positively influenced by the supply of organic fertilizer. The results of Idinger & Kromp (1997) establish that the saprophagous groups like certain collembolans as well as the nematoceran families of sciarids, cecydomiids and chironomids occurred more abundantly in the compost-fertilized fields than in nonfertilized and inorganic fertilized field.

Collembola are common inhabitants of soil, ground vegetation and tree trunks. Water surfaces are also colonized, especially when vegetation is present. Collembola communities have been analysed by numerous authors (Gisin 1943, Zmudczyńska-Skarbek 2015, da Silva et al. 2016, Rendoš et al. 2016), but our knowledge of the effect of environmental changes on collembolan communities is very poor. Results of these studies give evidence of strong relationships of species composition with soil conditions. The aim of this study was to establish the quality differences between the four types of mature compost by studying the structure of Collembola communities in them.

Materials and Methods

The characteristics of the compost

Various types of compost (forest and agricultural) were used for the purposes of the study. The composts in this work will be called conditional: forest compost, types A and B and agricultural compost, types C and D. The wood waste materials from deciduous trees (branches and bark) were used for compost A and B as "brown" material (carbon source). The sorted waste materials from the household and freshly mown grass were used as a "green" material (source of nitrogen), and as activator of composting process- forest litter. The difference between the compost types A and B was the size of the substrate particles. The waste

materials from agricultural production were used for C and D, as "green" material was stems of tomatoes and peppers, but as "brown" material shoots of vines, and as activator -soil. The difference between the compost types C and D was the size of the substrate particles. The duration of the composting process was terminated after 145 days, with a temperature peak of 68°C.

Sampling for investigation of compost

Samples were taken from the piles of mature compost. For the representative sample, an amount of about 100 cm^3 has been repeatedly taken from all sides of each compost heap to give average sample of 1L. After mixing of composting materials, the samples were placed in plastic bags, sealed and stored in a refrigerator at 4-5 °C to carry out laboratory analysis.

Extraction of the collembolans from the compost

The extraction of collembolans followed the concept of Macfayden (1961). All adult specimens were bleached in lactic acid for 24 hours and then determined to species level by Gisin (1960) and Palissa (1964). Collembola were identified according to Fjellberg (1998, 2007).

Results and Discussion

Species composition and abundance of individuals

When processing of the soil samples during the whole period of the studies were extracted a total number of 2106 (Σ collembolans/100 cm3), of which 1575 adults belonging to 14 species belonging to 10 genera and 6 families

The identified species were arranged systematically:

Kingdom: Animalia
Subkingdom: Eumetazoa
Phylum: Arthropoda
Subphylum: Hexapoda
Class: Entognatha
Order: Collembola
Suborder: Entomobryomorpha
Family: Tomoceridae
Tomocerus minor (Lubbock 1862)
Family: Entomobryidae
Lepidocyrtus curvicollis Bourlet, 1839
Lepidocyrtus cyaneus Tullberg, 1871
Heteromurus nitidus (Templeton, 1835)
Orchesella flavescens (BOURLET, 1839)
Orchesella villosa (Geoffroy 1762)
Family: Isotomidae
Folsomia candida Willem, 1902
*Folsomia fimetaria (*Linnaeus, 1758)
Folsomia quadrioculata (Tullberg, 1871)
Isotoma viridis Bourlet, 1839
Proisotoma minima (Absolon, 1901)
Suborder: Poduromorpha
Family: Onychiuridae
Onychiurus Gisin, 1952
Onychiurus spp. (armatus-group) *
Suborder: Symphypleona
Family: Katiannidae
Sminthurinus aureus (Lubbock, 1862)
Family: Sminthuridae
Sminthurus viridis (Linnaeus, 1758)

The classification and nomenclature of species of Collembola in the various compost types is based primarily on Gisin 1960, Hopkin (1997), Potapov (2001) and others.

Ecological characteristic. Life-forms of collembolans

According to Gisin (1943), the insects from the order of Collembola are divided into three life forms: atmobiont, hemiedaphic and euedaphic. The life forms of the species composition of collembola communities in the compost types are listed in Table 1. The hemiedaphic and euedaphic forms were dominated. The atmobiont collembolans were underrepresented.

Table 1: Life forms of established species of the order Collembola

Life forms	Species	Compost type
atmobiont		
	Orchesella flavescens	A; B; C
	Orchesella villosa	A; B; C
	Sminthurus viridis	C
	Lepidocyrtus curvicollis	B;C
hygrophilic hemiedaphon		
	Tomocerus minor	B; C
mesophilic hemiedaphon		
	Sminthurinus aureus	A; C
	Folsomia quadrioculata	A;B; C;D
	Lepidocyrtus cyaneus	A; B;C; D
	Isotoma viridis	C; B; D
	Proisotoma minima	A; B; C
euedaphon		
	Onychiurus spp. (*Onychiurus armatus* - group Gisin, 1952)	A; B; C; D
	Folsomia candida	A; B; C; D
	Folsomia fimetaria	A; B; C; D
	Heteromurus nitidus	C; B; D

Food specialization of collembolans

With regard to food sources the established Collembola species were divided into three ecological functional groups: herbivores, predators and detritivores according to (Hopkin 1997).The group of predators was smallest and the most numerous was the group of saprofagige. Depending on conditions, some phytophage species can be saprophages, especially as mycophages (Ulber 1982). The species identified by us were assigned to different groups according to feeding type only available literature data, which relate mainly to Western and Central Europe and part of North America and Australia (Table. 2).

Table 2: Feeding specialization (first trophic level) of identified species of the order Collembola

Feeding specialization	Species	Compost type
herbivores		
	Lepidocyrtus curvicollis	B; C
	Lepidocyrtus cyaneus	A; B; C; D
	Heteromurus nitidus	B; C; D
	Isotoma viridis	B; C; D
	Proisotoma minima	A; B; C
	Sminthurus viridis	C
detritivores		
	Orchesella flavescens	A; B; C
	Folsomia candida	A; B; C; D
	Folsomia fimetaria	A; B; C; D
	Folsomia quadrioculata	A; B; C; D
	Sminthurinus aureus	A; C
herbivore/detritivore		
	Onychiurus spp. (*Onychiurus armatus* - group Gisin, 1952)	A; B; C; D
detritivores/fungivore		
	Tomocerus minor	C; B
predator		
	Orchesella villosa	A; B; C

The taxonomic characterization showed that the representatives of the Collembola fauna in the compost types belong to the following families: Entomobryidae, Isotomidae, Neanuridae, Onychiuridae, Katiannidae, Sminthuridae. Species richness was highest /12 species/ in the forest compost (B) and lowest /7 species/ in the agricultural compost (D).

The data on abundance of the species demonstrated that the number of the dominant species was from 3 to 5 species; 39.2 % of the identified species were detritivores, 46.4 % herbivores and 7.1 % predators. The herbivore species of genus *Onychiurus* were in high abundance in agricultural compost (D) and the herbivores of family Isotomidae: *Isoloma viridis* in the agricultural compost (C).

The present study has analysed the structure of collembola communities in the four types of mature compost. The results has shown that compost type have an influence on Collembola species. According to Beare et al. (1992, 2014) functionally similar organisms often have different tolerance ranges with regard to certain environmental parameters as well as their physiological requirements microhabitat preferences. It can also be inferred that different species in the same habitat may fulfill different functions in the ecosystem. Mebes and Filser (1998) found differences in the influence of various Collembola species on the nitrate leaching and also assumed this for the organic matter decomposition. Cragg and Bardgett (2001) also found that the number of species or the species diversity is not decisive for the organic matter decomposition, promoting of microbial activity and release of organic carbon and nitrate, but only the species composition of the Collembola communities. In the open field, the hemiedaphic species are more exposed to microclimatic changes than the euedaphic species living in deeper soil layers (Heimann-Detlefsen et al. 1994).

According to Dunger et al. (2004), the euedaphone reacts more slowly to environmental changes than the hemiedaphone.In the present experiments, the animals were exposed to largely constant environmental conditions. An exception is the compost humidity. The drying out of the substrates during the experimental periods may have different effects on the species. According to

Fountain and Hopkin (2005), however, *F. candida* is also exceptionally resistant to dehydration.

It is possible that differences in the species composition of Collembola can occur due to processes arising from the physical and chemical composition of the different compost types and biological activity in the various substrate types. But these differences could be explained by established life forms of collembolans that are bioindicators of changes in soil and substrate quality. The vegetation structure plays an important role in the life cycle of atmobionts, and the soil substrate – in euedaphic species. Of undoubted interest is the further development of study, including the establishment of interactions between Collembola fauna and various properties of compost.

Conclusions

The most common were species of *Folsomia, Onychiurus, Lepidocyrtus* and *Heteromurus;*

The biodiversity was greater in the compost B and lowest in the compost D;

The hemiedaphic life forms were dominated in compost type, and the euedaphic life forms in in compost type. The atmobiont collembolans were underrepresented;

The established life forms of the Collembola communities can be used as bioindicators for the maturity status of the compost.

References

Bagyaraj, D.J., C.J. Nethravathi and K.S. Nitin, 2016. Soil Biodiversity and Arthropods: Role in Soil Fertility. In Economic and Ecological Significance of Arthropods in Diversified Ecosystems Springer Singapore, 17-51.

Beare, M.H., R.W. Parmelee, P.F. Hendrix, W. Cheng, D.C. Coleman, and D.A Crossley, 1992. Microbial and faunal interactions and effects on litter nitrogen and decomposition in agroecosystems. *Ecological Monographs*, *62*(4): 569-591.

Chertov, O., A. Komarov, C. Shaw, S. Bykhovets, P. Frolov, V. Shanin and M. Shashkov, 2017. Romul_Hum—A model of soil organic matter formation coupling with soil biota activity. II. Parameterisation of the soil food web biota activity. Ecological Modelling, *345*: 125-139.

Coleman, D.C., and D.H. Wall, 2015. Soil fauna: occurrence, biodiversity, and roles in ecosystem function. *Soil Microbiology, Ecology and Biochemistry*, 111-149.

Cragg, R.G. and R.D. Bardgett, 2001. How changes in soil faunal diversity and composition within a trophic group influence decomposition processes. Soil Biology and Biochemistry, 33(15):2073-2081.

da Silva, P.M., F. Carvalho, T. Dirilgen, D. Stone, R. Creamer, T. Bolger and J.P. Sousa, 2016. Traits of collembolan life-form indicate land use types and soil properties across an European transect. Applied Soil Ecology, 97: 69-77.

Dunger, W. and K. Voigtländer, 2009. Soil fauna (Lumbricidae, Collembola, Diplopoda and Chilopoda) as indicators of soil eco-subsystem development in post-mining sites of eastern Germany–a review. *Soil organisms*, *81*(1): 1-51.

Fjellberg, A. 1998. The Collembola of Fennoscandinavia and Denmark. Poduridae.– Fauna Entomological Scandinavica 35. Brill, Leiden.

Fjellberg, A. 2007. Collembola of Fennoscandia and Denmark: Entomobryomorpha and Symphypleona. Part II. Brill.

Fountain, M.T. and S.P. Hopkin, 2005. *Folsomia candida* (Collembola): a "standard" soil arthropod.Annu. Rev. Entomol*. 50*: 201-222.

Gisin, H. 1943. Ökologie und Lebensgemeinschaften der Collembolen im schweizerischen Exkursionsgebiet Basels: Inauguraldissertation... vorgelegt der philosophisch-naturwissenschaftlichen Fakultät der Universität Basel von Hermann Gisin A. Kundig.

Gisin, H. 1960. Collembolenfauna Europas. Geneva, Switzerland: Museum d'histoire naturelle, 243-243

Harrison-Kirk, T., M.H. Beare, E.D Meenken and L.M. Condron, 2014. Soil organic matter and texture affect responses to dry/wet cycles: Changes in soil organic matter fractions and relationships with C and N mineralisation. Soil Biology and Biochemistry, *74*: 50-60.

Heimann-Detlefsen, D., S Theiss and U. Heimbach, 1994. Auswirkungen unterschiedlich intensiver Bewirtschaftungsintensitäten auf die Collembolenfauna des Ackerbodens.

Mitteilungen aus der Biologischen Bundesanstalt für Land-und Forstwirtschaft, 230-273.

Hopkin, S.P. 1997. Biology of the springtails: (Insecta: Collembola). OUP Oxford.

Idinger, J., B. Kromp, 1997. Ground photoeclector evaluation of different arthropod groups in unfertilized, inorganic and compost-fertilized cereal fields in eastern Austria. Biological agriculture & horticulture, 15(1-4): 171-176.

Macfayden, A. 1961. Improved tunnel-type extractors for soil arthropods. Journal of Animal Ecology 30: 171–184.

Mebes, K.H. and J. Filser, 1998. Does the species composition of Collembola affect nitrogen turnover?. Applied Soil Ecology, *9*(1): 241-247.

Palissa, A. 1964. Apterygota – Urinsekten. In: P. BROHMER (Hrsg.): Die Tierwelt Mitteleuropas.4. Lieferung, Teil Ia. Leipzig, 1-407.

Potapov, M. 2001. Synopses on Palaearctic Collembola: Isotomidae. W. Dunger (Ed.). Staatliches Museum für Naturkunde Görlitz.

Rendoš, M., N. Raschmanová, Ľ. Kováč, D. Miklisová, A. Mock and P. Ľuptáčik, 2016. Organic carbon content and temperature as substantial factors affecting diversity and vertical distribution of Collembola on forested scree slopes. European Journal of Soil Biology, 75: 180-187.

Ulber, B. 1982 Einfluss von *Onychiurus fimatus* Gisin (Collembolla, Onychiuridae) und *Folsomia fimetaria* (L.) (Collembola, Isotomidae) auf *Pythium ultimum* Trow., einen Erreger des Wurzelbrandes der Zuckerrübe; In: Lebrun, P. H. et al. (eds.), Proc VIII Int. Coll. Soil Zoology, 261-268.

Zmudczyńska-Skarbek, K., A. Zwolicki, P. Convey, M. Barcikowsk, and L. Stempniewicz, 2015. Is ornithogenic fertilisation important for collembolan communities in Arctic terrestrial ecosystems?. Polar Research, 34.

The Species of Superfamily *Chalcidoidea* – Parasites of Aphidophagous Hoverflies

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The species of the family Syrphidae are one of the most important predators of the peach leaf aphids on tobacco. In the complex food chain, aphidophagous hoverflies are attacked by a wide range of parasites. During tobacco vegetation, in the Prilep area, we applied the following methods: survey of 20 tobacco stalks and survey of 100 tobacco leaves, in 10 days interval, 2003-2005. The prime material collected from the nature, further is cultivated and analysed under laboratory conditions. Parasited larva of Syrphidae, feeds with aphids and perform its bioregulatory role. Parasite species are activated after the larva of hoverflies is transformed into a puppet stage. They are feeding on tissues and organs of the hoverflies and destroy them. Were identified three parasite species from superfamily Chalcidoidea, eclosed from pupae of hoverflies: *Pachyneuron grande* (Pteromalidae), *Pachyneuron* cf. *grande* (Pteromalidae) and *Syrphophagus aeruginosus* (Encyrtidae). The most numerous is the parasitic specie *P. grande*. It was registered as a parasite on: *Sphaerophoria scripta*, *Sphaerophoria rueppelli*, *Episyrphus balteatus*, *Scaeva pyrastri* and *Eupeodes corollae*. *P.* cf. *grande* is a parasite on: *S. scripta* and *S. rueppelli. S. aeruginosus* is a parasite on: *S. scripta*, *S. rueppelli*, *S. pyrastri* and *E. balteatus*. The emergence of parasitic species depends on the occurrence of host-hoverflies, while the emergence and development of aphidophagous hoverflies depends on the occurrence of the peach leaf aphids on tobacco and climate conditions of the area. This study may contribute in strategies of biological control of plant pests and IPM implementation.

Keywords: Aphids, *Syrphidae*, parasites, *Chalcidoidea*

Introduction

Superfamily Chalcidoidea is one of the largest groups of parasitoid wasps within the order Hymenoptera, with several thousand known species, and many others which have yet to be described. They range in size from 0.13 mm to 30 mm, usually under 3 mm. Most of the species are parasitoids or super-parasitoids of other insects. A few species are phytophagous.

Family Pteromalidae includes 588 genera with 3506 species, belonging to 31 subfamilies. These subfamilies also include Pteromalinae, with 314 genera and 2073 species. The life-cycle of the species in this family is variable. There are solitary and gregarious species, ectoparasitoids and endoparasitoids, koinobionts and idiobionts, primary and secondary parasitoids and even predators.

Palaearctic species of the genus Pachyneuron (Pteromalidae) were revised from Szelényi (1942), while the European species of the genus Pachyneuron were revised by Delucchi (1955 b). Since then some synonyms and nomenclature are changed. Pek (1963), gave a list of 14 species of the genus Pachyneuron in North America (cit. Graham, 1969). According to Cheek et al. (1974), more than 50% of hoverflies are attacked by parasites, one of which is *Pachyneuron sp.*

The *P. grande* species is present in Croatia, Bosnia and Herzegovina, Serbia, Montenegro, Kosovo, Austria, Czech Republic, Hungary, parts of the former USSR, Slovakia, Moldova, France, Germany, Switzerland, Belgium, Sweden, Spain, Argentina, Iran (Graham, 1969; Boucek, 1977). Delucchi (1955), reported that the parasitic species could be found almost everywhere in the north of the Alps. The species has been recorded in Sweden and eastern Switzerland.

The family Encyrtidae currently includes 460 genera and 3735 species placed in 2 subfamilies: Encyrtinae and Tetracneminae. Encyrtinae subfamily includes 353 genera with 2920 species and Tetracneminae subfamily includes 107 genera with 815 species. The Encyrtidae greatest diversity is in tropical and subtropical areas. About half of the Encyrtidae species are associated with scale-insects (Homoptera: Coccoidea), generally as endoparasitoids of immatures or less commonly

adults, but with egg predation practised by some species of Microterys (De Bach, 1939). Almost all species belonging to the Tetracneminae are parasitoids of Pseudococcidae, whilst species of Encyrtinae are known to be parasitoids of a wider variety of coccoids (occasionally also of Pseudococcidae) and other insects, mites, ticks and spiders (Tachikawa, 1981).

According to Trjapitzin (1989), there are 77 species of the genus Syrphophagus (Encyrtidae), of which 44 are determined in Palaearctic and 17 species are recorded in parts of the former USSR. According to Trjapitzin (1989), the species of the genus Syrphophagus are hyper-parasites of pupas of hoverflies and sometimes Lonchaeidae (Diptera), and also they are hyper-parasites of aphids (Homoptera, Aphidoidea) and jumping plant lice (Homoptera, Psylloidea).

S. aeruginosus is determined in Serbia, Montenegro, Kosovo, Vojvodina, Macedonia, Croatia, Bulgaria, Austria, Czech Republic, Armenia, Moldova, Romania, Slovakia, Ukraine, Hungary, parts of the former USSR, France, Switzerland, Germany, UK, Madeira, India, Azerbaijan, Armenia, Georgia, the Asian part of Turkey, Lebanon, Syria, Israel, Jordan, Iran, Iraq, the Sinai Peninsula (Egypt), Arabian Peninsula (Boucek, 1977; Trjapitzin, 1978).

Materials and Methods

Field research

For realization of our goals the following methods were applied:

a. Method of survey of 20 randomly selected tobacco stalks infested with aphids. Tobacco stalks were sampled from the whole area of the trial at 10-days interval, starting from June 1, up to the end of September. The tests were performed on targets tobacco (leaves, tobacco flower and flower seed capsules). 10 checks were made by this method in each of the three years of investigations, i.e. 200 stalks per year, or 600 stalks in total. 5813 tobacco leaves were examined in 2003, 5851 in 2004 and 5944 in 2005 or 17608 tobacco leaves in total.

b. Method of Davies- survey of 100 randomly selected tobacco leaves infested with aphids. Tobacco leaves were sampled from the whole area of the trial at 10-days interval, during vegetation. 10 checks were made by this method in each of the three years of investigations, i.e. 1000 leaves per year, or 3000 leaves in total.

These two methods of analyses are simple and secure to be performed; the advantage of this method is that the data can be collected by one person only.

Laboratory research

The prime material is collected in the nature, after which it is further nourished, cultivated and analysed under laboratory conditions. The eggs, larvae and pupae of Syrphidae, are placed in special containers and raised until adult eclodes. Larvae were reared on tobacco leaves infested with *M. persicae* in Petri- dishes. The pupae which were brought up and those collected were separately placed in test-tubes. Later we have monitored the internal modifications of the pupae, the length of its developing stadium, the degree of eclosion of imago, the percentage of parasitism and the eclosion of different types of parasites.

Among ecloded parasites derived from the Syrphidae, we have determined their species and their morphological characteristics.

Results and Discussion

During laboratory analyzes we have identified three species of parasites of order Hymenopterae, suborder Apocrita, superfamily Chalcidoidea ecloded from the pupae of the aphidophagous Syrphidae: -from the Pteromalidae family, subfamily Pteromalinae, genus Pachyneuron Walker (1833) we have detected *Pachyneuron grande* Thomson (1878) and *Pachyneuron cf. grande* Thomson (1878),-from Encyrtidae family, subfamily Encyrtinae, genus Syrphophagus Ashmead, (1900): *Syrphophagus aeruginosus* Dalman (1820).

In investigation of parasitic species of the family Syrphidae, according to Jankowska (2004), similarity was found between two species: *P. grande* and *S. aeruginosus*. Harizanov, Babrikova (1990), identified seven species of hoverflies parasites in Bulgaria, including *P. grande* and *S. aeroginosus*, they were also included in our investigations. According to Adashkevich (1975), more than 28 species of syrphid parasites have been observed in the USSR, including *S. aeruginosus* and *P. grande.* Resende et *al.* (2006) also listed *Pachyneuron sp*. and *Syrphophagus sp*. as parasites of aphidophagous hoverflies.

We should have in consideration that aphidophagous larva still feeds with aphids and perform its bioregulatory role in the destruction of aphids. Parasite species are activated after the larva of hoverflies is transformed into a puppet stage. They destroy the host-hoverfly. In the beginning the pupa of the hoverfly has its specific larval color. Few days later its color becomes more whitish and dull.

According to Krüger (1926), larvae of *S. pyrastri* are often attacked by the parasites and they can be recognized by their pale color.
If you carefully cut the hoverfly pupa, inside you will notice a few small white larvae of the parasitic specie. Then the pupa turns dark and it is a sign of a parasitism.
Whether it is *P. grande*, *P. cf. grande* or *S. aeruginosus,* in the parasited pupa of hoverfly were developed larger number of parasites. When the adults of parasite eclode, they make many holes through the thick cuticle of the hoverfly pupa, on all sides of the pupa.The pupa usually looks like a grid.

Pachyneuron grande Thomson, 1878 is a widespread species of parasite, ecloded from the Syrphidae pupae. During investigations 2003-2005 in tobacco biocenosis in Prilep, *P. grande* was identified as a parasite on the aphidophagous hoverflies *S. scripta, S. rueppelli, E. balteatus, S. pyrastri* and *E. corollae.*

In Macedonia, this species was first identified by Hoffer (1970 d), in Struga and Popova Sapka (cit. Boucek, 1977). According to this author, *P. grande* is found in the forest zone of Europe. According to Jankowska (2004), Pteromalidae family was represented by *P. grande* and this species was responsible for parasitism on 29 species of hoverflies. *P. grande* was described by Evenhuis (1966), Malinowska (1973) and Wnuk (1974), as a parasite of hoverflies (cit. Jankowska, 2004).

In our investigations *P. grande* was registered on 5 hoverfly species. It was found in large number on *S. scripta, S. rueppelli* and *E. balteatus* pupae in 2003-2005. While in 2004, individual parasitised pupae were identified on *S. pyrastri* and *E. corollae*. Feraru et al. (2004) reported that the parasite *P. grande* emerged from the pupae of *E. balteatus.* In France, according to Delucchi (1953 b), *P. grande* is recorded as a parasite of *E. balteata* (cit. Graham, 1969).

After the hoverfly will reach the pupation stage, the parasite *P. grande* emerges in 12.1 days in *S. scripta*, 12.4 days in *S. rueppelli*, 10 days in *E. balteatus* and *S. pyrastri* and 11 days in E. corollae. During investigations we found that 1 - 20 parasites, or in average seven parasites, ecloded from one parasitised pupa of *P. grande*. According to Jankowska (2004), from 3 to 34 imagos of *P. grande* (females and males) emerge from the hoverfly pupae, while Resende et *al.* (2006), reported that the number of emerged *Pachyneuron sp* imagos is 7 – 11.

In tobacco fields, appearance of parasitised larvae was first determined on the 10th of August. They were most frequently recorded from 25th August to 15th September and then their number gradually decreased. Highest number of parasitised individuals was recorded from 20th August to 10th September and it accounts for 55.26% of the total parasitism of this species. During our ivestigations, the earliest registereded pupae parasitised with *P. grande* was the pupae of *S. rueppelli*, on 22.07.2004, and the last one was that of *S. scripta* and *S. rueppelli,* on 30.09.2003. From studies of Graham (1969), *P. grande* is found from July to September. According to Delucchi (1955) adults of this species are determined from June to July.

The imagos are small, considerably short. The length of females varies from 2.3 to 3 mm. Males are somewhat smaller than females, with a length of 2 to 2.5 mm. Body color of females is green and the thorax is bronze. The body of the males is green to bluish green.

Figure 1. *P. grande* ♀ (dorsal view)

Figure 2. *P. grande* ♂ (dorsal view)

Head is wider than its length, with big complex eyes, antennae are brighter and a little hairy, inserted above the ventral edge of the eyes. The propodeum is dotted, with weak irregular hairs. Hanging stomach is approximately half the length of the propodeum, just slightly longer than its width, slightly narrowed in front and dorsal part is obliquely underlined. The abdomen is shorter than the thorax and seen from above it is flat. The stomach is rounded the first abdominal tergum occupies 1/3 to 1/2 of the abdomen length. In females the abdomen is oval, while in males it is narrower. The wing nervature is rather simple, costal forewing cells are with a row of hairs extending from the distal third to half of the wings. The female legs are brown and tarsus and femur are darker, sometimes with greenish shade. In males the legs are yellow, except the tarsus.

Figure 3. *Pachyneuron cf. Grande*

Syrphophagus aeruginosus Dalman, 1820 is a widespread parasite ecloded from the *Syrphidae* pupae. *S. aeruginosus* is polyphagous species and during our trials it was registered as a parasite of four hoverflies species: *S. scripta, S. rueppelli*, *S. pyrastri* and *E. balteatus.*

In Macedonia it was first identified by Hoffer (1970 d), in Struga and Popova Sapka (cit.
Bouček, 1977). According to Bouček, *S. aeruginosus* is distributed in Palearctic, as a parasite of aphidophagous hoverfly pupae. According to Jankowska (2004), Encyrtidae family was represented by *S. aeruginosus* as a parasite of hoverfly pupae. The species was described by Scott (1939), Malinowska (1973), Wnuk (1974), as a parasite of hoverflies (cit. Jankowska, 2004).

Pachyneuron cf. grande Thomson, 1878 is a polyphagous parasite and in our investigations it was identified in 2003 as a parasite of two hoverfly species: *S. scripta* and *S. rueppelli*. *Pachyneuron cf. grande* is very similar to the previous species, but they differ in their marginal nerve. Development of the parasite from pupation until eclosion lasts 10 to 12 days. During investigations we found that minimum 1 to maximum 10 parasites of *P. cf. grande* eclode from the parasitised pupa, i.e. in average four parasites The first occurrence of parasitised larvae was recorded in August 10 and parasitism could be observed until September 18.

During investigations 2003-2005, in tobacco biocenosis in Prilep, *S. aeruginosus* was identified as a parasite of *S. scripta* and *S. rueppelli* in 2003 and *S. pyrastri* and *E. balteatus* in 2004. In 2005 no parasitized individuals of this species were identified. According to Adashkevich (1975), the parasite *S. aeruginosus* ecloded from the pupae of *S. rueppelli, M. corollae*, *S. pyrastri* and *S. balteatus*. In Leningrad area, *S. aeruginosus* was obtained by Talickiÿ (1966 a), from the pupae of *E. corollae* and in Moldova it was marked as a parasite of *E. balteatus* and *S. ribesii* (cit. Trapicìn, 1989). In Serbia (Belgrade) according to Vukasovic (1926 d, 1928 d), the species was found on *Syrphus sp*. and *Paragus sp*. (cit. Boucek, 1977).

Development of the parasite from pupation of the host until eclosion lasts 12 days. We determined that 15 to 31 imagos of *S. aeruginosus* ecloded from one parasitised pupa. According to Jankowska (2004), 17 imagos emerge from hoverfly pupae of

S. aeruginosus, while Resende et *al.* (2006), reported 7 to 11 imagos from the pupae of *Syrphophagus* sp.

During tobacco vegetation, the parasitised larvae emerged from15th August to 15th September.

Figure 4. *S. aeruginosus* ♀(side view)

Figure 5. *S. aeruginosus*♂(dorsal view)

The length of females of S. aeruginosus varies from 1 to 1.6 mm and of the males from 1 to 1.4 mm. The average length of imagos is 1.4 mm. The imago body color is black, with dark green reflection. Head is wider than its length, with complex, large eyes; antennae are inserted near oral margin. The width of the vertex is about 1/3 of the maximum width of the head. Both sexes have very elongated mesopleuron, which often takes up more than half of the thorax from side view. Abdomen is shorter than thorax; ovipositor is hidden. Hips of the second pair of legs are placed in the middle level of the mesopleuron. The yellow part of the rear tibia is clearly expressed.The wings are with very simplified veins.

Conclusions

P. grande is polyphagous parasite and during our trials it was registered on five hoverflies species: *S. scripta, S. rueppelli, E. balteatus, S. pyrastri* and *E. corollae.* After hoverfly will reach the pupation stage, the parasite *P. grande* emerges in 12.1 days in *S. scripta*, 12.4 days in *S. rueppelli*, 10 days in *E. balteatus* and *S. pyrastri* and 11 days in *E. corollae*. During investigations we found that minimum 1 to maximum 20 parasites, or in average seven parasites, ecloded from one parasitised pupa of *P. grande*.

During our investigations, *Pachyneuron cf. grande* was registered as a parasite of *S. scripta* and *S. rueppelli*. After hoverfly will reach the pupation stage, development of the parasite from pupation until eclosion lasts 10 to 12 days. In our investigations we found that minimum 1 to maximum 10 parasites of *P. cf. grande* eclode from the parasitised pupa, i.e. in average four parasites

S. aeruginosus is polyphagous species and during our trials it was registered as a parasite of four hoverflies species: *S. scripta, S. rueppelli*, *S. pyrastri* and *E. balteatus.* Development of the parasite from pupation of the host until eclosion lasts 12 days. We determined that 15 to 31 imagos of *S. aeruginosus* ecloded from one parasitised pupa.

The occurence of parasite depends on the occurrence of host-hoverflies, while the occurence and development of aphidophagous hoverflies depends on the appearance of green peach aphid on tobacco and climate conditions in the region of Prilep.

References

Adashkevich, B.P. 1975. Entomophagous insects on vegetable crops Kolos, Moscow. Commonwealth Bureau of Plant Breeding, 190, pp. 88-121.

Boucek, Z. 1977. A faunistic review of the Yugoslavian Chalcidoidea (Parazitic hymenoptera). Acta Entomologica Jugoslavica 1977, 13 Suppl.

Carrillo, LI.R., Z.M. Mellado and B. Pino, 1974. Alos afidos Citobion avenae (Фab.) y Metopolophium dirhodum (Walk.), su influencia en el rendimiento, ubicacion en la planta y sus enemigos naturales. Agro Sur Vol.2 (2) 71-85.

DeBach, P. 1939. Microterys titani Gir., an egg predator of Lecanium corni Bouch. Journal of Economic Entomology 32:728.

Delucchi, V. 1955. Biträge zur Kenntnis der Pteromaliden (Hym., Chalcioidea). Mit 7 Abbildungen, davon 2 auf 1 Farbtafel. Zeitschrift für angewandte Entomologié Zwölfer.

Feraru, E. and G. Mustata, 2004. www.geocities.com/brisbane_wasps/ICHNEUMONIDAE.htm. Graham M., 1969. The Pteromalidae od North-Western Europe (Hymenoptera :Chalcidoidea). The Billetin of the british museum (Natural history), Entomology supplement 16 London.

Харизанов, А. & Т. Бабрикова, 1990. Биологична борба срешу непријателите по растенијата. Издателство "Земја", Софија.

Jankowska, B. 2004. Parasitoids of Aphidophagous Syrphidae occurring in cabbage aphid (*Brevicoryne brassicae* L.) colonies on cabbage vegetables. Journal of plant protection research, vol 44, No 4.

Krsteska, V. 2007. Afidofagni osoliki muvi (Diptera, Syrphidae) na tutunot vo Prilepsko. Doktorska disertacija, Fakultet za zemjodelski nauki i hrana-Skopje, R. Makedonija.

Krüger, F. 1926. Biologie und Morphologie einiger Syrphiden larven. Z Morph Okol Tiere 6: 83-149, www.scienzemfn.uniroma1.it/faunait/F97.DOC.

Resende, A.L.S., E.E. Silva, V.B. Silva and R.L.D. Ribeiro, 2006. Primeiro Registro de *Lipaphis pseudobrassicae* Davis (Hemiptera: Aphididae) e sua Associaзro com Insetos Predadores, Parasityides e Formigas em Couve (Cruciferae) no Brasil. Neotropical Entomology 35(4):551-555.

Szelényi, G. Von, 1942. Über die Chalcididen-Gatung Pachyneuron Walk. (Hymen.). Zentralblatt für das Gesamte Forstwesen, Wien.

Tachikawa, T. 1981. Hosts of encyrtid genera in the world (Hymenoptera: Chalcidoidea). Mem. Coll. Agr., Ehime Univ. 25 (2) : 85-110.

Trjapitzin, V.A. 1978. Hymenoptera II. Chalcidoidea 7. Encyrtidae. Opredeliteli Nasekomykh Evropeyskoy Chasti SSR 3:287.

Трапицин, В.А. 1989. Наездники-Енциртиди (Hymenoptera, Encyrtidae) Палеарктики. Ленинград "Наука" Ленинградское отделение.

Preliminary Checking of Some Turkish Diatomaceous Earth Similarities with Commercial Diatomaceous Earths under Scanning Electron Microscope (SEM)

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Diatoms are dead bodies of unicellular algae's and made up of fossilized diatoms in aquatic ecosystems. Diatomaceous earth (DE) is a dust varying in color depending on composition, from white-grey to yellow to red and active ingredient is amorphous silicon dioxide. DEs are commonly used for purification of water, the purification of juices, separation of various oils and chemicals and also used as an insecticide. Mode of action as insecticide which damage occurs to the insects protective wax coat on the cuticle, mostly by sorption and to a lesser degree by abrasion, or both. The result is the loss of water from the insect's body through desiccation resulting in death. The efficacy of DE against insects depends on different physical and morphological characteristics of the diatoms. In present study, image properties of 10 different Turkish DE samples under Scanning Electron Microscope (SEM) were checked and compared similarities with commercial DEs, namely Protector, SilicoSec, Insecto and Pyrisec. SEM image analysis indicated that there were variations in shape and size of dead bodies of diatoms in Turkish and commercial DEs. The shapes of dead bodies of diatoms in Turkish local DE's named as CBN and BGN were found very similar with those in commercial DE, Silicosec. Local DE coded as DC has a round shape and looks similar to commercial DE of Pyrisec while local DE coded as CAN has triangle shape and its shape was different from those of all other DE samples.

Keywords: Turkish Diatomaceous earth, SEM, Diatom, composition, insecticide

Introduction

Diatomaceous earth is the naturally occurring fossilized remains of diatoms. When diatomite is crushed into a powder, it is usually called "diatomaceous earth," or abbreviated DE (Calvert, 1930). Diatoms are single-celled aquatic algae. They belong to the class of golden brown algae known as Bacillariophyceae. Diatomite is a near pure sedimentary deposit consisting almost entirely of silica. The properties which make diatomite valuable include low density, high porosity, high surface area, abrasiveness, insulating properties, inertness, absorptive capacity, brightness, and high silica content. Diatomite has a wide variety of uses, and is a component in hundreds of products or vital to the manufacturing process of thousands more.

The most important use relative of high-quality diatomite is as a filtering media. The naturally occurring fossilized remains of diatoms have innate filtering characteristics due to their unique honeycomb structure. Their filtering qualities are used in beer and wine making, pharmaceutical manufacturing, motor oil processing, and to filter swimming pool water. For almost 100 years diatomite has been the workhorse of food and beverage processing. Almost every shelf in the grocery store contains a product which has been filtered by diatomite.

In paints, diatomite alters glass and sheen, extends primary pigments, adds bulk and strength, controls permeability and enhances coating adhesion. In plastics, diatomite serves as an antiblocking agent which helps in the separation of plastic parts in manufacturing, and in the separation of plastic bags by the consumer. Due to such characteristics as porosity and high surface area, diatomite is highly absorbent and is very useful in the clean-up of spills in the automotive, industrial, janitorial and waste remediation industries. When diatomite is incorporated into soil, it serves to reduce compaction, and increase water and air permeation. It also increases plant available water, firms soggy soils, loosens hard to work soils, provides better drainage, aids in nutrient transfer, and improves root growth. In such applications as golf courses, and other landscaped areas it helps absorb and hold water, reducing the amount of water used.

As natural insecticide when insects come in contact with diatomaceous earth, it absorbs their protective wax coating and their shells are damaged by the glassy diatoms. This combination causes them to die by dehydration. There is no survival and no built-up immunity as there is with chemical insecticides. Also, it does not break down as chemicals do.

Currently, the control of stored product pests in durable stored food products, such as grains and legumes, is based on the use of chemical methods such as fumigants and residual insecticides. However, the use of these substances is directly related with toxic residues on the final product, as well as serious environmental hazards. These factors, along with the consumers' demand for residue-free food and the development of resistance by several insect pests, have made essential the evaluation of alternative, low-risk and environmentally-friendly control methods. One of the most promising alternatives over the use of traditional pesticides in durable stored products is the use of diatomaceous earths (DEs). DEs are composed by the fossil skeletons of phytoplankton's, which occur in fresh and salt water since the Eocene period and produce a soft sedimentary rock, which is composed mainly by amorphous silica (SiO_2 + H_2O). The DEs currently mined vary remarkably in their insecticidal activity, depending upon species composition, geological and geographical origin as well as certain chemical characteristics, such as SiO_2 content, pH and tapped density (Korunic, 1997).

DEs act in the insects' exoskeleton (cuticle) causing rapid desiccation resulting in death through water loss. They are non-toxic to mammals (rat oral LD_{50}>5000 mg/kg of body weight), leave no toxic residues on the product and according to the US EPA they are classified in the category of GRAS (Generally Recognized As Safe) since they are used as food or feed additives (FDA, 1995). Regarding their insecticidal use, DEs can be applied with the same application technology with traditional grain protectants, which means that no specialized equipment is required (Athanassiou et al. 2005). Moreover, since they are inert (silicaceous) materials, no interaction with the environment occurs. Thus, DEs persist in the treated substrate, providing a long-term protection against pests, which is currently a 'red flag' for the use of conventional pesticides. The efficacy of DE from different sources (mines) on insects is not the same (Snetsinger, 1988; Katz, 1991; McLaughlin, 1994). DE from salt water is more common, cheaper and supposedly less efficacious as an insecticide (Snetsinger, 1988). However, efficacy of DE against insects depends on different physical and morphological characteristics of the diatoms rather than on its origin (Korunic,1998).

The mining and processing of natural diatomite is delicate and complicated. It requires large processing facilities but minimize to costs, diatomite is usually mined in open-pit, surface mines. In surface mining, a considerable thickness of earth, known as overburden, may have to be removed. Once this layer is removed and the purest of the diatomite strata is exposed, it is then cut from the bed with powerful scrapers and stockpiled. Diatomite does not need to be blasted as it is a soft, friable ore. The stockpiled material is then hauled to the processing plant for crushing, drying, milling and often calcining. Going into the crusher, the pieces may be as large as a small car, but coming out they will be the size of a pea. At this point the ore will still contain moisture. In order to dry the ore, significant amounts of heat must be applied in flash dryers. The ore is then milled gently to preserve the structure. It is critical that the ore be completely pure. A small amount of foreign matter can greatly downgrade the materials performance. These impurities are removed via a series of separators and traps. Finally, the material is classified, packaged and sent to customers. (Anonymous, 2017a). Several DEs, based on natural deposits, are now commercially available, and have proved very effective against stored grain pests (Subramanyam and Roesli, 2000, Athanassiou et al., 2011). Some of the formulation names presently available as insecticides on the market are:, Bug Resistor, Crop Guard, DE Insect Killer, Dicalite, Diacide, Diasecticide, Diatom Dust, Diatomic Earth, Dryacide, Pyrisec, Insecto and Silicosec.

Based on the first evidence and preliminary samplings, it seems that Turkey is considered to have rich natural DE deposits, and there is clear evidence for the existence of large DE deposits at some areas of Turkey (Özbey and Atamer, 1987; Mete, 1988; Sıvacı and Dere, 2006; Çetin and Taş, 2012). Diatomite reserve of Turkey is approximately 125 million tons. The largest diatomite reserve (106 million tons) known in Turkey is Hırka (Kayseri) (Çetin and Taş, 2012). However, there is no local DEs commercially available in Turkey for usagainst stored grain insects.

In this study, 10 different Turkish and 4 commercial DE samples were scanned under Scanning Electron Microscope (SEM) to examine their image properties and compare their similarities.

Materials and Methods

Local diatomaceous earth formulations: Ten local diatomaceous earth samples (coded as BCN, BGN, BHN, CAN, CBN, DC, DN, FBN, GBN, NN)collected from different locations of Turkey and four commercial DE samples (namely Protector, Pyrisec, Insecto, Silicosec) were selected for scanning electron microscopy (SEM) analysis. Local diatomaceous earth samples were mostly collected from DE reserves located at middle Anatolia of Turkey and commercial DEs were provided from agricultural market.

Scanning electron microscopy (SEM): A scanning electron microscope (SEM) is a type of electron microscope that produces images of a sample by scanning the surface with a focused beam of electrons. The electrons interact with atoms in the sample, producing various signals that contain information about the sample's surface topography and composition (Anonymous, 2017b). Some physical properties of Turkish diatomaceous earth formulations and commercial diatomaceous earths were examined under Stereozoom electron microscope (SEM) at 5000x to 20000x magnification. Tiny DE rock samples were prepared to withstand the vacuum conditions and high energy beam of electrons, and size fits on the specimen stage. Samples were mounted rigidly to a specimen holder using a conductive adhesive. Afterwards DE samples were scanned under SEM at Namık Kemal University Central Laboratory (NABILTEM).

Results and Disscussion

Alternative methods are being emphasized to reduce the use of insecticides to reduce human exposure and to decrease the development of insecticide resistance. Diatomaceous earths (DEs) are among the most promising alternatives to chemical insecticides and fumigants, because they have low mammalian toxicity, do not break down rapidly, and do not affect grain end-use quality (Korunic et al., 1996).

Diatomaceous earth (DE) is a dust varying in color from white, grey and yellow to red. Dust is formed from fossilized diatoms, single-celled algae of various shapes and sizes which are composed almost entirely of amorphous silicon dioxide. The cell walls of diatoms are known as frustules. Diatom frustules are highly ornamented, forming an amazing range of forms. The shapes of the diatom frustule are species specific. The frustules have a broad variety of delicate, lacy, perforated shapes, including rods, disks, feathers, ladders, needles, and spheres (Raound et al.,1990; Bhishma et al.,2017). Diatoms are usually classified in to two main groups based on the symmetry of their frustules, namely, Centric Diatoms and Pennate Diatoms. Centric Diatoms are radially symmetrical, while Pennate Diatoms are elongated and generally have parallel striae (furrows or rows of holes in the silica) arranged normal to the long axis (Parkinson and Gordon,1992). Commercial DEs such as Protector and Pyrisec have radially symmetrical while Insecto and Silicosec have rod shape cell wall (Figure 1).

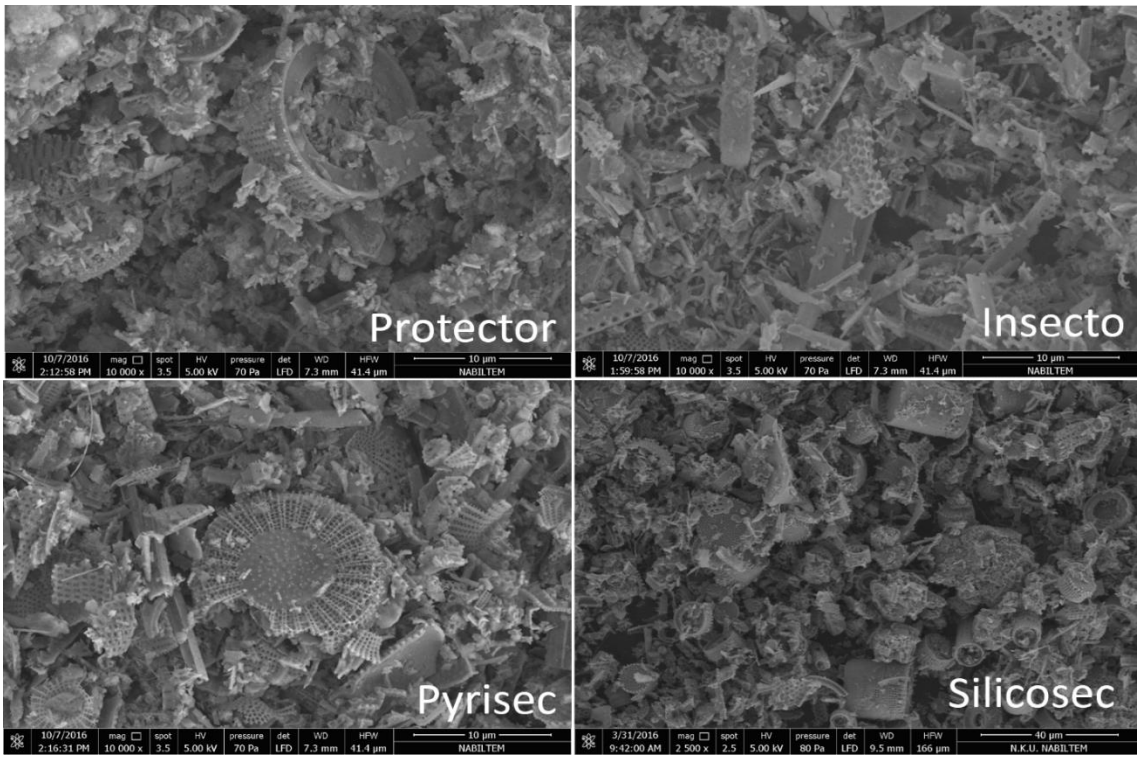

Figure 1. Scanning electron microscope images of fossilized bodies of different shapes of commercial diatomaceous earths.

The dust base of DEs is made of the dead bodies of diatoms in various shapes and size (Korunic,1998) SEM image analysis indicated that there were variations in shape and size of dead bodies of diatoms in Turkish DEs.The shapes of Turkish local DEs coded as CBN and BGN, collected from Middle Anatolia were found very similar to that of commercial DE of Silicosec. Local DE coded as DC has a round shape and looks similar to commercial DE of Pyrisec. Local De coded as CAN has triangle shape and its shape was tottaly different from those of all other DE samples (Figure 2).

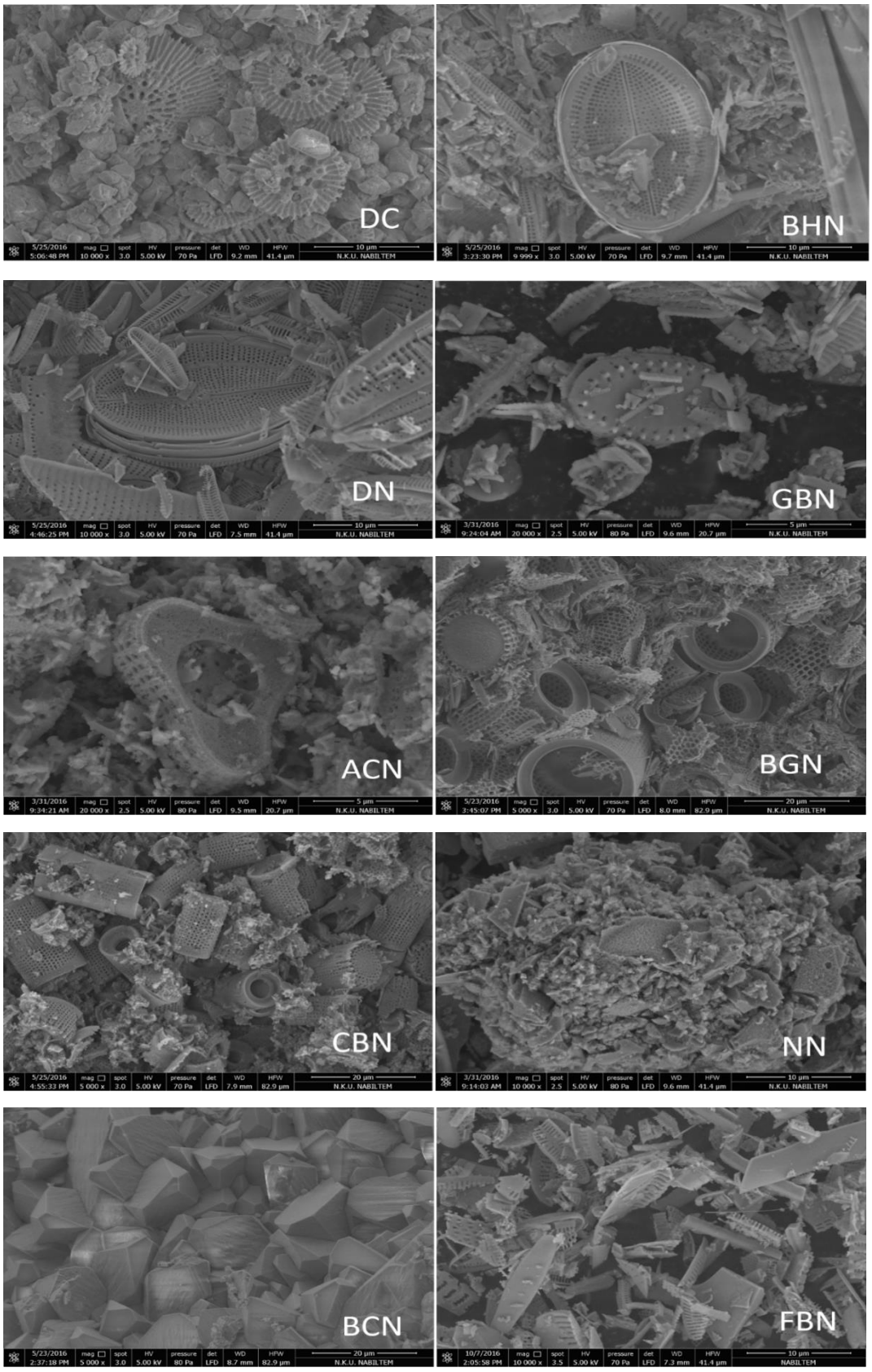

Figure 2. Scanning electron microscope images of fossilized bodies of different shapes of Turkish diatomaceous earths.

In conclusion, this preliminary SEM study indicated there were variations in shape and size of dead bodies of diatoms in Turkish and commercial DEs. While some Turkish DEs had similar shape with commercial DEs, some of them had different shape with commercial ones. Although a few studies have been published (Doğanay et al,2014; Işıkber et al. 2015; 2016; Ertürk, 2014), there is a limited data on efficacy of Turkish diatomaceous earths deposit against stored product pests in the literature. These similarities and differences between Turkish and commercial DEs can provide information on efficacy of Turkish diatomaceous earths deposit against stored product pests. Therefore, further studies are required to clarify interaction or relationship between their shape and size and biological efficacy of DEs against stored product insect pests.

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References

Anonymous, 2017a. Diatomite Mining and Processing,http://diatomite.org/Diatomite-Mining-and-Processing.IDPA, International diatomite producers association. (Accessed date: 05.05.2017).

Anonymous, 2017b. Scanning Electron Microscopy (SEM). https://en.wikipedia.org/wiki/Scanningelectronmicroscope (Accessed date: 05.05.2017).

Athanassiou, C.G., B.J. Vayias, C.B. Dimizas, N.G. Kavallieratos, A.S. Papagregoriou and C.Th. Buchelos, 2005. Insecticidal efficacy of diatomaceous earth against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and *Tribolium confusum* du Val (Coleoptera: Tenebrionidae) on stored wheat: influence of dose rate, temperature and exposure interval. Journal of Stored Products Research, : 41- 47.

Athanassiou, C.G., N.G. Kavallieratos, B.J. Vayias, Z. TomanoviC, A. Petrovic, V. Rozman, C. Adler, Z. Korunic and D. Milovanovic, 2011. Laboratory evaluation of diatomaceous earth deposits mined from several locations in central and southeastern Europe as potential protectants against coleopteran grain pests. Crop protection 30: 329-339.

Bhishma, R.S., S.H. Alavi, S.P. Harimkar, M. McCollum, J.F. Donoghue and F.D. Blum, 2017. Particle morphology dependent superhydrophobicity in treated diatomaceous earth/ polystyrene coatings, Applied Surface Science 416:947–956.

Calvert, R. 1930. Diatomaceous earth Journal of Chemical Education, 7: 28-29.

Çetin, M. and B. Taş, 2012. Biyolojik orjinli tek mineral: Diyatomit. Türk Bilim Araştırma Vakfı (TÜBAV) Bilim Dergisi, 5(2): 28-46.

Parkinson, J. and R. Gordon, 1999. Beyond micromachining: the potential of diatoms. Trends Biotechnology, 17:190–196.

Round, F., R. Crawford and D. Mann, 1990. The Diatoms: Biology and Morphology of the Genera, Cambridge University Press, UK.

Katz, H. 1991. Desiccants: dry as dust means insect's death. Pest Control Technology, 82-84.

Korunic, Z., P.G. Fields, M.I.P. Kovacs, J.S. Noll, O.M. Lukow, C.J. Demianyk and K.J. Shibley, 1996. The effect of diatomaceous earth on grain quality. Postharvest Biology and Technology, 9(3): 373-387.

Korunic, Z. 1997. Rapid assessment of the insecticidal value of diatomaceous earths without conducting bioassays. Journal of Stored Products Research, 33, 219-229.

Korunic, Z. 1998. Diatomaceous earths, a group of natural insecticides. Journal of Stored Products Research, 34: 87–97.

Doğanay, İ.Ş. , A.A. Işıkber, Ö. Sağlam and Y. Bilgili, 2014. Efficiency of some Turkish diatomaceous earth deposits against stored-grain insects, *Sitophilus granarius* (L.) and *Rhyzopertha dominica* (F.). International Conference on Biopesticide (ICOP 7), p.91.

Ertürk, S. 2014. Farklı diyatom toprağı formülasyonlarının depolanmış çeltikte zararlı böceklere etkinliği üzerinde araştırmalar. Ankara Üniversitesi, Fen bilimleri Enstitüsü, Bitki Koruma Anabilim Dalı, Doktora Tezi, s.120.

FDA (Food and Drug Administration, USA), 1995. Specifications for diatomaceous earths as a maximum 2 % animal feed additive. 21 CFR Section, 573.340.

Işıkber, A.A., Ö. Sağlam, M.K. Er and H. Tunaz, 2016. Potential of Turkish diatomaceous earth formulations as natural grain protectants for control of stored grain insects. 15th International Cereal and Bread Congress, p.42.

Işıkber, A.A., Ö. Sağlam, M.K. Er, H. Tunaz and R. Şen, 2015. Toxicity of Turkish diatomaceous earth deposits against some stored-grain insects on wheat. 10th Conference of IOBC/WPRS Working Group "Integrated Protection of Stored Products" Congress, p.29.

McLaughlin, A. 1994. Laboratory trials on desiccant dust insecticides. 6th InternationalWorking Conference on Stored-Product Protection. CAB, Wallingford, Canberra, Australia, pp. 638–645.

Mete, Z. 1998. Kütahya-Alayunt Yöresi Diyatomit Yataklarının Zenginleştirilmesi, Akdeniz Üniversitesi Isparta. Mühendislik Fakültesi Dergisi, 184-201.

Özbey, G. and N. Atamer, 1987. Kizelgur (Diatomit) hakkında bazı bilgiler. 10. Türkiye Madencilik Bilimsel Teknik Kongresi, Ankara, s.493-502.

Round, F., R. Crawford and D. Mann, 1990. The Diatoms: Biology and Morphology of the Genera, Cambridge University Press, UK.

Sıvacı, R. and Ş. Dere, 2006. Melendiz Çayı'nın (Aksaray-Ihlara) Epipelik Diyatome Florasının Mevsimsel Değişimi. C.Ü. Fen-Edebiyat Fakültesi Fen Bilimleri Dergisi, 27(1): 1-12.

Snetsinger, R. 1988. Report on Shellshock insecticide. Report of Department of Entomology, Pennsylvania State University, pp. 1–7.

Subramanyam, B. and R. Roesli, 2000. Inert dust. In Subramanyam Bh, Hagstrum, D.W. (Eds), Alternatives to Pesticides in Stored - Product IPM. Kluwer Academic Publishers, Boston, MA: p.321-379.

Impact of Some Mixtures between Foliar Fertilizers and Combined Herbicides on the Sowing Properties of the Durum Wheat Sowing-Seeds

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The research was conducted during 2010 - 2012 on pellic vertisol soil type. Under investigation was Bulgarian durum wheat cultivar Predel, which belongs to var. valenciae. Factor A included no treated check and 3 foliar fertilizers - Lactofol O - 8 l/ha, Terra-sorb - 3 l/ha, Humustim - 1 l/ha. Factor B included weeded, no treated check and 3 combined herbicides – Axial one (pinoxaden + florasulam) - 1 l/ha, Hussar max OD (mesosulfuron + iodosulfuron) – 1 l/ha, Palace 75 WG (pyroxulam) - 250 g/ha. Because of the low adhesion of the herbicide Palace it was used in addition with adjuvant Dassoil - 500 ml/ha. All of foliar fertilizers, herbicides and their tank-mixtures were treated in tillering stage of the durum wheat and are applied in a working solution of 200 l/ha. Mixing was done in the tank on the sprayer.
Tank mixtures of combined herbicide Axial one with complex foliar fertilizers Lactofol and Terra-sorb decreases germination energy of the durum wheat seeds. Tank mixture Lactofol + Hussar max decreases lab seed germination. Investigated foliar fertilizers, combined herbicides and their tank mixtures increase lengths of primary roots and coleoptile and decrease waste grain quantity. Combination between herbicide Hussar max and foliar fertilizer Lactofol not influences on lengths of primary roots and coleoptile. There is antagonism of combined use by herbicide Hussar max with foliar fertilizers Lactofol and Humustim and by herbicide Palace with foliar fertilizer Lactofol. There is synergism by tank mixtures of herbicide Axial one with the three foliar fertilizers, by tank mixtures of herbicide Palace with foliar fertilizers Lactofol and Humustim, by tank mixtures of herbicide Hussar max with foliar fertilizer Terra-sorb. The highest grain yield is obtained by tank mixture Terra-sorb + Axial one.

Keywords: Durum wheat, foliar fertilizers, herbicides, grain yield, germinative energy, seed germination, roots and coleoptiles length, waste grain

Introduction

Receiving more high-quality production of durum wheat with lower cost and resource consumption, free from residues of fertilizers and pesticides requires continuous improvement of the various units of the technology of growing and connecting them in science-based system (Lalev et al., 2000). During the last years has considerably increased the the number of registered biologically active substances that are used to regulate the growth and development of plants (Rapparini et al. 1987; Radišič et al., 1997; Sharma and Kumar, 1998), the quantity and quality of the obtained production (Wu et al., 1993; Vildflush and Gurban, 1999; Taniguchi et al., 1999). They are used more and in durum wheat to increase the yield and grain quality (Yanev et al., 2008). There are still less researches concerning the effects of mixtures of preparations on seeds.

One of the important conditions for obtaining normal sown fields and a good harvest is the use of quality seeds. Furthermore, highly productive cultivar that has several conditions such as resistance to lodging, diseases and pests, the seeds must have the necessary sowing properties, the main of which are highly germinative energy and seed germination (Panayotov and Stoeva, 2000). Depending on soil and climatic conditions, lodging and seed attack from diseases and pests has been observed to obtain seeds with different germination (Bhaskara et al., 1998). In its determination should be recorded and the time when seeds in a rest after harvest. It varies depending on cultivar and condition in which the seeds were during the harvest.

These studies do not provide enough light to questions about the impact of mixtures between different pesticides on durum wheat.

Considering these achievements, we set the aim of this investigation to establish the influence of some foliar fertilizers, combined herbicides and their tank mixtures on sowing properties of the durum wheat seeds and the quantity of waste grain.

Materials and Methods

The research was conducted during 2010 - 2012 on pellic vertisol soil type. Two-factor field experiment was carried out with durum wheat cultivar Predel (*Triticum durum var. valenciae*). The experiment was conducted under the block method, in 4 repetitions; the size of the crop plot was 15 m^2.

Factor A included no treated check and 3 foliar fertilizers - Lactofol O - 8 l/ha, Terra-sorb - 3 l/ha, Humustim - 1 l/ha. Factor B included weeded, no treated check and 3 combined herbicides – Axial one (pinoxaden + florasulam) - 1 l/ha, Hussar max OD (mesosulfuron + iodosulfuron) – 1 l/ha, Palace 75 WG (pyroxulam) - 250 g/ha.
Because of the low adhesion of the herbicide Palace it was used in addition with adjuvant Dassoil - 500 ml/ha. All of foliar fertilizers, herbicides and their tank-mixtures were treated in tillering stage of the durum wheat and are applied in a working solution of 200 l/ha. Mixing was done in the tank on the sprayer.

Complex fertilizers Lactofol O and Terra-sorb contain nitrogen in amide, ammonium and nitrate forms, easily absorbable phosphorus and potassium, trace elements, amino acids, physiologically active substances, and organic fertilizer Humustim - potassium salts of humic acids and fulvia acids. Both complex foliar fertilizers differ mainly in the nature of the complexing agent – in Lactofol O it is lactic acid, and in Terra-sorb it is ethylene-diamine-tetra-acetic acid (EDTA).

The grain gained after every variant was cleaned through a sieve with holes size 2.2 mm and the quantity of the waste grain was defined (siftings). All version seeds for sowing were defined for their germination energy and lab seed germination. It was studied intensity of early growth of seeds, expressed by the length of primary roots and coleoptile definite on the eighth day after setting the samples. Each index was determined in two repetitions of the year. Averages in each of the years of experience were used as repetitions in mathematical data processing were done according to the method of analysis of variance.

Results and Discussion

One of the important conditions for obtaining a normal crop and a good harvest is the use of quality seeds. Apart from the high-yield cultivar which is resistance to diseases and pests, it must have the necessary sowing properties, the main of which are high germination energy and seed germination. Germination energy is one of the most important characteristics of the sowing properties of the seed. The low germination energy is the reason for slower development of primary roots and coleoptile after seed germination and is associated with later germination in field conditions, less tempering of plants and a higher risk of frost in the winter. Its lead to lower grain yields. The obtained results show that the treatment of the durum wheat with tank mixtures of combined herbicide Axial one with complex foliar fertilizers Lactofol O and Terra-sorb during tillering stage of durum wheat lead to decrease in the germination energy (Table 1). Analysis of variance, in which the years have taken for replications, shows that these decreases are mathematically proven. Combinations of herbicide Axial one with organic foliar fertilizer Humustim do not lead to decrease in the germination energy.

Germination is the most important index who characterizing the sowing properties of the seed. At low laboratory germination sowing should be done with higher sowing rate, which increases the cost production. Laboratory germination of the seeds at all variant during the three years of study above the requirements of the standard for over 85% germination, although in different years account for some variation of its values. This is the positive effect of their use, because it is not necessary to increase the sowing rate (in kg/ha) and the cost of necessary seeds. Tank mixture Lactofol O + Hussar max decreases seed germination. Tank mixture Terra-sorb + Hussar max do not decrease proven seed germination. The durum wheat seeds germinate normally by influence of this tank mixture, although the initial rate of development is lower due to lower germination energy. Foliar fertilizers, combined herbicides and another tank mixtures increase proven the indexes germination energy and seed germination. This means that they help for joint and fast germination of the durum wheat sowing-seeds.

The obtained results for germination energy and seed germination are a prerequisite continue to investigate the effect of stimulators, herbicides and their tank mixtures on initial intensity of the growth of seeds, expressed by the length of roots and coleoptiles. It was found that the length of coleoptiles and length of primary roots of durum wheat have tendency of decrease by combinations between herbicide Hussar max with foliar fertilizer Lactofol O. This tank mixture difficult young plants developments, reduces their resistance to cold and increase risk of frost damages during winter months. Other tank mixtures between investigated foliar fertilizers and combined herbicides stimulate the growth of the length of primary roots and coleoptiles of the durum wheat and recommended for use in seed production crops of durum wheat.

Table 1. Sowing properties of the seeds (mean 2010-2012)

Variants		Germinative energy (%)	Germination (%)	Length (cm)		Waste grain (%)
Foliar fertilizers	Herbicides			Coleoptile	Root	
-	-	73	85	8.02	13.65	14.0
	Axial one	89	90	8.73	14.09	13.2
	Hussar max	82	94	8.47	14.31	13.4
	Palace	82	94	8.63	14.49	13.9
Lactofol O	-	86	96	9.83	15.04	13.8
	Axial one	80	95	9.00	14.47	10.6
	Hussar max	78	79	8.07	13.72	10.9
	Palace	84	85	9.97	14.19	10.7
Terra-sorb	-	88	96	8.50	14.72	11.3
	Axial one	87	97	8.45	14.89	10.4
	Hussar max	78	90	9.68	15.00	10.8
	Palace	83	97	8.62	15.02	10.1
Humustim	-	82	94	9.14	14.61	13.8
	Axial one	83	98	9.12	14.70	10.2
	Hussar max	84	96	8.61	14.03	10.9
	Palace	87	97	8.15	15.30	10.9
	LSD 5%	4.6	4.0	2.6	3.4	3.0
	LSD 1%	6.1	5.5	4.5	5.8	5.8
	LSD 0.1%	7.9	7.4	6.9	7.7	7.8

At the evaluation of the sowing characteristics we have to consider not only the characteristics of the sowing seeds but also the quantity of the waste grain (siftings) which are gained at the preparation of these seeds. Bigger quantity screenings lead to higher cost of the seed and reduce the economic effect of seed production of durum wheat. All tank mixtures of combined herbicides Axial one, Hussar max and Palace with foliar fertilizers Lactofol O, Terra-sorb and Humustim lead to decreasing in the quantity of waste grain. Differences between them and untreated control are mathematically proven.

Decreases in the values of germination energy and laboratory seed germination, changes in the intensity of the initial growth, expressed by the length of the root and coleoptile at germination and changes in the quantity of waste grain under the influence of the combination between foliar fertilizers and combined herbicides are explained by the depressing effects on growth and development of the durum wheat during its vegetative period.

To make a full evaluation of the sowing properties needed to establish not only the quality of seeds, but also the quantity of grain which will be received this seeds. Data for the influence of foliar fertilizers, combined herbicides and their tank mixtures on grain yield (Table 2) show that the lower yield is obtained in untreated control. The alone application of herbicides Axial one, Hussar max and Palace increases grain yield because the weeds are destroyed. The differences are small, due to superior efficacy of the three combined herbicides against grassy and broadleaved weeds. Differences in mean grain yields are from 10 kg/ha to 33 kg/ha.

The alone application of complex foliar fertilizers Lactofol and Terra-sorb and organic foliar fertilizer Humustim also increases grain yields because they stimulate the growth and development of durum wheat. The increases are 3.4 – 3.9 %. The increase by alone application of foliar fertilizers is less than the increase by combined herbicides because present weeds neutralize a part of positive effect.

It is established manifestations of antagonism by combined application of herbicide Hussar max with foliar fertilizers Lactofol and Humustim and by combined application of herbicide Palace with

foliar fertilizer Lactofol. This antagonism leads to a decrease in grain yield in tank mixtures when they compared with alone application of the combined herbicides and fertilizers. Probable cause of antagonism between herbicides Hussar max and Palace by one hand and complex fertilizer Lactofol by other hand is the lactic acid, which is a complexing in this foliar fertilizer. There is not antagonism by tank mixtures of Hussar max and Palace with complex fertilizer Terra-sorb with complexing EDTA. Both complex foliar fertilizers differ mainly in the nature of the complexing agent - in Lactofol it is lactic acid, and in Terra-sorb it is ethylene-diamine-tetra-acetic acid (EDTA).

Table 2. Grain yield (2010-2012)

Variants		2010		2011		2012		Mean	
Foliar fertilizers	Herbicides	kg/ha	%	kg/ha	%	kg/ha	%	kg/ha	%
-	-	4444	100	3943	100	5004	100	4464	100
	Axial one	4603	103.6	4190	106.3	5262	105.2	4685	105.0
	Hussar max	4597	103.4	4180	106.0	5207	104.1	4662	104.4
	Palace	4600	103.5	4133	104.8	5223	104.4	4652	104.2
Lactofol O	-	4587	103.2	4033	102.3	5223	104.4	4614	103.4
	Axial one	4707	105.9	4343	110.1	5405	108.0	4818	107.9
	Hussar max	4377	98.5	4093	103.8	5317	106.3	4596	102.9
	Palace	4730	106.4	3487	88.3	5571	111.3	4600	103.0
Terra-sorb	-	4603	103.6	4007	101.6	5249	104.9	4620	103.5
	Axial one	4770	107.3	4460	113.1	5590	111.7	4934	110.5
	Hussar max	4623	104.0	4363	110.7	5327	106.5	4771	106.9
	Palace	4723	106.3	4444	112.7	5498	109.9	4888	109.5
Humustim	-	4593	103.4	4093	103.8	5222	104.3	4636	103.9
	Axial one	4716	106.1	4373	110.9	5502	110.0	4864	109.0
	Hussar max	4353	98.0	4003	101.5	5299	105.9	4552	101.2
	Palace	4707	105.9	4430	112.4	5557	111.1	4898	109.7
	LSD 5%	110	2.5	170	4.3	164	3.3		
	LSD 1%	148	3.3	229	5.8	221	4.4		
	LSD 0.1%	197	4.4	303	7.7	293	5.9		

There is synergism in 2011 and 2012 by combined application of herbicide Axial one with the three foliar fertilizers, by combined application of herbicide Palace with foliar fertilizers Terra-sorb and Humustim and by combined application of herbicide Hussar max with foliar fertilizer Terra-sorb. Grain yield and herbicidal efficacy by these tank mixtures are higher in comparison with the alone application of the foliar fertilizers and combined herbicides. There is additive effect in 2010 by these tank mixtures. Grain yield is approximately equal to the aggregate effect of individual preparations. The reason for these differences is large differences in the weather during the three years of the investigation.

Conclusions

Tank mixtures of combined herbicide Axial one with complex foliar fertilizers Lactofol and Terra-sorb decreases germination energy of the durum wheat seeds.

Tank mixture Lactofol + Hussar max decreases lab seed germination.

Investigated foliar fertilizers, combined herbicides and their tank mixtures increase lengths of primary roots and coleoptile and decrease waste grain quantity. Combination between herbicide Hussar max and foliar fertilizer Lactofol not influences on lengths of primary roots and coleoptile.

There is antagonism of combined use by herbicide Hussar max with foliar fertilizers Lactofol and Humustim and by herbicide Palace with foliar fertilizer Lactofol.

There is synergism by tank mixtures of herbicide Axial one with the three foliar fertilizers, by tank mixtures of herbicide Palace with foliar fertilizers Lactofol and Humustim, by tank mixtures of herbicide Hussar max with foliar fertilizer Terra-sorb. The highest grain yield is obtained by tank mixture Terra-sorb + Axial one.

References

Bhaskara, M., G. Raghavan, A. Kushilapa and T. Paulitz, 1998. Effect of microwave treatment on quality of wheat seeds infected with Fussarium graminearum. Journal of Agricultural Resources, 71 (2) 333-338.

Lalev, Ts., Gr. Delchev, G. Panayotova, D. Nikolov, I. Saldzhiev, Sh. Yanev and M. Deneva, 2000. Successes of research in area of technology for growing durum wheat. Plant Science, 9 (37) 682-687.

Panayotov, N. and N. Stoeva, 2000. Seed quality and some physiological behaviour in presowing treatment. Progress in Botanical Research, 1-st Balkan Botanical Congress, 345-349.

Radišič, M., D. Stajkovič and K. Kolev, 1997. Influence of natural growth regulator Agrostemine on the wheat seed germination. Casopis za procesnu tehniku i energetiku u poljoprivredi, 1 (1-2) 34-35.

Rapparini, G., D. Benussi and F. Bassi, 1987. Verifica dell`inpiego di fitoregulatori di crescida sui cereali vernini. Informatore Agrario, 43 (5) 29-35.

Sharma, S. and R. Kumar, 1998. Effects of DCD on growth and yield of wheat. Journal of Agricultural Science, 131 (4) 389-394.

Taniguchi, Y., M. Fujita, A. Sasaki, K. Ujihara and M. Ohnushi, 1999. Effect of top dressing of growth regulators at booting stage on crude protein content of wheat in Kyushu district. Japanese Journal of Crop Science, 68(1) 48-53.

Vildflush I. and K. Gurban, 1999. Yield and quality of spring wheat in an integrated application of mineral fertilizers, trace elements and new growth regulators. In: International scientific conference. Minsk, Belarus, 16-19. 02. 1999. Bogdevich, I. M.; Smeyan, N. I.; Ciganov, A. R.; Lapa, V. V.; Shkurinov, P. I.; Citron, G. S.; Levitan, T. V., 84-85.

Wu, Z.L., Y.H. Shi, Z.G. He and Y.I. Li, 1993. Studies grain yields and physiological effects of the plant growth regulator Harmaline on wheat. Acta Agronomica Sinica, 19 (4) 380-383.

Yanev, Sh., D. Dechev, Ts. Lalev, I. Saldzhiev, D. Panajotova, G. Delchev, T. Kolev and S. Rashev, 2008. Technology for the cultivation of durum wheat. "Temko," Stara Zagora.

Effects of Four Different Crops Harvest Processes on Soils Compaction

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The rapid population growth in the developing world in order to meet the food demands, agricultural land is exposed to high traffic in the year. The composed traffic influences negatively the physical properties of the soil. As a result of unconsciously processing the soil; the loss of organic matter increases, the compaction occurs at the top and bottom of soils, the structure breaks down and thereby, soil degradation begins. Because of the degradation, there is a significant decline in productivity. Water storage property of the soil in Konya plain, where it is located in the arid and semi-arid climate zone, has great important. The connection between soil particle array and pore structures is important in terms of soil water holding capacity. In this study, the effects of agricultural machinery on the soils, which are under different cultures (corn, sugar beet, sunflower and wheat), was examined at the pre-harvest and post-harvest stage. Bulk density and soil compaction were measured in the clay soils. The bulk density values showed an alteration between 1.21-1.46. Showed significant differences between pre and post-harvest the bulk density values. In the measurements of penetration resistance values of 0-20 and 20-40 cm, the values measured before harvest were observed to increase after harvest.

Keywords: Penetration resistance, bulk density, Konya plain

Introduction

Productivity should be increased for humans to be able to be nourished balanced and with higher quality. The mechanization operations made in the agricultural lands within the period increase gradually and machines became bigger and heavier (Bennett et al. 2017). Mechanizing the agriculture increased productivity of the crop production. On the other hand, the axle load of farm machines increased over the last decades so much that questions regarding the long-term detrimental effects of soil compaction were revealed. Agricultural production techniques on the industrialized countries within the period of the last decades changed dramatically. Growing economic crops, makes machine power, vehicle weight and implementation size to increase continuously. For example, the rate of tractors bigger than 50 hp which are registered in Turkey increased from 50% to 90% between 1988 and 2015 (TÜİK. 2015). During the recent years, the number of tractors in Turkey increased 32% and became a total of 1,260358. Continuous increase of the heaviness of agricultural machines and the need of using heavy machines in inappropriate ground conditions increased the compaction in soils. Soil compaction was found to have detrimental effects on lots of soil characteristics regarding the soil cultivation, drainage, crop growth and environment. For example, the subsoil compaction blocked the infiltration and increased the runoff and erosion (Fullen. 1985).

The degree of soil compaction depends on soil texture, soil moisture, organic substance content as well as the axle loads, tyre pressure and contact area of the vehicle to the soil (Alakukku et al. 2003; Bygdén et al. 2003; Jansson & Johansson. 1998). The harvesters cause superficial or substratum soil compaction. The highest mechanization operation occurs within the harvest period. The fact that the harvesters are too heavy and their maximum loads show an increase between 5-10 tons with the harvest cause burdens on the lands. As the soil compaction increases, an increase occurs on the bulk density values (Bertrand & Kohnke. 1957; Phillips & Kirkham. 1962). With the increasing bulk density values, the aggregates were broken up and porosity, ventilation and infiltration decreases (Kozlowski. 1999). For this reason, depending on the soil compaction on the lands, soil compaction also affects the nitrogen content and uptake of crops (Bertrand & Kohnke. 1957; Lowery & Schuler. 1991; Phillips & Kirkham. 1962).

In this study, the changes in the physical characteristics of lands under different plant growth were examined as a result of field traffic during the harvest period. The harvesters were compared to determine in which plant pattern the soil compaction affects the soil within the superficial or substratum.

Materials and Methods

The research was carried out in the application farm of Selcuk University agricultural faculty. In this

study made in 2015, samplings and measurements were implemented on the lands where planting of different plant species (corn, sugar beet, sunflower and wheat) were executed. In order to determine some physical and chemical characteristics of the soils, 0-20 cm disturbed soil samples were taken from the lands. In order to determine the pre-harvest and post-harvest situation in the lands, the penetration measurements were made in 2 periods with 10 repetitions from each land. Undisturbed soil samples were also taken with 4 repetitions at the same time with the penetration measurements. Moisture adjusting should be made in order to compare the penetration resistance values measured in different periods (Şeker. 1999). For this reason, the function developed by Busscher and Bauer (2003), was used.

The samples' textures were found according to the Bouyoucos hydrometer method (Gee & Bauder. 1986); the field capacity (FC) was determined using a pressure plate at 33 kPa (FC) pressure (Klute. 1986), as a percentage of moisture retained in the weight of soil; the wilting point (WP), using a pressure plate at 1500 kPa pressure, as the percentage of moisture retained in the weight of soil (Klute. 1986); available water capacity (AWC) was found, by subtracting the PWP value from the FC values. Penetration resistance (PR) measurements were made by using a penetrologger produced by Eijkelkamp, where the device is inserted into the soil by pushing with hands. Bulk density (Pb) was measured by the core sampling method (Blake & Hartge. 1986). Organic matter contents were measured by "Smith-Weldon" (Nelson & Sommers. 1982).

Results and Discussion

Some physical characteristics of the research subject soils are given in Table 1. The soils contain clay between 53.25-58.85 %, silt between 32.70-36.60 % and sand between 8.45-10.32 %. All of the soils take place in the class of clay. The FC and WP values show a change between 33.25-34.67 % and 15.84-17.28 % respectively. The AWC were found to be between 17.36-18.38 %. When the OM content is examined, all of the soils take place in the middle class organic substance (Ülgen & Yurtsever. 1974). The soil characteristics are observed to be compatible with the previous studies.

Table 1. Soil physical and chemical properties at different plants samples.

Soil properties		Sunflower	Corn	Sugar beet	Wheat
Sand (%)		10.15	8.88	8.45	10.32
Clay (%)		53.25	55.35	58.85	53.85
Silt (%)		36.60	35.77	32.70	35.83
Texture class		Clay	Clay	Clay	Clay
FC (g g^{-1})		34.10	34.65	33.25	34.67
WP (g g^{-1})		15.84	17.28	15.89	16.29
AWC (g g^{-1})		18.26	17.37	17.36	18.38
OM (%)		2.36	2.74	2.95	2.55
Moisture (%)	0-10 cm	12.44	9.74	18.39	9.17
	10-20 cm	15.22	11.87	20.55	13.14
	20-30 cm	17.35	12.19	20.85	15.49

FC: field capacity; WP: wilting point; AWC: available water capacity; OM: organic matter

Pre-harvest and post-harvest Pb values within the soils of study area are given in Table 2. The comparisons in each plant species were made in the pre-harvest and post-harvest same depths. When the sunflower plant is examined, no differences are observed to occur cyclically in the depths. Since the plant was left to dry in the sunflower harvest, the mechanization operation for the harvest was carried out on dry soil and did not generate differences statistically on Pb values ($P>0,05$). When the Pb values of corn were examined and pre-harvest and post-harvest values in the same depths were compared, an increase was observed on the post-harvest values. Significant differences occurred statistically in all of the values ($P<0.01$). Even though the land conditions in the corn harvest were dry, this increase is considered to occur from the high-weight harvesters. The weight of harvester used is 14400 kg and its carrying capacity is 7600 liters. When the Pb values of the sugar beet plant were examined, statistically significant differences were found between the values ($P<0.01$). When the Pb values were examined, the highest increase was

observed to occur in the sugar beet agriculture. When all of the depths were examined, an average increase of 13% was observed to occur. The increase of Pb value in the sugar beet agriculture was originated from the harvest works made in high moisture content. When the wheat was examined, no significant differences were observed statistically in 0-10 and 10-20 cm within the Pb values ($p>0.05$) while statistically significant difference was found in 20-30 cm ($p<0.01$).

Table 2. The values of bulk density before and after harvesting of different plants.

Crop pattern / Period	Deep (cm) 0-10 BH (g cm^{-3})	0-10 AH (g cm^{-3})	10-20 BH (g cm^{-3})	10-20 AH (g cm^{-3})	20-30 BH (g cm^{-3})	20-30 AH (g cm^{-3})
Sunflower	1.14±0.01	1.17±0.03	1.20±0.03	1.21±0.02	1.29±0.05	1.34±0.05
Corn	1.15±0.02^{b}	1.26±0.02^{a}	1.16±0.02^{b}	1.30±0.02^{a}	1.24±0.04^{b}	1.34±1.02^{a}
Sugar beet	1.17±0.02^{b}	1.31±0.04^{a}	1.22±0.01^{b}	1.38±0.02^{a}	1.24±0.01^{b}	1.42±0.03^{a}
Wheat	1.14±0.02	1.15±0.10	1.19±0.01	1.21±0.04	1.24±0.03^{b}	1.34±0.05^{a}

BH: before harvesting; AH: after harvesting. ^{a,b}: $P<0.01$.

The measurements of PR are usually being used as an indicator of the density of soil cultivation and field traffic (Salem et al. 2015; Soane & Van Ouwerkerk. 1994; Van Ouwerkerk & Soane. 1994). The values regarding the penetration measurements are given in Table 3. The comparison of the PR in the plant species was carried out in 3 different depths to examine the effect the post-harvest values make to the depths. When the penetration values of the sunflower plant were examined, it was not found statistically significant among the PR in 0-10 and 10-20 cm depth statistically ($P>0,05$). Its PR values have a positive relationship with its Pb values. When its Pb values were examined, no increases were observed in the 0-10 and 10-20 cm values. When its 20-40 cm PR values were examined, post-harvest values were observed to increase in comparison with pre-harvest values ($P<0.01$). When the PR values of corn, sugar beet and wheat samples were examined, statistically significant results were obtained in 0-10, 10-20 and 20-40 cm ($P<0,01$). In each 3 plant varieties, pre-harvest values were observed to be lower than post-harvest values. The PR values of wheat in the pre-harvest values were observed to be smaller than >3MPa, which is the value that will limit the plant root development. However, pre-harvest values for the sugar beet and the corn plants were observed to be bigger than >3 MPa in the 20-40 cm depth (Gugino et al., 2009). The fact that the field traffic which occurs in the development period of both plant species has effects on the PR is also indicated in the previous study (Negiş, 2014). Picture 1 is constituted for the PR values to be examined in terms of the soil profile in 0-40 cm. As is also understood from this, an increase is observed in the post-harvest penetration values in all of the plant samples.

Table 3. The values of penetration resistance before and after harvesting of different plants.

BH: before harvesting; AH: after harvesting; MPa: megapascal. a,b: $P<0.01$

Crop pattern / Period	Deep (cm) 0-10 BH (MPa)	0-10 AH (MPa)	10-20 BH (MPa)	10-20 AH (MPa)	20-40 BH (MPa)	20-40 AH (MPa)
Sunflower	1,40±0,55	1,98±0,61	2,71±0,37	2,96±0,22	3,71±0,43^{b}	4,26±0,42^{a}
Corn	0,94±0,53^{b}	2,31±0,94^{a}	2,60±0,22^{b}	3,60±0,22^{a}	3,95±0,56^{b}	4,43±0,45^{a}
Sugar beet	0,99±0,34^{b}	2,27±0,81^{a}	2,12±0,09^{b}	3,27±0,34^{a}	2,99±0,40^{b}	4,47±0,39^{a}
Wheat	0,85±0,37^{b}	2,15±0,45^{a}	1,88±0,17^{b}	2,56±0,07^{a}	2,24±0,11^{b}	2,86±0,08^{a}

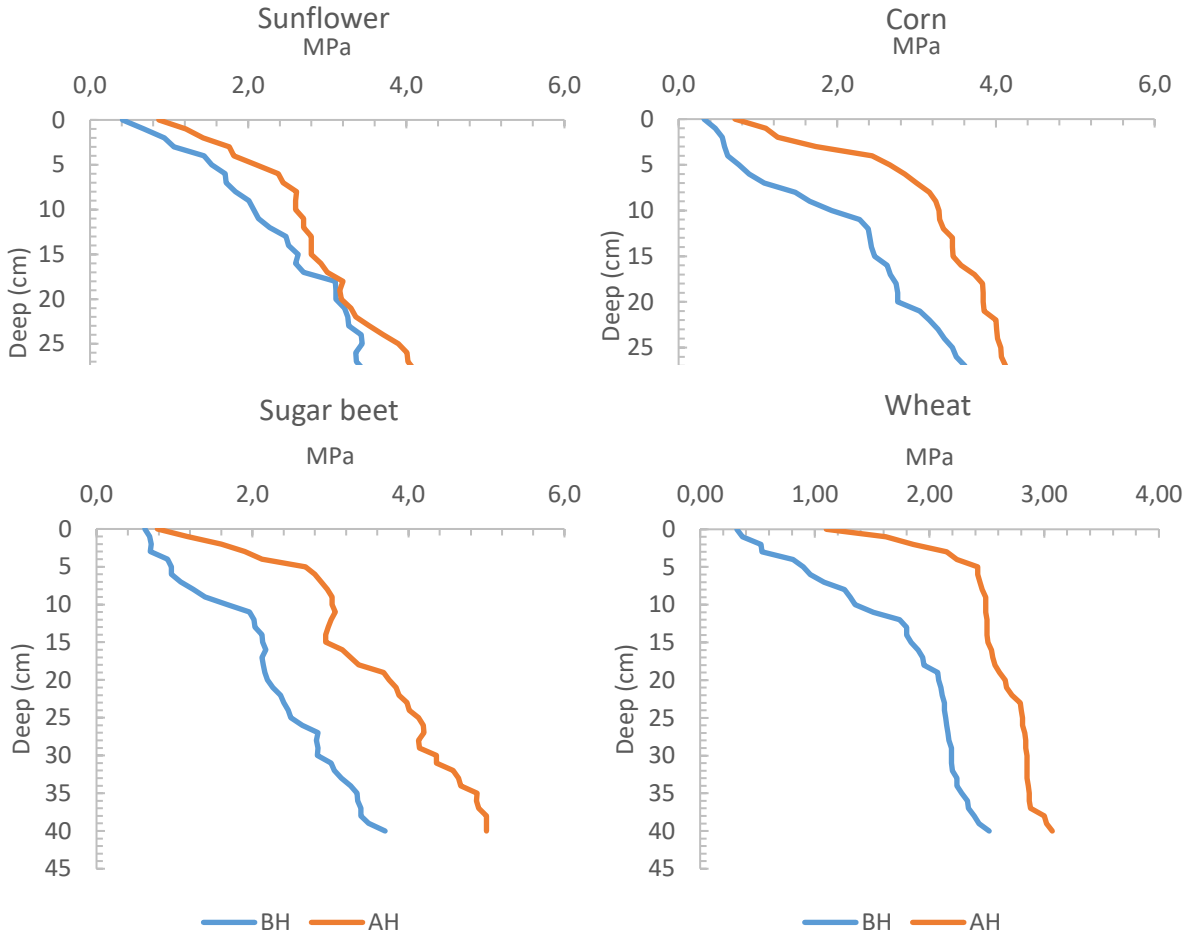

Figure 1. Before and after harvest under four different crops 0-40 cm depth penetration measurements

Conclusions

This study provided the assessment of effect of the compaction formed as a result of harvesting different plant species on the soil. In the post-harvest measurements, the harvesters were observed to leave the lands under too heavy loads. Our findings revealed that the physical soil characteristics were greatly influenced following the field traffic. The wetness of the soils decreases the carrying capacity of the soil.

The most serious sources for soil compaction are drives on heavy wheel loads implemented in soft ground conditions with high ground contact pressure. Machines and equipment's used in the field in critical conditions should be used after controlling their wheel loads and with low tire inflation pressures.

References

Alakukku, L., P. Weisskopf, W. Chamen, F. Tijink, J. Van der Linden, S. Pires, C. Sommer and G. Spoor, 2003. Prevention strategies for field traffic-induced subsoil compaction: a review: Part 1. Machine/soil interactions. Soil & Tillage Research, 73(1): 145-160.

Bennett, J.M., S.D. Roberton, T.A. Jensen, D.L. Antille and J. Hall, 2017. A comparative study of conventional and controlled traffic in irrigated cotton: I. Heavy machinery impact on the soil resource. Soil & Tillage Research, 168, 143-154.

Bertrand, A. and H. Kohnke, 1957. Subsoil conditions and their effects on oxygen supply and the growth of corn roots. Soil Science Society of America Journal, 21(2): 135-140.

Blake, G. and K. Hartge, 1986. Bulk density. Methods of Soil Analysis: Part, 1, 363-375.

Busscher, W. and P. Bauer, 2003. Soil strength, cotton root growth and lint yield in a southeastern USA coastal loamy sand. Soil and Tillage Research, 74(2): 151-159.

Bygdén, G., L. Eliasson and I. Wästerlund, 2003. Rut depth, soil compaction and rolling resistance when using bogie tracks. Journal of Terramechanics, 40(3): 179-190.

Fullen, M.A. 1985. Compaction, hydrological processes and soil erosion on loamy sands in east Shropshire, England. Soil and Tillage Research, 6(1): 17-29.

Gee, G.W. and J.W. Bauder, 1986. Particle-size analysis. Methods of soil analysis: Part 1—Physical and mineralogical methods(methodsofsoilan1), 383-411.

Gugino, B.K., G.S. Abawi, O.J. Idowu, R.R. Schindelbeck, L.L. Smith, J.E. Thies, D.W.Wolfe and H.M. Van Es, 2009. Cornell soil health assessment training manual: Cornell University College of Agriculture and Life Sciences.

Jansson, K. and J. Johansson, 1998. Soil changes after traffic with a tracked and a wheeled forest machine: a case study on a silt loam in Sweden. Forestry, 71(1): 57-66.

Klute, A. 1986. Water retention: laboratory methods. Methods of soil analysis: Part 1—Physical and mineralogical methods(methodsofsoilan1), 635-662.

Kozlowski, T. 1999. Soil compaction and growth of woody plants. Scandinavian Journal of Forest Research, 14(6): 596-619.

Lowery, B. and R. Schuler, 1991. Temporal effects of subsoil compaction on soil strength and plant growth. Soil Science Society of America Journal, 55(1): 216-223.

Negiş, H. 2014. Şeker pancarı tarımında dönemsel toprak sıkışmasının belirlenmesi. Selçuk Üniversitesi Fen Bilimleri Enstitüsü.

Nelson, D. and L.E. Sommers, 1982. Total carbon, organic carbon, and organic matter. Methods of soil analysis. Part 2. Chemical and microbiological properties (methodsofsoilan2), 539-579.

Phillips, R. and D. Kirkham, 1962. Soil compaction in the field and corn growth. Agronomy journal, 54(1): 29-34.

Salem, H.M., C. Valero, M.Á. Muñoz, M.G. Rodríguez and L.L. Silva, 2015. Short-term effects of four tillage practices on soil physical properties, soil water potential, and maize yield. Geoderma, 237, 60-70.

Soane, B. and C. Van Ouwerkerk, 1994. Soil compaction problems in world agriculture. Soil compaction in crop production, 11, 1-2.

Şeker, C. 1999. Farklı toprakların penetrasyon dirençleri üzerine su içeriklerinin etkisi ve regresyon modelleri. Tr. J. of Agriculture and Forestry, 23, 467-471.

TÜİK, 2015. Tarım Alet ve Makine Sayıları. Türkiye İstatistik Kurumu Haber Bülteni. Retrieved from www.tuik.gov.tr/PreIstatistikTablo.do?istab_id=305

Ülgen, N. and N. Yurtsever, 1974. Türkiye gübreler ve gübreleme rehberi. Toprak ve Gübre Araştırma Enstitüsü Müdürlüğü, Teknik Yayınlar (28).

Van Ouwerkerk, C. and B. Soane, 1994. Conclusions and recommendations for further research on soil compaction in crop production. Soil compaction in crop production (Ed. Soane, BD-Van Ouwerkerk, C.), Elsevier Sci, 627-642.

Choise of Explant Material and Media for *in vitro* Callus Regeneration in Sultana Grape Cultivar *(Vitis vinifera* L.*)*

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The study was carried out to choise of explant material and culture media for callus regeneration in 'Sultana' (*Vitis vinifera* L.). Leaf disc and node explants were the main explant materials which were taken from *in vitro* shoots which were obtained from macroshoot tip explants. The initial macroshoot tip were cultured on MS (Murashige and Skoog) medium including BAP (6-benzylaminopurine) (1 mg L^{-1}) and *in vitro* shoots were subcultured on shoot multiplication medium with BAP (1 mg L^{-1}) + IBA (Indole-3-butyric acid) (0.1 mg L^{-1}) In order to investigate callus regeneration potential of Sultana grape cultivar, leaf disc explants were cultured on two different MS medium including BAP in combination with 2,4-D (2,4-dichlorophenoxyacetic acid) whereas node explants cultured on four different MS medium including BAP in combination with 2,4-D and NAA (naphthalene acetic acid). The intensity of callus proliferation was greater in leaf disc culture than in node culture. In all media combinations, MS medium including BAP (1 mg L^{-1}) + 2,4-D (0.1 mg L^{-1}) were found to be the most effective on callus regeneration. In this medium, callus regeneration rate was found to be 100% and the average diameter of callus was found to be 6.3 mm.

Keywords: 'Sultana' (*Vitis vinifera* L.), tissue culture, callus, leaf disc, node

Sultani Çekirdeksiz Üzüm Çeşidinde (*Vitis vinifera* L.) *in vitro* Kallus Rejenerasyonunda Eksplant ve Ortam Seçimi Üzerinde Araştırmalar

Bu çalışmada Sultani Çekirdeksiz üzüm çeşidinde başarılı bir kallus rejenerasyonu sağlamak için eksplant materyali ve kültür ortamı seçimi üzerinde çalışılmıştır. Ana eksplant materyalleri olarak, *in vitro* makro sürgün ucu materyallerinin *in vitro* sürgünlerinden elde edilen, yaprak diskleri ve boğum parçaları kullanılmıştır. Başlangıçta, makro sürgün ucu 1 mg L^{-1} BAP (6-benzylaminopurine) içeren MS (Murashige ve Skoog) besin ortamına kültüre alınmıştır. Bunun ardından *in vitro* sürgünler BAP (1 mg L^{-1}) + IBA (Indole-3-butyric acid) (0.1 mg L^{-1}) içeren sürgün çoğaltma besin ortamına alt kültüre aktarılmıştır. Sultani Çekirdeksiz üzüm çeşidinde kallus rejenerasyon potansiyelini araştırmak için; yaprak diskleri 2,4-D (2,4-dichlorophenoxyacetic acid) ile BAP kombinasyonlarını içeren iki farklı MS ortamında, boğum parçaları ise 2,4-D ve NAA (naphthalene acetic acid) ile kombinasyon halinde BAP içeren dört farklı MS ortamında kültüre alınmıştır. Kallus çoğaltım oranı, yaprak disk eksplantında boğum eksplantına göre daha yüksek oranda gerçekleşmiştir. Tüm uygulama kombinasyonlarının içinde kallus rejenerasyonunda en etkili sonuç BAP (1 mg L^{-1}) + 2,4-D (0.1 mg L^{-1}) içeren MS ortamından elde edilmiştir. Bu ortamda ortalama kallus çapı 6.3 mm ve kallus rejenerasyon oranı ise 100% oranında bulunmuştur.

Anahtar Kelimeler: 'Sultani Çekirdeksiz' **(***Vitis vinifera* L.), doku kültürü, kallus, yaprak disk, boğum

Introduction

Grapevine has been the subject of callus culture studies aimed at obtaining the best callus production for somatic embryogenesis, genetic transformation and cell suspension culture. Callus culture based procedures also represent opportunities for breeding studies. In the literature, different organs such as anther (Perl *et al*. 1995; Nakajima *et al*. 2000; Martinelli *et al*. 2001; Lopez-Perez et al. 2005; Cutanda *et al*. 2008; Zhang et al. 2009), immature ovule (Xu et al. 2005), immature inflorescences (Lopez-Perez *et al*. 2005; Cutanda *et al*. 2008; Acanda *et al*. 2013), ovary (Lopez-Perez *et al*. 2005), leaf (Passos *et al*. 1999; Keskin and Kunter 2007-2008), leaf petiole (Tassoni *et al*. 2005), nodal and stem segments (Jaskani *et al*. 2008; Chao *et al*. 2015), tendrils (Salunkhe *et al*. 1997) were used for callus regeneration. However differences in the rate of callus initiation and proliferation intensity have been reported depending on the genotype, explant type and culture media used. (Lopez-Perez *et al*. 2005, Jaskani *et al*. 2008; Diab *et al*., 2011; Khan *et*

al. 2015). Because there is not a general protocol for grapevine callus culture, improvements are needed in cultivar specific base.

'Sultana' (*Vitis vinifera* L.) is the well-known stenospermocarpic grapevine genotype. It is used as either seedless table or dried grape. Also 'Sultana' is the progenitor of seedless hybrids. Therefore there is a particular interest in the area of *in vitro* researches in 'Sultana'. On the other little is known about callus proliferation in local research area. The aim of this study was to choice of explant material and culture media led to *in vitro* regeneration of callus in *Vitis vinifera* L. cv. Sultana.

Materials and Methods

Plant material

The study was carried on *Vitis vinifera* L. cv. Sultana, the well-known ancestral genotype of seedless table grapes. The initial materials were one year old dormant cuttings of *Vitis vinifera* L. cv. Sultana which were obtained from Kalecik Viticultural Research Station of Faculty of Agriculture, Ankara University. Two or three budded dormant canes were cultured to initiate shoot proliferation. Buds were forced to burst in controlled growth cabinet at 24 °C. After shoots appeared macroshoot tips were used as explant material. Explants (macroshoot tip) were washed in tap water during 120 min, followed by immersion in 5 and 10% (v/v) actijen supplemented with a few drops of Tween 20 for 5 and 10 min, and rinsed three times with sterile distilled water for 5 min each.

Shoot regeneration and multiplication

After sterilization macroshoot tip were excised from shoot tip then placed onto shoot regeneration medium individually in 32 x 150 mm culture tubes with 20 ml shoot regeneration medium containing of MS medium (Murashige and Skoog, 1962) with 1 mg L^{-1} BAP and 30 g L^{-1} sucrose. pH was adjusted to (5.6-5.8) before sterilization at 121 °C and 102.97 kPa for 20 min. Plant growth regulators (BAP, IBA, NAA, 2,4-D) added to the nutrient medium were sterilized using a filter syringe, 0.2 µm in size, in a laminar flow hood. For regeneration, the culture tubes were incubated in culture room at 24±1 ºC with 16h photoperiod for 4 weeks. After 3-4 weeks of establishment of *in vitro* shoots regeneration, the healthy shoots were transferred on shoot multiplication MS medium supplemented with BAP (1 mg L^{-1}) + IBA (0.1 mg L^{-1}). Cultures were incubated at 24±1 °C with a 16-h photoperiod under 2.000-2.500 lux light intensity provided by cool-white fluorescent. Cultures were evaluated after four weeks of culture and observed on a weekly basis for necrosis, bacterial and fungal contamination, and explant survival rate.

Callus regeneration

After subculture, callus cultures were formed with leaf disc and node explants from the 'Sultana' (*Vitis vinifera* L.). Callus was initiated in 15 x 90 mm petri dishes containing 20 ml of MS medium supplemented with different concentrations and combinations of plant growth regulators. Callus incubated in dark at 24±1 °C were subcultured twice with 4-6 weeks. In order to investigate callus regeneration potential of 'Sultana', leaf disc explants were cultured on two different MS medium including BAP in combination with 2,4-D, whereas node explants cultured on four different MS medium including BAP in combination with 2,4-D and NAA.

Statistical analysis

All analyses were performed in 3 replications. All data were subjected to analysis by two-way analysis of variance (ANOVA) of SPSS Version16.0 (Snedecor and Cochran, 1980).

Results and Discussion

Callus regeneration was attempted by used various initial explants on different basal nutrient media and PGR's *in vitro*. In the present study, the optimum conditions for callus culture was determined by applying two different explant type (leaf disc and node) and three different callus culture medias. In first application, leaf disc explants were cultured in MS medium including 5, 10 and 15 µM NAA. After 30 days, no callus formation was found in either medium type (Table 1). The findings of Jaskani *et al*. (2008) have shown percentage of callus induction rate differs to explant type and the media used for culture. In their study, callus induction rate derived from node explant was found to be 70% and 80% in media containing 5 and 10 µM NAA respectively whereas 40% and 50% for leaf disc explants.

In the second application, leaf disc explants were planted in MS medium modified by Babalık and Baydar (2008) containing BAP (0,5 mg L^{-1}) + 2,4-D (1 mg L^{-1}) and BAP (1 mg L^{-1}) + 2,4-D (2 mg L^{-1}). Callus culture was incubated under dark conditions at 24 °C and no callus formation was observed 15

days later. Callus regeneration rate (57%) was observed on MS medium supplemented with 2,4-D (2 mg L^{-1}) + BAP (0.3 mg L^{-1}) + NAA (0.2 mg L^{-1}) derived from with node explant under dark conditions (Table 2). In contrast to our results Babalık and Baydar (2008), were found higher callus regeneration rate from stem and leaf stalk explants (62.50% and 100%, respectively) under dark conditions on this medium 2,4-D (2 mg L^{-1}) + BAP (0.3 mg L^{-1}) + NAA (0.2 mg L^{-1}). Babalık and Baydar (2008) also reported to the best callus regeneration (97.56%) was obtained in the MS medium supplemented with BAP (1 mg L^{-1}) + 2,4-D (2 mg L^{-1}) + casein hydrolysate (1 g L^{-1}) derived from leaf stalk explants for 'Kalecik Karası' (*Vitis vinifera* L.) under dark conditions. Passos *et al*. (1999) described to found to rate of callus regeneration (100%) derived from leaf disc explants in the MS medium supplemented with NAA (4 mg L^{-1}) and TDZ (0.9 mg L^{-1}) for 'Seyve Villard 5276' (*Vitis* sp.).

In the third application, callus culture was established using leaf and node explants in two different MS medium including BAP (1 mg L^{-1}) + 2,4-D (0.1 mg L^{-1}) modified by Keskin and Kunter (2007) and 2,4-D (2 mg L^{-1})+ BAP (0.3 mg L^{-1}) + NAA (0.2 mg L^{-1}) (Khan *et al*., 2015) and 2,4-D (4 mg L^{-1}) + BAP (0.6 mg L^{-1}) + NAA (0.4 mg L^{-1}). In the third application, callus regeneration rates were %100, %70 and %93, respectively in 'Sultana' (*Vitis vinifera* L.) (Table 2). Callus regeneration rate (70%) was found in the MS medium including 2,4-D (2 mg L^{-1}) + BAP (0.3 mg L^{-1}) + NAA (0.2 mg L^{-1}) derived from with node explant (Table 2). Results of this study were similar with the findings of Khan *et al*. (2015), whose also observed callus regeneration rate at 73% on MS medium including 2,4-D (2 mg L^{-1}) + BAP (0.3 mg L^{-1}) + NAA (0.2 mg L^{-1}).

In our study, among media combinations, the most effective callus texture was found in the MS medium including BAP (1 mg L^{-1}) + 2,4-D (0.1 mg L^{-1}) from node explant. Also callus size was larger than the other MS medium. Callus induction was initiated in the 4^{th}-6^{th}. weeks of culture and average callus size was 6.3 mm in this medium. Calluses derived from node explant type were green or white and friable whereas those derived from leaf disc explant type were became brown and necrotic after subcultures. Best callus regeneration response (93%) derived from with leaf disc explant was observed callus cultured on MS medium supplemented with BAP (0.5 mg L^{-1}) + 2,4-D (1 mg L^{-1}) (Table 1). Therefore the quality of regenerated callus materials could be used for cell culture.

Table 1. Effect of different concentrations of various PGRs on leaf disc explant on callus regeneration

Growth regulator combinations	**Number of explants cultured****	**Number of calluses induced****	**Frequency (%) of callus regeneration****
5 mg L^{-1} NAA	20	no callus formation	0.00
10 mg L^{-1} NAA	20	no callus formation	0.00
15 mg L^{-1} NAA	20	no callus formation	0.00
0,5 mg L^{-1} BAP + 1 mg L^{-1} 2,4-D	14	13 a	93.00 a
1 mg L^{-1} BAP + 2 mg L^{-1} 2,4-D	14	8 b	57.00 b
F			no results (*p*=0,000)

** p<0.01, Data represent mean of 3 repeats.

Table 2. Effect of different concentrations of various PGRs on node explant on callus regeneration

Growth regulator combinations	**Number of explants** cultured**	**Number of calluses** induced**	**Frequency (%) of callus** regeneration**
1 mg L^{-1} BAP + 2 mg L^{-1} 2,4-D	15	14	93.00 b
1 mg L^{-1} BAP + 0,1 mg L^{-1} 2,4-D	33	33	100.00 b
2 mg L^{-1} 2,4-D + 0,3 mg L^{-1} BAP + 0,2 mg L^{-1} NAA	33	23	70.00 a
4 mg L^{-1} 2,4-D + 0,6 mg L^{-1} BAP + 0,4 mg L^{-1} NAA	98	93	97.00 b
F			69,790 (*p*=0,000)

**p<0.01, Data represent mean of 3 repeats.

Conclusions

The findings obtained from this study and discussions of these findings with the results of the previous literature were supported that callus regeneration ratio and quality depend on the explant material and media. In this study, node explant was found to be the best explant type for callus regeneration. Callus regeneration rate of node explant has been 100% on the MS medium including BAP (1 mg L^{-1}) + 2,4-D (0.1 mg L^{-1}). As the result node explant regenerated on MS medium including BAP (1 mg L^{-1}) + 2,4-D (0.1 mg L^{-1}) could be recommended for callus regeneration study in Sultana grape cv.

References

Acanda, Y., M.J. Prado, M.V. González, and M. Rey, 2013. Somatic embryogenesis from stamen filaments in grapevine (*Vitis vinifera* L. cv. Mencía): changes in ploidy level and nuclear DNA content. In Vitro Cellular & Developmental Biology-Plant, 49(3):276-284.

Babalık, Z and N. Göktürk Baydar, 2008. Asmada (*Vitis vinifera* L.) Gövde ve Yaprak Sapı Eksplantlarından Adventif Sürgün Oluşumu Üzerine Bir Araştırma. Medıterranean Agrıcultural Scıences, 21(2):231-240.

Chao, Y., J.C. Feng, W. Yan, Y.A.N.G Xiao, Y.Y. Jun and J. Jun., 2015. Effects of exogenous growth regulators on cell suspension culture of "Yin-hong" grape (*Vitis vinifera* L.) and establishment of the optimum medium. Pak J. Bot, 47:77-81.

Cutanda, M.C., A. Bouquet, P. Chatelet, G. Lopez, O. Botella, F.J. Montero and L Torregrosa. 2008. Somatic embryogenesis and plant regeneration of *Vitis vinifera* cultivars' Macabeo'and'Tempranillo'. VITIS-Journal of Grapevine Research, 47(3):159.

Diab, A.A., S.M. Khalil and R.M. Ismail, 2011. Regeneration and micropropagation of grapevine (*Vitis vinifera* L.) through shoot tips and axillary buds. IJABR, 2(4):484-491.

Jaskani, M.J., H. Abbas, R. Sultana, M.M. Khan, M. Qasim and I.A. Khan, 2008. Effect of growth hormones on micropropagation of *Vitis vinifera* L. cv. Perlette. Pakistan J. Bot., 40:105-109.

Keskin, N and B. Kunter, 2007. Induction of Resveratrol via UV Irradiation Effect in Ercis Callus Culture. Journal of Agricultural Science, 13(4):379-384.

Keskin, N and B. Kunter, 2008. Production of *trans*-resveratrol in 'Cabernet Sauvignon' (*Vitis vinifera* L.) callus culture in response to ultraviolet-C irradiation. Vitis, 47(4):193-196.

Khan N., M. Ahmed, I. Hafız, N. Abbası, S. Ejaz, and M. Anjum, 2015. Optimizing the concentrations of plant growth regulators for in vitro shoot cultures, callus induction and shoot regeneration from calluses of grapes. OENO One, 49(1):37-45.

López-Pérez, A.J., J. Carreño, A. Martinez-Cutillas and M. Dabauza, 2005. High embryogenic ability and plant regeneration of table grapevine cultivars (*Vitis vinifera* L.) induced by activated charcoal. VITIS-Journal of Grapevine Research, 44(2):79.

Martinelli, L., I. Gribaudo, D. Bertoldi, E. Candioli, and V. Polettı, 2001. High efficiency somatic

embryogenesis and plant germination in grapevine cultivars Chardonnay and Brachetto a grappolo lungo. Vitis, 40:111-115.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol Plant, 15(3):473-497.

Nakajima, I., S. Kobayashi and Y. Nakamura, 2000. Embryogenic callus induction and plant regeneration from unfertilized ovule of 'Kyoho' grape. Journal of the Japanese Society for Horticultural Science, 69(2):186-188.

Passos, I.R.S., B. Appezzato-Da-Glória and M.L.C. Vieira, 1999. Embryogenic responses of *Vitis* spp.: Effects of genotype and polyvinylpyrrolidone. Vitis, 38:47-50.

Perl, A., S. Saad, N. Sahar and D. Holland, 1995. Establishment of long-term embryogenic cultures of seedless *Vitis vinifera* cultivars – a synergistic effect of auxins and the role of abscisic acid. Plant Science., 104:193-200.

Salunkhe, C.K., P.S. Rao and M. Mhatre, 1997. Induction of somatic embryogenesis and plantlets in tendrils of *Vitis vinifera* L. Plant cell reports, 17(1):65-67.

Snedecor, G.W. and W.G. Cochran, 1980. Statistical Methods. Seventh Edition. The Iowa State University Press.

Tassoni, A., S. Fornalè, M. Franceschetti, F. Musiani, A.J. Michael, B. Perry and N. Bagni, 2005. Jasmonates and Na-orthovanadate promote resveratrol production in *Vitis vinifera* cv. Barbera cell cultures. New Phytologist, 166(3):895-905.

Xu, X., J. Lu, Z. Ren, H. Wang and S. Leong, 2005. Callus induction and somatic embryogenesis in muscadine and seedless bunch grapes (*Vitis*) from immature ovule culture. In Proc. Fla. State Hort. Soc , 118:260-262.

Zhang, J., H. Ma, S. Chen, M. Ji, A. Perl, L. Kovacs and S. Chen, 2009. Stress response proteins' differential expression in embryogenic and non-embryogenic callus of *Vitis vinifera* L. cv. Cabernet Sauvignon—a proteomic approach. Plant Science, 177(2):103-113.

Polymorphism in Exon 7 of β- Lactoglobulin (*β-LG*) Gene and Its Association with Milk Yield in Saanen Goats

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β-Lactoglobulin (*β-LG)* is one of milk protein and has important function on technological properties of milk such as cheese making. The relations between whey protein genes and milk yield/ composition have been investigated in previous researches. *β-LG* can be utilized as a candidate gene for selection and breeding programs to increase milk yield and protein quality. The aim of this study is to investigate the *β-LG* gene polymorphism and relation between *β-LG* genotypes and milk yield. In this study, a total of 74 purebred Saanen goats originated from Australia were used to detect polymorphism with PCR-RFLP method. *SacII* digestion in 427 bp of *β-LG* exon 7 (GenBank: Z33881.1) was revealed. Two alleles (S_1, S_2) and 3 genotypes (S_1S_1, S_1S_2, S_2S_2) were determined in *β-LG*/ *SacII* locus. The *β-LG*/S_1S_1 genotype with only one restriction site revealed two bands (347 bp and 80 bp). The *β-LG*/S_1S_2 heterozygote genotype generated three bands (427 bp, 347 bp and 80 bp). An undigested product, 427 bp was β-LG/S_2S_2 genotype which was due to a single nucleotide substitution at position g.4601G>A. S_1S_2 with genotype frequency (43.3%) higher than the other genotypes. S_1 allele frequency was determined predominantly. Deviation from Hardy-Weinberg equilibrium was not identified in the Saanen breed. In *β-LG/ SacII* locus, a significant relationship was not found between genotypes and lactation milk yield. However, *β-LG*/ S_1S_1 genotype was observed to have higher lactation milk yield. It is concluded that *β-LG* gene could be used as a molecular marker for economic traits such as milk yield and composition.

Keywords: *β-LG*, PCR-RFLP, Saanen, SNP, *SacII*, milk yield

Saanen Keçilerinde β- Laktoglobulin (*β-LG*) Geni Ekzon 7 Polimorfizmi ve Süt Verimi ile İlişkisi

Süt proteinlerinden biri olan B-Laktoglobulin *(β-LG*), peynir yapımı gibi sütün teknolojik özellikleri üzerinde önemli bir fonksiyona sahiptir. Serum protein genleri ile süt verimi/bileşimi arasındaki ilişkiler daha önceki çalışmalarda araştırılmıştır. *β-LG*, süt verimi ve protein kalitesini artırmak için seleksiyon ve ıslah programları için aday bir gen olarak kullanılabilmektedir. Bu çalışmanın amacı, *β-LG* gen polimorfizmini ve *β-LG* genotipleri ile süt verimi arasındaki ilişkiyi araştırmaktır. Bu çalışmada, PCR-RFLP yöntemi ile polimorfizm belirlenmesi amacıyla Avustralya kökenli toplam 74 safkan Saanen keçi kullanılmıştır. *SacII* restriksiyon enzimi ile *β-LG* geni 427 baz çiftlik yedinci ekzonu (GenBank: Z33881.1) genotiplenmiştir. *β-LG/SacII* lokusunda iki alel (S_1, S_2) ve üç genotip (S_1S_1, S_1S_2, S_2S_2) belirlenmiştir. *β-LG*/S_1S_1, genotipi tek kesim bölgesine sahip olduğundan iki bant vermektedir (347 bç ve 80 bç). *β-LG*/S_1S_2 heterozigot genotipi üç bant oluşturmaktadır (427 bp, 347 bp ve 80 bp). *β-LG*/S_2S_2 genotipi g.4601G> A pozisyonunda tek nükleotid değişiminden dolayı 427 bç uzunluğunda kesilmemiş ürün vermektedir. S_1S_2 genotip frekansı (% 43.3) diğer genotiplerden daha yüksektir. S_1 allel frekansı predominant olarak belirlenmiştir. Saanen ırkında Hardy-Weinberg dengesinde sapma gözlenmemiştir. *β-LG/ SacII* lokusunda, genotipler ile laktasyon süt verimi arasında önemli bir ilişki bulunmamıştır. Ancak *β-LG*/ S_1S_1 genotipinin daha yüksek laktasyon süt verimine sahip olduğu belirlenmiştir. *β-LG* geninin süt verimi ve kompozisyonu gibi ekonomik özellikler için moleküler marker olarak kullanılabileceği sonucuna varılmaktadır.

Anahtar Kelimeler: *β-LG*, PCR-RFLP, Saanen, SNP, *SacII*, süt verimi

Introduction

The world goat population, 95% of which is found in Asian and African countries, is estimated to be about 1 billion in 2015. In the last 10 years, world goat population has increased by 10% and similarly increased by 27% in Turkey (FAO, 2016). Especially goat breeding is localized intensively in Mediterranean, Aegean and South-Eastern Anatolia regions of Turkey. Generally, indigenous breeds are reared such as Hair, Angora, Cashmere, Norduz, Honamlı goat but Saanen and its hybrids with local breeds have become widespread in recent years. Saanen goat is used for the breeding of native breeds since the milk yield is high.

Milk is an important food for human nutrition. Milk proteins consist of 80 % casein (α-s1, α-s2, β and κ-casein) and 20% serum proteins (β-lactoglobulin, α-lactalbumin and others). serum protein occurs; β-lactoglobulin (50%), α-lactalbumin (20%), serum albumin (10%), immunoglobulins (10%) and proteose-peptones (10%) (Gür et al., 2010). *β-LG* is a protein that has dimer form and its molecular weight is 36.4 kDa in ruminant milk (Hambling et al., 1992). *β-LG* gene is localized on chromosome 11 in goat and cattle genome, on chromosome 3 in sheep genome (Hayes and Petit, 1993). The *β-LG* transcription unit consists of 7 exons (42-178 bases), 6 introns (213-1116 bases) and 4.7 kb length (Jain et al., 2012). Various investigations have been made to elucidate the variation in the gene regions coding for milk proteins (α-s1, α-s2, β- and *κ-casein*, *β-lactoglobulin*, *α-lactalbumin*) and to identify variants in these genes (Folch et al., 1993;1994; Sánchez et al., 2005). One of these milk protein, *β-LG,* was investigated on protein and DNA levels (Aschaffenburg and Drewry 1955; Eigel et al., 1984; Gaye et al., 1986; Erhardt, 1989; Özdil and Asal, 2002; Lekerpes et al., 2014). Firstly, two different variants have been identified for the goat-lactoglobulin gene at the molecular level by Pena *et al.,* (2000) in Spanish and French goats. Pena *et al.,* (2000) examined on exon 7 and 3 'flanking region by PCR-RFLP analysis and they detected the presence of a G> A base transition (5 'CCAC'GG 3') which caused the *SacII* restriction enzyme target site (5 'CCGC'GG 3'). Then, Graziano et al., (2003) identified a new transition (T>C) mutation at 341. position in the promoter region of the goat *β-LG* gene. Kumar et al., (2006) investigated *β-LG* gene polymorphism in eight different goat breeds grown in India and identified three different genotypes. Rout et al., (2010) observed two different variants of this gene, A and B, in the same breeds. Chen et al., (2005) detected a variant of the *β-LG* gene in the 5' flanking region in Xinong Saanen goats. At the same time, polymorphisms of *β-LG* gene and their effect on milk yield and composition have been studied in farm animal (Chen et al., 2005; El-Hanafy et al., 2010; El Shazly et al., 2012; Kahilo et al., 2014; Selvaggi et al., 2015; Gharedaghi et al., 2016). Also, polymorphism of *β-LG* was studied by Elmacı *et al.* (2009), Ağaoğlu et al. (2012), Yüksel and Akyüz (2014) in some Turkish local goat breeds.

The aim of this study is to investigate the *β-LG* gene polymorphism and relation between *β-LG* genotypes and milk yield by using PCR-RFLP method in Saanen goats.

Materials and Methods

Blood samples and DNA isolation

A total of 74 blood samples were collected from Saanen goats around İzmir province, Turkey. Blood samples from goats were placed into an EDTA evacuated blood collection tubes, transported to the laboratory and storaged in -20 °C until analysis. Genomic DNA was isolated from whole blood using genomic DNA Purification Mini Kit (GeneJET Whole Blood Genomic DNA Purification Mini Kit, Thermo Fisher Scientific) following the manufacturer's protocol. DNA concentration and purity were examined on 1% agarose gels and UV spectrophotometer at 260/280 nm.

Milk recording and statistical analysis

Goats were reared at Ege University, Faculty of Agriculture, Department of Animal Science, Small Ruminant Animal Application and Research Unit. Milk yield was recorded 2 times per day during the lactation. Total milk yield was statistically analysed by ANOVA at significance level ($P<0.05$). Genotypes were determined by direct counting of restriction fragments observed in the gel. Genotype frequency was estimated. The allele frequency of *β-LG* and Hardy-Weinberg equilibrium of the population were calculated in the PopGene (Yeh et al., 2000). Association between *β-LG* genotypes and milk yield was estimated in SPSS program using one way ANOVA (SPSS Inc. V. 18.0, IBM, Chicago, IL, 2009).

DNA amplification and genotyping

A region of the *β-LG* gene spanning over exon 7 to 3' flanking area was amplified by Polymerase Chain Reaction according to Pena et al., 2000. The primer sequences of the β-lactoglobulin gene (accession number Z33881.1) were: forward 5'-CGG GAG CCT TGG CCC CTC TGG-3'; reverse 5'-CCT TTG TCG AGT TTG GGT GT-3'. PCR amplification of *β-LG* gene was carried out in 25 µl reaction mixture, containing 2 mM $MgCl_2$, 200 µM of each dNTPs, 0.5 µM each primer, 1 X PCR buffer, 1U Taq polymerase (i-star Taq DNA Polymerase, Intron) and 100 ng of genomic DNA template. The thermal cycling conditions were as follows: Pre-denaturation at 95°C for 5 min, 35 cycles; denaturation at 94°C for 30 sec, annealing at 65°C for 60 sec and extension at 72°C for 90 sec, followed by a final extension at 72°C for 5 min. Then, for the RFLP analysis on seventh exon which is 427 base pairs length of *ß-LG* gene was digested using *SacII* restriction enzyme (ER0201, Thermo Fisher Scientific) at 37°C

for at least 3 h. PCR products and restriction fragments were electrophoresed on 2,5 % agarose gel stained with SafeView™ Classic (abm) and visualised on UV transilluminator.

Sequencing analysis

In order to verify the *β-LG* fragments which revealed different genotypes, PCR products were sequenced on capillary electrophoresis (ABI 3130XL Genetic Analyzer, USA), Genmar Laboratories (İzmir). The sequences of three genotypes were analyzed using the Molecular Evolutionary Genetics Analysis (MEGA6) software and ClustalW sub-programme of the BioEdit Sequence Alignment Editor (BioEdit Version 7.2.5, 2013).

Results and Discussion

β-LG is one of milk protein and has important function as major whey protein technological properties of milk (such as cheese making) in ruminants and several non-ruminant species (Schaar, 1985; Perez and Calvo, 1995). After the genetic polymorphism in milk proteins was described by Aschaffenburg and Drewry (1955), researches focused on association between *β-LG* polymorphism and milk production. Polymorphisms of some protein variants have been found not only in the open reading frame of the *β-LG* encoding gene (Godovac-Zimmerman et al., 1996) but also in non-coding areas such as the 3' flanking region (Wagner et al., 1994).

In this study, genetic polymorphism of the *β-LG* gene and the relations between the *β-LG* genotype and milk yield were investigated by PCR-RFLP method in Saanen goats. Exon 7 to the 3'flanking region of *β-LG* gene (427 bp) was amplified and digested with restriction endonuclease *SacII* to detect S_1 or S_2 variants. As a result of amplification product with *SacII* digestion, two alleles (S_1 and S2) with three different restriction patterns or genotypes (S_1S_1, S_1S_2 and S_2S_2) were observed. S_1S_1, S_1S_2 and S_2S_2 genotypes have been entitled as BB, AB, AA respectively in some studies (Ağaoğlu et al., 2012; Yüksel and Akyüz 2014).

The *β-LG* genotype S_1S_1 with only one restriction site revealed two bands of sizes 347 bp and 80 bp. An undigested product of size 427 bp termed as *β-LG* S_2S_2 genotype was also obtained (Fig. 1). The small restricted fragment at 80 bp do not appear on agarose gel. The presence of 427 bp and 347 bp bands makes it possible to identify heterozygous individuals.

The results of sequence analysis of a heterozygous genotype (S_1S_2) and homozygous genotypes (S_1S_1 and S_2S_2) were shown in Figure 2.

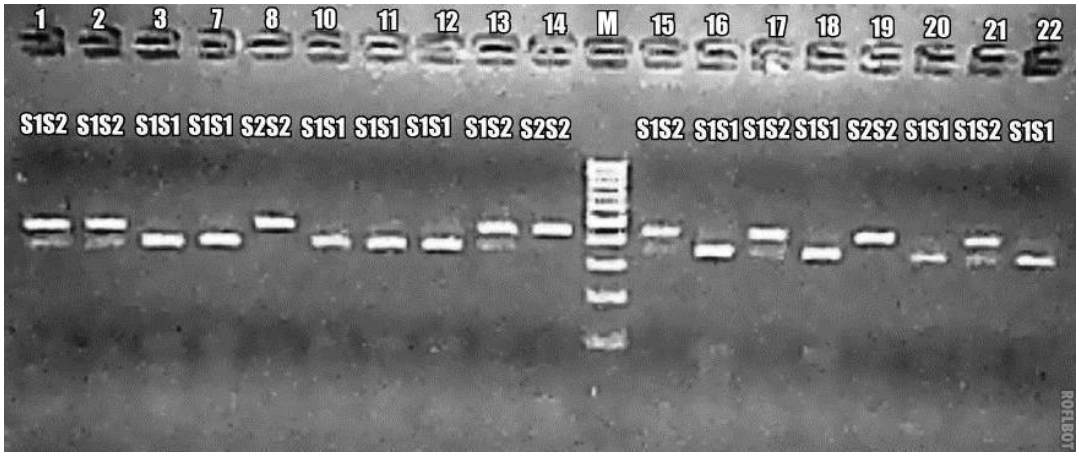

Fig 1. Electrophoresis of RFLP of caprine *β-LG* gene after digestion by *SacII* of animals with S_1S_2 (Lane 1,2,13,15,17,21; 427bp/347bp/80bp), S_1S_1 (Lane 3,7,10,11,12,16,18,20,22; 347bp/80bp), S_2S_2 (Lane 8,14,19; 427bp) genotypes. Lane M, molecular size marker (100 bp DNA ladder)

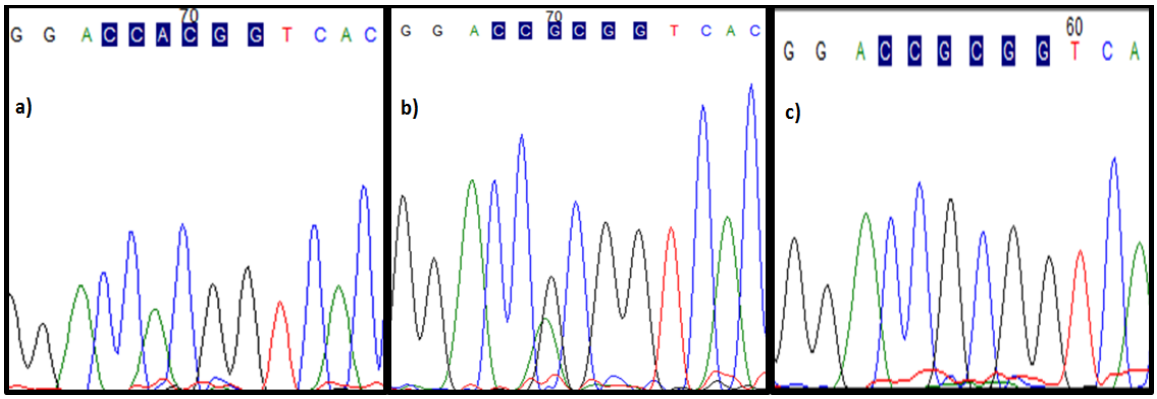

Fig 2. *SacII* restriction enzyme cleavage site a) S_2S_2: Sequence analysis of a homozygous genotype b) S_1S_2: Sequence analysis of a heterozygous genotype c) S_1S_1: Sequence analysis of a homozygous genotype

The allele and genotype frequencies at the exon 7 to the 3'flanking region of *β-LG* gene in the Saanen goats were given in Table 1. The allele frequencies were determined as 0.59 and 0.41 for S1 and S2. Genotype frequencies of S1S1, S1S2 and S2S2 were 37.8 %, 43.3 % and 18.9 %, respectively. A significant deviation from Hardy-Weinberg equilibrium was not observed in the investigated breed. The result of Chi-square statistics reflected that in the investigated breed was in Hardy-Weinberg equilibrium.

Table 1. Allele and genotype frequencies of *β-LG* gene for *SacII* site in Saanen goats.

		B-LG Genotype Frequency			*B-LG* Allele Frequency (%)		χ2
		S_1S_1	S_1S_2	S_2S_2	S_1	S_2	
Saanen	Observed	28 (37.8)+	32 (43.3)+	14 (18.9)+	0.59±0.04	0.41±0.04	0.89
	Expected	26.04 (35.2)+	35.92 (48.5)+	12.04 (16.3)+			

+ Observed and expected genotype frequency (%), χ2: Chi-square

Goat β-LG polymorphism has been investigated with 28 Saanen goat breeds by Elmacı et al. (2008) and 41 Saanen goat breeds by Ağaoğlu *et al.* (2012). Elmacı et al. (2008) revealed that the frequency of S_2S_2 (0.14) genotype was found to be lower than S_1S_1 (0.41) similar with present study. In paralell with these results, observed genotype frequency of S2S2 (AA, 0.12) was found to be lower than S1S1 (BB, 0.39) by Ağaoğlu et al. (2012). In the present study, the S_1 allele frequency was 0.59 and higher than the S2 allele frequency (0.41). A similar observation has been revealed by Elmacı et al., (2008) and by Agaoglu *et al.,* (2012) in Saanen goat (0.64, 0.63) respectively. Lekerpes et al. (2014) have identified the S_1 allele frequency (between 0.76-0.86) as predominant in two native goat breeds of Kenya, similar to our study. In contrast to our study, Kumar et al., (2006) reported that of the 13 indigenous goat breeds reared in India, the S_1 allele frequency ranged from 0.03 to 0.41 and was lower than the S_2 allele frequency. Similar to Kumar et al. (2006), the frequency of the S_2 allele (0.81) was observed to be high in the study performed by Yüksel and Akyüz (2014) on Hair goat in Turkey.

In recent years, many researches have been conducted to investigate association *β-LG* polymorphism in DNA level with milk yield and composition in farm animal (Chen et al., 2005; Selvaggi et al., 2015; El Shazly et al., 2012; Kahilo et al., 2014). Investigations conducted to study the polymorphism in different goat breeds showed significant association between *β-LG* genotypes and milk yield/composition. Lactation milk yields of goats were 812.09 kg, 777.42 kg and 791.60 kg for S1S1, S1S2 and S2S2 genotypes, respectively. No

significant association was established between β-LG genotype and milk yield. But S1S1 genotype had higher milk yield (812.09 kg) than the other genotypes (p>0.05) in Saanen goats under the study (p>0.05). El-Hanafy et al. (2015) reported that S_1 allele frequencies ranged from 0.74 to 0.57 and were higher than the S2 allele frequency in three goat races reared in Saudi Arabia. The relationships between genotypes and milk yields were examined and it was reported that milk yield of S_2S_2 genotypes significantly higher than other genotypes (p<0.05).

Conclusions

The results of this study indicate that the relationship between *β-LG* polymorphism and milk yield is very important for enhancing the productive and genetic performance of farm animals. There were conflict results about association between *β-LG* polymorphism and milk yield, these results may depend on the breed and number of animal under the study. As it is obvious from the current study, more studies including large number of animals must be carried out. It is suggested to determine the relationship between *β-LG* polymorphism in the promoter and other exon regions and milk yield especially in native goat breeds.

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References

Ağaoğlu, Ö.K., B.Ç. Kul, B. Akyüz, Ö. Elmaz, M. Ö. Metin, M. Saatçı and O. Ertuğrul, 2012. Identification of β- Lactoglobulin gene SacII polymorfphism in Honamlı, Hair and Saanen goat breeds reared in Burdur vicinity. Kafkas Univ Vet Fak Derg. 18 (3) 385-388

Aschaffenburg, R. and J. Drewry, 1955. Occurrence of different β-lactoglobulins in cow's milk**,** *Nature,* 176 218–219

Chen, H., X.Y. Lan, R.B. Li, C.Z. Lei, W.B. Sun, R.F. Zhang, Y.L. Zheng and B.C. Zhu, 2005. The effect of CSN1 S_2, CSN3 and beta-lg genes on milk performance in Xinong Saanen dairy goat. Yi Chuan Xue Bao. 32 (8), 804–810

Eigel, WN., J.E. Butler, C.A. Ernstrom, H.M. Farrell, V.R. Harwalkar, R. Jenness and R.M. Whitney, 1984. Nomenclature of proteins of cows milk: 5th revision. J. Dairy Sci. 67:1599-1631

El-Hanafy, A.A., M.A. El-Saadani, M. Eissa, G. Maharem and M.Z.A. Khalifa, 2010. Polymorphısm of β-lactoglobulın gene in Barki and Damascus and their cross bred goats in relation to milk yield. Biotechnol in Anim. Husbandry. 26 (1-2), 1-12

El-Hanafy, A.A.M., M.I. Qureshi, J. Sabir, M. Mutawakil, M.M.M. Ahmed, H. El Ashmaoui, H.A.M.I. Ramadan, M. Abou-Alsoud and M.A. Sadek, 2015. Nucleotide sequencing and DNA polymorphism studies of beta-lactoglobulin gene in native Saudi goat breeds in relation to milk yield. Czech J. Anim. Sci. 60, (3): 132–138

El-Shazly, S.A., M.E. Mahfouz, S.A. Otaibi and M.M. Ahmed, 2012. Genetic Polymorphism in Beta-lactoglobulin Gene of Some Sheep Breeds in KSA and its Influence on Milk Composition. African J. of Biotech. 11 (19):4330-4337

Elmaci, C., Y. Öner and M. Koyuncu, 2008. Determination of β-lactoglobulin Genotype Using by PCR - RFLP in Saanen Goats. Hayvansal Üretim. 49(1): 1-4

Elmacı, C., Y. Öner and M. Koyuncu, 2009. Allelic frequencies of a *SacII* RFLP at exon 7 of the goat b-lactoglobulin gene in Turkish breeds. Asian Journal of Animal and Veterinary Advances. 4(3):130-133

Erhardt, G. 1989. Evidence for a third allele at the β- lactoglobulin (*β-LG*) locus of sheep milk and its occurrence in different breeds. Anim Genet. 20:197-207

FAO, 2016. Food and Agriculture Organization of the United Nations. http://www.fao.org/economic/ess/en/ (Erişim tarihi: 10. 06. 2016)

Folch, J.M., A. Coll and A. Sa´nchez, 1993. Rapid communication: Cloning and sequencing of the cDNA encoding goat β-lactoglobulin. J Anim Sci. 71:2832.

Folch, J.M., A. Coll and A. Sanchez, 1994. Complete sequence of the caprine β-lactoglobulin gene. J. Dairy Sci. 77, 3493-3497

Gaye, P., D. Hue-Delhaie, J.C. Mercier, S. Soulier, J.L. Vilotte and J.P. Furet, 1986. Ovine β-lactoglobulin messenger RNA: nucleotide sequence and mRNA levels during functional

differentiation of the mammary gland. Biochimie. 68: 1097-1107

Gharedaghi L., H. Moradi Shahrbabak and M. Sadeghi, 2016. Identification of novel SNP in caprine β-lactoglobulin gene. J. Genet. 95, 485–490

Godovac-Zimmerman, J., I. Krause, M. Barany, S. Fisher-Fruhloz, J. Juszczak, G. Erhardt, J. Buchberger and H. Klostermeyer, 1996. Isolation and rapid sequence characterization of two novel β-lactoglobulins. J. Protein. Chem. 5: 743-750

Graziano, M., M. D'Andrea, A.R. Lagonigro and F. Pilla, 2003. Short communication: A new polymorphism in goat β-lactoglobulin promoter region. Ital. J. Anim. Sci. 2: 67-70

Gür, F., M. Güzel, N. Öncül, Z. Yıldırım and M. Yıldırım, 2010. Süt serum proteinleri ve türevlerinin biyolojik ve fizyolojik aktiviteleri. Akademik Gıda. 8 (1) 23-31

Hambling, S., A. McAlpine and L. Sawyer, 1992. Beta-lactoglobulin, In: Advanced Dairy Chemistry, Ed. Fox PF, London, Elsevier, Appl. Sci., 141-190 p.

Hayes, H.C. and E.J. Petit, 1993. Mapping of the b-lactoglobulin gene and of immunoglobulin heavy chain-like sequence to homologous cattle, sheep and goat chromosomes. Mamm. Gen. 4, 207–210

Jain, A., D.S. Gour, P.S. Bisen, P.P. Dubey, D.K. Sharma, R.P. Tiwari, N. Gupta and D. Kumar, 2012. Allele mining in β- lactoglobulin gene of capra hircus. Afr J. Biotechnol. 11(50), 11057-11064

Kahilo, K., S. EL-Shazly, A. El-Khadrawy and I. Fattouh, 2014. Genetic Polymorphism in β-lactoglobulin gene of some goat breeds in Egypt and its influence on milk yield. Life Sci J. 11(10):232-238

Kumar, A., P.K. Rout and R. Roy, 2006. Polymorphism of β -lacto globulin gene in Indian goats and its effect on milk yield. J Appl Genet. 47(1), 49-53

Lekerpes, S.S., J.O. Jung'a, M.S. Badamanaa and D.I. Rubenstein, 2014. Genetic polymorphism of beta-lactoglobulin in kenyan small east African goat breed using PCR-RFLP and sequencing. Scientific Journal of Animal Science. 3(9) 233-239

Özdil, F. and S. Asal, 2002. The polymorphism of β-Lactoglobulin. Proceedings of the 3rd Annual Meeting on Animal Science. September, 14-16

Pena, R.N., A. Sa´nchez and J.M. Folch, 2000. Characterization of genetic polymorphism in the goat β-lactoglobulin gene. J. Dairy Res. 67: 217-24

Perez, M.D. and M. Calvo, 1995. Interaction of β-lactoglobulin with retinol and fatty acids and its role as a possible biological function for this protein: A review. J. Dairy Sci. 78: 978

Rout, P.K., A. Kumar, A. Mandal, D. Laloe, S.K. Singh and R. Roy, 2010. Characterization of casein gene complex and genetic diversity analysis in Indian goats. Anim Biotechnol. 21: 122–134

Sánchez, A., H. İlahi, E. Manfredi and J.M. Serradilla, 2005. Potential benefit from using the αs1-casein genotype information in a selection scheme for dairy goats. J. Anim. Breed. Genet. 122: 21-29

Schaar, J. 1985. Effects of genetic variants of k-casein and β- lactoglobulin on cheesemaking. J Dairy Res. 52: 429-437

Selvaggi, M., V. Laudadio, C. Dario and V. Tufarelli, 2015. β-Lactoglobulin gene polymorphisms in sheep and effects on milk production traits: A Review. Adv. Anim. Vet. Sci. 3(9): 478-484.

Wagner V.A., T.A. Schild and H. Geldermann, 1994. DNA variants within the 5'-flanking region of milk-protein-encoding genes II. The β-lactoglobulin-encoding gene. Theor Appl Genet 89: 121-126.

Yeh, F., R.C. Yang and T. Boyle, 2000. Popgene (v.1.32), Microsoft Windows-based freeware for Population Genetic Analysis. http://www.ualberta.ca/~fyeh/ Pop32.exe.

Yüksel, M. and B. Akyüz, 2014. Detection of βeta-lactoglobulin gene polymorphism with PCR-RFLP method in hair goat breed raising at Kayseri and Province. Journal of Health Sciences. 23 (2) 62-66.

Effects of Grafting on Organic Seedling Quality and Tomato Production in Greenhouse

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Grafting is used as an essential cultural technique to increase tolerance of vegetables against biotic and abiotic stresses. Since grafting contributes to sustainable agriculture by reducing the amount of agrochemicals used for soil disinfection, it is an important strategy in particular in organic production. However, grafted organic seedling production is not a common practice. Thus, this study was conducted in order to determine seedling growth and crop performance of organically grown grafted tomato plants in spring-summer season of 2016. Seeds of rootstocks namely 'Beaufort' and 'Sarafin' and scion cv. 'Melis' were sown in vermicompost:local peat (1:1.5 v/v) mixture and after germination in growth chamber, seedlings were moved to a greenhouse. When they had 3-4 fully developed leaf, they were grafted with tube grafting method. Self-grafted seedlings were used as control. Grafted seedlings were left in a healing unit for 10 days and placed again into greenhouse for adaptation. One week later, ten seedlings from each replicate were harvested to measure seedling performance and others were transplanted in greenhouse in order to determine their biomass, yield and fruit quality performance. The results confirmed that the use of rootstock affected root and stem length, stem diameter, shoot and root fresh and dry weights of seedlings; yield and plant growth of plants significantly. However, fruit quality did not change. The use of rootstocks increased the seedling quality, total and marketable yield and plant growth. Among the tested rootstocks 'Beaufort' was found more appropriate due to the highest performance on organic seedling growth and tomato production under greenhouse conditions.

Keywords: Rootstock, scion, biomass, yield, quality.

Introduction

Grafting of vegetable seedlings is a practical horticultural technology used for many years in the world. Grafted vegetables can improve the resistance to soil borne diseases and abiotic stress factors like drought, salinity, chilling etc. and enhance the resistance ability (King et al., 2008; Lee et al., 2010; Jianping, 2011). Since the root systems of rootstocks are usually much larger and more vigorous, they can absorb nutrients more efficiently as compared to non-grafted plants (Huang et al., 2015). Using rootstock also improve plant growth and yield, influence product quality (Ruiz et al., 1997; Khah, 2005; Martorana et al., 2007; Rouphael et al., 2010; Lee et al., 2010; Colla et al., 2011). The effects of rootstocks can be related to the synthesis and translocation of plant growth regulators, to water and nutrient uptake by vigorous roots of rootstock, as well as their assimilation (Lee et al., 2010).

Grafting is also a key technique for sustainable crop production to overcome the continuous cropping obstacle and to keep the vegetable products safe (Maršić and Osvald, 2004; Khah et al., 2006; Rivard and Louws, 2008; Louws et al., 2010; Schwarz et al., 2010). Thus, the use of grafting in greenhouses has become widespread because soil borne diseases such as Fusarium wilt, Verticillium wilt, bacteria wilt and root knot nematodes are more serious under protected cultivation compared to open fields. The second reason is that vegetables often suffer from low temperature and low light intensity (from late fall to early spring). In addition, the soil secondary salinization caused by excess fertilizer input and cover of protected facilities is also serious under protected cultivation. Through the selection of appropriate rootstock, the grafted plant tolerance to low temperature and salinity in greenhouses can be enhanced (Huang et al., 2010; Li et al., 2014; Huang et al., 2015).

In this regards, grafting could be used as a vital and clear strategy in order to avoid soil-borne diseases, especially in organic production under greenhouse (Oztekin and Tuzel, 2015). According to Council Regulation (EEC) No 2092/91 of 24 June 1991, seed and plant propagation material should be organically produced in organic farming (EUR-Lex, 1015) however, grafted organic seedling production is not a commercial practice due to necessity for intensive care, the requirement of knowledge and skill, availability of inputs (i.e.

organic seeds). The high cost of organically produced scion and rootstock seeds and also all other inputs (growing medium, fertilizers, pesticide, biological agents etc.), relatively lower amount of demand compared to conventional ones and lack of production protocols are other the most important drawbacks.

This study aimed to determine seedling growth and crop performance of organically grown grafted tomato plants in spring-summer season under greenhouse.

Materials and Methods

This research was conducted during the spring season of 2016 at the Department of Horticulture at Ege University in Izmir, Turkey (38°27'17"-N, 27°14'17"-E) in order to determine the performance of organically grown grafted tomato seedlings and plants grafted on different rootstocks.

Non-treated seeds of tomato (Lycopersicon esculentum) cultivar Melis (AG Seeds, Antalya-Turkey) were used as scion. Two interspecific tomato hybrid (L. esculentum × L. hirsutum) rootstocks namely Beaufort (De Ruiter, The Netherlands) and Sarafin (AG Seeds, Antalya-Turkey) were used for grafted tomato seedling production and compared with self-grafted plants used as control. The seeds of rootstocks 'Sarafin' were organically certified, but Beaufort seeds were washed before used since organic or non-chemical treated seeds were not available in the market.

Local peat (provided from Denizli peatlands from Aegean Region/Turkey) and vermicompost (Ekosol, Istanbul-Turkey) mixture (1:1.5 v/v) were used as organic growing medium (Tuzel et al., 2014) and Melis and Sarafin seeds were sown on the 13th of January 2016 while Beaufort seeds were sown 19th of January 2016 due to differences of seed germination period. Scion and rootstock seeds were sown into three trays having 128 plugs in each.

After sowing, trays were left in a germination room at 24/24°C and 80% RH under dark for 3 days and then moved to a climate controlled (24-26°C) greenhouse which is specialized for seedling production for 3 weeks. Seedlings were fertilized with liquid composted farmyard manure (Botanica, Camli Yem Besicilik, Izmir-Turkey) (30 L ha^{-1}) (Tuzel et al., 2014) every day with boom system. In this period, germination rate and germination period of seeds were noted. Germination rate was calculated by counting the number of germinated seeds in cells and expressed as %. Germination period was determined as the number of days required for 50% of seed emergence (Tuzel et al., 2014).

When seedlings had 3 to 4 fully developed leaf, tomato scions were grafted with tube grafting method at the date of 22.02.2016. Grafting was performed manually and then water was sprayed immediately and grafted seedlings were left in growing room for healing at 24-26°C, 80-90% RH, 16 h LED light/8 h dark for 10 days by covering each rack with PE cover (40 micron thickness, thermal curtain) in order to conserve humidity. PE cover was totally closed at the beginning and after 5 days it started to open half during the day.

Seedlings were placed again into greenhouse after healing for adaptation for a week. During this period they were fertilized as mentioned above. When seedlings were ready for planting, 10 seedlings were harvested from each replicate of treatments in order to measure root, rootstock and scion length, root and shoot diameter, shoot and root fresh and dry weights. Root length (cm) was determined with a ruler from the longest root. Rootstock length (cm) was measured from root collar to grafting point through ruler; scion length (cm) was the distance between grafting and growth point; rootstock and scion diameter (mm) was measured from the middle of seedling with a digital caliper. Roots were washed and cleaned from growing medium and separated from shoots. Root and shoot samples were weighed for fresh weight (g) and then dried in a thermo ventilated oven at 65°C and weighed for dry weight (g).

Seedlings were transferred into the greenhouse on 08th of March, 2016 with a plant density of 2.86 plants per m^2 with the randomized blocks experimental design with 8 replicates; each replicate include 14 plants. Organic growing standards prevailed throughout production. Sticky yellow traps (one per 15 m^2) were placed above the plant level and moved up as they grew. Pests and diseases were monitored weekly and bumblebees were used for pollination. Drip irrigation used and irrigation amount was based on Class-A-Pan. Irrigation intervals varied from 3 to 4 days. Plants

were topped on the 6th trusses and removed on 27th of June, 2016.

Fruits harvest started on 23th of May and continued until 26th of June, 2016. Harvested fruits were weighed and counted to determine the total and marketable fruit yield (kg m^{-2}), fruit number (no m^{-2}) and average fruit weight (g). Fruits were separated in terms of diameters as ∅>5.5 cm, 4.5>∅<5.5cm, 3.5<∅<4.5 cm and ∅<3.5 cm (non marketable) and fruits in each group was counted. In order to determine fruit quality, ten fully ripened tomato fruits were taken from each grafting combinations with 4 replicates on 09th of June, 2016. Penetrometer (Nippon FHR-1) with a conical tip (base diameter 8 mm and length 10 mm) was used to measure rind strength (firmness) and the results were expressed in Newtons (N). The detailed surface colour of tomatoes was assessed with a colorimeter (Minolta, CR-300, Japan) by measuring opposite sides of randomly selected fruit using a 8 mm diameter viewing area. Measurements were recorded as L (lightness, from white = 100 to black = 0), a (red-green) and b (yellow-blue) CIE (Commission Internationale de l'Eclairage) colour co-ordinates. Fruit juice was extracted using a kitchen juicer and filtered. EC (dS m^{-1}) and pH values were measured with an EC and pH meter (Seven Easy, Mettler Toledo, Istanbul, Turkey). Total soluble solids (TSS, %) content was measured using a digital refractometer (Euromex RD 645, Arnhem, The Netherlands). Titratable acidity (TA) was determined by titration with 0.1 N NaOH to pH 8.1 and expressed as mval 100 ml^{-1}. The dry weight (DW, % of fresh weight) was determined by drying samples in an oven at 65°C until a constant weight was obtained. Nitrate (mg kg^{-1}) and vitamine C (mg 100 ml^{-1}) were determined according to Fresenius et al. (1998) and Pearson (1970), respectively. At the end of the growing cycles, measurements of plant height (cm) from soil to top with ruler; stem diameter (mm) with digital calipers; root, fruit and shoot fresh and dry weights (g $plant^{-1}$) at 65°C in a thermoventilated oven were also made on 4 plants in each treatment.

Data were subjected to analysis of variance to determine any statistically significant differences among rootstock by using the JMP statistical analysis package program (SAS Institute, USA). Tukey range test was conducted at 5% importance level ($P \leq 0.05$) in order to identify the differences between the means.

Results and Discussion

Seedling performance

Beaufort, Sarafin and Melis seeds completed 50% of emergence 2, 5 and 4 days after sowing, respectively. The germination rate was the highest in Beaufort (100%) compared with Sarafin (95%) and Melis (97%) (data not shown).

Effect of grafting on scion length and rootstock diameter; shoot fresh and dry weights and root dry weight were found statistically different, however, root and rootstock lengths did not change significantly in treatments. Scion stem length accepting as one of the most important seedling quality parameters was higher in Melis/Beaufort, followed by Melis/Sarafin and self-grafted plants. Beaufort gave the highest rootstock diameter while rootstock did not affect scion diameter at seedling stage. Rootstocks statistically affected root fresh and shoot fresh and dry weights. Melis/Beaufort gave the highest seedling biomass with the highest root and shoot fresh weights followed by Melis/Sarafin and self-grafted ones (Table 1).

Seedling quality has a vital role in the success of crop production and it is particularly related to well root and shoot development (Kubota et al., 2013). The results obtained from the study have shown that rootstocks had better performance than self-grafted seedlings especially in terms of root and shoot fresh weights, shoot dry weight and stem length and diameter. Although rootstocks showed more vigour and vegetative growth, it was depending on rootstock genotype. Among the tested rootstocks, the performance of Beaufort as a rootstock on seedling quality was obvious. This result showed similarity with our previous findings (Oztekin and Tuzel, 2016). Overall results confirms that Beaufort could be more appropriate for organic seedling production compared to other rootstocks due to its higher performance.

Table 1. Effects of treatments on organic tomato seedling growth (2 weeks after grafting)

Grafting Combinations	Length (cm) Root	Length (cm) Rootstock	Length (cm) Scion	Diameter (cm) Rootstock	Diameter (cm) Scion	Root (g plant^{-1}) Fresh weight	Root (g plant^{-1}) Dry weight	Shoot (g plant^{-1}) Fresh weight	Shoot (g plant^{-1}) Dry weight
Melis/Melis	9.26	3.42	10.04 c	2.33 b	3.11	0.20 b	0.02	2.68 b	0.29 b
Melis/Beaufort	10.21	3.32	19.15 a	3.03 a	2.93	0.46 a	0.05	5.44 a	0.52 a
Melis/Sarafin	9.79	2.88	15.94 b	2.61 b	2.85	0.27 ab	0.02	4.12 ab	0.38 ab

Mean data followed by the same letter are not significantly different within each column (P≤0.05, Tukey test).

Plant performance

Grafting combinations affected total and marketable yields significantly. Beaufort increased total and marketable yields by 12.7% and 13.6% respectively compared to self-grafted ones. Sarafin took a place between Beaufort and Melis and self-grafted plants had the lowest yield performance (Table 2).

Table 2. Effects of treatments on fruit yield of grafted organic tomato plants

Grafting Combinations	Total yield (kg m^{-2})	Marketable yield (kg m^{-2})	Mean fruit weight (g)	Total fruit number (no plant^{-1})	Fruit diameter ratio (%) <3.5 cm	3.5-4.5 cm	4.5-5.5 cm	>5.5 cm
Melis/Melis	11.93 b	11.73 b	107.93	110.54	9.31	12.76	12.54	65.39
Melis/Beaufort	13.45 a	13.32 a	116.71	115.35	7.28	11.27	13.15	68.30
Melis/Sarafin	12.27 ab	12.08 ab	110.44	111.11	8.35	13.02	13.43	65.20

Mean data followed by the same letter are not significantly different within each column (P≤0.05, Tukey test).

Grafting did not affect fruit skin colour and lightness (Table 3) and also dry weight, firmness, titratable acidity, total soluble solids; nitrate and vitamine C content (Table 4). EC and pH values of fruit juice only changed within measured quality parameters. EC and pH was the lowest in fruit juices from self-grafted and Beaufort plants, respectively.

Table 3. Effect of different grafting combinations on fruit skin colour

Grafting Combinations	L	a*	b*	a/b	Hue°	Croma°
Melis/Melis	41.07	22.19	26.44	0.84	49.99	34.52
Melis/Beaufort	41.37	21.54	26.11	0.82	50.49	33.85
Melis/Sarafin	41.44	21.70	26.20	0.83	50.42	34.03

Table 4. Effect of different grafting combinations on some fruit quality parameters

Grafting Combinations	DW (%)	Firmness (N)	EC (dS m^{-1})	pH	TA (mval 100 ml^{-1})	TSS (%)	NO_3-N (mg kg^{-1})	Vitamin C (mg 100 ml^{-1})
Melis/Melis	7.89	48.95	5.40 b	4.77 a	4.93	5.08	28.00	24.90
Melis/Beaufort	6.45	49.10	5.67 ab	4.74 b	4.89	5.08	31.26	25.06
Melis/Sarafin	6.85	51.80	5.77 a	4.75 ab	4.92	5.28	35.64	26.04

Mean data followed by the same letter are not significantly different within each column (P≤0.05, Tukey test).
DW: dry weight, EC: electrical conductivity, pH: power of Hydrogen, TA: titratable acidity, TSS: total solube solids

Among plant biomass parameters scion diameter, vegetative, generative and root fresh and dry weights were affected from different rootstocks. Plants on Beaufort gave the highest stem diameter

and fresh and dry weights. Rootstock Sarafin showed the same performance with self-grafted plants (Tables 5 and 6)

Tablo 5. Effets of rootstocks on grafted tomato plant growth

Grafting Combinations	Plant height (cm)	Root lenght (cm)	Stem diameters (mm)	
			Rootstock	Scion
Melis/Melis	206.4	29.7	17.5	12.8 c
Melis/Beaufort	193.6	29.7	19.1	15.8 a
Melis/Sarafin	198.6	32.1	18.5	14.2 b

Mean data followed by the same letter are not significantly different within each column (P≤0.05, Tukey test).

Table 6. Effect of different grafting combinations on fresh and dry weights of tomato plants

Grafting Combinations	Vegetative parts			Generative parts			Root		
	FW (g)	DW (g)	DW (%)	FW (g)	DW (g)	DW (%)	FW (g)	DW (g)	DW (%)
Melis/Melis	643.1 b	106.6 b	16.8	4069.6 b	268.9 b	6.6	30.9 b	8.2 b	26.5
Melis/Beaufort	1099.4 a	172.5 a	15.7	4775.6 a	313.2 a	6.6	55.3 a	16.2 a	28.7
Melis/Sarafin	678.4 b	108.0 b	15.9	4310.4 b	276.1 ab	6.4	31.5 b	8.2 b	26.0

Mean data followed by the same letter are not significantly different within each column (P≤0.05, Tukey test).
FW: fresh weight, DW: dry weight

Grafted plants have been used to improve plant growth and yield, provide earliness, extend harvesting period, influence product quality, improve water and nutrient use of efficiency etc. (Ruiz et al., 1997; Khah, 2005; Martorana et al., 2007; Rouphael et al., 2008; Rouphael et al., 2010; Colla et al., 2011). These may be caused by signals such as water, nutrients and especially hormones and nucleic acids that move through the graft union to affect scion growth (Perez Alfocea, 2015). When they are compared with the self-rooted plants, grafted plants have stronger and denser root structure, which increases internal plant hormones and as a result the rate of photosynthesis, thus inciting plant growth and development, and positively contributing to resistance against stress conditions (Ahn et al., 1999; Lee et al., 2010). Grafted plants reflect these advantageous properties more under stress conditions and against soil-borne disease problems. In this study there was no stress factor however especially total and marketable yields and plant stem diameter, fresh and dry weights of plant parts increased by the use of rootstock. This higher performance most probably came from the seedling stage due to increased seedling quality with higher fresh weight and stem diameter.

The response changed according to the rootstock genotype. As in the seedling phase, rootstock Beaufort showed better performance compared to Sarafin and Melis. These results are consistent with other studies showing that the grafting effect changed depending on the rootstock genotype (Santa Cruz et al., 2002; Lee and Oda, 2003; Abdelmageed et al., 2004; Khah, 2005; Öztekin, 2009).

Conclusions

Rootstocks showed more vigour in seedling stage, yield and vegetative growth in greenhouse condition. These parameters differed according to grafting combination; among the tested rootstocks Beaufort was found more appropriate for organic seedling and crop production compared to other rootstocks due to the higher performance. It was concluded that grafting could be used in organic agriculture providing organic inputs are supplied.

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References

Abdelmageed, A.H., N. Gruda and B. Geyer, 2004. Effects of temperature and grafting on the growth and development of tomato plants under controlled conditions. (www.tropentag.de).

Ahn, S.J., Y.J. Im, G.C. Chung, B.H. Cho and S.R. Suh, 1999. Physiological responses of grafted-cucumber leaves and rootstock roots affected by low root temperature. Sci. Hortic. 81, 397-408.

Colla, G., Y. Rouphael, C. Mirabelli and M. Cardarelli, 2011. Nitrogen-use efficiency traits of mini-watermelon in response to grafting and nitrogen-fertilization doses. J Plant Nutr Soil Sci. 174 (6): 933–941.

EUR-Lex, 2015. Free access to European Union Low. The Official Journal of the European Union. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:1991R2092:20060506:EN:PDF

Fresenius, W., K.E. Quentin and W. Schneider, 1998. Water Analyis. A practical Quide to Physicochemical, Chemical and Microbiological Water Examination and Quality Assurance. Springer-Verlag, Berlin.

Huang, Y., Z.L. Bie, S.P. He, B. Hua, A. Zhen and Z.X. Liu, 2010. Improving cucumber tolerance to major nutrients induced salinity by grafting onto *Cucurbita ficifolia*. Environ. Exp. Bot. 69:32-38.

Huang, Y., Q.S. Kong, F. Chen and Z.L. Bie, 2015. The history, current status and future prospects of vegetable grafting in China. Acta Hort. 1086:31-40.

Jianping, Y. 2011. The advantage of Grafted Tomato cultivation and grafting techniques. Modern Agri. Sci. and Tech. 18:131-132.

Khah, E.M. (2005). Effects of grafting on growth, performance and yield of aubergine in the field and greenhouse. J. Food Agric. Environ. 3, 92-94.

Khah, E., E. Kakava, A. Mavromatis, D. Chachalis and C. Goulas, 2006. Effect of grafting on growth and yield of tomato (*Lycopersicon esculentum* Mill.) in greenhouse and open-field. J. Appl. Hort. 8:3-7.

King, S.R., A.R. Davis, W. Liu and A. Levi, 2008. Grafting for disease resistance. HortScience 43:1673-1676.

Kubato, C., A. Balliu and S. Nicola, 2013. Quality of Planting Materials. Good Africultural Practices for Greenhouse Vegetable Crops: Principles for Mediterranean climate Areas. FAO Plant Production and Protection Paper, 217,355-378.

Lee, J.M. and M. Oda, 2003. Grafting of herbaeous vegetable and ornamental crops. Horticultural Reviews, 28, 61-124.

Lee, J.M., C. Kubota, S.J. Tsao, Z.L. Bie, E.P. Hoyos, L. Morra and M. Oda, 2010. Current status of vegetable grafting: diffusion, grafting techniques, automation. Scientia Hort. 127:93-105.

Li, H., F. Wang, X.J. Chen, K. Shi, X.J. Xia, M.J. Considine, J.Q. Yu and Y.H. Zhou, 2014. The sub/supra-optimal temperature-induced inhibition of photosynthesis and oxidative damage in cucumber leaves are alleviated by grafting onto figleaf gourd/luffa rootstocks. Physiol. Plant. 152:571-584.

Louws, F.J., C.L. Rivard and C. Kubota, 2010. Grafting fruiting vegetables to manage soilborne pathogens, foliar pathogens, arthropods and weeds. Scientia Hort. 127:127-146.

Maršić, K. and J. Osvald, 2004. The influence of grafting on yield of two tomato cultivars (Lycopersicon esculentum Mill.) grown in a plastichouse. Acta Agric. Slov. 83:243-249.

Martorana, M., F. Giuffrida, C. Leonardi and S. Kaya, 2007. Influence of rootstock on tomato response to salinity. Acta Hort. 747,555-561.

Öztekin, G.B. 2009. Response of Tomato Rootstocks to Salinity Stress (in Turkish). Ege University, Graduate school of Natural and Applied Science, PhD Thesis, Bornova-İzmir/Turkey, 342 p.

Öztekin, G.B. and Y. Tuzel, 2016. Grafted organic seedling production of tomato and watermelon. III International Symposium on Organic Greenhouse Horticulture, 11-14 April, 2016. İzmir-Turkey.

Pearson, D. 1970. The Chemical Analysis of Foods. Chemical Publishing Co Inc, New York, USA.

Perez Alsocea, F. 2015. Why should we investigate vegetable grafting? Acta Hort. 1086:21-30.

Rivard, C.L. and F.J. Louws, 2008. Grafting to manage soilborne diseases in heirloom tomato production. HortScience 43:2104-2111.

Ruiz, J.M., A. Belakbir, I. López-Cantarero and L. Romero, 1997. Leaf-macronutrient content and yield in grafted melon plants. A model to evaluate the influence of rootstock genotype. Sci Hortic, 71 (3-4), 227–234.

Rouphael, Y., M. Cardarelli, G. Colla and E. Rea, 2008. Yield mineral composition, water relations, and water use efficiency of grafted mini-watermelon plants under deficit irrigation. HortSci. 43, 730–736.

Rouphael, Y., D. Schwarz, A, Krumbein and G. Colla, 2010. Impact of grafting on product quality of fruit vegetables. Sci Hortic. 127 (2), 172–179.

Tuzel, Y., G.B. Oztekin and E. Tan, 2014. Use of different growing media and nutrition in organic seedling production. Acta Hort. 1107:165-175.

Santa-Curz, A., M. Martinez-Rodriguez, F. Perez-Alfocea, R, Romero-Aranda and C.M. Bolarin, 2002. The rootstock effect on the tomato salinity response depends on the shoot genotype. Plant Science 162: 825-831.

Schwarz, D., Y. Rouphael, G. Colla and J.H. Venema, 2010. Grafting as a tool to improve tolerance of vegetables to abiotic stresses: Thermal stress, water stress and organic pollutants. Scientia Hort. 127:162-171.

The Effect of Water Deficit on some Physiological Properties of *Abelmoschus esculentus* (L.) Moench cv. "Sultani"

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This study was conducted to determine the some physiological changes after the artificial drought stress in okra (*Abelmoschus esculentus* (L.) Moench cv."Sultani") which is widely cultivated in Turkey and well adapted to Trakya region. After germination, the seedlings were grown under normal growing conditions in an unheated plastic greenhouse until they reached to 2-4 leaf. They were planted in the field with a distance of 50 cm between rows and 25 cm in rows. The plants normally irrigated until flowering time. After then, water constraint applied for drought stress. Control plants were irrigated to bring to field capacity, when they lost 50% of usable water capacity in root region. Other plots were irrigated to 0%, 25% and 50% of applied water in control parcel. Water restriction was done once a week and then measurements were made. During the experiment, leaf water potential (-MPa), leaf relative water content (%), membrane damage in leaf cells (%) and total chlorophyll (SPAD value) in leaves were determined. As results, it has been determined that as the amount of water restriction goes from control to 0%, the leaf water potential decreases and the plants grown at 0% water constraint show severe damage symptoms. It has also been found that the amount of leaf-relative water content and the total amount of chlorophyll are reduced in a manner contrary to the increase in drought stress. Contrary to other criteria, membrane damage and leaf temperatures increase in leaf cells due to the increase in the amount of water restriction.

Keywords: *Abelmoschus esculentus* cv. Sultani, water stress, leaf water potential, leaf relative water content, membrane damage, total chlorophyll

Introduction

As in the whole world, our country has also been affected with the results of global warming such as weakening of water resources, drought, desertification and ecological degradation. Turkey is definitely one of the risky countries in terms of the potential effects of global warming.

Because of the global climate change, arid and semi-arid areas in the world have been expanding continuously. The duration and severity of droughts which can trigger other processes such as salinization and erosion are getting bigger (Turkes, 1997).

One of the continuing problems in crop production is lack of water. Cultivated plants usually require too much water for their growth. Water shortage often leads to significant loss of quality as well as yield losses. The traditional solution to the drought struggle is irrigation. Nowadays, however, qualified water resources are declining and many users, such as farmers, industrialists and municipalities, compete for the same water. Even if the farmers can afford high costs of the irrigation and necessary equipment for irrigation, it is not always a reasonable solution. This conviction is becoming increasingly widespread and there is growing interest in plants that have the capacity to provide good yield under arid conditions (Cirak and Esendal, 2006).

Okra originates from West Africa. It is scientifically named "*Abelmoschus esculentus*" or "*Hibiscus esculentus*" and belongs to the family Malvaceae. In Trakya region, Okra is usually planted in the first week of May and after 57-60 days it can be harvested. Harvest is usually done in the morning hours. After this time, the fruit does not easily pull off and the harvest becomes difficult (Salk et al. 2008).

In our research, physiological morphological and chemical changes brought about by different water constraints in okra cultivation were determined.

Materials and Methods

In this study, Sultani (*Abelmoschus esculentus* L) variety which is well adapted to Trakya Region and widely grown in Turkey was used as material. The test was established in the Edirne-Kesan district. GPS coordinates of the location are follows as 40°52'25.43" N, 26°36'43.01"E.

In the experiment, the okra seeds placed between a moist cloth were soaked for two days. The seeds were planted in multipots and then they were kept

in plastic greenhouse without heating until 2-4 leaf seedlings and grown under normal care and irrigation conditions. After then, the plants were planted in experimental area with a distance of 50 cm between the rows and 25 cm above the rows (Salk et al. 2008). Then plants had been given normal water by drip irrigation. We started to apply artificial drought stress after the first flowering period. Control plants were irrigated to bring to field capacity, when they lost 50% of usable water capacity in root region. Other plots were irrigated to 0%, 25% and 50% of applied water in control parcel. Water restriction was done once a week until harvesting period. (Yildirim and Kodal, 1998).

Experimental design was completely randomized block design with 5 replicates. There were 4 different treatments of water deficiency levels and there were 20 plants in each treatment plot.

Statistical analyzes of the data obtained from the experiment were made using the MSTAT version 3.00/EM package program. Significant statistical differences were determined with the L.S.D. control method (Akdemir et al. 1994).

Water restriction was initiated during the first flowering period and after one week from this date, first leaf water potential measurements were started and a total of 6 measurements were made for this purpose. In the last harvest period of the experiment, leaf relative water content, membrane damage in leaf cells and total chlorophyll content were determined by the following methods.

Leaf water potential measurement (-MPa)

Leaf water potential was measured by Scholander Pressure Chamber. The measurements were made 2 hours before (Ψ_{pd}: Pre-dawn leaf water potential) and 6 hours after (Ψ_{md}: Midday leaf water potential) sun rise respectively. The measurements (in 40 atmospheric pressure was using pure nitrogen) were made on most developed leaves of plant (Scholander et al. 1965). They were repeated six times with 1 week intervals after flowering.

Leaf relative water content (RWC) (%)

RWC was calculated by below equation (1) (Sanchez et al. 2004).

$$\mathbf{RCW} = \frac{100(\mathrm{FW}-\mathrm{DW})}{\mathrm{TW}-\mathrm{DW}} \qquad (1)$$

FW=Fresh weight, DW= Dry weight, TW= Turgid weight

Membrane damage index (%)

Membrane damage index (MDI) was calculated by measuring the electrolyte released from the cell (Fan and Blake, 1994). In each vegetation period, disks with diameter of 17 mm taking from leaves of stress and control plants were kept in ionized water for 5 hours and then their electricity conductivities (EC) were measured. Same disks were kept in autoclave at 100 °C for 10 minutes and then the EC value of the solution was measured again. The membrane damage in leaf cells (%) was calculated with the help of the below equation (2).

$$\mathrm{MDI} = \frac{100(\mathrm{Lt}-\mathrm{Lc})}{1-\mathrm{Lc}} \qquad (2)$$

Lt: EC before autoclaving / EC after autoclaving of the leaf which is under drought stress

Lc: EC before autoclaving / EC after autoclaving of the control leaf.

Determination of total chlorophyll content (SPAD)

The chlorophyll content of the okra leaves was measured by “Konica Minolta SPAD-502” portable chlorophyll-meter. In each period, same readings were made from two regions of the leaf (close to midrib) and from five plants in each parcel (Geravandi et al. 2011).

Results and Discussion

The effects of different water constraints on pre-dawn (Ψ_{pd}) and mid-day (Ψ_{md}) leaf water potentials are shown in Table 1, 2 and Figure 1, 2.

In Figures 1 and 2, the background is colored according to general plant physiology and scale values that many researchers have found in studies of different species (Taiz and Zeiger 2008). Significant differences were found between 0% and control applications on the basis of pre-dawn and mid-day leaf water potential measurements. Initial measurements were made one week after the start of water restriction, and pre-dawn leaf water potential (Ψ_{pd}) values were found to be gradually decreasing when the stress level increases (Table 1 and Figure 1).

During the experiment, Ψ_{pd} values ranged between 0.09 MPa and 2.24 MPa. stress was not determined in control, 50% and 25% irrigation

regimes (-0.09, -0.13 and -0.20 MPa), otherwise in the 0% irrigation medium stress conditions were measured (-0.29 MPa).
In the 100% irrigation regime, pre-dawn leaf water potential (Ψ_{pd}) at the 6th week after flowering decreased to -0.24 MPa and remained at the level of low-medium stress. The Ψ_{pd} values after 6 weeks later from the flowering time showed that okra plants are at a severe stress levels (-0.78, -1.26 and -2.24 MPa) in 50%, 25% and 0% irrigation plots (Table 1 and Figure 1).

Table 1. The effect of different water constraints on the mean values of pre-dawn leaf water potential (-MPa)

Water Deficiency Level	Number of weeks after flowering					
	1	2	3	4	5	6
% 0	-0.29	-0.58	-1.26	-1.76	-1.96	-2.24
% 25	-0.20	-0.43	-0.57	-0.81	-1.14	-1.26
% 50	-0.13	-0.28	-0.33	-0.48	-0.65	-0.78
% 100 (Control)	-0.09	-0.11	-0.13	-0.17	-0.23	-0.24

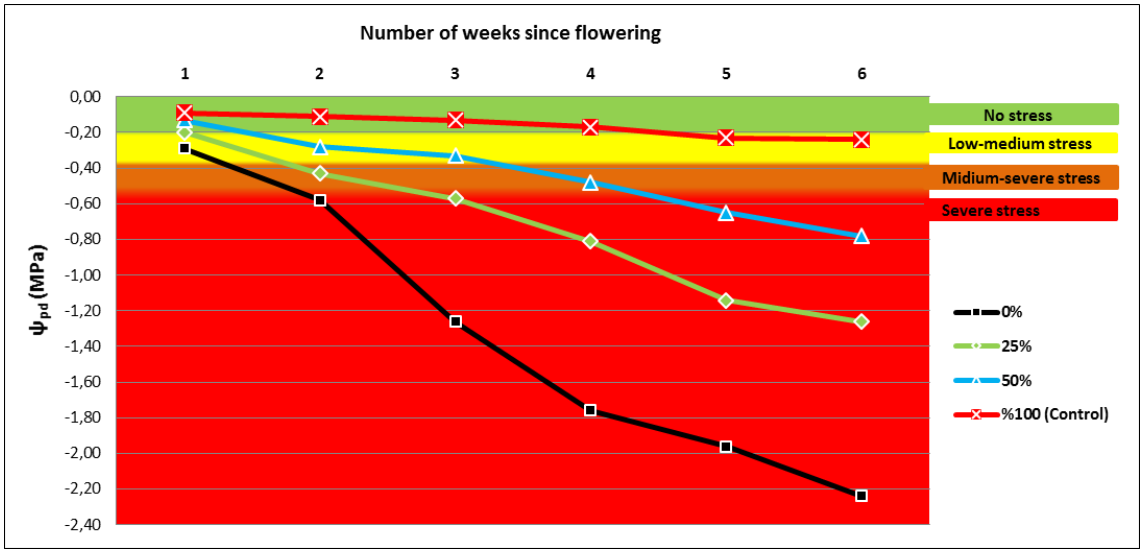

Figure 1.The effect of different water constraints on the mean pre-dawn leaf water potential (-MPa).

It was determined that the mid-day leaf water potentials (Ψ_{md}) tend to decrease gradually and the stress level increases accordingly (Table 2 and Figure 2).

According to first mid-day leaf water potential values (Ψ_{md}) which were measured after 1 week from the start of water restrictions, it was observed that control, 50% and 25% irrigation regimes had no stress (-0.48, -0.66 and -0.93 MPa), otherwise the Ψ_{md} value (-1.13 MPa) in the first measurement in the 0% parcel where no irrigation was performed remained under the low stress conditions. In parallel with the continuation of the stress conditions, in the control application values were observed in the low stress zone (-1.01 MPa), 25% in the high stress zone (-1.45 MPa) and in the severe stress zone (-2.39 and -3.16 MPa)

As a result of stress conditions, control were in the low- stress region (-1.01 MPa), 50% treatment was in high stress region (-1.45 MPa), 25% and 0% treatments were in severe stress (-2.39 and -3.16 MPa) region (Table 2 and Figure 2).

Table 2. The effect of different water constraints on the mean values of mid-day leaf water potential (-MPa)

Water Deficiency Level	Number of weeks after flowering					
	1	2	3	4	5	6
% 0	-1.13	-1.52	-1.89	-2.37	-2.54	-3.16
% 25	-0.93	-1.17	-1.26	-1.79	-2.03	-2.39
% 50	-0.66	-0.72	-0.98	-1.18	-1.29	-1.45
% 100 (Control)	-0.48	-0.6	-0.68	-0.76	-0.91	-1.01

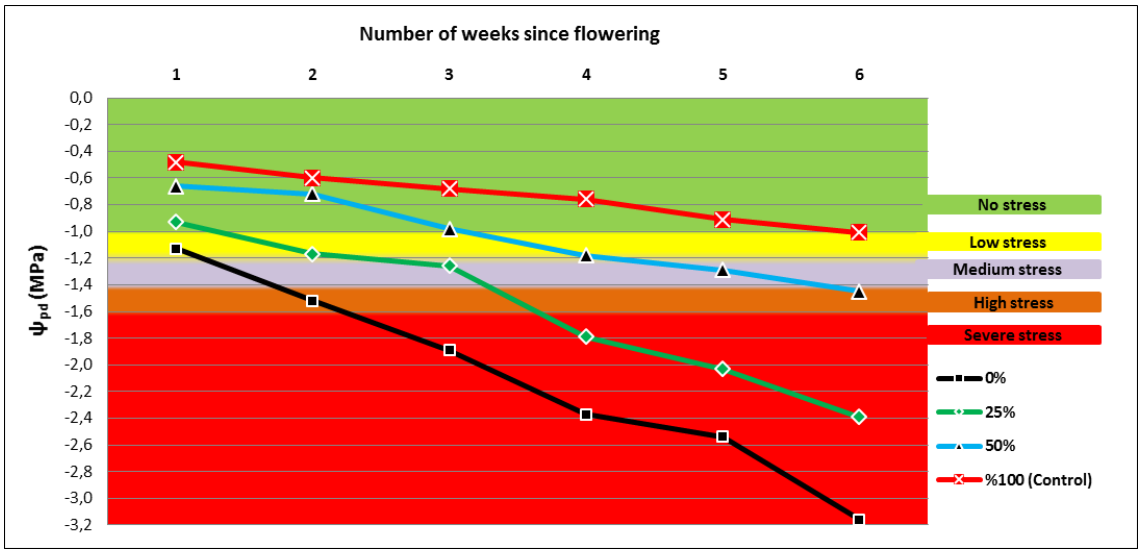

Figure 2. The effect of different water restraints on the mid-day leaf water potential (-MPa).

Deveci and Uyan (2011) stated that spinach plant has the highest amount of water at the harvesting period. So the most water is needed to sustain its activities in that period. They also added that water stress will occur in this period, As result, only control and 75% irrigation group plants were not affected, but 0%, 25% and 50% irrigation group of the plants were not able to survive agains the stress. Kucukkomurcu (2011) who was studied on the screening of okra genotypes for tolerance to salinity and drought stresses stated that, the damage to the cell is more pronounced than in drought stress conditions. Deveci and Celik (2016) determined that the leaf water potential was reduced due to the decrease in irrigated water by up to time from sowing to blossoming in the ground cherry According to this, while the lowest leaf water potential was obtained from the water restriction increased, the leaf water potential increased as the irrigation rate increased.

Mean values of the leaf-relative water content (LRWC) measured in this study are given in Table 3. There appears to be a direct relationship between the different watering rates applied to the plants and the leaf-proportional water content (Table 3). The leaf-proportional water content has been found to fall in parallel to the water restrictions imposed on the plants. The greatest decrease in leaf-proportional water content was seen at 0% water restraints. The control group has the highest leaf-relative water content (98.11%).

In the studies dealing with drought and salinity carried out by Kucukkomurcu (2011) in okra , Kaya and Higgs (2003) in pepper, Tuna et al. (2010) in melon, Turkan et al. (2005) in pea, water stress significantly reduced leaf-proportional water content and vegetative growth. The result of our study was similar to those of researchers working with different plants.

Table 3 and Figure 3 shows that the membrane damage of leaf cells varies between 0.34% and 22.83% depending on the water stress levels. While the lowest value was obtained from the control application, plants grown without irrigation had the highest value.

Arslan (2011) stated that tissue membrane permeability is an expression of the ability of plants to maintain membrane integrity under stress. As a result of cell membrane damage of the plants grown under stress conditions, the water-soluble substances migrate into intercellular spaces, which increase the tissue electrical conductivity value. So it is known that there is an inverse relationship between tissue electrical conductivity values and membrane integrity. Kucukkomurcu (2011) reported that in the okra leaves had damage at different proportions resulted in drought. Deveci and Celik (2016) and Kaya and Dasgan (2013) reported that decreases in water use increased electrolyte leakage.

The total chlorophyll amount of okra plants which are grown under different water constraints and the statistical significance rankings of these averages are shown in Table 3 and Figure 3. l, the highest total chlorophyll amount was obtained from the control application (55.76 SPAD) and the lowest total chlorophyll amount was found in 0% application (31.02 SPAD)

Table 3. Effect of different water restraints on leaf-relative water content (%), membrane damage index (%) and total chlorophyll content (SPAD) and groups according to LSD test*

	Water constraint Levels			
	% 0	% 25	% 50	% 100
Leaf relative water content (%)	47.58 d	59.35 c	77.74 b	98.11 a
Membrane damage index (%)	22.83 a	5.85 b	1.24 b	0.34 b
Total chlorophyll content (SPAD)	31.02 d	37.48 c	44.56 b	55.76 a

*There is no difference between the averages carrying the same letter as 0.01

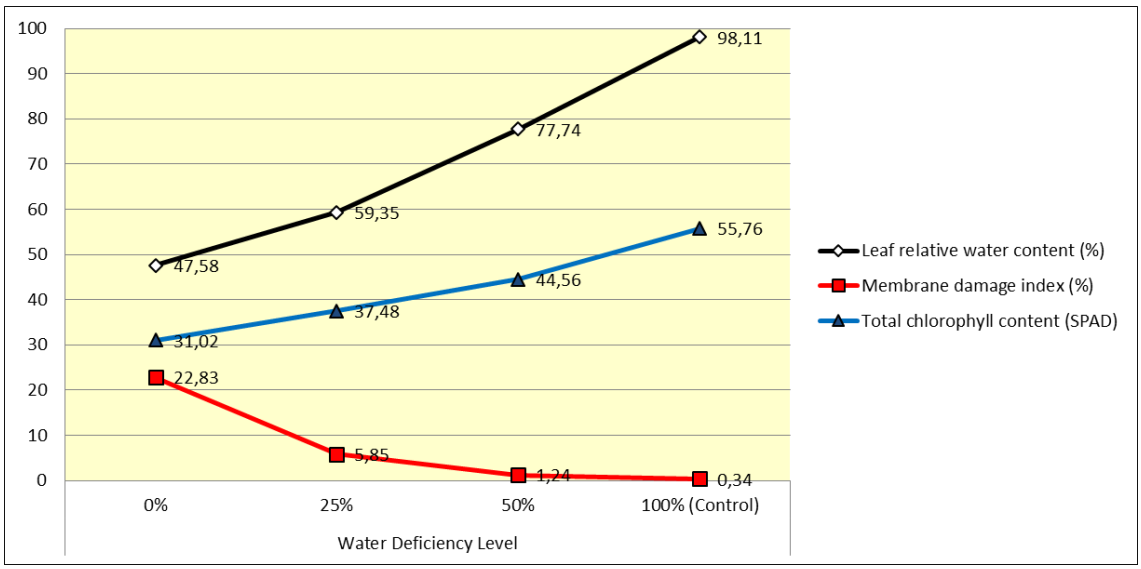

Figure 3.Effect of different water restraints on leaf-relative water content (%), membrane damage index (%) and total chlorophyll content (SPAD) of okra plants

It has been found that the total chlorophyll amount decreases with increasing water stress compared to the control application. Reductions in the amount of chlorophyll with water stress are generally caused by the damage of the chlorophyll membranes (Yagmur, 2008). Changes in the total

amount of chlorophyll are consistent with the findings of other investigators (Deveci and Celik 2016).

Conclusions

As results, it has been determined that as the amount of water restriction goes from control to 0%, the leaf water potential decreases and the plants grown at 0% water constraint show severe damage symptoms. It has also been found that the amount of leaf-relative water content and the total amount of chlorophyll are reduced in a manner contrary to the increase in drought stress. Contrary to other criteria, membrane damage increased when the amount of applied water was decreased.

References

Akdemir, B., B. Kayisoglu and I. Kavdir, 1994. The use of MSTAT statistic package program. Trakya Univ. Faculty of Agriculture Publication No: 203, Supplementary Course Book No: 7, Tekirdag.

Arora, A., R.K. Sairam and G.C. Srivastava, 2002. Oxidative stress and antioxidative systems in plants, Curr. Sci., 82: 1227–1238.

Arslan, A. 2011. Enhancing drought stress tolerance in pepper at seedling stage by 24-epibrassinolid (EBL) applications. MSc Thesis, 105 p. Sutcu Imam University, Institute of Natural and Applied Sciences, Department of Horticulture, Kahramanmaras, Turkey.

Blum, A. 1986. Breeding crop varieties for stress environments. Critical Reviews in Plant Sciences, 2: 199-237.

Cirak, C. and E. Esendal, 2006. Soy disease stress. Ondokuz Mayıs University Journal of Faculty of Agriculture 21 (2): 231-237

Deveci, M. and A. Celik, 2016. The Effect of different water deficiency on physiological and chemical changes in cape gooseberry (*Physialis peruviana* L.) which were grown in greenhouse conditions. Scientia Agriculturae, 14(2): 260-265.

Fan, S. and T. Blake, 1994. Absisic acid induced electrolyte leakage in woody species with contrasting ecological requirements. Physiologia Plantarum, 90(2): 414-419.

Geravandi, M., E. Farshadfar and D. Kahrizi, 2011. Evaluation of some physiological traits as indicators of drought tolerance in bread wheat genotypes. Russian Journal of Plant Physiology, 58 (1): 69–75

Kalefetoglu, T. and E. Ekmekci, 2005. The effects of drought on plants and tolerance mechanisms, G.U. Journal of Science, 18(4): 723-740.

Kaya, C. and D. Higgs, 2003. Supplementary KNO_3 improves salt tolerance in bell pepper plants, J. of Plant Nutr., 26(7): 1367-1382.

Kaya, E. and H.Y. Dasgan, 2013. Screening of the bean genotypes for their tolerance to salinity and drought stresses at the early plant growth phase. Cukurova University, Journal of Science and Engineering, 29 (2): 39-48.

Kucukkomurcu, S. 2011. Screening of the okra genotypes for their tolerance to salinity and drought stresses. MSc Thesis. Çukurova University Institute of Natural and Applied Sciences Department of Horticulture, Adana, Turkey.

Scholander, P.F., H.T. Yamel, E.D. Bradstreet and E.A. Hemmingsen, 1965. Sap pressure in vascular plants. Science, 148: 339-346.

Salk, A., L. Arin, M. Deveci and S. Polat, 2008. Special vegetable growing. In Turkish, Tekirdag, p.488, ISBN 978-9944-0786-0-3.

Sanchez, F.J., E.F. Andres, J.L. Tenorio and L. Ayerbe, 2004. Growth of epicotyls, turgor maintenance and osmotic adjustment in pea plants (*Pisum sativum* L.) subjected to water stress. Field Crops Research, 86: 81-90.

Taiz, L. and E. Zeiger, 2008. Plant physiology (translation from third edition, translation editor Ismail Turkan). Palme Publishing. 893p. Ankara, Turkey.

Tuna, A. L., C. Kaya and M. Ashraf, 2010. Potassium sulfate improves water deficit tolerance in melon plants grown under glasshouse conditions. Journal of Plant Nutrition, 33(9): 1276-1286.

Turkes, M. 1997. On the concepts of weather and climate. TUBITAK Science and Technical Journal, 355: 36-37.

Turkan, I., M. Bor, F. Ozdemir and H. Koca, 2005. Differential Responses of Lipid Peroxidation and Antioxidants in the Leaves of Drought-Tolerant *P.acutifolius* Gray and Drought Sensitive *P. vulgaris* L. Subjected to Polyethylene Glycol Mediates Water Stres. Plant Science, 168: 223-231.

Yagmur, Y. 2008. Investigation of some physiological and biochemical tolerance parameters against drought stress of different grapevine (*Vitis vinifera* L.) cultivars. MSc Thesis, 108 p. Ege University Institute of Natural and Applied Sciences Department of Biology, İzmir, Turkey.

Yildirim, Y.E. and S. Kodal, 1998. Effect of irrigation water on corn grain yield in Ankara Conditions, Tr. J. of Agriculture and Forestry, 22: 65-70.

Effects of Seedling Age, and Different Levels of N, K and K/N on Quality and Yield of Tomato Grown in Perlite Bag Culture

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The object of the study was to investigate the effects of seedling age, and different levels of N, K and K/N on quality and yield of tomato grown in perlite bag culture. Seeds of Big Boy F1 sown in perlite and subsequently seedlings were planted in ten-liter PE bag filled with coarse perlite. Four fertigation regimes were applied to the plants concerning four N (103, 110, 113 and 120 mg l^{-1}), four K (186, 195, 258 and 275 mg l^{-1}) and two K/N (1,6 and 2,5) levels. Thrace element levels were the same for all the fertigation regimes. Seedling were planted at three different growth stages; *1)*appearance of first inflorescence (7 week-old), *2)*50% flower opening on first truss (8,5 week-old) and *3)*fruit set on first truss (10,5 week-old). Although effects of seedling age on total, marketable and early yield, and cracking and blossom-end rot occurrence were not statistically significant, total, marketable and early yield were higher when the seedling were planted at the time of fruit set on first truss. The highest total, early and marketable yields were obtained from the transplants planted at fruit set on first truss with FR1 (plants were fertigated with the high K/N starter solution: K/N= 2,5; 110 mg l^{-1} N and 275 mg l^{-1} K until the fruits on the first truss reached 2cm in diameter, then with the low K/N mainfed solution; K/N= 1,6; 120 mg l^{-1} N and 195 mg l^{-1} K for the rest of the vegetation).

Keywords: Seedling age, K/N ratio, soilless culture, tomato, nitrogen, potassium

Introduction

A common problem associated with hydroponically grown plants is the excessively strong growth can occur after planting the seedlings. Strong plants are difficult to train and suffer from poor flower set.

The seedlings should be planted when the first truss is well in flower, or a little earlier for late crops, to encourage good early fruit development. It may be possible to delay planting a little longer providing the seedling in perlite pots are given adequate amount of nutrient in bright weather. But in our country, seedlings are grown in modules filled with peat and are planted at 3-4 true leaf stage. This makes the control of early season vigour difficult.

The proposed remedies to control the vegetative vigour are; to restrict water availability by increasing the conductivity of the nutrient solution until the fruit load takes over, then reduce the conductivity gradually (Day, 1991) or to use nitrogen restriction or low salt nutrient regime by using solution relatively low in nitrogen but high in potassium. In perlite growing systems, the high substrate capillary makes it very difficult to restrict water and once salts built up they are difficult to displace by flushing out with plain water or low nutrient solution.

Therefore stage of planting, different levels of N, K and N/K ratios are tried for curbing excessive early season vigour, for improving fruit yield and quality and for encouraging good early fruit development of tomato grown in perlite bag culture in this experiment.

Materials and Methods

Experiment was conducted in spring-early summer growing period in polythene house. Seeds of Big Boy F1 were sown in black PE bag filled with coarse perlite.

Four fertigation regimes concerning various N and K levels and K/N ratios applied to the plants were:
Fertigation regime 1 (FR1): plants were fertigated with the high K/N ratio starter nutrient solution (NS1: 110 mg l^{-1} N and 275 mg l^{-1} K; K/N ratio= 2,5; pH=5,8; EC=2000 µmhos cm^{-1}; 4,9% of total N is NH_4-N) until the fruits on the first truss reached 2cm in diameter, then with the low K/N ratio mainfed nutrient solution (NS2: 120 mg l^{-1} N and 195 mg l^{-1} K, K/N ratio= 1,6; pH=6,2; EC=2200 µmhos cm^{-1}; 4,5% of total N is NH_4-N) for the rest of the vegetation period.
Fertigation regime 2 (FR2): plants were fertigated with the low K/N ratio nutrient solution (NS2: 120 mg l^{-1} N and 195 mg l^{-1} K, K/N ratio= 1,6; pH=6,2; EC=2200 µmhos cm^{-1}; 4,5% of total N is NH_4-N) for the entire vegetation period.
Fertigation regime 3 (FR3): plants were fertigated with the high K/N ratio nutrient solution (NS3: 103

mg l^{-1} N and 258 mg l^{-1} K; K/N ratio= 2,5; pH=5,5; EC=2000 µmhos cm^{-1}; 5,3% of total N is NH_4-N) until the fruits on the first truss reached 2cm in diameter, then with the low K/N ratio nutrient solution (NS4: 113 mg l^{-1} and 186 mg l^{-1} K, K/N ratio= 1,6; pH=6,2; EC=2200 µmhos cm^{-1}; 4,8% of total N is NH_4-N) for the rest of the vegetation period.

Fertigation regime 4 (FR4): plants were fertigated with the low K/N ratio nutrient solution (NS4: 113 mg l^{-1} N and 186 mg l^{-1} K, K/N ratio= 1,6; pH=6,2; EC=2200 µmhos cm^{-1}; 4,8% of total N is NH_4-N) for the entire vegetation period.

Nutrition solution (NS) contents, after injection, including ions from water and diluted acid solution of HNO_3;%3+H_3PO_4;%7 (used for balancing the pH of nutrient solution; injection rate: 1/100) were, (mg l^{-1});

NS1:110N; 51P; 275K; 125Ca; 31Mg; 1,5Fe; 111S; 0,7Mn; 0,3B; 0,2Zn; 0,2Cu; 0,05Mo (K/N=2,5; K/Ca=2,2; Ca/Mg=4,0; EC=2000µmhos cm^{-1}; pH=5,8 and 4,9% of total N is NH_4-N)

NS2: 120N; 51P; 195K; 125Ca; 39Mg; 1,5Fe; 79S; 0,7Mn; 0,3B; 0,2Zn; 0,2Cu; 0,05Mo (K/N=1,6; K/Ca=1,5; Ca/Mg=3,2; EC=2200µmhos cm^{-1}; pH=6,2 and 4,5% of total N is NH_4-N)

NS3: 103N; 51P; 258K; 125Ca; 25Mg; 1,5Fe; 104S; 0,7Mn; 0,3B; 0,2Zn; 0,2Cu; 0,05Mo (K/N=2,5; K/Ca=2,0; Ca/Mg=5,0; EC=2000µmhos cm^{-1}; pH=5,6 and 5,3% of total N is NH_4-N)

NS4: 113N; 51P; 186K; 125Ca; 34Mg; 1,5Fe; 75S; 0,7Mn; 0,3B; 0,2Zn; 0,2Cu; 0,05Mo (K/N=1,6; K/Ca=1,5; Ca/Mg=3,7; EC=2200µmhos cm^{-1}; pH=6,2 and 4,8% of total N is NH_4-N)

Chemical contents of water used for diluting stock solutions were; pH=8,2; EC=400 µmhos cm^{-1}; HCO_3=189 mg l^{-1}; Mg^{++}=7 mg l^{-1}¸ Ca^{++}=36 mg l^{-1}

Seedlings were planted at three different growth stages; *1)*appearance of first inflorescence; 7 week-old (AI), *2)*50% flower opening on first truss; 8,5 week-old (FO) and *3)*fruit set on first truss; 10,5 week-old (FS).

All side shoots of plants, in all seedling ages, removed periodically when shoots were 3-5cm long. Plants were topped to leave 4 trusses on a plant and fruits were thinned to leave 5 fruits.

7g N m^{-2} (calcareous ammonium nitrate, 26% N) and 12g K m^{-2} (K_2SO_4, 42% K) was applied in the soil parcel every 15 days as dry fertilizers.

Results and Discussion

Seedling age and fertigation regimes main effects did not significantly influenced total, marketable and early yield. The effect of seedling age on yield differed depending on fertigation regimes. Total and early yields of FS seedling were higher from fertigation regime 1 (FR1) and fertigation regime 2 (FR2) than those from fertigation regime 3 (FR3) and fertigation regime 4 (FR4). It should be noted that FR1 was the one of the nutrient regimes which K/N ratio was reduced from 2,5 to 1,6 when the fruits on the first truss reached 2cm in diameter and FR2 was the the one of the nutrient regimes which K/N ratio was 1,6 for the entire vegetation period and only differences in same K/N ratio regimes were N and K content of the solution (Table 1).

Table 1. Effects of seedling age and fertigation regimes on total, marketable, and early yield of tomato (g $plant^{-1}$)

	Total yield				Early yield				Marketable yield			
	AI	*FO*	*FS*	mean	*AI*	*FO*	*FS*	mean	*AI*	*FO*	*FS*	mean
FR1	2478^{ab}	2306^{ab}	3154^{a}	2646	1283	1115	1540	1313	1942	2140	2982	2355
FR2	2563^{ab}	2573^{ab}	3007^{a}	2714	1270	1258	1363	1297	2234	2276	2911	2474
FR3	2448^{ab}	2371^{ab}	2589^{ab}	2469	1168	1175	1223	1189	2099	2168	2371	2213
FR4	2511^{ab}	2666^{ab}	2450^{ab}	2543	1210	1208	1118	1179	2268	2326	2170	2255
soil	-	-	-	1834^{b}	-	-	-	1065	-	-	-	1770
mean	2500	2479	2800	-	1233	1189	1311	-	2136	2228	2609	-

AI:appearance of first inflorescence (7 week-old); *FO*: 50% flower opening on first truss (8,5 week-old); *FS*: fruit set on first truss (10,5 week-old); *FR1*: K/N ratio of nutrient solution decreased from 2,5 (110 mg/l N and 275 mg/l K) to 1,6 (120 mg/l N and 195 mg/l K); *FR2*: K/N ratio of nutrient solution was 1,6 (120 mg/l N and 195 mg/l K) for the entire vegetation period; *FR3*: K/N ratio of nutrient solution decreased from 2,5 (103 mg/l N and 258 mg/l K) to 1,6 (113 mg/l N and 186 mg/l K); *FR4*: K/N ratio of nutrient solution was 1,6 (113 mg/l N and 186 mg/l K) for the entire vegetation period. %5 LSD for combinations= 1211.

Similar total and marketable yields with FR1 and FR2, as in with FR3 and FR4, indicate that, in addition to K/N ratio, N and K content of the solutions seems to have an influence on the yield.

The effect of K/N ratio of solution on yield was depended on N and K content of the solutions. In FR1 and FR3, K/N ratio of the nutrient solutions were 2,5 until the fruits on the first truss reached 2cm in diameter, then decreased to 1,6. On the other hand, in FR2 and FR4, K/N ratio of solution was 1,6 for the entire vegetation period. Although, both in FR1 and FR3, K/N ratios were the same, N and K concentrations of nutrient solutions were different. When the K/N ratio was dropped to 1,6 in FR1, N content of solution increased from 110 mg/l to 120 mg/l and K content decreased from 275 mg/l to 195 mg/l, and in FR3, N content of solution increased from 103 mg/l to 113 mg/l and K content decreased from 258 mg/l to 186 mg/l. In the fertigation regimes, in which K/N ratio was 1,6 throughout the vegetation period; N and K content of the solutions were 110 mg/l and 195 mg/l, respectively, in FR2 and were 113 mg/l and 186 mg/l, respectively, in FR4.

Evaluating the effect of fertigation regime and seedling age on yield revealed more information. Highest yields were observed when seedling were transplanted at fruit set on first truss with FR1 and FR2 regimes (Figure 1 and Figure 2).

When seedlings were transplanted at appearance of first inflorescence and 50% flower opening on first truss, although statistically not significant, total and marketable yields were lower with the increasing N concentration of nutrient solution when fruits on the first truss reached 2cm in diameter, related to higher N concentration throughout the vegetation period. On the other hand when seedlings transplanted at the fruit set on first cluster yields were similar whether N content increased or remained the same throughout the vegetation period.

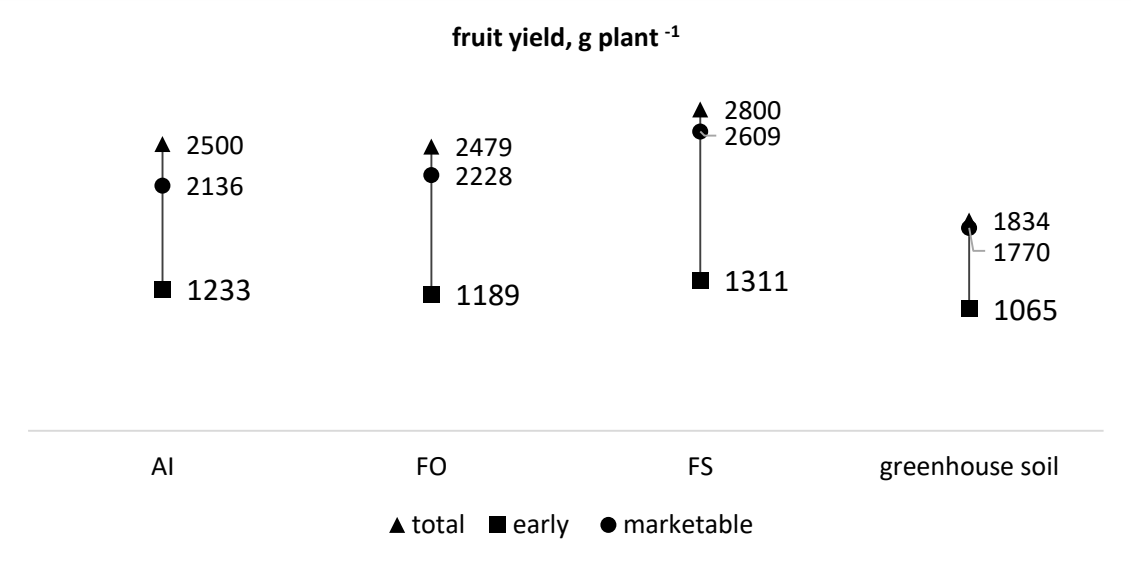

Figure 1. Main effect of seedling age on yield.

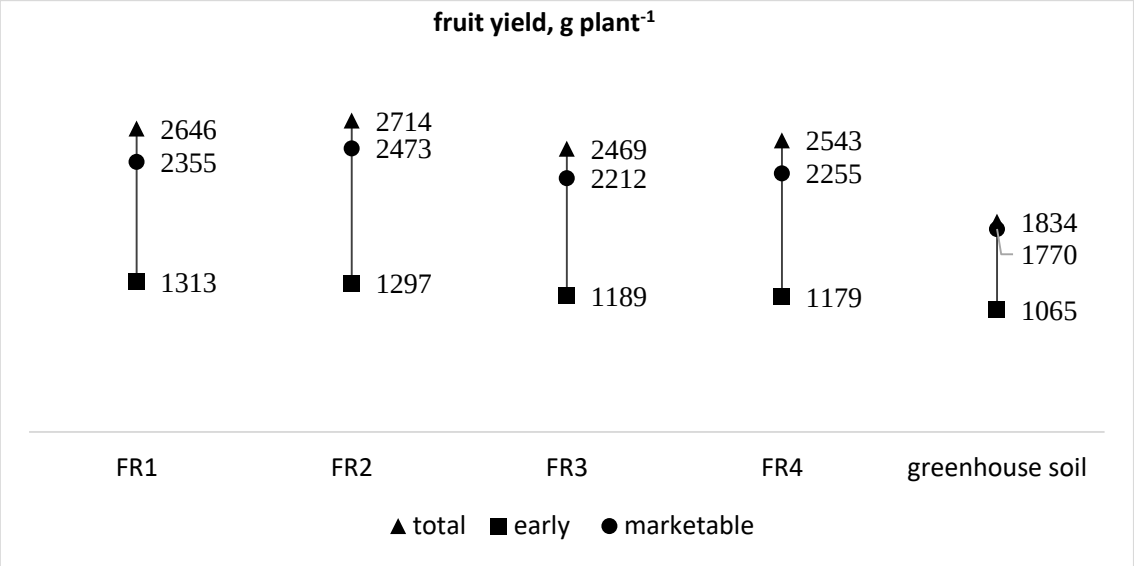

Figure 2. Effect of fertigation regime on yield.

It seems from the results that; restricting nitrogen approach in order to control vegetative vigour and promote early yield also needed to be evaluate together with the N and K contents of solution, as well as with seedling age. Because relative increases in N content (9,0% in FR1 and 9,7% in FR3) and reductions in K content (28% in FR1 and 29% in FR3) were similar in treatments from which similar early yields were observed. As oppose with the suggestion by Vavrina (1991) growth restriction in container with the older seedlings seems to be positively affected early yield. But higher early yields with the older seedlings may be result of fertigation regimes. Transplanting the seedlings at the time of fruit set on first truss and lowering K/N ratio as long as N and K concentrations of the nutrient solution kept relatively higher, promoted early yield.

Due to the scarce of cheap energy sources, one of the most important advantages of the greenhouse growing for the region is early harvest. In this study early yield refers to the fruits harvested until the beginning of the harvest in open field. Seedlings of all ages in this study were spaced more widely and youngest seedling age was considerably older than commerciall ones. Although 7-week old seedlings may seem impractical on a commercial scale as suggested by Vavrina and Orzolek (1993), it may be necessary to use relatively older seedlings to control excessively strong growth after transplanting and to encourage good early fruit development in hydroponic growing. Our findings indicate that adjusting of nutrient regimes according to specific growing period may be beneficial to obtain earlier fruit development with relatively older seedlings.

Fertigation regimes and seedling age affected individual fruit weight. The highest individual fruit weights were observed with FR1 in all seedling ages (Figure 3).

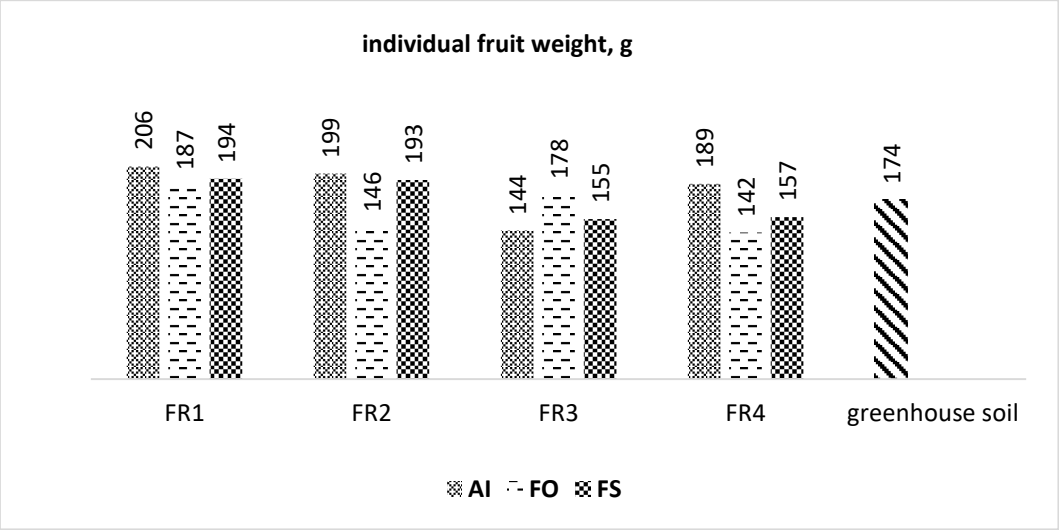

Figure 3. Effects of seedling age and fertigation regime combinations on individual fruit weight

Though similar marketable fruit numbers obtained from seedlings transplanted at fruit set on first truss with FR1, FR2 and FR3 (Figure 4), marketable yield was 25% higher in FR1 and FR2 than it was in FR3.

It seems that, higher marketable yields in FR1 and FR2 were result of higher individual fruit weight. Despite the fact that, seedlings transplanted at at fruit set on first truss with FR3 were manifested in more first class fruit, fruit sizes in this group were either large or small (data not presented). And despite the similar marketable fruit weight, marketable fruit number, individual fruit weight and relative percentage of BER incidence, seedlings transplanted at fruit set on first truss with FR1 produced more first class fruit (Figure 4).

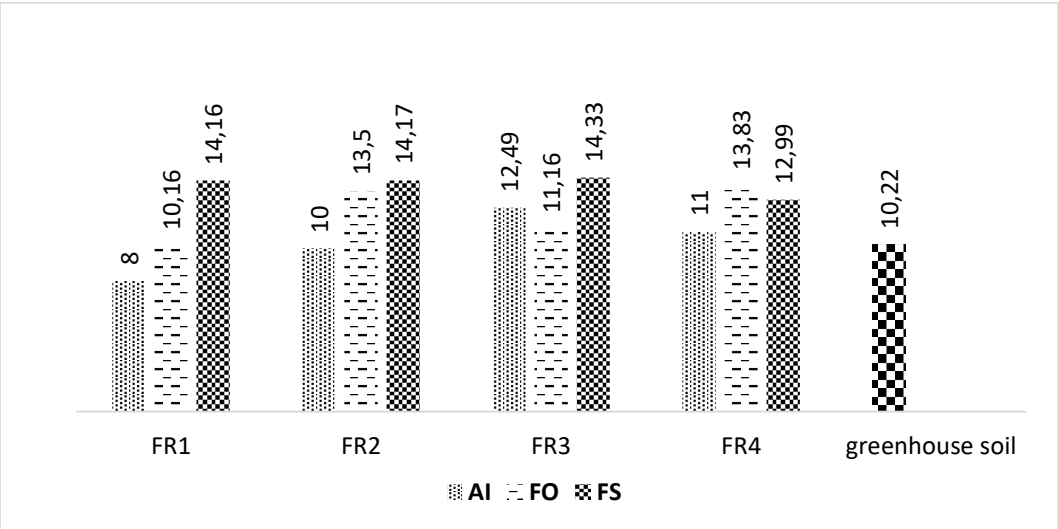

Figure 4. Effects of seedling age and fertigation regime combinations on marketable fruit number

The impact of fertigation regimes on blossom-end rot (BER) incidence varied in accordance with seedling age. It was varied from 5%, to 22% in FR1, from 3%, to 13% in FR2, from 8%, to 14% in FR3 and from 10%, to 11% in FR4 (Table 2).

Table 2. Relative percentage of first class fruit weight, and fruit cracking and blossom-end rot incidence to total fruit weight (%)

	Blossom-end rot				Fruit cracking				First class fruit			
	AI	*FO*	*FS*	mean	*AI*	*FO*	*FS*	mean	*AI*	*FO*	*FS*	mean
FR1	22	7	5	11	53	76	53	60	24,9	17,3	41,6	29
FR2	13	12	3	9	79	52	67	66	8,2	36,8	29,7	25
FR3	14	9	8	10	52	78	44	58	33,6	13,0	48,0	32
FR4	10	13	11	11	78	62	69	69	11,6	25,6	20,2	19
soil	-	-	-	3	-	-	-	12	-	-	-	84
mean	15	10	7	-	66	66	58	-	19	24	35	-

AI:appearance of first inflorescence (7 week-old); *FO*: 50% flower opening on first truss (8,5 week-old); *FS*: fruit set on first truss (10,5 week-old); *FR1*: K/N ratio of nutrient solution decreased from 2,5 (110 mg/l N and 275 mg/l K) to 1,6 (120 mg/l N and 195 mg/l K); *FR2*: K/N ratio of nutrient solution was 1,6 (120 mg/l N and 195 mg/l K) for the entire vegetation period; *FR3*: K/N ratio of nutrient solution decreased from 2,5 (103 mg/l N and 258 mg/l K) to 1,6 (113 mg/l N and 186 mg/l K); *FR4*: K/N ratio of nutrient solution was 1,6 (113 mg/l N and 186 mg/l K) for the entire vegetation period.

Blossom-end rot occurrence was lowest when the seedling were planted at the time of fruit set on first truss (Figure 5). As in BER incidence, the effect of fertigation regimes on fruit cracking varied in relate to seedling age. Fruit cracking ratios were between 53%-76% in FR1, between 52%-79% in FR2, between 44%-78% in FR3 and varied from 62%, to 78% in FR4. Reducing K/N ratio from 2,5 to 1,6 when fruits on the first truss reached 2cm in diameter had an improving effect on fruit cracking when seedling transplanted either Aİ or FS (Table 2).

First class fruit ratio was lower with treatments that of fruit cracking ratios were higher. The highest first class fruit ratio was observed with transplanting at fruit set stage in reducing K/N ratio fertigation regimes.

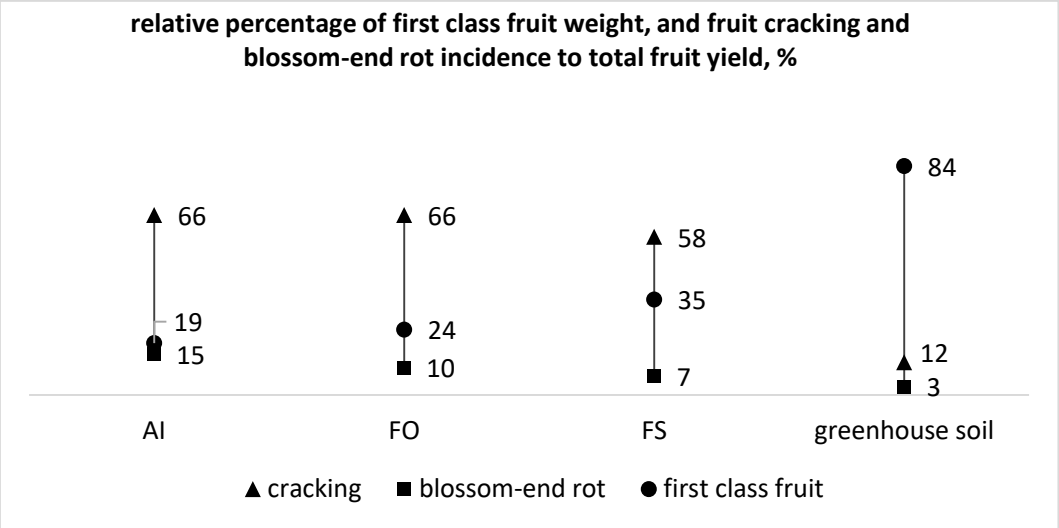

Figure 5. Main effect of seedling age on blossom-end rot incidence, fruit cracking and first class fruit ratio

Conclusions

Regardless of seedling age, it can be speculated that, whether K/N ratio was decreased from 2,5 to 1,6 or remained 1,6 throughout the vegetation period, the effect of K/N ratio on yield relates to N and K content of the solution. And higher yields observed when N and K content of solution were higher, since the N and K content of the solution in FR1 were higher than those of FR3 and similarly they were higher in FR2 than those of FR4.

With regard to seedling age, it may be suggested that younger transplants produce higher marketable fruits when K/N ratio of solution was 1,6 throughout the vegetation period while older transplants produce higher marketable yields when K/N ratio of solution reduced from 2,5 to 1,6 when the fruits on the first cluster reached 2cm in diameter.

Transplanting the seedlings at the time of fruit set on first truss and lowering K/N ratio as long as N and K concentrations of the nutrient solution kept relatively higher, promoted early yield. As the highest total, early and marketable yields were obtained from the transplants planted at fruit set on first truss with FR1 (plants were fertigated with the high K/N starter solution: K/N= 2,5; 110 mg l^{-1} N and 275 mg l^{-1} K until the fruits on the first truss reached 2cm in diameter, then with the low K/N mainfed solution; K/N= 1,6; 120 mg l^{-1} N and 195 mg l^{-1} K for the rest of the vegetation period), this combination can be recommended for the growers. Hydroponic growers in our country also can benefit using transplants which have fruit set on the first truss instead of seedling at 3-4 true leaf stages to achieve early harvest and get more early yield. This also makes the control of early season vigour easy.

Further researches can be made with low conductivity (1500 µmhos cm^{-1} or lower) mainfeed solutions for the summer to keep the conductivity of the solution less than 3000 µmhos cm^{-1} in perlite bag culture. Since the effect of seedling age varied according to nutritional regimes, further research is needed to evaluate pre and post transplant nutritional regimes. And to keep the fruit craking and blossom-end rot ratio down, beneficial practices should be investigated on tank aeration and greenhouse temperature reduction as well as investigation of varieties resistant to fruit cracking and blossom-end rot.

References

Day, D. 1991. Tomatoes. Grower Digest 9, Grower Publications Ltd., London.

Vavrina, C.S. 1991. Effect of transplant age on tomato production. Proc. Fla. State Hort. Soc., 104:225-226.

Vavrina, S.C., M.D. Orzolek, 1993. Tomato transplant age: A review. HortTechnology, 3(3):313-316.

Vine and Berry Responses to Severe Water Stress in Different Stages in cv. Syrah (*Vitis vinifera* L.)

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This experiment was carried out during the 2009 growing season in order to evaluate the ecophysiology and quality characteristics of *cv*. Syrah (*Vitis vinifera* L.) grafted onto SO4 in the ECOTRON vineyard in Montpellier SupAgro/INRA in France. The aim of this study was to analyse the effect of sudden and severe water stress (SWS) on the ecophysiological changes, volume losses and shrivellings in grape berries. Also possibilities of existence of recovery, and its possible relationship with SWS and final grapes composition at different phenological stages in cv. Syrah were studied. Three water regime levels; Control (only very mild water stress) and 2 severe water stress levels, SWS1 and SWS2 were established depending on the Ψ_{pd}. Stressed periods were started at the BV, MM and EM phenological stages. A randomized block design was used. The experimental plots consisted of 54 vines totally. All data analyses were performed with MSTAT-C Statistical Software (Michigan State University) and LSD tests were done for significant differences of measured traits between groups. Minimum Ψ_{pd} values in BV were -0.28MPa, -1.79MPa, -2.16MPa¸ in MM were -0.14MPa; -1.58MPa, -2.72MPa, in EM were -0.16MPa; -2.37MPa, -2.76MPa for Control, SWS1 and SWS2 respectively. Notice that such low predawn total leaf water potential are really exceptional, and at that level the regulation of the osmotic potential is critical. In the berry volume a limited recovery determined after about 13.00-26.00% volume loss for SWS1 and SWS2 respectively along stressed periods. At harvest; berry volume (cm^3), 100-berry weight (g), total soluble solids (°Brix), pH, total acidity (g tartaric acid L^{-1}), Tartaric acid (g L^{-1}), K (g L^{-1}), TPI and Anthocyanins (mg L^{-1}) were analysed. Berry volume loss rate (%) and mg sugar per 1g berry were calculated. SWS had a negative effect on the sugar per 1g berry, 100 berry weight, berry volume, TSS and positive effect on the anthocyanin concentrations and TPI at three phenological stages. This can open new ways for irrigation monitoring in combination with too high berry sugar content due to the climate change.

Keywords: Syrah, leaf water potential (Ψ_{leaf}), water stress, volume loss, recovery.

Introduction

Climate irregularity and consequently water stress are some of the most important worldwide problems in recent years. Water stress may greatly influence grape and vine metabolism. Furthermore, stress levels at different stages may lead to important physiological alterations that will affect vine yield and grape composition.

The water status of the vines has a significant influence on berry growth, at the herbaceous growth stage and during the period from veraison to maturity (Ojeda et al., 2001; Carbonneau and Bahar, 2009). Early water deficits modify the structural properties of the cell components and consequently cell wall extensibility, thereby limiting the subsequent enlargement of pericarp cell. After veraison berry size reduction due to water deficits is thought to be a consequence of a limitation of cell enlargement (Ojeda et al., 2001). Since development of the pressure chamber (Scholander et al., 1965), measurement of leaf water potential (Ψ_L) has been used as a tool to assess the water status of plants (Jones, 1990). Also in vineyard leaf water potential is considered the most practicable method for the control vine water status**.** Carbonneau (1998) and Deloire et al. (2004) use both pre-dawn (Ψ_{pd}) and mid-day (Ψ_{md}) leaf water potential as a criterion to evaluate vine water status at different developmental stages. Moreover Deloire et al. (2005) and Rogiers et al. (2015) proposed different levels of Ψ_{pd} for various vine styles. Depending on the intensity of the water stress and the period at which it occurs, it may or not be favourable for the harvest and the wine it is used to produce (Deloire et al., 2004).

Severe water stress is known for affecting negatively berry ripening (Coipel et al., 2006). Intensity of water stress is also correlated to production levels. Also berry solutes like organic acids, sugars, anthocyanins and soluble phenolic compounds are sensitive to vine water status (De la Hera Orts et al., 2005; Carbonneau and Bahar, 2009).

Many researches were conducted as to determine the effect of sudden and severe water stress on vine and berry metabolism. Some water fluxes

were simulated in Ecotron (SupAgro/Montpellier) by Carbonneau and Bahar (2009). The manipulation of water limitation towards some extreme values and short periods around veraison, allowed the control of berry size and the differentiation in primary metabolites such as sugars and secondary metabolites such as polyphenols. Bahar et al. (2011a) revealed the importance of sudden and extreme water stress (EWS) on vine physiology and berry composition of three cultivars, namely Chardonnay, Merlot and Cabernet-Sauvignon. Their results showed that, both Ψ_{pd} and Ψ_{md} values were similar when Ψ_{pd} was reduced to -2.1MPa in all three varieties. The results also indicated that all the leaves were dropped and the clusters were fully exposed to sun after EWS treatment, these lead to smaller berries, thus increase of anthocyanin concentration, FCI and PTI values at harvest time. Also there was an increase in pH and total acidity values. On the contrary a decrease in 100 berry weight, berry volume, TSS, sugar concentration, sugar content per berry, K and tartaric acid was dermined. Bahar et al. (2011b) analyzed the effects of Severe Water Stress (depend on Ψ_{leaf}) to determine the ratio of berry shrivelling, possibilities of recovering, changes of berry composition during the stress period in the lag phase and its relationship with yield and grape composition. Because of SWS more than 50-60% of the leaves dried from the base to upper part of shoots in vines. Despite SWS the vines did not die and there was recovering after irrigation. Full recovery of berry sugar loading and concentration was not possible just after the period of stress.

The response of polyphenol in terms of hue attesting the real change in colour at veraison (days following the period of stress) was quite independent of the evolution of sugars which lead to berries rich enough in polyphenol with less sugars. Thus berry characteristics at harvest which correspond to a sugar maturity for both SWS1 and SWS2 water limitation between bunch closure and veraison stages leads to a significant increase of total polyphenol and anthocyanin.

The manipulation of water limitation towards some extreme values and short periods between bunch closure and veraison, allows to control berry size, primary metabolites and secondary metabolites. Because of SWS after 20% volume loss (shrivelling) there was a possibility of recovering of the shrivelled berries. The growth of berries continued but at harvest time almost 50% of the clusters shrivelled after the stress period. According to Korkutal et al. (2011) pollen viability and pollen germination ratios were not affected by early water stress conditions, but berry set ratio, phenologic stages and berry development were negatively affected. It was determined that in cv. Merlot, Ψ_{pd} values below -0.4MPa, should be avoided between 19th to 29th stages of Eichhorn and Lorenz (1977). Bahar et al. (2012) indicated that under severe water stress yield levels significantly and sustainably affected sugar concentration, titratable acidity and cluster weights. During the period of decrease of berry volume, berry sugar loading was stabilized by any stress during its application meanwhile it was increasing for the control. Because of water loss there was an increase in berry density and decrease in berry volume. It looks like there was an increase in sugar concentration but actually there was no increase in sugar content per berry.

The manipulation of water limitation towards some extreme values and short periods between bunch closure and veraison, allows to control berry size and to differentiate primary metabolites. According to yield level and SWS level and SWS period there was a possibility of recovering after about 15-20% volume loss in the shrivelled berries. After the stress period the growth of berries continued but at harvest time almost 58-81% of the clusters were partly or completely damaged according to stress levels.

As it was seen there are many possibilities for the development of new strategies in combating drought in vineyards. The aim of this study was to analyse the effect of sudden and severe water stress (SWS) on the ecophysiological changes, volume losses and shrivellings in grape berries. Also possibilities of existence of recovery, and its possible relationship with SWS and final grapes composition at different phenological stages in cv. Syrah were studied.

Materials and Methods

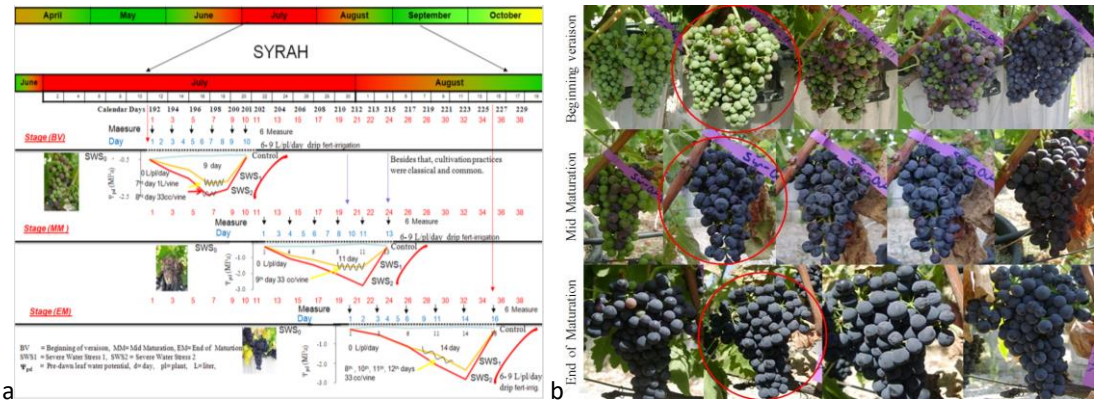
Plant material and location

The experiment was carried out during the 2009 growing season on Syrah (*Vitis vinifera* L.) grafted onto SO4 in the ECOTRON of the campus of Montpellier SupAgro/INRA in France inserted in the general experimental vineyard. The eight-year-old grapevines were grown in pots under natural conditions which had 70L volume. The pots were isolated from rainfall and had a controlled drainage and potting media with a mixture of coarse sand

and perlite. Row spacing was 3.5-0.8m and rows were N–S oriented and bilateral cordon trained vines were pruned on a Lyre architecture. Six arms and each had 2-3 nodes which remained, for a shoot load of 10-12 shoots per vine.

The calculated volumes of nutrient solution (6-9 L day^{-1}) were applied regularly every 6 hours for a day through two drip emitter for each plant except during water stressed period of growing season in severe water stress 1 (SWS1) and severe water stress 2 (SWS2) while its permanent for Control [well irrigated (WI)] group. Stress periods of vines were started in 192nd(BV), 203rd(MM) and 211th(EM) days of growth under well watered conditions in the ECOTRON at the Beginning Veraison (BV), Mid Maturation (MM) and End of Maturation (EM) phenological stages (stages 35, 36, 37) (Eichhorn and Lorenz, 1977). Ψ_{pd} of control vines was maintained consistently between -0,30MPa and -0,05MPa during the experimental periods in all three developmental stages.

Two stress levels Severe Water Stress1 (SWS1) and Severe Water Stress2 (SWS2) were established in relation to the reference of maximal transpiration, and monitored in function of the vine response measured as the pre-dawn leaf water potential (Ψ_{pd}) (Carbonneau, 2001). The Ψ_{pd} values for the SWS1 in BV stage were nЄ[-1,83, -0,34] MPa, for MM nЄ[-1,58, -0,27] MPa, as for EM the values were nЄ[-2,35, -0,26] MPa. For the SWS2 the Ψ_{pd} values in BV stage were nЄ[-2,46, -0,41] MPa, for MM nЄ[-2,72, -0,31] MPa, as for EM the values were nЄ[-2,81, -0,27] MPa. The changes in Ψ_{pd} values for all treatments were indicated in detail according to certain days in (Figure 1). For two stress level, vines were irrigated with negligible quantity water during the stressed period. The experimental period for BV was 10 days, as for MM it was 13 days and for EM was 16 days. After the stressed period, potted vines were irrigated one time on the saturation point. Besides that, cultivation practices were classical and common (Figure 1).



a b

Figure 1. Experimental design of research (a) and general appearance of shrivelled grape berries (b).

Physiological measurement

The Ψ_{leaf} of each vine was determined with a Scholander pressure chamber (Scholander et al., 1965). Pre-dawn (Ψ_{pd}) and mid-day (Ψ_{md}) leaf water potentials were measured 6 times from beginning to the end of stress period for each growing stage (total 18-times measurement). Measurements were carried out about 35 days on freshly cut, healthy and fully expanded (mature) leaves from each vines for each of the two stress levels and control. After the first measurements, water and mineral supply cut off during days until pre-dawn leaf water potential (Ψ_{pd}) to be approximately equivalent with mid-day leaf water potential (Ψ_{md}). Those modalities and their distribution over time were illustrated in Figure 1.

Grape and juice analysis

During stressed period in early morning (06:00 to 07:00 AM), 10 berries per vines were sampled from different parts of various clusters and transported to the laboratory. Berry volumes (cm^3) were measured immediately after sampling by Dyostem apparatus (Sferis technology). After that, classical measurements were made on berries. Berries were weighed with an electronic balance and processed

to determine 100-berry weight (g) and then juice extractions were analyzed for total soluble solids [(TSS) (Brix°)], pH and total acidity (g L^{-1}). Total soluble solids [(TSS) (Brix°)] were measured using an Abbé-type refractometer. Juice pH was measured using a pH-meter. Total acidities were measured by pH-meter with a base to an end point of pH 7.0 (20°C), and results were expressed as a g-tartaric acid L^{-1}. Potassium (K) analysis were conducted by flame photometer and expressed as a g L^{-1}. Tartaric acid (g L^{-1}) were analysed according to Cemeroglu (2007). TPI was quantified according to Ribéreau-Gayon (1970). Anthocyanins (mg L^{-1}) were analysed according to Mode d'Opératoire MO-LAB-23 UE Pech Rouge INRA-France (Anonymous, 2007).

Berry volume loss rate was calculated according to the following formula:
Berry volume loss rate (%)= [100 x (Berry volume 2, 3...n – Berry volume 1)]/Berry volume 1

Miligram sugar per 1g berry was calculated according to the following formulas:
Sugar (mg $berry^{-1}$) = [(1/3) x sugar cons. (g L^{-1})] x [(1/100) x 100 berries weight (g)] Carbonneau and Bahar (2009) and mg sugar per 1g berry= (Sugar (mg $berry^{-1}$) x 1)/Berry weight (g)

Experimental design and statistical analysis

A randomized block design was used. There were three blocks of 3 rows. The experimental plots consisted of 54 vines totally (18 vines for SWS1, 18 vines for SWS2, and 18 vines for Control) (Table 1). All data analyses were performed with MSTAT-C Statistical Software (Michigan State University) and LSD tests were done for significant differences of measured traits between groups.

Results and Discussion

During beginning of veraison stage (BV) in Control vines Ψ_{pd} values changed between -0,27MPa and -0,48MPa. Similarly, in other stages [mid-maturation (MM) and end of maturation (EM)] Control vines were non stressed or moderate stressed. Throughout MM and EM stages in Control vines Ψ_{pd} values were between -0,14MPa : -0,30MPa and -0,12MPa : -0,25MPa respectively (Figure 2 a; 3 a and 4 a). In relation to these values average Ψ_{md} in Control (wel irrigated) vines were remained almost -1,25MPa and changed between -0,99MPa and-1,47MPa; -0,84MPa and -1,51MPa; -1,19MPa and -1,53MPa in BV, MM and EM stages respectively (Figures 2 b; 3 b and 4 b).

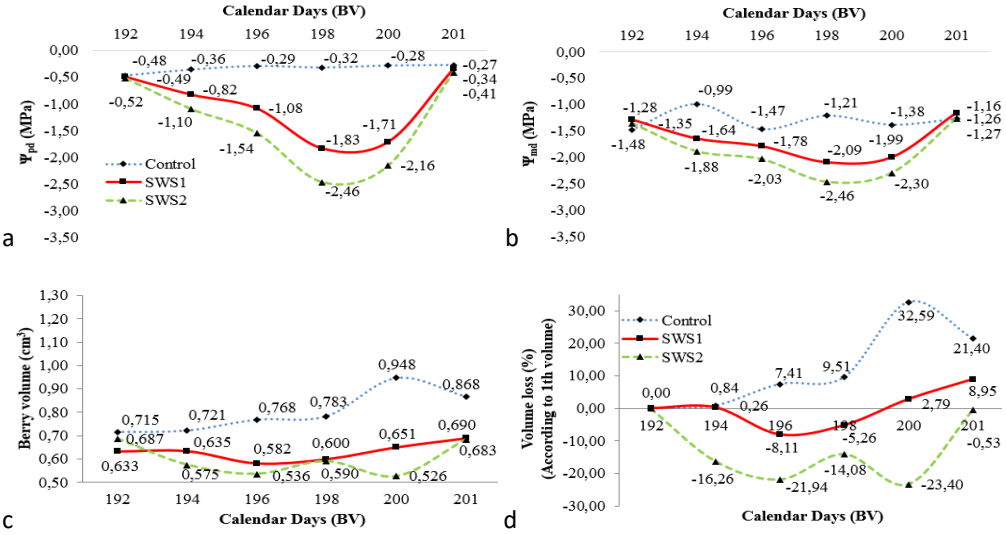


Figure 2. Changes in Ψ_{md}, berry volumes and berry volume losses, depending on Ψ_{pd} during BV.

In addition to Ψ_{pd} levels Ψ_{md} values were also affected by daily conditions (sunlight intensity, sunlight exposure duration, temperature, relative humidity, wind speed and direction, etc.) and in control, they did not fall below the critical limit of -1.6 MPa (Ψ_{md}) and remained compatible with Ψ_{pd} (Figure 2 a, b; 3 a, b and 4 a, b). First water stresses were seen after 194th, 204th and 213th calendar days in BV, MM and EM stages respectively after irrigation cut of. Severe water stresses were started in SWS1 and SWS2 at 5th (BV) and 6th (MM and EM) days after irrigation stopping and were

finished at 200th, 211th and 224th calendar days. When Ψ_{pd} values decreased below to -1.6 MPa Ψ_{md} values were generally below to -2.0MPa in SWS1 and SWS2. Also when Ψ_{pd} decreased below to -2.1 MPa both Ψ_{pd} and Ψ_{md} values were similar in all three phenological stages (BV: Ψ_{pd}= -2.46MPa and Ψ_{md}= -2.46MPa); MM: Ψ_{pd}= -2.72MPa and Ψ_{md}= -3.40MPa and EM: Ψ_{pd}= -2.81MPa and Ψ_{md}= -2.95MPa) in SWS2. 1-2 days after re-irrigation water stresses finished and Ψ_{leaf} values returned to normal at all periods and stress levels. There was a recovery after re-irrigation in stressed vines (Figures 2 a,b,3 a,b and 4 a,b). Similar findings obtained by Carbonneau and Bahar (2009), Bahar et al. (2011a), Bahar et al. (2011b), Korkutal et al. (2011), Bahar et al. (2012).

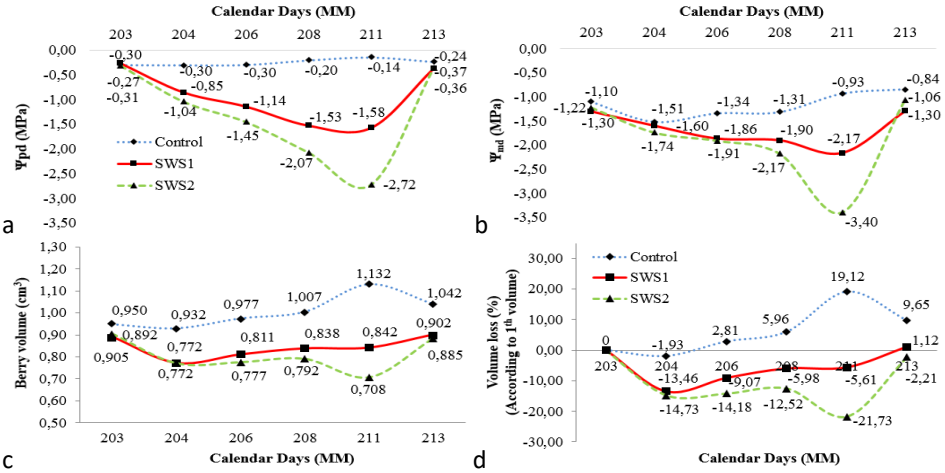


Figure 3. Changes in Ψ_{md}, berry volumes and berry volume losses, depending on Ψ_{pd} during MM.

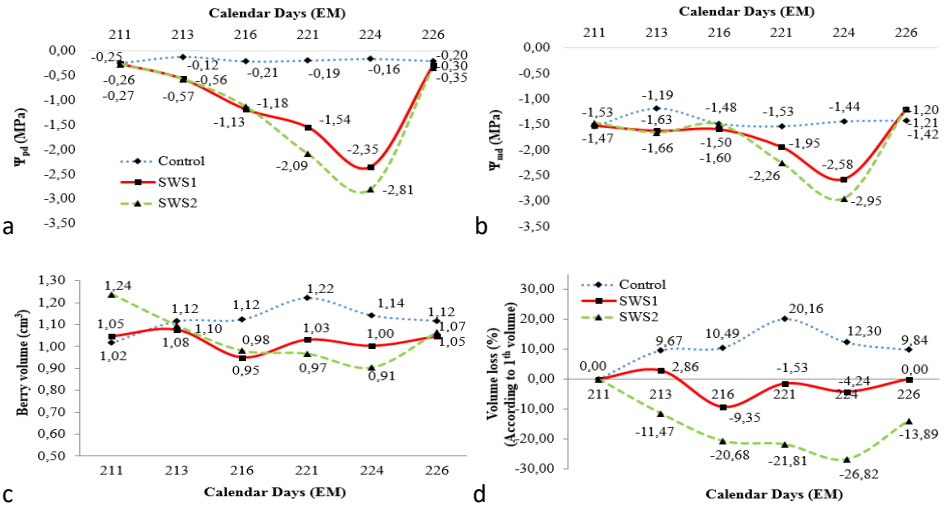


Figure 4. Changes in Ψ_{md}, berry volumes and berry volume losses, depending on Ψ_{pd} during EM.

There were considerable volume losses in berries according to stress levels while berry volumes continued to increase normally in non-stressed vines (Figures 2 c, 3 c and 4 c). These volume losses range from 8.11% (BV) to 9.35% (EM) in SWS1.

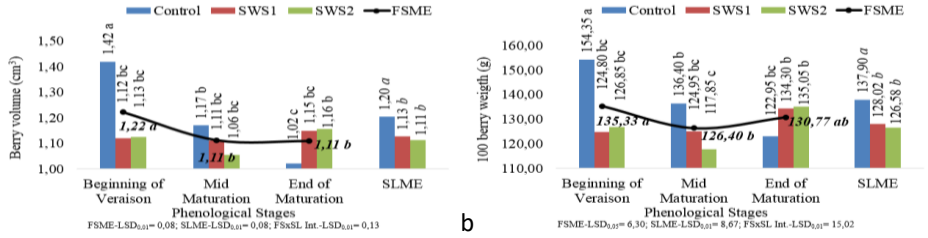


Figure 5. Changes of berry volumes (a) and 100 berry weights (b) in BV, MM and EM stages according to stress levels.

In SWS2, the volume losses were greater than SWS1 and changed from 21.73% (MM) to 26.82% (EM). Shrivelling finished after re-irrigation but there were a limited recovery in all grape berries according to stress levels (Figures 2 d, 3 d and 4 d). Therefore, the final berry volumes and berry weights were also negatively affected by both stress levels (SWS1: 1.13 b and 128.02 b; SWS2: 1.11 b and 126.58 b) during BV (1.22 a; 135.33 a) MM (1.11 b; 126.40 b) and EM (1.11 b; 130.77 ab) stages. (Figure 5 a, b).

Photosynthetic radiation use efficiency strongly depended on both, pre-dawn leaf water potential and light-satured stomatal conductance (Escalona, 2003). TSS ratios decreased with increases in stress levels during BV (21.67 a), MM (19.67 b) and EM (19.87 b) periods. These declines in concentration were reflected to the mg sugar/1 g berry. Especially in SWS2, the decline in sugar concentration (19.77 b) and sugar quantity per 1 g berry (63.12 b) has become very apparent in MM (18.10 e; 56.80 e) and EM (19.10 de; 60.57 de) stages respectively (Figure 6 a, b).

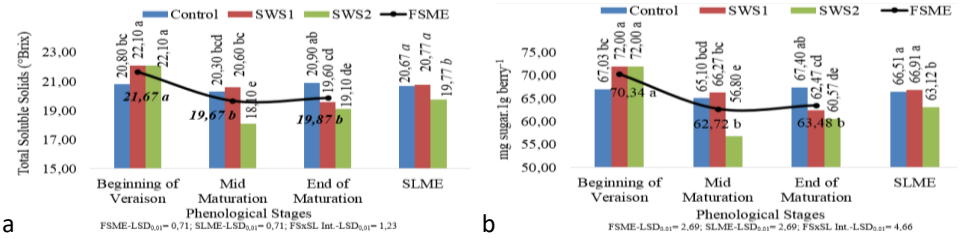


Figure 6. Changes of total soluble solids (TSS) (a) and mg sugar/1 g berry (b) in BV, MM and EM stages according to stress levels.

The final pH and K content of grape juice increased in parallel with the increase in stress levels during BV, MM and EM stages. Therefore, the highest pH was observed in SWS2 (4.11 a), followed by SWS1 (4.09 a) and similar results obtained by Bahar et al. (2011a). The highest potassium content was determined during the BV period in SWS1 (3.88 a). Higher water stresses in the early period (in BV) increased the pH (4.09 a) and K levels (3.51 a) in grape juices. Grape berry potassium accumulation is important because elevated levels of berry potassium can have a negative effect on wine quality by increasing berry and wine pH (Gawel et al., 2000).

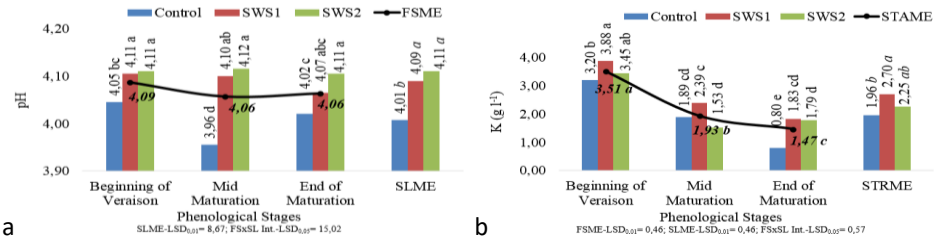


Figure 7. Changes of pH (a) and K (Potassium) (b) in BV, MM and EM stages according to stress levels.

Total acidity was higher in SWS1 (4.36 b) and SWS2 (4.54 a) than control vines (4.11 c). It also varied depending on the periods of stress applied. Especially the effect of stress applied in the later stages of maturation is more apparent (BV: 4.20 b; MM: 4.50 a; EM: 4.31 b). Bahar et al. (2011b) obtained similar results. As noted by Carbonneau and Bahar (2009) depending on the stress level tartaric acid also increased and SWS2 (2.51 a) was the highest.

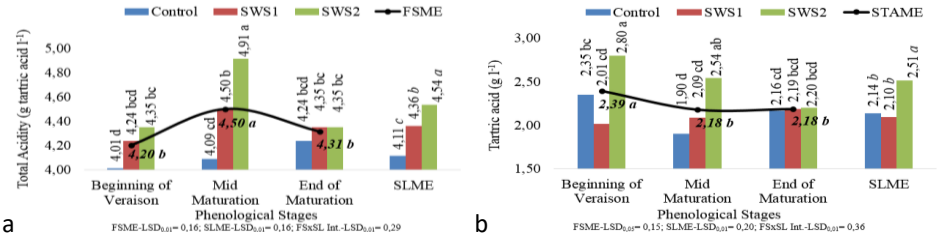


Figure 8. Changes of total acidity (a) and tartaric acid (b) concentrations in BV, MM and EM stages according to stress levels.

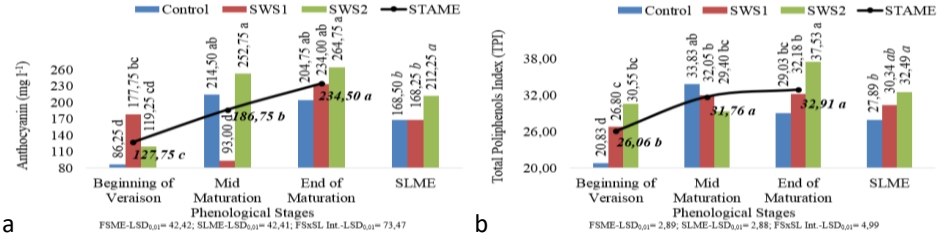


Figure 9. Changes of anthocyanin concentrations (a) and TPI (b) in BV, MM and EM stages according to stress levels.

Conclusions

As a result of evaluating the data obtained from this study: It was determined that sudden and severe water stresses applied at different stages caused significant shrivelling (about 27.00%) in the grape berries. After the sudden and severe water stresses, there was a recovery in the vines and limited one in grapes berries. There was also a decrease in berry volume, 100 berry weight, TSS and mg sugar per 1 g berry depending on periods and stress levels. Furthermore there was an increase in pH, K, total acidity, tartaric acid, anthocyanin and TPI quantities depending on periods and stress levels. In conclusion the stress levels applied at different stages can be strategically used to decrease or increase some problematic criterias depending on terroir in wine varieties such as re-equilibrating the ratio polyphenols/sugars of the grape berry.

References

Anonymous, 2007. Détermination d'Anthocyanes en échantillons de raisin. Mode opératoire MO-LAB-23 UE Pech Rouge INRA-France. 1-2.

Bahar, E. and A.S. Yasasin, 2010. The yield and berry quality under different soil tillage and clusters thinning treatments in grape (*Vitis vinifera* L.) *cv.* Cabernet-Sauvignon. African J Agric Res., 5(21): 2986-2993.

Bahar, E., A. Carbonneau and I. Korkutal, 2011a. The effect of extreme water stress on leaf drying limits and possibilities of recovering in three grapevine (*Vitis vinifera* L.) cultivars. African J Agric Res., 6(5): 1151-1160.

Bahar, E., Carbonneau A. and I. Korkutal, 2011b. The Effect of Lag Phase Water Stress on Berry Shrivelling and Recovering After Irrıigation in *cv*. Cabernet-Sauvignon (*Vitis vinifera* L.) 17th International Symposium GiESCO 2011 Asti - Alba, Italy August 29th - September 2nd.

Bahar, E., I. Korkutal and A. Carbonneau, 2012. Lag phase water stress effect on berry shrivelling and recovering in *cv*. Chardonnay (*Vitis vinifera* L.) Bulletin de l'OIV, 85(974-975-976): 141-154.

Carbonneau, A. 1998. Aspects qualitatifs, 258-276. In Tiercelin, JR (Ed.), Traite d'irrigation. Tec & Doc. Lavosier Ed., Paris, p. 1011.

Carbonneau, A. 2001. Gestion de l'eau dans le vignoble: théorie et pratique. CR GESCO 12 (Comité de Lecture), tome. 'J. Professionale', pp. 3-21.

Carbonneau, A. and E. Bahar, 2009. Vine and berry responses to contrasted water fluxes in Ecotron around Veraison. Manipulation of berry shrivelling and consequences on berry growth, sugar loading and maturation. Proceedings of the 16th International GiESCO Symposium. July 12-15, University of California, Davis, USA. pp. 145-155.

Cemeroglu, B. 2007. Food Analyses. Food Technology Ass. Publication. Number 34, Ankara.

Coipel, J., L.B. Rodriguez, C. Sipp and C. Van Leeuwen, 2006. Terroir effect, as a result of environmental stress, depends more on soil depth than on soil type (*Vitis vinifera* L. cv. Grenache noir, Cotes du Rhone, France, 2000). J Int. Sci. Vigne Vin., 40(4): 177-185.

De La Hera Orts, M.L., A. Martinez-Cutillas, J.M. Lopez-Roca and E. Gomez - Plaza, 2005. Effect of moderate irrigation on grape composition during ripening. Spanish J. Agric. Res., 3(3): 352-361.

Deloire, A., A. Carbonneau, Z. Wang and H. Ojeda, 2004. Vine and water, a short review. J. Int. Sci. Vigne Vin., 38(1): 1-13.

Deloire, A., H. Ojeda, O. Zebic, N. Bernard, J.J. Hunter and A. Carbonneau, 2005. Influence de l'etat hydrique de la vigne sur le style de vin. Le Progres Agricole et Viticole, 122(21): 455-462.

Eichhorn, K.W. and D.H. Lorenz, 1977. Phenological development stages of the grapevine. Nachrichtenbl. Dt. Pflanzenschutzd, 29: 119-120.

Escalona, J.M., J. Flexas, J. Bota and H. Medrano, 2003. Distribution of leaf phytosynthesis and transpiration within grapevine canopies under different drought conditions. Vitis, 42(2): 57-64.

Gawel, R., A. Ewart and R. Cirami, 2000. Effect of rootstock on must and wine composition and the sensory properties of Cabernet Sauvignon grown at Langhorne Creek, South Australia. Aust. N.Z. Wine Ind. J., 15: 67-73.

Jones, J.A. 1990. Valuable information from accurate crop estimation. Adelaide. Aust. Grapegrow. Winemaking, (316): 59-60.

Kok, D. and E. Bahar, 2015. Effects of different vineyard altitudes and grapevine directions on some leaf characteristics of *cv.* Gamay (*Vitis vinifera* L.). Bulgarian J Agric Sci., 21(2): 320-324.

Kok, D. and E. Bal, 2017. Chemical and non-chemical thinning treatments influence berry growth and composition of cv. Shiraz wine grape (*V. vinifera* L.). Erwerbs-Obstbau Doi 10.1007/s10341-017-0321-2.

Korkutal, I., A. Carbonneau and E. Bahar, 2011. Effects of early water stress levels on berry set and berry development in Merlot cv. (*Vitis vinifera* L.). African J Biotech., 10(71): 15921-15932.

Kotseridis, Y., A. Georgiadou, P. Tikos, S. Kallithraka and S. Koundouras, 2012. Effects of severity of post-flowering leaf removal on berry growth and composition of three red *Vitis vinifera* L.

cultivars grown under semiarid conditions. J Agric Food Chem. 13; 60(23): 6000-10.

Ojeda, H., A. Deloire and A. Carbonneau, 2001. Influence of water deficits on grape berry growth. Vitis, 40(3): 141-145.

Ribereau-Gayon, P. 1970. Determination of total phenols in red wines Chim. Anal. (Paris), 52: 627-631.

Rogiers, S., A. Deloire, J. Smith and S. Tyreman, 2015. Monitoring vine water status, Part:1 Some physiological principles. Grapevine management guide 2014-15 NSW Government Department of Primary Industries 16-19.

Scholander, P.F., H.T. Yamel, E.D. Bradstreet and E.A. Hemmingsen, 1965. Sap pressure in vascular plants. Science, 148: 339-346.

Evaluating Mineral Contents of Selected Bread Wheat Landrace Pure Lines Derived from West Anatolia and Marmara Regions and Cultivars by GGE Biplot

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Bread wheat landraces are important resources for micronutritent improvement in plant breeding programs. This research aims to evaluate 9 macro and micronutrient contents (Fe, Zn, B, K, Mn, Cu, Mg, Ca, Mo) of 37 bread wheat pure lines derived from landraces of West Anatolia (Eskişehir and Kütahya) and Thrace (Edirne and Kırklareli) regions and compare with 11 bread wheat cultivars by using GGE Biplot. Field trials are conducted by incomplete block design with two replications in 2011-2012 growing season in Dardanos Agricultural Facility of Çanakkale Onsekiz Mart University. According to the biplot graphics, iron and zinc contents of genotypes were involved into the same section when boron and molibden contents were also highly correlated. Pure line L4 (TR57999/5) were the most prominent genotype for iron and zinc contents when L36 (TR38316/2) were superior by both boron and molibden contents. Copper contents of grains were found negatively correlated with iron and zinc contents. Results indicate notable variability among pure lines and lesser variability for cultivars for all micronutrients. Wheat cultivars had relatively higher Mo content while could be improved by their Fe, Zn, B, K and Ca contents. Possible candidates are introduced to be used in a future study.

Keywords: Bread wheat, landraces, GGE biplot, pure line, West Anatolia, mineral contents

Introduction

Wheat is a worldwide cultivated staple food and has always been one of the most widely practiced crops in plant breeding programs. Extensive breeding efforts have been made to improve older wheat lines and genotypes into modern high input cultivars with much higher yields from the beginning of 20th century. Reduced plant height increased plants resistance of lodging (especially grown under high nitrogen fertilization) and increased proportion of dry matter partition into the grain (Hedden, 2003). Today, high – yielding modern wheat cultivars are grown widely worldwide, replacing landraces that could no longer satisfy wheat farmers increasing expectations. This replacement almost caused extinction of landrace populations with a rapid reduction of wheat's natural germplasm that has been gradually reducing since the very beginning of its domestication (Reif et al. 2005, Cavanagh et al. 2013, Fu 2015).

Plant breeding programs usually requires high genetic variability among their material. Wheat landraces are considered as important sources of variation. (Kaya et al. 2006, Akçura et al. 2011, Hocaoglu and Akcura 2014). Previous studies showed that modern wheat cultivars tend to have lower mineral concentrations when compared to landraces or older cultivars (Fan et al. 2008, Ficco et al. 2009, Hussain et al. 2012, Akcura et al. 2013, Guzman et al. 2014, Akcura and Kokten 2017) which can be attributed to their significant reduction in quantity after the introduction of semi-dwarf cultivars (Zhao et al. 2009) or fertilization (Kirchmann et al. 2009, Martínez-Ballesta et al. 2010). Thus, improving important nutrient contents of bread wheat such as Fe, Zn, Cu, Ca would have great effects on human nutrition (Kashian and Fathivand 2015). Breeding more nutritious crops through plant breeding are essential (Ortiz-Monasterio et al. 2007, Bouis and Welch 2010) but nutrifiaction is only the beginning of an ongoing struggle to battle human malnutrition, which is growing to become an even bigger challenge with population growth and climate change (Godfray et al. 2010).

This study aims to evaluate macro and micronutrient contents of 37 bread wheat pure lines derived from landraces of West Anatolia and Thrace regions and compare with 11 registered cultivars.

Materials and Methods

37 bread wheat lines which are derived from landraces of West Anatolia (Eskişehir and Kütahya) and Thrace (Edirne and Kırklareli) regions are compared with commonly cultivated bread wheat cultivars of the same regions by their Fe, Zn, B, K, Mn, Cu, Mg, Ca and Mo contents (Table 1).

Table 1. Mineral concentrations (ppm) of bread wheat landrace derived pure lines and cultivars.

Genotype		Fe	Zn	B	K	Mn	Cu	Mg	Ca	Mo
L1	TR55155/6	48.12	34.17	8.96	2745.72	38.83	5.26	1243.75	409.99	0.98
L2	TR57999/6	48.35	34.30	9.71	2795.72	38.67	5.36	1167.91	490.66	1.00
L3	TR57999/2	36.92	25.00	10.46	5195.63	33.33	7.11	1620.41	429.32	1.09
L4	TR57999/5	51.68	44.00	10.96	4986.00	36.83	5.05	1554.16	433.98	1.05
L5	TR55154/4	46.25	30.17	12.96	3624.94	31.50	5.43	1533.75	346.64	1.00
L6	TR55155/2	42.29	33.16	9.71	2574.90	49.50	5.36	1559.16	434.64	0.97
L7	TR55164/2	40.96	30.92	8.17	4770.60	34.00	6.25	1530.41	472.64	0.94
L8	TR57999/3	38.00	26.42	8.08	4920.64	32.50	5.03	1636.25	454.64	1.05
L9	TR55140/5	46.04	30.17	12.96	2599.90	35.67	5.39	1521.66	483.32	1.24
L10	TR55138/6	48.33	34.25	9.50	3166.62	38.67	5.51	1342.50	461.98	1.05
L11	TR55148/3	38.00	24.50	9.83	2441.57	33.83	6.17	1033.75	437.31	0.97
L12	TR55149/6	36.00	24.33	9.62	4699.81	34.67	4.89	1564.58	411.32	0.93
L13	TR55125/6	43.75	29.17	9.79	3737.35	30.83	5.79	1517.91	441.32	1.09
L14	TR55142/1	42.29	33.17	9.75	2437.40	49.33	5.88	1576.25	440.65	0.92
L15	TR55174/3	48.75	34.66	8.96	2391.57	38.66	5.60	1565.00	433.32	0.93
L16	TR55125/1	42.92	32.33	10.42	2458.24	37.83	4.69	1082.92	432.65	1.10
L17	TR55146/7	40.73	33.00	9.62	3916.59	49.67	4.97	1378.75	473.99	0.94
L18	TR55142/3	48.00	34.08	10.46	3154.04	38.83	5.51	1344.29	496.65	1.00
L19	TR55144/3	39.17	26.50	9.67	3370.78	32.50	5.47	1249.58	441.32	1.09
L20	TR55167/1	39.58	28.68	8.96	3895.76	32.00	4.75	1158.75	551.00	1.05
L21	TR55148/4	42.08	33.33	9.83	3766.60	49.17	5.25	1591.66	443.31	0.94
L22	TR55128/2	50.00	35.91	12.96	4970.79	36.00	5.42	1514.58	469.98	1.08
L23	TR55127/1	42.29	33.00	11.04	3833.26	49.83	5.51	1356.66	424.65	0.92
L24	TR55146/4	39.71	33.00	9.75	4866.47	49.66	5.43	1278.75	443.98	0.90
L25	TR55212/2	43.75	29.25	10.96	4183.25	44.00	5.31	1144.58	477.30	0.94
L26	TR55143/5	43.65	29.08	10.46	4020.75	31.83	5.97	1330.41	486.61	0.97
L27	TR55174/5	41.00	32.33	11.21	4551.60	37.83	5.66	1514.58	335.97	1.08
L28	TR55167/2	41.46	31.92	10.29	4445.82	37.50	5.48	1550.00	489.98	0.94
L29	TR55141/2	43.33	26.00	10.96	3312.45	32.50	4.92	1593.75	405.99	1.00
L30	TR55144/5	49.37	35.33	10.50	4549.60	38.50	4.95	1608.33	499.31	0.94
L31	TR55166/6	44.87	29.83	9.79	4552.30	35.66	6.48	1076.25	479.99	1.00
L32	TR55138/5	38.00	26.42	10.46	4770.80	32.66	5.47	1175.41	401.32	1.09
L33	TR33419/2	42.58	32.50	8.04	4495.65	38.16	6.48	1555.41	482.64	1.05
L34	TR33257/3	40.42	31.08	8.46	2279.08	37.00	6.35	1356.25	503.98	0.97
L35	TR33419/5	35.50	24.33	10.46	3737.35	34.66	6.15	1509.58	416.65	0.89
L36	TR38316/2	44.17	29.33	14.25	2666.56	35.33	5.05	1464.16	434.65	1.23
L37	TR33521/3	45.42	30.08	10.96	2679.06	31.16	6.00	1512.50	414.65	0.92
ALTAY		33.00	24.33	7.71	3720.76	34.00	6.62	1610.00	423.98	1.09
FLAMURA		36.40	30.75	12.12	2391.57	32.33	5.10	1536.25	414.65	1.19
GELİBOLU		41.25	28.00	12.00	3466.53	34.33	6.40	1398.75	435.30	1.22
GEREK 79		38.00	28.58	9.00	5008.09	32.00	5.62	1527.50	483.31	1.05
HARMANKAYA		37.00	28.67	11.92	4179.08	32.16	4.20	1095.83	479.31	1.11
KIRAÇ		36.21	28.67	11.62	3420.78	33.66	4.50	1116.67	465.98	1.09
KIRGIZ		35.04	29.00	11.92	2799.89	33.33	4.82	1560.41	459.99	1.21
MÜFİTBEY		38.60	28.00	11.83	3029.05	37.00	5.87	1592.08	482.66	1.23
PEHLİVAN		39.70	24.65	8.42	4545.65	36.33	5.36	1476.66	437.99	1.23
SÖNMEZ		37.75	31.58	12.25	3724.93	37.17	5.27	1611.66	415.98	1.17
TEKİRDAĞ		41.25	31.75	12.21	2404.07	32.66	5.88	1468.75	419.32	1.20

Field trials are conducted by incomplete block design with two replications in 2011-2012 growing season in Dardanos Agricultural Facility of Çanakkale Onsekiz Mart University. Plots were 1.6 m^2 each and contained 4 rows with 20 cm space between lines; sown in 2 November 2011 with hand. Plant density were 550 plants per squaremeter. 2.7 kg da^{-1} N and 6.9 kg da^{-1} P_2O_5

fertilizer were applied with sowing, followed by 4.3 kg da^{-1} N application as topdress at the beginning of tillering phase. All agronomical procedures were in accordance with usual applications in wheat cultivation in Çanakkale, with the only exception of avoiding the use of herbicides and pestiticides. Weeds around and within experiment area are controlled by hand. Plants were harvested by hand and threshed. Seed samples are grinded by using a laboratuary-type grinder. Obtained whole bread flour put under 0.05 cm sieve before chemical analysis. Mineral contents of seed samples are analysed by atomic absorption spectroscopy (Kacar and Inal, 2008).

GGE Biplot method is introduced to identify genotype, environment and genotype – environment interaction effects on multi-environmental data (Yan et al. 2000) which is projected on biplot graphic (Gabriel 1971). Ever since its development, GGE Biplot method gained popularity among plant breeders and agronomists for its capability of visualizing genotype performances on different environments by reducing the dimentionality of the data, making it possible to evaluate genotypes, variables and environments on the same graphic statistically. Furthermore, it is also used to rank genotypes and environments, provides information about stability of genotypes (Yan and Tinker 2006).

The GGE Biplot model used in this study is:

$$Y_{ij} - \mu - \beta_j = \lambda_1 \xi_{i1} \eta_{1j} + \lambda_2 \xi_{i2} \eta_{2j} + \varepsilon ij$$

Where Y_{ij} is the expected mineral j content of genotype i, μ is the grand mean, β_j is main effect of mineral j, λ1 and λ2 are the singular values of first and second principal components, ξ_{i1} and ξ_{i2} are eigenvectors of mineral j for PC1 and PC2, and εij is the residual effect for genotype i and mineral j. Biplot analysis was made and graphics were created in "GGEbiplot" software created by Weikai Yan (Yan et al. 2001).

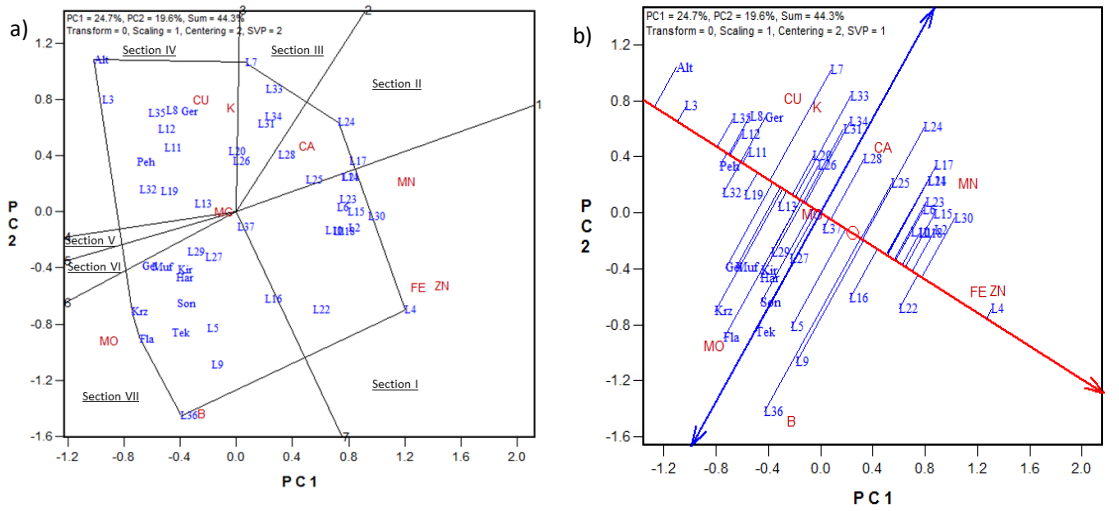

Figure 1. Polygon view of GGE Biplot – which is best for what (a) and Genotypes ranking based on average mineral axes (details of genotypes were given in Table 1) with genotype projections on AMC (Average mineral coordination) (b)

Results and Discussion

Polygon view of the biplot graphic is drawn by connecting the most distant genotypes from biplot origin to form a polygon, then drawing perpendicular lines from each side of this polygon to stop at the origin, which separates genotypes and environments into seven sections (Figure 1a). Vertex genotypes contributes overall variation most, delivering the most marjinal results that either the best or the worst (Yan and Tinker 2006). In our study, L4, L30, L17, L24, L7, Altay, Kırgız, Flamura and L36 were the vertex genotypes. Vertex genotypes with each sector is considered having relatively higher mineral contents that is included in the same sector (Yan et al. 2000). Therefore, L4 was a possible candidate for both iron and zinc content when L24 for calcium and L36 for boron and molibden (Table 1).

High variation of mineral concentrations of pure lines can be seen in Figure 1a. Contrarily, cultivars except Altay, Pehlivan and Gerek seemed relatively closer to each other and placed in lower-left part of the biplot, showing much lesser variation compared to landrace pure lines. These cultivars also had remarkably higher molibden and lower calcium contents than the rest of the genotypes. Projections of genotypes on AMC (average mineral coordinate) are shown in Figure 1b. L4, L37 and Pehlivan had little variation for different mineral contents because they were located near the AMC when L36 had the most.

Biplot graphics suggests a high variation among genotypes by their mineral contents due to the distance of mineral vectors from biplot origin except magnesium (Figure 2a). Additionally, mineral vectors are scattered in almost every direction across the biplot, making acute and obtuse angles with each other (Figure 2a). High correlation of iron and zinc contents were explicit (figure 2a), due to acute angle between these two vectors indicates high positive correlation (Yan et al. 2000), which were also found in previous studies (Hussain et al. 2010, Moreira-Ascarrunz et al. 2016, Akcura and Kokten 2017). Iron and zinc concentrations of wheat genotypes had strong negative correlation with copper while as weak negative correlation with potassium, which were also positively correlated with each other. Another significant negative correlation is seen between molibden and calcium (figure 2a).

Mineral vectors which are located far away from the biplot origin also contribute greatly to overall variation, having higher discriminativeness than others. In Figure 2a, iron, zinc, boron and molibden were very good examples of discriminitiveness and followed by magnesium which is located on the edge of the last circle. Discriminativeness reduces with minerals such as copper, potassium and calcium respectively, which are placed in inner circles closer to the origin. Magnesium is located near the center of the origin, being the least discriminating mineral in our study, in a way that its contents among all landraces and cultivars contributes little to overall variation. Acute angle made by both iron and zinc with average environment axis indicated their high representetiveness which are followed by magnesium. It is clearly seen that iron and zinc contents were placed near AMC in figure 2a, which is an axis represents all mineral concentrations combined. Given that, iron and zinc were highly representetive for all mineral concentrations in addition to their high discriminitiveness, which means iron and zinc contents would make good criteria for selecting genotypes for generally good mineral contents (Yan and Tinker 2006).

In theory, the ideal genotype should be included by the concentric circle in figure 2b. Accordingly, L4 were placed near ideal genotype circle, suggesting L4 were the most desireable genotype to be selected by its overall mineral content. Other desireable genotypes were L22, L18 and L3 where cultivars were not more desireable than half of the landraces used in this study, which means landraces pure lines derived from West Anatolia and Thrace regions could contribute future breeding programs aimed to imrove seed mineral contents of winter wheat.

Conclusions

GGE Biplot provides insights and simplifies otherwise complicated two-way data, hovewer its statistical power in our study were limited due to combined rate of explained variation (PC1 and PC2) were 44,31%, which is under 50% (Yan et al. 2000). Still, explicit results such as L4's promising Zn and Fe content could be confirmed from Table 3. We believe presented biplot graphics provides information by its valuable visualization capabilities.

Results shows that registered cultivars had lesser variation among different mineral contents of landrace pure lines of northwest Anatolia and Thrace regions. These lines could provide candidates for biofortification of bread wheat. Landrace L4 were the most desirable genotype while having high mineral contents, especially iron and zinc. Landraces L22, L18 and L30 could also be candidates for future studies. L36, L24, L7 and L13 could be specifically used as plant materials to enrich boron, calcium, potassium and magnesium contents of future bread wheat cultivars, respectively. All registered cultivars had especially lower iron, zinc, manganese and calcium contents. This indicates importance of investigating and improving nutrification value of bread wheat in future.

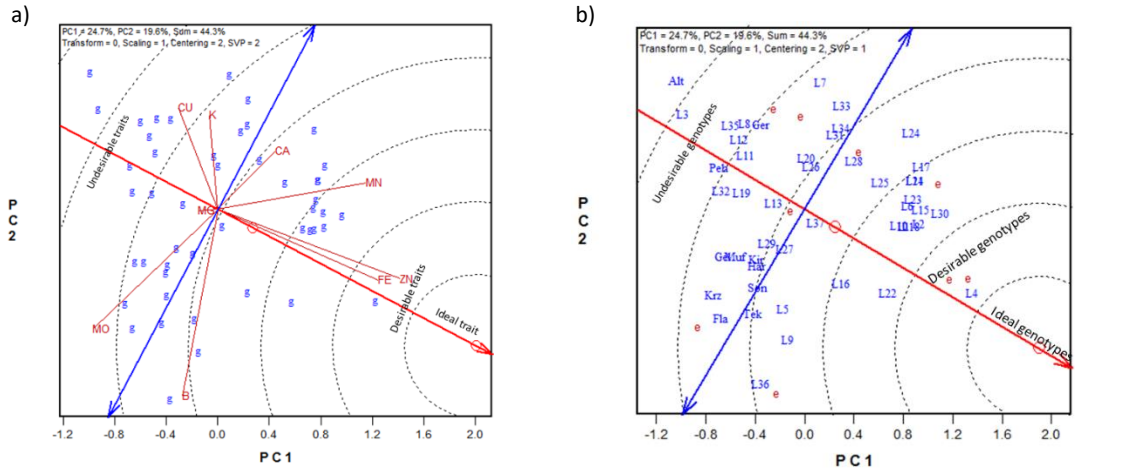

Figure 2. Ranking mineral concentrations on discriminating abilities and representativeness (a) and ranking genotypes based on their concentrations for all minerals altogether (b).

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References

Akcura, M. 2011. The Relationships of some traits in Turkish winter bread wheat landraces. Turk J Agric For, 35 (2) 115-125.

Akcura, M., O. Hocaoglu, H. Kılıc and K. Kokten 2013. Comparing wheat pure lines from Karadeniz region landraces with registered wheat cultivars by elemental content of the grain. 10th Field Crops Congress, 10-13 September 2013, Konya (In Turkish).

Akcura, M. and K. Kokten 2017. Variations in grain mineral concentrations of Turkish wheat landraces germplasm. Quality Assurance and Safety of Crops & Foods, 2017; 9 (2): 153-159.

Bouis, H.E. and R.M. Welch. 2010. Biofortification: a sustainable agricultural strategy for reducing micronutrient malnutrition in the global south. Crop Science 50: S-20-S-32.

Cavanagh, C.R., S. Chao, S. Wang, B.E. Huang, S. Stephen, S. Kiani and K. Forrest. 2013. Genome-Wide Comparative Diversity Uncovers Multiple Targets of Selection for Improvement in Hexaploid Wheat Landraces and Cultivars. Proceedings of the National Academy of Sciences of the United States of America 110 (20): 8057–62.

Fan, M.S., F.J. Zhao, S.J. Fairweather-Tait, P.R. Poulton, S.J. Dunham and S.P. McGrath. 2008. Evidence of decreasing mineral density in wheat grain over the last 160 years. Journal of Trace Elements in Medicine and Biology 22 (4): 315–24.

Ficco, D., C. Riefolo, G. Nicastro, V. De Simone, A.M. Di Gesù, R. Beleggia, C. Platani, L. Cattivelli and P. De Vita. 2009. Phytate and mineral elements concentration in a collection of Italian durum wheat cultivars. Field Crops Research 111 (3): 235–42.

Fu, Y.B. 2015. Understanding crop genetic diversity under modern plant breeding. Theoretical and Applied Genetics 128 (11). Springer Berlin Heidelberg: 2131–42.

Gabriel, K.R. 1971. The biplot graphic display of matrices with application to principal component analysis. Biometrika, 58:453-467.

Godfray, H.C.J., J.R. Beddington, I.R. Crute, L. Haddad, D. Lawrence, J.F. Muir, J. Pretty, S. Robinson, S.M. Thomas and C. Toulmin. 2010. Food security: the challenge of feeding 9 billion people. Science 327 (5967): 812–18.

Guzman, C., A.S. Medina-Larque, G. Velu, H. Gonzalez-Santoyo, R.P. Singh, J. Huerta-Espino, I. Ortiz-Monasterio and R.J. Pena. 2014. Use of wheat genetic resources to develop biofortified wheat with enhanced grain zinc and iron concentrations and desirable processing quality. Journal of Cereal Science 60 (3): 617–22.

Hedden, P. 2003. The genes of green revolution. Trends Genet 2003; 19 : 5-9.

Hocaoglu, O. and M. Akcura. 2014. Evaluating yield and yield components of pure lines selected from bread wheat landraces comparatively along with registered wheat cultivars in Canakkale ecological conditions. Turkish Journal of Agriculture and Natural Sciences, no. Special Issue 2: 1528–39.

Hussain, A., H. Larsson, R. Kuktaite and E. Johansson. 2010. Mineral composition of organically grown wheat genotypes: contribution to daily minerals intake. International Journal of Environmental Research and Public Health 7 (9): 3442–56. doi:10.3390/ijerph7093442.

Kacar, B. and A. Inal 2008. Plant analysis. Nobel Publication No: 1241. Applied Sciences, 63, 879 (In Turkish).

Kashian, S. and A.A. Fathivand. 2015. Estimated daily intake of Fe, Cu, Ca and Zn through common cereals in Tehran, Iran. Food Chemistry 176: 193–96.

Kaya, Y., M. Akçura and S. Taner. 2006. GGE-Biplot analysis of multi-environment yield trials in bread wheat. Turkish Journal of Agriculture and Forestry 30 (5): 325–37.

Kirchmann, H., L. Mattsson and J. Eriksson. 2009. Trace element concentration in wheat grain: results from the Swedish long-term soil fertility experiments and national monitoring program. Environmental Geochemistry and Health 31 (5): 561–71.

Martinez-Ballesta M. C., R. Dominguez-Perles, D. A. Moreno, B. Muries, C. Alcaraz-López, E. Bastias, C. Garcia-Viguera and M. Carvajal. 2010. Minerals in plant food: effect of agricultural practices and role in human health. A review. Agronomy for sustainable development, 30(2), 295-309.

Moreira-Ascarrunz, Sergio, Hans Larsson, Maria Prieto-Linde and Eva Johansson. 2016. Mineral nutritional yield and nutrient density of locally adapted wheat genotypes under organic production. Foods 5 (4): 89.

Ortiz-Monasterio, J.I., N. Palacios-Rojas, E. Meng, K. Pixley, R. Trethowan and R.J. Peña. 2007. Enhancing the mineral and vitamin content of wheat and maize through plant breeding. Journal of Cereal Science 46 (3): 293–307.

Reif, J.C., P. Zhang, S. Dreisigacker, M.L. Warburton, M. Van Ginkel, D. Hoisington, M. Bohn and A.E. Melchinger. 2005. Wheat genetic diversity trends during domestication and breeding. Theoretical and Applied Genetics 110 (5): 859–64. doi:10.1007/s00122-004-1881-8.

Yan, W., L.A. Hunt, Q. Sheng and Z. Szlavnics. 2000. Cultivar evaluation and mega-environment investigation based on the GGE biplot. Crop Science 40(3): 597-605.

Yan, W., P.L. Cornelius, J. Crossa and L.A. Hunt. 2001. Two types of GGE Biplots for analyzing multi-environment trial data. Crop Science 41 (3): 656–63.

Yan, W. and N.A. Tinker. 2006. Biplot analysis of multi-environment trial data: principles and applications. Canadian Journal of Plant Science 86 (3): 623–45.

Zhao, F.J., Y.H. Su, S.J. Dunham, M. Rakszegi, Z. Bedo, S.P. McGrath and P.R. Shewry. 2009. Variation in mineral micronutrient concentrations in grain of wheat lines of diverse origin. Journal of Cereal Science 49 (2): 290–95.

Stability Parameters for Yield and Yield Component of the Bread Wheat Genotypes under Various Drought Stress Condition

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Drought is the main abiotic stress factor and low rainfall during grain filling period affect bread wheat yield and yield component. This experiment was carried out in the experimental field of Trakya ARI, Edirne (Turkey), in 2008-2009 and 2009-2010 years. Totally, 15 genotypes were planted in RCBD in a split-plot with three replications. The main plots were assigned to five moisture regimes, which included 3 drought stress environments, one non-stress and one non-treatment environment. Droughts were created under mobile rain shelter at various plant growth stages from shooting up to maturating stage. Stability parameters were determined for grain yield, biological yield, harvest index, spike number per square meter, kernel and spikelet number per spike were investigated. A joint regression analysis was applied to grain yield and other yield component to estimate the stability parameters; mean yield (x), determinations coefficient (R^2), regression coefficient (b), deviation from regression coefficient (S^2d), and intercept value (a). The highest yield (658.3 kg/da) was determined in Bereket and biological yield (2539.4 kg/da) in Kate A-1 cultivar. The highest grain and biological yield was obtained under non-stress condition. For grain yield, it was determined that Kate A-1 and BBVD7 were adapted to well environmental conditions, Bereket was well adapted to all environment condition. For biological yield Pehlivan, BBVD7 and Bereket were well adapted to all environmental conditions. Wide range of stability statistics was determined among cultivars for all the parameters. Gelibolu and Bereket were the stable cultivars on the basis of overall mean yield and stability parameters.

Keywords: Bread wheat, genotypes, yield component, stability parameters

Introduction

Trakya region is located in the North western part of Turkey and covered about 3% of Turkey. Mostly winter and facultative bread wheat cultivars are grown average wheat yield is over 4.5 tons/ha. The amount of the rainfall (589.1 mm) during growing season is enough for wheat production but the distribution of this rainfall is not regular. Fluctuation of rainfall causes reducing grain yield and quality (Öztürk and Korkut, 2015). Wheat is the world most important cereal crop and it has been grown in a wide range of arid and semi-arid areas, where drought occurs frequently because of rainfall fluctuations in rain-fed regions (Mardeh et al. 2006). Drought stress tolerance is a complex trait that is obstructed by low heritability and deficiency of successful selection approaches (Blum 1988). Almost all breeding programs in the world aim to improve varieties with stable yields. The yield stability is generally grouped as static or dynamic stability (Pfeiffer and Braun, 1989). Therefore, selection of wheat genotypes should be adapted to drought stress. In addition, drought tolerance mechanism should be identified during the development of new cultivars in order to increase the productivity (Rajaram et al. 1996). Stable yield performance of genotypes under both favorable and drought stress conditions is vital for plant breeders to identify drought tolerant genotypes (Pirayvatlou, 2001). Moreover, high-yielding genotypes under optimum conditions may not be drought tolerant (Blum, 1996; Mardeh et al. 2006); therefore, many studies preferred the selection under stress and non-stress conditions (Clarke et al. 1992; Fernandez, 1992; Rajaram and Van Ginkle, 2001). In the same pattern, the selection in the current study was conducted under optimum, moderate, and sever stress conditions. The objective of this study was to evaluate the performance of the advance genotypes and cultivars and to investigate their yield stability across various drought stress condition over two consecutive years.

Materials and Methods

This research was carried out in the experimental field of Trakya ARI, Edirne (Turkey) (41 m above sea level, 41º64´ N, 26º59´ E), during two years (2008-2009 and 2009-2010) and 15 genotypes were evaluated in randomized completely blocks design in a split-plot with three replications. Plot size was 6 rows, 6 m long and 17 cm between the rows. Kate A-1, Gelibolu, Pehlivan, Tekirdağ, Selimiye, Aldane, Bereket, Flamura-85, Golia cultivars and 6

advanced lines were used. The main plots were assigned to five moisture regimes, which included 3 drought stress environments, one non-stress and one non-treatment environment. Drought treatments are placed on main parcel and genotypes on the sub-plot. Droughts were created under mobile rain shelter at various plant growth stages from shooting up to maturating stage. A mobile rain shelter was used to exclude rain and induce drought stress. In this experiment, all parcels were covered only when raining. A drip irrigation set was used and each plot was irrigated separately by controlling dripping irrigation system. Grain yield, biological yield, spike length, spike number per square meter, kernel and spikelet number in spike and harvest index with stability parameters were investigated under different drought stress conditions. Drought treatments are placed on main parcel and genotypes on the sub-plot. The Zadoks Decimal Code (GS) was used to describe plant growth stages. The described plant development stages are; Drought stress applied from GS31 (stem starts to elongate) to GS51 (10% of spikes visible), Drought stress applied from GS51 (10% of spikes visible) to GS94 (over-ripe, straw brittle), Drought stress applied from GS31 (stem starts to elongate) to GS94 (over-ripe, straw brittle), non-treatment and non-stress. Stability analysis (genotypes x environment interaction) was done according to Eberhardt and Russel (1966) and Finlay and Wilkinson (1963). Mean yield (x), regression coefficient (b), coefficient of determination (R^2), deviation from regression coefficient (S^2d), intercept value (a) and determination coefficient (CV) were evaluated as stability parameters. The statistical analyses of measurements were performed by using statistics program for and the differences among the means were compared with L.S.D at a 5% significant level. The entire statistical analysis was done using the computer (Gomez and Gomez, 1984; Kalaycı 2005).

Results and Discussion

The stability parameters analysis for the yield and some of the selected yield components was performed and given in Table 1, 2 and 3. The mean yield and biological yield was 583.0 and 2252.9 kg/da, respectively.

Table 1. Determined stability parameters for yield and biological yield of the genotypes

No	Genotypes	stability parameters for grain yield					Stability parameters for biological yield				
		X	R^2	S^2d	A	b	X	R^2	S^2d	a	b
1	Kate A-1	631.5	0.99	52.3	-54.7	1.18	2539.2	0.98	2433.9	-391.3	1.30
2	Gelibolu	613.0	0.99	47.3	32.3	1.00	2205.8	0.99	448.1	170.4	0.90
3	Pehlivan	587.7	0.99	34.9	-0.6	1.01	2417.4	0.99	800.1	142.9	1.01
4	Tekirdağ	594.5	0.95	147.8	71.2	0.90	2193.2	0.92	3525.1	476.9	0.76
5	Selimiye	608.9	0.97	111.9	9.5	1.03	2382.0	1.00	335.5	-438.6	1.25
6	Aldane	551.1	0.96	135.1	11.5	0.93	2356.0	0.96	3947.8	-406.2	1.23
7	Flamura-85	518.9	0.96	108.2	-14.3	0.91	2028.4	0.93	5066.2	-187.3	0.98
8	Golia	610.4	0.99	35.0	113.8	0.85	2037.8	0.91	4004.5	329.1	0.76
9	BBVD7	651.0	0.96	249.4	-129.3	1.34	2439.4	0.95	3683.0	192.1	1.00
10	Bereket	658.3	0.99	35.4	25.8	1.08	2364.6	0.97	2289.0	55.9	1.02
11	ÖVD26-07	579.1	0.97	103.2	-17.5	1.02	2218.4	0.90	4758.6	479.1	0.77
12	ÖVD2/21-07	563.0	0.90	235.3	112.2	0.77	2017.2	0.99	1166.0	-419.4	1.08
13	ÖVD2/27-07	617.8	1.00	8.5	114.7	0.86	2259.4	0.97	1228.3	551.9	0.76
14	EBVD24-07	577.4	0.99	87.3	-205.1	1.34	2228.4	0.96	2833.6	140.1	0.93
15	BBVD21-07	383.0	0.94	144.0	-69.3	0.78	2106.6	0.93	8318.6	-695.5	1.24

The results of variance analyses showed that there were significant differences (P<0.01) among genotypes and treatments for investigated yield and yield components under varied drought stress condition. The highest grain yield with 658.3 kg/da was determined in Bereket cultivar and also BBVD7 with 651.0 kg/da and Kate A-1 with 631.5 kg/da were other highest yielding cultivars (Table 1). A genotype having stabile grain yield across the environment condition is very important in wheat. Genotype x environment interaction is a mainly issue for plant breeders in improving high yield across variable environments. Stability analysis showed a wide variation among genotypes. Some genotypes exhibited wide adaptation and other showed specific adaptation to favorable or un-favorable environments. The regression coefficients (b) ranged between 0.77-1.34 in grain yield, 0.76-1.30 in biological yield among genotypes. It was determined that Kate A-1 and BBVD7 were adapted to well environmental conditions while Bereket was well adapted to all environment condition. Cultivar Bereket produced the highest yield (658.3 kg/da) in all environments averaged for two years, and had regression coefficient (b) close to unity (1.08) and R^2 close to zero (0.99) and positive intercept value (a). This result indicated wide adaptation and stability of performance of Bereket in all environments conditions. Gelibolu (R^2=0.99, b=1.00), Pehlivan (R^2=0.99, b=1.01), Selimiye (R^2=0.97, b=1.03), and Aldane (R^2=0.96, b=0.93) cultivars were medium adaptable to all environmental conditions with suitable determination coefficient and regression coefficient.

Genotypes Pehlivan, Bereket, and BBVD7 produced high biological yield over two years and five environments showed regression coefficient (b=1.01; b=1.02, b=1.00) close the unity and suitable determination coefficient (R^2=0.99, R^2=0.97, and R^2=0.95). Also, Kate A-1, Selimiye, and Aldane were well adapted to all environment condition with their biological yield and regression coefficient over average.

Table 2. Determined stability parameters for spike number in square meter and harvest index

No	Genotypes	Stability parameters for spike in square meter					Stability parameters for harvest index				
		X	R^2	S^2d	a	b	X	R^2	S^2d	a	b
1	Kate A-1	457.3	0.86	92.82	10.7	1.04	36.9	0.32	0.53	14.8	0.60
2	Gelibolu	435.8	0.96	30.14	-55.5	1.14	39.5	0.83	0.38	-21.3	1.65
3	Pehlivan	436.0	0.93	73.79	-130.5	1.32	36.0	0.87	0.14	-6.4	1.15
4	Tekirdağ	411.0	0.77	72.37	122.5	0.67	37.5	0.02	0.67	32.9	0.13
5	Selimiye	455.9	0.78	243.28	-88.2	1.27	37.5	0.94	0.05	-2.9	1.10
6	Aldane	433.8	0.99	3.93	11.2	0.98	35.6	0.32	2.21	-9.0	1.21
7	Flamura-85	371.2	0.97	12.48	26.5	0.80	36.4	0.11	1.87	14.7	0.59
8	Golia	486.6	0.86	116.70	-14.0	1.16	40.8	0.50	0.77	1.8	1.06
9	BBVD7	448.1	0.91	57.59	4.7	1.03	36.6	0.17	0.47	22.8	0.37
10	Bereket	451.4	0.99	5.21	-101.4	1.29	37.5	1.00	0.00	2.3	0.96
11	ÖVD26-07	429.6	0.93	30.16	68.5	0.84	39.3	0.95	0.12	-29.6	1.87
12	ÖVD2/21-07	409.9	0.79	95.04	56.7	0.82	38.1	0.68	0.87	-21.2	1.61
13	ÖVD2/27-07	433.4	0.62	97.77	200.2	0.54	38.2	0.64	0.13	17.1	0.57
14	EBVD24-07	460.1	0.98	11.27	-16.3	1.11	36.0	0.93	0.12	-20.0	1.52
15	BBVD21-07	328.8	0.98	13.11	-94.9	0.99	26.9	0.29	0.67	4.1	0.62

Stability analysis for spike in square meter, kernel number in spike and spikelet number in spike showed there was wide variation among genotypes. The mean value of the spike in square meter was 429.9, kernel number in spike was 34.6 and spikelet number in spike was 16.0. Some other tested cultivars, Selimiye and Golia were able to adapt to favourable conditions, as their spike number in square meter were stable only under favourable conditions. Also, Bereket, Tekirdağ and

Gelibolu were could be adapt to fertile environment conditions, with their kernel number in spike were stable only under favourable conditions. Cultivars Bereket, Gelibolu and Tekirdağ produced high kernel number in spike over range of environments showed over regression coefficient (b=1.59, b=1.17, b=1.16 and respectively) and higher determination coefficient (R^2= 0.97, 0.95 and 0.95 respectively), and higher deviation from regression (S^2d= 0.50, 0.41 and 0.45 respectively) indicated specific adaptability of these cultivars to favourable environmental conditions.

For spikelet number in spike the highest determinations coefficient (R^2) was obtained in Pehlivan and Tekirdağ. There was variation in regression coefficients (b) and ranged between 0.80 and 1.20, and optimal regression coefficient (b) determined in Aldane, Selimiye and Kate A-1cultivar. According to spikelet number in spike it could be seen that optimal determinations coefficient (R^2) determined in Pehlivan followed by Tekirdağ, Aldane and Golia cultivars.

Table 3. Determined stability parameters for kernel number and spikelet number in spike

No	Genotypes	Stability parameters for kernel number in spike					Stability parameters for spikelet number in spike				
		X	R^2	S^2d	a	b	X	R^2	S^2d	a	b
1	Kate A-1	36.5	0.93	0.47	-0.12	1.06	16.31	0.62	0.185	1.71	0.91
2	Gelibolu	36.4	0.95	0.41	-4.13	1.17	15.89	0.96	0.021	-2.12	1.12
3	Pehlivan	30.2	0.91	0.26	7.51	0.66	15.94	1.00	0.000	0.34	0.97
4	Tekirdağ	36.0	0.95	0.45	-4.18	1.16	16.65	0.99	0.007	-0.92	1.10
5	Selimiye	33.2	1.00	0.01	-4.31	1.09	16.02	0.97	0.007	3.26	0.80
6	Aldane	31.3	0.94	0.41	-3.95	1.02	14.90	0.98	0.006	-1.48	1.02
7	Flamura-85	34.8	0.96	0.19	2.75	0.93	15.44	0.86	0.051	0.56	0.93
8	Golia	33.9	0.99	0.05	7.75	0.76	15.46	0.98	0.008	0.35	0.94
9	BBVD7	34.3	0.91	0.47	2.73	0.91	16.39	0.91	0.029	1.69	0.92
10	Bereket	36.9	0.97	0.50	-17.99	1.59	16.92	0.94	0.031	-2.38	1.20
11	ÖVD26-07	34.4	0.93	0.40	2.46	0.92	14.36	0.89	0.029	1.51	0.80
12	ÖVD2/21-07	34.5	0.89	0.64	1.83	0.95	17.47	0.91	0.047	-0.44	1.12
13	ÖVD2/27-07	38.3	0.98	0.16	-4.84	1.25	14.89	0.98	0.006	0.25	0.91
14	EBVD24-07	34.0	0.89	0.67	0.63	0.97	15.67	0.90	0.056	-3.58	1.20
15	BBVD21-07	33.4	0.86	0.30	13.85	0.57	18.22	0.66	0.211	1.25	1.06

Note: X: mean, R^2: determinations coefficient, S^2d: deviation from regression, a: intercept value, b: regression coefficient, CV: variation coefficient

Table 4. Correlation coefficients among stability parameters based on investigated characters

Grain yield						**Biological yield**				
	X	**R^2**	**S^2d**	**CV**	**a**	**X**	**R^2**	**S^2d**	**CV**	**a**
R^2	0.518*					0.456				
S^2d	-0.241	-0.832**				-0.357	-0.778**			
CV	-0.241	-0.832**	1.000**			-0.357	-0.778**	1.000		
A	0.156	-0.136	-0.215	-0.215		-0.029	-0.352	-0.215	-0.215	
B	0.510	0.452	0.032	0.032	-0.771**	0.405	0.494	0.062	0.062	-0.925**
Harvest index						**Spike number in square meter**				
R^2	0.306					-0.165				
S^2d	-0.160	-0.638*				0.371	-0.677**			
CV	-0.161	-0.639*	1.000**			0.371	-0.677**	1.000**		
A	-0.153	-0.727**	0.124	0.125		-0.025	-0.653**	0.015	0.015	
B	0.312	0.750**	-0.146	-0.147	-0.987**	0.411	0.529*	0.133	0.133	-0.921**
Spikelet number in spike						**Kernel number in spike**				
R^2	-0.435					0.350				
S^2d	0.548*	-0.977**				0.140	-0.635*			
CV	0.548*	-0.977**	1.000**			0.140	-0.635*	1.000**		
A	0.013	-0.315	0.227	0.227		-0.507	-0.532*	-0.206	-0.206	
B	0.476	0.065	0.067	0.067	-0.873**	0.678*	0.540*	0.210	0.210	-0.977**

Note: X: mean, R^2: determinations coefficient, S^2d: deviation from regression, a: intercept value, b: regression coefficient, CV: variation coefficient

Correlation analysis was used to study the relationships between mean yield and stability parameters, between studied stability parameters. The results of coefficient of rank correlations showed that mean yield was statistically significant ($P<0.05$) and positively correlated with determinations coefficient (R^2=0.518*). Also, the correlation was negative between mean yield and S^2d, but this correlation was statistically nonsignificant. The results of correlations coefficient of the stability parameters showed that biological yield was statistically nonsignificant ($P<0.05$) with determinations coefficient (R^2=0.456), and negatively correlated with determinations coefficient (S^2d=-0.357). Furthermore, the spikelet number in spike was statistically significant and positively correlated with determinations coefficient (S^2d=0.548*), and negatively nonsignificant correlated with determinations coefficient (R^2=-0.435).

Conclusions

Drought is the main abiotic stress factor and low moisture during grain filling period affected bread wheat yield and yield component. The highest yield was determined in Bereket and biological yield in Kate A-1 cultivar. The highest grain and biological yield was obtained under non-stress condition. For grain yield, it was determined that Kate A-1 and BBVD7 were adapted to well environmental conditions, Bereket was well adapted to all environment condition. For biological yield Pehlivan, BBVD7 and Bereket were well adapted to all environmental conditions. Wide range of stability statistics was determined among cultivars for all the parameters. Gelibolu and Bereket were the stable cultivars on the basis of overall mean yield and stability parameters.

References

Blum, A. 1988. Plant breeding for stress environments. CRC Press, Boca Raton, FL, 38–78.

Blum, A. 1996. Yield potential and drought resistance: are they mutually exclusive? In: Reynolds M.P., Rajaram S., Mc Nab A. (Eds.) Increasing yield potential in wheat. Breaking the barriers, 90–101.

Clarke, J.M., R.M. De Pauw and T.F. Townley-Smith, 1992. Evaluation of methods for quantification of drought tolerance in wheat. Crop Science 32, 423–428.

Eberhart, S.A. and W.A Russell, 1966. Stability parameters for comparing varieties. Crop. Sci.6: 36-40.

Finlay, K.W. and G.N. Wilkinson, 1963. The Analysis of Adaptation in a Plant Breeding Programme. Aust. J. Agric.Res., 14: 742-754.

Fernandez, G.C.J. 1992. Effective selection criteria for assessing stress tolerance. In: Kuo, C.G. (Ed.) Proceedings of the International Symposium on Adaptation of Vegetables and Other Food Crops in Temperature and Water Stress. Tainan, Taiwan.

Kalaycı, M. 2005. Örneklerle Jump Kullanımı ve Tarımsal Araştırma için Varyans Analiz Modelleri. Anadolu Tarımsal Araştırma Enst. Müd. Yayınları, Yayın No: 21, Eskişehir.

Mardeh, A.S.S., A. Ahmadi, K. Poustini and V. Mohammadi, 2006. Evaluation of drought resistance indices under various environmental conditions. Field Crops Research 98, 222-229.

Öztürk, İ. and K.Z. Korkut, 2015. Effect of Drought Consist of Different Plant Growth on Some Physiological Traits in Bread Wheat (*Triticun aestivum* L.) Genotypes. 2. International Plant Breeding Congress (2. IPBC), 1-5 Nov., 2015. Antalya, Turkey.

Pfeiffer, W.H. and H.J. Braun, 1989. Yield stability in bread wheat. In J.R. Anderson and P.B Hazel, eds. Variability in Grain Yields. Washington D.C.: John Hopkins Univ. and the Int. Food Policy Res. Inst.

Pirayvatlou, A.S. 2001. Relations among yield potential, drought tolerance and stability of yield in bread wheat cultivars under water deficit conditions. Proceedings of the 10th Australian Agronomy Conference. Hobart, January 29, 2001

Rajaram, S. and M. Van Ginkel, 2001. Mexico, 50 years of international wheat breeding. Bonjean A.P., Angus W.J., (Eds.) The world wheat book: A history of wheat breeding. Lavoisier Publishing, Paris, France, 579–604.

Rajaram, S., H.J. Braun and M. Van Ginkel, 1996. CIMMYT's approach to breed for drought tolerance. Euphytica 92, 147-153.

Lin, C.S., M.R. Binns and L.P. Lefkovitch, 1986. Stability analysis: Where do we stand? Crop Sci., 26: 894-900.

Zadoks, J.C., T.T. Chang and C.F. Konzak, 1974. A decimal code for growth stages of cereals. Weed Res. 14: 415-421.

Analysis and Assessment of Yield Ranking Models in Triticale (x*Triticosecale* Wittm.) in Contrasting Environmental Conditions

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The analysis and evaluation of yield is an important stage of the breeding process in cereals. There are various methods for grouping and ranking of the tested genotypes by their yield, which allows for a correct interpretation of the interaction of the environment with the genotype. Triticale as a product of wide hybridization is characterized by certain features, which requires the applicable models for yield evaluation in other cereals to be analyzed in this crop. For this purpose five yield ranking models were assessed in 16 triticale varieties for a three-year period. The three monitored periods were characterized by contrasting agro-climatic conditions of the environment. With the highest efficiency was the model using average standard value formed by values of check varieties in the experiment. With a good performance for yield evaluation are also models in which the yield is adjusted by the variation caused by different environmental conditions – heritability adjusted (HA) model and Hi-model. In spite of this fact, HA-grouping is very similar to the grouping of the varieties by their absolute yield. This is related to the absence of many locations of the study, regardless of the contrasting agro-climatic conditions. On the other hand, the Hi-model enables interpretation of yield and grouping of yield reaction in different varieties without multilocation trials. Despite some of their disadvantages, each of the used models could be applied to the analysis of the yield in periods of different conditions, depending on the specific purpose of the breeding program.

Keywords: Contrasting conditions, environment, triticale, yield model

Introduction

The development of new crop plant cultivars is a complex task rather than a one-sided process and involves finding solutions for multiple problems. One of the most significant factors seriously limiting the yield from the cereals is the influence of the environment. In this respect, contemporary breeding is trying to develop genotypes that can be simultaneously very stable under contrasting environments and highly productive. There are various investigations, which point out that the ecological plasticity of a given genotype is in negative correlation with its potential for yield (Lozano del Rio et al., 2009; Becker and Leon, 1988). On the other hand, Tsenov et al. (2013) have reported a good combination of high stability and high productivity.

As a product of wide hybridization, triticale possesses stability, which is closely related to the investigated genotype (Baychev, 2013; Stoyanov and Baychev, 2016b). Different studies on the crop (Stoyanov and Baychev, 2016b; Dhindsa et al., 2002) have reported that the interaction of the factors environment x genotype determines about 10-15 % of the total variation. In comparison to other cereals (Tsenov et al., 2014), such reaction is very high. Therefore the investigated cultivars and breeding materials have to be ranked by their yield and its stability.

There are different methods for ranking of the investigated genotypes (Tsenov et al., 2014). Some of them are based on the conventional statistical procedures and models - PCA, ANOVA, Duncan test, etc. (Gabriel, 1971; Zobel et al., 1988). Other approaches are related to the use of models developed especially for the evaluation of the effect of the environment on the genotype - AMMI, GGE, HARV, Hi (Yan and Kang, 2003; Karimzadeh, 2012; Farshadfar and Farhadi, 2002). Last but not least are the methods for analysis of the genotype's stability and plasticity (Becker and Leon, 1988).

The use of all these models allows grouping (ranking) of a given set of investigated genotypes according to the phenotypic reaction of the yield. The efficiency of each of the applied approaches is directly related to both the involved genotypes and the conditions of the environment (Tsenov et al., 2014). Therefore careful analysis and evaluation of each approach is necessary when applying it to a certain crop. The specific peculiarities of triticale with regard to the stability of its yield have been subject of a limited number of investigations (Stoyanov and Baychev, 2016b; Alljarah et al.,

2014; Dhindsa et al., 2002; Goyal et al., 2011; Goyali and Dhindsa, 2003; Motzo et al., 2001). This is the reason why ranking models of the cultivars should be applied very carefully, analyzing in detail the respective models to be applied (Stoyanov and Baychev, 2016b). Such a necessity arises from the fact that sharp deviations from the normal meteorological conditions cause distortion of the results from certain statistical approaches (Tsenov et al., 2013). Since triticale is considered a crop with enhanced tolerance to abiotic stress (Baychev, 2013), the ranking of the specific genotypes according to their yield and stability can be of high practical value.

The aim of this investigation was to analyze the yield of Bulgarian triticale cultivars through various approaches for ranking and to assess their applicability under contrasting environments.

Materials and Methods

In order to realize the above aim, 11 Bulgarian cultivars (Kolorit, Atila, Akord, Respekt, Bumerang, Irnik, Dobrudzhanets, Lovchanets, Doni 52, Blagovest, Borislav) were used.

The investigated 11 cultivars were grown as a whole-surface crop in trial plots of 10 m^2, in four replications according to a standard block design within a competitive varietal trial. The trial was carried out in 3 successive cropping seasons: 2013/2014, 2014/2015, and 2015/2016. Planting was done using mechanical equipment within the standard dates (10th – 15th October) at density 550 seeds per m^2. Besides the above cultivars, the standard triticale varieties AD-7291, Vihren and Rakita, as well as the world standards Lasko and Presto were involved in the investigation.

The yield data (Y) were summarized by calculating the mean values according to cultivar and year. Each genotype was ranked on the basis of five different models:

1. RY-model. It is based on the ranking of cultivars by their relative yield according to the accepted mean standard between check varieties Vihren and Rakita.

2. RV-model. This model is based on the ranking of the cultivars by their relative value between the absolute yield from each cultivar and the mean yield from all cultivars (Yan and Holland, 2010).

3. RE-model. The cultivars are ranked on the basis of their score by the relative value of the yield as follows: below 90% (1); 90-94% (2); 95-100% (4); 101-105% (6); 106-110% (8); 111— 115% (10), and above 116% (12), according to Tsenov et al. (2014).

4. HA-model. The cultivars are ranked on the basis of the corrected relative value of yield according to the conditions of the environment. The HARV parameter is calculated according to Yan and Holland (2010).

5. Hi-model. The model is based on the Hi parameter according to Martynov (1990) and gives idea about the stability of the absolute yield according to the direct effect of the growing year.

Each model was assessed on the basis of the correlation between its values and the values of the absolute yield. To analyze the efficiency of the models, cluster and regression analyses and Duncan test were carried out. For data processing, the software Microsoft Excel 2003 was used, and for correlation analysis, cluster analysis, regression analysis and Duncan test – IBM SPSS Statistics 19.

Results and Discussion

The investigated periods differed significantly with regard to the weather (Table 1). This was valid both for the average monthly temperatures and the rainfalls. The data from the three investigated periods allowed evaluating the cultivars by their stability due to the rather contrasting conditions of the environment. The great variations during the growing period strongly changed the values of the yield components because the weather had direct effect on them. Especially high was this effect on the weight parameters because proper development of the plants was necessary for the formation of high values (Baychev, 2013).

The results from the ANOVA (Table 2) unequivocally emphasized the serious influence of the interaction of the environment with the genotype on the variation as a result from the contrasting conditions. The environment x genotype interaction accounted for 14 % of the total variation. Such high values are related to a wide genetic basis of adaptability of the yield components, which implies different mechanisms with regard to the values of productive tillering, number of grains in spike and absolute weight of grain (Tsenov et al., 2013).

In fact, the investigated cultivars formed their productivity in different ways. Previous investigations indicate that such cultivars as Atila

and Borislav form their yields mainly from the productive tillering and the thousand kernel weight (Stoyanov and Baychev, 2016a). The other cultivars formed their yield mainly from the number of grains per spike and the productive tillering (Stoyanov and Baychev, 2016a).

Table 1. Meteorological data during the period of investigation

Parameter/Months		Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul
AMT, °C	2013/2014	10.9	9.0	1.1	2.4	4.0	7.7	10.7	15.2	18.8	21.7
	2014/2015	11.2	5.6	3.1	1.4	2.0	5.0	10.1	16.4	19.4	22.4
	2015/2016	10.9	9.3	3.4	-0.8	7.3	6.8	13.2	14.7	20.9	22.8
TMP, mm	2013/2014	156.3	18.6	9.2	94.8	6.9	37.7	29.6	78.2	192.5	50.9
	2014/2015	57.9	33.2	87.0	33.2	79.5	67.7	8.5	12.9	31.3	27.2
	2015/2016	78.3	55.1	0.4	86.3	40.7	52.7	20.8	117.1	55.7	2.8

AMT – average monthly temperatures; TMP – total monthly precipitation

Table 2. Two way ANOVA of the yield from the investigated triticale cultivars

Parameter	SS	df	MS	F	Sig.	F%
Total	3883247,250	191	20331,137	-	-	-
Genotypes (G)	432112,917	15	28807,528	6,197	0,000	11,13
Environments (E)	2191414,781	2	1095707,391	235,694	0,000	56,43
G x E	543684,552	30	18122,818	3,898	0,000	14,00
Blocks	60545,750	3	20181,917	4,341	0,006	1,56
Err	655489,250	141	4648,860	-	-	-

Such type of genetic basis allows clearly differentiating the separate genotypes according to their adaptability to specific contrasting environments, and ranking them by their productivity. Depending on their origin, the set of genotypes involved in a certain trial, demonstrated different reactions, as indicated by the greater part of the investigations on triticale (Alljarah et al., 2014; Dhindsa et al., 2002; Goyal et al., 2011; Goyali and Dhindsa, 2003) and common winter wheat (Banjack et al., 2014; Tsenov et al., 2013). The 8 groups formed according to the Duncan test additionally confirmed the differences in the response of the genotypes.

Table 3. Yield values and numerical parameters of the applied ranking models

Cultivar	Y, kg/dca	RY, %	RV, %	RE	HARV	H_i
AD-7291	572^{bcd}	103,4	102,5	6	98,2	0,16
Vihren	548^{abc}	98,9	97,9	4	93,8	-0,75
Rakita	570^{bcd}	101,1	99,3	4	95,2	-0,37
Lasko	548^{abc}	96,2	94,0	2	90,2	-1,38
Presto	537^{ab}	94,0	91,7	2	87,9	-1,94
Kolorit	580^{bcd}	103,6	102,1	6	97,9	0,40
Atila	609^{cde}	107,7	106,0	8	101,8	0,74
Akord	611^{cde}	108,6	106,7	8	102,3	1,61
Respekt	490^{a}	86,3	84,0	1	80,4	-3,20
Bumerang	550^{abc}	96,5	94,1	4	90,2	-1,10
Irnik	602^{bcde}	106,3	104,2	6	99,9	1,11
Dobrudzhanets	538^{ab}	96,3	94,7	4	90,7	-1,16
Lovchanets	494^{a}	88,0	86,3	1	82,6	-2,91
Doni 52	661^{e}	117,8	116,0	12	111,2	3,88
Blagovest	616de	110,0	108,2	8	103,7	2,18
Borislav	646^{e}	114,2	112,1	10	107,6	2,73

Table 3 shows the data by cultivars obtained as a result from the application of the above ranking models. In all calculated parameters, a clear tendency was outlined toward forming of groups which included genotypes with identical productivity. In spite of the different methodologies used in each model, the results clearly emphasized their similar ranking under the specific growing conditions. The present high effect of the environment x genotype interaction implies variable stability and ecological plasticity. From this point of view, the conditions of the environment allowed following the genotypes with highest interaction with the sharp changes during the growth period with regard to total productivity. This information is being visualized through the parameters HARV and Hi. Their values corrected the absolute yield according to the expression of the cultivars during the investigated period and provided practical data on the behavior of the complex index yield-stability. On the other hand, the presence of an identical tendency of HARV and Hi with the rest of the models underlined the fact that a negative correlation between the productive potential and the genotype's stability was not observed. This fact is also confirmed by previous investigations on the same cultivars (Stoyanov and Baychev, 2016b), in which it was found on the basis of different methods that the most productive cultivars (Akord, Doni 52, Blagovest and Borislav) possessed highest stability. The different ways for formation of productivity of the individual genotypes, however, were difficult to differentiate. This is so because the stability of the separate yield components does not follow the stability of the yield itself (Tsenov et al., 2014).

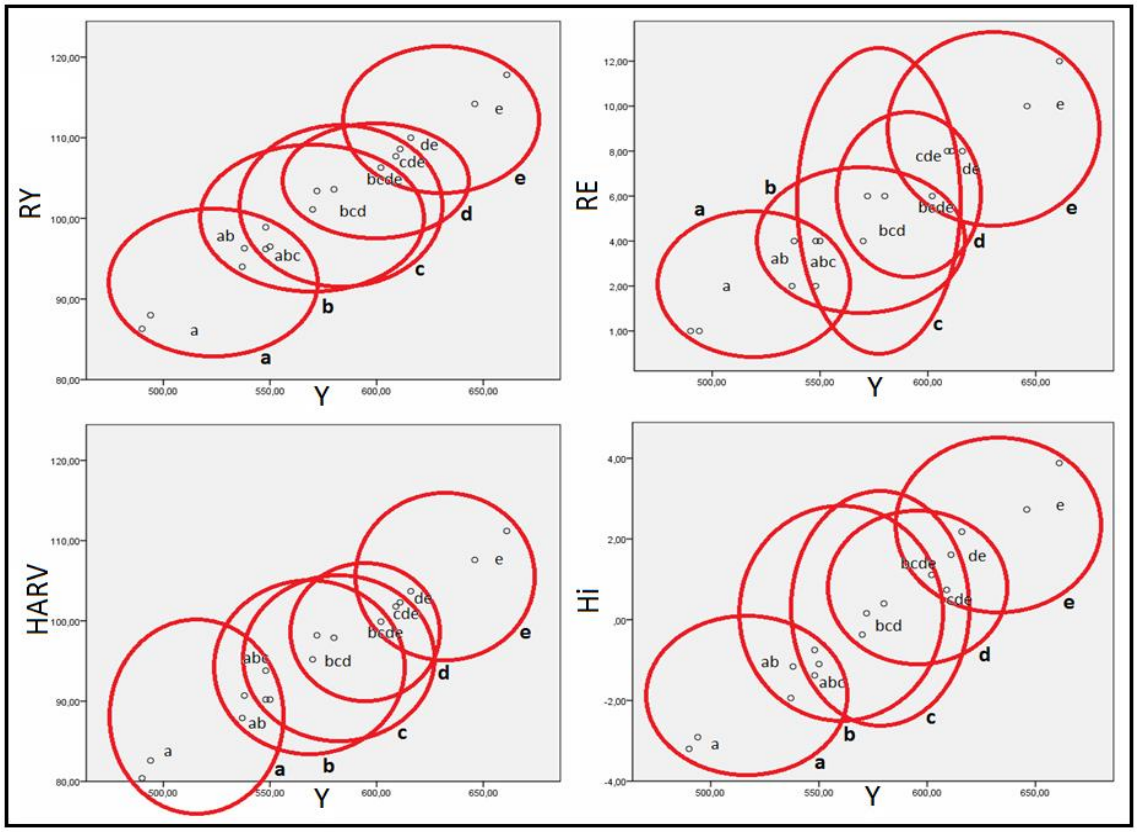

Figure 1. Regression between yield and the applied ranking models

The high correlation observed between the yield and the values of the respective models (Table 4) emphasized the applicability of each of them. The RY-model was with the highest correlation coefficient, and the RE-model – with the lowest. The parameters RV, HARV and Hi had similar values. The parameter HARV was with the highest coefficient of determination, which emphasized its practical applicability because apart from yield, it was also taking into account the effect of the environment. Tsenov et al., (2014) have pointed out that in experiments with multiple locations, the applicability of Hi significantly decreased. Since this parameter is aimed at trials carried out in a single location (Martynov, 1990), its high correlation and applicability for ranking of the investigated genotypes is in accordance with such investigations.

Table 4. Correlation of yield with the numerical parameters of the applied ranking models

Parameter	Y, kg/dca		
	r	sig	R^2
RY, %	0,994	0,000	0,989
RV, %	0,989	0,000	0,977
RE	0,964	0,000	0,929
HARV	0,989	0,000	0,978
H_i	0,986	0,000	0,973

A confirmation for the high applicability of all models is the graphic presentation of the linear regression of each of them with the yield. Such type of correlation is related to the efficiency of the used ranking models in the investigated set of genotypes. Figure 1 allows clearly following the regression lines and the slight deviations from the main tendency. Nevertheless, it was observed when using the RE-model that the ranking was less precise. It can be claimed that the coefficient of determination also confirm that, this model was less efficient than the rest. In these models the groups can be easily distinguished as a result from the Dunkan test, while in the RE-model certain values can not be clearly differentiated due to the discrete nature of the index. Concerning the two models related to the effect of the environmental conditions, the HA-model gave better ranking than the Hi-model. Nevertheless, the absence of multiple locations in the HA-model is related to higher identity of the environmental conditions regardless of the presence of contrasting environments (Yan and Holland, 2010). On the other hand the high correlation with the absolute yield makes it suitable for ranking since it takes into account the actual variation of the yield within the entire investigated set of cultivars.

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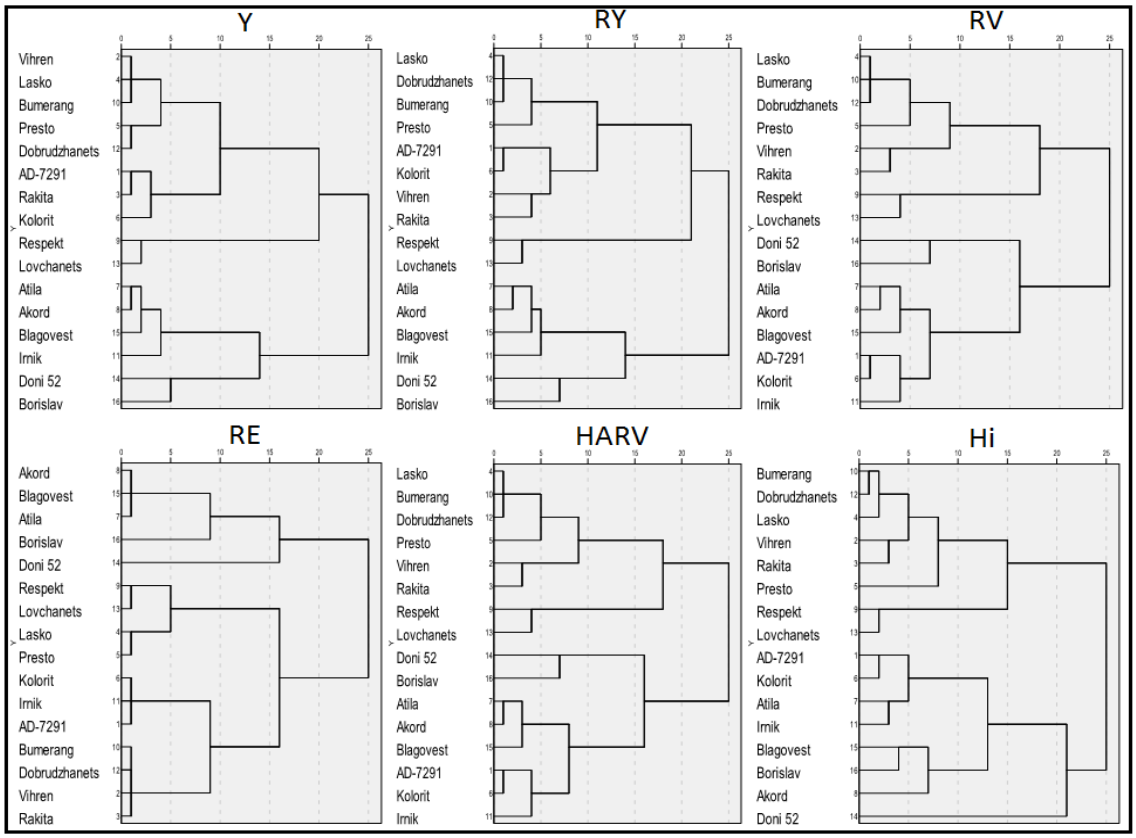

Figure 2. Cluster analysis by yield and parameters of the applied ranking models

Figure 2 presents dendrograms from the cluster analysis by values of each applied model. This analysis gives a real idea about the ranking of the genotypes taking into account the similarities between them. It is worth mentioning that the high-yielding and stable varieties ranked in identical clusters regardless of the method used. Cultivars Doni 52 and Borislav comprised a separate cluster in each of the applied models. This emphasized their unique productivity and high ecological plasticity.

In triticale, such ranking is essential due to the complexity in the response of the genotypes to the environment. The investigated genotypes allowed, even under very contrasting environments, the forming of groups which combined certain productivity with stability. All applied models are in this respect suitable for identification of this

ranking and can be efficiently used in the breeding of triticale.

Conclusions

Most efficient was the model using the mean standard from the check varieties in the trial. The models, in which the yield was being corrected with the variation caused by the variable environment (HA and Hi models), were also with good efficiency for yield evaluation. Nevertheless, the HA-model was very similar to the ranking of the cultivars by their absolute yield. This was related to the single location of investigation regardless of the contrasting agro-climatic conditions. On the other hand, the Hi-model allowed interpreting the yield and ranking the response of the yield from the different cultivars. The score based on the relative yield according to the mean of the trial turned out to be with lowest efficiency because the ranking of the cultivars was too subjective according to the accepted scale and did not correctly take into account the effect of the contrasting conditions. In spite of some disadvantages, however, each model can be successfully used for analysis of the yield during periods of strongly contrasting conditions depending on the specific purpose of the breeding program.

References

Aljarrah, M., L. Oatway, S. Alberts and C. Bergen, 2014. Variability, heritability and genetic advance in some agronomic and forage quality characters of spring triticale in western Canada. Communications in Agricultural and Applied Biological Sciences, Ghent University, 79(4): 9-18.

Banjac B., V. Mladenov, M. Dimitrijevic, S. Petrovic and J. Bocanski, 2014. Genotype × environment interactions and phenotypic stability for wheat grown in stressful conditions- Genetika, Vol 46, No. 3, 799-806

Baychev, V. 2013. Triticale lines and varieties grown under contrasting meteorological conditions. Scientific Works of Instiute of Agriculture – Karnobat, 2(1): 79-86.

Becker, H.C. and J. Leon, 1988. Stability analysis in plant breeding. Plant Breeding, 101, 1-23.

Dhindsa, G.S., A.S. Dosanjh, V.S. Sohn, J.S. Dhindsa and J.C. Goyali, 2002. Genotype x environment interaction for yield components in hexaploid triticale. Proceedings of the 5th International Triticale Symposium, Volume II, June 30 - July 5, 2002, Radzikow, Poland, 199-200.

Farshadfar, E. and M. Farhadi, 2014. AMMI and AMMI based analysis of phenotypic stability in wheat-Agropyron disomic addition lines. Journal of Biodiversity and Environmental Sciences, 5(4), 548-557.

Gabriel, K.R. 1971. The biplot graphic display of matrices with application to principal component analysis. Biometrika, 58: 453-467.

Goyal, A., B.L. Beres, H.S. Randhawa, A. Navabi, D.F. Salmon and F. Eudes, 2011. Yield stability analysis of broadly adaptive triticale germplasm in southern and central Alberta, Canada for industrial end-use suitability. Can. J. Plant Sci., 91: 125-135.

Goyali, J.C. and G.S. Dhindsa, 2003. Stability behaviour of some triticale (xTriticosecale Wittmack) genotypes for yield and yield components. Triticale Topicos, 19: 17-21.

Karimizadeh ,R., M. Mohammadi, N. Sabaghnia, M. K. Shefazadeh and J. Pouralhossini, 2012. Univariate stability analysis methods for determining genotype × environment interaction of durum wheat grain yield. African Journal of Biotechnology, 11(10): 2563-2573.

Martynov, S. 1990. A method for the estimation of crop varieties stability, Biometrical Journal, 7: 887-893.

Lozano-del Río, A.J., V.M. Zamora-Villa, L. Ibarra-Jiménez, S.A. Rodríguez-Herrera, E. de la Cruz-Lázaro and M. de la Rosa-Ibarra, 2009. AMMI analysis of genotype-environment interaction and production potential of forage triticale (X Triticosecale Wittm.). Universidad y Ciencia Tropico Humedo, 25(31): 81-92.

Motzo, R., F. Giunta and M. Deidda, 2001. Factors affecting the genotype x environment interaction in spring triticale grown in a Mediterranea environment. Euphytica, 121, 317-324.

Stoyanov, H. and V. Baychev, 2016a, Achievements and trends in the breeding of triticale in Bulgaria. 9th International Triticale Symposium, Szeged, Hungary, May 23-27, 2016 Book of Abstracts: 20.

Stoyanov, H. and V. Baychev, 2016b. Analysis on "genotype x environment" interaction in Bulgarian triticale (×Triticosecale Wittm.)

cultivars. Scientific works of Institute of Agriculture – Karnobat, (in press)

Tsenov, N., D. Atanasova and T. Gubatov, 2013, Genotype x environment effects on the productivity traits of common wheat. I. Nature of interaction. Scientific Works of Institute of Agriculture – Karnobat, 3(1): 57-70.

Tsenov N., D. Atanasova, M. Nankova, A. Ivanova, E. Tsenova, P. Chamurliiski and G. Raykov, 2014. Approaches for grading breeding evaluation of winter wheat varieties for grain yield. Scientific Works of Instiute of Agriculture – Karnobat, 3(1): 21-35

Yan, W. and J. Holland, 2010. A heritability-adjusted GGE biplot for test environment evaluation, Euphytica: 171, 355-369.

Yan, W. and M.S. Kang, 2003. GGE Biplot Analysis. A Graphical Tool for Breeders, Geneticists, and Agronomists. CRC PRESS, Boca Raton, London, New York, Washington, D.C.

Zobel, R.W., M.J. Wright and H.G. Gauch, 1988. Statistical analysis of a yield trial. Agronomy Journal, 80: 388-393

Seasonal Variation of the Nutrient Contents of *Sarcopoterium spinosum* (L.) Spach

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The research was conducted to find out the feeding potential of *Sarcopoterium spinosum* (L.) Spach, commonly found in Mediterranean climate dominated areas and also have an important place in rangeland livestock farming as well as in erosion of these areas. The study was carried out in Yıldız Koyu of Gökçeada Island in 2013. The nutrient contents of plant were determined seasonally (winter, summer, spring and fall) as well as in different organs (stalk and leaf) of the plant. Total of 10 plants were taken during each sampling period. Only the crude protein and ADF contents of plant leaf had found statistically important in terms of season, but there were not any significant importance in respect to other characteristics of the plant. The leaf and stalk ratios were changed between 37.57% (fall)–42.38% (winter) and 57.62% (winter)–62.49% (fall), respectively. Highest leaf/stalk ratio (0.74) has been observed in winter while the least (0.60) in fall. Average crude protein (6.39%), ash (9.66%) and fat (6.33–7.60%) of leaves were found more as compared to the crude protein (3.81%), ash (4.95%) and fat (4.37–5.80%) of stalk. On the other hand, NDF (36.19–43.41%), ADF (22.31–27.54%) and ADL (9.50–13.35%) of leaves were found less as compared to stalks. Consequently, *Sarcopoterium spinosum* has an importance in terms of soil protection and shrub formation into the degraded parts of Mediterranean flora, also found as a feed source for animals. However, supplementary feed should be given to the animals grazing in these areas for overcoming their poor performances.

Keywords: *Sarcopoterium spinosum* L., crude protein, crude ash, crude lipid, NDF, ADF, ADL

Introduction

Broad areas of maquis scrubland are found in Marmara, Aegean and Mediterranean regions of Turkey where the Mediterranean climatic conditions are dominant. This situation is due to the long hot and dry summer season of the Mediterranean climatic zone. A portion of Mediterranean shrubby (especially in disrupted areas) is composed of dwarf shrubs called as garrigue. In addition to climatic factors, soil conditions were also found very important for dwarf shrubs (Diamantopoulos et al., 1994). Thus in areas with drought season and particularly shallow soils, woody plants with roots close to the surface become dry quickly. In this situation shrubs with deeper roots become emplaced and preserve their greenery during the drought season. Because of this, the dominant plant vegetation cover in the Mediterranean climatic zone are the shrubby lands (maquis and garrigue). Maquis describes shrubs growing up to 5 meters, while the plant vegetation cover of garrigue describes the plants growing to a height of 1 meter. Maquis and garrigue plant vegetation cover encompasses 100 million hectares of the earth's surface in the world, while around 7 million hectares in Turkey (Baytekin et al., 2005).

Maquis plant cover is distributed in karstic areas, while garrigue plant vegetation cover grows in poor, arid and shallow soils. According to Yılmaz (1993), the most significant species found in maquis plant vegetation cover are: strawberry tree (*Arbutus unedo*), Greek strawberry tree (*Arbutus andrachne*), tree heath (*Erica arborea*), common myrtle (*Myrtus communis*), turpentine tree (*Pistacia terebinthus*), carob tree (*Ceretonia siliqua*), bay laurel (*Laurus nobilis*), evergreen oak (*Quercus ilex*), mock privet (*Phillyrea latifolia*), Judas tree (*Cercis siliquastrum*), juniper (*Juniperus* sp.), Spanish broom (*Spartium junceum*, *Genista* spp.), oleander (*Nerium oleander*), spiny broom (*Calicotome villosa*) and rockrose (pink rockrose) (*Cistus* spp.). In case of garrigue plant cover, kermes oak (*Quercus coccifera*), mock privet (*Phillyrea latifolia*), thyme (*Thymus* spp.), sage (*Salvia* spp.), rockrose (*Cistus* spp.), thorny burnet (*Sarcopoterium spinosum*), lavender (*Lavandula* spp.), rosemary (*Rosmarinum* spp.) and milkvetch (*Astragalus* spp.) are common (Yilmaz, 1996). Garrigue plant cover is commonly found in disrupted maquis areas. Many researchers mentioned that these are the peak plant cover in the Mediterranean climatic zone and they minimize the erosion risk than may occur in these areas (Bakir, 1987; Atalay et al., 2003). The westernmost point of Turkey in Gökçeada (Imbros)

is covered by 27% maquis and 33% thorny burnet (garrigue) stated by Cengiz et al., 2009. Within the garrigue pant cover, the amount of thorny burnet is very high. These areas on Gökçeada represent an important place in terms of pasture animal husbandry. As the characteristic trait of the Mediterranean belt is the short and dry green forage period, in these areas sheep and goat farming has increased. In general, cattle farming increases in areas with a long humid and green forage period (Seligman, 1996). By hiding the grazed portion of the leaves under the thorny portions, in semi-arid Mediterranean shrub pastures plants have become resistant to grazing (Osem et al., 2007). Due to these characteristics of plants, grazing is at very limited levels. That is why, the most favourable grazing periods were the winter and autumn months when the plants become soften (Gökkuş et al., 2009). Some studies showed that in periods when tasty species are not found into the vegetation cover then the animals are compelled to graze on ***Sarcopoterium spinosum*** with low flavor (Kababya et al., 1998; Aharon et al., 2007). ***S. spinosum*** is best consumed by sheep and especially goats. However, there is a reduction observed in goat population in island since 1980. On the other hand, the Gökçeada (Imbros) sheep has adapted well to this plant. The thorny burnet (*Sarcopoterium spinosum* (L.) Spach. syn: *Poterium spinosum* L.) belongs to the Rosaceae family and is within the scrub bush (nanophanerophyte) group (Smirin et al., 2010). Globally, it is spreading between the Mediterranean and Iran-Turanian regions (Lanteri et al., 2012).

In Turkey, it is generally observed in the Aegean, Mediterranean and Marmara regions (Adana, İstanbul, Antalya, Aydın, Çanakkale, İzmir and Sinop) (Davis, 1972; Anonymous, 2014). Its habitat is between 0-1000 meters (Kaya and Aladağ, 2009). ***S. spinosum*** requires shallow soil that is poor in phosphorus, and develops in practiced but abandoned areas and on limestone bedrock (Alphan, 2006; Reisman-Berman et al., 2006; Cengiz et al., 2009; Kaya and Aladağ, 2009; Henkin and Seligman, 2011). Garrigue plant cover develops after the disruption of maquis areas and is generally observed in areas with semi-arid climatic conditions (Atalay, 1994; Sternberg and Shoshany, 2001). The reason for its development and widespread cover in these areas are proposed as its adaptation ability, and high ability for sexual and asexual proliferation (Seligman and Henkin, 2009). It is a perennial plant, and may reach a height of 100-150 cm. Leaves are oppositely set and there are 9-15 leaves. Seeds do not have distribution characteristics that is why it forms an excess amount of seeds. Seeds may only spread up to 50 cm from the main plant. The amount of seeds left under the main plant is about 2000-3000 seeds/m^2. Thus it proliferates in a very rapid fashion. Fruits have 2 carpels and each carpel contains one seed (Mishkinsky, 1965; Osem et al., 2007; Seligman and Henkin, 2007; Metz et al., 2010). Flowers are bisexual, with a flowering duration of 3 months generally (February-March-April). Roots may reach a depth of 40 cm. The competitive power of the plant against other plants in terms of competition and aridity is very weak, especially in their seedling period (Litav et al., 1963; Reisman-Berman, 2007; Seifan et al., 2010). The thorny burnet is used in Middle Eastern countries for diabetes, to solve digestive problems, in cancer treatment and in religious services (Tovit et al., 2010). In Greece, it is used for bio-fuel technology (Margaris and Vokou, 1985). This plant is used in some Middle Eastern countries like Palestine as fencing material (Figure 9). A large proportion of the essential oil acids in the roots is elemol (66.65%) with the remainder α-eudesmol (33.26%) (Hudifa et al., 2013).

The pasture quality is an important factor in estimation of the productivity power of animals (Tatlı Seven and Çerçi, 2006). For this reason, the main aim of this study was to determine nutritional content of the grazable portions of thorny burnet (investigation of seasonal variations) and provide a good description of 33% vegetation covering of the grazing area.

Materials and Methods

The research was conducted in 2013 in the Yıldız Village of Gökçeada in Çanakkale Province. The soils used in the experiments were slightly alkaline (pH: 7.92), with low soluble salt (94.46 ppm) and carbonate (2.32%), moderate organic matter (2.68%), low phosphorus (2.42 ppm), high calcium (42.015 ppm), magnesium (4275 ppm) and potassium (2823 ppm) and low sodium (675 ppm) have been identified. According to the data records obtained from Çanakkale Provincial Directorate of Meteorology, in last 20 years (1982-2012) showed that the average monthly temperature in Gökçeada was 15.3 °C. Whether in the long term or in the experimental year, the highest monthly mean temperatures were in July and August while

the lowest temperatures have been measured in January and February. The 20 year mean annual total precipitation in Gökçeada was recorded as 722.1 mm, with mean total precipitation in 2010, 2012 and 2013 (except December) of 791.8, 792.7 and 869.1 mm with higher precipitation as compared to the long-term records. The year of 2011 was found more arid as compared to previous years (640.8 mm). During the experimental period, drought was generally effective in June-September. The plant materials used in this research work have been collected from a total of 10 plants selected randomly in each time. Plant samples were collected in the months of May, August, November and February to represent the spring, summer, autumn and winter seasons. Plant samples were taken to the laboratory and separated into leaf and stalk, and then necessary analyses have been done. The research determined leaf/stalk ratio, crude oil and ash analyses (AOAC, 2000), crude protein (Bremner, 1960) and NDF-ADF and ADL analyses (Van Soest et al., 1991). Statistical analysis in the research used the mean multiple comparison test with the SAS 9.0 statistical program.

Findings and Discussion

Leaf, stalk (%) and leaf/stalk ratio

There was no statistically significant variation in leaf, stalk and leaf/stalk ratios of thorny burnet according to season (Table 1). The highest leaf percentage was 42.38% in winter with lowest leaf percentage of 37.57% recorded in autumn for thorny burnet. The mean leaf percentages in the spring and summer months were very close to each other i.e., 41.28 and 41.72%, respectively. The mean leaf percentage produced during the year was noted as 40.88%. The stalk percentage within the seasonal mean grazable plant component produced was as 59.12%. The highest stalk percentage (62.43%) was measured in autumn while stalk percentages quite similar in the other months (between 57.62-58.28%). The year-long mean leaf/stalk ratio was identified as 0.69%. Within the year, the highest leaf/stalk ratio was 0.74% in winter, but the lowest has been identified in autumn (0.60). The ratio of leaf was found correlated to the life cycle of plant. As the plant leaves became dry in summer period it enters the autumn with the lowest leaf percentage and begins green again with autumn rains, increasing toward the winter period and reaching its highest point. After winter, the leaf percentages continuously reduced. Thus, the stalk percentage exhibits an inverse conditio

Table 1. Leaf, stalk and leaf/stalk ratio according to seasons

Seasons	Leaf ratio (%)	stalk ratio (%)	Leaf/stalk ratio (%)
Autumn	37,57	62,43	0,60
Winter	42,38	57,62	0,74
Spring	41,84	58,16	0,72
Summer	41,72	58,28	0,72
Mean	**40,88**	**59,12**	**0,69**
Pr>F P_{leaf} ratio=0,7944, $P_{stalk\ ratio}$=0,7944, $P_{leaf/stalk\ ratio}$ = 0,8871			

Ratio of crude protein (%)

In terms of crude protein content of different parts of the plant, the stalk had little difference in the periods (Pstalk=0.6773), while the leaf portion produced completely adverse results ((Pleaf=0.0017). The crude protein percentage in stalk part of the plant was recorded as 3.81%, having highest in spring (4.13%) and lowest (3.71%) in autumn. The mean protein percentage in the leaves was noted as 6.39%. The crude protein content in leaves were observed as 7.46% and 7.10% in winter and spring, respectively; while in autumn (6.38%) and summer (4.63%). The mean crude protein content in case of the whole plant was found highest in spring (5.37%) and lowest in summer (4.10%). As all portions of the plant dry out in the summer season, the crude protein percentage reaches at lowest levels. The mean crude protein content per season and plant part was identified as 4.87% shown in Table 2. For sheep and goat breeders, the protein content in feed for survival should be at least 8.9% (Welch, 1989). Studies have found the crude protein contents of thorny burnet are in between 3.8-5.6% (Özaslan-Parlak et al., 2011), but in case of another study findings, a variation of 6-8% has been mentioned (Kababya et al., 1997). Thus the thorny

burnet does not supply necessary protein requirements for sheep and goat survival in any season within the all parts of the plant.

Table2. Variation in crude protein ratio according to seasons and plant parts

Seasons	Ratio of crude protein (%)		
	stalk	Leaf	Mean
Autumn	3,71	6,38 **a**	4,71
Winter	3,69	7,46 **a**	5,29
Spring	4,13	7,10 **a**	5,37
Summer	3,72	4,63 **b**	4,10
Mean	**3,81**	**6,39**	**4,87**
Pr>F P_{stalk}=0,6773, P_{leaf}=0,0017			

Ratio of crude ash (%)

Differences between crude ash content of plant parts depending on season were not found statistically significant (Pstalk=0.2986, Pleaf=0.3854). The stalk part of thorny burnet had mean crude ash percentage of 4.92% along with the leaf content of 9.66%. However, the crude ash content of stalk part was highest in winter valued by 6.13%, while for leaves, it was observed highest in summer season with 11.35%. The lowest ash contents were recorded in stalk part in autumn with 3.99%, while the lowest was determined in leaves in autumn with a ratio of 8.39%. According to season, the crude ash content of the whole plant (stalks and leaves) was recorded highest in summer (7.63%), but the lowest was found in autumn (6.01%). Seasonal mean crude ash content of thorny burnet was determined as 6.85% shown in Table 3. The crude ash content found in the leaves of taken plant samples during the year was above the mineral requirements of animals. In this way, thorny burnet may be a good inorganic matter source for grazing animals.

Table 3. Variation in crude ash ratio according to seasons and plant parts

Seasons	Ratio of crude ash (%)		
	stalk	Leaf	Mean
Autumn	3,99	9,38	6,01
Winter	6,13	9,53	7,57
Spring	4,61	8,39	6,19
Summer	4,95	11,35	7,63
Mean	**4,92**	**9,66**	**6,85**
Pr>F P_{stalk}=0,2986, P_{leaf}=0,3854			

Ratio of crude oil (%)

Variance analysis found that the crude oil content of thorny burnet had no statistically significant difference according to season. The mean crude oil percentage in stalks was noted as 5.33% and in leaves it was recorded as 7.16%. Depending on season, the crude oil percentage in stalk varied from 4.73-5.80%, while in leaves it varied from 6.33-7.60%. The mean crude oil percentage in stalk and leaves varied from 5.89% (in autumn) and 6.37% (in spring) according to season (Table 4). For grazing animals, the consumed forage should not contain less than 2.70% oil (NRC, 2001). Accordingly, the grazable portions of thorny burnet contain sufficient levels of crude oil for animals throughout the year.

Table 4. Variation in crude oil ratio according to seasons and plant parts

Seasons	Crude fat ratio (%)		
	Stalk	Leaf	Mean
Autumn	5,62	6,33	5,89
Winter	5,18	7,56	6,19
Spring	5,80	7,16	6,37
Summer	4,73	7,60	5,93
Mean	**5,33**	**7,16**	**6,10**
Pr>F P_{stalk}=0,3475, P_{leaf}=0,1622			

Ratio of cell membrane matters (NDF, ADF, ADL)

The NDF ratio in the thorny burnet shrub did not have a statistically significant difference according to season. The stem section of the plant has NDF percentage of 69.52%, with this percentage 40.79% in leaves. Additionally, when season is examined, the highest NDF percentage in the stem was found in the summer season (71.85%) with highest percentage for leaves found in the autumn (43.41%). The lowest NDF percentage in the stem was 66.93% in the autumn and in leaves was 36.19% in the winter period. The NDF percentage for the whole plant varied from 59.81% (summer) to 54.83% (winter) and mean NDF percentage was determined as 57.75% (Table 5). For grazing animals, the daily based consumed forage should not contain NDF content more than 45.80% (NRC, 2001). According to this, the NDF content in stalk of the plant was above this level, but leaves had NDF content below the desired level.

Table 5. Variation in NDF ratio according to seasons and plant parts

Seasons	NDF oranı (%)		
	Stalk	Leaf	Mean
Autumn	66,93	40,60	57,03
Winter	68,53	36,19	54,83
Spring	70,77	43,41	59,32
Summer	71,85	42,98	59,81
Mean	**69,52**	**40,79**	**57,75**
Pr>F P_{stalk}=0,1884, P_{leaf}=0,2339			

The percentage of ADF in thorny burnet only had a statistically significant difference in leaves according to season. Accordingly, the mean ADF percentage in the stalk of the plant was recorded as 52.14%, while in leaves this percentage was gone down less than half (24.17%). The ADF percentage in plant stalks was lowest in autumn (49.21%), increasing by significant levels in spring and summer to 52.88% and 54.74%, respectively. The ADF percentage in leaves was significantly higher in spring as compared to other seasons (27.54%). It varied from 22.31-23.89% in other seasons. The seasonal mean varied from 42.28% (spring) and 39.25% (summer) for the whole plant (stalk + leaves). The mean ADF percentage was recorded as 40.67%, shown in Table 6. For grazing animals, the highest acceptable level of mean ADF is taken as 25%, and accordingly the stalk part of the plant had high ADF percentages throughout the year. However, the leaf ADF content was remained below at acceptable levels.

Table 6. Variation in ADF ratio according to seasons and plant parts

Seasons	ADF ratio (%)		
	stalk	Leaf	Mean
Autumn	49,21	23,98 **b**	39,73
Winter	51,72	22,31 **b**	39,25
Spring	52,88	27,54 **a**	42,28
Summer	54,74	22,83 **b**	41,42
Mean	**52,14**	**24,17**	**40,67**
Pr>F P_{stalk}=0,0582, P_{leaf}=0,0087			

The ADL content of stalk and leaf parts of the thorny burnet did not show any significant importance according to season. The stalk section of the plant had mean ADL as 20.26%, while the leaf portion had a much lower percentage of 11.36%. The ADL percentage in stalk varied from 18.83% (autumn) to 21.27% (summer), while the ADL percentage in leaves varied from 9.50% (spring) to 13.35% (autumn). The seasonal means varied from 17.56% (summer) to 15.98% (spring) for the whole plant (leaf + stem) along with the mean ADL percentage identified as 16.59% (Table 7). The ADL level in forage consumed by rangelands livestocks should not be above 10%. Accordingly, this level remained above into stalk section in all seasons, while the ADL content was slightly kept above the desired level in leaves.

Table 7. Variation in ADL ratio according to seasons and plant parts

Seasons	ADL ratio (%)		
	stalk	Leaf	Mean
Autumn	18.83	13.35	16.78
Winter	20.30	10.25	16.04
Spring	20.65	9.50	15.98
Summer	21.27	12.36	17.56
Mean	**20.26**	**11.36**	**16.59**
Pr>F P_{stalk}=0,6968, P_{leaf}=0,1128			

Conclusions

The research was conducted aim to reveal the nutritional potential of different plant parts (stalk and leaf) of the thorny burnet plant commonly found in shrubland vegetation cover (garrigue) in Mediterranean countries. The leaf percentage mean was found 40.88% with stalk percentage as 59.12% and leaf/stalk ratio as 0.69. The mean crude protein percentage in leaves was determined as 6.39%, but this ratio was found as 3.18% in stalks. The protein content of the leaves reached a maximum of 7.46% in winter, but with the lowest percentage of 4.63% in summer. The mean crude protein content for the whole plant was 4.87%. The mean crude ash percentage in leaves was found as 9.63%, while this value was 4.92% in stalks. The highest crude ash content in leaves was noted in summer (11.35%) with lowest in autumn (8.39%). The mean crude ash content for the whole plant was identified as 6.85%. The mean crude oil content of leaves was obtained as 7.16%, with stalk having mean of 5.33%. The highest oil content in the leaves was found in summer (7.60%) with lowest in autumn (6.33%), and mean oil content for the whole plant noted as 6.10%. The mean ratios of NDF, ADF and ADL found in the leaves were 40.79%, 24.17% and 11.14%, respectively, while this ratio was recorded as 69.52, 52.14 and 20.26% in stalks. According to these results, the nutritional content of the leaves of plant may not be an alternative source of forage for pasture-based animal farming in Mediterranean countries with short green forage period as the nutritional potential of the stalk is below desired levels and is not appropriate for animal nutrition. Thus, animals generally take more benefit from this plant in spring when new leaves start emerging.

References

Alphan, H. 2006. Ekosistem dinamiklerinin izlenmesine bir araç olarak peyzaj değişimlerinin analizi. Ekoloji, 58: 8-15.

Anonim, 2014. Türkiye bitkileri veri serisi. http://turkherb.ibu.edu.tr/index.

AOAC, 2000. Official Methods of Analysis. 17th Edition, Association of Analytical Chemists, Gaithersburg, MD, USA.

Atalay, I. 1994. Türkiye Vejetasyon Coğrafyası. Ege Üni. Basımevi, İzmir, s, 195.

Atalay, I., A. Semenderoğlu, H. Çukur and N. Gümüş, 2003. Driving forces of rangeland degradation in Turkey. The RICAMARE Workshop on Land Use Changes and Cover and Water Resources in the Mediterranean Region, 17 Feb., Toulouse, France, pp 8.

Bakır, Ö. 1987. Çayır Mera Amenajmanı. Ankara Üni. Ziraat Fak. Yay.: 992, Ders Kitabı: 292, 362 s.

Baytekin, H., İ. Y. Yurtman and T. Savaş, 2005. Süt keçiciliğinde kaba yem üretim organizasyonu. Süt Keçiciliği Ulusal Kongresi, 26-27 Mayıs 2005, İzmir.

Bremner, J.M. 1960. Determination of nitrogen in soil by Kjeldahl method. J. Agri. Sci. 55: 11-33.

Cengiz, T., H. Özcan, H. Baytekin, Ü. Altınoluk, A. Kelkit, F. Özkök, C. Akbulak and A.Ç. Kaptan, 2009. Gökçeada Arazi Kullanım Planlaması. TÜBİTAK ÇAYDAĞ Hızlı Destek Projesi (Proje No: 107Y337) Sonuç Raporu, s 146.

Davis, P.H. 1972. Flora of Turkey and the East Aegean Islands. Uni. of Edinburg Press, Vol. 4.

Diamantopulos, J., S.A. Pirintsos, N.S. Margaris and G.P. Stamou, 1994. Variation in Greek phrygana vegetation in relation to soil and climate. J. Vegetation Sci., 5: 355-360.

Gökkuş, A., A. Özaslan Parlak, H. Hakyemez, H. Baytekin and M. Parlak, 2009. Maki örtüsünde yer alan bitki türlerinin botanik özellikleri ile besleme değerlerindeki değişimin belirlenmesi. TÜBİTAK Proje No: 106O458, Sonuç Raporu, 147 s.

Henkin, Z. and N.G. Seligman, 2011. The role of management on the rate of secondary succession in Mediterranean shrubland after fire. Plant Biosystems, 145(3): 708-714.

Kababya, D., A. Perevolotsky, I. Bruckental and S. Landau, 1997. Nutritional potential of woody vegetation for local goats in Israel, In: Recent Advances in Small Ruminant Nutrition (Eds; Lindberg J.E., Gonda H.L., Ledin I.), Options Méditerranéennes: Série A, Séminaires Méditerranéens, n. 34, 47-52.

Kaya, B. and C. Aladağ, 2009. Maki ve garig topluluklarının Türkiye'deki yayılış alanları ve ekolojik özelliklerinin incelenmesi. Selçuk Üni. Sosyal Bil. Enst. Dergisi, 22: 67-80.

Lanteri, A., A. Guglielmo, P. Pavone and C. Salmeri, 2012. Seed germination in *Sarcopoterium spinosum* (L.) Spach from South-Eastern Sicily. Plant Biosystems, 147(1): 60-63.

Litav, M., G. Kupernik and G. Orshan, 1963. The role of competition as a factor in determining the distribution of dwarf shrub communities in the Mediterranean territory of Israel. J. Ecology, 51: 467-480.

Margaris, N. and D. Vokou, 1985. Latex producing plants in Greece. Biomass, 7: 161-170.

Metz, J., P. Liancourt, J. Kigel, D. Harel, M. Sternberg and K. Tielbörger, 2010. Plant survival in relation to seed size along environmental gradients: A long-term study from semi-arid and Mediterranean annual plant communities. J. Ecology, 98: 697-704.

Mishkinsky, J., E. Menczel and F. Sulman, 1965. Hypoglycemic Effect of *Poterium spinosum* L. (Rosaceae). Archives Internationales de Pharmacodynamie ET de Thérapie, 161(2): 306-313.

Nelson, C.J. and L.E. Moser, 1994. Plant factors affecting forage quality. In: Forage Quality, Evaluation and Utilization Fahey, Jr G.C., Ed., ASA, CSSA, SSSA, Wisconsin, 115-154.

NRC, 2001. Nutrient Requirements of Dairy Cattle. 7th Rev. ed. *Natl. Acad. Sci*., Washington, DC.

Osem, Y., I. Konsens, A. Perevelotsky and J. Kigel, 2007. Soil seed bank and seedling emergence of *Sarcopoterium spinosum* as affected by grazing in a patchy semiarid shrubland. Israel J. Plant Sciences, 55(1): 35-43.

Özaslan-Parlak, A., A. Gökkuş, B.H. Hakyemez and H. Baytekin, 2011. Shrub yield, forage quality in Mediterranean shrublands of West Turkey during a year. African J. Agric. Research, 6(7), 1726-1734.

Reisman-Berman, O. 2007. Age-related change in canopy traits shifts conspecific facilitation to interference in a semi-arid shrubland. Ecography, 30: 459-470.

Reisman–Berman, O., R. Kadmon and M. Shachak, 2006. Spatio-temporal scales of dispersal limitation in the recolonization of a semi–arid Mediterranean old–field. Echography, 29: 418-426.

Seifan, M., K. Tielbörger and R. Kadmon, 2010. Direct and indirect interactions among plants explain counterintuitive positive drought effects on an Eastern Mediterranean shrub species. Oikos, 119: 1601-1609.

Seligman, N. and Z. Henkin, 2007. Survival of *Sarcopoterium spinosum* seedlings growing on terra rossa soil. Israel J. Plant Sciences, 55(1): 45-51.

Seligman, N.G. 1996. Management of Mediterranean grasslands. In: The Ecology and Management of Grazing Systems, J. Hudgson, A.W. Illuis (eds.), CAB Int., 359-391.

Seligman, N.G. and Z. Henkin, 2009. Regeneration of a dominant Mediterranean Dwarf–Shrub after Fire. *J.* Vegetation Science, 11(6): 893-902.

Smirin, P., D. Taler, G. Abitbol, T. Brutman-Barazani, Z. Kerem, S. Sampson and T. Rosenzweig, 2010. *Sarcopoterium spinosum* extract as an antidiabetic agent: in vitro and in vivo study. J. Ethnopharmacology, 129: 10-17.

Sternberg, M. and Shoshany, 2001. Influence of slope aspect on Mediterranean woody formations: comparison of a semiarid and an arid site in Israel. Ecological Research, 16: 335-345.

Tatlı Seven, P. and I.H. Çerçi, 2006. Relationship between nutrient composition and feed digestibility determined with enzyme and nylon bag (in situ) techniques in feed sources. Bulg. J. Vet. Med., 9(2), 107−113.

Tovit, R., K. Zohar and D. Dvir, 2010. Pharmaceutical compositions comprising extracts of *Sarcopoterium spinosum*, components thereof and uses thereof. Patent: PCT/IB2010/052551.

Van Soest, P.J., J.D. Robertson and B.A. Lewis, 1991. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. J. Dairy Science, 74: 3583-3597.

Welch, B.L. 1989. Nutritive Value of Shrubs. In: The Biology and Utilization of Shrubs (Ed. C.M. McKell), Academic Press, Inc., 405-424.

Yılmaz, K.T. 1996. Akdeniz Doğal Bitki Örtüsü. Çukurova Üni. Ziraat Fak. Genel Yayın No: 141, Yardımcı Ders Kitapları Yayın No: 13, s 179.

Yılmaz, O. 1993. Maki Bitkileri. Ankara Üni. Ziraat Fak. Yayın No: 1326, Ders Kitabı: 325, s 60

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Nutritional Changes of Sour Cherry (*Prunus cerasus*) Kernel Subjected to *Aspergillus niger* Solid-state Fermentation

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This study was carried out to investigate the effects of *Aspergillus niger* solid-state fermentation on main nutritional content of cherry (*Prunus cerasus*) kernel. Three *Aspergillus niger* strains (ATCC 52172, ATCC 200345, ATCC 9142) were used in this study. Cherry kernels were analyzed for crude protein (CP), total ash (TA), total fat (TF), crude fiber (CF), nitrogen free extract (NFE), neutral detergent fiber (NDF) and acid detergent fiber (ADF) before and after fermentation to see nutritional change. CP level of the sour cherry increased by 14.1% and reached up to 41.66% from 27.56%. Fungal fermentation changed also TA, TF, CF, NFE, NDF, ADF contents of cherry kernel. These results suggest that solid-state fermentation with *Aspergillus niger* can be used for utilization nutritional properties of cherry kernels to make having potential in animal nutrition.

Keywords: Solid-state fermentation, cherry kernel, *Prunus cerasus*, *Aspergillus niger*, animal nutrition

Introduction

The demand for protein foods will increase because world population is growing rapidly. It is expected that world animal product needs will increase by 58% in 2050 (Makkar et al. 2014). As it is necessary to produce more animal product (meat, egg, milk etc.) for feeding the growing population, the demand for feedstuffs used in animal nutrition will also increase. Thus, the use of feedstuffs competing with human nutrition in animal feeding rather than feeding the world population expected to rise to 9 billion in 2050 will become controversial (Cohen, 2003). For this reason, it is considered that the feed resources with low competition with human consumption should be made available in animal nutrition.

Feed costs constitute 60-70% of total costs in animal production. In feed costs, protein sources have a large share because of being very expensive and using at high levels in the ration. It is necessary that the new protein sources should be found for animal nutrition in order to reduce the pressure on the protein sources used animal nutrition made by increasing animal product demand in parallel with rising in human population.

Cherry (*Prunus cerasus* L.) is a seed fruit of Rosaceae family. The world's total production of cherry, which is being cultivated in many parts of the world, especially in Russia, Ukraine and Turkey, have reached 1.3 million tons per year (FAO, 2014). According to the National Agricultural Statistics Service, 99% of the sour cherries are consumed as processed products such as fruit juice, jam or canned cherries (USDA, 2012). After the sour cherry has been harvested, the plant is brought to the factory and the seeds are separated by passing through the seed separating machines. 60.4-71.4% by weight of the sour cherry constitutes the fleshy portion, 13.5-18.1% the peel and 5.5-7.9% the seed (Chaovanalikit and Wrolstad, 2003). 23.5% of the sour cherry seed, which is separated as a by-product during the conversion of sour cherry to processed products, is composed of the kernel. Cherry kernel contains 30.4% crude protein, 17.6% total fat, 3.2% total ash and 9.5% crude fiber (Popa et al. 2011; Yılmaz and Gökmen, 2013). Although the sour cherry kernel is rich in lysine (5.28%), it is poor in terms of essential amino acids such as methionine, threonine and tryptophan (Yılmaz and Gökmen, 2013). It has been reported that sour cherry oil contains 46.8% oleic and 40.58% linoleic acid and thereby is also rich in unsaturated fatty acids (Yılmaz and Gökmen, 2013).

Fermentation method can be used to increase protein, amino acid, fat, mineral and vitamin contents of feed raw materials, waste and by-products (Cao, 2012; Zhang et al. 2013; Xie et al. 2016). Fermentation in which microorganisms such as bacteria, fungi or yeast are used is generally divided into liquid-state and solid-state fermentation. Solid-state fermentation is the preferred method for liquid culture fermentation because of the being economical, using abundant and cheap substrates in and having relatively less risk of contamination, which indicate the development of microorganisms on moistened

solid substrates without free water (Perez-Guerra et al. 2003; Osma et al. 2007).

The filamentous fungi, such as *A. niger*, are suitable for solid-state fermentations because they can rapidly grow in the low-water environment (Raimbault, 1998). *A. niger* is used as a probiotic in animal nutrition and is accepted as "Generally Recognized as Safe" (GRAS) by the US Food and Drug Administration (FDA) (Harimurti and Hadisaputro, 2015). *A. niger* can increase the content of protein, amino acid and mineral of feedstuffs and can lose its anti-nutritional components (Iyayi and Losel, 2001; Dei et al. 2008; Okpako et al. 2008; Cao, 2012; Zhang et al. 2013). *A. niger* can also increase the digestibility of feed by producing enzymes protease, amylase, lipase, cellulase and xylanase (Milala, 2005; Betini et al. 2009; Oyeleke and Oyewole, 2011; Oliveira et al. 2016). These features of *A. niger* make it possible to enrich the cherry kernel for protein, amino acids and minerals, and to improve the digestibility of possible anti-nutritional factors. With these properties of *A. niger* it is thought that the sour cherry kernel can be enriched with respect to protein, amino acid and mineral, and also its digestibility can be increased by eliminating the possible anti-nutritional factors. In this study, the effect of solid-state fermentation with *A. niger* on crude protein (CP), total ash (TA), total fat (TF), crude fiber (CF), nitrogen free extract (NFE), neutral detergent fiber (NDF) and acid detergent fiber (ADF) content of sour cherry kernel was investigated.

Materials and Methods

Cherry kernel supply and storage

Cherry kernels were supplied from a fruit juice factory in Turkey. The cherry kernels were stored at -20 ° C until fermentation.

Microorganisms

The microorganisms used in the study were obtained from the American Type Culture Collection (ATCC). The microorganisms were ATCC® 9142™, ATCC® 200345™, ATCC® 52172™.

Culture media and culture conditions

A. niger strains obtained from ATCC were left in incubation in Potato-Dextrose-Agar (PDA, Oxoid Ltd., Basingstoke, UK) on 28 °C for 7 days according to agar plate technique. After incubation, *Aspergillus* spores were harvested by turning the plate upside down and gently hitting the top. Spore counting was conducted according to Fuch-Rosenthal technique using a hematocytometer. After the formed spores were counted when they were inoculated into cherry kernels the same day.

Solid-state fermentation preparation

Before the fermentation, cherry kernels were milled to a size of 2 mm (RETCH ZM200) and sterilized by autoclaving on 121 °C for 15 minutes. After that, the cherry kernels were divided in two groups, namely, before fermentation and after fermentation. Environment used for fermentation of cherry kernels was 1 kg cherry kernel and 1.6 l nutritional salt (glucose: urea:$(NH4)_2SO_4$:peptone:KH_2PO_4:MgSO4.7H_2O 4:2:6:1:4:1). The pH of fermentation environment was calibrated into 5 using 1N NaOH and HCl. Starting humidity was 60% and, after adding nutritional salt, for each kg of the solid environment $1{,}4x10^4$ *A. niger* spores were inoculated inside a sterile cabin and left in incubation on 28-30 °C. After incubation, fermented cherry kernels were placed in plastic containers, gently pressed and left for 48 hours on its heat. Since *A. niger* is a microaerobic organism, there will be enough microaerobic conditions for its growth and development even if its left in a closed environment (David et al. 2003). At the end of this period, cherry kernels were spread over a polyethylene paper in a room with 30-40 °C temperature for 6 days until reaching approximately 90% of dry matter upon which they were splintered into 0.15 mm pieces. After the fermentation period, *A. niger* strains were exposed to 60 °C temperature for 48 hours in order to be rendered inactive.

This study was designed from 3 treatment consisting of different *A. niger* strains. ATCC 52172 used group was called C1, ATCC 200345 used group was called C2, ATCC 9142 used group was called C3 and unfermented cherry kernel (control) was called CK.

Determination of chemical composition

Ash (method, 942.05), CP (method, 976.06), ether extract (EE, method, 920.29), crude fiber (CF, method, 973.18) analyses of cherry kernels before and after solid-state fermentation were conducted (AOAC, 2000). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) analyses were conducted according to Van Soest (1991) using the ANKOM$^{200/220}$ fiber analyzer (ANKOM corporation®

Technology Fairport, NY). Measurement for each sample was conducted 3 times and the average was taken.

Statistical analysis

All of the experiments were carried out in triplicate, and the data were expressed as an average value and pooled SEM. Comparisons between different groups were used by one-way ANOVA and Duncan (SPSS 21.0 Statics). The level of statistical significance was preset at $P < 0.05$.

Results and Discussion

Solid-state fermentation of cherry kernel with A. niger resulted in significant changes in its nutrient content (Table 1). CP level of the sour cherry increased by 14.1% and reached up to 41.66%. The CP level was highest in the C3 group, followed by the C2 and C1 groups. This result is in line with the results of studies on solid-state fermentation of cassava (Iyayi and Losel, 2001; Aderemi and Nworgu, 2007; Aro, 2008; Okpako et al. 2008), ginkgo leaves (Cao et al. 2012), shea nut (Dei et al. 2008), olive leaves (Xie et al. 2016), pomegranate peel and creosote bush leaves (Aguilar et al. 2008) with *A. niger*. This protein increase may be due to micelles and/or enzymes produced by A. niger (Onilude, 1994; Raimbault, 1998).

There was no difference in TF contents of treatments compared to the control, except a decrease in C3 group. This result is consistent with studies in which the fat content of cassava (Aro, 2008), pomegranate peel and creosote bush leaves (Aguilar et al. 2008) was not affected by fermentation while inconsistent with the studies that increased fat content of shea nut (Dei et al. 2008) and cassava (Okpako et al. 2008). Differences in the results obtained may be due to differences in the substrate used for fermentation. Besides, when the study results are interpreted, it can be seen that different *A. niger* strains can have different effects on crude oil content even in the same substrate. While the crude oil level of the sour cherry kernel was unchanged in C1 and C2, decreased in C3.

Table 1. Changes of main nutritional ingredients in sour cherry kernel with *Aspergillus niger* solid-state fermentation (%, on dry matter basis)

Component	C1	C2	C3	CK	SEM	P-value
Crude Protein	39.70^{c}	41.24^{b}	41.66^{a}	27.56^{d}	2.19	<0.001
Total Fat	26,00^{a}	26.93^{a}	22.39^{b}	25.78^{a}	0.67	0.005
Total Ash	6.90^{b}	7.89^{a}	7.79^{a}	2.60^{c}	0.71	<0.001
NFE	20.10^{b}	18.05^{c}	19.35^{b}	38.30^{a}	3.15	<0.001
Crude Fiber	7.32^{ab}	5.82^{b}	8.78^{a}	5.84^{b}	0.49	0.032
NDF	22.03^{a}	17.14^{c}	19.82^{b}	17.74^{c}	0.74	0.002
ADF	13.01^{a}	10.97^{c}	11.79^{b}	9.67^{d}	0.46	<0.001

^{abcd}Means within a row lacking a common superscript differ ($p < 0.05$); C1: cherry kernel fermented with ATCC 52172, C2: cherry kernel fermented with ATCC 200345, C3: cherry kernel fermented with ATCC 9142, CK: non-fermented cherry kernel, SEM: standard error of means

TA level was higher in all groups than control group. While there is an equal and highest TA increase in groups C2 and C3, the increase in C1 group is less than that of the others. This result is consistent with studies on cassava (Okpako et al. 2008), shea nut (Dei et al. 2008), pomegranate peel and creosote bush leaves (Aguilar et al. 2008), but inconsistent with a study on cassava (Aro, 2008) in which the amount of TA was not changed. Increase in TA level by fermentation may indicate that mineral content has been increased. It can be assumed that the enzymes produced by A. niger cause the sour cherry to be hydrolyzed and the mineral substances within it to become liberated and therefore the increase in TA level is caused by this. As a matter of fact, it has been reported that the phytase secreted by *A. niger* increases the phosphorus level of the substrate by releasing the phosphorus bound in phytic acid form (Dei et al. 2008).

NFE level decreased in all groups compared to control and the highest decrease was observed in the C2 group. The decrease in the NFE may be due to the degrading of sugars by the enzymes secreted by *A. niger* for use as a carbon source (Oboh, 2006). This result is in line with the fermentation studies

on cassava (Aro, 2008, Okpako et al. 2008) and pomegranate peel (Aguilar et al. 2008).

CF was increased in group C3 but remained the same with the control group in C1, C2. CF was decreased with *A. niger* fermentation in a study on cassava (Okpako et al. 2008), while CF was increased in studies on cassava (Aderemi and Nworgu, 2007) and pomegranate peel and creosote bush leaves (Aguilar et al. 2008) in parallel with this work.

NDF and ADF were increased in all groups compared to control, except NDF in C2 group. NDF in C2 group remained the same as the control may because of having the same CF level with the control group. NDF and ADF decreased in fermentation study on cassava (Aderemi and Nworgu, 2007; Dei et al. 2008).

Conclusions

The protein, ash and cellulose contents of Cherry kernel can be enhanced by *Aspergillus niger* solid-state fermentation. Thus, it is thought that cherry kernel, a waste product, can be fermented for increasing its protein and mineral content in making an available feedstuff for animal feeding. Besides, there has been a need to make advanced animal experiments of this fermented product in order to obtain more detailed information for suggesting animal feding operations.

References

Aguilar, C.N., A. Aguilera-Carbo, A. Robledo, J. Ventura, R. Belmares, D. Martinez, R.R. Herrera and J. Contreras, 2008. Production of antioxidant nutraceuticals by solid-state cultures of pomegranate (*Punica granatum*) peel and creosote bush (*Larrea tridentata*) leaves. Food Technology and Biotechnology, 46(2): 218-222.

AOAC, 2000. Official Methods of Analysis of AOAC International (17. Edition). ABD: AOAC International.

Aro, S. 2008. Improvement in the nutritive quality of cassava and its by-products through microbial fermentation. African Journal of Biotechnology, 7(25): 4789-4797.

Betini, J., M. Michelin, S. Peixoto-Nogueira, J. Jorge, H. Terenzi and M. Polizeli, 2009. Xylanases from *Aspergillus niger*, *Aspergillus niveus* and *Aspergillus ochraceus* produced under solid-state fermentation and their application in cellulose pulp bleaching. Bioprocess and Biosystems Engineering, 32(6): 819-824.

Cao, F., X. Zhang, W. Yu, L. Zhao and T. Wang, 2012. Effect of feeding fermented *Ginkgo biloba* leaves on growth performance, meat quality, and lipid metabolism in broilers. Poultry Science, 91(5): 1210-1221.

Chaovanalikit, A. and R. Wrolstad, 2004. Total anthocyanins and total phenolics of fresh and processed cherries and their antioxidant properties. Journal of Food Science, 69(1).

Cohen, J.E. 2003. Human population: The next half century. Science, 302(5648): 1172-1175.

David, H., M. Akesson and J. Nielsen, 2003. Reconstruction of the central carbon metabolism of *Aspergillus niger*. European Journal of Biochemistry, 270: 4243–4253.

Dei, H., S. Rose, A. Mackenzie and R. Amarowicz, 2008. Growth performance of broiler chickens fed diets containing shea nut (*Vitellaria paradoxa*, gaertn.) meal fermented with *Aspergillus niger*. Poultry Science, 87(9): 1773-1778.

FAO, 2014. Food and Agricultural Commodities Production. http://www.fao.org/faostat/

Harimurti, S. and W. Hadisaputro, 2015. Probiotics in Poultry. Beneficial Microorganisms in Agriculture, Aquaculture and Other Areas: Liong M. İsviçre: Springer.

Iyayi, E.A. and D.M. Losel, 2001. Protein enrichment of cassava by-products through solid state fermentation by fungi. Journal of Food Technology in Africa, 6(4): 116-118.

Makkar, H.P., G. Tran, V. Heuzé and P. Ankers, 2014. State-of-the-art on use of insects as animal feed. Animal Feed Science and Technology, 197: 1-33.

Milala, M., A. Shugaba, A. Gidado, A. Ene and J. Wafar, 2005. Studies on the use of agricultural wastes for cellulase enzyme production by *Aspergillus niger*. Research Journal of Biological Sciences, 1(4): 325-328.

Oboh, G. 2006. Nutrient enrichment of cassava peels using a mixed culture of Saccharomyces cerevisae and *Lactobacillus spp* solid media fermentation techniques. *Electronic Journal of Biotechnology*, *9*(1).

Okpako, C., V. Ntui, A. Osuagwu and F. Obasi, 2008. Proximate composition and cyanide content of cassava peels fermented with aspergillus niger and *Lactobacillus rhamnosus*. Journal of Food Agriculture and Environment, 6(2): 251.

Oliveira, F., C. Moreira, J.M. Salgado, L. Abrunhosa, A. Venâncio and I. Belo, 2016. Olive pomace valorization by Aspergillus species: Lipase production using solid-state fermentation. Journal of the Science of Food and Agriculture, 96(10): 3583-3589.

Onilude, A.A. 1994. Production Characteristion and utilization of some dietary fiber degrading enzymes as additives in Broilerdiets. Ph.D. Thesis, Department of Botany and Microbiology University of Ibadan, Nigeria.

Osma, J.F., J.L.T. Herrera and S.R. Couto, 2007. Banana skin: A novel waste for laccase production by trametes pubescens under solid-state conditions. Application to synthetic dye decolouration. Dyes and Pigments, 75(1): 32-37.

Oyeleke, S. and O. Oyewole, 2011. Production of protease and amylase from *Bacillus subtilis* and *Aspergillus niger* using *Parkia biglobossa* (africa locust beans) as substrate in solid state fermentation. Advances in Life Sciences, 1(2): 49-53.

Pérez-Guerra, N., A. Torrado-Agrasar, C. López-Macias and L. Pastrana, 2003. Main characteristics and applications of solid substrate fermentation. Electronic Journal of Environmental, Agricultural and Food Chemistry, 2(3).

Popa, V., C. Misca., D. Bordean, D.N. Raba, D. Stef and D. Dumbrava, 2011. Characterization of sour cherries (*Prunus cerasus*) kernel oil cultivars from banat. Journal of Agroalimentary Processes and Technologies, 17: 398-401.

Raimbault, M. 1998. General and microbiological aspects of solid substrate fermentation. Electronic Journal of Biotechnology, 1(3): 26-27.

USDA, 2012. United States Department of Agriculture, National Agricultural Statics Service, Noncitrus Fruits and Nuts.

Xie, P., L. Huang, C. Zhang and Y.L. Zhang, 2016. Nutrient assessment of olive leaf residues processed by solid-state fermentation as an innovative feedstuff additive. Journal of Applied Microbiology, 121(1): 28-40.

Yılmaz, C. and V. Gökmen, 2013. Compositional characteristics of sour cherry kernel and its oil as influenced by different extraction and roasting conditions. Industrial Crops and Products, 49: 130-135.

Zhang, X., L. Zhao, F. Cao, H. Ahmad, G. Wang and T. Wang, 2013. "Effects of feeding fermented *Ginkgo biloba* leaves on small intestinal morphology, absorption, and immunomodulation of early lipopolysaccharide-challenged chicks", Poultry Science, 92(1): 119-130.

Influence of Pasture Composition and Weather Conditions on the Productivity and Behavioral Reactions of Pigs of East Balkan Breed

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In the Agricultural Experimental station- Sredets, in Strandzha region, a study was conducted with two groups of 10 pigs of East Balkan breed. The experiment began after weaning at 60 days of age (8,91 and 8,81 kg) and continued to the age of 123 days (28,02 and 26,48 kg live mass). The period from May (spring) to the end of August (summer) was covered. The purpose of this study was to investigate the productivity and some behavioral reactions of East Balkan pigs depending on the composition of the pasture and weather conditions. The pastures in Strandzha are not highly productive - from 890 to 1320 kg/daa and with low nutritional value - the protein content ranges from 8,89 to 12,1 % in the spring and from 5.53 to 5.91% during the summer. Growing pigs in the control group have achieved average daily gain of 0.155 kg throughout the whole period and the pigs from the experimental group 0.144 kg. This indicating that the various chemical and botanical composition of the herbageas well as herbage yield did not significantly affect the live weight of animals. Major influence on the productivity and behavior of piglets from the East Balkan breed have weather conditions during the season. The behavior of the two groups of pigs was observed for two consecutive days for 8 hours during the spring season (May) and summer (July). In the spring, the activity of the animals of both groups (83% and 89%), was higher compared to the summer (62% and 73%).

Keywords: East Balkan swine, grassland, climatic factors, growth, etology.

Introduction

The relationshipland - animals is twofold. The natural pastures are a single source of cheap and complete fodder for livestock, and they in turn enrich the soil with natural fertilizers, which determines the positive effect of this interaction (Horsted et al., 2012). Fertile and clean land, grass composition of pastures, diverse chemical composition and variation of nutritional value are factors that determine their behavior and productivity. Kirilov et al. (2013) indicate that by the eating behavior of animals one can judge for the preservation of pasture grass - if most of the animals are lying down two hours after putting them out to pasture, that means there is enough grass. According to other authors practically the complete feeding of animals can be determinedby their behavior - when they start to slow their movements and start looking for a place to lie down and rest, that means that they are completely fed and should be taken out from the pasture and brought to their place for rest (Slanev et al ., 2006). The nutritional contribution made by pasture will depend on the availability, nutrient composition, grazing intake and digestive utilisation of herbage(Edwards, 2003). A number of authors have found that the quantity and quality of herbage and climatic factors determine the behavior of pigs on pasture and their productivity (Andresen and Redbo, 1999; Olczak et al., 2015; Horsted et al., 2012; Velazco et al., 2013; Allwin et al., 2016; Palova, 2006; Palova, 2008.

East Balkan pig is the only preserved until now in a clean condition Bulgarian pasture aboriginal breed of pigs. Hlebarov (1922) and Danchev (1984) stated that it has the ability to utilize different trophic sources with low nutritional value. Vittoz and Hainard (2002) reached the conclusion that pigs should use pastures with less sensitive plant communities. Ivanova-Peneva (2010) conducted a study on the behavioral responses of pigs of free-range East Balkan breed and results obtained are similar to the behavior of feral pigs atthe pasture.

Objective

To explore some behavioral reactions and productivity of pigs from East Balkan breed depending on the composition of the pasture and weather conditions.

Materials and Methods

The study was conducted in the Agricultural ExperimentalStation – Sredetsfor a period of four months during the spring-summer season. Two groups of animals were formed– control group (n=10) and experimental group (n=10), each of them contains 5 male and 5 female, equalized by

gender, age, body weight. The animals were taken daily to controlled grazing in the forests of Strandzha. The animals use every day different pasture from 8:00am to 16:00 pm. and after return they were fed with 0.500 kg/head of milled barley. The pigs had unlimited access to drinking water. The weight development of pigs was observed during adolescence. The average live weight and average daily gain by months in different seasons were registered. The climatic factors air temperature (t^0C) and rainfall mm/sq.m were reported, taking into account their impact on the growth of animals and their behavior during the study. Natural pastures were used, wherethe botanical composition was studied in terms of cereals, legumes and weeds. When examining the chemical composition of herbage, dry and organic matter, crude protein, fat, fiber, minerals, Ca and Pwere defined. Protein was determined by the Kjeldahl method, Soksle fat, fibersas perWeende (methods described by Sandev, 1964). Herbage yield wasdetermined by the method of Shanin (1977). Observations were carried out using the method of group timingon two consecutive days. The parameters standing, grazing, rest (lying), movement were controlled in 10 minutes and deviations from the actual values did not exceed 2%.

The results obtained were processed by modules of a computer program for statistical analysis StatSoft 6 (Microsoft Corp.1984-2000Inc.). The options ANOVA and Other Significance Test were used.

Results and Discussion

Data for the change of botanical composition of pastures as well as the yield from it are shown in Table 1 and the chemical composition of herbage - in Table 2. Herbage yield of the pasture used by the first group is 1320 kg/daa, and drymass yield - 570 kg/daa. In the pasture used by the second group herbage yield is 890 kg/daa and dry mass yield is 360 kg/daa. Palova et al., (2011) obtained results that are closed to our results. They found that the pastures used by the East Balkan swine in the area ofAgricultural ExperimentalStation - Strandzha mountain are not highly productive- from 550 kg/daa to 840 kg/daaof herbage. Protein content ranges from 8.60 to 13.76%. The herbage contains cereal (twitch grass, poa bulbous panicle, false sheep's fescue, common bent, perennial ryegrass,cocksfoot), legumes (species of clover, yellow alfalfa, trefoil, wild vetch) and other grass families (mullein, green shield bug, dandelion, plantain etc.). The botanical composition of herbage in the first pasture is as follows: grasses - 57.2%, beans - 25% other grass families - 17.8%. Less weeds contentis due to the greater amount of benign cereal and leguminous grasses. The second pasture has a more balanced composition of herbage: cereal grasses - 41.1%, bean grasses - 12.5% other grass families - 46.4%.

It was found that the grass composition of natural pastures in Strandzha used by East Balkan pig breed is characterized by a low nutritional value- protein from 8,89 to 12,1%, fat from 1,61 to 2,78%, fiber from 23,09 to 25,57 and minerals from 6,54 to 7.94%. Our data is similar to that derived by Nedev et al., 2009; Stoeva and Vateva, 2008. Based on the results of the chemical composition of herbage the conclusion is that the nutritional value of pasture is higher during May-June and it is decreased in July-August. We think this is due to the period in the development of grasses, as well as more favorable weather conditions- temperature and rainfall in the spring, predisposing the development of rich grass cover.
The values of temperature and rainfall during the studied period are shown in Fig1. The results indicate that the weather conditions determine the herbage condition of pastures. Climatic conditions during the spring were suitable for development of pastures. The amount of rainfall during the period was 113,2 mm/sq.m., in May the rainfall was 24.5 mm/sq.m., and in July -the least amount - 0.9 mm/sq.m. The average monthly air temperature was highest in July 24.2 ^0C, with deviation from 11.8 to 37.3^0C. The lowest average monthly temperature was recorded in May18.3^0C, ranging from 6.0 to 30.5^0C. The combination of rainfall and temperatures in the different months determines the productivity of pastures. During the months of May and June the rainfall was high and temperatures were lower than in July and August, and July is determined as the most unfavorable month. During this month herbage is poor in nutrients and digestion is more difficult for pigs, because vegetation has passed pasture maturity.

Table 1. Botanical composition, %, green and dry mass yield kg/dasward from natural pastures in Strandzha

Indicators	Pasture 1	Pasture 2
Green mass yield kg/dasward	1320	890
Dry mass yield kg/dasward	570	360
Cereal, %	57,2	41,1
Legumes,%	25,0	12,5
Weed,%	17,8	46,4

Table 2. Chemical composition of the nature sward from Strandzha

Indicators	Sward 1 Spring	Sward 2 Spring	Sward 3 Summer	Sward 4 Summer
Water, %	74,81	74,92	7,15	7,96
Dry matter,%	25,19	25,08	92,85	92,04
Crude protein, %	12,1	8,89	5,91	5,53
Crude fat,%	2,78	1,61	1,93	2,69
Crude fiber,%	23,09	25,57	30,81	27,55
Mineral traces,%	7,94	6,54	12,23	8,04
Crude ash, %	7,68	7,31	6,52	6,70
NFE	54,35	56,62	54,83	57,53

Table 3. Average live weight of pigs in the control and test group by months

Group	Control group			Test group		
Month	n	X	±SX	n	X	±SX
1 May	10	8,92	±1,40	10	8.81	±1.63
1 June	10	14,73	±2,09	10	14,01	±1,63
1 July	10	19,96	±1,64	10	18,48	±2,71
1 August	10	23,52	±1,64	10	21,93	±1,79
1September	10	28,02	±2,10	10	26,48	±3,04

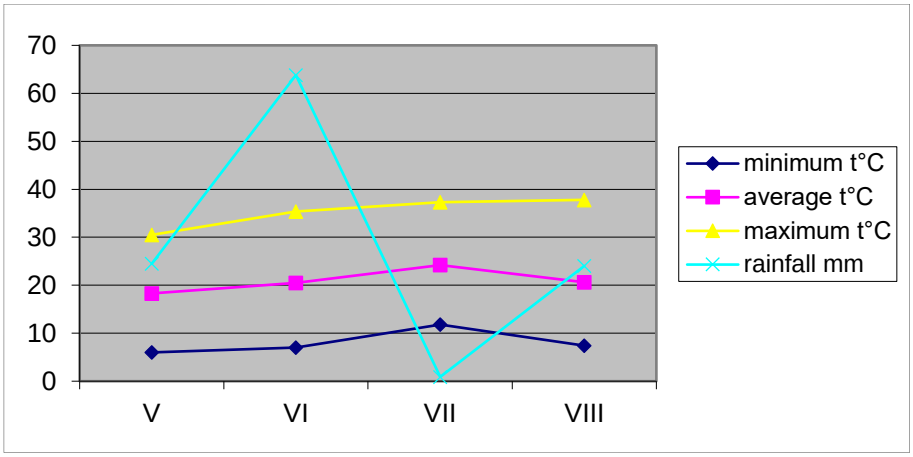

Fig.1 Weather conditions during the period of study

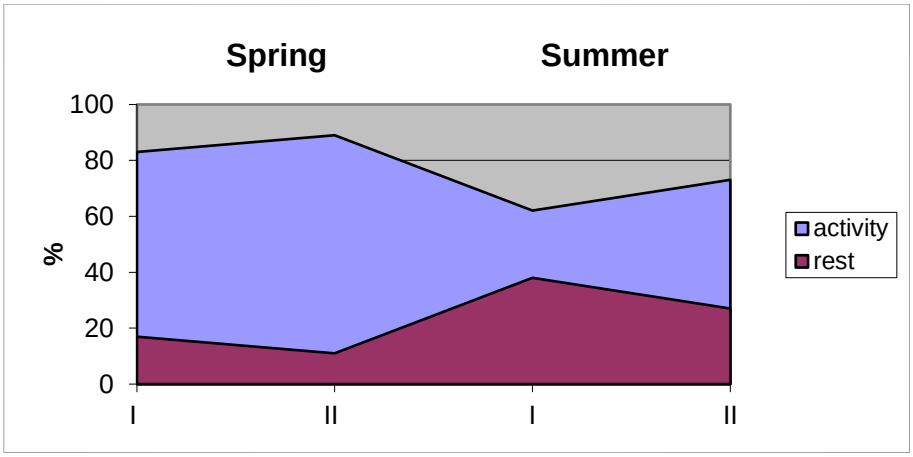

Fig.2 Behavior of pigs from East Balkan breed depemding on the season

Data on weight development of the pigs are shown in Table 3. Average live weight kg and average daily growthrate during the individual months were registered. The influence of climatic factors and pasture composition on pigs growth was determined. A trend was found for lower average live weight of pigs in the experimental group compared with the control group, but statistically proven differences between the two groups were missing. The average live weight at the end of the test period (123 days) reached 28,2 $\pm$ 2,10 for the control group and 26,48 $\pm$ 3,04 for the experimental group. These differences are insignificant and reliable influence of the pasture on average live weight was not registered. The data obtained show that the major influence on the average daily gain for pigs ofthe East Balkan breed have the climatic factors. The values in different seasons differ at $p < 0.05$. The lowest average daily gain in both groups received in July was 0,115 kg for the control groupand 0,111 for the experimental group and the highest in May –0,187 and 0,168 kgrespectively. The higher average daily gain in May may result from a combination of favorable agro-meteorological conditions and the availability of abundant grass cover. The development of most types of plants in the herbage depends mainly on the presence of rainfall–they require higher moisture and lower temperaturesfor their development. In the spring grasses are in the most favorable phase of development for the utilization by animals - beginning of tasseling in wheat and budding to beginning of flowering in beans. The negative correlation between temperature and rainfall during the summer months justify the insufficient pasture with poor quality of herbage, increasing the proportion of its indigestible or difficult to digest components (lignin and cellulose). This respectively results in a low average daily gain during this period. Marchev and Nedeva (2003) also came to the conclusion that the deteriorating agro-meteorological conditions have an unfavorable impact on average daily gain.

There have been observations on the behavior of pigs of East Balkan breed according to the nutritional resources of the area and weather conditions. The term "Activity" includes active movement such as walking, standing, grazing, digging, waterdrinking, excretory and sexual behavior, which inherently represent the changes in body position or part of the body in space. The social behavior (aggression) and hierarchical relationships between animals in the herd are also attributed to activity. Tranquility and rest contain the elements lying and sleeping. In all cases of studying animal behavior thequantitative assessment method was used to quantify the behavior reactions,different in duration, recorded in 10 minutes. Above all the duration of the underlying behavior was covered - activity and rest, including all their elements, measured with a stopwatch in minutes. Observations were carried out in spring and summer for two consecutive days in both groups of animals. According to the results, both groups of animals showed a similar pattern of behavior depending on the preservation of pasture and weather conditions.

The summarized results in Fig.2 show that in the spring (May) the animals from both groups have spent in movement 83% and 89%, and this includes walking, standing, grazing, digging, drinking water,

excretory behavior, mudding (some movements are performed simultaneously - such as standing, digging, eating). The rest that involves lying under the shade and in mud is 17% and 11% for both groups. In the summer (July) the movement in both groups was 62% and 73% and rest time 38% and 27%. The activity was mainly in the morning, and lying mostly in the afternoon and it is probably due to the higher temperatures. It is quite possible that this was due to the grazing up. Actually, the grazing up of animals was found in their behavior - slow movement and they were seeking a place to lie down and rest. Slanev et al. (2006) also came to the same conclusion.

In terms of territory that pigs walk to meet theirfeeding needs we can say that it is highly dependent on the availability of pasture during the relevant period and the weather conditions of the environment. Movement and feeding in the spring is more intensive, which is probably due to the greater amount of grass cover and acceptable temperatures for normal thermoregulation in pigs. In July pigs spend more time lying in the shade and in mudding in wet areas and less time in feeding, which we believe is due to the high temperatures and depletion of of pastures from green mass. Our results are similar to those of Andresen and Redbo (1999). They found that the length of time for feeding in adolescents is associated with the quantity and quality of herbage and it increases in good preservation of pasture. The authors register decrease infeeding activity at elevated temperatures. Olczak et al. (2015) reached the conclusion that weather conditions (temperature, humidity, solar radiation, atmospheric pressure, wind strength, wind direction and rainfall) have a significant impact on the behavior of pigs. With increasing temperature the most characteristic behavior of pigs is reduced activity and seeking places for mudding.

Conclusions

The pastures in Strandzha used by the East Balkan swine are not highly productive - from 890 to 1320 kg/daaherbage yield and low nutritional value - the protein content ranges from 8,89 to 12,1 % in spring and to during the summer.

Adolescent pigs in the control group have achieved average daily gain of 0.155 kg throughout the period and the experimental group 0.144 kg, which indicates that various chemical and botanical composition of herbage and herbage yield did not significantly affect weight development and behavior of the animals from both groups.
Weather conditions during the relevant season have basic impact on productivity and behavior of adolescent pigsof East Balkan breed.

References

Allwin, B., R. Swaminathan, A. Mohanraj, N. Gokhan, S. Vedaminckam, G. Sathish and M Kumar, 2016. The Wild Pig (Sus scrofa) Behavior – A Retrospective Study. J Veterinar Sci Techno 7:333. doi:10.4172/2157-7579.1000333

Andresen, N. and I. Redbo, 1999, Foraging behaviour of growing pigs on grassland in relation to stocking rate and feed crude protein level, 1999, Applied Animal Behaviour Science, V. 62, I.2–3: 183–197

Danchev, Y. 1984. Aboriginal primitive breed East Balkan pigin Strandzha, Strandzha-Sakar collection, Malko Tarnovo, 2.

Edwards, S.A. 2003. Intake of nutrients from pasture by pigs. Proceeding of the Nutrition Society, Vol. 62 (2): 257-265.

Graves, H. 1984. Beaviour and ecology of wild and feral swine /Sus scrofa*, Journal of Animal science, Vol.58: 482-492.

Hlebarov, G.S.T. 1922. East Balkan swine, Sofia.

Horsted, K., A.G. Kongsted, U. Jorgesen and J. Sorensen, 2012. Combined production of free-range pigs and energy crops—animal behaviour and crop damages, Livestock Science, V.150 no.1-3: 200-208.

Ivanova-Peneva, S. 2010. Study on the behavior of free-range East Balkan pig, Livestock Science, 5: 43-47.

Kirilov, A., Z. Shindarska and E. Vasilev, 2013. Meadows and pastures - an important forage resource for Bulgaria and the EU. Publishing House at the University of Forestry, Sofia.

Marchev, Y. and R. Nedeva, 2003. Investigation of the impact of temperature and the level of protein on the productivity of adolescent pigs, Livestock Science, 1-2:22-24.

Nedeva, R., Y. Marchev, N. Palova and K. Stoeva, Chemical composition of natural forage resourses used by the East Balkan pig. Journal of Mountain Agriculture on the Balkans, 3: 443 – 454.

Palova, N., R.Nedeva, K. Stoeva and Y. Marchev, 2011. Influence of different types of forage on the productivity of pigsof East Balkan breed, Agricultural Sciences, 6:95-98.

Palova, N., R. Nedeva, K. Stoeva and Y. Marchev, 2011. Influence of different types of forage on the productivity of pigsof East Balkan breed, Agricultural Sciences, 6:95-98.

Olczak, K., J. Nowicki and C. Klocek, 2015. Pig behaviour in relation to weather conditions – a review, Annals of Animal Science. Volume 15 (3):601–610.

Palova, N. 2006, A fattening abilities of pigs from Eastbalkan breed natural accordingly breeding the Strandzha. Journal of Animal Science, 5:19-22

Palova, N. 2008. Investigation of the body weight development of pig from East Balkan breed acrding to sex and season with two levels of protein. Bulgarian Journal of Veterinary Medicine, vol.12, supl.1:130 – 134.

Ricardo Blumetto Velazco, O., S. Calvet Sanz, F. Estellés Barber and A. Villagrá García, 2013. Comparison of extensive and intensive pig production systems in Uruguay in terms of ethologic, physiologic and meat quality parameters, Revista Brasileira de Zootecnia, Vol.42 (7).

Slanev, S., A. Stoykov, P. Petrov, A. Apostolov, M. Kirov, R. Nedeva, S. Ivanova-Peneva, Zh. Nakev and D. Zlateva, 2006. Efficient pig breeding.

Stoeva, K. and V. Vateva, 2008. Productivity and nutritional value of natural pastures in the region of Strandzha. Jubilee Conference of Experimental Station of Livestock breeding and Agriculture - Smolyan "80 years Agrarian Science in the Rhodopes", 176-179.

Vittoz, P. and P. Hainard, 2002. Impact of free-range pigs on mountain pastures in the Swiss Jura, Applied Vegetation Science 5(2):247-254

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YAZIM KURALLARI

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Özet ve Anahtar Kelimeler: Türkçe ve İngilizce özetlerin her biri 200 kelimeyi geçmemelidir. Türkçe ve İngilizce özetlerde "Özet" ve "Abstract " kelimeleri kullanılmamalarıdır. Özetin altına küçük harflerle, mümkünse başlıkta kullanılmayan, çalışmayı en iyi şekilde tanımlayacak 4-6 anahtar kelime, sola dayalı olarak yazılmalıdır.

Giriş: Bu bölümde; çalışma konusu, gerekçesi, önceden yapılmış çalışmalar ve amacı verilmelidir.

Materyal ve Yöntem: Kullanılan Materyal ve Yöntem aynı başlık altında verilmelidir. Yeni veya değiştirilmiş yöntemler aynı konuda çalışanlara araştırmayı tekrarlama olanağı verecek nitelikte olmalıdır.

Bulgular ve Tartışma: Bu bölümde elde edilen bulgular verilmeli, gerekirse çizelge, şekil ve grafiklerle de desteklenerek açıklanmalıdır. Ayrıntılı istatistik analiz tabloları yerine sonuçları gösteren özet tablolar tercih edilmelidir. Bulgular tartışılmalı, ancak gereksiz tekrarlarda kaçınılmalıdır. Bulguların başka araştırmalarla benzerlik ve farklılıkları verilmeli, nedenleri tartışılmalıdır.

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Teşekkür: Gerekli ise mümkün olduğunca kısa olmalıdır.

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Örnekler:

Klich, M.A. 1993. Morphological studies of Aspergillus section Versicolores and related species

Mycologia. 85: 100-107.

McDonald, M.,I.Mila and A.Scalbert,1996. Precipitation of metal ions by plant polypheneols: Optimal conditions and origin of precipitation.J.Agric.Food Chem.44:599-606

Kitap: Yazarın soyadı, adının baş harfi, basıldığı yıl, kitabın adı (varsa derleyen veya çeviren ya da editör), cilt numarası, baskı numarası, basım evi, basıldığı şehir, sayfa yapısı.

Baenett, H.L. and B.B. Hunter, 1999. III ustrated Genera of Imperfect Fungi 4th. Ed., APS Press, The American Phytopathological Society, St. paul, Meinesota, USA, 218 p.

Kitapta Bölüm: Yazarın soyadı, adının baş harfi, basıldığı yıl, bölüm adı, yayınlandığı kitabın adı, (kitabın editörleri), yayınlanan şirket veya kurum, yayınlandığı yer, sayfa numaraları.

Klich, M.A. and T.E. Cleveland, 2000. Aspergillus systematics and and moleculer genetics of mycotoxin biosyntesis. In: integration of Modern Taxonomic Methods for Penicillium and Aspergillus Classification. (Ed(s): R.A. Samson and J.I. Pitt). Harwood Academic Publishers. Singapore. Pp: 425-434.

Kongre, Sempozyum: Yazar(lar)ın soyadı, adının baş harfi, yıl veya bildiri kitabının basıldığı yıl, makale başlığı, kongre adı, kitapçık adı, varsa cilt numarası, kongrenin yapıldığı yer, kongre tarihi ve sayfa numarası, basıldığı yer.

Arıkan, S. G. Sağıroğlu, S. Yıldız ve D. Turgut, 1994. Bazı hayvan yemlerinden izole edilen funguslar ve bunların ürettiği toksinlerin biyolojik ölçüm metodu ile saptanması. XII. Ulusal Biyoloji Kongresi. Moleküler Biyoloji, Genetik ve Mikrobiyoloji Seksiyonu Bildiriler Kitabı, Cilt V. Edirne, 25-27 Mayıs 1994, s. 48-54.

Tezler: Yazarın soyadı, adının baş harfi, yıl, Tezin adı, Tezin Niteliği (YL veya Dr), Tezin Yapıldığı Kurum, Sayfa Sayısı.

Dikmen, i. 1968. Zeytin Çeliklerinin Köklendirilmesi Üzerine Araştırmalar. Yüksek Lisans Tezi. Ege Üniversitesi, Fen Bilimleri Enstitüsü, 98 s.

Internet: Eğer bir bilgi herhangi bir internet sayfasından alınmış ise (internetten alınan ve dergilerde yayınlanan makaleler hariç), kaynaklar bölümüne internet sitesinin ismi, tam olarak yazılmalıdır.

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Kaynak gösterilecek yayında kaç isim varsa, kaynaklar bölümünde tümü belirtilmelidir. " Landen ve ark., 2002" şeklinde kısaltma yapılmamalıdır.

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References are given in the following forms:

Journal articles: Last name of the author, initials, year of publication, title of article, title of journal, volume number, page numbers.

Klich, M.A. 1993. Morphological studies of Aspergillus section Versicolores and related species

Mycologia. 85: 100-107.

McDonald, M., I. Mila and A. Scalbert, 1996. Precipitation of metal ions by plant polypheneols: Optimal conditions and origin of precipitation. J. Agric. Food Chem. 44: 599-606.

Whole book: Last name of the author, initials, year of publication, title of book (name of the editor(s), translator, etc.), volume number, publication number, publisher, place, page numbers.

Baenett, H.L. and B.B. Hunter, 1999. III ustrated Genera of Imperfect Fungi 4th. Ed., APS Press, The American Phytopathological Society, St. paul, Meinesota,USA, 218 p.

Chapter in a book: Last name of the author, initials, year of publication, title of chapter, title of book (name of the editor), publisher, place, page numbers.

Klich, M.A. and T:E: Cleveland, 2000. Aspergillus systematics and and moleculer genetics of mycotoxin biosyntesis. In: integration of Modern Taxonomic Methods for Penicillium and Aspergillus Classification. (Ed(s): R.A.Samson and J.I.Pitt). harwood Academic Publishers. Singapore. Pp: 425-434.

Congress/symposium article: Last name of the author, initials, year of the meeting or publication of the proceeding, title of article, title of meeting, title of proceeding, volume number, place of meeting, date of meeting, page members.

Arıkan, S. G. Sağıroğlu, S. Yıldız ve D. Turgut, 1994. Bazı hayvan yemlerinden izole edilen funguslar ve bunların ürettiği toksinlerin biyolojik ölçüm metodu ile saptanması. XII. Ulusal Biyoloji Kongresi. Moleküler Biyoloji, Genetik ve Mikrobiyoloji Seksiyonu Bildiriler Kitabı, Cilt V. Edirne, 25-27 Mayıs 1994, s.48-54.

Thesis: Last name of author. Initials, year, title of thesis, type of thesis (PhD, DMSc), Institute or university thesis was obtained, number of pages.

Dikmen, i. 1968. Zeytin Çeliklerinin Köklendirilmesi Üzerine Araştırmalar. Yüksek Lisans Tezi. Ege Üniversitesi, Fen Bilimleri Enstitüsü, 98 s.

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