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**THE AUTHORS ARE RESPONSIBLE FOR THE CONTENT AND FOR THE LANGUAGE OF THEIR CONTRIBUTION*

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CONTENTS

WATER - GRAIN AND BIOMASS YIELD RELATIONS OF WINTER WHEAT IN A SEMI-ARID CONDITIONS OF TURKEY

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Abstract
The aim of this study was to determine the effect of different irrigation regime on winter wheat yield and water-use efficiency (WUE). The field experiment was carried out between the year 2009 and 2011 at Murted Basin in Turkey with 4 different irrigation treatments which was Rainfed (RF), Full irrigation (FI), Moderate irrigation (MI), and Deficit irrigation (DI). Soil moisture was measured with neutron probe. According to results average wheat yield was obtained 3.35 t ha^{-1}, 4.54 t ha^{-1}, 4.22 t $ha^{-1}$and 4.31 t ha^{-1} and average harvest index was 29%, 31%, 32% 31% and 32% for RI, FI, MI and DI irrigation treatments respectively. The highest WUE value with 9.1 kg m^{-3} was calculated at DI treatment. A significant negative correlation was found between grain yield, total harvested biomass and the WUE. The results presented in this work suggest that the amount of soil water content affects grain yield and water use efficiency. It might be recommended that irrigation concentrated in the after heading period (DI) increase WUE in semi-arid climate condition such as Central Anatolia Region of Turkey.

Keywords: Wheat, water use efficiency, irrigation.

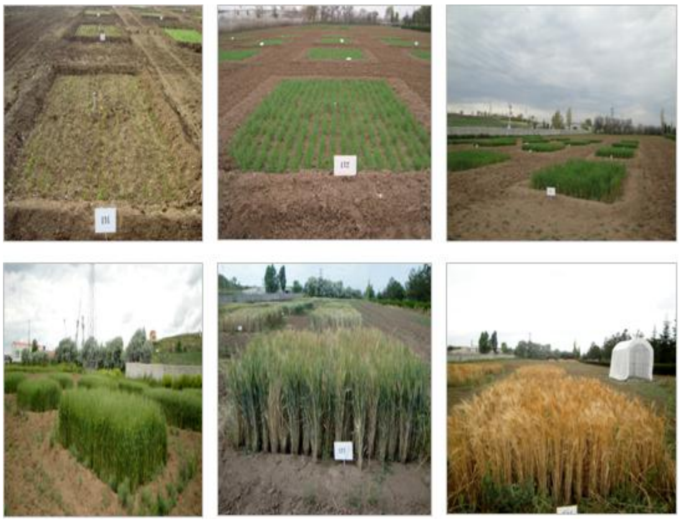
Introduction

One of the most important consequences of the climate change, perhaps the most important one, is its One of the most important consequences of the climate change, perhaps the most important one, is its negative effects on water sources (Agnew, 2011; Barnett et al. 2005). Turkey, particularly the Central Anatolia Region, is adversely affected due to high temperature and lack of precipitation. (Turkes and Akgunduz, 2011). Wheat is the main crop in this area. Approximately 90% of the agricultural lands do not compensate sufficient precipitation during the crop growing period (Cayci et al. 2009). Irrigation is necessary for this lands to avoid water stress and maximize wheat yield. Several researcher frequently emphasized that wheat has different sensitivity to water stress at different growth stages such as booting and heading (Zhang et al. 1998; Varga et al. 2013). Several studies have shown that the water use efficiency (WUE) is very essential indices for identifying the optimum water management strategies (Taylor et al. 1983; Musick et al. 1994; Kharrou et al. 2011). Consequently, the increase in WUE commonly causes to a decrease in the amount of total dry matter (Oweis et al. 1998). The aim of this study was to determine the impact of different irrigation regime on winter wheat crop and water-use efficiency.

Material and methods

The experiment was carried out at Saraykoy research station of Soil, Fertilizer and Water Resources Central Research Institute (39° 57′N and 32° 53′E) of central part of Turkey during the growing season 2009 to 2011. The soil of the research areas is mostly silty clay and field capacity about 35%, and wilting point 20% by volume for 90 cm soil profile. The climate is characterized as semi-arid where annual rainfall and evaporation is about 360 mm and 1300 mm respectively as an average for

long time period. Wheat (*Triticum aestivum* L.) is major crop which is generally planted in the late fall.
The experiment was conducted with four different irrigation water strategies and a completely randomized block design with four replications. Treatments were; RF; Rainfed, FI; Full irrigation MI; Moderate irrigation (2 irrigation maximum) DI; No irrigation after establishment until heading. Each plot area was 17.5 m^2 (Picture 1). The wheat cultivated at 2009-2010 and 2010-2011 growing season.



Picture 1. Field experiment design

The sowing date was late October in all study period. Commercial N fertilizers was divided into two, half was applied at planting and the other half was at early tillering stage (110 kg N ha^{-1}). Approximately 50 kg P ha^{-1} were applied to ensure adequate phosphorous nutrition. Meteorological data were measured on hourly basis from automatic meteorological station at the field site. Irrigation water were applied with basin method. Soil moistures contents were monitored using a neutron probe (CPN) from soil surface to 90 cm soil depth with 20 cm interval twice a week. ET value was calculated according to the "Soil Water Budget".

$$ET = I + P + \Delta S - R - D$$

I = Irrigation water (mm),
P = Precipitation (mm),
ΔS = Change in soil water content (mm),
R = Surface flow (mm),
D = Percolation from the root zone to depth.

Results and discussion

Daily precipitation and ET_o values from the year 2008 to 2012 (including experimental period 20 October 2009 -15 July 2011) was given at Figure 1. As apparent from the graph, although the amount of rainfall during the winter months in 2010-2011 was not high, it reached high levels in autumn and spring season.

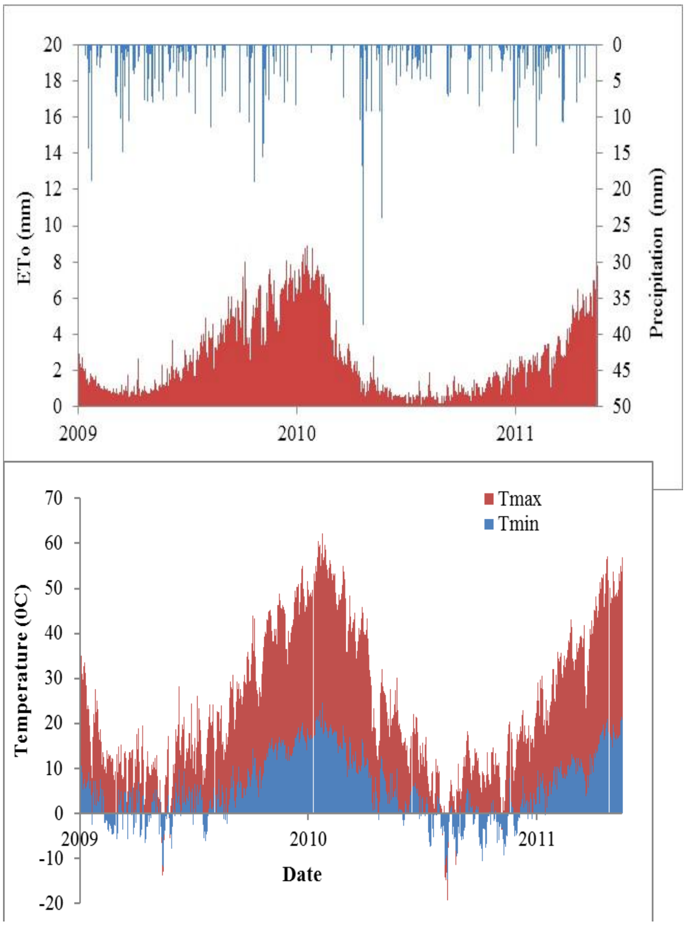


Figure 1. Daily precipitation and ET_o distribution for growing seasons

Average applied irrigation water amount was about 247 mm, 151 mm and 133 mm for FI, MI and DI treatments respectively. The changes of soil water content for whole growing period were presented in Figure 2.

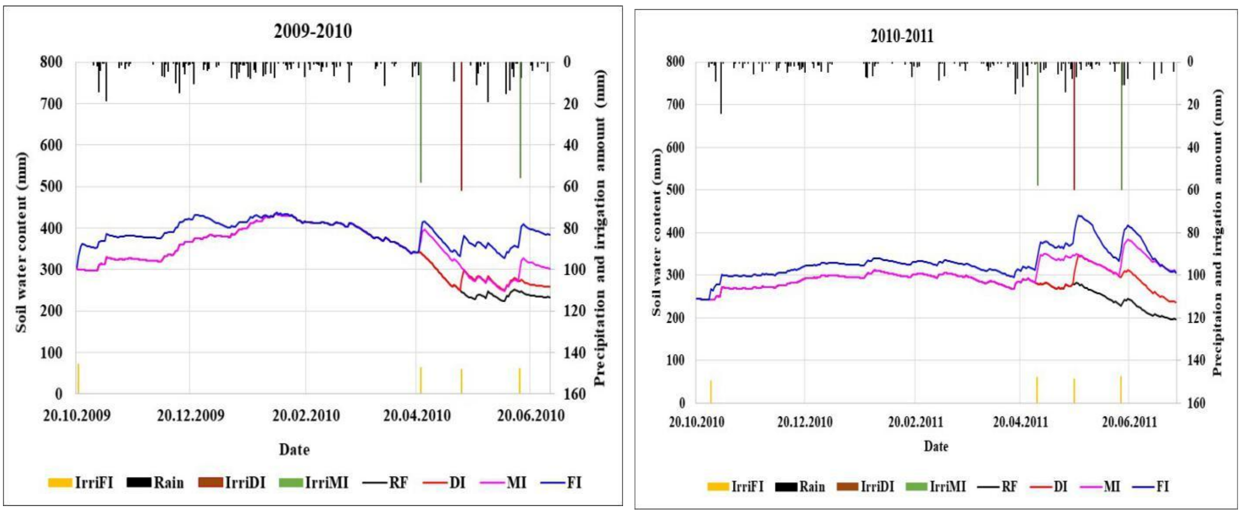


Figure 2. Soil water content data (for the growth period of 2009-2010 and 2010-2011)

The changes in soil profile moistures showed a compatible change depending on the rainfall and irrigation treatments. Irrigation significantly affected to soil water content. Irrigation water had been applied until field capacity level at each irrigation event. Monthly and seasonal plant water consumptions are given in the Table 1 in line with the applied irrigation water and rainfalls. Soil water changes in the soil of 0-90 cm depth were used for the plant water consumption calculations. The highest water consumption was occurred at full irrigation treatments.

Table 1. Monthly and seasonal water consumption of treatments

Years	Treatments	ET (mm)							
		Oct.*	Nov.	Decem.	Apr.	May	June	July**	Total
2009-2010	RF	33.39	61.78	112.44	60.61	58.01	70.27	25.84	422.34
	FI	49.47	77.96	112.43	125.67	120.14	95.29	27.89	608.85
	MI	37.48	49.88	110.55	122.64	119.75	112.35	21.98	574.63
	DI	33.31	57.82	106.37	68.60	92.25	107.23	20.71	486.29
2010-2011	RF	17.53	58.33	59.31	72.50	68.16	76.92	56.23	408.98
	FI	25.27	63.82	82.65	101.48	129.42	96.25	54.71	553.60
	MI	14.42	50.40	57.39	105.21	99.39	105.27	57.48	489.56
	DI	15.14	53.21	52.47	86.14	103.81	93.53	56.34	460.64

*, **; For 10 and 20 days

The highest grain yield (GY) and above ground biomass (BM) (4.55 t ha^{-1} and 14.71 t ha^{-1}) was obtained from FI treatment. Average grain yield values of full irrigated treatment was 26.37%, 3.30% and 8.79% higher than RF, DI and MI treatments respectively. Harvest index (HI) values of the plots were calculated using the grain yield and biomass values (HI = Grain yield / Biomass yield). The highest average harvest index was found in DI, with the percent of 32.2. Average grain yield, abouve ground biomass and harvest index values of treatments were given at Figure 3.

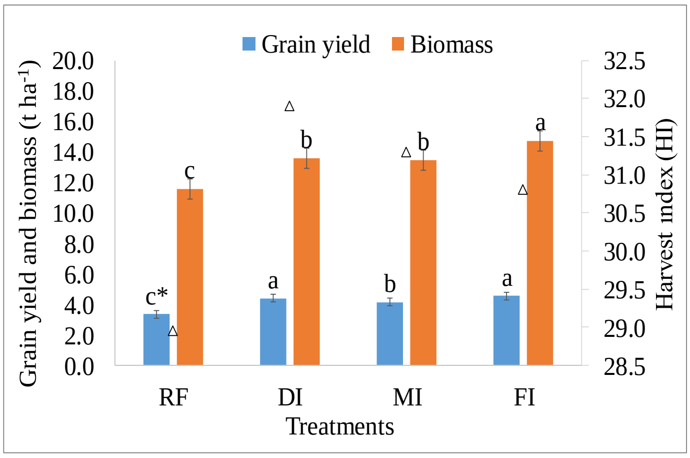


Figure 3. Average yield, biomass and harvest index values and (*) indicated that Duncan classes of treatments

Results of variance analysis showed that the difference between GY and BM of treatments was statistically significant (P <0.05). Additionally, GY and BM and HI was also positively correlated. Determination coeeficient (R^2) was 0.88 for GY, BM relationship and 0.66 for GY, HI relationship as given at Figure 5. Sevaral researcher reported similar results (Yagbasanlar et al., 1995; Villagas et al., 2001).

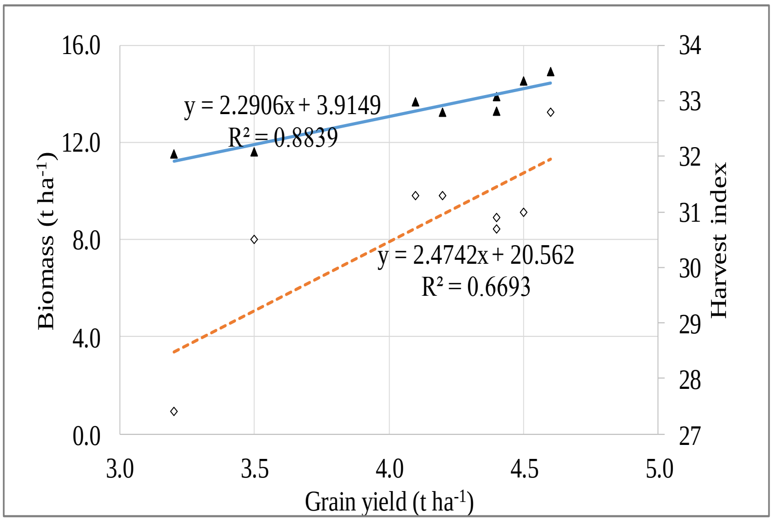


Figure 4. Relationship between grain yield, biomass and harvest indexes

WUE was calculated as grain yield (kg ha^{-1}) divided by seasonal ET (mm). According to treatments calculated WUE values, irrigation amount and ETo values were presented in Table 2. Some researcher reported that the winter wheat WUE ranges from 4.0 to 18.3 kg ha^{-1} mm^{-1} globally on a yield basis (Anderson, 1992; Oweis, et al., 2000).

Table 2. WUE values by treatments

Years	Treatments	ET (mm)	Yields (t ha^{-1})	WUE (kg ha^{-1} mm^{-1})
2009-2010	RF	422	3.54	8.38^{b}
	FI	609	4.58	7.52^{b}
	MI	575	4.15	7.22^{b}
	DI	486	4.36	8.97^{a}
2010-2011	RF	409	3.16	7.72^{b}
	FI	554	4.49	8.10^{b}
	MI	490	4.28	8.73^{b}
	DI	461	4.25	9.22^{a}

The water use had the lowest efficiency in the treatments MI and FI treatments. Applied irrigation water was positively affected to season ET_o (Zhang et al., 2008; 2011). The highest ET_o and grain yield values were obtained from FI treatment. As it was reported by Chang et al. (2014) while increasing ET values WUE was decreased and grain yield was increased. However according to results the highest WUE value was achieved at DI treatment (winter wheat irrigated only heading period). It was respectively followed by RF. This shows that there is no need to apply more water for wheat irrigation. Thus, especially in semi-arid regions, more areas can be irrigated with the same amount of water.

Relationship between grain yield and WUE

The relationships between WUE and grain yield are shown in Figure 5. A significant positive relationship was found between the irrigation water efficiency and grain yield with correlation coefficient 0.65 (R^2). Several researchers were reported similar positive relationships between the grain yield, biomass and WUE under arid and semi-arid climate (Oweis, et al., 2000; Kharrou, et al., 2011; Azizi, et al., 2014; Chen, et al., 2014; Bian, et al., 2016).

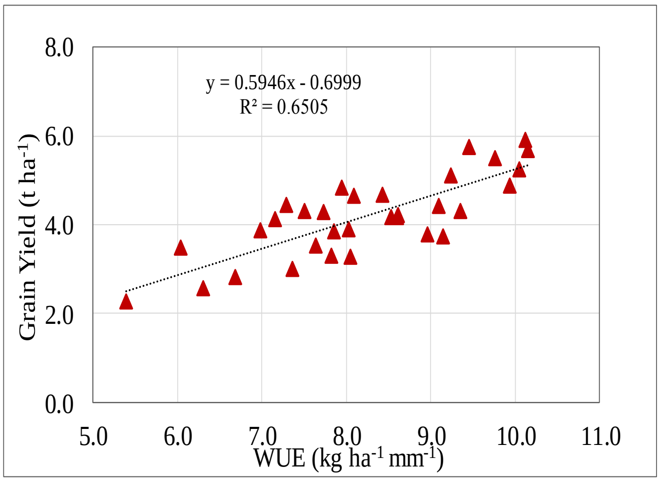


Figure 5. Relationship between grain yield and WUE

As a conclusion; efforts to get more crops per unit area are quite important and necessary for human nutrition in today's world having limited resources as well as a rapidly growing population. At the end of the research study conducted during the wheat growth period between the years 2009-2011 in average wheat yield was found to be 3.35 t ha^{-1}, 4.54 t ha^{-1}, 4.14 t $ha^{-1}$and 4.37 t ha^{-1} respectively according to the treatments (RF, FI, MI, DI). The highest yield was obtained from the full-irrigation treatment while the lowest yield one from the rainfed treatment. The highest average harvest index was found in DI, with the percent of 32. The treatment DI that was irrigated in the same way after the period of heading for both years appeared to have the highest value in the water use efficiency. It might be recommended that irrigation concentrated in the after heading period increase WUE in semi-arid regions such as Central Anatolia Region of Turkey. Two or three irrigation can be sufficient to minimize yield loss from water stress and to improve WUE.

Acknowledgments

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References

1. Agnew, R. (2011). Dire forecast: A theoretical model of the impact of climate change on crime. Theor. Criminol. 16: 21-42.
2. Anderson, W.K. (1992). Increasing grain yield and water use of wheat in rainfed Mediterranean type environment. Australian J. of Agri. Res. 43:1-17.
3. Azizi, T., W. Saidi, M. Rezgui and M. Mechri. (2014). Periodic variation of the water use efficiency in durum wheat. Pakistan J. Biol. Sci. 17:1141–1151.
4. Bian, C., C. Ma, X. Liu, C. Gao, Q. Liu, Z. Yan, Y. Ren and Q. Li. (2016). Responses of winter wheat yield and water use efficiency to irrigation frequency and planting pattern. PLoSONE.11(5):1-13.
5. Cayci, G., L.K. Heng, H.S. Ozturk, D. Surek, C. Kutuk and M. Saglam. (2009). Crop yield and water use efficiency in semi-arid region of Turkey. Soil and Til. Res. 103:65-72.
6. Chen, S., H. Sun, L. Shao and X. Zhang. (2014). Performance of winter wheat under different irrigation regimes associated with weather conditions in the North China Plain. Australian J. of Crop Sci. 8:550–557.
7. Kharrou, M.H., S. Er-Raki, A. Chehbouni, B. Duchemin, V. Simonneaux, M. Lepage, L. Ouzine and L. Jarlan. (2011). Water use efficiency and yield of winter wheat under different irrigation regimes in a semi-arid region. Agr. Sci. 2 (3): 273–282.
8. Musick, J.T., O.R. Jones, B.A. Stewart and D.A. Dusek. (1994). Water-Yield relationship for irrigated and dryland wheat in the US southern plains. Agron J. 86:980-986

9. Oweis, T., M. Pala and J. Ryan. (1998). Stabilizing rainfed wheat yields with supplemental irrigation and nitrogen in a Mediterranean climate. Agron. J. 90:672-681.
10. Oweis, T., H. Zhang and M. Pala. (2000). Water use efficiency of rainfed and irrigated bread wheat in a Mediterranean environment. Agron. J. 92:231–238.
11. Turkes, M. and A.S. Akgunduz. (2011). Assessment of the desertification vulnerability of the Cappadocian district (Central Anatolia, Turkey) based on aridity and climate-process system. Intern. J. Hum. Sci. 8:1230–1268.
12. Yagbasanlar T., H. Ozkan and I. Genc. (1995). Relationships of growth periods, harvest index and grain yield in common wheat under Mediterranean climatic conditions. *Cereal Res. Comm.* Vol. 23: 59-62.
13. Zhang, H., T.Y. Oweis, S. Garabet and M. Pala. (1998). Water-use efficiency and transpiration efficiency of wheat under rainfed conditions and supplemental irrigation in a Mediterranean-type environment. Plant Soil. 201:295–305.
14. Zhang, X.Y., S.Y. Chen, H.Y. Sun, D. Pei, Y.M. Wang. (2008). Dry matter, harvest index, grain yield and water use efficiency as affected by water supply in winter wheat. Irrigation Sci. 27:1-10.
15. Zhang, X.Y., S.Y. Chen, H.Y. Sun, L.W. Shao and Y.Z. Wang. (2011). Changes in evapotranspiration over irrigated winter wheat and maize in North China Plain over three decades. Agri. Water Management 98:1097-1104.

DETERMINATION OF GENETIC VARIABILITY OF EASTERN AND SOUTHEASTERN ANATOLIA REGION TOBACCOS

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Abstract
Tobacco establishing various ecotypes in different ecological zones is one of the crop plants having high genetic variability. In previous years, tobacco ecotypes were conserved by tobacco farming controlled governmentally. But now tobacco sector is privatized. As a result of privatization, companies in tobacco sector have adopted a few ecotypes based on tobacco farming. This phenomenon has increased the risk of extinction for the present genetic variability and now some cultivars are nearly extinct. In this study, conducted to prevent genetic resource erosion, different tobacco ecotypes were determined by visiting the tobacco fields in Eastern and Southeastern Anatolia Region. Some morphological, technique and yield characters of the cultivars were compared by farming them in field via seed. 12 characters of the cultivars were tabulated. Seeds of cultivars, characters of which were determined were delivered to Seed Gene Bank of Turkey.

Keywords: Ecotypes, genetic variation, *Nicotiana tabaccum* L.

Introduction
Tobacco is a different crop plant when compared to the others as the alkaloid nicotine is synthesized in its root and it is also an international legal tips plant. Despite of its all harmful effects on health, it has been used for the same purpose since the first person from Mayan tribe in Yucatan Peninsula. Tobacco plant having high adaptation ability has established ecotypes, suitable for the region where farmed. There are many of tobacco ecotypes in worldwide as it can be farmed in a broad area from 56° N to 38° S latitudes (Esendal, 1986). Turkey has production areas where oriental tobaccos have been farmed successfully for 400 years and experienced producers. Oriental tobacco lines and cultivars, adapted to different ecological conditions have good reputation in worldwide and are indispensable parts of the most appreciated tobacco blends. According to world tobacco production statistics, Turkey is first in oriental tobacco production in country base (FAO, 2015). Yılmaz (1990) separated tobacco production areas of Turkey in 4 regions. The regions are Aegean (Gavurkaya, Akhisar, Ligda, Muğla origins), Black Sea (Trabzon, Maden, Evkaf, Canik, Bafra, Alaçam, Sinop, Gümüşhacıköy, Taşova, Tokat, Erbaa, Niksar origins), Marmara (Düzce, Hendek, İzmit, Bursa, Agonya, Gönen, Yenice, Edirne origins), Eastern and Southeastern Regions of Anatolia (Malatya, Adıyaman, İskenderun, Yayladağ, Bitlis, Silvan, Muş, Şemdinli origins). He mentioned the differences in some morphological and quality characters such as dimension, shape, stem type, tissue, color, rigidity, odor, chemical content among these tobaccos. For example, leaf number per plant was reported to be 17-100 depending on cultivars, ecological conditions and the adopted cultural applications (İncekara, 1979; Emiroğlu et al. 1987; Uz, 1988; Yazan, 1989; Otan and Apti 1989). According to Peksüslü (1998) the oriental tobacco cultivar Bitlis 52 produced the lowest leaf number per plant, but the highest levels for the parameter were observed in the cultivars Agonya 6-1/A, Bafra 6391, Karabağlar 6265 and Düzce Özbaş 190/5. Otan and Apti (1989) reported that Eastern and Southeastern part of Anatolia are the region having the highest variability and ecotypes for oriental tobacco. There were many of indigenous tobaccos in the region as tobacco had been farmed in

many fields of the region, different to each other both ecologically and geographically. Broad leaf webby butt and less webby butt types as well as small leaf webby butt types could be encountered in the region and they had been farmed in their own populations. These researchers indicated the presence of Yayladağ, Malatya, Adıyaman, İskenderun, Diyarbakır, Siirt, Mardin, Muş, Bitlis and Şemdinli populations. The mentioned populations were different from those of Aegean, Marmara and Black Sea regions in terms of their physical and blend characters. "Tombeki" and "Hasankeyf" tobaccos of *Nicotiana rustica* were also present in the region. Ketenci (1985) reported that tobacco was farmed by using the unaccredited seeds and tobaccos having different appearance from different origins were farmed in the same field in Malatya and Adıyaman-Çelikhan 676, a certificated breeding cultivar for the region was degenerated over time and the cultivar could satisfy neither producer nor management in that its yield and quality. Virginia tobacco, substituted for eastern tobaccos, was tried to farm in Urfa surrounding not to cause the unemployment of farmers from eastern regions, farming tobacco for many years. Results indicated that it was possible to farm Virginia tobacco in this region, but not possible to reach the same quality of that, farmed in its own ecology. Researcher concluded that appropriate farming applications as trashing and using of high quality seed were key factors to increase quality of our eastern tobaccos. On the other hand, it is possible to use thick veined eastern tobaccos in blends at the rate of 10% by paying more attention the farming applications. Tobacco fields of the region constituting significant rate of Turkish tobacco production in total are generally mountainous terrains with high altitude. Tobaccos of region have generally stronger flavor and amber in color. It is true that the first tobaccos introducing to Anatolia were sessile but webby butt types were also present (Yılmaz, 1998). Each tobacco region in Turkey where tobacco was either farmed in the past or is being farmed presently has many of ecotypes. Ecotypes are genetic materials which are not possible nearly to find in other ecologies than theirs. For that reason, they are important for both farming and breeding. In this study, it was aimed to determine the biodiversity of Eastern and Southeastern Anatolia region and to take this biodiversity under preservation by re-identifying the present genetic variation. Thus, it would be possible to determine the tobacco genetic diversity of region and to establish data base for future breeding studies.

Material and methods

Plant Material

Production centers of tobacco in our country have been known from past to present very well and based on the knowledge, we determined the provinces and their districts where research trips were conducted. In Eastern and Southeastern Anatolia Region, a total of 57 samples were taken as shown in Table 1. Geographical info on the sampling sites is shown in Table 2.

Table 1. The sampled provinces and their districts from Eastern and Southeastern Anatolia Region

Province	District
Adıyaman	Central district, Kahta, Savsat, Besni, Çelikhan
Batman	Central district, Hasankeyf, Kozluk, Sason
Bitlis	Central district, Güroymak, Hizan, Mutki
Diyarbakır	Central district, Hazro, Kulp, Lice, Silvan
Gaziantep	Şahinbey, Şehitkamil, Araban, Islahiye, Karkamış, Nizip, Nurdağ, Oğuzeli, Yavuzeli
Hakkari	Central district, Şemdinli
Hatay	Central district, İskenderun, Yayladağ, Altınözü
Malatya	Doğanşehir
Mardin	Central district, Mazıdağı, Samur
Muş	Central district, Bulanık, Hasköy

Table 2. Geographical info on the sampling sites from Eastern and Southeastern Anatolia Region

Code	District	Locality	Altitude/Latitude/ Longitude (A/L/L)	*Code*	District	Locality	A/L/L)
D1	Yayladağ	Sebenoba	370-360326-360052	*D37*	Hatay	Altınözü	*
D2	Yayladağ	Sebanoba	369-360325-360053	*D38*	Muş	Merkez	*
D3-4	Yayladağ	Karaköse	463-360107-360204	*D39*	Adıyaman	Çelikhan	*
D5	Yayladağ	Gözlüce	340-360036-355947	*D40*	Muş	Merkez	*
D6-7	Yayladağ	Gözlüce	326-360036-355947	*D41*	Muş	Merkez	*
D8	Samandağ	Meydan	11-360115-355854	*D42*	Hatay	Altınözü	*
D9	Hassa	Merkez	378-364639-363142	*D43*	Batman	Merkez	*
D10-11	Hassa	Yolluklar	694-664712-362716	*D44*	Batman	Merkez	*
D12-13-14	Adıyaman	Alibeyköy	645-374450-382836	*D45*	Batman	Merkez	*
D15-16-17	Kömür	MYÖz. Mh.	747-375206-382638	*D46*	Batman	Merkez	*
D18	Adıyaman	Doğanlı	1272-375942-381334	*D47*	Batman	Merkez	*
D19-20	Doğanşehir	Kurucuova	1479-375847-380619	*D48*	Batman	Merkez	*
D21-22	Hazro	Ormankaya	1024-381750-404620	*D49*	Batman	Merkez	*
D23	Hazro	Ormankaya	1008-381754-404623	*D50*	Batman	Merkez	*
D24	Hazro	Ormankaya	1005-381753-404623	*D51*	Batman	Merkez	*
D25	Diyarbakır	Silvan	*	*D52*	Batman	Merkez	*
D26-27	Batman	Bıçaklı köyü	586-375817-410832	*D53*	Muş	Merkez	*
D28	Kızıltepe	Kahraman	582-371641-403821	*D54*	Malatya	Kurucuova	*
D29-30-31	Muş	Kızılağaç	1315-384739-411932	*D55*	Malatya	Kurucuova	*
D32	Muş	Suvaran	1305-384638-122437	*D56*	Gaziantep	Hasankeyf	*
D33	Bitlis	Bölükyazı	1583-381943-421034	*D57*	Hakkari	Şemdinli	*
D35	Muş	Merkez	*	*D58*	Hakkari	Çukurca	*
D36	Kurucuova	Sürgü	*				

*For these populations, seeds were not collected in field but supplied by farmers not sowing but storing them for many years

Methods

Material Collecting: Field trips were made to Tobacco production areas in Eastern and Southeastern Anatolia Region in 2013. During the trips, plants were selected at the flowering of 10 % stage, sampling form was filled, inflorescences were boxed with isolation bags and the plants were sealed. Before plants were sealed, plant height (cm), leaf long/width (cm), leaf number per plant and flower color were recorded. The second trip was made in seed formation stage and seeds were collected from the boxed capsules for each plant. The old seeds, not sowed but stored by farmers in localities where tobacco was produced in the past were also provided.

Field Studies: After collected, the seeds for each ecotype, collected from different localities were sowed as two rows, 4 m in length in testing site of Ondokuz Mayis University, High School of Bafra in second year. The tested parameters were plant height (distance between plant crown and inflorescence), leaf number per plant (economically important), leaf length / width (2nd hand), stem diameter (center of stem), angle between leaf and stem, angle for the top leaf, number of flowering day (at least 50% of flowering), leaf yield, invert sugar rate, nicotine rate and quality grade. Quality grade was determined based on mean values from scales, established for organoleptic observations. Mean values of these scales, 1 is the best, are 1-4 for leaf dimensions, 1-6 for color and brightness, 1-5 for leaf thickness, 1-4 leaf grainy, 1-5 for strength and flexibility and 1-5 for odor.

Chemical Analyses: Nicotine rate (%): Nicotine content was determined spectrophotometrically as described by Eğilmez (1988). Invert sugar rate (%): Invert sugar content was determined spectrophotometrically as described by Sekin (1979).

Results and discussion

According to the results of the study on Eastern and Southeastern Anatolia Region tobaccos (Bitlis, Yayladağ and Silvan), the average plant height varied with 29-134.6 cm, leaf number per plant 12-42, leaf width 8.2-21.5 cm, leaf length 18.44.2 cm, number of flowering day 48-74 day, the angle between leaf and stem 35-61° (Peksüslü, 1998). Reducing sugar ratio for Turkish tobaccos was reported as between 2-21% and nicotine 0.4-2.5% by Er et al., 2014. Eastern and Southeastern Anatolia tobaccos were also reported to contain 3-5% nicotine (Şahin and Taşlıgil, 2013).

Measurements and Observations for Harvest Period: The lowest and highest values of measurements and observations for harvest period on the basis of province are shown in Table 3.

Table 3. The lowest and highest values of measurements and observations for harvest period on the basis of province.

Province	Locality number	Plant height (cm)	Leaf number	Leaf width (cm)	Leaf length (cm)	Flower color
Adıyaman	8	49.1-103	27-39	11.7-19.5	27-57	white-pink-light pink
Batman	12	126-142	19-27	29.3-31.5	51.7-52.2	pink-light pink
Bitlis	1	41	12	18.3	31.5	red
Diyarbakır	5	47-107	16-20	18-27.5	27-39	white-yellow- pink-light pink
Gaziantep	1	-	-	-	-	-
Hakkari	2	-	-	-	-	-
Hatay	13	66-152	14-41	7.8-50	15.5-70	white-yellow - pink-light pink
Malatya	5	95-105	28-29	19.4-26	45.5-47.5	light pink
Mardin	1	124	18	27	49.5	white- light pink
Muş	9	47-115	15-18	18.5-24.2	32.39-6	white- light pink -pale pink

Plant height (PH, cm): Mean values of tobaccos from eastern region for plant height varied with 19.9 and 104.2 cm. Mean values for this parameter was calculated as 57.8 cm (Table 4). The space values of plant height cumulated was 38-76 cm. It was observed that plant height for 89% of tobaccos from eastern region was under 85.9 cm cumulatively. This region has quite a change of climatic and soil conditions. It is difficult to find similarity/closeness among the cultivars farmed in the broad area where mixed populations are prevalent. This phenomenon affected plant height significantly and as a result the difference between the lowest and highest values for this parameter was found to be bigger than that of other regions (Table 5).

Leaf Number per Plant (NP, number/plant): Mean values for leaf number per plant varied with 12 and 51 among lines (Table 4). Data for the parameter was recorded between 12.1 and 29.6 and the mean was determined as 23. Tipping is the reason why leaf number per plant such fluctuated. 10% of the studied lines produced 34-51 leaves per plant. It is noteworthy to note that short lines produced low leaf per plant but tall ones produced higher values for the parameter. This phenomenon was attributed the abundance of tobacco ecotypes in this region and tipping applied to some lines (Table 5).

Leaf Width (LW, cm): Among lines, leaf width varied with 7.28 and 21.56 cm (Table 4). Generally lines yielded leaves whose widths were between 10.5 and 15.5 cm. Mean value for the parameter was recorded as 13.16 cm. The reason why leaf width values were higher in this region than those of the other ones is development of the leaves, left following tipping. Leaf length and width are characters belonging to given cultivar; as a result, different ecotypes of the region were differed with leaf dimensions as it is expected. Besides, leaf development increased in tipped tobacco, thus leaf length and width also increased in parallel (Table 5). *Leaf length (LL, cm)*: It was observed that leaf length was differed with 13.58 and 47.18 cm (Table 4). Mean values centered between 17 and 32.5 cm cumulatively. Tipping was considered again the reason why eastern tobaccos yielded taller leaves than tobaccos from other regions. It was observed that the leaves, left following tipping continued to develop (Table 5). *Stem Diameter (SD, mm)*: Mean values for stem diameter of eastern tobaccos varied with 6.76 and 16.61 mm (Table 4). It was observed that stem diameters centered 10.9 and 14.4 mm. Mean value for stem diameter was found to be 11.2 mm. In tipped lines, stem diameter was affected and increased as shown in Table 5. *Angle between Leaf and Stem (AS, ^{0})*: This parameter varied with 31.8 and 77.4° (Table 4). Most of the means were recorded between 43 and 66°. The great variation in the mean values could be attributed to abundance of tobacco cultivars in the region as shown in Table 5. *Angle for the Top Leaf (AL, ^{0})*: Angle for the top leaf of eastern tobaccos varied with 43.2 and 87.8° and mean value for the parameter was recorded as 72.14° (Table 4). Lines yielding angle for the top leaf from 79 to 87° has constituted 50% of total lines studied. Variation in angle for the top leaf was a result of the great cultivar variability in the region (Table 5).

Table 4. Statistical data for Eastern and Southeastern Anatolia Region tobaccos

	Plant height (cm)	Leaf number per plant	Leaf width (cm)	Leaf length (cm)	Stem diameter (mm)	Stem angle (^{0})
Mean	57.8	23.16	13.16	26.38	11.18	53.72
Standard error	2.654	1.131	0.394	1.011	0.321	1.304
Median	57.8	20.6	13.36	26.1	11.272	54.8
Standard deviation	20.043	8.533	2.979	7.633	2.416	9.846
Genotype number	57	57	57	57	57	57
The highest	104.2	51	21.56	47.18	16.61	77.4
The lowest	19.9	12	7.28	13.58	6.76	31.8
	Top angle (^{0})	Number of flowering day (day)	Yield (kg/da)	Invert sugar (%)	Nicotine (%)	Yield (%)
Mean	72.14	53.86	129.59	2.33	2.6	48.405
Standard error	1.788	1.724	5.0129	0.101	0.088	1.054
Median	78.4	51	121.869	2.16	2.685	48.61
Standard deviation	13.499	13.022	37.846	0.766	0.665	7.963
Genotype number	57	57	57	57	57	57
The highest	87.8	102	220.13	4.23	3.69	70.56
The lowest	43.2	46	73.73	1.29	0.74	34.17

Number of Flowering Day (FD): Eastern tobaccos flowered during a period from 46 to 102 days as shown in Table 4. Among the studied lines, one flowered in 92th and 3 lines flowered in 101-102th days. The difference in flowering days of the lines was not surprising when considering the great cultivar variability in this region (Table 5). *Leaf Yield (LY, kg/da)*: Leaf yield for the studied lines varied with 73.73 and 220.13 kg/da (Table 4). 80% of the lines produced leaf per decare from 73 to 147 kg. Mean value for the parameter was recorded as 129.59. It should be noted that leaf yield was found to be higher than the level of 160 kg/da for 11 lines. Higher leaf yields for eastern tobaccos were probably caused by different agricultural practices namely, tipping, fertilization and irrigation (Table 5). *Invert Sugar Rate (IS, %)*: Invert sugar rates varied with 1.29 and 4.23 (Table 4). It should be noted that invert sugar rate in 80% of the studied lines was under 2.92% level (Table 5). *Nicotine Rate (N, %)*: Nicotine content of eastern tobaccos varied with 0.74 and 3.69% and mean value for the parameter was found to be 2.6% (Table 4). 2/3 of the lines examined yielded nicotine in rates of 2.4-3.5%. It can be concluded that fertilization and irrigation, applied to eastern tobaccos, but not the tobaccos from other regions increased nicotine content of these tobaccos (Table 5).
Quality Grade (QG, %): Quality grade of eastern tobaccos varied with 34.17 and 70.56 (Table 4) and was found to be 40-59 averagely (Table 5).

Table 5. Mean values for yield, the morphologic, chemical and some technologic characters of tobacco lines from Eastern and Southeastern Anatolia Region

Code	PH	NP	LW	LL	SD	AS	AL	FC	FD	LY	IS	N	QG
D1	81.60	26.80	18.16	29.36	15.32	57.00	76.20	l.pink	92	199.09	2.14	2.83	36.94
D2	87.40	31.00	12.40	23.84	12.38	59.60	85.40	pink	101	140.78	1.87	2.63	36.94
D3	86.20	32.20	11.58	24.70	11.76	53.80	84.20	l.pink	102	190.37	2.44	2.72	35.83
D4	91.20	31.00	12.60	21.40	12.21	58.00	85.40	l.pink	101	120.82	1.93	3.15	39.72
D5	76.20	25.80	15.10	41.44	13.94	49.60	65.00	white	55	134.48	1.54	3.08	47.22
D6	88.90	26.60	13.84	39.84	13.99	42.80	58.80	white	53	220.14	1.78	2.49	49.17
D7	81.20	20.60	13.48	34.94	12.12	63.60	69.00	l.pink	54	212.71	2.85	2.63	36.94
D8	52.60	17.20	18.82	30.54	13.92	52.20	72.60	l.pink	54	106.37	3.52	2.99	34.17
D9	48.30	16.60	12.92	13.58	10.07	54.80	81.60	l.yel.	46	96.82	2.54	2.69	55.56
D10	33.60	20.20	17.40	24.58	6.76	32.60	83.40	l.yel.	46	99.09	3.21	2.57	45.28
D11	32.80	20.00	17.10	23.54	6.76	32.20	81.20	l.yel.	46	124.50	2.94	2.06	45.28
D12	73.40	26.20	13.78	35.04	13.51	50.40	53.40	l.pink	56	220.10	1.47	1.09	48.61
D13	62.40	24.60	11.90	33.82	12.36	41.20	54.20	l.pink	47	122.92	2.52	2.29	45.28
D14	75.60	27.80	21.56	47.18	16.61	44.80	55.00	l.pink	51	190.68	1.43	0.91	48.61
D15	39.10	24.60	14.28	36.74	13.80	48.00	45.60	l.pink	47	121.87	1.91	1.77	49.72
D16	39.60	20.00	15.34	39.50	13.40	41.80	53.00	white	53	135.53	3.34	1.74	44.72
D17	35.00	16.60	9.92	23.72	9.43	51.60	51.80	l.pink	53	145.90	2.71	1.75	45.28
D18	47.10	21.40	13.36	39.74	14.37	42.00	48.40	l.yel.	51	195.94	1.72	3.36	44.44
D19	53.40	18.20	9.86	30.68	12.24	59.40	49.80	pink	46	107.42	1.77	1.86	49.44
D20	42.20	18.00	12.94	31.08	10.64	45.60	63.20	pink	46	112.35	2.76	2.89	45.28
D21	44.90	13.60	14.94	26.56	8.95	42.80	86.00	l.pink	51	101.85	3.21	2.51	45.28
D22	38.60	15.40	15.66	26.70	11.11	50.40	80.20	l.pink	46	102.99	1.81	2.82	51.94
D23	40.20	16.20	16.74	20.02	11.61	49.60	74.00	l.pink	48	86.26	2.66	3.18	43.61
D24	59.40	15.20	15.46	25.66	8.19	57.20	87.80	l.pink	47	170.20	1.90	1.56	48.61
D25	53.10	14.60	16.74	32.54	11.56	53.40	83.00	l.pink	53	125.92	2.59	2.44	45.28
D26	56.00	17.40	14.84	27.00	10.85	50.60	79.40	l.pink	53	96.54	2.85	2.27	39.17
D27	47.00	16.40	14.74	26.10	9.43	59.40	82.60	l.pink	53	171.77	3.84	2.21	41.94
D28	46.60	15.40	12.20	26.94	8.33	55.00	61.20	l.pink	49	99.09	1.93	2.76	48.61
D29	40.20	14.20	14.02	26.68	11.51	45.60	81.00	l.pink	49	94.95	1.49	2.21	50.28
D30	41.20	15.20	14.53	27.54	10.81	50.40	73.40	l.pink	50	73.73	3.67	2.25	46.67
D31	39.00	12.40	13.64	24.32	11.00	56.60	82.60	l.pink	54	89.99	1.34	2.39	45.83
D32	60.40	15.20	13.34	23.00	9.02	49.60	82.20	l.pink	46	113.99	3.78	2.77	49.44
D33	19.90	12.00	10.80	18.40	7.77	69.60	78.40	pink	46	93.62	4.23	0.74	54.17
D35	65.30	22.40	12.78	25.62	11.27	59.80	72.80	white	54	104.01	1.84	3.69	54.72
D36	51.00	26.00	10.70	31.90	13.13	49.20	48.80	l.pink	46	130.54	1.37	2.75	41.94
D37	71.20	27.00	13.54	22.56	10.30	49.80	79.60	l.pink	56	136.32	1.57	3.24	56.94
D38	74.42	27.80	14.16	32.04	13.37	77.40	51.60	l.pink	54	123.97	1.91	3.46	37.78
D39	48.30	26.80	12.86	35.92	14.23	64.20	43.20	l.pink	55	172.30	1.32	3.38	48.61
D40	63.20	26.80	15.00	28.00	13.48	58.00	81.20	l.pink	56	140.04	1.48	3.32	48.61
D41	85.40	25.60	15.84	31.00	13.39	53.80	75.80	l.yel.	52	140.44	2.33	3.04	58.89
D42	68.50	30.40	14.90	19.80	12.82	59.20	83.80	l.pink	51	99.61	2.33	3.08	55.56
D43	59.20	18.80	17.44	33.24	11.70	58.20	76.70	l.pink	52	130.54	1.60	2.63	45.83
D44	58.50	42.60	10.56	18.34	15.36	66.00	84.20	l.yel.	51	142.16	2.26	2.43	63.61
D45	65.10	36.40	7.28	13.96	7.40	56.00	72.40	l.pink	46	131.08	2.22	2.53	57.5
D46	72.28	51.00	9.78	20.22	13.07	55.40	85.60	white	53	214.82	2.30	2.86	70.56
D47	104.20	43.00	10.46	20.92	10.79	76.80	81.40	pink	47	143.75	1.96	2.42	36.67
D48	83.20	33.00	8.82	17.74	9.66	70.00	84.70	pink	46	101.38	3.63	3.33	48.61
D49	85.10	35.00	9.00	17.40	9.24	55.80	83.80	l.pink	50	103.97	1.87	2.81	64.72
D50	89.80	27.40	10.96	19.50	9.10	60.40	86.20	l.pink	46	113.46	3.77	3.49	48.06
D51	68.00	18.00	14.36	26.66	10.00	56.00	68.40	l.pink	50	118.07	1.29	2.43	48.61
D52	57.80	39.80	11.42	17.74	12.05	64.40	83.00	l.pink	58	106.63	2.89	3.48	67.22
D53	61.60	18.60	9.16	16.32	7.65	56.00	78.40	l.pink	55	138.68	2.16	1.31	40.28
D54	31.30	19.20	7.52	22.62	10.20	66.00	52.40	l.pink	51	89.47	1.74	2.86	55.28
D55	25.60	17.40	9.34	27.71	10.95	62.00	48.80	l.pink	54	92.83	1.81	3.17	51.39
D56	31.40	20.60	11.80	14.08	7.16	31.80	79.40	l.yel.	47	91.16	3.39	2.27	44.44
D57	29.40	15.20	7.68	13.92	6.77	35.40	83.00	l.yel.	47	112.27	2.26	3.55	64.44
D58	34.40	13.00	11.02	19.58	8.20	49.00	77.80	l.pink	48	90.61	2.01	3.09	53.61

*PH; plant height, NP; leaf number per plant, LW; leaf width, LL; leaf length, SD; stem diameter, AS; angle between leaf and stem, AL; angle for the top leaf, FC; flower color, FD; number of flowering day, LY; leaf yield, IS; invert sugar rate, N; nicotine rate, QG; quality grade, l.; light, l.yel.; light yellow

Conclusions

Turkey is one of the most important geographic areas of the world having high plant diversity as it has three different phytogeographic regions (Europe-Siberia, Persian-Turan, Mediterranean) as well as distinctive climatic and soil characters and is a junction of two gene centers (Mediterranean and the Near East). Tobacco is a very adaptive plant which can establish idiocratical ecotypes in response to geographic and climatic conditions of its environment. In the present study, variation limits of tobacco ecotypes from Eastern and Southeastern Anatolian region was revealed in detail and seeds of the examined ecotypes were delivered to Seed Gene Bank of Turkey.

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References

1. Eğilmez, Ö. (1988). Physical, Chemical and Smoke Analysis Methods for Tobacco and Cigarettes. Monopoly Institutes Publication No: 28, İstanbul, Turkey.
2. Emiroğlu Ü., Sekin S. and Bürün, B. (1987). Development of new line by another culture for Aegean region. Doga Scientific Journal, 11:2 334-347.
3. Er, C. and Yıldız, M. 2014. Merriment Plants. Ankara University Faculty of Agriculture Press No: 1616. 247 p. Ankara.
4. İncekara, F. (1979). Industrial Crops-4 (Merriment Plants). Ege University Faculty of Agriculture Pub. No: 84. İzmir, Turkey.
5. Ketenci, K. (1985). A New Type of Tobacco 500/24 to the Area of Malatya and Adıyaman. Monopoly Institutes Publication Pub. No: EM/19, İstanbul, Turkey.
6. Otan, H. and R, Apti. (1989). Tobacco. T.C. T.O.K.İ.B. Ege Agricultural Research Institute Pub. No: 83, İzmir, Turkey.
7. Peksüslü, A. (1998). The Morphological phsiological and biochemical characteristics of some Turkish tobacco varieties in İzmir-Bornova conditions. Doctoral thesis. Ege University, İzmir, Turkey.
8. Sekin, S. (1979). Some Analysis Methods Research on Tobacco. Chemical Composition and Changes Occurring During Fermentation in Aegean Region Tobacco. Readership Thesis, Ege University, İzmir, Turkey.
9. Şahin, G. and Taşlıgil, N. (2014). Le Developpement Hıstorıque et la Dıspersıon Geographıque de la Cultıvatıon de Tabac en Turquıe. Eastern Geographical Review, 18 (30): 71-102.
10. Uz. E. (1988). Effects on Yield and Quality of Differences in Plant Development in Tobacco Field Period. Master's thesis. Ege University, İzmir, Turkey.
11. Yazan, G. (1989). Performances of the Aegean region saribaglar group tobacco resistant to blue mold (*Perenospora tabacina* Adam). Anadolu Journal of AARI, 22 (2): 49-58.
12. Yılmaz, G. (1998). Merriment Plants. GOP University Faculty of Agriculture Pub. No: 11, Tokat, Turkey.

BREEDING OF SUNFLOWER (*HELIANTHUS ANNUUS L.)* AT DOBRUDZHA AGRICULTURAL INSTITUTE – GENERAL TOSHEVO

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Abstract
Dobrudzha Agricultural Institute – General Toshevo is the only breeding center of sunflower in Bulgaria. Since its establishement in 1951, over 50 varieties and hybrids of this crop have been developed here. More than 15 joint hybrids have been registered abroad, the result from cooperation with our European partners. The institute has a rich and variable collection of initial breeding materials. The methods we use in our breeding work are intraspecific, interlinear, interspecific and intergeneric hybridization, experimental mutagenesis, embryoculture, somaclonal variation, *in vitro* screening and selection. The methods for evaluation of resistance to economically important diseases and the parasite *Orobanche* have been adapted to the working conditions of the Institute. Morphological, biological, technological, biochemical and phytopathological characterizations of the released and registered hybrids and their parental lines have been made. Many new materials have been developed during the last decade, which possess valuable breeding properties. Over 6000 inbred lines are involved in the breeding work. Annually, 1400 new hybrid combinations are being tested in Bulgaria and abroad. Hybrids have been developed, which possess very good productivity and adaptability potential, and which have been registered in Bulgaria, EU and other foreign contries. Some foreign companies included our new hybrids (Velko, Veleka, Yana, Divna, Valin, etc.) in their catalogs and are successfully organizing their seed production and marketing in the respective countries. A new direction of our breeding is the devlopment of sunflower hybrids resistant to herbicides. Several hybrids - Enigma, Sunny imi, Desi, Viyani, Danaya, etc., are now within the system of official testing in Bulgaria and abroad. Their registration is forthcoming. The aim of this investigation was to present the current status and achievements of the breeding work on sunflower at DAI-General Toshevo during the last decade.

Keywords: Hybrids, lines, productivity, resistance.

Introduction

Sunflower breeding in Bulgaria began as early as the 1920's. It was initially carried out only as individual family selection breeding method with the aim to develop varietal populations (Petrov et al., 1994). Thus the first large-seeded cultivar Stadion was developed, which has high protein content, low percent of oil and is suitable for direct consumation. This cultivar was followed by another large-seeded variety Favorit, which is currently widely distributed in Bulgaria and abroad. The improvement and breeding work by the method of interlinear hybridization for development of hybrids started in 1963. Distant hybridiuzation, experimental mutagenesis and biotechnology have been used as auxiliary methods. One of the first breeding tasks was increasing the oil content and reducing the percent of husk. Later, with the occurrence and wide distribution of broomrape, breeding for resistance to this parasite also began. During 1970 – 2000, the breeding work by the method of heterosis gradually increased. Significant results were obtained both in the breeding of inbred lines and in the development of hybrids. This became possible after the discovery of the first CMS source in sunflower by Leclercq (1969) and of effective restorers of fertility (Enns et al., 1970; Kinman, 1970; Leclercq, 1971; Vranceanu and Stoenescu, 1971). The testing of the first Bulgarian hybrids began in 1973 (Stoyanova et al., 1977; Ivanov et al., 1988). The first Bulgarian hybrid Start was released and distributed on the entire teritory of the country in 1979. It was resistant to downy mildew and exceeded variety Peredovik, its contemporary standard of highest mass distribution, with more than 12 % (Gotsov et al., 1981). In the 1980s, a new group of early hybrids was developed, among them Albena, Super Start, Dobrich and SantaFe. They gradually occupied over 90 % of the areas sown with sunflower in Bulgaria. In 1988, hybrid Albena was registered and distributed in France, and in 1993 it became the main hybrid there, with 40 % of the sunflower areas. Albena was also acknowledged as a world standard from the group of the early maturing hybrids. The fast spreading of the parasite *Orobanche* became the main reason for the development of a new group of hybrids at the end of the last century and the beginning of the new one, which possessed parallel resistance to downy mildew and *Orobanche* (Christov et al., 2009). Hybrids San Luka, Maritsa, Mussala, Rada, Yana, Merkuriy, Perfekt, etc., were released and distributed. The new Bulgarian hybrids occupied at that time more than 80 % of the sunflower areas in Bulgaria. Unfortunately, in the last decade the percent of the own hybrids has decreased dramatically. The major reason for this is the flexible, aggressive and financially well supported policy of the foreign companies. Nowadays over 50 sunflower hybrids and varieties are registered in Bulgaria, which have been developed at DAI – General Toshevo. The aim of this investigation was to present the current status of sunflower breeding at DAI – General Toshevo and its achievements during the last decade.

Material and methods

The investigations subject to this publication were carried out at DAI – General Toshevo; they are the result from the implementation of several 4-year projecs the Sunflower Breeding Department has been working on. The main aim of these projects was the development of new sunflower hybrids resistant to biotic and abiotic factors through combined use of conventional and biotechnology methods in breeeding. The initial breeding materials were Bulgarian and foreign direct and hybrid sunflower varieties, land races and foreign populations, own old lines – maintainers of sterility, their sterile analogues and fertility restorers, wild species of genus *Helianthus*, species from other genera of *Compositae* family, hybrids, derived through intraspecific, interspecific and intergeneric hybridization, forms obtained by using experimental mutagenesis, lines produced through various bio technology methods applied independently or in combination with induced mutagenesis. A permanen stationary collection of 250 accessions from the perennial species of genus *Helianthus* with official registration at FAO, which is unique for Bulgaria, is being maintained at DAI – General Toshevo under natural conditions. The collection includes also about 200 accessions from 7 annual species. The methodologies for interspecific and intergeneric hybridization are being constantly improved. The methods for evaluation of the resistance to the economically important diseases on sunflower and the parasite broomrape have been adapted to the conditions of the Institute's location (Панченко, 1975; Maric et al., 1981; Vear and Tourvielle, 1987; Encheva and Kiryakov, 2002). A new methodology for testing of the resistance to sclerotiona has been elaborated (Christov et al., 2004), as well as a method for irradiation of immature sunflower embryos with gamma radiation or ultrasonic (Encheva et al., 1997). Oil percent in seed is being determined by improved methodologies of Rushkovskiy,1967, Stoyanova and Ivanov, 1968, Ivanov et al., 1996. Equipment for fast evaluation is also used (Newport Instruments Ltd., 1972). Out of the 4 schemes of hybrid seed production developed at our Institute (Velkov and Stoyanova, 1974), only the method of inter linear hybridization is being used for production of simple two-linear male fertile hybrids with full restoration of fertility. The new hybrid combinations undergo through several-year testing according to a scheme and technology adopted at our Institute; they are then forwarded for official variety testing to our EU partners and other countries. All new released and registered lines and hybrids were given morphological descriptions according to UPOV (2002).

Results and discussion

The higher efficiency of production is a main task of the bereeding programs in the different crops. A key factor for stable yields is the resistance to different types of stress (Mihova et al., 2015; Dimitrova-Doneva et al., 2016). In this relation, the most important task of the project on which the Sunflower Breeding Department is working is the development of high-yielding hybrids resistant to the economically important diseases and the parasite *Orobanche*, which are adaptable to constantly changing environments. This is primarily dependent on the efficiency of the breeding of lines. DAI – General Toshevo has a rich collection of inbred lines derived through conventional methods of breeding, which possess various biological and morphological traits (Table 1).

Table 1. Volume of inbred sunflower lines during the last decade

Inbred lines	Number during 2006-2016 years
R lines – restorers of fertility	3300
B lines – maintainers of sterility	2400
A lines – sterile analogs of B lines	320

Since the above volume of lines is constantly being increased and enriched, and due to the practical impossibility to work with such a great number of materials, the larger part of them are being stored in the genetick stock center of DAI, regularly reproducing the older and more valuable ones.

Breeding of inbred lines with normal cytoplasm

A large part of this group of lines were obtained from old Russian high oleic cultivars, others are with origin from Argentina, USA, Hungary, etc. The percent of the lines obtained through interlinear hybridization of already well established lines, followed by selection, is getting higher. In the past years a large number of lines were also developed by using interspecific hybridization and experimental mutagenesis (Christov, 2002). When developing inbred lines, the selection is carried out for the following traits: productivity, combining ability, duration of vegetative growth, plant height, head diameter, resistance to lodging, 1000 kernel weight, number of seeds per plant, oil and protein content in seed, resistance to diseases, parasites and pests. The new genotypes are tested for combining ability with A-lines; the development of their sterile analog begins simultaneously using citoplasmic male sterility (CMS) type Petiolaris (PET 1). This type of sterility is shown to be stable, it is inherited in the next generaions without being affected by the growing conditions. The sterile analogs obtained on the baisi of this CMS type have normal female fertiliuty. To test the new B-lines, branched sterile fertility restorers of cytoplasmic type ssp. Falax are also used.

Breeding of fertility restorer inbred lines

The first fertility restorers in our institute were obtained in several ways – by selection and selfing of cultivars with established presence of Rf genes; by crossing of two lines, one of which is a carrier of Rf genes; crossing of a line to a cultivar; developing of synthetic populations, etc. In the recent years there are two methods, which are used most frequently – crossing of R lines with valuable traits and selection and selfing of high-yielding hybrids well established on the market. Another less frequent but a valuable way of developing fertility restorer lines is the interspecific and intergeneric hybridization. A large number of R lines are obtained from crosses of male sterile cultural sunflower lines with different *Helianthus* species and species from other genera of *Compositae* family and selfing of the obtained hybrid fertile plants, which is repeated 8-9 times with the aim to render the *Rf* gene in homozygous condition, accompanied with multiple and successive selection. These R lines possess other valuiable properties, as well, inherited from the two parental forms involved in the hybridization (Christov et al., 1996; Christov, 2002). The majority of the new lines possess excellent combining ability, full resistance to downy mildew and the parasite *Orobanche* and high resistance to the diseases phoma, phomopsis and sclerotinia. Significant is the percent of the materials obtained by using distant hybridization and experimental mutagenesis and by combining distant hybridization with bio technology methods and techniques (Table 2).

Table 2. Species, varieties, forms and lines obtained through distant hybridization, experimental mutagenesis and some bio technology methods and techniques

Species, forms, lines, varieties	Number
Lines obtained through distant hybridization	3500
- R lines	3300
- B lines	200
Lines obtained by combining distant hybridization with bio technology techniques	1100
- R lines	1030
- B lines	70
Lines obtained through experimental mutagenesis	650
Herbicide resistant lines	200
- Distant hybridization + conventional breeding	170
- Biotechnology techniques	30
Initial breeding material	
- Varieties, lines, hybrids and populations	1400
- Accessions from species of genus *Helianthus*	660
- New forms for lines of wild *H. annuus*	50
- Accessions from species of other genera of *Compositae* family	70
- New CMS sources	29
- New sources of Rf genes	230

The results from Table 2 show that a large volume of initial material has been collected and developed, which was multiplied and evaluated for presence of properties important for breeding. Diffeernt methods and techniques were studied and applied in the process of investigation. It is evident that the number of the fertility restorer lines is significantly predominat; this is due to the improved methodology for their development. Furthermore, almost all species from *Helianthus* genus and the species form other genera of *Compositae* family involved in the hybridization are carriers of Rf genes. For developing of herbicide-resistant sunflower lines, six different origins are used at DAI – two from USA, one from Serbia, one from BASF and two own. We are annually working with about 1000 items, including primarily R lines and a smaller number of B lines and their sterile analogs. About 80 % of these materials are imi-tolerant, and 20 % are Express-tolerant. The work on herbicide-resistant materials at DAI started later in comparison to other breeding centers and therefore certain lagging behind is observed in this direction. However work is gong on at accelerated rates and the volume of hybrids is increasing annually, already giving very good results. The main method applied here is the experimental mutagenesis for development of B lines (Christov and Nikolova, 1996). The greater part of the materials were obtained after treatment of seeds, kernels and germs with gamma rays, ultrasonic and EMS. The fertility restorer lines were obtained mainly by selfing of hybrids and selection. All developed and stabilized sunflower lines are tested for their combining ability according to a preliminary approved scheme. The testers used are B lines, R lines or R lines with Falax cytoplasm. Thousands of lines are annually evaluated at DAI, and over 1400 hybrid combinations are tested in Bulgaria and abroad. Table 3 presents the amount of experimental hybrids tested during the past 4 years in Bulgaria and in other countries.

The most important stage from the testing process of the experimental hybrids is the unified varietal trial (UVT), where the best breeding of the Sunflower breeding department is put together (Table 4). The collected information allows determining the yield structure under changeable environment and developing appropriate technological solutions for growing (Baychev and Mihova, 2014). After their testing in this type of trial, the best hybrids are subjected to official testing and registration in Bulgaria, or are provided to foreign partners, who organize their official testing in the respective countries and eventually enlist them in their catalogs before initiating their seed production and distribution.

Table 3. Volume of experimental hybrids tested in the last 4 years

Stages of testing	Number of hybrids
1. Preliminary varietal trial	1360
2. Competitive varietal trial	4080
3. Unified varietal trial	136
4. Joint hybrids with foreign participation tested at DAI	120
5. Foreign hybrids tested at DAI	110
6. Hybrids under official testing in Bulgaria and abroad	26
7. Bulgarian hybrids tested abroad	90

Table 4. Characteristics of some experimental hybrids under UVT testing in the last 4 years, which exceeded the mean standard with more than 5%.

Hybrid	*Yield kg/ha*	*% from the mean standard*	*Oil %*	*Resistance to downy mildew and Orobanche*
		2013		
3607A x 31R	*4113*	*110.2*	*44.1*	*100+100*
3607A x 240R	*4036*	*108.2*	*44.6*	*100+100*
217A x 242R	*3971*	*106.4*	*46.7*	*100+100*
217A x 12R	*3999*	*107.2*	*48.2*	*100+100*
3607A x 253R	*4275*	*114.6*	*48.2*	*100+100*
Mean standard	*3732*	*100.0*	*47.2*	*100+100*
		2014		
217A x 150R	*4336*	*105.5*	*46.8*	*100+100*
217A x 169R	*4348*	*105.8*	*45.8*	*100+100*
T6A x C60	*4367*	*106.3*	*47.0*	*100+100*
Mean standard	*4110*	*100.0*	*44.5*	*100+100*
		2015		
217A x 10594R	*3724*	*106.4*	*49.2*	*100+100*
217A x 10595R	*3724*	*106.4*	*48.0*	*100+100*
3607A x 10595R	*3764*	*107.6*	*44.4*	*100+100*
T6A x BTI1/1R	*4047*	*115.7*	*48.4*	*100+100*
A5 x 66R	*3882*	*110.9*	*46.1*	*100+100*
Mean standard	*3499*	*100.0*	*45.0*	*100+100*
3607A x 10596R	*4142*	*110.5*	*43.5*	*100+100*
3607A x 509PR	*4008*	*106.9*	*45.1*	*100+100*
846A x 10681R	*3969*	*105.9*	*46.5*	*100+100*
217A x KM85-2	*4236*	*113.0*	*46.0*	*100+100*
Mean standard	*3748*	*100.0*	*44.2*	*100+100*
		2016		
217A x 99R	*4486*	*105.9*	*51.0*	*100+100*
712A x 1216R	*4509*	*106.4*	*50.8*	*100+100*
217A x 278R	*4513*	*106.5*	*52.3*	*100+100*
4645A x 417 x 4151R	*4515*	*106.6*	*49.2*	*100+100*
Mean standard	*4237*	*100.0*	*46.5*	*100+100*
3607A x 99R	*4635*	*117.6*	*45.9*	*100+100*
2017A x PR427	*4184*	*106.1*	*47.8*	*100+100*
813A x 4204R	*4226*	*107.2*	*46.9*	*100+100*
813A x HA335 x 99-392R	*4327*	*109.8*	*44.8*	*100+100*
Mean standard	*3942*	*100.0*	*46.8*	*100+100*

Many of the hybrid combinations from Table 3 are already given names and are now within the systems of official testing in Bulgaria and abroad. Some of them are already registered in official catalogs, while others are currently undergoing the obilatory field tests. Table 5 shows the most recent sunflower hybrids of DAI – General Toshevo developed during the last 10 years, which have been released in the respective country or are currently under official testing.

Table 5. Sunflower hybrids registered in the respective country or under process of official testing

Hybrid	Bulgaria	Romania	Ukraine	Moldova	Russia	Belarus	Serbia	Kazachstan
Alpin		Registered	++	Registered	++	Registered		Registered
Veleka		Registered	Registered	Registered	++		++	
Vokil		Registered	Registered	Registered	++		Registered	
Mihaela		Registered	++		+			
Velko		Registered	Registered		Registered		Registered	
Gabi		Registered	Registered		+			
Divna			Registered		++			
Deya		Registered						
Sevar		Registered	Registered		+			
Yana	Registered			+	++		++	Registered
Valin	Registered		Registered					
Maritsa	Registered		Registered	Registered				
Kameliya					+			
Enigma imi	++							
Dessi imi	+							
Danaya imi	++							
Tedi	Registered							
Deveda	Registered							
Linzi	Registered			+				
Vessi			++					
Sunny imi		++	++					
Giga imi		++						
Vyara				Registered				
Kaliya imi	+			+	+			
Pavlina				+				
Rumyana				+				
Yanitsa				+				
Viliya	+							
Viyani imi	++							
St. George					+			
Rada	Registered				++			
Zhanina					+			
Lyubov					+			
Baikal					+			

+ First year of official testing ++ Second year of official testing

In last few years, the interest of seed production companies from Ukraine and Russia increased considerably; they are intensively testing our materials with the aim to register and promote them. Our comparatively old hybrid San Luka is still successfully grown in Russia; it was one of the first *Orobanche*-resistant hybrids develoiped in collaboration with the company Syngenta. Hybrid Maritsa is recently also being distributed in Russia. The cooperation of DAI with other research centers of sunflower breeding is ongoing. We are constantly exchaning materials which are involved in different breeding programs.

Conclusions

A rich and variable collection of initial breeding material has been built up as a result from the use of different breeding methods. Methods for evaluation of the resistance to the economically important diseases for Bulgaria and the parasite *Orobanche* have been developed and adapted to the working conditions of DAI. New high-yielding sunflower hybrids resistant to biotic and abiotic stress factors were developed. Morphological, biological, technological, bio chemical and phyto pathological evaluation of the released and registered hybrids and their parental forms were made. The hybrids realized their high production potential not only in Bulgaria, but also in other countries with traditionally large-scale sunflower production and specific soil and climatic conditions. Many of the new hybrids were registered in Bulgaria and abroad, while others are currently involved in official testing in different countries. The joint projects of DAI with other research centers and companies dealing with breeding and production of sunflower are constantly increasing.

References

1. Gotsov, К., A. Karaivanov, F. Tsvetkova, St. Tsvetkov, V. Velkov, P. Radkov, (1981). Achievements and problems of the breeding at IWS – General Toshevo. Section „Sunflower“. Scientific conference on the problems of breeding. NAPS, Sofia, 32-36 (In Bg).
2. Ivanov, P., V. Velkov, P. Petrov, I. Georgiev, P. Shindrova, F. Tsvetkova, (1988). Directions of contemporary breeding work on sunflower. Agricultural science. XXVI, No 1, 40-50 (In Bg)
3. Panchenko, A. Y.,(1975). Newsletter of agricultural sciences, No 2 (In Ru).
4. Petrov P., F. Tsvetkova, V. Velkov, P. Ivanov, A. Piskov, M. Christov, P. Shindrova, D. Petakov, N. Nenov, V. Encheva (1994). Current status and problems of sunflower breeding in Bulgaria. Plant breeding sciences, No 3-4, 72-76 (In Bg).
5. Rushkovskiy, S. V., (1957). Research methods in the breeding of oil seed crops for oil content and quality. Pishtepromizdat. (In Uk).
6. Stoyanova. Y., P. Ivanov, (1968). Investigations on the preparation of sunflower seeds for laboratory analyses to determine their oil percent. Plant breeding sciences, 5, No 4, 49 – 57 (In Bg).
7. Stoyanova, Y., B. Simeonov, G. Sabev, D. Petrov, I. Georgiev, I. Dimitrov, Y. Georgieva-Todorova, L. Rangelov, M. Petrova, P. Ivanov, P. Palazov, H. Kontev, (1977). Sunflower in Bulgaria. BAS (in Bg).
8. Hristov, M., (2002). Results from the use of distant hybridization and experimental mutagenesis in the improvement work on sunfllower. Jubilee session on the 50th anniversary of DAI, vol. I, 315 – 335 (In Bg).
9. Baychev V. and G. Mihova, (2014). Variations in the production potential of barley and triticale under contrasting conditions of the environment.. Scientific Works of the Institute of Agriculture – Karnobat , vol. 3, № 1, 107-120.
10. Christov M., V. Nikolova, (1996). Increasing of the Sunflower Genetic Diversity by Mutagenesis. In: Proceedings of 14th International Sunflower Conference, Beijing/Shenyang, China, pp. 19-30.
11. Christov M., P. Shindrova, V. Entcheva, (1996). Transfer of new characters from wild *Helianthus* species to cultivated sunflower. Genet. a Slecht., 32, (4): 275- 286.Christov, M., I. Kiryakov, P.
12. Shindrova, and V. Encheva, M. Christova, (2004). Evaluation of new interspecific and intergeneric sunflowerhybrids for resistance to Sclerotinia sclerotiorum. In: Proceedings of 16th International Sunflower Conference, Fargo, North Dakota, USA, Vol. II, p. 693-698.
13. Christov, M., Al. Piskov, J. Encheva, D. Valkova, M. Drumeva, N. Nenova, V. Nikolova, V. Encheva, P. Shindrova, P. Petrov, G. Georgiev, (2009). Developing sunflower hybrid cultivars with increased productive potential, resistant to economic important for the country diseases and parasite broomrape using classical and biotechnological methods. Proceedings of International Scientific Conference, Zaporozhie, 74-87.
14. Dimitrova-Doneva M., D. Valcheva, G. Mihova, B. Dyulgerova, (2016). Genotype-environment interaction and stability analysis for grain yield of winter barley in the conditions of North-East and South Bulgaria. Agricultural Science and Technology, vol. 8, № 1, 19-23.
15. Encheva, J. and P. Ivanov, (1997). Sunflower genotype reaction to direct and indirect organogenesis and somatic embryogenesis using three media and gamma ray treatment. Helia, 20, Nr 27, 135-142.
16. Encheva, V. and I. Kiryakov, (2002). Method for evaluation of sunflower resistance for *Diaporthe (Phomopsis) helianthi* Munt. Cnet. Et al. Bulgarian Journal of Agricultural Science 8:219-222.

17. Enns H., D. G. Dorrel, J. A. Hoes, and W. O. Chubb, (1970). Sunflower research, a progrees report, p. 162-167. In: Proc. 4 th Inter. Sunflower Conf., Memphis, Tennessee.
18. Ivanov, P., M. Christov, I. Ivanova and V. Nikolova, (1996). Stady of seed oil and protrin quality of some *Bidens tripartita* accessions. Helia, 19, N 25, pp. 79-85.
19. Kinman M. L., (1970). New development in the USDA and State experiment stations sunflower breeding programs. Proc. Of the 4th Int. Sunfl. Conf. Memphis, 181-184.
20. Leclercq P., (1969). Une sterile male cytoplasmatique du tournesol. Ann. Amelior Plantes, 19 (2), 99-106.
21. Leclercq P., 1971. La sterilite male citoplasmatiaque du tournesol. I. Premieres etudes sur la restauration de la fertilite. Ann. Amelior Plantes, 21, 45-54.
22. Marić, A., S. Maširević, and F. El Sayed, (1981). Pojava *Leptosphaeria lindquisti* Frezzi, savršenog stadija gljive *Phoma macdonaldii* Boereme prouzrokovača crne pegavosti suncokreta u Jugoslaviji. Zaštita bilja 32(4): 329-334.
23. Mihova G., D. Valcheva, D. Vulchev, M. Dimitrova-Doneva, D. Dimova, (2015). Response of Bulgarian winter barley varieties to different types of stress. 2nd International Symposium for Agriculture and Food, 07-09 October 2015, Ohrid, Republic of Macedonia, 977-984
24. Newport Instrument (1972). Use of the Newport quantity analyzed as a replacement for solven extraction for measuring the oil, fat content of oil seeds, chocolate, meat and other material. Newport Pagnell, England.
25. UPOV, (2002). Protocol for distinctness uniformity and stability tests (Helianthus annuus L.) European Union Community plant variety office, 10-28.
26. Vear, F. and D. Tourvielle, (1987). Test the resistance au Mildiou chez le tournesol. CETIOM. Information techniques, vol.98,p.p.19-20.
27. Velkov V., Y. Stoyanova, (1974). Biological peculiarities of cytoplasmic male sterility and schemes of its use. Proceedings of 6th International Sunflower Conference Bucharest, 361-365.
28. Vranceanu V., F. Stoenescu, (1971). Pollen fertility restorer gene from cultivated sunflower. Euphytica, vol. 20, 536-541.

THE IMPACT OF INSERTION ON SOME TECHNOLOGICAL CHARACTERS OF BASMAK TOBACCO VARIETIES IN THE REPUBLIC OF MACEDONIA

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Abstract
Technological characters determine the so-called technological - commercial quality of tobacco and tobacco products during fabrication. Three-year trials were performed with variety YK 7-4/2 (check) and three Basmak varieties - MK-1, MB-2 and MB-3. The trial was designed in randomized blocks with five replications. The aim of investigation was to determine water retention capacity, density, filling capacity and fractional composition of tobacco by insertions. WRC was determined by the method of (Boceski, 2003), filling capacity (cm^3/g) and density (g/cm^3) were determined using the Borgwaldt densimeter by the method of Shuto (1972) and fractional composition by the method of Dorahova and Dikker. During the three years of investigation, WRC ranged from 16.87% in the lower leaf of variety MB-2 to 23.70% in the middle leaf of MB-3, which proves that the investigated varieties of Basmak tobacco have higher WRC compared to the check. The lowest density by insertions and years and the highest filling capacity was recorded in the middle leaf of MB-2 in 2009 (0.195 g/cm^3). In the three years of investigation, the lowest average density was recorded in lower leaves of the variety MB-3 (0.213 g/cm^3) and the highest in top leaves of the check variety YK 7-4/2 (0.303 g/cm^3). The filling capacity was the highest (5.13 cm^3/g) in middle leaves of the variety MB-2 and the lowest (2.91 cm^3/g) in undertop leaves of the variety MB-3 in 2009. In average, the lowest positive fraction was measured in lower leaves of the variety MB-2 (78.99%) and the highest in middle leaves of the variety MB-3 (93.23%), while the negative fraction ranges from 10.16% in MB-3 to 21.11% in the check variety JK 7-4/2. The data obtained show that the characteristics of the investigated Basmak varieties are typical for the oriental tobacco.

Keywords: density, filling capacity, water retention capacity, faction.

Introduction
The structure of tobacco production in Macedonia consists of oriental tobacco types Prilep, Yaka and Djebel, presented with several varieties which account for about 95% of the total tobacco production. In recent years, new tobacco varieties of the type Basmak were included in the production. The yield and quality of tobacco raw material obtained from Basmak varieties meet the criteria and quality standards of both manufacturers and tobacco purchase companies.Tobacco is a very important industrial crop in our country, especially in areas where conditions for production of other crops are difficult. Of the total area planted with industrial crops, 82% belong to tobacco, most of it being grown in the southwestern and southeastern regions. Because of its good quality, Macedonian tobacco is highly appreciated in the international market and, along with other tobacco products, it is important export product. Republic of Macedonia accounts for 14% of the total oriental tobacco production in the Balkans in the period 2005-2011, with the types Yaka, Prilep, Djebel and Basmak. According to (Boceski, 2003), technological characters are divided into morphological, physical and organoleptic. Technological characters are determined in a laboratory, after curing of tobacco. This paper refers to some technological (physical) characters of tobacco raw,

presented by insertions: water retention capacity, positive and negative fraction, density and filling capacity. Physical characters of tobacco are particularly important in manufacture of final products.

Material and methods

Three-year investigations were carried out with four tobacco varieties YK 7 - 4/2 (ø) and Basmak varieties MK-1, MB-2 and MB-3. Seedling was produced in the Scientific Tobacco Institute - Prilep in traditional way, in seedbeds covered with polyethylene. Investigations were made in randomized blocks with five replications, at 45 × 12 cm spacing, on previously treated soil. Tobacco leaves were harvested manually at 7 primings in the stage of technical maturity and then sun cured on horizontal frames. Technological characters of fermented tobacco (water retention capacity, density, filling capacity and fractional composition were investigated in Tobacco Company - Prilep, LTD Cigaretttes. Water retention capacity was tested on tobacco powder by the method of (Boceski, 1984). Filling capacity was expressed in cm^3/g and tobacco density (g/cm^3) was determined using Borgwaldt densimeter. Fractional composition was analysed by the method of Dorahova and Dikker, using vibrator with sieve apertures of 5.0 mm, 3.14 mm, 2.0 mm, 1.0 mm and 0.5 mm and horizontal vibration of 3 minutes. Fractions of the test material from each sieve were measured on analytical scale and the share of positive and negative fractions was expressed in percentage. The humidity of cut tobacco ranged from 13 to 15% and the cutting width was 0.7-0.8 mm.

Results and discussion

Water retention capacity

Water retention capacity (WRC) is one of the most important technological properties which affect the condition of tobacco during its treatment and processing. The ability of tobacco to receive and retain the moisture at certain temperature and relative humidity has an influence on many technological properties of tobacco, such as fractional composition, filling capacity and the ability of tobacco to withstand a pneumatic transport.

Table 1. Water retention capacity by leaf position, in%

Variety	Year	Insertions							Average
		Lower primings	Upper primings	Lower middle leaf	True middle leaf	Upper middle leaf	Under top leaf	Top leaf	
JK 7-4/2 Ø	2009	15.81	17.62	18.79	18.99	19.32	21.57	19.72	18.48
	2010	17.63	19.47	20.24	21.47	18.73	21.36	20.59	17.24
	2011	21.60	22.59	20.48	20.68	20.64	20.38	22.31	21.24
	Average	18.35	19.89	19.84	20.38	19.56	21.10	20.87	18.99
	Index	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
MK-1	2009	14.28	17.45	17.76	19.02	18.61	20.32	20.07	14.81
	2010	17.04	18.62	19.00	20.37	20.83	21.56	21.77	18.30
	2011	19.96	20.87	20.07	26.51	24.37	24.20	21.64	22.52
	Average	17.09	18.98	18.94	21.97	21.27	22.03	21.16	18.54
	Index	93.13	95.42	95.46	107.80	108.74	104.41	101.39	97.63
MB-2	2009	13.65	18.69	18.27	18.85	18.75	19.53	21.21	18.39
	2010	17.22	19.15	18.54	19.79	21.10	22.09	20.66	18.80
	2011	19.73	22.69	21.56	22.63	20.61	20.45	23.39	21.58
	Average	16.87	20.18	19.46	20.42	20.15	20.69	21.75	19.59
	Index	91.93	101.46	98.08	100.20	103.02	98.06	104.20	103.16
MB-3	2009	16.56	20.80	20.44	19.76	22.32	22.33	21.00	17.50
	2010	16.44	17.78	17.80	20.17	22.57	22.60	23.44	16.67
	2011	19.73	23.02	23.42	23.65	26.20	22.66	21.78	22.92
	Average	17.58	20.53	20.55	21.19	23.70	22.53	22.07	19.03
	Index	95.80	103.22	103.58	103.97	121.17	106.78	105.73	100.21

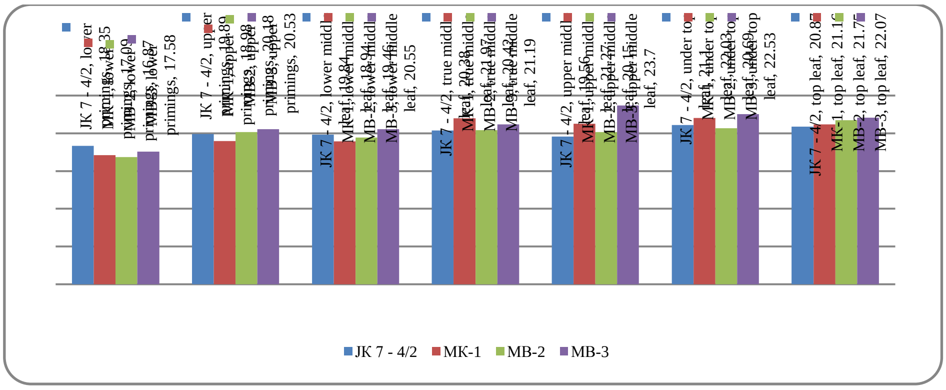

Figure 1. Water retention capacity of tobacco leaves by leaf position (in %)

The ability of tobacco to receive and retain moisture differs from variety to variety. Due to this property, different tobaccos have different percentage of humidity. Water retention capacity depends on chemical composition (pectic substances) and leaf structure. The results on water retention capacity of the varieties tested (Table 1 and Figure 1) range from 13.65% in lower primings of the the variety MB-2 in 2009 to 26.20% in the upper middle leaf of the MB-3 variety in 2011.
(Patche and Gjorgjioski.1967) reported that carbohydrates, organic acids and their salts, amino acids etc. have a positive impact on water retention capacity of tobacco. (Nuneski, 1975) points out that water retention capacity of Prilep tobacco from the region of Krusevo amounts to 19.78% in lower middle leaf, 21.3% in true middle leaf and 20.14% in upper middle leaf. According to (Nuneski, 2008) and his studies on Izmir Basma tobacco from Turkey, the highest WRC was recorded in the under top leaves (29.51%) and top leaves (29.95%). The average water retention capacity in our investigations, however, ranged from 16.87% in MB-2 variety to 23.70% in the upper middle leaf of MB-3 variety, which is 6.78% higher than the check. Varieties MK-1, MB-2 and MB-3 showed higher WRC compared to the check. Higher water retention capacity compared to the other varieties was recorded in variety MB-3, ranging from 17.58% in the lower primings, 20.53% in the upper primings, 20.55% in lower middle leaf, 21.19% in true middle leaf, 23.70% in the upper middle leaf, 22.53% in under top leaf and 7.22% in 22:53 the top leaf.

Density

In manufacture of tobacco, the weight and volume of cut tobacco are very important. They largely determine the use value and the commercial value of the raw material. Tobacco density was measured by leaf positions and years at 13% absolute humidity Table 2. The lowest density (0.195 g/cm^3) and the highest water retention capacity was recorded in the true middle leaf of the variety MB-2 in 2009. The highest density (0.344 g/cm3) and the lowest water retention capacity was recorded in the under top leaves of the variety MB-3 in 2009. During the three years of investigation, the lowest density was observed in the lower primings of MB-3 (0.213 g/cm^3) and the top leaves of the check variety YK 7-4/2 had the highest density (0.303 g/cm^3). With regard to leaf position, there are no significant differences in density and water retention capacity among the varieties tested. According to (Boceski, 2003), density is a bulk weight of cut tobacco, expressed in g/l or g/cm^3. The reciprocal value of density is filling capacity, expressed in cm^3/g. Tobacco with lower density has higher water retention capacity and in fabrication it gives higher number of cigarettes from 1 kg of tobacco, i.e. it has higher economic value compared to tobacco with higher density.

Table 2. Tobacco density by leaf position, in g/cm^3

Variety	Year	Insertions							Average
		Lower primings	Upper primings	Lower middle leaf	True middle leaf	Upper middle leaf	Under top leaf	Top leaf	
JK 7-4/2 Ø	2009	0.211	0.231	0.207	0.213	0.239	0.307	0.318	0.247
	2010	0.227	0.249	0.246	0.308	0.299	0.300	0.284	0.273
	2011	0.259	0.235	0.255	0.274	0.296	0.258	0.308	0.269
	Average	0.232	0.238	0.236	0.265	0.278	0.288	0.303	0.263
	Index	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
MK-1	2009	0.212	0.225	0.213	0.202	0.252	0.292	0.308	0.243
	2010	0.247	0.261	0.274	0.270	0.327	0.308	0.296	0.283
	2011	0.197	0.228	0.240	0.272	0.291	0.299	0.311	0.262
	Average	0.219	0.238	0.242	0.248	0.290	0.300	0.305	0.263
	Index	94.12	100.00	102.54	93.58	104.32	104.17	100.66	100.01
MB-2	2009	0.205	0.231	0.214	0.195	0.250	0.308	0.287	0.241
	2010	0.241	0.279	0.310	0.300	0.335	0.312	0.299	0.297
	2011	0.231	0.283	0.282	0.282	0.312	0.297	0.266	0.279
	Average	0.226	0.264	0.269	0.259	0.299	0.306	0.284	0.272
	Index	97.41	110.92	113.98	97.74	107.55	106.25	93.73	103.42
MB-3	2009	0.201	0.250	0.261	0.258	0.283	0.344	0.329	0.275
	2010	0.232	0.236	0.281	0.267	0.251	0.277	0.252	0.257
	2011	0.206	0.247	0.255	0.269	0.301	0.298	0.287	0.266
	Average	0.213	0.244	0.266	0.265	0.278	0.306	0.289	0.266
	Index	91.81	102.52	112.71	100.00	100.00	105.21	95.38	101.14

The values for density by belts and years are presented in Table 2. The lowest density was recorded in the lower belt of MB-2 in 2009 (0.218 g/cm^3) and the highest density in the upper belt of MB-3 2009 (0.337 g/cm^3). According to the average values, the lowest density was recorded in the lower belt of MK-1 (0.228 g/cm^3) and the highest in the upper belt of the same variety (0.302 g/cm^3), which is 2.03% more than the check variety. It can be stated that the tested varieties are characterized by density increase from the lower to the upper belt.

Water retention capacity

Water retention capacity is reciprocal value of density and is expressed in cm^3/g tobacco. Actually, it is the volume in cm^3 which is occupied by 1 g of tobacco under certain test conditions. The data on water retention capacity of tobacco (in cm^3/g) by leaf position are presented in Table 3 and Figure 3. The lowest water retention capacity was measured in the under top leaves of MB-3 in 2009 (2.91 cm^3/g) and the highest water retention capacity was found in MB-2 in 2009 (5.13 cm^3/g). The average filling capacity during the three-year investigations ranged from 3.30 cm^3/g in top leaves of the check variety to 4.71 cm^3/g in variety MB-3, which is 8.78% higher compared to the check. The obtained data show that filling capacity in the four varieties tested, with some variations, gradually decreases from the lower to the upper primings.

Fractional composition

After cutting, tobacco material consists of fractions with different dimensions. Fractional composition of cut tobacco has a major impact on the filling capacity of tobacco blends in cigarette production and efficiency. The fraction coefficient is the ratio between positive and negative fraction. Positive faction consists of tobacco particles that remain on a screen mesh of over 2 mm and negative faction consists of tobacco particles below 2 mm. The lowest percentage of positive fraction in the varieties tested (Table 4, Figure 4) was recorded in the lower primings of MK-1 in 2010 (72.58%) and the highest in the true middle leaf of MB-3 in 2009 (96.20%). During the three-year investigations, climate conditions had a big impact on the share of positive fraction of cut tobacco. In dry conditions of 2011, the share of positive fraction was smaller. On average, the lowest positive fraction was recorded in lower primings of the variety MB-2 (78.99%) and the highest in the upper middle leaf of MB-3 (93.23%), which is 1.44% higher compared to the check variety. The results show that raw material of the varieties MK-1 and MB-3 has a good fractional composition, which exceeds 85%.

Table 3. Filling capacity of the cut tobacco by leaf position, in cm^3/g

Variety	Year	Insertions							Average
		Lower primings	Upper primings	Lower middle leaf	True middle leaf	Upper middle leaf	Under top leaf	Top leaf	
JK 7-4/2 Ø	2009	4.74	4.33	4.83	4.69	4.18	3.26	3.14	4.17
	2010	4.39	4.02	4.06	3.25	3.34	3.33	3.52	3.70
	2011	3.86	4.25	3.92	3.65	3.38	3.87	3.25	3.74
	Average	4.33	4.20	4.27	3.86	3.63	3.49	3.30	3.87
	Index	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
MK-1	2009	4.71	4.48	4.69	4.95	3.97	3.42	3.24	4.21
	2010	4.05	3.83	3.65	3.70	3.06	3.25	3.38	3.56
	2011	5.12	4.38	4.17	3.68	3.44	3.34	3.21	3.91
	Average	4.63	4.23	4.17	4.11	3.49	3.34	3.28	3.89
	Index	106.93	100.71	97.66	106.48	96.14	95.70	99.39	100.43
MB-2	2009	4.88	4.33	4.67	5.13	4.00	3.25	3.48	4.25
	2010	4.15	3.58	3.23	3.33	2.98	3.20	3.34	3.40
	2011	4.13	3.54	3.55	3.55	3.21	3.37	3.76	3.62
	Average	4.45	3.82	3.82	4.00	3.40	3.27	3.53	3.76
	Index	102.77	90.95	89.46	103.63	93.66	93.70	106.97	97.16
MB-3	2009	4.97	4.00	3.83	3.88	3.53	2.91	3.04	3.74
	2010	4.31	4.24	3.56	3.75	3.98	3.61	3.97	3.92
	2011	4.85	4.05	3.92	3.72	3.32	3.35	3.48	3.81
	Average	4.71	4.10	3.77	3.78	3.61	3.29	3.50	3.82
	Index	108.78	97.62	88.29	97.93	99.45	94.27	103.03	98.71

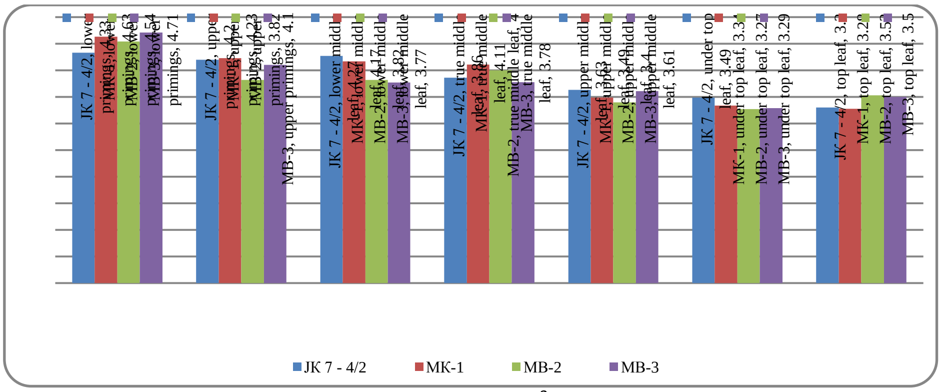

Figure 3. Filling capacity of cut tobacco by leaf position, in cm^3/g (average)

(Najdoski, 1980) in his four-year investigations of Prilep tobacco reported that cut tobacco from the middle belt has the highest percentage of positive faction, with 85.80 % in 1976 and 78.80% in 1973. In other harvests this percentage varies depending on the climate conditions.

Negative faction by varieties and leaf position, presented in Table 5, showed that the lowest percentage (3.80%) was recorded in the true middle leaf of MB-3 in 2009 and the highest (27.42%) in MK-1 in 2010. On average, the negative fraction has the lowest percentage in the upper middle leaf of MB-3 (6.77%) and the highest percentage in the lower primings of MB-2 (21.01%), which is

24.39% higher than the check variety. The average negative fraction for the seven leaf positions in the four varieties ranges from 16.10% in MB-3 to 11.21% in the check variety YK 7-4/2.

Table 4. Positive fraction in the cut tobacco by leaf position, in %

Variety	Year	Insertions							Average
		Lower primings	Upper primings	Lower middle leaf	True middle leaf	Upper middle leaf	Under top leaf	Top leaf	
JK 7-4/2 Ø	2009	86.76	89.94	92.18	93.12	94.44	90.44	91.98	91.27
	2010	83.50	90.62	91.92	93.44	92.28	90.66	89.54	90.28
	2011	79.06	79.58	86.00	88.00	89.00	88.32	84.00	84.85
	Average	83.11	86.71	90.03	91.52	91.91	89.81	88.51	88.80
	Index	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
MK-1	2009	88.12	91.12	90.70	91.54	91.36	91.78	89.78	90.63
	2010	72.58	87.18	88.34	89.86	94.63	93.02	90.52	88.02
	2011	77.60	78.34	88.00	86.40	87.20	84.10	81.42	83.29
	Average	79.43	85.55	89.01	89.27	91.06	89.63	87.24	87.31
	Index	95.58	98.66	98.87	97.54	99.08	99.80	98.57	98.32
MB-2	2009	78.76	85.76	88.22	89.42	92.50	90.44	89.16	87.75
	2010	77.74	86.38	88.32	91.72	93.02	92.16	90.58	88.56
	2011	80.46	80.86	85.90	89.00	87.70	83.34	86.54	84.83
	Average	78.99	84.33	87.48	90.05	91.07	88.65	88.76	87.05
	Index	95.04	97.26	97.17	98.39	99.09	98.71	100.28	98.03
MB-3	2009	80.80	88.96	92.80	96.20	94.78	93.30	90.70	91.08
	2010	83.50	90.62	91.92	93.44	92.28	90.66	89.54	90.28
	2011	80.04	84.06	87.68	89.66	92.62	92.54	90.54	88.16
	Average	81.45	87.88	90.80	93.10	93.23	92.17	90.26	89.84
	Index	98.00	101.35	100.85	101.73	101.44	102.63	101.98	101.17

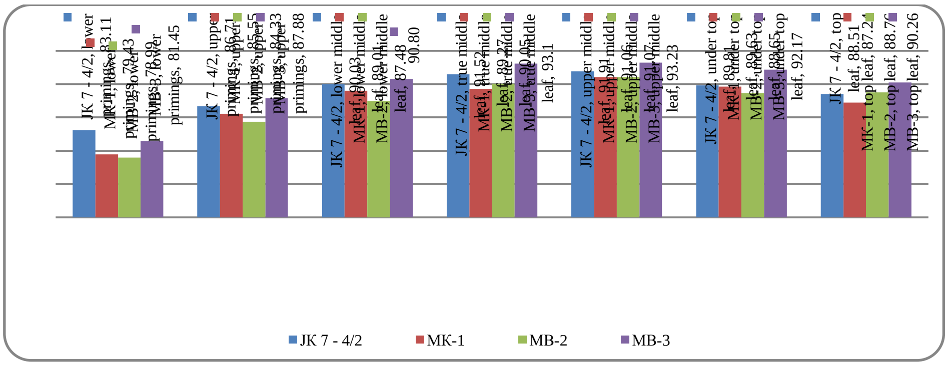

Figure 4. Positive fraction in the cut tobacco by leaf position, in % (average)

Table 5. Negative fraction in the cut tobacco by leaf position, in %

Variety	Year	Insertions							Average
		Lower primings	Upper primings	Lower middle leaf	True middle leaf	Upper middle leaf	Under top leaf	Top leaf	
JK 7-4/2 Ø	2009	13.24	10.06	7.82	6.88	5.56	9.56	8.02	8.73
	2010	16.50	9.38	8.08	6.56	7.72	9.34	10.46	9.72
	2011	20.94	20.42	14.00	12.00	11.00	11.68	16.00	15.15
	Average	16.89	13.29	9.97	8.48	8.09	10.19	11.49	11.21
	Index	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
MK-1	2009	11.88	8.88	9.30	8.46	8.64	8.22	10.22	9.37
	2010	27.42	12.82	11.66	10.14	5.37	6.98	9.48	11.98
	2011	22.40	21.66	12.00	13.60	12.80	15.90	18.58	17.68
	Average	20.57	14.45	10.99	10.73	8.94	10.37	12.76	13.01
	Index	121.79	108.73	110.23	126.53	110.51	101.77	111.05	116.06
MB-2	2009	21.24	14.24	11.78	10.58	7.50	9.56	10.84	12.25
	2010	22.26	13.62	11.68	8.28	6.98	7.84	9.42	11.44
	2011	19.54	19.14	14.10	11.00	12.30	16.66	13.46	15.17
	Average	21.01	15.67	12.52	9.95	8.93	11.35	11.24	12.95
	Index	124.39	117.91	125.58	117.33	110.38	111.38	97.82	115.52
MB-3	2009	19.20	11.04	7.20	3.80	5.22	6.70	9.30	8.92
	2010	16.50	9.38	8.08	6.56	7.72	9.34	10.46	9.72
	2011	20.94	15.94	12.32	10.34	7.38	7.46	9.46	11.74
	Average	18.55	12.12	9.20	6.90	6.77	7.83	9.74	10.16
	Index	109.83	91.20	92.28	81.37	83.68	76.84	84.77	90.63

Conclusions

Water retention capacity ranged from 16.87% in the middle primings of the variety MB-2 to 23.70% in the upper-middle leaf in MB-3, which is 6.78% higher than the check. -The lowest density was recorded in lower primings of MB-3 (0.213 g/cm^3) and the highest in the top leaf of the check variety YK 7-4/2 (0.303 g/cm^3). The average filling capacity ranged from 3.30 cm^3/g in the top leaf of the check variety to 4.71 cm^3/g in the lower primings of MB-3, which is 8.78% higher compared to the check. From the data obtained it can be concluded that the filling capacity of the varieties tested, with few exceptions, gradually decreases from lower primings to the top. Positive faction was the highest in the upper middle leaf of MB-3 (93.23%), which is 1.44% higher than the check, and the lowest in lower primings of MB-2 (78.99%). The results show that raw material of the varieties MK-1 and MB-3 has a good fractional composition, which exceeds 85%. The lowest share of negative fraction was recorded in the upper middle leaf of the variety MB-3 (6.77%) and the highest in lower primings of MB-2 (21.01%), which is 24.39% higher than the check variety.

References

1. Боцески Д. (2003). Познавање и обработка на тутунската суровина. Институт за тутун – Прилеп, II дополнително издание, 677.
2. Лазароски Т. (1976). Придонес кон запознавањето на поважните физички и хемиски карактеристики на тутунската суровина (средни берби) од типот прилеп, реон Битола. Тутун/ Tobacco, Vol 36, N ˚ 11-12, стр. 59-62, Институт за тутун-Прилеп.
3. Lazaroski T. (1984). Uticaj navodnjavanja na prinos i tehnoloska svojstva orijentalnog aromaticnog duvana sorte prilep. Poljoprivredni fakultet. Beograd-Zemun. Doktorska disertacija, pp 280.

4. Најдоски Ј. (1980). Придонес кон запознавањето на поважните физички, хемиски и дегустативни својства на суровината од типот прилеп, потекло Прилеп. Земјоделски факултет- Скопје. Докторска дисертација, pp180.
5. Нунески И. (1975). Придонес кон запознавањето на поважните физички и хемиски особини на типот ”прилеп” потекло Крушево. Земјоделски факултет – Скопје, Магистерски труд, pp. 190.
6. Нунески И. (1986). Придонес кон запознавањето на полнечката способност на тутунот во зависност од типот, потеклото, инсерцијата и некои технолошки својства. Земјоделски факултет - Скопје. Докторска дисертација. pp 240.
7. Нунески И., Нунески Р. (2009). Дегустациони својства како еден од методите за вреднување на тутунот и тутунските преработки. Тутун/Tobacco, Vol. 58, N ˚ 7 - 8, стр. 181-192. Научен институт за тутун – Прилеп.
8. Патче Л., Георгиевски К. (1987). Познавање на тутунската суровина - Стокознаење. Стопански весник. Скопје.

THE EFFECT OF COVER CROPS ON THE CONTENT OF PROTEIN IN GRAIN OF SWEET MAIZE

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Abstract
The study was conducted in the experimental field of the Maize Research Institute Zemun Polje, Serbia, during 2013/14-2014/15. The experiment was established as a block design with four replications. As winter cover crops-CC (factor A) the following plants were grown: CV–common vetch (*Vicia sativa* L.), FP-field pea (*Pisum sativum* L.), WO-winter oats, (*Avena sativa* L.), FK-fodder kale (*Brassica oleracea* (L.) convar. *acephala*), two mixture variants of legume crops with oats (CV+WO and FP+WO) and two control treatments: a variant in which the surface was covered with dead organic mulch (DOM) and traditional variant: after ploughing in the fall plot stayed uncovered during the winter (TV). Green biomass of the cover crops was incorporated in the soil, immediately after, half of the elementary plot was infested with bio-fertilizer (BF) - Uniker (mobilizer of nutrients) in an amount of 10 l ha^{-1} (factor B), which contains the strains of cellulolytic and proteolytic bacteria to support the mineralization of entered crop residues. The seeds of sweet maize 'ZPSC 421*su* (FAO 400) were sown at the arrangement of 70 cm between rows and 22 cm between plants in the row (65,000 plants per ha). Preceding crop in both years was winter wheat. The kernel protein content was measured on infrared analyser. The data were processed by ANOVA. The investigated factors (CC and BF) showed significant effect on protein content in sweet maize kernel in both years. As it was expected, the greatest impact on protein content was exhibited in leguminous species grown alone, or in mixtures with oats, particularly in the dry, 2015. Small grains intercropped with legumes obtained higher values of protein content than small grain grown as monocrops.

Keywords: sweet maize, content of protein, microbiological fertilizer.

Introduction

Over the last decade, substantial effort has been devoted to implementing alternative practices in agriculture (conservation and minimal tillage, permanent soil cover and diverse crop rotation) under sustainable and organic production because a combination of both strategies could have synergistic effects and further improve soil quality. From this point of view it is important to examine relations between applied cropping practices and nutritional value of produced crops. The overall strategies include growing of healthy plants with good defense capabilities, stressing pests and enhancing populations of beneficial organisms (Magdoff, 2007). From that point, sustainable agriculture includes combination of different crops at the same field and application of organic and microbiological fertilizers, enabling better utilization of space and nutrients, with lower inputs. On the other hand, organic farming means production of high quality food or raw material with maintaining and improving the soil quality. It is based on controlled input of allowed agrochemicals that are mainly natural products, like plant extracts, fertilizers originating from decomposed organic products or manure from organic cattle production (Dragičević et al. 2013). The application of reduced or no tillage practices in sustainability or organic production often increases problems related to weed control and crop nutrition (Armengot et al. 2015). One way to tackle these issues is the inclusion of cover crops in the crop rotation. Cover crops are implemented between two main crops and are known to provide various ecological services in agro-ecosystems, such as protection against soil erosion (Hartwig and Ammon 2002), reduction of nutrient losses (Janošević et al. 2017), improvement of soil and water quality, and to some extent (Dabney et al. 2001), the reduction of weeds and pests (Dorn et al. 2015). In adition, adding nitrogen (N) fixing legume species as a cover crop can improve N nutrition of the succeeding main crop and increase the soil N organic pool (Thorup-Kristensen et al. 2003). Despite these advantages, cover crops are generally not widely used by farmers, mainly due to additional costs and labour requirements. Cover crop effects on productivity, crop nutrition, soil erosion or weed control are variable and depend on cover crop species, soil type, and meteorological conditions. A very large number of plants have been used for cover-crops (Oljača and Dolijanović 2013). The selection of compatible crops depends on their growth habit, land, light, water and fertilizer utilization (Thayamini and Brintha, 2010). These may be divided into two groups, viz., the legumes, or nitrogen-gatherers, and the non-legumes, or those which are sometimes distinguished as nitrogen-consumers. The kind of crop to plant must be determined by the local conditions and the local needs; that is, whether a grass, cereal, legume, or cruciferous plant shall be used, will depend on whether the habits of growth and characteristics of the plant will accomplish the purpose desired. Weather conditions, in particular air temperature during the growing season, have the greatest influence on sweet maize growth, yield and yield quality (Stone et al. 1999). The chemical composition of the grain of the main crop depends to weediness. Cover crops contribute to the reduction of number and fresh weight of weeds in different ways. Incorporation of cover-crop plant material as a green manure is done at the expense of harvestable grain or seed yield, but maximizes the input to the soil of both slowly degraded organic N and secondary chemicals that may affect soil microbiological community structure. Many secondary metabolites of plants are inhibitory to a wide range of pathogenic fungi such as *Fusarium culmorum* and *Rhizoctonia solani* (Lovett, 1991). The concentrations of these metabolites are generally highest in plants at the flowering stage (Chen et al. 2012), and the incorporation of green manure at this stage offers the further advantage that it is unlikely to interfere with the sowing time of the next crop. Bio-fertilizers have an important role in keeping high soil fertility and crop yields increasing (Janošević et al. 2017). The positive impact of microbiological fertilizers is also observed in regards to quality of plant products, as for example increase in lycopene and vitamin C accumulation in sweet maize (Rosa, 2015) and higher glutathione content in maize grain (Dragicevic et al. 2013). Data obtained in this study from field experiments provides valuable knowledge regarding the (i) influences of different type of cover crops and (ii) the applied form of fertilizers (i.e. microbiological) on content of protein in grain of sweet maize.

Material and methods

A field experiment was carried out in 2013/14–2014/15 growing seasons, at the Experimental Field of Maize Research Institute in Zemun Polje near Belgrade (44°52'N; 20°20'E). The soil was slightly calcareous chernozem with 47% of clay and silt and 53% of sand. The soil at 0-30-cm layer were contained 3.22% of organic matter, 0.19% of total N, 1.9% of organic C, 16.2 and 22.4 mg per 100 g soil of available P and extractable K, respectively, 1.38% of total $CaCO_3$ and had pH 7.3. The experiment was established as a block design with four replications. As winter cover crops (factor A) the following plants were grown: CV–common vetch (*Vicia sativa* L.), FP-field pea (*Pisum sativum* L.), WO-winter oats, (*Avena sativa* L.), FK-fodder kale (*Brassica oleracea* (L.) *convar. acephala*), two mixture variants of legume crops with oats (CV+WO and FP+WO) and two control treatments: a variant in which the surface was covered with dead organic mulch (DOM) and traditional variant: after ploughing in the fall plot stayed uncovered during the winter (TV). The cover crops (CC) were sown in the amount: common vetch – 120 kg, field pea – 150 kg, oat – 160 kg, and fodder kale 15 kg per ha, and in mixture relation between legume and oats was 70:30. The plot size was 17.5 m^2. The seeds of the Institute for Forage Crops Institute of Field and Vegetable Crops in Novi Sad was used for planting in both years. The seeds of sweet maize ‘ZPSC 421*su* (FAO 400) were sown at the arrangement of 70 cm between rows and 22 cm between plants in the row (65,000 plants per ha).

Preceding crop in both years was winter wheat. The autumn soil preparation (ploughing and seedbed preparation) was performed immediately before sowing, when also soil samples were taken for available N analysis at depths of 0-20 cm and 20-40 cm. Further soil sampling from all CC and control treatments was done in the spring, after CC harvest, as well as after sweet maize harvesting. Before the sowing of CC (autumn) and sweet maize (spring) mineral fertilization was applied in order to obtain 120 kg ha^{-1} N, 90 kg ha^{-1} P and 60 kg ha^{-1} K. The total amount of P and K fertilizer was applied in autumn with mono-potassium phosphate fertilizer (a.m. 0:52:34) and the required N amount was incorporated together with sweet maize sowing (urea 46% a.m). Nitrogen fertilization followed: for non-legume crops and control treatments it was 120, for sole legume it was 80 and for mixture it was 90 kg ha^{-1} N. The remaining 40 or 30 kg ha^{-1} N was considered to be provided by nitrogen fixation. Green biomass of the cover crops was incorporated in the soil, immediately after, half of the elementary plot was infested with bio-fertilizer (BF) - Uniker (mobilizer of nutrients) in an amount of 10 l ha^{-1} (factor B), which contains the strains of cellulolytic and proteolytic bacteria to support the mineralization of entered crop residues. The ears were harvested at the stage of milk maturity of kernels. The schedule of the main works on the experiment is shown in Table 1.

Table 1. Chronology of field operations and length of vegetation period of sweet maize

Cover crops sowing	October, 30	November, 13
	2014	2015
Cover crops sampling	April, 23	May, 12
Cover crops and microbiological fertilizer incorporated	May, 12	May, 21
Sweet maize sowing	May, 20	May, 21
Hand weeding 1	June, 27	June, 22
Hand weeding 2	July, 17	July, 15
Sweet maize harvest	August, 14	August, 21
Length of vegetation period of sweet maize (in days)	86	92

The content of protein, starch and oil in grain was determined after drying in ventilation dryer at 80 °C, on infrared analyser (Infraneo, Chopin Technologies, France). The difference between fresh and dry mass (after drying at 60 °C, 105 °C and 130 °C) referred to contents of free, bulk and chemically bound water, calculated by free energy by sorption isotherm (Sun, 2002):

$$\Delta G = -RT\ln(a_w)$$

where a_w is the relative water content achieved after drying at T (60, 105 and 130 °C), R is the gas constant (8.3145 J mol^{-1} K^{-1}) and ΔG is differential free energy. The obtained data were processed using analysis of variance for two-factorial experiments (ANOVA). Statistical analysis was performed by SPSS 15.0 (IBM Corporation, Armonk, New York, USA) for Windows Evaluation version. For the individual comparisons, the least significant difference (LSD test) was used.

Meteorological conditions

The meteorological conditions during the investigation period are presented in Table 2. The first year investigation characterized by optimal air temperatures, enough of quantity and favourable distribution of precipitation. It has not had an effect on the investigation parameter of sweet maize, expect in traditional variant of growing. One of the many reasons for of introducing alternative systems of growing is the change weather conditions in recent years, such as the case in 2015. Air temperatures were higher and the quantity of precipitation were very lower, a particularly significant lack of precipitation for crop during the critical period for water. Precisely, in drought years, cover crops showed greater efficiency, while microbiological fertilizers affected the increase in quantity but not the yield quality of the main crop.

Table 2. Average air temperatures and precipitation sums from April to September at Zemun Polje

Months	Temperature (°C)		Precipitation (mm)	
	2014	2015	2014	2015
April	13.7	12.9	84.8	19.7
May	17.4	19.1	192.5	97.8
June	21.1	22.1	71.2	31.1
July	23.2	26.4	187.4	7.2
August	22.6	25.7	41.0	56.0
September	18.0	20.2	75.6	73.6
Average/Sum	19.3	21.1	652.5	285.4

Results and discussion

Results about the effects of different cover crops and microbiological fertilizer on the content of protein in grain of sweet maize are presented in Table 3. The investigated factors (CC and BF) showed significant effect on protein content in sweet maize kernel in both years (table 3). As it was expected, the greatest impact on protein content was exhibited in leguminous species grown alone, or in mixtures with oats, particularly in the dry, 2015. Advantages of cover crops are more pronounced in drought years, which is a very important fact in today's weather, because we are witnessing the existence of climate change. Small grains intercropped with legumes obtained higher values of protein content than small grain grown as monocrops (Kadžiulienė et al. 2011). Microbiological fertilizer application in the first and second year of investigation did not increase the protein content in the sweet maize grain, except in the variants with a mixture of leguminous crops and winter oats. In previous studies on the same location (Dolijanović et al. 2012, Janošević et al. 2017) treatment with a microbiological fertilizer had a positive impact on the yield, the chlorophyll content and content of vitamin C in the grain. In investigation with cover crops in Poland, the average protein content of sweet corn kernels was 3.62% FM (Rosa, 2015). The crop following hairy vetch, white clover and Italian ryegrass as well as farmyard manure contained more protein than the remaining catch crops. It was probably due to the fact that nitrogen was more available for corn as it was more rapidly released from farmyard manure, leguminous catch crops (hairy vetch and white clover) and Italian ryegrass. Numerous investigations have demonstrated that incorporation of leguminous catch crops (crimson clover, hairy vetch, common vetch, fodder kale, subclover, red clover) preceeding popcorn and sweet maize resulted in increased N content in kernels compared with growing without cover crops (Salmerón et al. 2011, Kramberger et al. 2014, Rosa, 2015, Janošević et al. 2017). Tejada et al. (2008) cited that protein contents in kernels of maize following oilseed rape and red clover cover crops were similar when the same mineral fertilisation of cover crops had been applied. Since the highest seed yield (Yeganehpoor et al. 2015) and oil, starch and protein contents of corn seeds (Yeganehpoor et al. 2013) were obtained from synchronic cultivation of companion crops with corn, especially red clover, corn plants cultivated simultaneously with clover had also higher oil, starch and protein yields. Kramberger et al. (2014) in their investigation found a decline in N content in maize kernels harvested from an Italian ryegrass-manured treatment compared with the non-manured control and crimson clover manured plots. Błażewicz-Woźniak and Mitura (2004), Błażewicz-Woźniak et al. (2008) observed a positive effect of leguminous biomass on nitrogen accumulation of other vegetable species (onion, parsley, white cabbage).

Table 3. The protein content (%) in sweet maize grain

Treatments	2014			2015		
	BFØ	BF	Average	BFØ	BF	Average
CV	10.92	10.87	10.58	11.68	11.16	11.42
FP	11.23	10.68	10.96	11.54	11.36	11.45
WO	10.68	10.09	10.39	11.56	10.04	10.80
FK	11.08	10.79	10.94	11.18	10.87	11.03
CV+WO	11.14	11.82	11.48	11.32	11.33	11.33
FP+WO	10.22	10.14	10.18	10.58	11.64	11.11
DOM	10.80	10.29	10.55	10.42	10.27	10.35
TV	11.39	10.81	11.10	11.01	11.36	11.19
Average	10.93	10.68	10.81	11.16	11.00	11.08

LSD 0.05	CC**	BF**	CC x BF**
Protein content 2014	0.063	0.032	0.090
LSD 0.05	CC**	BF**	CC x BF**
Protein content 2015	0.021	0.010	0.029

p<0.01 very significant (**); p<0.05 significant (*); p>0.05 no significant (ns)

Salmerón et al. (2011) noticed that N content in kernels of corn grown after oilseed rape and winter rape cover crops was significantly lower compared with common vetch although it did not differ significantly from the control without cover crop. Caporali et al. (2004) reported a decline in N content after ryegrass compared with cultivation after subclover and hairy vetch cover crops, the N content being similar to the control. According to investigation of Dragičević et al. (2016), the highest protein content was in field pea + winter oats treatment, followed by common vetch and field pea, what could be explained by the positive response of sweet maize to nitrogen enrichment caused by leguminous plants present in those treatments (Idikut et al. 2009). Leguminous cover crops were important for protein accumulation, together with decrease in available sorption sites and decrease in endergonic reactions, what could in result contribute to the increased nutritional quality of produced kernel (Dragičević et al. 2016).

Conclusions

Based on results, obtained effects of the cover crops, with and without microbiological fertilizer, on content of protein in grain of sweet maize grown on chernozem under rain fed conditions, the following can be concluded: Meteorological conditions during the investigation had an important impact on content of protein in all the cropping systems. In both years, microbiological fertilizer not have an effect on the content of protein, except in mixtures treatments. The greatest impact on protein content was exhibited in variant of mixture legumes and winter oats but the lowest in variant with winter oats and dead organic mulch.

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References

1. Armengot, L., Berner, A., Blanco-Moreno, J.M., Maeder, P. and Sans, F.X. (2015). Long-term feasibility of reduced tillage in organic farming. Agronomy for Sustainable Development 35, 339–346.
2. Błażewicz-Woźniak, M. and Mitura, R. (2004). Wpływ uprawy konserwującej na zawartość składników mineralnych w glebie i w korzeniach pietruszki. Rocz. AR Poznań, Ogrodnictwo, 356(37): 3–11.

3. Błażewicz-Woźniak, M., Kęsik, T., Wach, D. and Konopiński, M. (2008). The influence of conservation tillage on the mineral elements content in soil and chemical composition of onion. Acta Sci. Pol. Hortorum Cultus, 7(2): 61–72.
4. Caporali, F., Campiglia, E., Mancinelli, R. and Paolini, R. (2004). Maize performances as influenced by winter cover crop green manuring. Ital. J. Agron., 8(1): 37–45.
5. Chen, Y., Zhu, Z., Guo, Q., Zhang, L. and Zhang, X. (2012). Variation in concentrations of major bioactive compounds in *prunella vulgaris* L. related to plant parts and phenological stages. Biol Res. 45: 171–5. pmid:23096361
6. Dabney, S., Delgado, M.J.A. and Reeves, D.W. (2001). Using winter cover crops to improve soil and water quality. Communications in Soil Science and Plant Analysis 32, 1221–1250.
7. Dolijanovic, Z., Momirovic, N., Mihajlovic, V., Simic, M., Oljaca, S., Kovacevic, D. and Kaitovic, Z. (2012). Cover crops effects on the yield of sweet corn. Third International Scientific Symposium, "Agrosym Jahorina 2012" Jahorina, November 15-17, Bosnia and Herzegovina, Proceedings: 104-110.
8. Dorn, B., Jossi, W. and van der Heijden, M.G.A. (2015). Weed suppression by cover crops: comparative on-farm experiments under integrated and organic conservation tillage. Weed Research 55, 586–597.
9. Dragičević, V., Janošević, B., Simić, M., Brankov, M., Mesarović, J. and Dolijanović, Ž. (2016). Relations between free energy and grain composition of sweet maize from ecological production. 13th International Conference on Fundamental and Applied Aspects of Physical Chemistry. Belgrade, September 26-30, 2016, Proceedings, Volume II: 885-888.
10. Dragicevic, V., Spasojevic, I., Oljaca, S., Simic, M. and Dolijanovic, Z. (2013). Grain quality in organic and ecological cropping systems. IV International Symposium „Agrosym 2013“, Jahorina 03.-06. October 2013, Bosnia and Herzegovina, Proceedings: 700-705.
11. Hartwig, N.L. and Ammon, H.U. (2002). Cover crops and living mulches. Weed Science 50, 688–699.
12. Idikut, L., Boga, M., Atalay, A.I., Kara, S.N. and Kamalak, A. (2009). Effect of previous plant on chemical composition of sweet corn grain. J Anim. Vet. Adv., 8 (10): 1979-1981.
13. Janosevic, B., Dolijanovic, Z., Dragicevic, V., Simic, M., Dodevska, M., Djordjevic, S., Moravcevic, Dj. and Miodragovic, R. (2017). Cover crop effects on the fate of N in sweet maize (Zea mays L. saccharata Sturt.) production in a semiarid region. International Journal of Plant Production 11 (2): 285-294.
14. Kadžiulienė, Ž., Šarūnaitė, L. and Deveikytė, I., (2011). Effect of Pea and Spring Cereals Intercropping on Grain Yield and Crude Protein Content. Field and Veg. Crops Res. 48, 183-188.
15. Kramberger, B., Gselman, A., Kristl, J., Lešnik, M., Šuštar, V., Muršec, M. and Podvršnik, M. (2014). Winter cover crop: the effects of grass–clover mixture proportion and biomass management on maize and the apparent residual N in the soil. Eur. J. Agron. 55, 63-71.
16. Lovett, J.V. (1991). Changing perceptions of allelopathy and biological control. Biol. Agric. Hortic. 8: 89–100.
17. Magdoff, F. (2007). Ecological agriculture: Principles, practices, and constraints, Renew. Agric. Food Systems, 22, 109–117.
18. Oljaca, S. and Dolijanovic, Z. (2013). Ecology and cultural practice of intercropping. Monograph. University of Belgrade, Faculty of Agriculture. 173p. (In Serbian)
19. Rosa, R. (2015). Quality of sweet corn yield depending on winter catch crops and weed control method, Acta Sci. Pol. Hortorum Cultus, 14(2): 59-74 .
20. Salmerón, M., Isla, R. and Cavero, J. (2011). Effect of winter cover crop species and planting methods on maize yield and N availability under irrigated Mediterranean conditions. Field Crops Res., 123, 89–99.

21. Stone, P.J., Sorensen, I.B. and Jamieson, P.D. (1999). Effect of soil temperature on phenology, canopy, development, biomass and yield of maize in a cool-temperate climate. Field Crops Res., 63(1): 169–178.
22. Sun, W.Q. (2002). Drying Without Dying. In Black, M. and Pritchard, H.W. (eds), Desiccation and Survival in Plants. CABI Publishing, New York, USA, 47-91.
23. Tejada, M., Gonzales, J.L., García-Martínez, A.M. and Parado, J. (2008). Effects of different green manures on soil biological properties and maize yield. Biores. Technol., 99, 1758–1767.
24. Thayamini, H.S. and Brintha, I. (2010). Review on maize based intercropping. Journal of Agronomy 9(3): 135-145.
25. Thorup-Kristensen, K., Magid, J. and Jensen, L.S. (2003). Catch crops and green manures as biological tools in nitrogen management in temperate zones. Advances in Agronomy. Vol 79, 227–302.
26. Yeganehpoor, F., Zehtab-Salmasi, S., Ghassemi-Golezani, K., Valizadeh, M. and Moeinirad, A. (2013). Effect of weeding and by forage and medicinal plants as companion crops on some of quality traits of Corn (*Zea mays*). International Journal of Farming and Allied Sciences 2(20): 821-825.
27. Yeganehpoor, F., Zehtab-Salmasi, S., Abedi, G., Samadiyan, S. and Beyginiya, V. (2015). Effects of cover crops and weed management on corn yield. Journal of the Saudi Society of Agricultural Sciences 14, 178-181.

ASSIMILATION OF NUTRIENTS FROM COMMON WHEAT (*TRITICUM AESTIVUM* L.) DEPENDING ON SOME AGRONOMY FACTORS

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Abstract
The genotype specificity in the uptake of the main nutrients according to the fertilization rate was investigated in *Triticum aestivum* L. varieties under conditions of a vegetation experiment. Four fertilization rates were tested: $N_0P_0K_0$, $N_{200}P_{200}K_{200}$, $N_{400}P_{200}K_{200}$ and $N_{600}P_{200}K_{200}$. The response of the varieties to the nutrition conditions provided was studied during several stages of growth and development. The different nutrition regimes used lead to manifestation of the specific abilities of the varieties to take up nutrients. Genotype and mineral fertilization had a higher effect on nutrients uptake than stage of development. During the initial stage of wheat development, the differences established in the uptake of macro elements were greater between the varieties than between the individual fertilization rates. The differences were most evident at maturation. This specificity was well expressed in nitrogen and phosphorus uptake in total biomass and to a lesser extends - in potassium uptake. Varieties Slaveya and Milena had highest amounts of nitrogen uptake in grain.

Keywords: Wheat, mineral fertilization, uptake, nutrients.

Introduction
The variation of grain yield when growing winter wheat cultivars is always different as a result from the strong and constant effect of the environment. (Hagos and Abay, 2013, Tsenov and Atanasova, 2013a). This is the main reason for the various degrees to which the productive potential of each cultivar is being realized. Grain yield depends directly on the expression of several traits which are considered essential for productivity (Yagdi, 2009, Anderson et al, 2011). Varieties and hybrids of different cultures differ not only in yield potential, but also in requirements for nutrients and behaviour fertilization (Clark, 1990, Klimashevskiy, 1991). These differences are referred to the growth or genetic specificity of mineral nutrition. Wheat genotypes adaptive differently to various levels of fertilization and their productivity are connected mainly to the absorption of major macro elements - nitrogen, phosphorus and potassium (Ivanov et al, 1993; Le Gouis et al, 2000). The amount of absorbed nutrients depends on the plant genotype, the energy balance within it, the internal nutritional status, and the needs of the plants and the presence of nutrients in the soil (Misas et al, 2003). Energetically rational varieties absorb high levels of nutrients from fertilizers and soil (Klimashevskiy, 1990). One way to increase the wheat yielding is to create new varieties which grow fastly and have high potential and effective productivity (Dimova et al, 2002). The aim of the study was to characterize genotypic specificity in absorbing the main macronutrients (nitrogen, phosphorus and potassium) depending on the variety and level of fertilization.

Material and methods
In terms of vegetation experiment, 12 varieties of common wheat (*Triticum aestivum* L.) were tested: Sadovo 1 (st), Aglika, Iveta, Bolyarka, Milena, Slaveya, Enola, Kristi, Pryaspa, Todora, Karat and Pobeda (st).

The experiment is displayed in plastic containers each of them contains 1 kg of soil in three repetitions. The varieties are grown in Luvic Faeozem (FAO) in 4 types of diets: $N_0P_0K_0$ (a), $N_{200}P_{200}K_{200}$ (b), $N_{400}P_{200}K_{200}$ (c) и $N_{600}P_{200}K_{200}$ (d). Nutrients were applied in soluble condition, before sowing the whole quantity of phosphorus and potassium and 200 mg of nitrogen were added per 1000 g soil. The remaining amounts of nitrogen rate were imported at the end of tillering stage. The reaction of varieties to the established conditions is studied in stages 31 (I phase), 57-59 (II phase) and 94-95 (III phase) by Zadoks (Zadoks et al, 1974). In each of these phases the plant organs are separated and analysed singly. The total amount of nitrogen (Kjeldahl), phosphorus (colorimetric by the yellow colour reaction) and potassium (flame photometry) was determined after preparing the samples for analysis. The conclusion was based on the received sample results and the NPK content in different organs and phases defined absorption of macronutrients in both separately organs and in total biomass.

Results and discussion

The analysis of variances shows the influence of the factors investigated on the nutrients uptake in the common wheat organs (table 1). The influence of the variety is strongest in the nitrogen uptake by the leaves (248.8**). The influence of fertilization is strongest in the nitrogen uptake by the stems (469.1**) and the spikes (64.9**). Nitrogen is the most important element in plants' nutrition. Its full effect over yield and quality of production can be observed only when the other nutrients are applied optimally. Fertilization has a very strong influence over phosphorus uptake by the leaves (1139.8**) and the stems (575.7**). Factors Variety (431**) and Fertilization (426.4**) are close effect over phosphorus uptake by the spikes. The influence of fertilization is strongest concerning potassium uptake by the leaves (925.7**), the stems (543.3**) and the spikes (1344.6**) of the wheat genotypes investigated. The stage of development has strongest influence over potassium uptake by the leaves (45.5**) and the stems (28.3**). The effect of this factor is less pronounced. Of all combined influences the most meaningful one for the assimilation of nitrogen and phosphorus by the leaves and the stems and the assimilation of the three macro elements by the spikes is AxC (Variety x Fertilization).

Table 1. Analysis of variances of factor interaction for amount of N, P, K uptake (mg/pot) by stage of development.

Organs \ Factors		A (Variety)	B (Stage)	C (Fertilization)	AxB	AxC	BxC	AxBxC
Leaves	N	**248.8****	11.1**	113.3**	7.3**	**23.4****	1.4	2.1**
	P	270.6**	0.5	**1139.8****	13.1*	**19.8***	1.2	2.6
	K	142.5**	**45.5****	**925.7****	18.4**	9.9**	3.1*	1.7*
Stems	N	258.3**	16.5**	**469.1****	11.83**	**60.9****	5.8**	4.5**
	P	381.2**	6.6	**575.7****	7.9*	**24.5****	1.1	2.2
	K	156.4**	**28.3****	**543.3****	11.5**	5.2**	2.2*	2.5**
Spikes	N	46.1**	2.3	**64.9****	1.1	**36****	0.1	1.1
	P	**431****	4.2	**426.4****	3.2*	**18.4****	1.3	2.0
	K	928**	0.1	**1344.6****	22.1**	**52.2****	0.5	4.8**

** Significance at the 0.01 level,, * Significance at the 0.05 level

Depending on the stage of development and the fertilization rate, the varieties grown under controlled conditions show considerable differences in relation to yield from separate organs and from the total biomass. Similar results are received by Nankova et al (1999). At the end of tillering and the beginning of booting stage (31 according to Zadoks) the differences among the investigated varieties in the uptake of nitrogen, phosphorus and potassium of the total biomass are insignificant. In the variants without mineral fertilization $N_0P_0K_0$ investigated varieties assimilate similar quantities of all elements. After fertilization the differences between the separate genotypes in nutrients uptake increase. The accumulated nitrogen, phosphorus and potassium increase parallelly with the increase of the fertilization rate. In these conditions the varieties Sadovo 1, Aglika, Bolyarka and Karat distinguish with increased nutrients uptake. At the stage of heading (57-59 according to Zadoks) greater differences in the assimilation of macro elements by the total biomass in comparison with the previous stage are found out. In the variants without mineral fertilization $N_0P_0K_0$ the variation in the values between the extracted quantities of nutrients is not very significant. With mineral fertilization genotypes investigated reach their maximum in nitrogen, phosphorus and potassium uptake when different fertilization rate are applied. The varieties Sadovo 1, Aglika, Iveta and Bolyarka distinguish with the greatest quantities of accumulated macro elements. At the full maturity stage (94-95 according to Zadoks) the differences between the investigated varieties in the nutrients uptake are the strongest. This peculiarity is extremely expressed at nitrogen uptake by the total biomass (figure 1). The quantity of the assimilated nitrogen by the leaves and the stems is decreases sharply, but its quantity in the spikes and in the total biomass increases. The trend for increasing the quantity of assimilated nitrogen by the spikes in similar experiments is found by Nankova (1994). The varieties differentiate in the accumulated quantities of nitrogen at the different fertilization rates. Among the variants without mineral fertilization $N_0P_0K_0$ (a) the differences between the genotypes are not very important. When using mineral fertilization these differences become deeper. This means that the genotypes researched can be divided into a few groups. The varieties Aglika, Iveta, Bolyarka and Slaveya accumulate higher than average quantities when balanced mineral fertilization $N_{200}P_{200}K_{200}$ (b) applied, and Milena, Enola, Pryaspa and Todora when fertilized with $N_{400}P_{200}K_{200}$ (c). The increasing nitrogen fertilization leads to increase of the assimilated quantities of nitrogen above the average for the standard sort Sadovo 1 and Kristi, and with Karat and Pobeda this suppresses the assimilation. The varieties Sadovo 1, Milena and Slaveya extract the greatest quantities nitrogen with the three fertilizer rates researched.

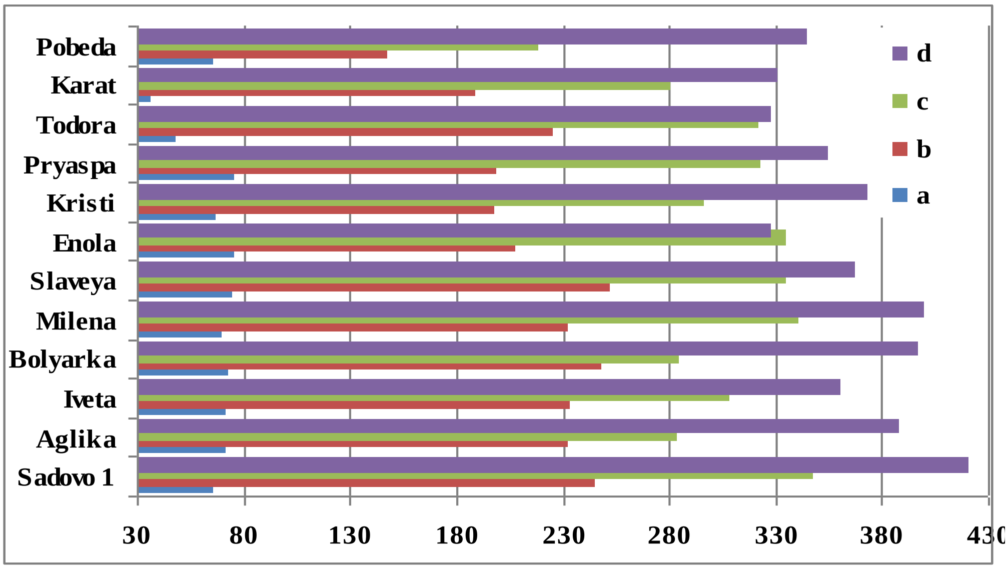

Figure 1. Utilization of nitrogen (mg/pot) of the total biomass in the phase of full maturity - $N_0P_0K_0$ (a), $N_{200}P_{200}K_{200}$ (b), $N_{400}P_{200}K_{200}$ (c) и $N_{600}P_{200}K_{200}$ (d).

In phosphorus uptake by the total biomass the differences between the varieties are clear still with the variants without mineral fertilization $N_0P_0K_0$ (a) (figure 2). The greater part of the genotypes investigated assimilate maximal quantities when balanced mineral fertilization $N_{200}P_{200}K_{200}$ (b) used – Sadovo 1, Aglika, Iveta, Bolyarka and Milena. The further increase of the nitrogen fertilization rate does not lead to increased phosphorus uptake. According to the quantity of uptake of this macro element, the investigated genotypes can be divided into two groups. Sadovo 1, Aglika, Iveta, Bolyarka and Milena that assimilate higher than average quantities of phosphorus with all fertilizer rates belong to the first group. When fertilizing with $N_{400}P_{200}K_{200}$ (c) the varieties Sadovo 1, Iveta and Bolyarka accumulate less phosphorus in comparison with the accumulated quantities in the variants with zero fertilization (a). Slaveya, Enola, Kristi, Pryaspa, Todora, Karat and Pobeda belong to the second group. With all researched fertilizing rates these genotypes accumulate less than the average quantities of phosphorus. The varieties Sadovo 1, Aglika, Iveta, Bolyarka and Milena extract the greatest quantity phosphorus with the three fertilizer rates investigated.

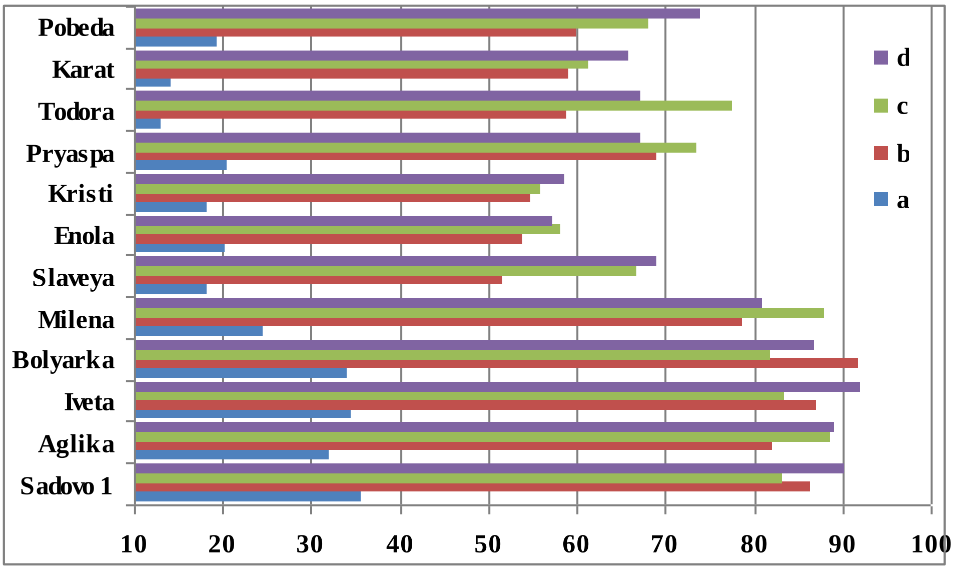

Figure 2. Utilization of phosphorus (mg/pot) from the total biomass in the phase of full maturity - $N_0P_0K_0$ (a), $N_{200}P_{200}K_{200}$ (b), $N_{400}P_{200}K_{200}$ (c) и $N_{600}P_{200}K_{200}$ (d).

At the full maturity stage the genotype specificity in the potassium uptake is not very outstanding (figure 3). With the variants without mineral fertilization $N_0P_0K_0$ (a) there are not meaningful differences between the varieties in the uptake of this macro element. At these conditions of nutrient poor regime only Sadovo 1 standard is distinguished with fewest quantities of potassium in their biomass above the ground. When fertilizing no meaningful differences between the genotypes are found out independently of the fertilizer rate. Increasing nitrogen fertilization leads to parallel increase of the values of the extracted quantities of this nutrient. Here the varieties can also be divided into a few groups. Milena, Slaveya and Pryaspa that accumulate the largest quantities of potassium compared to the average quantities when $N_{400}P_{200}K_{200}$ (c) used, belong to the first group. Aglika, Iveta, Bolyarka, Todora and Karat belong to the second group. In this group the increasing nitrogen fertilization leads to increase of the assimilated quantities of potassium above the average and reaching the maximum at the last stage of fertilization with $N_{600}P_{200}K_{200}$ (d). Sadovo 1, Enola, Kristi and Pobeda belong to the third group. These varieties extract close quantities of potassium independently of the fertilization rate applied. The varieties Aglika, Iveta and Bolyarka extract the greatest quantity potassium with the three fertilizer rates investigated. Among the variants without mineral fertilization $N_0P_0K_0$ (a) the varieties Aglika, Iveta and Bolyarka distinguished with the greatest quantities of assimilated nutrients at this final stage of their individual development. Among the variants with mineral fertilization these are the varieties Aglika, Iveta, Bolyarka, Milena and Slaveya. The increasing nitrogen fertilization influences strongly nitrogen uptake in grain of the investigated varieties (table 2). Previous research show that the increase of the nitrogen fertilization rate influences productivity and the assimilated quantities of nitrogen in the grain (Kostov et al, 1999; Nankova et al, 1999). The varieties Enola (62.7 mg/pot) and Pryaspa (63.4 mg/pot) accumulate the largest quantities of nitrogen in the control variants without fertilization $N_0P_0K_0$ (a). Fertilization with $N_{400}P_{200}K_{200}$ (c) leads to increase of nitrogen in the grain compared to the balanced proportion $N_{200}P_{200}K_{200}$ (b). Further increase of the nitrogen proportion leads to increase of the positive effect, i.e. increases the addition of accumulated nitrogen in the grain compared to the previous fertilizer rate. This trend concerns all varieties except for Enola which shows certain decrease of the quantity of nitrogen uptake in the grain. The varieties Slaveya and Milena distinguish with highest quantities of nitrogen uptake and Pobeda with lowest at all three levels of investigated nitrogen fertilization. The average increase in comparison to control samples that are not fertilized (a) is with 319,4% (b), 407,7% (c) and 366,7% (d) correspondingly. Fertilization in proportion N:P:K=2:1:1 (c) leads to accumulation of nitrogen in the grain 27,7% more than the balanced proportion (b).

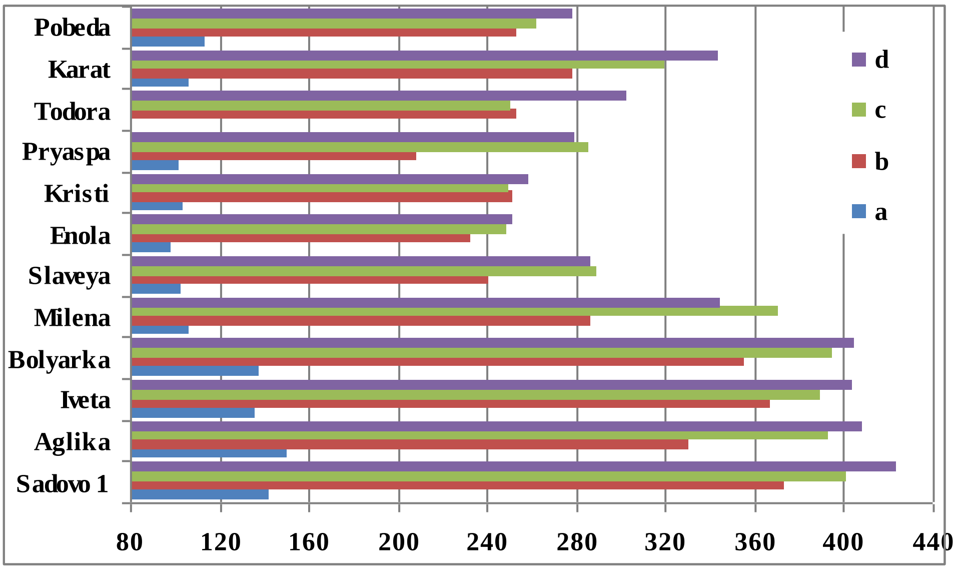

Figure 3. Utilization of potassium (mg/pot) from the total biomass in the phase of full maturity - $N_0P_0K_0$ (a), $N_{200}P_{200}K_{200}$ (b), $N_{400}P_{200}K_{200}$ (c) и $N_{600}P_{200}K_{200}$ (d).

Table 2. Nitrogen uptake in grain

Varieties	Fertilization rate – mg/1000 g soil			
	NoPoKo - a	$N_{200}P_{200}K_{200}$ - b	$N_{400}P_{200}K_{200}$ - c	$N_{600}P_{200}K_{200}$- d
Sadovo 1 – St.	52,335	186,633	205,884	239,677
Aglika	57,459	174,012	187,833	230,524
Iveta	60,092	185,061	176,085	222,804
Bolyarka	58,953	191,618	193,972	237,561
Milena	58,114	183,260	**220,909**	**281,064**
Slaveya	60,142	207,879 *	**269,388** *	**286,004** *
Enola	**62,727**	170,145 *	247,046	244,099
Kristi	54,472	160,524 *	233,631	277,524
Pryaspa	**63,390**	163,971 *	248,570	256,479
Todora	30,590 *	162,549 *	218,621	248,553
Karat	28,081 *	145,894 **	220,587	239,302
Pobeda	51,027	103,965 **	175,817	210,777
Average	*53,115*	*169,626*	*216,528*	*247,864*

** Significance at the 0.01 level, * Significance at the 0.05 level

Conclusions

The different diets lead to the expression of specific characteristics in terms of their ability to absorb nutrients through vegetation process. The factors "genotype" and "fertilization" have stronger influence on the absorption of nutrients compared to the phase development. In the initial phase of wheat development (end of tillering-beginning of spindling), the differences in the absorption of macronutrients are larger among the varieties than between different levels of fertilization. The genotypic specificity in the nutrients absorption is most pronounced in phase ripe. This feature is well expressed in the accumulation of nitrogen and phosphorus from total biomass and less from the potassium extraction. Except the variety Enola all other reach a maximum absorbed nitrogen in grain at fertilization ratio of N:P:K=3:1:1. The varieties Slaveya and Milena have the largest amount of nitrogen absorbed in their grains.

References

1. Anderson, W.K.,Van Burgel, A.J., Sharma, A.J., Shakcley, B. J., Zaicou-Kunesch, C.M., Miyan, M.S. and Amjad, M., (2011). Assessing specific agronomic response of wheat cultivars in a winter rainfall environment, Crop & Pasture Science, 62: 115-124.
2. Clark, R.B. (1990). Physiology of cereals for mineral nutrient uptake, use and efficiency, Academic Press, San Diego, California, USA, 234-245.
3. Dimova, D., Rachovska, G., and Ganuscheva, G.,(2002). Characteristics of the new wheat lines selected, Plant science, 5-6: 255-261.
4. Hagos, H.G. and Abay, F., (2013). AMMI and GGE Biplot analysis of bread wheat genotypes in the Northern part of Ethiopia, J. Plant Breed. Genet, 1: 12-18.
5. Ivanov, P., Djendova, R., and Dikova, P., (1993). Genotypic specificity in nitrogen feeding 13 varieties of winter wheat, Soil science, agricultural chemistry and ecology, 4: 7-12.
6. Klimaschevskii, L. (1990). Theory agrochemical efficiency of plants, Agrochemicals, 1: 131-145.
7. Klimaschevskii, L. (1991). Genetic aspects of mineral nutrition of plants, Agropromizdat, Moscow.
8. Le Gouis, J., Beghin, D., Heumez, E., and Pluchard, P., (2000). Genetic differences for nitrogen uptake and nitrogen utilization efficiencies in winter wheat, European Journal of Agronomy, 12, 3-4: 163-173.
9. Misas, J., Kostadinova, Sv. and Tomov, T, (2003). Absorption and distribution of nitrogen, phosphorus and potassium in wheat variety Prelom, Soil Science, Agro-chemistry and ecology, 1: 24-27.
10. Nankova, M., (1994). Seed N-content and its relation to some nitrogen metabolism elements in common wheat cultivar Pryaspa, XXIVth Annual meeting of ESNA, September 12-16, Varna.
11. Nankova, M., Kostov, K. and Penchev, E., (1999). Genetic variations in the dynamics of dry matter accumulation, nitrogen assimilation and translocation in new *T. aestivum* L. varieties. II. Nitrogen assimilation and translocation in relation to grain yield and protein content, XXIXth Annual meeting of ESNA, October.
12. Tsenov N. and Atanasova, D., (2013a). Influence of environments on the amount and stability of grain yield in today‘s winter wheat cultivars, I. Interaction and degree of variability, Agricultural Science and Technology, 5:153-159.
13. Yagdi, K. (2009). Path coefficient analysis of some yield components in durum wheat (*Triticum durum* Desf,), Pak. J. Botany, 41: 745-751.
14. Zadoks, J. C., Chang, T. T. and Zonzak, C. F., (1974). A decimal code for the growth stages of cereal, Weed Res, 14: 415-421.

STUDIES ON SOME QUALITY COMPONENTS IN A LANDRACE COLLECTION OF CLIMBING BEANS (*PHASEOLUS VULGARIS* VAR. *COMMUNIS*)

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Abstract
The study aimed at evaluating a landraces collection of climbing beans regarding some characters that contribute to quality of grains or green pods. The biological material was formed of 56 landraces and 2 varieties of climbing beans from which the grains or green pods can be used in food. The landraces were collected from western and southwestern Romania. The experimentation was performed over three years in a collection-type experience, arranged in three repetitions. As elements of quality we determined: the percentage of husks from beans, boiling coefficient, the percentage of bean total protein, 1000 grain weight, the percentage of sugar in green pods. The experimental data were processed by analysis of variance and applying the t-test for determination of differences from the variety 'Aurie de Bacau' used as a control. The collection includes precious populations for all studied characters, but their number is reduced. For the percentage of grain husks and the protein content of grain, the most populations are below the control variety. For sugar percentage in green pod and the coefficient of boiling, the landraces are similar to control variety. In the collection, there are populations that can be processed by selection or can be used as parents in hybridization programs.

Keywords: climbing beans, landraces, quality.

Introduction
Phaseolus vulgaris L. is one of the traditional legumes in many countries in the world, that is why there is a very large number of varieties and landraces. The importance of food is given by the quality of the grains and pods which can be evaluated from various points of view. The study of nutritional value shows great diversity in minerals and organic substances. The lipid content may be 0.57-2.86 g /100 g DW, 18.55-29.69 g /100 g DW protein, 23.40-52.65 g /100 g DW sugar. In addition, there are significant amounts of N, P, K, Mg, Fe, Cu, Zn, Mn, B. Among the cultivated forms, we can also find some low-sugar content, good for diabetics, or others rich in protein (Gouveira Carla et al., 2014). Besides proteins, beans are a major source of dietary fiber and minerals, very useful in human nutrition (Poujola et al., 2007). Lately, cultivated varieties are less and less different in terms of quality. The landraces are more diverse in this respect, but they are about to disappear from culture (Singh, 2001). The studies show that the biochemical composition of bean seeds depends on genetic and environmental factors (Kigel, 1999). In quality study, it is important to know the genetic determinism of each component. The protein content is controlled by additive and non-additive genes. When selecting with a selection rate of 10%, an increase of up to 10.16% can be expected after a selection cycle (Noubissie et al., 2012). The protein content depends on the area from which the germplasm is. In some populations collected from Kosovo, the protein is in a smaller quantity. The accumulation of proteins and minerals varies depending on environmental conditions (Aliuet et al., 2014). To improve the bean quality of beans, the biological material should be well

studied. In addition to the study of the biochemical composition, there is a need to study some of the characters related to the boiling process. But, the beans rich in protein and minerals boil harder and remain hard after boiling (Saha et al., 2009). For cooking, it is necessary to study the ability of grains to absorb water. They can be used as indicators: grain hydration coefficient, grain swelling coefficient. These characters have a high heritability and may be imposed on new varieties by choosing the genitors correctly in hybridization programs (Saba et al., 2016). Regarding the cooking skills, the boiling time is important. The swelling and hydration capacity of the beans is correlated with the boiling time. Dried beans can take up to 85 minutes to boil. Swollen beans are boiled much faster, requiring up to 30 minutes (Wani et al., 2017). The mass of 100 grains, the cooking time and the mineral content are correlated and can be used as selection criteria to improve cooking capacity. Some genotypes with a short cooking time (less than 24 minutes) have a high content of potassium, iron, zinc, copper (Dalfollo Ribeiro Nerinéia et al., 2014). The culinary quality elements can also be correlated with morphological characters of pods or grains. The genotype × environment interaction was significant for the characters of the raw pods: length, width, thickness and texture. The interactions between genotype and environment also appear for the characters of dried grains: length, width, thickness, volume, hardness and water absorption (Escribano et al., 1997). Regarding the quality of raw pods, the harvesting period is very important. The highest percentage of sugar is found in 15 days from blooming. At the same harvest period, obtain the highest content of carotene, and ascorbic acid is maximum at 7 days of blooming (Harunor and Hossain, 2014). The study of some varieties of reniform grains has revealed the existence of correlations between different types of sugars from the immature pods. There is a positive correlation between simple glucose and fructose. These studies lead to the recommendation of certain types of pods in different diets (Nienhuis et al., 2016). Studies on water absorption in grains were performed on genotypes of African origin. There are also positive correlations between the grain yield of the pods and the number of pods per plant, or between the shell content of the grains and water absorption (Balcha, 2010). The boiling time is correlated with the grain size. The water absorption is related to grain quality. The hydration index close to 100% indicates that the seeds absorb a quantity of water equal to their weight. This index may exceed 90%. The moisture capacity depends on the hardness of the bean skin (Lioi Lucia et al., 2012).

Material and methods

The purpose of the studies was to evaluate the variability for some characters that are quality components of the local garden bean germplasm, in order to find possible genitors, for use in breeding programs or in finding some populations which will be recommended to be grown in the areas of origin. The biological material used is the majority of bean garden landraces. They come from very different pedoclimatic zones in terms of soil and climate. Procurement of local populations was carried out by collecting in the field, from the counties of western and southwestern Romania. The basic points in the collecting activity were the localities with a traditional agriculture. The collection included 58 variants, of which 56 landraces and 2 varieties. The best known climbing beans variety is Aurie de Bacau, an old variety, used as a control. Along with this, we also studied the Juliska variety, from Hungary. The experimentation was organized as a collection field, with parcels sized to provide representative samples for each genotype studied. They were organized in two repetitions per plot. The samples were made up of 50 pods from each plot. The plants were obtained under traditional technological conditions, without any application of chemical fertilizers and without irrigation. The observations on yield quality have targeted both the quality of the grains and the pods. To determine the quality of the grains have been tested shells percentage, the protein content of grain, boiling coefficient, weight of 1000 grains, and for pods quality was determined the percentage of sugar in the raw pods. For all these attributes, were performed three determinations for each repetition. The percentage of shells was determined on samples of 10 g. The percentage of protein was determined using the Kjeldahl system. The boiling coefficient has been set by determining the average boiling time of a grain, each sample was constituted by 10 grains. The weight of 1000 grains was determined on samples consisting of 200 grains. The percentage of sugar in the raw pods was determined using the electronic refractometer. The obtained data were statistically processed by analysis of variance (Ciulca, 2006).

Results and discussion

The study of climbing bean landraces has led to some results which highlight a great variability of the collection. The shells percentage has varied within very wide limits, from 4.60% in the Pocola 1 landrace, up to 13.37% in Otelu Rosu 2 landrace. Valuable are the landraces with a low shells percentage, below the 7% limit. Of the evaluated populations, 17 populations showed the shells percentage below 7%. As valuable populations from this point of view, we may also note the landraces Oţelu Roşu 3, Tărcaia 3, Voiteg, Joia Mare 1 and Faget, populations where the shells percentage is below 6% (table 1). Compared to the control variety Aurie de Bacău, variety that has a fairly high shells percentage, it is found that in 15 landraces this character has significantly lower values. From figure 1 it is noted that of the 58 variants, most landraces fall within the limits of 7 and 10% (33 landraces). A percentage of shells above 10% was found in only 7 populations. From the general evaluation we can say that there is a representative set of valuable genotypes for low shell content in the collection. The boiling coefficient expresses the ability of the grains to boil in a shorter or longer time interval. The variability for the boiling coefficient was not very pronounced, the extreme values were 5.36 for the Vinga landrace and 10.56 for the Vinga 3 landrace. Very high values were shown by few populations. Over the boiling coefficient 10 were registered another 3 populations: Becicherecu Mic, Barsa and Sebis. Small values were recorded for Ortisoara and Vanatori 1 landraces (table 1). The control, being cultivated mainly for pods, has a high boiling coefficient. With regard to this character, the landraces are valuable, the vast majority having a boiling coefficient lower than the control; 35 of the landraces are statistically significant. Few landraces had higher boiling coefficient than the control. Regarding the boiling coefficient, in the studied collection, 40 of the landraces showed this character with values between 7 and 9. Low values (under 7) were reported in only 3 landraces, and very high values were reported for 4 landraces. According to estimations, we may observe that the population in the collection is valuable for the boiling coefficient, constituting a useful germplasm for improving this character (figure 2). The protein percentage of grain variability was between 22.10% for the Becicherecu Mic landrace and 28.04% for the Buteni 1 landrace. The most common values were between 24% and 26%. High values, over 27% protein in grain, were also presented for the Julita 1, Olari 1 and Fizis 2 landraces. The Aurie de Bacau variety, used as a control, had a 24.85% protein content in grains; compared to this, there are many superior but also inferior genotypes. Of the superior ones, statistical assurance exists for the differences between the control and other 18 genotypes. The inferior cases are 15. The results do not indicate a very big protein content, compared to the values reported in the literature. The protein content may exceed 30% of grain weight. The genetic variability for quality is very high, just that genes sources have to be searched for (Andrade Silva Camila et al., 2010). Generally, the percentage of protein is within the limits 18.55-29.69 g / 100 g DW (Gouveira Carla et al., 2014). The distribution of genotypes within the limits of variability shows that in the climbing bean collection, 28 of the 58 genotypes have a protein content of 24 - 26%. Populations rich in protein are a few, only 6 genotypes have a content above 27%. Neither those invaluable for this character are numerous, only in 5 cases the protein is below 23%. Although valuable forms are few, the collection is useful for the breeding process (figure 3). The sugar content in raw pods showed a fairly wide variability, the limits of the observed interval being 1.96% for the Julita 2 landrace and 7.29% for the Semlac landrace. In most genotypes, the recorded values were between 3% and 5%. More than 5% sugar content in raw pods was available in the case of the control variety Aurie de Bacau, but also in Paulean 2, Tarcaia 3, Bobda, Vanatori, Bolvasnita, Svinita, Joia Mare 1, Julita 3, Barsa, Cornereva landraces (Table 1).

Table 1. Results regarding the shells percentage, boiling coefficient, protein percentage and sugar percentage in raw pods in climbing bean genotypes

No.	Genotype	Shells percentage (%)		Boiling coefficient		Protein percentage (%)		Sugar percentagein raw pods (%)	
		Average	Difference from the control / significance	Average	Difference from the control / significance	Average	Difference from the control / significance	Average	Difference from the control / significance
1	Aurie de Bacau	8.26	Control	8.99	Control	24.85	Control	5.03	Control
2	Tarcaia 1	6.29	-1.97^{000}	8.62	-0.36	23.86	-0.99^{0}	4.25	-0.78^{00}
3	Tarcaia 2	7.86	-0.40	9.53	0.54*	27.36	2.51***	3.87	-1.16^{000}
4	Julita 1	8.65	0.39	9.35	0.36	27.35	2.50***	4.05	-0.98^{000}
5	Julita 2	10.69	2.43***	8.10	-0.88^{0}	21.65	-3.20^{000}	3.76	-1.27^{000}
6	Julita 3	8.95	0.69	7.46	-1.53^{000}	24.85	0.00	3.89	-1.14^{000}
7	Vinga 1	10.07	1.81***	5.36	-3.63^{000}	22.07	-2.78^{000}	3.02	-2.01^{000}
8	Vinga 2	6.59	-1.67^{000}	7.53	-1.46^{00}	26.49	1.64***	4.07	-0.96^{000}
9	Secusigiu	7.76	-0.50	8.00	-0.99^{0}	24.96	0.11	3.16	-1.87^{000}
10	Buteni 1	8.86	0.60	8.25	-0.73^{0}	28.04	3.19***	4.19	-0.84^{000}
11	Paulean 1	8.76	0.50	9.47	0.48	25.72	0.87*	3.13	-1.90^{000}
12	Paulean 2	8.54	0.28	7.25	-1.74^{000}	26.33	1.48**	5.04	0.01
13	Sebis 1	8.43	0.17	7.53	-1.46^{00}	24.63	-0.22	4.40	-0.63^{0}
14	Olari 1	7.07	-1.19^{00}	8.39	-0.60^{0}	27.01	2.16***	4.30	-0.73^{00}
15	Olari 2	8.37	0.11	7.07	-1.2^{000}	25.17	0.32	4.08	-0.95^{000}
16	Bata	10.07	1.81***	7.86	-1.2^{00}	23.67	-1.18^{00}	4.19	-0.84^{000}
17	Birchis 1	6.20	-2.06^{000}	9.35	0.6	26.22	1.37**	4.08	-0.95^{000}
18	Birchis 2	7.45	-0.81^{0}	7.03	-1.5^{000}	25.85	1.00*	4.93	-0.10
19	Otelu Rosu 1	6.69	-1.57^{000}	7.40	-1.9^{000}	26.31	1.46**	3.89	-1.14^{000}
20	Otelu Rosu 2	13.37	5.10***	8.66	-0.3	23.08	-1.77^{00}	3.34	-1.69^{000}
21	Otelu Rosu 3	5.82	-2.44^{000}	7.08	-1.90^{000}	25.61	0.76	4.08	-0.95^{000}
22	Caransebes 3	6.75	-1.51^{000}	8.24	-0.75^{0}	25.51	0.66	3.24	-1.79^{000}
23	Ortisoara	6.81	-1.45^{000}	6.88	-2.10^{000}	25.81	0.96*	4.30	-0.73^{00}
24	Semlac	5.87	-2.39^{000}	9.36	0.37	23.56	-1.29^{00}	7.29	2.26***
25	Tarcaia 3	5.92	-2.34^{000}	9.89	0.90^{0}	25.35	0.51	5.88	0.85***
26	Voiteg 1	5.94	-2.32^{000}	8.55	-0.44	25.14	0.29	3.48	-1.55^{000}
27	Voiteg 2	7.55	-0.71	7.55	-1.44^{00}	23.85	-1.00^{0}	4.19	-0.84^{000}
28	Ionis 1	9.61	1.35***	9.24	0.25	24.55	-0.30	3.99	-1.04^{000}
29	Becicherecu Mic	10.36	2.09***	10.02	1.03***	22.10	-2.75^{000}	2.91	-1.04^{000}
30	Bobda	7.06	-1.20^{00}	9.87	0.88*	25.14	0.29	3.23	0.01
31	Ionis 2	6.48	-1.79^{000}	7.65	-1.34^{00}	26.83	1.98***	4.19	-0.94^{000}
32	Beius	10.41	2.15**	9.61	0.62*	24.19	-0.66	4.19	-2.12^{000}
33	Fizis 1	7.40	-0.86^{0}	8.00	-0.99^{00}	25.44	0.59	3.66	-1.80^{000}
34	Fizis 2	8.81	0.55	7.99	-1.00^{00}	28.01	3.16***	3.98	-0.84^{000}
35	Pocola 1	4.60	-3.66^{000}	8.24	-0.75^{0}	24.65	-0.20	5.25	-0.84^{000}
36	Pocola 2	9.30	1.04**	8.33	-0.66^{0}	27.36	2.51***	5.04	-1.37^{000}
37	Pietrani	8.47	0.20	7.98	-1.01^{00}	25.47	0.62	4.09	-1.05^{000}
38	Vanatori	7.26	-1.00^{0}	6.96	-2.02^{000}	26.29	1.44**	7.19	0.22
39	Buteni 2	7.78	-0.48	7.33	-1.66^{000}	26.78	1.93***	7.16	0.01
40	Buteni 3	9.11	0.85*	9.89	0.90*	24.35	-0.50	5.14	-0.94^{000}
41	Barsa	7.82	-0.45	10.35	1.36**	26.51	1.66**	5.26	2.16***
42	Cornereva	7.06	-1.20^{00}	8.66	-0.33	25.44	0.59	4.89	2.13***
43	Bolvasnita	8.58	0.32	8.95	-0.04	23.86	-0.99^{0}	3.99	0.11
44	Svinita	9.61	1.34***	7.53	-1.46^{00}	24.16	-0.69	6.95	0.23
45	Begheiu Mic	7.99	-0.28	7.68	-1.31^{00}	25.19	0.34	4.66	-0.14
46	Carasova	8.25	-0.01	7.78	-1.21^{00}	24.67	-0.18	4.86	-1.04^{000}
47	Joia Mare 1	5.18	-3.09^{000}	8.16	-0.82^{0}	25.36	0.52	3.98	1.92***
48	Joia Mare 2	8.72	0.45	8.39	-0.60^{0}	23.88	-0.97^{0}	4.19	-0.37
49	Vinga 3	1.20	1.94***	10.56	1.57***	25.30	0.45	2.70	-0.17
50	Nadab	1.50	4.24***	9.43	0.44	22.32	-2.53^{000}	4.17	-1.05^{000}
51	Sebis 2	8.37	0.11	10.25	1.26***	24.54	-0.31	3.98	-0.84^{000}
52	Vălisoara 1	7.03	-1.23^{00}	8.63	-0.36	23.75	-1.10^{00}	5.29	-2.33^{000}
53	Valisoara 2	9.81	1.54***	7.37	-1.62^{000}	22.56	-2.29^{000}	1.96	-0.86^{000}
54	Caransebes 1	7.39	-0.87^{0}	8.35	-0.63^{0}	24.38	-0.47	5.04	-1.05^{000}
55	Caransebes 2	8.38	0.12	7.39	-1.60^{000}	24.49	-0.36	4.38	0.26
56	Sacu	6.81	-1.45^{000}	9.25	0.26	26.01	1.16**	1.96	-3.07^{000}
57	Juliska	10.40	2.13***	9.83	0.84*	23.63	-1.22^{00}	5.04	0.01
58	Faget	5.18	-3.09^{000}	8.36	-0.63^{0}	24.07	-0.78^{0}	4.38	-0.65^{00}
		LSD 5%= 0.75%; LSD 1%= 1.00%; LSD 0,1%= 1.30%		LSD 5%= 0.52; LSD 1%= 0.90; LSD 0,1%= 1.49		LSD 5%= 0.76%; LSD 1%= 1.08%; LS SD 0,1%= 1.82%		LSD 5%= 0.75%; LSD 1%= 1.00%; LSD 0,1%= 1.30%	

The control is a valuable variety regarding the presence of sugars in raw pods and the comparison between these reveals that most local landraces are inferior for this character. Following the statistical calculation, we may notice positive differences compared to control only in the case of 5 landraces. In all cases, the differences are very significant. The lower populations in terms of this character are much more numerous and in many cases the differences are statistically assured. Very significant negative differences were recorded for 34 populations. Carrying out an overview of the collection in terms of sugar percentage in raw pods, we may observe that almost half of the evaluated genotypes have a sugar content of between 4 and 5%, a limit quite close to the value recorded in the control variety. Remarkable are the four populations where the sugar percentage exceeds the limit of 6%, of which 3 exceed the limit of 7%. This situation highlights the fact that, in the collection studied, there are valuable forms for breeding the quality of raw pods (figure 4). Similar results have been obtained in the study of some genotypes in Brazil. There are large differences between genotypes in terms of boiling time and shell hardness or water absorption in grains (Marques Correa Mariana et al., 2010).

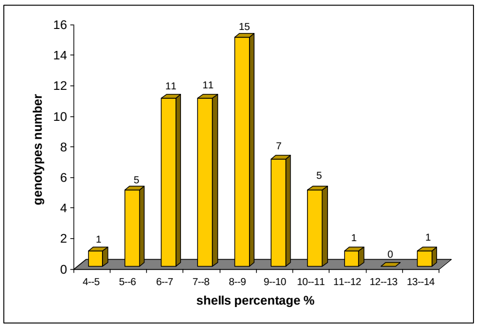

Figure 1. Shells percentage situation for climbing bean collection

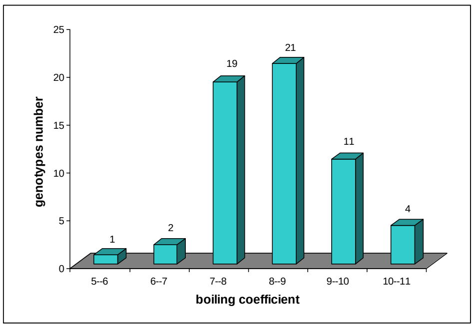

Figure 2. Boiling coefficient situation for climbing bean collection

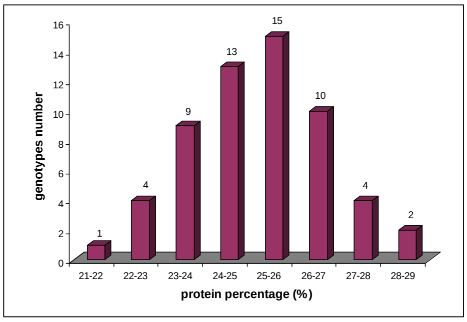

Figure 3. Protein content situation for climbing bean collection

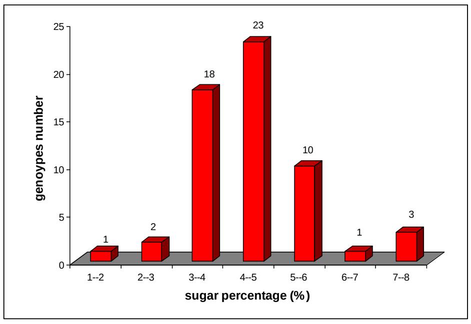

Figure 4. Sugar percentage situation for raw pods for climbing bean collection

In populations studied, the grains are quite large, the weight of 1000 grains variability ranging from 160.67 g in the Nadab landrace and a maximum value of 2032.00 g for the Vinga 2 landrace. The control, Aurie de Bacau, has the weight of 1000 grains of 430 g, value present in several of breedings bean garden varieties. Very big grains were also found at Vanatori 1 (1538.00 g) (Table 1). Studying the values of the weight of 1000 grains as compared to the control variety shows that the majority of studied landraces are above its value with statistically ensured differences. Thus, 22 landraces showed highly significant positive differences. Statistical insurance exists for other 23 populations. Only 14 populations were recorded under the value of the control variety. The results obtained are similar to those obtained in other studies. The quality of the grains and their size varies with the growth conditions. Studies on the Perola variety show that, depending on the crop area, the mass of 100 grains ranges from 17.3 to 33.5 g. Such variations are also found for biochemical content. Between the two, grain size and quality are quite strong correlations (Silveira et al., 2016).

Table 2. Results regarding the weight of 1000 grains in climbing bean genotypes

no.	Genotype	Average (%)	Difference from the control / significance	No.	Genotype	Average (%)	Difference from the control / significance
1	Aurie de Bacau	430.33	Control	30	Bobda	383.00	-47.33^{0}
2	Tarcaia 1	506.00	75.67***	31	Ionis 2	570.33	140.00***
3	Tarcaia 2	535.67	105.33***	32	Beius	373.00	-57.33^{00}
4	Julita 1	395.00	-35.33*	33	Fizis 1	602.00	171.67***
5	Julita 2	441.00	10.67	34	Fizis 2	535.00	104.67***
6	Julita 3	474.00	43.67*	35	Pocola 1	537.00	106.67***
7	Vinga 1	2032.00	1601.67**	36	Pocola 2	544.33	114.00***
8	Vinga 2	553.00	122.67***	37	Pietrani	487.33	57.00*
9	Secusigiu	502.00	71.67**	38	Vanatori	1538.00	1107.67***
10	Buteni 1	426.33	-4.00	39	Buteni 2	877.00	446.67***
11	Paulean 1	497.00	66.67**	40	Buteni 3	427.33	-3.00
12	Paulean 2	621.00	190.67***	41	Barsa	497.00	66.67***
13	Sebis 1	503.33	73.00**	42	Cornereva	334.00	-96.33^{000}
14	Olari 1	399.33	-31.00^{000}	43	Bolvasnita	875.33	445.00***
15	Olari 2	507.00	76.67**	44	Svinita	453.33	23.00
16	Bata	444.33	14.00	45	Begheiu Mic	478.00	47.67*
17	Birchis 1	494.00	63.67**	46	Carasova	401.00	-29.33
18	Birchis 2	695.33	265.00***	47	Joia Mare 1	547.00	116.67***
19	Otelu Rosu 1	581.00	150.67***	48	Joia Mare 2	440.00	9.67
20	Otelu Rosu 2	502.33	72.00**	49	Vinga 3	174.33	-256.00^{000}
21	Otelu Rosu 3	646.00	215.67***	50	Nadab	160.67	-269.67^{000}
22	Caransebes 3	541.33	111.00***	51	Sebis 2	365.00	-65.33
23	Ortisoara	712.00	281.67***	52	Vălisoara 1	435.33	5.00
24	Semlac	577.00	146.67***	53	Valisoara 2	361.00	-69.33^{00}
25	Tarcaia 3	517.00	86.67***	54	Caransebes 1	335.33	-95.00^{000}
26	Voiteg 1	595,.0	164.67***	55	Caransebes 2	309.00	-121.33^{000}
27	Voiteg 2	515.00	84.67**	56	Sacu	259.00	-171.33^{000}
28	Ionis 1	490.00	59.67**	57	Juliska	351.00	-79.33^{00}
29	Becicherecu Mic	387.00	-43.33^{0}	58	Faget	398.00	-32.33^{0}
LSD 5%= 30.30 g; LSD 1%= 57.00 g; LSD 0,1%= 85.13 g							

Conclusions

The studied landraces are an important source of genes for the quality amelioration process in beans. The percentage of shells varied within very wide limits. The total value of the collection is mediocre. There are important populations with a low percentage of shells: Pocola, Otelu Rosu 3, Tarcaia 3, Voiteg, Joia Mare 1 and Faget. The weight of 1000 grains ranged from an average of 160.67 g in the Nadab landrace to a maximum of 2032.00 g in the Vinga 1 landrace. Given the great variability of this character, we consider the collection to be valuable for the breeding process. For the boiling coefficient, in the collection there were values close to the one recommended as an improvement objective, some of the collected populations being valuable. Protein is the main biochemical compound in bean grains. The populations very rich in protein are few. From this point of view, the populations: Buteni, Julita 1, Olari 1 and Fizis 2 are remarkable. Even if they were not selected from this point of view, because the local growers had no opportunity to assess this attribute, these populations may be recommended to improve the percentage of protein. The raw pods are a seasonal food rich in vitamins, carbohydrates and a very low protein and fat content. Within genotypes from the collection, the sugar content of the raw pods showed wide variability, almost half of the evaluated populations have a percentage of sugar close to the value recorded in the control variety Aurie de Bacau. The climbing bean collection comprises a great variability for the quality characters studied. Many of the landraces are not valuable for all characters, but we can also find genitors to improve each of the characters studied.

References

1. Aliu S., Rusinovci I., Fetahu S., Bislimi K., Thaqi M., Recica X. (2014) Chemical compostion of common bean (*Phaseolus vulgaris* L.) grown in Kosovo, 49th Croatian & 9th International Symposium on Agriculture, Dubrovnik, Croatia: 275-279.
2. Andrade Silva Camila, de Fatima Barbosa Abreu Angela, Patto Ramalho M.A., Duarte Correa Angelita, Silva Maia L.G. (2010) Genetic variability for protein and minerals content in common bean lines (*Phaseolus vulgaris* L) Annual Report of the Bean Improvement Cooperative, 53: 144-145.
3. Balcha Alemayehu (2010) Genetic variation for grain yield and water absorption in common bean (*Phaseolus vulgaris* L.), African Journal of Food Science and Technology, 1(6): 128-131.
4. Ciulca S. (2006) Metodologii de experimentare în agricultură şi biologie. Ed. Agropirnt, Timişoara.
5. Correa Mariana Marques, Jaeger de Carvalho M Lucia., Nutti Marilia Regini, Viana de Carvalho J.,L., Hohn Neto A.R., Gomes Ribeiro M. Ediane (2010) Water Absorption, Hard Shell and Cooking Time of Common Beans (*Phaseolus vulgaris* L.), African J.Food Sci.Tech., 1(1): 13-2.
6. Dalfollo Ribeiro Nerinéia, de Abreu Rodrigues Josana, Prigol Marina, Wayne Nogueira Cristina, Storck L., Muller Gruhn E. (2014) Evaluation of special grains bean lines for grain yield, cooking time and mineral concentrations. Crop Breed. Appl. Biotechnol.,14 (1),15-22.
7. Escribano M.R. , Santalla M. , de Ron A.M. (1997) Genetic diversity in pod and seed quality traits of common bean populations from northwestern Spain, Euphytica, 93 (1): 71–81.
8. Gouveia S. S. Carla, Freitas G., de Brito H.J., Slaski J.J., Pinheiro de Carvalho M.A.A., (2014) Nutritional and Mineral Variability in 52 Accessions of Common Bean Varieties (*Phaseolus vulgaris*L.) from Madeira Island, Agricultural Sciences, 5: 317-329.
9. Harunor Rashid A.S.M., Hossain M.M. (2014), Yield and Quality of Green Pod Production of Bush Bean (*Phaseolus vulgaris* L.) As Influenced by Harvesting Time, American-Eurasian J. Agric. & Environ. Sci., 14 (11): 1221-1227.
10. Kigel J. (1999) Culinary and Nutritional Quality of Phaseolus vulgaris Seeds as Affected by Environmental Factors. Biotechnology,Agronomy,Society and Environment, 3: 205-209.
11. Lioi Lucia, Nuzz A., Campion B., Piergiovanni Angela Rosa (2012) Assessment of genetic variation in common bean(*Phaseolus vulgaris* L.) from Nebrodi mountains (Sicily, Italy), Genet. Resour. Crop. Evol., 59:455–464.
12. Nienhuis J., Kisha T., Bethke P. (2016) Understanding and improving flavor in snap beans: Screening the USDA Phaseolus core collection for pod sugar and flavor compounds. Bean Improvement Cooperative Annual Report. 59:31-32.
13. Noubissie J.B.T., Bell J.M., Yadji H.T. (2012) Studies on variability and gene effects of seed protein contents in Cameroonian bean (*Phaseolus vulgaris* L.) cultivars. J. Agric. Soc. Sci., 8: 17–23.
14. Pujola M., Farreras A., Casanas F. (2007) Protein and Starch Content of Raw, Soaked and Cooked Beans (*Phaseolus vulgaris* L.). Food Chemistry, 102: 1034-1041.
15. Saba I., Sofi P.A., Zeerak N.A., Bhat M.A., Mir R.R. (2016) Characterisation of a core set of common bean (*Phaseolus vulgaris* L.) germplasm for seed quality traits, Sabrao Journal of Breeding and Genetics 48(3): 359-376.
16. Saha Supradip, Singh Gyanendra, Mahajan Vinay, Gupta H.S. (2009) Variability of Nutritional and Cooking Quality in Bean (*Phaseolus vulgaris* L) as a Function of Genotype. Plant Foods for Human Nutrition 64(2):174-80.
17. Silveira P.M., Mesquita A.M., Gonzaga A.C.O., Bernardes T.G., da Cunha P.C.R (2016) Variability of mass and content of mineral elements in grains of the common bean cultivar Pérola , Jaboticabal, 44 (3),: 446–450.

18. Singh S.P., (2001) Broadening the Genetic Base of Common Bean Cultivars: A Review. Crop Science, 41: 1659-1675.
19. Wani I.A., Sogi D.S., Wani A.A, Singh Gill B.(2017)- Physical and cooking characteristics of some Indian kidney bean (*Phaseolus vulgaris* L.) cultivars, Journal of the Saudi Soc.Agr.Sci., 16 (1): 7–15.

HYBRIDIZATION BETWEEN CULTIVATED SUNFLOWER AND WILD SPECIES *HELIANTHUS BOLANDERI* A. GRAY

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Abstract
Interspecific hybridization was carried out between sterile analogues of cultivated sunflower lines with normal cytoplasm and wild annual *Helianthus bolanderi* accession E-009 from collection of DAI-General Toshevo. Hybrid plants were produced using classical breeding methods and the biotechnological method *embryo rescue*. The degree of crossability and the inheritance of some morphological traits were determined. The obtained F1 progenies were characterized from morphological and phytopathological point of view. Hybrid forms distinguished with resistance to stem canker, phoma and downy mildew were obtained. The hybrid plants, carriers of Rf genes for CMS Pet 1, could be used in sunflower breeding programs for developing restorer lines.

Keywords: interspecific hybridization, sunflower, *Helianthus bolanderi*, embryo rescue, resistance.

Introduction

Sunflower is the main oil crop in Bulgaria. The planting areas have increased in recent years because of higher profitability, low input requirements and better exporting possibilities, but higher rates of disease and pests have severely limited the sunflower production in some years. Genetic variability of the cultivated sunflower may be increased by interspecific hybridization with wild sunflower species. Wild species from genus *Helianthus* possess not only considerable variability for most of the traits but also excellent survival environmental mechanisms (Thompson et al., 1981). They possess genes for resistance to diseases (biotic stress), tolerance to abiotic stresses (drought, cold, soil salinity, certain herbicides) and high quality of proteins and oil (Hladni N. and Mikljč V., 2012; Seiler, 1992; Skoric, 1992). That is why they were widely used in sunflower breeding programs. Whelan (1978) and Christov (1996) established that interspecific hybrids could be obtained, more or less easily, in crossings between annual wild species of the section *Helianthus* and cultivated sunflower, with or without embryo rescue techniques. In such interspecific hybrids, semi-sterility is a common trait due to strong genetic barriers: chromosomal translocations, inversions, etc. Sterility in F1 sunflower interspecific hybrids limits utilization of wild *Helianthus* species for the improvement of cultivated sunflower. According to Rieseberg *et al*., (1998) viable hybrids and fertile interspecific progenies could be produced and phenotype of obtained F1 hybrids was very close to the female parent. Jan C.C. and Chandler J.M. (1989) improved backcross seed set and the effectiveness of the colchicine chromosome doubling technique, analyzing the resultant tetraploids of H. annuus x H. bolanderi Gray. According to Sukno S. *et al.,* (1999) interspecific crosses *cultivated × wild sunflower*, showed higher proportion of fully developed embryos. Embryo culture proved to be a useful tool to overcome post-zygotic hybrid incompatibility (Nenova N., 2002). The aim of this study was to obtain interspecific hybrid progenies with participation of wild species *H. bolanderi*, determine the rate of crossability and inheritance mode of some traits as well as to find a resistant initial material for breeding purposes.

Material and methods

The investigation was carried out at Dobrudzha agricultural institute during 2011-2015. The cultivated sunflower was represented by seven CMS lines– HA 382, 325A, 217A, 704A, 349A, 353A, 383A. The wild species *Helianthus bolanderi*, accession E-009 was included in the investigation. The interspecific hybridization on the scheme *cultivated sunflower x wild species* was successfully applied in field conditions. The isolated sterile inflorescences of cultivated lines were pollinated by pollen from the inflorescences of *H. bolanderi*, previously excised. Seeds from interspecific crosses were obtained applying the methods of classical breeding and embryo rescue (Azpiroz *et al.*, 1988; Nenova N., 2002). Morphological and phenological characters were conformable with descriptors of IBPGR. The inheritableness *d/a* was calculated for F_1 progeny, using the coefficient of Mather and Jinks (1982). Phytopathological evaluations of F_1 hybrid progenies were carried out in laboratory conditions and in artificial infection plot. Evaluation for resistance to downy mildew (*Plasmopara halstedii* Farl. Berlese et de Toni) was carried out on the method of Vear and Tourvieille (1987). Evaluation for resistance to grey spots on sunflower (*Phomopsis/Diaporthe helianthi* Munt.-Cvet. *et al*.) was carried out on the method of Encheva and Kiryakov (2002) in field conditions on artificial infection plot. Evaluation for resistance to black spots on sunflower (*Phoma macdonaldii* Boerema/*Phoma oleracea* var. *helianthi-tuberosi* Sacc) was carried out on the method of Fayralla and Maric (1981) in field conditions on artificial infection plot.

Results and discussion

Interspecific crosses *cultivated sunflower x wild species* were performed and the obtained hybrid plants were grown in field conditions. As paternal component in the realized crosses was the accession of wild *H. bolanderi*. The sterile analogues of fertile sunflower lines with normal cytoplasm were used as maternal parents. The data connected to crossability rate and seed set were presented on table 1. The results of hybridization showed that the crossability varied from 33% to 66% and the average percentage for all crosses was 42,8%. The seed set of one head (the percentage of insemination) wase very low and varied from 4,55% for the cross HA 382A x E-009 to 12,78% for the cross 353 A x E-009.

Table 1. Crossability of wild species H. bolanderi (E-009) and cultivated sunflower lines

Hybrid combination	Pollinated inflorescences			Obtained seeds			Hybrid plants obtained	
	Total number	with seeds		Average per head	Total number	Seed set, %	Total number	compared to seeds,%
		Num ber	%					
HA 382A x E-009	3	1		12	12	4,55	7	58,3
325 A x E-009	3	1		13	13	5,82	10	76,9
217 A x E-009	3	1		14	14	6,33	11	78,5
704 A x E-009	3	2		17	34	11,75	29	85,3
349 A x E-009	3	1		18	18	7,94	10	55,5
353 A x E-009	3	2		18	36	12,78	17	47,2
383 A x E-009	3	1		17	17	7,58	9	52,9
H.annuus x E-009	21	9	42,8	16	144	8,1	93	64,9

Some differences were established in the viability of hybrid seeds. The percentage of obtained F_1 plants varied from 47,2% for the cross 353 A x E-009 to 85,3% for the cross 704 A x E-009. At the average, hybrid plants were obtained from 64,9% of all obtained seeds. For overcoming the difficulties in applying of classical methods of breeding, connected to incompatibility of cultivated sunflower and with aim to obtain maximum number of hybrid plants, the method of *embryo rescue* was applied. The initial crosses were done in field conditions. The obtained 211 embryos from hybrid combinations were cultivated on firm plant tissue culture. The most suitable period for detachment of embryos were 10-12 days after pollination. F_1 hybrid plants were obtained from all crosses. The percentage of received hybrid plants varied from 65% to 100 %. This showed, that *embryo rescue* could be successfully applied for obtaining of more than one generation per year. Hybrid plants from all crosses were characterized morphologically. They had erect and branched stem with weak or heavy expressed anthocyanin coloration. Thin greyish-white hairs covered stems, leaves, bracts and petioles. These traits were not observed in cultivated sunflower, but they were typical for wild species. Their presence were suitable morphological markers for early determining of hybrid type of obtained F_1 plants. The central stem was longer than the branches. Plants had larger central inflorescence and many smaller heads, formed on branches. The number of branched varied from 7 to 14. Leaves were green. Their shape was similar to that of cultivated sunflower, but their size was smaller and the serration was different and well expressed. The inheritance of some morphological traits was presented on table 2. The lowest were the indices of variation coefficient for traits, characterized the cultivated sunflower, which was presented by morphologically uniform lines. The paternal form and its F_1 combinations were characterized by higher indices of VC for the character number of inseminated disk florets, and that of the progeny was the highest.

Table2. Variation in characters and type of inheritance for parents and F_1 progeny

Traits	cultivated sunflower (H. annuus L.)		Helianthus bolanderi		H. annuus x H. bolanderi			
	$\bar{x}$	VC	$\bar{x}$	VC	$\bar{x}$	VC	d/a*	H2
Plant height, cm	145	11,5	160,5	4,7	159,7	25,1	0,09i	0.85
Stem-diameter, cm	3,6	8,3	0,7	5,2	1,4	11,7	-0,49i	0.94
Number of branches	-	-	21	14,5	11,7	11,2	0,38i	0.89
Length of the longest branch, cm	-	-	99,5	14,6	113,5	13,3	3,24h	0.96
Leaf-length, cm	33	7,7	14	7,8	17	10,5	-0,23i	0.91
Leaf-width, cm	36	6,3	11	5,1	15,6	15,3	-0,57pd	0.92
Leaf petiole-length, cm	16,4	9,7	8,7	14,2	9,7	10,2	-0,59pd	0.92
Head-diameter, cm	19,7	11,2	2,5	6,4	7,3	21,8	-0,24i	0.89
Number of bracts	69	10,4	30	7,2	77,6	17,2	1,92h	0.94
Number of ray flowers	39	8,8	16	9,4	33,9	9,1	0,23i	0.95
Ray flowers-length, cm	6,8	8,2	2,6	6,7	3,8	23,5	-0,03i	0.9
Ray flowers-width, cm	2,9	6,1	0,6	5,6	1,5	15,4	0,68pd	0.93
Number of disk florets	1510,2	17,1	198	11,2	523,3	18	-0,69pd	0.87
Number of inseminated disk florets	1110,6	12,5	33,5	21,3	49,9	31,2	-0,66pd	0.86
1000 seeds weight, g	79,5	7,1	4,9	4,1	49,9	24,3	0,48i	0.79
*i- intermediate; pd- partial dominance; d- dominance; h- heterosis.								

Significant variation in plant height was not observed for the wild species, contrary to the variation for hybrid plants height. They were characterized by well displayed heterosis effect for the characters length of the longest branch and number of bracts. Partial dominance to maternal parent was established for the characters width of ray flowers. Regarding leaf width, length of petiole, number of disk florets and number of inseminated disk florets, the partial dominance to paternal parent was established. The indices of coefficient H^2 were high. The vegetation period of hybrids was shorter than that of wild species and varied from 94-110 days for early progenies to 115-135 days for the late ones.

The reaction of hybrid materials to the pathogens *Plasmopara helianthi, Phomopsis helianthi, Phoma macdonaldii* was studied with aim to establish the sources for resistance to these pathogens. The hybrid combinations 704 A x E-009 and 383 A x E-009 were resistant (100%) to downy mildew. The hybrid combinations 704 A x E-009, 349 A x E-009 and 353 A x E-009 were resistant (76%-100%) to *Phomopsis helianthi* and *Phoma macdonaldii*. Their vegetation period was 115-118 days. They could be successfully included in the sunflower breeding programs for developing new resistant restorer lines.

Conclusions

Wild *Helianthus* species have been included in sunflower breeding programs mainly as donors for resistance to diseases. Transfer of genes, controlling the resistance, into cultivated sunflower lines, gave the opportunity for diversification of cultivated sunflower and broadening its gene pool. E*mbryo rescue* could be successfully applied for overcoming the difficulties of classical breeding methods, connected to incompatibility of cultivated sunflower. Plants from hybrid combinations 704 A x E-009, 383 A x E-009, 704 A x E-009, 349 A x E-009 and 353 A x E-009, carriers of Rf genes for CMS Pet 1, could be used for obtaining of new resistant restorer lines and included as initial material in sunflower breeding programs.

References

1. Azpiroz H.S., Vincourt P., Serieys H., Gallais A. 1988. In vitro immature embryo culture for accelerating the breeding cycle of sunflower lines and its morphovegetative effects. Sci. Bull. FAO Res. Network Romania (1987). *Helia* (10) p. 35-38.
2. Christov, M. 1996. Hybridization of cultivated sunflower and wild *Helianthus* species. In: P.D.S. Caligari& D.J.N. Hind (eds). Compositae: Biology & Utilization. Proc. Int. Compositae Conference, Кew, 1994, vol.2, pp. 603-615, Royal Botanic Gardens, Кew.
3. IBPGR. 1985. Descriptors for cultivated and wild sunflower.AGPG. IBPGR/85/54, Roma, Italy.
4. Hladni N., Miklič V. 2012. Old and New Trends of Using Genetic Resources in Sunflower Plant Breeding With The Aim of Preserving Biodiversity. Proc. Int. Conf. on BioScience: Biotechnology and Biodiversity“,Novi Sad, Serbia.
5. Jan CC., Chandler JM. 1989. Sunflower interspecific hybrids and amphiploids of Helianthus annuus × H. bolanderi. Crop Science, 29: 643–646.
6. Mather K. J. Jinks. 1982. Biometrical genetics. Chapman & Hall, 430 p.
7. Nenova, N. 2002. Combining methods of interspecific hybridization with in vitro techniques to enrich the cultivated sunflower genome (*H. annuus* L.). PhD Thesis, Sofia, 2002.179 p. In Bulgarian.
8. Seiler, G. J. 1992. Utilization of wild sunflower species for the improvement of cultivated sunflower. Field Crops Research, 30: 195-230.
9. Rieseberg, LH., Baird S., Desrochers A. 1998. Patterns of mating in wild sunflower hybrid zones. Evolution, 52: 713-726.
10. Skoric, D., 1992. Results obtained and future directions of wild species use in sunflower breeding. Proc. of the 13th Inter. Sunfl. Conf., Pisa, Italy, p. 1317-1348.
11. SuknoS., J. Ruso, C.C. Jan, J.M. Melero-Vara, J.M. Fernández-Martínez.1999. Interspecific hybridization between sunflower and wild perennial Helianthus species via embryo rescue. Euphytica, Vol. 106 (1): 69–78.
12. Thompson, T.E., D.C. Zimmerman, and C.E. Rogers. 1981. Wild Helianthus as a genetic resource. Field Crops Res. 4:333–343.
13. Whelan, E.D.P. 1978. Cytology and interspecific hybridization. pp. 371-386. In: Carter JF. Editor. Sunflower Science and Technology. The American Society of Agronomy. Monograph, 505 pages.

USEFULNESS OF A LOCUS LEEF1Aa IN THE GENETIC DIFFERENTIATION OF TOMATO VARIETIES

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Abstract
The molecular techniques provide new possibilities to characterize advanced genetic materials for registration purposes and for the protection of breeders' rights. The microsatellites appear as suitable molecular markers due to their highly polymorphic character. Such microsatellites may generate polymorphism useful for the analysis of genetic diversity and relationships within the genus *Lycopersicon*. The focus of the following study was usefulness of the locus LEEF1Aa in the genetic differentiation among six morphologically different tomato varieties of *Lycopersicon esculentum* Mill. The fragment analyses were done using *Applied Biosystems* DNA analyzer (*ABI 3130*) and *GeneMapper®Software program*. The obtained data were analyzed using the specific program *Power Marker Software*. The number of detected alleles for the microsatellites locus LEEF1Aa was six in estimated tomato varieties (219-221-223-225-227-229bp). The allele with the length of 229 bp was noticed only in *Lycopersicon esculentum* subsp. *cultum* var. *grandifolium*, while the alleles (221, 225 and 227 bp) in 4 varieties, the allele of 219 bp in 3 varieties and the allele of 223 bp in 2 varieties. The average PIC value for the locus LEEF1Aa was 0.7552 and it belongs to the group of high informative markers. Obtained results showed that the locus LEEF1Aais good choice for genetic differentiation of tomato varieties in combination with other polymorphic microsatellite loci.

Keywords: DNA microsatellites, fragment analyses, locus LEEF1Aa, tomato.

Introduction

Molecular biology is a powerful tool to study genetic diversity, which allows a better understanding of the relationships between species within the same genus, successful taxonomic classification, and greater ability to identify species and cultivars (Aguirre et al. 2017). A number of molecular marker technologies exist, each with different advantages and disadvantages. Molecular markers have great potential to identify the structure and genetic diversity of accessions (Raveendar et al. 2016). According to Suresh et al. (2014) genetic diversity analysis is important for collections, conservations and sustainable utilization of Genbank accessions. The tomato is one of the most important vegetable crops globally. It is a dicot species belonging to the family of *Solanaceae*. Different types of DNA markers were used to estimate the genetic diversity and phylogenetic relationship among tomato genotypes (Klein-Lankhorst et al. 1991, Kwon et al. 2009, Geethanjali et al. 2011). Microsatellites are valuable as molecular markers, particularly for studies for closely related individuals. Microsatellites also known as simple sequence repeats (SSRs), or simple sequence length polymorphisms, consist of tandemly repeated motifs of 2 to 6 bp and are a common feature of most eukaryotic genomes. SSRs markers have several advantages. They are co-dominant, meaning that heterozygous can be discerned from the homozygous. The markers are easily automated and it is possible to multiplex several markers with non-overlapping size ranges on a single electrophoresis run. The obtained results are highly reproducible. Many studies have described the application of SSRs to reveal polymorphisms in tomatoes (Smulders et al. 1997, He et al. 2003, Villalta et al. 2005,Garcia-Martinez et al. 2006, Mazzucato et al. 2008, Geethanjali et al. 2010, Geethanjali et al. 2011). The informative amount of DNA markers can be quantitatively measured statistically by means of PIC (polymorphism information content). The locus LEEF1Aa was included in the research studies of Arens et al. (1995), Smulders et al. (1997), Bredemeijer et al. (1998), Villalta et al. (2005), Garcia-Martinez et al. (2006), Mazzucato et al. (2008). The aim of present study was to evaluate the potential of the locus LEEF1Aa in genetic differentiation among six different tomato varieties of *Lycopersicon esculentum* Mill.

Material and methods

In this research, the plant material was obtained from the GeneBank of the Agricultural Institute in Skopje.Six morphologically different tomato varieties of *Lycopersicon esculentum* Mill. (var. *grandifolium* from subsp. *cultum*; var. *cerasiforme* – red and yellow, var. *pruniforme*and var. *pyriforme* from subsp. *subspontaneum*; and var. *racemigerum* from subsp. *spontaneum*) were used. The DNA isolation and optimization of the PCR conditions were performed in the Laboratory for biochemistry and molecular biology within the Department of Biochemistry and Genetic Engineering at the Faculty of Agricultural Sciences and Food – Skopje (Miskoska – Milevska et al. 2012). The DNA isolation from fresh leaves was performed using Promega's Wizard ® Genomic DNA purification kit. The leaves were collected from ten individual plants per each variety. Also, DNA isolation was donefrom pooled seeds. The DNA isolation from seeds was performed using modified CTAB method (Doyle and Doyle 1987, Cullings, 1992, Miskoska – Milevskaet al. 2011). The quality of the isolated DNA was checked by running it on 0.8% agarose gel. The optimization of the PCR conditions for amplification of the locus LEEF1Aawas done using appropriate primers, produced by Operon, Huntsville, AL. Some general data for the locus LEEF1Aa and appropriate primer pair are showed in Table 1 (Miskoska-Milevska et al. 2012).The PCR products were visualized by running them on 2% agarose gel, stained with ethidium bromide and photographed under UV light by using G-Box system (Sygene).

Table 1. General data for microsatellite locus LEEF1Aa and primers used in this study

Locus	Repeat motif	Primer sequences (5'-3')
LEEF1	$Aa(TA)_8(ATA)_9$	F: M13-aaa taa tta gct tgc caa ttg
		R: ctg aaa gca gca aca gta ttt

F - Forward primer (5'-3') R - Reverse primer (5'-3') M13 tail: 5'-cac gac gtt gta aaa cga c-3'

The fragment analyses of the PCR products were performed on *Applied Biosystems* DNA analyzer (*ABI 3130*) using *GeneMapper®Software program*. The data analyzing was done by the specific program *Power Marker Software*.

Results and discussion

The analysed microsatellite primers gave amplification across all researched tomato varieties and were used for fragment analyses. The fragment analyses of the locus LEEF1Aa detected six allelic variances (219-221-223-225-227-229 bp)and were presented in the form of electropherograms (Fig. 1 and Fig. 2).One of these alleles (229 bp) was specific for *Lycopersicon esculentum* subsp. *cultum* var. *grandifolium*. For locus LEEF1Aa, Arens et al. (1995) detected six alleles (193-213 bp), even if they expected allelic variance in size 131 bp. According to Arens et al. (1995) generation of products which exceeded the expected sizes could be due to the deletions in the original sequences, or insertions in the cultivars used in their study. But, to be resolved this amplification should be performed on the cultivars from which the sequences were derived. Smulders et al. (1997) reported 8 different alleles in researched tomatoes, while Bredemeijer et al. (1998) detected 7 different alleles (198-200-202-204-206-208-213 bp). Only one allele (200 bp) was noticed by Villalta et al. (2005), while Garcia-Martinez et al. (2006) found 10 alleles in size range from 165 to 226 bp. The biggest number of alleles (13) was detected by Mazzucato et al. (2008). Many factors can be the reason for the differences in allele number and size, between this research and the researches mentioned above. Firstly, different plant material can be reason for that. In this research tomato varieties from subsp. *spontaneum*, subsp. *subspontaneum* and subsp. *cultum* were used, while Bredemeijer et al. (1998) and Arens et al. (1995) included only cultivated tomato accessions. Also, Smulders et al. (1997) and Mazzucato et al. (2008) worked on cultivated and wild tomatoes. A collection of traditional tomato cultivars was studied by Garcia-Martinez et al. (2006). The second reason for the differences in allele number and size could be the methodological approach. Working on same DNA analyser with the same working conditions is the best option (Miskoska-Milevska et al. 2017).

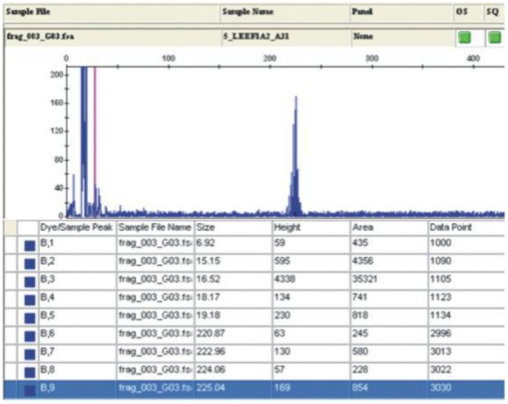

Fig. 1.Electropherograms of locus LEEF1Aa

The frequencies of allelic variances are presented in Fig. 3. The allelic variant of 219 bp was noticed for the locusLEEF1Aa in *Lycopersicon esculentum* subsp. *subspontaneum* var. *cerasiforme* (yellow), *Lycopersicon esculentum* subsp. *subspontaneum* var. *Pruniforme* and *Lycopersicon esculentum* subsp. *subspontaneum* var. *pyriforme.* The allelic variant in size of 221 bp was found for the locusLEEF1Aa in *Lycopersicon esculentum* subsp. *cultum* var. *grandifolium, Lycopersicon esculentum* subsp. *subspontaneum* var. *pruniforme, Lycopersicon esculentum* subsp. *subspontaneum* var. *pyriforme* and *Lycopersicon esculentum* subsp. *spontaneum* var. *racemigerum.*

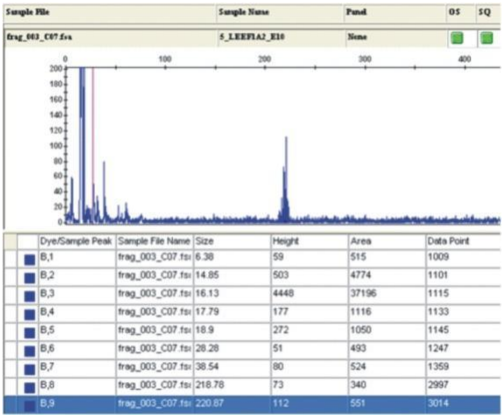

Fig. 2. Electropherograms of locus LEEF1Aa

The allele of 223 bp appeared on the locusLEEF1Aa in *Lycopersicon esculentum* subsp. *subspontaneum* var. *cerasiforme* (red) and *Lycopersicon esculentum* subsp. *spontaneum* var. *racemigerum.* The allelic variant in size of 225 bp was noticed for the locusLEEF1Aa across all analysed tomato varieties, with exception of *Lycopersicon esculentum* subsp. *subspontaneum* var. *pruniforme* and *Lycopersicon esculentum* subsp. *subspontaneum* var. *pyriforme.* The allele of 227 bp appeared on the locusLEEF1Aa across all analysed tomato varieties, only in *Lycopersicon esculentum* subsp. *subspontaneum* var. *cerasiforme* (red) and *Lycopersicon esculentum* subsp. *subspontaneum* var. *pyriforme* was not found. The allelic variant of 229 bp was detected for the locusLEEF1Aa only in *Lycopersicon esculentum* subsp. *cultum* var. *grandifolium*.

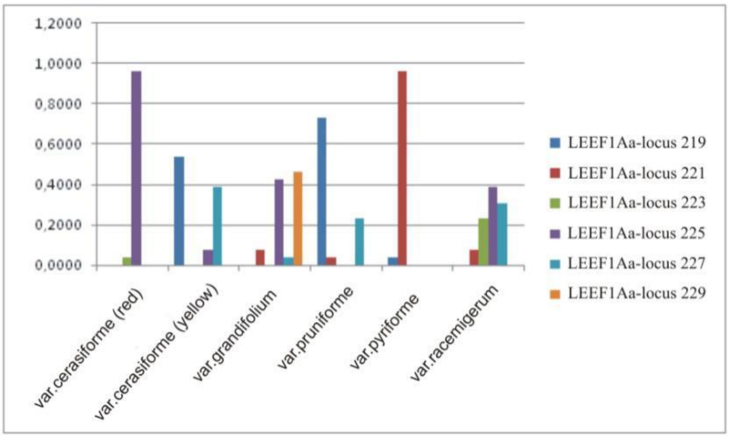

Fig. 3. Allelic variances and their frequencies for locus LEEF1Aa

In *Lycopersicon esculentum* subsp. *subspontaneum* var. *cerasiforme* (red) and *Lycopersicon esculentum* subsp. *spontaneum* var. *racemigerum* was detected the biggest allele frequency for allelic variant of 225 bp and its values were 0.9615 and 0.3846 respectively. For *Lycopersicon esculentum* subspvar. *Cerasiforme* (yellow) and *Lycopersicon esculentum* subsp. *subspontaneum* var. *pruniforme,* the biggest allele frequency was found for allele of 219 bp and its values were 0.5385 and 0.7308 respecively. In *Lycopersicon esculentum* subsp. *cultum* var. *grandifolium*, the biggest allele frequency was noticed for allelic variant of 229 bp and its value was 0.4615. For *Lycopersicon esculentum* subsp. *subspontaneum* var. *pyriforme,* the biggest allele frequency was found for allelic variant of 221 bp and its value was 0.9615 (Fig. 3). The average observed heterozygosity for the locus LEEF1Aa (0.1026) was lower than average expected heterozygosity (0.7872).This indicates on reduced level of heterogeneity in the analyzed tomatoes (Table 2). PIC-test determines informativeness of polymorphic DNA microsatellite loci and it is very important in analyses of the usefulness of microsatellite loci. In the researched tomato varieties average PIC value for the locus LEEF1Aa was 0.7552 (Table 2) and according to classification of Botstein et al. (1980), this locus belongs to the group of high informative markers.

Table 2. Genetic variability and polymorphism of locus LEEF1Aa in the researched tomato varieties

Locus	Number of genotypes	Number of alleles	*He*	*Ho*	PIC
LEEF1Aa	9.0000	6.0000	0.7872	0.1026	0.7552

He – expected heterosigosity;*Ho* – observed heterosigosityPIC-test for determination of informativeness for analysed DNA microsatellite locus

The genetic differentiation test in the investigated tomato varieties showedmajorgenetic differentiation for the locus LEEF1Aa (0.5006). Also, this test presentedmajordifferentiation for the locus LEEF1Aaon subspecies level (0.6167) (Miskoska-Milevskaet al. 2015).

Conclusions

Obtained results indicated that the locus LEEF1Aa gave amplification across all estimated tomato varieties. The number of detected allelic variants for this microsatellite locus was six in the researched tomato varieties. Only one specific allele of 229 bp in *Lycopersicon esculentum* subsp. *cultum* var. *grandifolium* was found. Received data showed that this microsatellitelocus isgood a choice for genetic differentiation of tomato varietiesin combination with other polymorphic microsatellite loci.

References

1. Aguirre, N.C., Lopez, W., Orozco-Cardenas, M., Coronado, Y.M. and Vallejo-Cabrera, F. (2017).Use of microsatellites for evaluation of genetic diversity in cherry tomato. Bragantia, 76 (2): 220-228.
2. Arens, P., Bredemeijer, G., Smulders, M.J.M. and Vosman, B. (1995). Identification of tomato cultivars using microsatellites. ActaHorticulturae, 419: 49-57.
3. Botstein, D., White, R.L., Skolinick, M. And Davis, R.W. (1980). Construction of genetic linkage hjn7u6my map in a man using restriction fragment length polymorphism. American Journal of Human Genetics, 32:314-331.
4. Bredemeijer, G.M.M., Arens, P. and Wouters, D. (1998).The use of semi-automated fluorescent microsatellite analysis for tomato cultivar identification. Theoretical and Applied Genetics, 97:584-590.
5. Cullings, K.W. (1992): Design an d testing of a plant-specific PCR primer for ecological and evolutionary studies.Molecular ecology, 1(4): 233-240.
6. Doyle, J.J and Doyle, J.L. (1987).A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phitochem. Bull., 19: 11-15.
7. Garcia-Martinez, S., Andreani, L., Garcia-Gusano, M., Geuna, F. and Ruiz, J.J. (2006). Evaluation of amplified fragment length polymorphism and simple sequence repeats for tomato germplasm fingerprinting utility for grouping related traditional cultivars, Genome, 49: 648-656.
8. Geethanjali, S., Chen, K.Y., Pastrana, D.V. and Wang, J.F. (2010).Development and characterization of tomato SSR markers from genomic sequences of anchored BAC clones on chromosome 6.Euphytica, 173: 85-97.
9. Geethanjali, S., Kadirvel, P., de la Pena, R., Rao, E.S. and Wang, J.F. (2011). Development and characterization of tomato SSR markers from genomic sequences of anchored BAC clones on chromosome 12 and their application for genetic diversity analysis and linkage mapping. Euphytica, 178: 283-295.
10. He, C., Poysa, V. and Yu, K. (2003). Development and characterization of simple sequence repeat (SSR) markers and their use in determining relationships among *Lycopersicon esculentum* cultivars. Theoretcial and Applied Genetics, 106(2):363-373.
11. Klein-Lankhorst, R.M., Vermunt, A., Weide, R., Liharska, T. and Zabel. (1991). Isolation of molecular markers for tomato (*L. esculentum*) using random amplified polymorphic DNA (RAPD). Theoretcial and Applied Genetics,83: 108-114.
12. Kwon, Y.S., Park. S.G. and Yi, S.I. (2009).Assesment of genetic variation among commercial tomato (*Solanum lycopersicum* L.) varieties using SSR markers and morphological characteristics. Gens Genom, 31: 1-10.
13. Mazzucato, A., Papa, R., Bitocchi, E., Mosconi, P., Nanni, L., Negri,V., Picarella, M.E., Siligato, F., Soressi, G.P., Tiranti, B. and Veronesi, F. (2008). Genetic diversity, structure and marker-trait associations in a collection of Italian tomato (*Solanum lycopersicon*L.) landraces.Theoretcial and Applied Genetics,116: 657-669.
14. Miskoska-Milevska, E.,Popovski, Z.T., Dimitrovska, B.R. and Porcu, K.D. (2011). Isolation of DNA for fragment analyses from tomato leaves and seeds. Conference of biotechnology with international participation. Faculty of Agronomy, Čačak, Serbia, 16(18): 59-64.
15. Miskoska-Milevska, E., Dimitrovska, B.R. and Popovski, Z.T. (2012). Optimization of PCR conditions to amplify DNA microsatellites in tomato (*Lycopersiconesculentum* Mill.). Internorational Symposium for Agriclture and Food, Faculty of Agricultural Sciences and Food, Skopje, Macedonia, 626-634.
16. Miskoska-Milevska, E., Popovski, T.Z., Dimitrievska, R.B. and Bandzo, K. (2015). DNA microsatellite analyses for tomato genetic differentiation.Genetika, 47(3):1123-1130.
17. Miskoska-Milevska, E., Popovski, T.Z. and Nestorovski, T. (2017).Usefulness of a locus LE21085 in the genetic differentiation of tomato varieties.NotulaeScientiaBiologicae, 9 (2): 209-213.

18. Raveendar, A., Jong-Wook, C., Gi-An, L., Jung-Ro, L., Kyung-Jun, L., Myoung-Jae, S., Yang-Hee, C. and Kyung-Ho, Ma.(2016). Genetic diversity and population structure of Asian tomato accessions based on simple-sequence repeats.Plant. Breed. Biotech., 4 (3): 306-314.
19. Smulders, M.J.M., Bredemeijer, G., Rus-Kortekaas, W., Arens, P. and Vosman, B. (1997). Use of short microsatellites from database sequences to generate polymorphisms among *Lycopersicon esculentum* cultivars and accessions of their Lycopersicon species.Theoretcial and Applied Genetic., 97: 264-272.
20. Suresh, S., Chung, J.W., Cho, G.T., Sung, J.S., Park, J.H., Gwag, J.G. and Baek, H.J. (2014).Analysis of molecular genetic diversity and population structure in Amaranthus germplasm using SSR markers. Plant Biosyst., 148: 635-644.
21. Villalta,I., Reina-Sánchez, A., Cuartero, J., Carbonell, A.E. and Asins, M.J. (2005). Comparative microsatellite linkage analysis and genetic structure of two populations of F_6 lines derived from *Lycopersicon pimpinellifolium* and *L. cheesmanii*. Theoretcial and Applied Genetics, 110: 881-894.

EVALUTION OF THE EFFECT OF THE NEW FRACTION PAIR 1Dx1.5+1Dy10 INHERITED FROM Glu-D^t LOCUS OF Ae. Tauschii (D^{t}D^t, 2n=14) ON SOME QUALITATIVE INDICES IN THE PROGENIES OF SYNTHETIC WHEAT VARIETIES (BBA^{u}A^{u}D^{t}D^t, 2n=42)

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Abstract
The effect of the new fraction pair 1.5+10, coded by *Glu-D1* locus, on the qualitative indices protein, lysine and sedimentation value in synthetic hexaploid wheat varieties was investigated in comparison to common winter wheat standards. For this purpose, in two successive cropping seasons (2013 and 2014), grains from plants of the progenies of the synthetic haploid wheat genotypes 106, 107 and 32 were analyzed; preliminary SDS-PAGE analysis determined that they were carriers of the new allele *ah* inherited from the *Glu-D^{t}1* locus of *Ae. tauschii*. Data were analyzed with the help of descriptive statistics, t-criterion, correlation and regression analyses, which were at the basis of the conclusions made. The obtained results allow the assumption that in the synthetic hexaploid wheat genotypes the new allele *ah*, coding for the expression of the new fraction pair 1.5+10 in D-genome, has positive effect on the qualitative indices protein, lysine and sedimentation in contrast to the standard common wheat cultivars, which do no carry this subunit.

Keywords: protein, lysine, sedimentation, HMW-GS.

Introduction

Common winter wheat *Triticum aestivum* L. (2n=6x=42, AABBDD) is distributed on large areas in many parts of the world due to its high plasticity to the growing environments and the universal usage of the wheat plant. After the 1960's, the efforts of the researchers have been directed toward intensive wheat breeding. The goal has been to induce genetic changes in *Triticum aestivum* in order to ensure more food of higher quality for the increasing global population. In the following years, a number of high-yielding cultivars with good adaptability to biotic and abiotic stress were developed as a result from the improved growing technology. Wheat production in China, India, Pakistan, Egypt, Canada, etc., increased considerably giving sustenance to millions of people. However, the intensive breeding of bread wheat based on a limited set of cultivars lead to loss of valuable genes in the new crosses and to a standstill in the yield potential of the crop. The major challenge to contemporary breeding is to preserve the valuable traits and genes from the wild species by applying the distant hybridization method, and to further transfer them in new bread wheat lines with a view of their use as initial breeding material in breeding (Spetsov, 2004; Jauhar and Peterson, 2006; Jauhar et al., 2009; Plamenov and Spetsov, 2011; Khalid, 2013; Gurmani et al., 2014; Daskalova, 2015). Thus the resistance to fungal diseases and insect pests can be successfully increased, as well as the productivity and quality of the cultivated wheat. In this relation, hexaploid wheat forms have been developed, which can be considered a bridge between the wild and the cultural forms (Carderini and Ortiz-Monasterio, 2003; Rana et al., 2013). They are carriers of valuable prolamin alleles (glutenin and gliadin) inherited from the parental forms *Ae. tauschii* and *T. turgidum* ssp. *durum* (Wieser et al., 2003; Yang et al., 2009; Lage et al., 2006; Hu et al., 2013), many of which have positive effect on the end-use technological indices of flour (Peña et al., 1995; Hsam et al., 2001; Wieser et al., 2003; Lage et al., 2006; Tang et al., 2008; Tang et al., 2010). The main aim of this investigation was to evaluate the effect of the new fraction pair 1.5+10, coded by locus *Glu-*

D1, on some qualitative indices (protein, lysine and sedimentation) in synthetic amphidiploids developed with the participation of the diploid species *Ae. tauschii*.

Material and methods

The materials (Table 1) subjected to this investigation were progenies of three synthetic hexaploid wheat forms (SHW) – No 106, 107 and 32; in two successive harvest years (2013 and 2014) grains from selected elite synthetic plants were analyzed for content of raw protein (%), lysine (mg/100 g absolute dry matter) and sedimentation (ml). By preliminary SDS-PAGE, it was found that the grains carried the new allele coding for the subunits pair 1.5+10 inherited from the *Glu-D*^{t} locus of accessions from the diploid species *Ae. tauschii* - No 22744 and No 19089. The qualitative indices (protein, lysine and sedimentation) of common wheat cultivars used as standards were also analyzed for comparison (Table 1). The progenies from SHW No 106, 107 and 32 were grown under field conditions during cropping seasons 2013 and 2014 together with standard common wheat cultivars. Planting was done manually in mid-October in plots of 1.5 m rows, and interspacing 5 cm between and plants and 20 cm between the rows. Eighty seeds, the progenies from four plants of SHW 106 were sown in two replications, as well as 140 seeds from 7 plants of SHW 107, 160 seeds from 8 plants of SHW 32 and bread wheat cultivars used as standards (Sadovska ranozreika-4, M. amber, No 301, ussalka, San Pastore, Bezostaya-1 and Mironovskaya-808). The previous crop was common bean without nitrogen fertilization.

Table 1. Harvested SHW No 106, 107 and 32 and standards for analysis of qualitative indices

Breeding No	Cross No	Grown progenies	Grown plants
107	45398 x *Ae. tauschii* 22744	7	112
106	F_2 (44961x Zagorka x 45432) x *Ae. tauschii* 22744	4	72
32	F_1 (44961x Zagorka x 45432) x *Ae. tauschii* 19089	8	136
T. aestivum:	San Pastore, M. amber, cultivar No 301, Russalka, Sadovska Ranozreika-4, Mironovskaya - 808, Bezostaya-1	2 rows per cultivars in two replications	

The extraction of the high molecular weight glutenins was carried out by the method of Singh et al. (1991). The electrophoresis was run in a vertical apparatus as standard monomeric polyacrylamide gel electrophoresis on 12 % separating gel (Laemmli, 1970) at constant electric current of 20 mA per plate and room temperature, for 10-20 hrs. The gels were stained in 1 % solution of Coomassie Brilliant Blue (CBB) R 250 in acetic acid, methanol and water at ratio (1:5:4) overnight, and destained in a solution of acetic acid, methanol and distilled water (1:2:7). The quality indices protein (%), lysine (mg/100 g abs. dry matter) and sedimentation (ml) were determined by: the classical Kjeldahl method for determination of total nitrogen using Keltec equipment (Cohen, 1910); the ninhydrin method for determination of lysine content of Museiko and Sysoev (1970), and the method of Pumpyanskyi (1971) for determination of the sedimentation value. To estimate the effect of the new fraction pair 1.5+10 from *Glu-D1* locus on the qualitative indices (protein, lysine and sedimentation), the statistical packages SPSS 17.0 and Biostat 7.0 (Penchev, 1998) were used. The methods of descriptive statistics, the t-criterion (criterion of Student), correlation and linear regression analyses were applied. Based on the data from the regression analysis, regression equations of the type $Y = b*X + a$ (a – regression constant; b – regression coefficient) were composed, which represented the functional relation between the two variables – protein (X) and sedimentation (Y). This relation was represented also through regression lines, which allowed determining the sedimentation values positioned on the ordinate through the values of the protein positioned on the abscissa.

Results and discussion

In two successive cropping seasons, 2013 and 2014, grains from selected plants of progenies of the synthetic aphidiploids No 106, 107 and 32 were analyzed through SDS-PAGE electrophoresis. It was found that all three synthetic forms carried the new allele *ah* coding for fraction pair 1.5+10, which was inherited from locus *Glu-D*^{t} of the diploid species *Aegilops tauschii* No 22744 (SHW No 107 and 106) and *Aegilops tauschii* No 19089 (SHW No 32) (Figure 1).

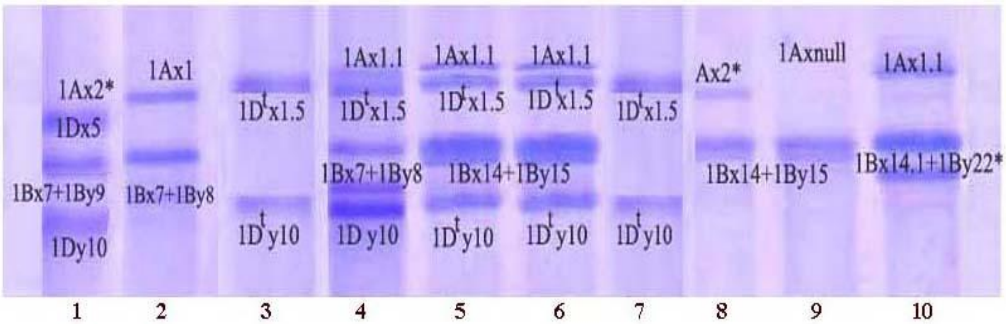

Figure 1. High molecular glutenin allelic composition of SHW: 1. Bezostaya1 (standard), 2. No 45398 (*T.dicoccum*, parent), 3. No 2744 (*Ae. tauschii*, parent), 4.SHW No 107, 5.SHW No 106, 6.SHW No 32, 7.No 19089 (*Ae. tauschii*, parent) 8. Zagorka (*T.durum*, parent), 9.No44961 (*T.dicoccum*, parent), 10. 45432 (*T.dicoccum*, parent)

Tables 2 and 3 present the statistical parameters of the qualitative indices obtained from the analysis of the grain from the three synthetic wheat forms and from the standard common wheat cultivars. The mean value of the index protein in the synthetic wheat forms significantly exceeded the standards in both years of the investigation. This is a confirmation of the findings of Peña et al. (1995) that the lines which carry fraction pair 1.5+10 in *Glu-D1* locus have higher protein content in the flour. Concerning the indices lysine and sedimentation, the tendency toward higher mean values of the SHW forms in comparison to the standard bread wheat cultivars was present in both harvest years; this was yet another proof of the positive effect of the new allele *ah*, inherited from the *Ae. tauschii* accessions in the synthetic forms. The obtained estimations about the variation coefficients (VC%; Tables 2-3) revealed stability with regard to protein, lysine and sedimentation in the aphidiploids. In contrast, a more significant variation of the separate parameters was observed in the standard cultivars, of sedimentation in particular. These results allow the assumption that the new fraction pair 1.5+10 has a positive effect on the analyzed qualitative indices. T-test was applied for examination of the differences between the mean values of protein, lysine and sedimentation in SHW and common winter wheat. The results are given in Table 4. The difference determined between the mean values of the index protein in the amphidiploids and the standards during the two harvest years was statistically significant at the highest level – 11.28*** (harvest year 2013) and 13.66*** (harvest year 2014). The same tendency was registered also for the index lysine – 12.23*** (harvest year 2013) and 12.64*** (harvest year 2014). For sedimentation, the difference between the means of the synthetic forms and the common wheat cultivars (2.69* - harvest year 2013; 2.80* - harvest year 2014) was less significant, at level P = 0.05 of the alternative hypothesis. The obtained results lead to the conclusion that the investigated allele (*ah*) in the amphidiploids was the main reason for the existing differences. To examine this hypothesis, the correlation coefficients between the qualitative indices of SHW and the standards were calculated (Table 5).

Table 2. Statistical parameters of protein, lysine and sedimentation in SHW and standard common wheat cultivars during harvest year 2013

Trait	SHW			Standards		
	2013					
	Xmean[1]	MS [2]	VC%[3]	Xmean[1]	MS [2]	VC%[3]
Raw protein, %	14.41±0.11	1.19	3.31	11.81±0.20	0.54	4.56
Lysine, mg/100g a.d.m.	305.31±2.14	9.31	3.05	247.00±4.26	11.28	4.56
Sedimentation, ml	63.68±0.84	3.67	5.76	54.57±3.29	8.70	15.93

[1] Means; [2] Mean square deviation; [3] Variation coefficient

Table 3. Statistical parameters of protein, lysine and sedimentation in SHW and standard common wheat cultivars during harvest year 2014

Trait	SHW			Standards		
	2014					
	Xmean[1]	MS [2]	VC%[3]	Xmean[1]	MS [2]	VC%[3]
Raw protein, %	15.75±0.06	0.27	1.74	12.32±0.24	0.64	5.23
Lysine, mg/100g a.d.m.	337.42±1.98	8.64	2.56	261.42±5.67	15.01	5.74
Sedimentation, ml	65.84±0.86	3.76	5.71	57.57±4.04	10.69	18.57

[1] Means; [2] Mean square deviation; [3] Variation coefficient

Table 4. Estimation of the differences between the means of the qualitative indices in SHW and the standards during harvest years 2013 and 2014 through t-test

	Protein SHW/standards				Lysine SHW/standards				Sedimentation SHW/standards			
	texp[1]	$t_{5\%}$[2]	$t_{1\%}$[2]	df[3]	t exp[1]	$t_{5\%}$[2]	$t_{1\%}$[2]	df[3]	t exp[1]	$t_{5\%}$[2]	$t_{1\%}$[2]	df[3]
1	11.28***	1.81	2.23	10	12.23***	1.83	2.26	9	2.69 *	1.89	2.36	7
2	13.66***	1.89	2.36	7	12.64***	1.86	2.31	8	2.80 *	1.89	2.36	7

[1] t-criterion; [2] t-table; [3] degrees of freedom; 1-2013; 2-2014
*,**,*** *at P ≤ 0.05;0.01;0.001, respectively*

In harvest year 2013, a high positive correlation was found in SHW and the standards between the qualitative indices protein and lysine – 0.903*** (SHW) and 0.999*** (standards). At a lower level of statistical significance, positive correlations were established between lysine and sedimentation (0.564**, P=0.01) and between protein and sedimentation (0.375*, P=0.05) in the synthetic forms, while in the standard cultivars the correlations between protein and sedimentation (-0.051) and between lysine and sedimentation (-0.074) were low and negative (Table 5).

Table 5. Correlations between the qualitative indices in SHW and the standards

Year	Synthetic hexaplod wheat			Standards		
2013		Lysine	Sedimentation		Lysine	Sedimentation
	Protein	0.903***	0.375*	Protein	0.999***	-0.051
	Lysine		0.564**	Lysine		-0.074
2014		Lysine	Sedimentation		Lysine	Sedimentation
	Protein	0.740***	0.385*	Protein	0.965***	0.476*
	Lysine		0.161	Lysine		0.416*

In harvest year 2014, the same tendency was observed toward high positive correlations between protein and lysine – 0.740*** (SHW) and 0.965*** (standards). In the synthetic forms, the correlations between raw protein and sedimentation (0.385*) and lysine and sedimentation (0.161) were less expressed than in 2013, although remaining stable and positive. In the standards, in contrast to 2013, there were positive correlations significant at a lower level between protein and sedimentation (0.476*) and lysine and sedimentation (0.416*) (Table 5). The estimation of the correlation coefficients revealed that they were largely dependant on the climatic conditions, i.e. the effect of the phenotype in the different cropping seasons on the correlation between the qualitative indices in SHW was much better expressed in comparison to the effect of the new fraction pair 1.5+10. In order to specify the investigated correlations, linear regression analysis was applied, in which sedimentation was the dependent variable (Y), and protein was the independent one (X). The established type of relationship between the two qualitative indices in both investigated cropping seasons showed that the trends in SHW and the standards were the same, i.e. the effect of the agro meteorological conditions was much stronger than the effect of the new alleles. The calculated regression equations were an evidence for this, as well as the graphic and analytical models of the amphidiploid and the common wheat cultivars in cropping seasons 2013 and 2014 (Figure 2A-B; Figure 3A-B).

Y = 2.9*X + 22.1 (A) Y = 2.4*X + 26 (B)

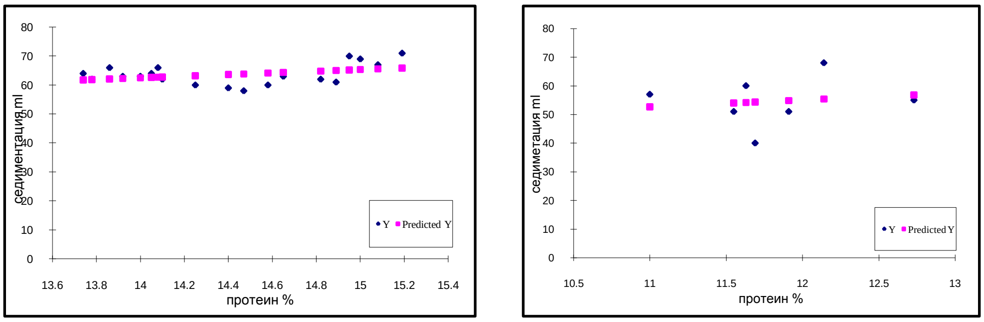

Figure 2. Graphic model sedimentation/protein in SHW (A) and standard (B), harvest year 2013

Y = 5.3*X – 17.1 (A) Y = 7.9*X – 39.9 (B)

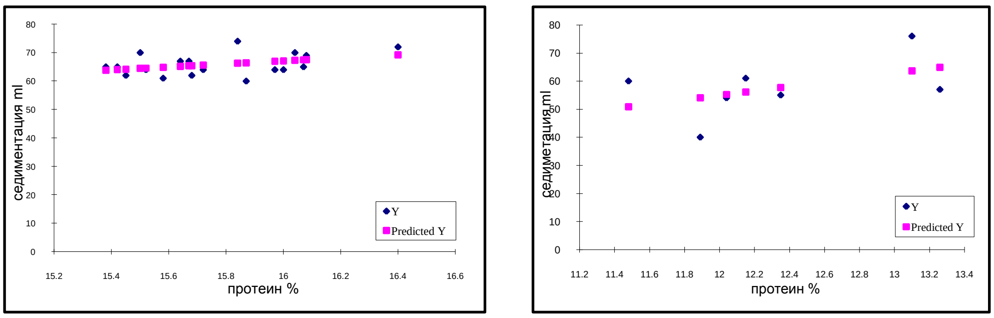

Figure 3. Graphic model sedimentation/protein in SHW (A) and standards (B), harvest year 2014

The evaluations in this investigation of the effect of the new allele *ah* from *Glu-D1* locus on the qualitative indices protein, lysine and sedimentation in the progenies of synthetic hexaploid wheat forms No 106, 107 and 32 were confirmed by the results from similar studies of other authors, as well. Ali et al. (2013) investigated the glutenin composition and the main qualitative parameters: raw protein content (%) and SDS-sedimentation volume of 24 conventional common wheat cultivars and 26 hexaploid wheat forms developed with the participation of synthetic forms; they found out that the synthetic hexaploid derivatives were characterized with considerable genetic variability in loci *Glu-A1* (0.38) and *Glu-D1* (0.59), and with higher protein content (13.8±0.9) and SDS-sedimentation volume (3.5±0.3) in comparison to the bread wheat cultivars. Analyzing 202 SHW lines, Rasheed et al. (2012[a]) identified a number of new *Glu-D1* alleles (*h*, *n*, *x*, *ae*, *ah*, *ai*, *aj*) coding for subunits 5+12, 2.1+10, 2+12.2, 2.1+12.2, 1.5+10, 2.1+10.5 and 1.5+12. When investigating their effect on the end breadmaking properties, they found out that the synthetic derivatives which possessed fraction pairs 2.1+10 and 1.5+10 had higher protein content in comparison to those possessing 2.1+12.2. On the other hand, the lines with fraction pairs 1.5+10, 2.1+12.2, 5+12 and 3+10 had higher bread volume and higher sedimentation. Wieser et al. (2003) investigated the correlations between the amount of the gluten proteins and the technological indices of synthetic hexaploid forms and found out that neither the raw protein content nor the total amount of gluten protein was in correlation with any of the studied quality parameters, although the protein content in flour is a very important factor in bread making. On the other hand, they obtained data on existing positive correlations between the high and low molecular weight glutenin subunits and the SDS-sedimentation volume, gluten index, maximum resistance of dough and bread loaf, while the gliadins to glutenins ratio was in negative correlation with some of the above indices (bread volume, maximum resistance of dough). The results from the investigations show that the researches on the genetics of the qualitative indices are important for the improvement of the breeding process since these results allow determining the relationships between the composition of the storage endosperm proteins and the technological properties of grain. (Tabasum et al., 2011; Carter et al., 2012).

Conclusions

The statistical data on the effect of the new fraction pair 1.5+10 on the content of raw protein, lysine and sedimentation allow drawing the following conclusions:

1. As a result from the positive influence of the new allele inherited in synthetic hexaploid wheat forms No 106, 107 and 32 from the D-genome *Aegilops tauschii*, higher mean values of protein, lysine and sedimentation were determined in comparison to the standard common wheat cultivars.
2. The variation coefficients of the investigated qualitative indices showed higher stability of the synthetic forms due to the effect of the new allele.
3. The correlations and the regression equations determined that the relationships between protein, lysine and sedimentation in SHW were unidirectional with the relationships in the standard cultivars, i.e. the effect of the phenotype on the correlations between protein, lysine and sedimentation was much better expressed as compared to the effect of the new fraction pair from locus *Glu-D1*.

References

1. Calderini D. F., Ortiz-Monasterio I. (2003). Are synthetic hexaploids a means of increasing grain element concentrations in wheat? Euphytica, 134: 169-178.
2. Carter A.H., Garland-Campbell K., Morris C.F., Kidwell K.K. (2012). Chromosomes 3B and 4D are associated with several milling and baking quality traits in soft white spring wheat (*Triticum aestivum* L.) population. Theor. Appl. Genet., 124: 1079-1096.
3. Cohen J. B. (1910). Practical Organic Chemistry.
4. Gurmani A.R.,, Khan S.U., Mabood F., Ahmed Z., Butt S., Din J., Mujeeb-Kazi A. and Smith D. (2014). Screening and selection of synthetic hexaploid wheat germaplasm for salinity tolerance based on physiological and biochemical characters. Int. J. Agric. Biol., 16: 681-690.
5. Hsam S.L.K., Kieffer R., Zeller F. J. (2001). Significance of *Aegilops tauschii* glutenin genes on breadmaking properties of wheat. Cereal. Chem., 78(5): 521-525.
6. Hu X., Dai S., Pu Z., Liu D., Pu Z., Jiang J., Wei Y., Wu B., Lan X., Zheng Y., Yan Z. (2013). Quality of synthetic hexaploid wheat containing null alleles at Glu-A1 and Glu-B1 loci. J. Genet., 92(2): 241-245.
7. Jauhar, P. P., Peterson T. C. (2006). Cytological analyses of hybrids and derivatives of hybrids between durum wheat and Thinopyrum bessarabicum, using multicolour fluorescent GISH. Plant Breed., 125:19-26.

8. Jauhar, P.P., T.S. Peterson, and S.S. Xu. (2009). Cytogenetic and molecular characterization of a durum alien disomic addition line with enhanced tolerance to Fusarium head blight. Genome 52: 467-483.
9. Khalid M., Mahmood T., Rasheed A., Kazi A., Ali A., Mujeeb-Kazi A. (2013). *Glu-D^{t}1* allelic variation in synthetic hexaploid wheats derived from durum "Decoy" x *Aegilops tauschii* accessional crosses. Pak. J. Bot., 45 (SI): 409-414.
10. Laemmli U.K. (1970). Clavage of structural proteins during the assembly of the head of bacteriofage T4. Nature, 227 (5259): 680-685.
11. Lage J., Skovmand B., Peña R. J., Anderson S. B. (2006). Grain quality of emmer wheat derived synthetic hexaploid wheats. Genet. Resour. Crop Evol., 53: 955-962.
12. Museiko A. S., Sysoev A. F. (1970). Determination of lysine in seeds, Diklad VASHNIL, 8-12.
13. Penchev E. (1998). Evaluation of productivity and quality indicators of wheat with mathematical models. Doctoral dissertation,Agricultural academy-Bulgaria.
14. Peña R.J., Zarco-Hernandez J., Mujeeb-Kazi A. (1995). Glutenin subunit compositions and breed-making quality characteristics of synthetic hexaploid wheats derived from *Triticum turgidum* x *Triticum tauschii* (coss.) Schmal Crosses. Journal of Cereal Science 21: 15-23.
15. Plamenov D., Spetsov P. (2011). Synthetic hexaploid lines are valuable resources for biotic stress resistance in wheat improvement. Journal of Plant Pathology, 93 (2): 251-262.
16. Pumpyanskyi A. Y. (1971). Micromethod of Determination of Swelling of Flour in Acidic Acid. Q: Technological properties of soft wheat. Ed. Kolos.L., 37-40.
17. Rana R.M., Bilal M., Rehman S., Iqbal F., Shah M. K. N. (2013). Synthetic wheats: a new hope for the hungry world. Asian J. Agri Biol., 1 (2): 91-94.
18. Singh N. K., Shepherd K. W, Cornish G. B. (1991). A simplified SDS-PAGE procedure for separating LMW subunits of glutenin. Journal of Cereal Science, 14: 203-208.
19. Tabasum A., Iqbal N., Hammed A., Arshad R. (2011). Evalution of Pakistan wheat germaplasm for bread quality based on allelic variation in HMW glutenin subunits. Pakistan journal of Botany, 43 (3): 1735-1740.
20. Tang Y., Yang W., Tian J., Li J., Chen F. (2008). Effect of HMW-GS 6+8 and 1.5+10 from Synthetic Hexaploid Wheat on wheat quality traits. Agricultural Science of China, 7: 1161-1171.
21. Tang Y., Yang W., Wu Y., Li C., Li J., Zou Y., Chen F., Mares D. (2010). Effect of high molecular weight glutenin allele, *Gu-B1d* from synthetic hexploid wheat on wheat quality parameters and dry, white Chinese noodle-making quality. Aust. J. Agric. Res., 61: 310-320.
22. Wieser H., Hsam S.L.K., Zeller F.J. (2003). Relationship between the qualitative and quantitative compositions of gluten protein types and technological properties of Synthetic Hexaploid Wheat derived from *Triticum durum* and *Aegilops tauschii*. Cereal Chem., 80 (3): 247-251.
23. Yang W.Y., Liu D., Li J., Zhang L., Wei H., Hu X., Zheng Y., He Z., Zou Y. (2009). Synthetic hexaploid wheat and its utilization for wheat genetic improvement in China. J. Genet. Genomics, 36: 539-546.

CHARACTERISTICS OF THE NEWLY CREATED GENOTYPE OF VIRGINIA TOBACCO LINE V-79/09 CMS F1 IN THE REGION OF PRILEP DURING 2012-2016

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Abstract
V-79/09 CMS F1 is a hybrid male sterile line created in the Scientific Tobacco Institute - Prilep in 2009 by intervariety hybridization. It was included in comparative trials in the period 2012/2016, performed at the Experimental field of the Tobacco Institute, along with introduced varieties as a check. Through the whole period of investigation, line V-79/09 CMS F1 showed better bio-morphological and productional properties compared to the check varieties. Due to its characteristics, it surpassed all other varieties and lines included in the investigation. Thus, the line starts to flower in 71.8 days, 50% flowering occurs in 76.6 days and end of flowering in 81.8 days from the date of planting. The average length and width of the 5th leaf are 52.1 cm and 36.8 cm, of the 10th leaf 69.3 cm and 39.9 cm, and of the 15th leaf 64.7 cm and 35.1 cm, respectively. Height of the stalk with inflorescence averages 213 cm and the number of leaves is 34.0. The yield was 3450 kg/ha, but it should be mentioned that throughout investigation, the line V-79/09 CMS F1 showed statistically significant differences compared to the check varieties. All these characteristics make this line interesting for tobacco industry.

Keywords: tobacco, Virginia, flowering, morphology, yield.

Introduction

Virginia tobacco raw is inevitable component in manufacture of blend cigarettes, accounting for about 60% of cigarette mixtures. This data clearly shows the great significance of this type of tobacco. According to Kalamanda (2009), Virginia tobaccos are basically skeletal, characterized by good technological properties, with higher sugar content and lower content of nitrogen. According to Risteski (2000), Virginia tobacco production in R. Macedonia was 1633 tons in the period 1976-1988 and 1475 tons in 1989 – 1997. Unfortunately, after 2002, Macedonian tobacco industry, despite its needs, ceized the production of Virginia and Burley tobacco. Today, Macedonian fabrication is completely dependent on the import of these two types. Little hope that the production will be restarted brought the Greek company SEKE, which organizes the production of several tens of hectares in Bitola. This should encourage other companies to follow their example. If producers are offered a good variety, the Virginia production should be increased to a greater extent. The optimism for rapid restarting of Virginia production should also be based on the fact that breeders at the Scientific Tobacco Institute - Prilep have already created several male sterile hybrid lines. According to Beljo (1996), in most of the properties, F1 hybrids are better than their parents. One of the successful male sterile hybrid lines is V-79/09 CMS F1 and it will be discussed in this paper.

Material and methods

In the period 2012-2016, comparative trials with Virginia tobacco varieties and lines of domestic and foreign origin were carried out in the field of the Scientific Tobacco Institute - Prilep, including the variety V-79/09 CMS F1 (Photo 1). During 2012/2013, the USA fertile variety K-324 was used as a check, in 2014/2015 the Australian fertile variety Ca-757 and in 2016 the American fertile variety Coker-348. The trials were set up on colluvial soil. The first plowing was done in the autumn at 40 cm depth. In spring, the plots were fertilized with 300 kg/ha N: P: K 8:22:20, and then ploughed two more times. Before transplanting, the plots were treated with selective herbicide and seedlings were planted in randomized block design with 4 replications at 90 × 50 cm planting density. Prior to second hoeing, 3 g / 26 % KAN was applied for nutrition of plants. During the periods of drought in July and August, additional irrigations and protection against diseases and pests were applied. During the growing season, three stages of flowering were recorded (beginning of flowering, 50% flowering and end of flowering). Morphological measurements in each variety were performed on 5 stalks and analysis was made of the 5th, 10th and 15th leaf, number of leaves and stalk height. After harvesting, the corrected yield per hectare was calculated using the the method of Rimker.

Photo. 1 Line V-79/09 CMS F_1

Results and discussion

The length of vegetation period (flowering)

The end of growth stage and the beginning of the reproductive stage, when flowers and seeds are formed, occurs at the beginning of flowering. According to Uzunoski (1985), tobacco flowering begins 10 days after butonization of the terminal bud. Naumoski et al. (1977) reported that tobacco plant has already built 90% of its mass by the end of flowering. S. N. Howks Jr. (1978) noted that some tobaccos with mammoth properties cause late flowering, forming a larger number of leaves on the stalk. Although the time of flowering is genetically regulated character in each tobacco variety, it can be strongly affected by climate conditions, applied cultural practices, irrigation, etc. The results for this character in line V-79/09 CMS F1 are presented in Table 1. According to the data, line V-79/09 CMS F1 had the earliest beginning of flowering in 2013 (66 days) and the latest in 2015 (77 days). In other years, this data ranges from 70 days in 2016 to 73 days in 2012 and 2014. The five-year average for this indicator is 71.8 days. The lowest number of days to 50% flowering was recorded in 2013 (73 days) and the highest in 2015 (81 days). In other sources this indicator ranges from 74 days in 2016 to 81 days in 2015 and the five-year average is 76.6 days. According to Beljo (1996), tobacco varieties which reach 50% flowering in 71-76 days belong to the group of tobaccos with moderate flowering time and those which need 76-80 days belong to the group of late-flowering tobaccos. Taking into account the average flowering time (76.6 days), it can be concluded that line V-79/09 CMS F1 is on the border between these two groups. The flowering time was completed in 79 and 85 days in 2016 and 2015, respectively. In other years of investigation this indicator ranges from 81 days in 2013 and 2014 to 83 days in 2012. The five-year average for this character is 81.8 days.

Table 1. The length of vegetation period of tobacco (flowering)

Year	Days from planting to the beginning of flowering, in days	Days from planting to 50% flowering	Days from planting to the end of flowering
2012	73	78	83
2013	66	73	81
2014	73	77	81
2015	77	81	85
2016	70	74	79
Average	71.8	76.6	81.8

Morphological properties

Morphological characteristics of tobacco are a very important indicator by which it is easy to determine the type of tobacco and the position of each leaf on the stalk. These characteristics are genetically controlled but they can be strongly affected by the agro-ecological conditions and applied cultural practices. The knowledge on morphological properties of tobacco type will help to easier determine its habitus, planting distance, more precise prognosis on its yield, etc. The carriers of yield in Virginia tobacco are the leaves from the middle belt (5th, 10th and 15th leaf). The number of leaves is also closely related to the yield, while stalk height shows the position and distribution of leaves and flowers on the stalk. Morphological properties of line V-79/09 CMS F1 are presented in Table 2.

Table 2. Morphological properties

Year	5th leaf		10th leaf		15th leaf		Stalk height, cm	Leaf number
	Length, cm	Width, cm	Length, cm	Width, cm	Length, cm	Width, cm		
2012	38.5	27.3	62.4	36.3	61.6	33.8	196	34.6
2013	44.4	32.4	65.6	37.4	62.8	33.8	198	34.8
2014	58.8	41.6	72.5	41.2	64.3	35.0	238	33.0
2015	60.3	42.5	74.3	42.8	66.3	36.5	228	34.3
2016	58.3	40.1	71.7	41.7	68.7	36.4	206	33.6
Average	52.1	36.8	69.3	39.9	64.7	35.1	213	34.0

According to the data presented in the table, all leaves analyzed during the five-year investigation exceed the length of 35 cm - the first condition to classify them as a first class-material. It can also be seen that the highest leaf length and width was achieved mostly in 2014 and 2015. The longest (60.3 cm) and widest (42.5 cm) 5th leaf was recorded in 2015. The five-year average shows that the 5th leaf is 52.1 cm long and 36.8 cm wide. The biggest length (74.3 cm) and width (42.8 cm) of the 10th leaf was also recorded in 2015. The average results show that the 10th leaf is 69.3 cm long and 39.9

cm wide. The biggest length and width of the 15th leaf was achieved in 2015 and 2016. Thus, in 2016 it was 68.7 cm long and 36.4 cm wide, while the average size of the 15th leaf was 64.7 cm in length and 35.1 cm in width. Kalamanda (2009), in his investigations in Republika Srpska with the varieties DH-17 and Hewisi-17 came to the conclusion that the average length and width of the middle belt leaves in DH-17 variety in 2006 was 49.20 cm and 25.40 cm, respectively. Devčić et al. (1982) reported average leaf width of 21 cm in varieties H-30 and H-31 and 20 cm in H-32. Practice have shown that the best length/width ratio of the leaves of Virginia tobacco is 2:1. According to Uzunoski (1985), morphological properties are highly variable under the influence of external environment and cultural practices. Thus, the height of the stalks can varies from 25 to 300 cm and more. However, these variations are also genetically controlled inside the type or variety of tobacco. The biggest stalk height in line V-79/09 CMS F1 was recorded in 2014 (238 cm) and in 2015 (228 cm). The average height of the stalk in the investigation period was 213 cm. According to Beljo (1996), tobaccos with a height of 201-220 cm belong to the group of high tobaccos. Risteski (1999) reported that stalks of MV-1 variety, which are planted at higher density, are higher. The highest leaf number was recorded in 2012 and 2013 (34.6 and 34.8, respectively). The average results in the test period show that the number of leaves in line V-79/09 CMS F1 is 34.0. According to Beljo (1996), tobacco varieties which number of leaves ranges between 33 and 36 belong to the group of tobacco with larger number of leaves. Hawks (1978) found that in most cases the varieties with higher stalk had higher number of leaves. This was especially expressed in non-flowering varieties with mammoth characteristics.

Yield per unit area, kg/ha

Carriers of tobacco yield are the leaves, with their number on stalk and their size. Although this character is genetically controlled, it is greatly affected by climate conditions and application of cultural practices. Drazic (1986) reported that the yield is directly influenced by the genotype and the external environment. Data on the corrected yield per hectare in line V-79/09 CMS F1 in the test period are presented in Table 3.

Table 3. Corrected yield per hectare, kg/ha

Year	Yield kg/ha
2012	2912
2013	3476
2014	3609
2015	3577
2016	3678
Average	3450

The above data reveal that the highest yields were recorded in 2014 (2009 kg/ha) and 2016 (3678 kg/ha). The average yield value obtained from the five-year results is 3450 kg/ha, which puts this line in the group of high-yielded genotypes. Risteski et al. (2012) reported that male-sterile genotypes of Virginia tobacco created in the Scientific Tobacco Institute – Prilep had a yield of 3549 kg/ha (line V-53 CMS F1). Devčić et al. (1982) reported that Croatian hybrid varieties H-30, H-31 and H-32, with appropriate cultural practice, can yield up to 2000 kg/ha. Hawks (1978) gives tabular presentation of movement of Virginia tobacco yields in the United States by periods. Thus, in the period 1934-1938, the average yield was only 959 kg/ha, and in 1964/1967 it was increased to 2224 kg/ha. It is worth mentioning that in the CORESTA report of 2013 on Blue Mold Pathogenicity in more than 20 countries worldwide, the line V-79/09 CMS F1 was described as significantly resistant to fungal disease *Peronospora tabacina* Adam (PTA) which causes major economic losses to tobacco.

Conclusions

According to the three stages of flowering (beginning, 50% flowering and end of flowering), line V-79/09 CMS F1 belongs to the group of moderate to late-flowering tobaccos.

According to the size of the 5th, 10th and 15th leaf, line V-79/09 CMS F1 can be grouped into Virginia tobaccos that have potential to produce middle belt leaves longer than 35 cm, the main condition for their classification into the first class tobacco.

With an average of 34.0 leaves per stalk, V-79/09 CMS F1 belongs to the group of tobacco with larger number of leaves.

By the average yield of 3450 kg/ha, line V-79/09 CMS F1 is characterized as high-yielding tobacco.

In the CORESTA report of 2013 on Blue Mold, V-79/09 CMS F1 is characterized as significantly resistant to *Peronospora tabacina* Adam (PTA).

References

1. Book Наумоски К. (1977) -Современо производство на тутун- Скопје.
2. Hawks N.S.Jr. (1978), Osnovi proizvodnje virginijskog duhana .Prevod - Zagreb.
3. Узуноски М. (1985). Производство на тутун. Стопански весник, Скопје.
4. Article in a journal Beljo, J. (1996). Postupak za identifikaciju kultivara duhana. Izvješče o znanstvenom stručnom radu u 1994 .Vol. 21 1996, pp.55-67. Duhanski instituit – Zagreb.
5. Devčić K., Triplat, J. (1982). Neka svojstva novih flue cured sorti izgajanih u Duhanskom institutu – Zagreb. Тутун/Tobacco, godina XXXI. No -11-12.5-10.
6. Ристески, И. (2000). Триесет години Вирџинија во Македонија-почетоци, состојби и перспективи.Тутун/ Tobacco, Vol. 50, N^0 7-8 , стр. 152-163.
7. Dissertations, reports, conference papers Dražić, S. (1986). Prilog proučavanja nasledivanja kvalitativnih svojstva duvana-Univerzitet u Beogradu. Poljoprivredni fakultet – Doktorska disertacija. pp 149.
8. Hawks N.S.Jr. (1978), Osnovi proizvodnje virginijskog duhana.Prevod - Zagreb.
9. CORESTA 2013-Norbert Bilenkamp: Satochi Katsuya: Sub-Group Collaborative Study on Blue Mold.
10. Каламанда О. (2009). Употребна вредност на тутунската суровина тип вирџинија од производното подрачје на Република Српска. Научен институт за тутун-Прилеп Докторска дисертација, стр. 151.
11. Ристески И., (1999). Корелација помеѓу хранливата површина и некои производно трхнолошки својства на тутунот од вирџиниската сортата МВ-1. ФЗНХ – Скопје. Магистерски труд. Pp.130.
12. Risteski I., Kočoska K., (2012). Results of broadleaf tobacco breeding Scientific tobacco institute-Prilep. International symposium for agriculture and food 12-14 December 2012 –Skopje, Republic of Macedonia pp. 643-648.

INVESTIGATION ON THE EFFECTS OF THE GENOTYPE X ENVIRONMENT INTERACTION IN SOME NEW BULGARIAN COMMON WINTER WHEAT CULTIVARS

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Abstract

Fifteen new Bulgarian common winter wheat cultivars were investigated for ecological plasticity and stability in four replicates of a randomized experiment during 2014 – 2016. The applied two-factor dispersion analysis proved the different genetic potential of the group of cultivars according to the investigated indices to the highest degree of statistical significance, as well as the effect of the climatic conditions. The genotype x ecological conditions interaction was significant for all indices, which was a necessary prerequisite to apply AMMI models for evaluation of the cultivars' ecological plasticity and stability according to the investigated indices. The use of the AMMI models allowed the evaluation of the ecological plasticity on the basis of the statistical parameters ASV, and of the ecological stability on the basis of the reliability index I. According to the index *yield*, cultivar Laska was with the highest plasticity and stability, ranking first among all cultivars. Cultivars Laska, Progress, Enola, Bezostaya 1, Kristi and Yantur demonstrated high ecological plasticity and stability according to the complex of all investigated traits.

Keywords: *Triticum aestivum* L , ecological plasticity and stability , AMMI models.

Introduction

Wheat is a main agricultural crop in Republic of Bulgaria. It is grown on millions of ha of arable land. The climatic changes are ostensible during the last decade in different regions of the country. The investigation on the response of the major cultivars used in production to these changes is important both for the farmers and the breeders. The estimation of the ecological plasticity and stability of winter wheat varieties is permanent investigated (Penchev 2004). Different models for evaluation of the interaction "genotype-enviroment" are compared and discussed their pryorities and disadvantages (Penchev 2005). Tsenov (10) discussed the importance for breeders and farmers to study the interaction "genotype x ecological conditions" and it influence at the productivity of winter wheat. A set of 25 advanced breeding lines and released varieties of wheat (*Triticum aestivum* L.) developed by different breeding centers in India were assessed for their adaptation in 18 different environments across the Indo-Gangetic plains. The study was aimed at identifying genotype(s) with high yield stability across the environments in general and heat stress environments in particular.(Rane J. at all). The contemporary methods for evaluation of the *genotype x environment* interaction such as the AMMI models (Abamu, J.; Nachit M, Lee) allow precise estimation of the ecological plasticity and stability of the investigated genotypes.

Material and methods

In our three years experiment the analyses of plasticity and stability were by using fifteen new Bulgarian winter – wheat varieties . The results of our research proved that the method, applied can be efficiently used to analyse the environmental responses, the behaviour under varying environmental conditions of different varieties. Varieties can adapt themselves differently to favourable and unfavourable environmental conditions. The cultivars were examined for ecological plasticity and stability in a randomized experiment of four replicates during 2014 – 2016.The experiment was taken in three different regions in Bulgaria at the north and south part of the

country: General Toshevo, Russe and Sadovo. The following actual for the praxis cultivars were investigated: Antitsa, Bezostaya 1, Galateya, Dobrudzhanka, Dona, Enola, Karat, Korona, Kristi, Kristora, Laska, Liliya, Ludogorie, Progress and Yantur. The indices yield, 1000 kernel weight, plant height, spike length, weight of grain per spike and number of grains per spike were subjected to analysis. These indices were measured on 10 randomly chosen spikes.
The dispersion analysis carried out according to the model:

$$Yijk = Y.. + Gi + Yj + Zk + (GY)ij + (GZ) ik + (YZ)jk + (GYZ)ijk + Eijk,$$

where Gi is the factor genotype, Yj is the climatic conditions ,Zk is the region and Eijk is the error. The linear regression models were calculated by last square method. The means for plasticity and stability of the studied indices and the varietal adaptability to the changeable environment were estimated by AMMI models (2). The experimental data were processed with statistical packages SPSS 19.0 and Biostat 6.0.

Results and discussion
Table 1 presents the results from the dispersion analysis carried out according to the model.

Table 1. Dispersion analysis of the investigated indices

	Yield	Height	Length of spike	Number of spikelets	Number of grains	1000 kernel weight
G	19089,8 ^{c}	5108,3 ^{c}	11 ^{c}	6306 ^{c}	518,8 ^{c}	471,4 ^{c}
Y	7793 ^{c}	776,8 ^{c}	38,8 ^{c}	5936 ^{c}	463,8 ^{c}	244,6 ^{c}
Z	47726,6 ^{c}	6643,6 ^{c}	101,55 ^{c}	20375,2 ^{c}	19523,1 ^{c}	7475,6 ^{c}
G x Y	1416,1 ^{c}	357 ^{c}	1,68 ^{c}	576,1 ^{c}	70,3 ^{c}	226,4 ^{c}
G x Z	438,1 ^{a}	87 ^{b}	0,32	97,8	46,2	2,8
Y x Z	4061,7 ^{c}	2915,2 ^{c}	2,55 ^{c}	714,8 ^{c}	479,7 ^{c}	112,2 ^{c}
G x Y x Z	474,3	82,8 ^{b}	0,22	158,7 ^{a}	25	24,1 ^{a}
Error	221,4	17,9	0,16	76	15,9	8,5

a – statistical significance at P=0,05, b – statistical significance at P=0,01, c – statistical significance at P=0,001

The applied F criterion proved the different genetic potential of the group of cultivars according to the investigated indices to the highest level of statistical significance, as well as of the other two investigated factors – climatic conditions and regime of drought. The *genotype x ecological conditions* interaction was significant for all indices and this was the necessary prerequisite to apply AMMI models for evaluation of the ecological plasticity and stability of the cultivars according to the investigated indices. Interesting results were obtained for the interaction *genotype x regime of drought*; it was significant only for the indices yield and plant height.
The application of the AMMI models allowed the estimation of the ecological plasticity on the basis of the statistical parameters ASV, and of the ecological stability on the basis of the reliability index I. According to the index *yield*, cultivar Laska demonstrated the highest plasticity and stability and ranked first among all cultivars. It was followed by cultivars Progress, Enola, Yantur, Galateya, Karat, Dona and Bezostaya 1; in these cultivars the productivity was less influenced by the variable climatic conditions and they can be evaluated as having good ecological plasticity and stability. Cultivars Kristi, Kristora and Dobrudzhanka were marginal, while Liliya, Ludogorie, Korona and Antitsa had susceptible reaction of this index under changeable climatic conditions and showed low ecological plasticity and stability. According to the index *plant height*, cultivars Liliya, Dona, Karat, Kristora, Progress and Bezostaya 1 had good ecological plasticity and stability, the values of the ASV parameters being below 0.200, and the reliability indices – higher than 1.5.

Table 2. Results from application of AMMI models

Cultivar	Yield			Height			Length of spike		
	Rank	ASV	I	Rank	ASV	I	Rank	ASV	I
Laska	1	0,022	2,432	12	0,327	0,744	9	0,289	0,976
Progress	2	0,084	2,213	5	0,178	2,121	1	0,085	2,433
Enola	3	0,111	1,945	15	0,356	0,627	8	0,252	1,255
Yantur	4	0,165	1,929	7	0,224	1,442	7	0,224	1,422
Galateya	5	0,180	1,896	10	0,318	0,795	13	0,348	0,855
Karat	6	0,187	1,854	3	0,108	2,224	3	0,128	2.261
Dona	7	0,208	1,805	2	0,088	2,247	6	0,198	1,724
Bezostaya 1	8	0,233	1,725	6	0,207	1,562	2	0,105	2,315
Kristi	9	0,276	1,664	9	0,284	0,829	10	0,312	0,955
Kristora	10	0,285	1,525	4	0,312	2,188	5	0,175	1,988
Dobrudzhanka	11	0,297	1,332	13	0,339	0,731	4	0,147	2,105
Liliya	12	0,332	1,031	1	0,003	2,571	15	0,379	0,832
Ludogorie	13	0,397	0,944	11	0,328	0,827	12	0,331	0,864
Korona	14	0,412	0,866	14	0,351	0,811	11	0,322	0,897
Antitsa	15	0,434	0,813	8	0,259	1,013	14	0,365	0,849

Cultivars Enola, Korona, Dobrudzhanka, Laska and Ludogorie showed susceptible reaction according to this index: the values of the AVS parameters were higher than 0.31, while the I indices were lower than 1. According to the index *length of spike*, cultivar Progress ranked first; it had only slight reaction to the changes of the climatic conditions. The value of the ASV parameter was 0.085, and the reliability index of this cultivar was 2.433. The cultivars with highest ecological plasticity and stability according to this index were Bezostaya 1, Karat, Dobrudzhanka, Kristora, Dona and Yantur; their ASV parameters were less than 0.24, and their reliability indices were higher than 1.5. Susceptible was the reaction of cultivars Galateya, Antitsa, Liliya, Ludogories, Korona and Kristi, the ASV parameters of which were higher than 0.31, and the reliability indices – lower than 1. Cultivars Progress, Karat, Dona, Yantur and Bezostaya 1 can be pointed out as having good ecological plasticity and stability by all three indices.

Table 3. Results from the use of the AMMI models

Cultivar	Number of spikes			Number of grains			1000 kernel weight		
	Rank	ASV	I	Rank	ASV	I	Rank	ASV	I
Laska	1	0.078	2,377	5	0.188	1.915	3	0.122	2.062
Progress	2	0.095	2.364	9	0.295	1.235	12	0.318	0.881
Liliya	3	0.117	2.107	11	0.327	0.915	15	0.345	0.794
Kristi	4	0.124	2.119	12	0.334	0.896	1	0.098	2.217
Korona	5	0.196	1.842	2	0.122	2.217	6	0.177	1.732
Enola	6	0.227	1.622	10	0.311	1.211	5	0.162	1.826
Bezostaya 1	7	0.271	1.327	4	0.182	2.080	8	0.194	1.527
Dobrudzhanka	8	0.286	1.248	1	0.118	2.235	10	0.258	1.466
Yantur	9	0.295	1.124	8	0.279	1.521	7	0.182	1.569
Dona	10	0.319	0.925	3	0.135	2.104	14	0.336	0.822
Karat	11	0.321	0.882	15	0.339	0.816	9	0.229	1.474
Galateya	12	0,327	0.880	6	0.209	1.742	4	0.147	1.953
Antitsa	13	0.372	0.628	13	0.347	0.884	11	0.289	0.973
Ludogorie	14	0.385	0.601	7	0.226	1.588	2	0.103	2.168
Kristora	15	0.394	0.595	14	0.356	0.852	13	0.329	0.846

According to the index *number of spikes*, high ecological plasticity was demonstrated by cultivars Laska, Progress, Liliya, Kristi, Korona, Enola, Bezostaya 1, Dobrudzhanka and Yantur with values of the statistical parameter ASV< 0.3 and of the reliability index I >1. Cultivars Dona, Karat, Galateya, Antitsa, Ludogorie and Kristora responded with susceptible reaction to the climatic conditions; their values of the statistical parameters were ASV > 0.3 , I < 1. According to the index number of grains per spike, high ecological plasticity and stability was observed in cultivars Dobrudzhanka, Korona, Dona, Bezostaya 1, Laska, Galateya, Ludogorie and Yantur, and the reaction of cultivars Progress, Enola, Liliya, Kristi, Antitsa, Kristora and Karat was susceptible. With regard to the index *1000 kernel weight*, the application of the AMMI models revealed that cultivars Kristi, Ludogorie, Laska, Galateya, Enola, Korona, Yantur and Bezostaya 1 were slightly influenced by the variable climatic conditions, while cultivars Karat, Dobrudzhanka, Antitsa, Progress, Kristora, Dona and Liliya exhibited more susceptible reaction.

Conclusions

According to the complex of all indices, cultivars Laska, Progress, Enola, Bezostaya 1, Kristi and Yantur demonstrated high ecological plasticity and stability. High susceptibility was found in cultivars Antitsa, Liliya, Ludogorie and Korona. According to the index *productivity*, cultivars Laska, Progress, Enola and Yantur were with high ecological plasticity. Cultivars Liliya, Ludogorie, Korona and Antitsa responded with susceptible reaction.

References

1. Abamu, J., E. Akinsola & K.Aliuri. Applying the AMMI models to understand genotype – by – environment (GxE) interactions in rice reaction to blast disease in Africa. Journal of Pest Management, pp 239-245, 2007.
2. Cornelius, L., J. Crossa. Prediction assessment of shrinkage estimators of multiplicative model for multi – environment cultivar trials. Crop Science, v.39, pp 998-1009, 1999.
3. Dias, S., W. Krzanowski. Model selection and cross validation in additive main effect and multiplicative interaction (AMMI) models. Crop Science, v 43, pp 865-873, 2003.
4. Nachit, M., G. Nachit, H.Ketata, G. Gauch & R. Zobel. Use of AMMI and linear regression models to analyze genotype x environment interaction in durum wheat. TAG v.83, N.5, pp 597-601, 2002.
5. Lee, Eun-Joo. Statistical Analysis Software for Multiplicative Interaction Models. Doctoral Dissertation. Kansas State University, 2004.
6. Miliken, A., E. Johnson. Analysis of Messy Data. Volume 2. Nonreplicated Experiments (2nd Edition), New York. Chapman & Hall, 1989.
7. Penchev E., I.Stoeva.Estimation the ecological plasticity and stability of a group winter wheat varieties. Field Crop Studies. Vol. , pp 30-33 . 2004.
8. Penchev E., M.Atanasova,I.Stoeva. Evaluation the ecological plasticity and stability of quality indices and productivity of winter wheat varieties by models of Shukla , Eberhart & Russel . International Scientific Conference , Agrar University Plovdiv . pp 61-65 . 2005.
9. Rane J. at all. Performance of Yield and Stability of Advanced Wheat Genotypes under Heat Stress Environments of the Indo-Gangetic Plains. Crop Science. Vol. 47 No. 4, p. 1561-1573, 2006.
10. Tsenov,N. , at all . Problems, achievements and perspectivies of the breeding of winter wheat by productivity . Field Crops - fcs.dai-gt.org ,2009 .

INVESTIGATION ON THE INHERITANCE OF THE TRAITS NUMBER OF SEEDS PER PLANT AND 1000 KERNEL WEIGHT IN SUNFLOWER HYBRIDS

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Abstract
The investigation was carried out during 2013 – 2016 at Dobrudzha Agricultural Institute – General Toshevo, Bulgaria. The traits number of seeds per plant and 1000 kernel weight are of primary importance; they influence the increase of yield. This investigation included 8 hybrid combinations and the parental forms involved in the crosses. The additive-dominant relations of the traits were analyzed through the ratio between the parameters d/a. In crosses 217A x 88R, 813A x 97R, 813A x 100R, and 2003A x 98R, super dominance according to the parental forms was observed. Higher number of seeds per plant and 1000 kernel weight were obtained in these hybrid combinations in comparison to the parental forms. Incomplete dominance of these traits was found in combinations 217 x RW666 and 217 x 138R.

Keywords: sunflower breeding, inheritance, quantitative traits.

Introduction

Sunflower, together with soybean and oil seed rape, amounts to 87 % of the production of oleaginous plants worldwide. It is a main agricultural crop grown on 23 million ha worldwide (FAO, 2012). Cultural sunflower (*Helianthus annuus* L.) ranks second after soybean by production of vegetable oils in the world. During the last 30 years, the world production has increased several times (Quresh *et al*., 1992). The developing of genetic diversity is directly related to the higher efficiency of the breeding process (Mihova and Dimova, 2012). The use of the wild species for developing of new fertility restorer lines is related to enhancement of the quantitative and qualitative indices of the hybrid combinations and their resistance to biotic and abiotic stress (Valkova, D., 2013, 2015). The thorough investigation of the main characters determining the yield of the parental sunflower lines is a good basis for more efficient breeding work. These statements have been confirmed in the investigations on other crops by a number of authors (Chamurliyski et al., 2012; Nenova et al., 2005). The number of seeds per plant is a character ensuring high yields. Kovacik and Skalound (1977) reported a high correlation between yield and number of seeds per plant. Thousand kernel weight is a variable value dependant on the effects of genetic factors and environmental conditions. This character, being an important qualitative index, gives an idea about the size, plumpness and fullness of the seeds. The character 1000 kernel weight is strongly influenced by the climatic factors, the used agronomy practices, and most of all – by the genotype. It is a main element of yield. The breeding for higher 1000 kernel weight leads to higher seed yield, so this character is used as a criterion for selection in the developing of sunflower hybrids (Miller and Fick, 1997; Kaya et. al., 2003; Goksoy and Turan, 2007; Hladni et al. 2008; Yasin and Singh 2010; Kholghi et al.2011). The aim of this investigation was to follow the type of inheritance of the main indices of F_1 in sunflower: number of seeds per plant and 1000 kernel weight. A direct effect was found depending on the type of inheritance of these characters.

Material and methods

The field experiment was carried out in the trial field of Dobrudzha Agricultural Institute – General Toshevo (DAI) during 2013 – 2016 according to a conventional technology for growing of sunflower

(Georgiev et al., 1997). Eight hybrid combinations were tested, which were obtained from the crossing of three lines with cytoplasmic male sterility to three fertility restorers. The hybrid combinations were tested in a field trial designed in three replications according to the Latin square method. The size of the plot was 7.35 м^2. The Bulgarian hybrids San Luka and Veleka were used as standards, as well as one of the most productive and popular foreign hybrid PR64F50. The indices number of seeds per plant and 1000 kernel weight were investigated in all hybrid combinations, and the heritability rate in F_1 was determined. The heritability rate of these two indices in the hybrid combinations was determined by the methodology of (Romero, G. & Gray, F., 1973). Correlation analysis was applied for statistical processing of the results using software XLSTAT Pro. ver 7.0.1.

Results and discussion

Figure 1 presents the results for the heritability rate of the index 1000 kernel weigh in the F_1 hybrid progenies. Super dominance of this index was found in all tested hybrid combinations. The highest values of heritability rate were obtained for crosses 813A x 98R (10.90), 2003A x 98R (11.30), 1017A x 98R (13.10). It is worth mentioning that the same fertility restorer line 98R was included in all of the above three hybrid combinations. The super dominance is a result from the accumulation and recombination of genes in the hybrid crosses. The high values of the index 1000 kernel weight in these three hybrid combinations were due to the fact that the mother and the father lines have similar values. Super dominance of this index was also found in the other hybrid combinations involved in the investigation (217A x 88R, 813A x 100R, 217A x RW666, 217A x 100R, 2003A x 84R); the difference was that their values were lower. This was a result from the values of the mother component significantly exceeding the values of the male parent. Similar results have been obtained also by Laureti, Gatto (2001) and Farroki et. al. **(**2008a).

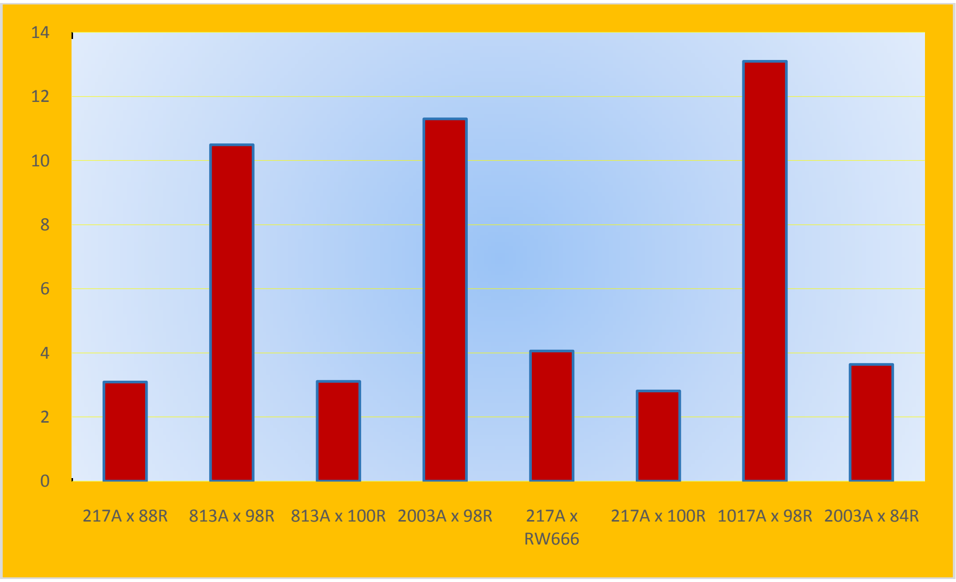

Figure 1. Type of inheritance of the character 1000 kernel weight in hybrid combinations (F_1).

The results presented in Figure 2 characterize the heritability rate of the index number of seeds per plant in the hybrid combinations. With regard to this character, highest values were obtained in crosses 217A x RW666 (3.34), 217A x 88R (2.56), 813A x 100R (2.45) and 813A x 98R (2.38). In all these hybrid combinations, super dominance was found according to the index number of seeds per plant. This super dominance was due to the established difference between the parental lines and the obtained hybrid combination in F_1. Incomplete dominance of this index was found only in one cross: 217A x 100R (0.79). It resulted from the obtained similar values between the mother parental form and the F_1 hybrid combination. Similar results have been reported by Joksimovic et al. (1992, 1995) and Josic (2004).

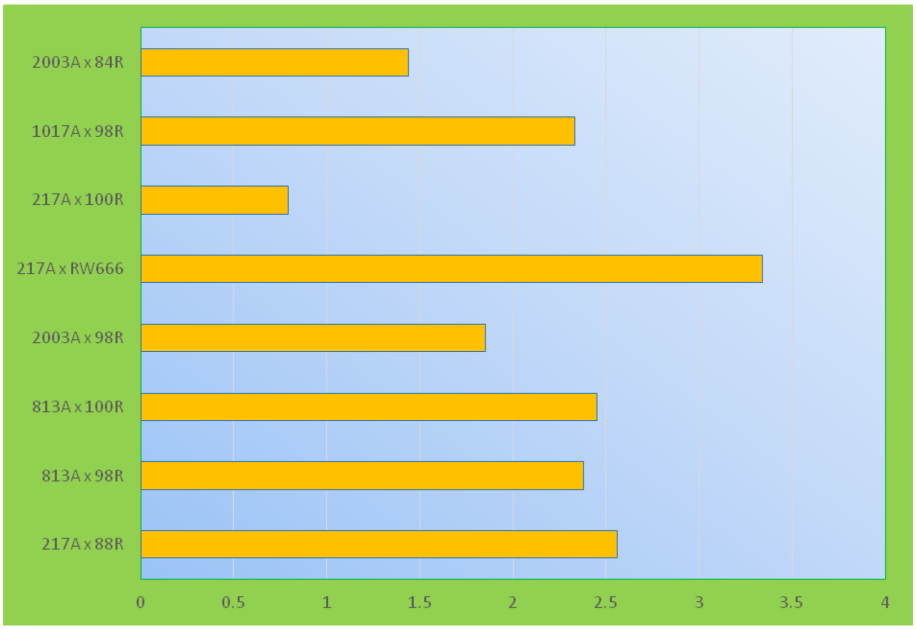

Figure 2. Type of heritability of the character number of seeds per plant in hybrid combinations (F_1)

Table 1 presents the correlation analysis of the characters included in the investigation – number of seeds per plant and 1000 kernel weight. The higher 1000 kernel weight (MC1000 = 0.519**) of the investigated hybrid combinations was in narrow positive correlation with the index number of seeds per plant. Similar results for a positive direct effect between number of seeds per plant and 1000 kernel weight have also been obtained by Marinkovic (1992), Goskoy & Turan (2007), Behradfar et.al. (2009).

Table 1. Correlation analysis

	NSP	*M1000C*
NSP	*1*	
M1000C	*0.519***	*1*

*** - p ≤ 0,01; ** - p ≤ 0,05; * - p ≤ 0,1; n.s. – non significant
Key: NSP – Number of seeds per plant; M1000C - 1000 kernel weight

Conclusions

In three hybrid combinations, 813A x 98R, 2003A x 98R and 1017A x 98R, super dominance of the index 1000 kernel weight was found. It was due to the fact that the mother and the father lines were with similar values. In the hybrid combinations, the genes recombined and accumulated. Lower values of super dominance were determined according to this index also in crosses 217A x 88R, 813A x 100R, 217A x RW666, 217A x 100R and 2003A x 84R; the difference in this case was that the values of the sterile lines significantly exceeded the values of the fertility restorers. According to the index number of seeds per plant, super dominance was found in hybrid combinations 217A x RW666, 217A x 88R, 813A x 100R and 813A x 98R. Incomplete dominance of this character was determined only in one cross: 217A x 100R. It resulted from the similar values of the mother parent and the hybrid combination F_1.

References

1. Behradfar, A., H. Gorttapeh, M. R. Zardashty, F. Talat (2009): Evaluation correlated traits for seed and oil yield in sunflower (*Helianthus annuus* L.). Through path analysis in under condition relay cropping. Res. J. Biol. *4*(1):82-85
2. Chamurliyski P., N. Tsenov, I. Stoeva, S. Doneva (2012). Economic characterization of some foreign common winter wheat cultivars (*Triticum aestivum* L.). Plant breeding sciences, No 5 – 49: 13-17 (In Bg).
3. FAOSTAT-FAO (2012). Area harvested. Food and Agriculture Organization of the United Nations. Available at http://faostat.fao.org/site/567/Desktop
4. Farokhi, E., Khodabandeh, A. and Ghaffari, M. (2008a). Studies on general and specific combining abilities in sunflower. Proc. of the 17th Intl. Sunflower Conf. Vol.2: 561-566. Cordoba, Spain, June 8-12. Intl. Sunflower Assoc. Paris, France.
5. Goksoy T. and Turan Z. M. (2007). Correlations and path analysis of yield components in synthetic varieties of sunflower (*Helianthus annuus* L.). Acta Agronomica Hungarica 55(3): 339-34.
6. Hladni, N., Skoric, D., Kraljevic-Balalic (2008): Line x tester analysis of morphophysiological traits and their correlations with seed yield and oil content in sunflower (*Helianthus annuus L.*) Genetika-Belgrade, Vol. *40*, No 2.
7. Joksimovic, J. (1992). Evaluation of combining abilities in some inbred sunflower lines, Ph.D. thesis, University of Novi Sad, Faculty of Agriculture: 1-157. (In Serbian)
8. Joksimovic, J., Marinkovic, R., Mihaljcevic, M. (1995). Genetic control of the number of flowers and percentage of pollination of sunflower (*Helianthus annuus* L.). Selection and seed production 2(1):71-74. (In Serbian)
9. Josic, S., and Skoric, D. (2004). Inheritance of some yield components in sunflower. In: Proc. 16th Intl. Sunflower Conf. Vol. 2: 503-510. Fargo, ND, USA, August 29-September 2. Intl. Sunflower Assoc. Paris, France.
10. Kaya Y. and Atakifli K. (2003). Path and correlation analysis in different yield characters in sunflower (*Helianthus annuus* L.). Anadolu J. 13: 31-45.
11. Kovacik A. and Skaloud V. (1972). Combining ability and production of heterosis in sunflower. Sci. Agr. Bohem. Vol.4 N.4
12. Kholghi M, Bernousi I, Darvishzadeh R, Pirzad A, Hatami Maleki H. (2011). Collection, evaluation and classification of Iranian confectionary sunflower (*Helianthus annuus* L.) populations using multivaraite statistical techniques. Afr. J. Biotech. 10: 5444-5451.
13. Laureti D., Del Gatto A. (2001). General and specific combining ability in sunflower (*Helianthus annuus* L.). Helia 24 (34): 1-16.
14. Marinkovic R. (1992). Path-coeficient analysis of some yield components of sunflower (*Helianthus annuus* L). Euphytica, *60*, 201-205.
15. Mihova G., D. Dimova (2012). Characterization of the yield components in different forms of feed barley. Field Crop Studies, VIII-1, 23-36 (In Bg).
16. Miller, J. F. and Fick, G. N. (1997). The genetics of sunflower. Sunflower Technology and Production. Schneiter, A.A (Ed.) Agronomy, American Society of Agronomy, Inc., CSSA, SSSA, Inc. Madison, Wisconsin, USA. 441-496
17. Nenova, N., E. Penchev, M. Christov, D. Petakov, M. Drumeva (2005). Structural analysis of important indices in promising sunflower hybrids. Agrarian University - Plovdid, Research communications, vol.L, No 5, 79-84 (In Bg).
18. Quresh, Z., H. Sabir and S. Ahmad (1992)**.** Annual report of NODP, Islamabad. 2: 20-21.
19. Valkova D**.,** N. Nenova, V. Encheva, J. Encheva. (2015). Creation of lines restorers of fertility originated from interspecific hybrids. Agrarian Sciences, Agrarian University - Plovdiv, VII, No. 18, 75-79.

20. Valkova D., J. Encheva, V. Encheva, P. Shindrova, M. Christov (2015). Study of hybrid material originated from interspecific crosses with wild *Helianthus annuus* L. for resistance to diseases and parasite broomrape. Plant Science, vol. LII, No. 4, 18-21.
21. Valkova, D. (2013). Investigation of the species from genus Helianthus as sources of important breeding characters. Ph.D. dissertation, 200 p (In Bg).
22. Yasin A. B. and Singh S. (2010). Correlation and path coefficient analyses in sunflower. J. of Plant Breeding and Crop Sci. 2(5): 129-133.

VARIABILITY OF THE MOST IMPORTANT QUANTITATIVE PROPERTIES IN SOME VARIETIES OF TOBACCO TYPE BURLEY

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Abstract
The morphological properties that characterize the type of tobacco or the variety in one type are known as qualitative and quantitative. The quantitative properties are regularly conditioned from the impact of a larger number of genes and also they depend from the environment factors. The investigations are done in 2015 on experimental field in the Scientific Tobacco Institute in Prilep, with four repetitions with following varieties: Posejdon (control), BD-1, B-1246 and one new perspective line DP-1710. The aim of this investigationes is to show the variability of the most important quantitative properties: height of the plant with inflorescence, the number of leaves per plant and the length and width of the biggest and the smallest leaf from the middle harvesting belt in tobacco type Burley. The results from the research are processed statistically by these parameters: average value ($\bar{x}$), average value error ($S\bar{x}$), standard deviation (S), variation coefficient (CV) and variation width (WV). From the research, we established that the subject varieties are stable enough, the variability is very low because everywhere the variation coefficient was lower than 10%. However, the newly created line DP -1710 is with the slightest variation in the tested properties. Among other things, it is on average the highest ($\bar{x}$ = 188 cm) and has the largest leaves ($\bar{x}$ = 63 cm), which is a positive feature in coarse tobacco of this type.

Keywords: tobacco, type Burley, quantitative properties, variability.

Introduction

Tobacco belongs to the group of strategic crops in the agricultural economy in the Republic of Macedonia. The cultivation of tobacco is conducted on an area between 12.000 and 15.000 ha with an annual production of 20 to 25 million kilograms of tobacco raw material with good quality. From the aspect of the typical representation, over 95% from this fields belong to aromatic oriental tobacco type Prilep and Yaka, while the large ones (Virginia and Burley) almost cannot be seen in the fields. By the end of the 90s of the last century these types were grown here which reduced the tobacco raw material import. But they were necessary for the production of the most popular "american blend" cigarettes. In this cigarette, the type Burley is involved with 35% (Mickovski, 2004). According to Devcic et al. (1984), the largest manufacturer in the world of this tobacco type are USA, Mexico, Japan, Italy and Spain. We need to point out that in Macedonia there are areas with perfect conditions for growing tobacco type Burley with good quality, so this makes a challenge for this type to be brought again in the production. Lately, the scientists form the Scientific Tobacco Institute - Prilep are picking and creating types that can meet all needs of the cigarette factories. That's why the subject and the purpose of this research is the variability of the most quantitative properties of the tobacco type Burley which have good combinational abilities and this is a condition for making new more productive and more qualitative from already existing. This is the only way to bring back the interest for this tobacco type and the factories will have no problem to absorb the tobacco raw material and plus the financial effects will be guaranteed for everyone in the tobacco industry in the Republic of Macedonia.

Material and methods

The researches were made on three varieties and one new line form the type Burley: Posejdon control variety (Ø), BD-1, B-1246 and the line DP-1710. The first three varieties are originally from other countries and some of them are still growing in their homelands and in other countries too (Posejdon was grown in Macedonia before). Despite now they are represented on smaller fields, they are still current because of its good quality and they are also used for selection about this tobacco type. The control type Posejdon (Photo 1) is created from Igor Bolsunov in the research station in Fürstenfeld in Austria. The type BD-1 (Photo 2) originally comes from Serbia and was created from Dobrivoje Jovanovik, the leader of the Department of Genetics and Selection within the Development Sector in Tobacco Industry in Nis. The type B-1246 (Photo 3) comes from Bulgaria and until recently it was fairly represented in the tobacco producing areas because of the recognizable quality of the obtained tobacco raw material. The new line DP-1710 (Photo 4) was created in the Scientific Tobacco Institute - Prilep by crossing and selecting of two Burley tobacco varieties. The experiment was set in the Experimental Field of the Scientific Tobacco Institute - Prilep in 2015, on a deluvial - colluvial soil in four repetitions. During the vegetation, the necessary agrotechnical operations are carried out to ensure normal growth and development of plants (feeding with nitrogen fertilizer, trampling and treating tobacco according to the program of the Tobacco Institute - Prilep for the protection of tobacco from diseases, pests and weeds). The tobacco in the experiment is sprinkled three times with a floating norm of 400 m^3 of water per hectare. We need to point out that the production of 2015 was assessed as a good year for tobacco production. Measurement of the quantitative properties (height of the plant with inflorescence, number of leaves and length and width of the largest and smallest leaf of the middle belt of the plant) were carried out in the field in the phase of full blossoming of tobacco according to standard methods in the selection, (the mean value $\bar{x}$) for each property is determined on the basis of fifteen randomly selected plants of each variety in the experiment. The obtained data from the measurements are statistically processed through parameters of variability of properties (Najcevska, 2002), and the results are shown in tables.

Photo 1. Posejdon

Photo 2. BD-1

Photo 3. B-1246

Photo 4. L. DP-1710

Results and discussion

The investigated quantitative properties of the Burley type tobacco varieties are also known as morphological. They have great meaning in tobacco selection and genetics because they determinate the type and the varieties that belongs to him. Despite this, the number and the size of the leaves are determined the yield and the quality of tobacco. They are dictated by their own genotype, but are also dependent on soil-climatic conditions in the region where tobacco is grown, as well as from applied agrotechnics during vegetation. We mentioned that the results from the research are shown in table, separately for each property just to be shown better, compared with the examined varieties and the new line and also coming up with a conclusion.

Height of the plant with inflorescence

The height of the tobacco plant is a varieties feature. According this property, Uzunoski (1985), separated tobacco varieties in three groups:

1. Variety with low growth, its height is up to 70 cm (Prilep, low Otlja);
2. Varieties with middle growth, with plant height with the inflorescence from 70 - 130 cm (Yaka, Dzebel);
3. Varieties with high growth, its height is over 130 cm (Virdzinija and Burley). Risteski et al. (2007), made a research on six varieties and they point out that the height of the plant with inflorescence approximately goes from 142 cm for variety B1317 to 194 cm for the variety B-98/N CMS.

The results for this quantitative property are shown in Table 1.

Table 1. Height of the plant with inflorescence (cm)

Varieties	n	$\bar{x}$	$S\bar{x}$	S	CV (%)	WV
Posejdon Ø	15	180	0.89	3.44	1.91	175-187
BD-1	15	172	0.88	3.42	1.99	164-175
B-1246	15	168	0.96	3.70	2.20	163-174
L. DP-1710	15	188	0.63	2.45	1.30	185-195

Number of leaves of the plant

The number of leaves of the plant depends on its genetic structure of the variety and the growing conditions. It is considered that the number of leaves is one of the most stable tobacco quantitative properties. According to Atanasov (1972), the number of leaves is a variety feature and represents highly consistent quantitative property. Dyulgerski (2016), examined the new lines from the type Burley in Bulgaria and confirmed that the highest number of leaves has line 1540 (average 31.6) and the lowest number of leaves has line 1525 (26.3 leaves per stalk). Standard variety Pliska with 27.7 leaves is on the fifth place from all eights examined varieties. Devcic et al. (1984), were researching domestic and foreign tobacco varieties from type Burley in more areas in Croatia and they came to a conclusion that the Posejdon variety has 30 leaves per plant, while Culinec 1 has 28 leaves. Here in our research (Table 2.), e also concluded that Posejdon has the highest number of leaves but we counted 29, DP-1710 has 28, BD-1 and B-1246 are with 27 leaves.

Table 2. Number of leaves of the plant

Varieties	n	$\bar{x}$	$S\bar{x}$	S	CV (%)	WV
Posejdon Ø	15	29	0.29	1.13	3.91	27 - 31
BD-1	15	27	0.30	1.16	4.30	25 - 29
B-1246	15	27	0.34	1.31	4.85	24 - 29
L. DP-1710	15	28	0.20	0.77	2.75	27 - 30

Length of the largest leaf of the plant

The length of the leaves in every tobacco type is very important property because it's in a relationship with the quality of tobacco raw material. In Burley, the larger the leaves are the higher the yield is and the quality is much better. Boceski (2003), points out that the length and the width and also the surface of the leaves during drying is reduced by 20 to 30%, which is very important in the technology of processing the tobacco. Thre results from our measuring and the variability are presented in Table 3.

Table 3. Length of the largest leaf (cm)

Varieties	n	$\bar{x}$	$S\bar{x}$	S	CV (%)	WV
Posejdon Ø	15	60	0.51	1.96	3.27	56-63
BD-1	15	61	0.47	1.83	3.00	58-63
B-1246	15	59	0.52	2.02	3.41	56-62
L. DP-1710	15	63	0.46	1.77	2.81	60-66

Width of the largest leaf of the plant

The width of the leaves as same as the length depends on the soil-climatic conditions and on the applied agrotechnics during the growing period. Risteski and Kocoska (2013), indicate that the largest leaf of the plant on six examined Burley varieties ranged from 28.2 cm to B-21, up to 40.2 cm in the variety Pelagonec CMS, which had the widest leaves in the experiment. The results that we have from our research are presented in Table 4.

Table 4. Width of the largest leaf (cm)

Varieties	n	$\bar{x}$	$S\bar{x}$	S	CV (%)	WV
Posejdon Ø	15	30	0.30	1.16	3.87	29-32
BD-1	15	31	0.25	0.96	3.09	30-33
B-1246	15	30	0.31	1.18	3.89	28-32
L. DP-1710	15	32	0.19	0.74	2.27	32-34

Length of the smallest leaf in the middle belt

The leaves of the middle belt of Burley tobacco type are the largest, the highest quality and have a high use value in the processes of processing and fabrication. Therefore, we have measured the length and width of the smallest leaf of this belt in order to determine the number of leaves that can be harvested, taking into account their utilization in the fabrication. In practice it is considered that tobacco dry leafs have length below 25cm and that they are useless, also are increasing production costs, and when purchasing tobacco, they are assessed in the lowest class or are not taken at all by purchasers. Bearing this in mind, it is not recommended harvesting the sum of the top insertions of tobacco of this type. The results of the examination of this property are presented in Table 5.

Table 5. Length of the smallest leaf in the middle belt (cm)

Varieties	n	$\bar{x}$	$S\bar{x}$	S	CV (%)	WV
Posejdon Ø	15	42	0.37	1.41	3.37	40-45
BD-1	15	43	0.36	1.39	3.32	40-45
B-1246	15	39	0.39	1.51	3.88	36-41
L. DP-1710	15	42	0.27	1.03	2.46	40-44

Width of the smallest leaf of the middle belt

The width of the smallest leaf of the middle belt, along with the length, has an impact on the overall yield and quality of the raw tobacco material from type Burley. The variability of this property is shown by the statistical parameters in Table 6.

Table 6. Width of the smallest leaf of the middle belt (cm)

Varieties	n	$\bar{x}$	$S\bar{x}$	S	CV (%)	WV
Posejdon Ø	15	21	0.21	0.80	3.79	19-22
BD-1	15	23	0.17	0.64	2.77	22-24
B-1246	15	20	0.22	0.83	4.14	18-21
L. DP-1710	15	22	0.18	0.70	3.19	20-23

References

1. Atanasov, D. (1972). Tobacco Production. ITTI Plovdiv.
2. Boceski, D. (2003). Knowledge and processing of tobacco raw material. Institute for Tobacco - Prilep, II supplemented edition, pp. 210.
3. Devčić, K., Triplat, J., Benković, F. (1984). Sorte Burley Selected at the Tobacco Institute of Zagreb. Tutun/Tobacco, No. 1-2, pp. 3-10.
4. Dyulgerski, Y. (2016). Assessment of biological indicators and production characteristics of perspective lines burley tobacco. Tobacco/Tobacco, 1-6, pp. 13-21.
5. Mickovski, J. (2004). Tobacco in the Republic of Macedonia. Society for Science and Art - Prilep, pp. 162.
6. Najcevska, C. (2002). Experimental statistics applied in agricultural and biological research. Book publishing house Bona-Skopje, pp. 49-71.
7. Risteski, I., Kochoska, K., Hristoski, Zh. (2007). Results of examinations of some foreign and newly created domestic Burley varieties of tobacco in CMS and fertile form. Tobacco / Tobacco, No. 9-10, pp. 200-208.
8. Risteski, I., Kochoska, K. (2013). Results of Comparative Experiment with Berleian Varieties of Tobacco. Report on the Project Tasks of the Scientific Research Work in 2011, University "St. Kliment Ohridski "- Bitola, Scientific Institute for Tobacco - Prilep, pp. 20-24.
9. Uzunoski, M. (1985). Tobacco production. Economic newspaper - Skopje, pp. 96.

PHOTOSYNTHESIS OF VEGETABLES AND FIELD PLANTS IN RESPONSE TO BIOCHAR TREATMENT

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Abstract
The requirements of modern agriculture include besides obtaining maximum yields of crops also maintaining and improving soil fertility and environmental protection. In recent years, interest of usage solid phase (charcoal or biochar) obtained by pyrolysis as soil improver, increases. Biochar is a solid material obtained from the thermo chemical conversion of biomass in anoxygen-limited environment. Additions of biochar to soil have generally been shown to be beneficial for growing crops. From an agronomic perspective it is suggested that biochar could improve soil health by increasing nutrient retention. The present pot experiment was conducted to investigate the effect of biochar application on the plants photosynthetic activity. The experiment was carrying out on two soil types, treated with different BC ratio. The plants grown on cinnamic pseudopozolic soil show a high content of plastid pigments in comparison with these on alluvial meadow soil. The aim of study is to evaluate the biochar agronomic impact on the plants growth, development and quality indicators.

Keywords: chlorophyll content, Brix, plant development.

Introduction
The requirements of modern agriculture include, besides obtaining maximum yields of crops also maintaining and improving soil fertility and environmental protection. Traditionally, post-harvest residues of cereals crops in past were used for animals feed. Nowadays, under the conditions of greatly reduced livestock and lack of market, they have to be exported from the field or burned. This practice leads to deterioration of soil fertility, loss of fresh organic matter, disrupting soil structure and its properties, as well as release of large amounts of CO_2. Biomass is a sustainable source of organic carbon. There are many ways of its processing and use. Transformation of biomass into biochar (BC) as a result of pyrolysis has various everyday life applications - heating and barbecues, as well as a soil improver and composting element. In recent years, interest of usage solid phase (charcoal or biochar) obtained by pyrolysis as soil improver increases. The number research concerning biochar applications in order to mimic the effect of fertile soils in the Amazon Terra Petra (Glaser, 2002) were carried out in last ten years. The biochar structure, porosity, chemical composition, influence of external factors on the BC properties and its application, distribution and movement in the soil profile is necessary to be studied. In Bulgaria are made research only to determining the influence of systematic plowing of crop residues on the soil fertility and crop productivity on gray forest soil (Donkova, 2005). The studies of BC influence on the soil properties and plant growth are less. The aim of study is to evaluate the biochar agronomic impact on the plants growth, development and quality indicators.

Material and methods
The present pot experiment was conducted to investigate the effect of biochar application on the plants photosynthetic activity, soil agrochemical properties and plant development. Various methods for biochar preparation are described in the literature (Thomas B. Reed, 2004).Our team

has developed a small pyrolysis stove according to the "Lucia Stove" methodology. The wooden residues used in the process of pyrolysis are from cherry, apple, plum, quince and oak. The process of pyrolysis runs at 300-500°C, the obtained BC is without impurities from petroleum products. The obtained biochar was crushed to 1 mm particles. The experiment was carried out on two soil types- cinnamic pseudopozolic from Sekirovo village and alluvial meadow soil from Tsalapitsa village. The tested crops were maize (*Zea mays*), after harvesting to determine the effect of biochar, was planted lettuce (*Lactuca sativa*) as a second crop. The biochar used for conducting the path experiment was in rate of 200 kg / ha and 400 kg / ha.

The following variants are tested:
1. Control 1 / clean soil /
2. Control + Biochar 1 / 6 g per pot/
3. Control + Biochar 2 / 12 g per pot /
4. Control 2 - (soil + NPK)
5. NPK + Biochar 1 / 6 g per pot /
6. NPK + Biochar 2 /12 g per pot/

Individual pots have a capacity of 2 kg and the total number of experimental pots is 36. Each variant is set in three replications. The mineral fertilizer rate was calculated based on our previous studies with both crops (Stoimenov, et al 2009). The fertilization norms are: nitrogen fertilizer - ammonium nitrate - 150 mg nitrogen per 1 kg of soil; Triple superphosphate - 120 mg P_2O_5 per 1 kg of soil and Potassium sulphate - 100 mg of K_2O per 1 kg of soil. In the first stage of experiments 5 maize seeds were planted per pot from variety Kn 209. Three plants are left after germination. Maize plants are harvested in the 6-7 leaf stage. After maize harvesting, lettuce from pre-produced seedlings was planted. Five plants per pot were planted leaving only one till the end of vegetation. The average duration of vegetation is 40 days. Throughout the growing season, irrigation is maintained close to the optimal. Phenological observations and biometric measurements were made during vegetation. The content of the soils macro- and microelements is determined by standard methodologies (Arinushkin, 1970). Ammonium and nitrate nitrogen were determined calorimetrically, mobile forms of phosphorus and potassium by method of P. Ivanov (1984), Movable forms of microelements with EDTA-extract, pH-potentiometrically, in aqueous extract and potassium chloride solution. Total nitrogen content in the plants was determined by the Keldahl method by decomposition with concentrated H_2SO_4 and 30% H_2O_2. The remaining macro- and microelements were determined by "dry" burning in muffle furnaces and subsequent dissolution in 20% HCl, followed by measuring on Atomic Absorption Spectrophotometer (Mincheva M., A. Brashnarova, 1975). In the study, the chlorophyll was determined in fresh mass (mg %), spectrophotometrically in 80% acetone leaching by the Vernon method, 1960. The total sugars content was determined refractometrically (%) (Digital Refractometer – 32145).

Results and discussion

The two soil types- cinnamic pseudopozolic from Sekirovo village and alluvial-meadow soil from Tsalapitsa village were used to carry out the vegetation pot experiments.The soil of the experimental field of Tsalapitsa village is with a light mechanical composition, which resulted in faster development of the processes. According to Stoycheva, (2007) soil chemical analysis of the Tsalapitsa village is characterized by a low content of total N (0.052%) and humus (0.70%), slightly acidic reaction throughout the profile and a sorption capacity of 22.5 meq / 100g soil. The soil from the Sekirovo village is characterized by a low humus content which gradually decreases in depth. The soil has a medium acidity in the surface horizon and low acidity in the ´By´ horizon. The availability of total nitrogen is very low. There is a very low sorption capacity. The distribution of cation exchanges is diffuse. There is an exchangeable acidity. The chemical composition -pH in KHL is a 5.5 for the surface horizon (tab.1).

Table 1. Chemical analysis of alluvial-meadow soil from the village of Tsalapitsa and cinnamic pseudopozolic from the of Sekirovo village used in pot experiments

	pH KCl	Sum N NH_4+NO_3 mg/kg	P_2O_3 mg/100g	K_2O mg/100g	Humus %
Tsalapica	7,3	23,6	25,2	43.7	2,98
Sekirovo	5,5	19	22,7	33,3	2,15

Soil analysis after harvesting of the experimental plants reported a slight increase in pH values due to the BC use, the highest value was recorded in variants with- 12 g/BC on both soil types (Tsalapica pH-7.5; Sekirovo pH-6.00). Similar results have also been obtained from many other authors (Nigussie et al. 2012; Arocena, J.M. and C. Opio, 2003; Khanna, et al. 1994). Nigussie et al. show a statistically proven (P <0.01) increase in pH after BC use in ratio- 10 t / ha. The residual NH4 + NO3 content in the test treatments ranges between 10.6-77.7 mg / kg of soil for Sekirovo and between 5.00-75.00 mg / kg of soil for Tsalapitsa. The content of mobile K and P on both soil types and both forms of fertilization increases as the fertilizer rate increases. In the maize growing experiment, results were obtained for the amount of plastid pigments in the leaves of each treatment. The content of plastid pigments in plants depends on the species, variety, development stage, temperature, lighting, feeding conditions, etc. Therefore, the chlorophyll content can serve as an indirect indicator of plant growth, as chlorophyll "a" is more sensitive to external influences in compeers with chlorophyll "b" (Petrova, 2010). Figure 1 shows the chlorophyll content of maize leaves in the 6-7 leaf stage. There is a trend of chlorophyll “a” growth from the control to the treatment with imported 12 g/ BC per kg/ soil. The chlorophyll "a" is ranges from 0.39 to 1.35 mg / g in fresh mass. The highest values on both soil types in both fertilization variants are reported for the variant with imported 12 g / BC per kg/ soil. Chlorophyll ´b” ranges from 0.19 to 0.65 mg / g in fresh mass. The ratio of Ch "a" to Ch "b" is 2 (3): 1, which is within the normal range.

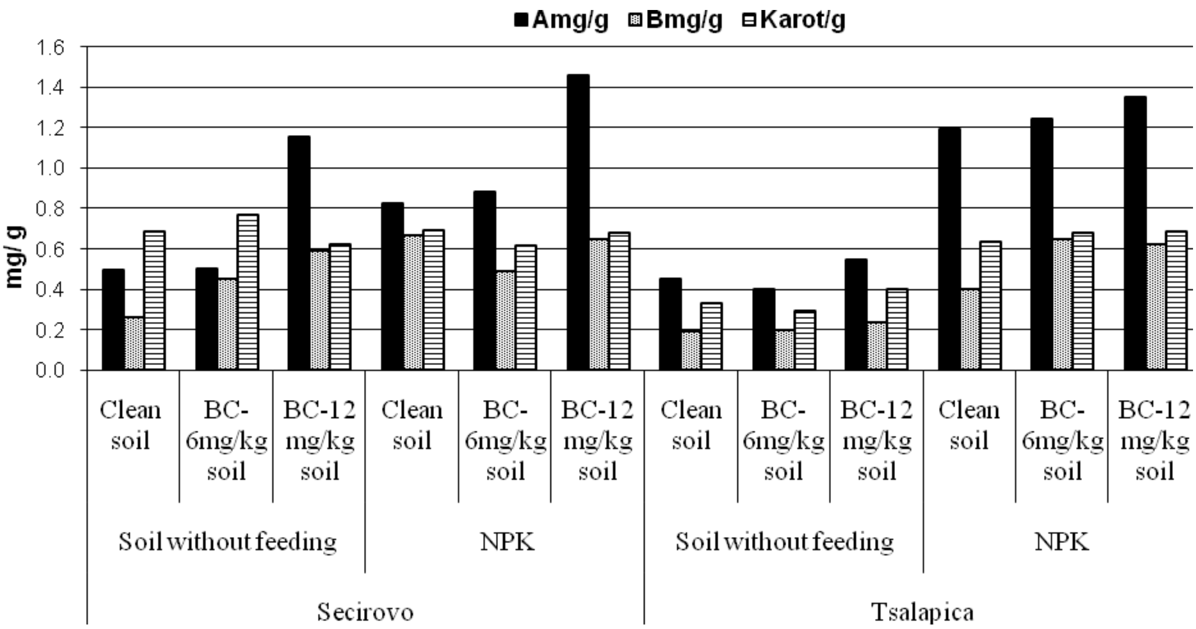

Figure. 1 Content of plastid pigments in pot experiment with maize 6-7 leaf phase

The content of plastid pigments is an important factor determining the growth and development of plants (Dinev, 1996; Stancheva, 2002; Stancheva, 2004). As an indirect indicator, it can be associated with the absorption of nutrients, especially nitrogen. The lower content of N in variant with BC on alluvial meadow soil from Tsalapitsa, compared to cinnamic pseudopozolic from Sekirovo leads to a lower content of plastid pigments. This is probably due to the fact that the nitrogen content of these soils has not ensured the optimal nitrogen nutrition regime. In both soil types the content of plastid pigments increases with increasing of fertilizer rate. The Brix percentage is a very important as it is a direct measurement of how the plant is performing, as all plants use 6 molecules of water and 6 molecules of carbon dioxide with the radiation from the sun to make 1 molecule of basic sugar and 6 molecules of oxygen. The one molecule of sugar produced in the photosynthesis process is the foundational building block for everything we see growing above and below ground in the form of leaves, stems, tree trunks, branches, fruit, bulbs, grain, roots and even algae. So, when we measure the sugar levels in plants it directly corresponds to how much sugar production has taken place in the plant. The reported total sugars content on both soil types ranges between 3.15 and 7.2 Brix % (fig.2). In the treatments with added BC and organic fertilizer, it was observed increasing of sugar content on both soil types. For NPK-added variants, the highest values were reported for the 6 g/ BC + NPK.

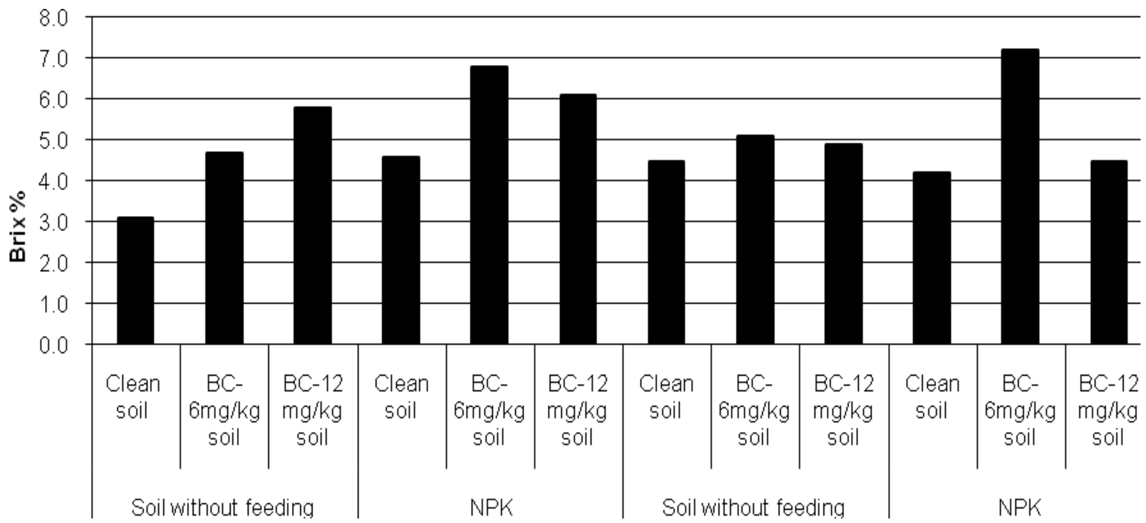

Figure 2. Total sugars content in maize 6-7 leaf phase

The yield of fresh biomass from the maize growing experiment varies between 12.08 g and 48.4 g (fig.3).

The results from fresh biomass measurements in the organic fertilizer variants shows, that the differences between the control and the two variants with BC are minimal. This proves that the chosen BC norm, as well as the step between them, is small. The highest yield on cinnamic pseudopozolic soil was observed in variant with 6 g/BC + NPK - 48, 4 g. While in the alluvial meadow soil, which has lower baseline nitrogen content, the yield increases from the control to the variation by12 g/ BC + NPK (from 26.9 to 42.13 g). The mass of the maize roots in variants without mineral fertilization increases with increasing of the BC norm. This relationship is reversed in the version with the addition of NPK. It is known that leafy vegetable crops are particularly suitable as indicators for testing certain factors (Dinev and Mitova, 2011, Dinev and Mitova, 2013, Mengel and Kirkby, 1982). That's why after harvesting maize, lettuce (*Lactuca sativa*) was planted as a second crop to determine the effect of biochar. The similar trends in yields obtained from maize are seen in the second crop. Fig. 4 shows the yields of lettuce fresh biomass.

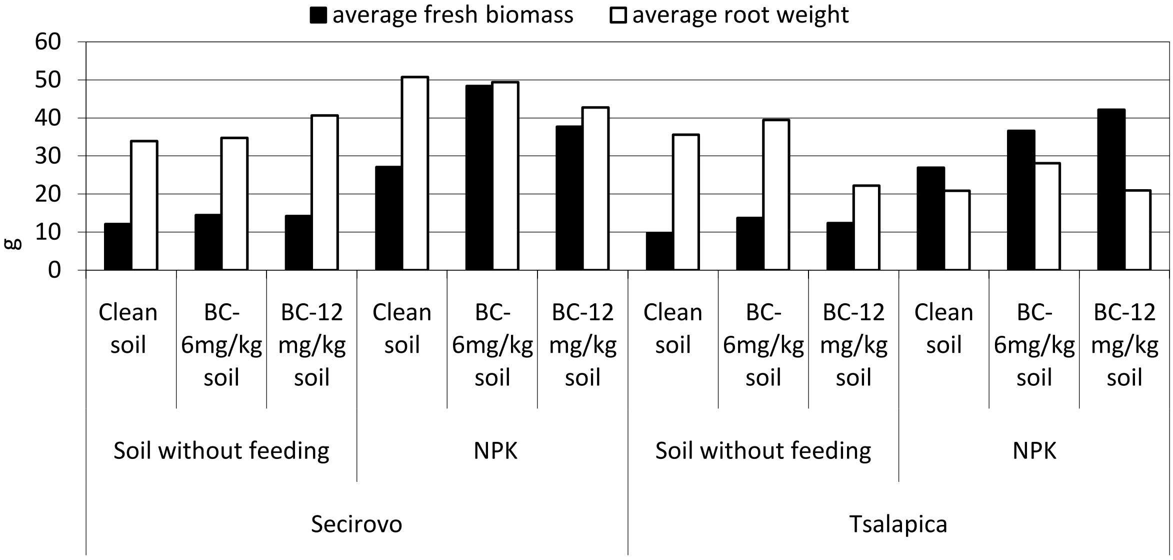

Figure 3. Average weights of fresh biomass and roots from pot experiment with maize on two soil types

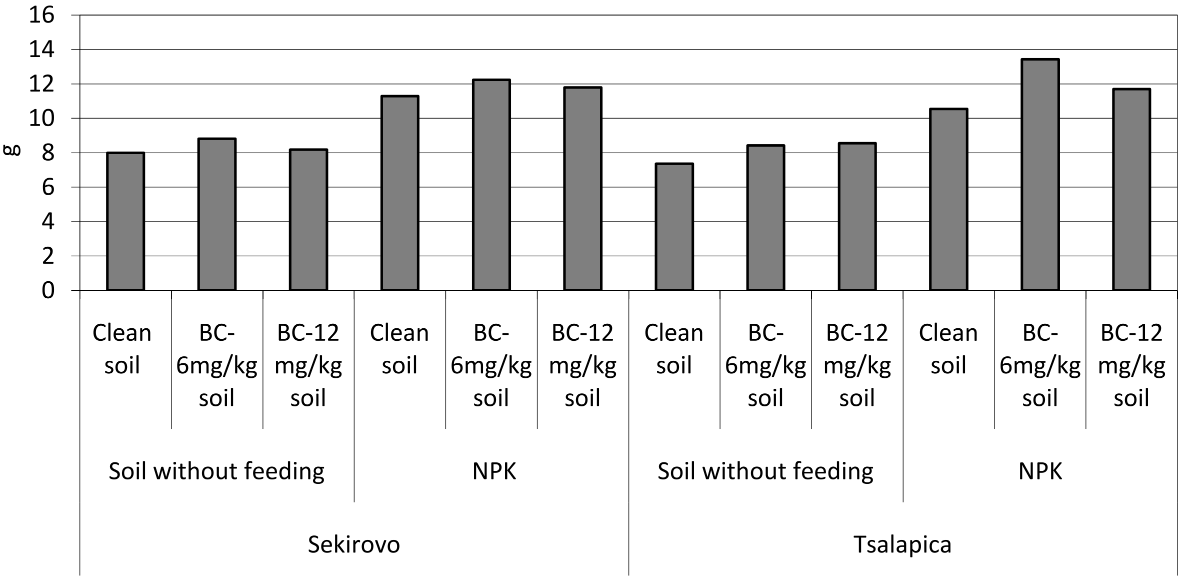

Figure 4. Average weight of fresh biomass from pot experiment with lettuce on two soil types

The largest yield was reported for variants with 6g/ BC on both soil types, which appears to be optimal for lettuce development. In variants with imported 12 g/ BC, the reported yield is slightly lower from 8.2 g to 11.8 g. It has been reported that leaf colour can indicate the amount and proportion of chlorophyll in leaves which are, in turn, closely related to plant nutrient status. Figure 5 presents the results obtained for the content of plastid pigments.

In the variants with imported BC and of both soil types, those treated with 6 g /BC +NPK appear to be optimal. The lowest value was recorded for the non-added BC variants on both soil types.

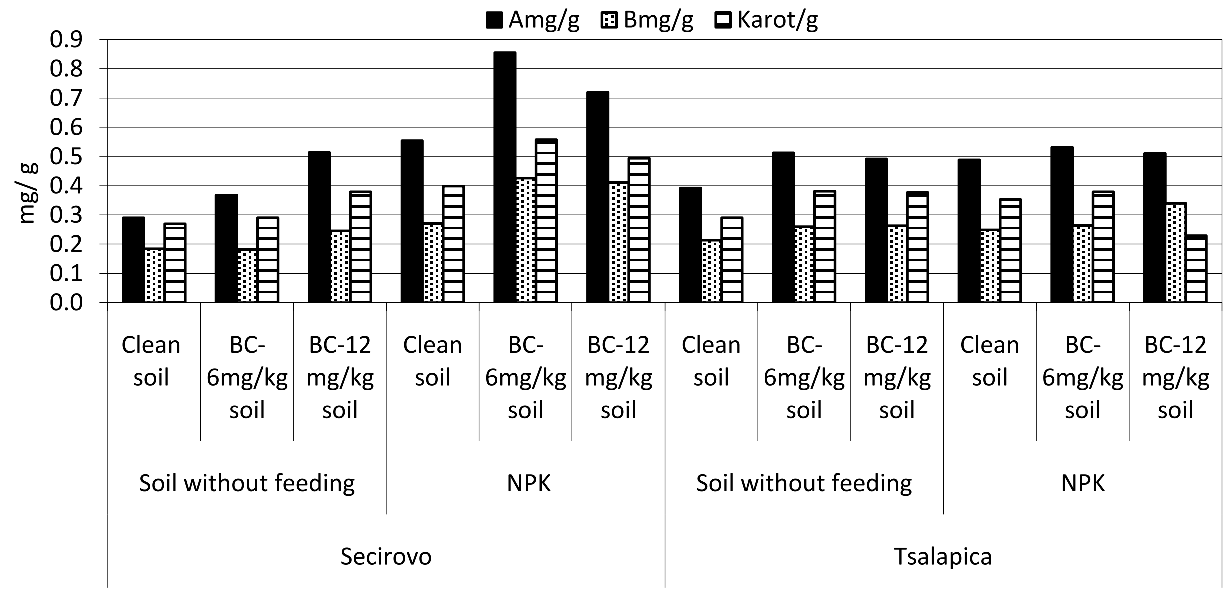

Figure 5. The content of plastid pigments in pot experiment with lettuce on two soil types

Conclusions

The biomass yield in both tested crops follow common pattern. While in the variants with different ratio BC, the differences in the obtained vegetative mass are lower, in the BC+ NPK treatments there was increase in yield in parallel with the increase of the quantity of BC applied. The variants with a higher content of total N in the plants tissues (maize and lettuce) on cinnamic pseudopozolic soil from Sekirovo village, a higher content of plastid pigments was measured as compared to the plants grown on alluvial meadow soil from Tsalapitsa village. In both tested cultures an increase in the BC norm also increases the chlorophyll content, which is particularly noticeable on the cinnamon pseudopozolic soil. The highest total sugars content in maize, for both soil types, was found in the variant with 12g/ BC. In the case of NPK + BC fertilization, the maximum is reached in variant with 6 g/BC + NPK. As a result of studies, the combined use of BC with mineral fertilization in the cultivation of trenches and vegetable crops can be recommended.

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References

1. Arocena, J.M. And C. Opio, 2003. Prescribed Fire-Induced Changes in the Properties of Sub-Boreal Forest Soils. Geoderma, 113: 1-16.
2. Dinev N., Iv. Mitova, 2011. Quality and safety of salad (Lactucasativa) depending on irrigation water. N. conf. With a foreigner. Participation »Food science, technology and technologies-2011», 14- 15.10.2011, Plovdiv, 415- 420.
3. Dinev N., Iv. Mitova, 2013. Extraction and quality of parsley produce (Petroselinumhortense var. Foliosum) depending on nitrate content in irrigated waters.
4. Dinev, N., 1996. Biochemical changes of cereal genotypes (triple-strand CBC, Sofia.
5. Donkova, R., 2005, Influence of systemic plowing of plant residues and mineral fertilization in crop rotation on soil fertility and productivity of crops in the conditions of gray forest soil, Dissertation work for acquisition of educational and scientific degree "Doctor".
6. Glaser, B., Lehmann, J. and Zech, W. (2002), "Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal", Biology and Fertility of Soils 35, 219-230 .

7. Khanna, P. K., R.J. Raison and R.A. Falkiner, 1994. Chemical properties of ash derived from Eucalyptus litter and its effects on forest soils. Forest Ecology Management, 66: 107-125. 50.
8. Mengel K. and E. A. Kirkby, 1982. Principles of plant nutrition, Intrn. Potash Inst. Bern, Switzerland
9. Mincheva M., A. Brashnarova, 1975. Certain Ways of Mineralization of Plant Material by Routine Analysis to Fefine K, Ca, Mg, Na, Zn, Mn, Cu and Fe by the Methods of Contemporary Spectrophotometry, Soil Science and Agrochemistry . Vol. X, N-1, p. 114-122.
10. Nigussie, A., Kissi, E., Misganaw, M., and G. Ambaw. 2012. Effect of biochar application on soil properties and nutrient uptake of lettuces (*Lactuca sativa*) grown in chromium-polluted soils. American-Eurasian Journal of Agriculture & Environmental Science 12 (3): 369-376. Raison, R.J., 1979. Modification of the soil environment by vegetation fire, with particular reference to nitrogen transformation: A Review. Plant Soil 51: 73-108.
11. Petrova, V., 2010. Dissertation for Acquiring Scientific and Educational Degree PhD.
12. Stancheva I. and I. Mitova, 2002. Effects of Different Sources and Fertilizer Rates on Lettuce Yield and Quality under Controlled Conditions. Bul. J. of Agricultural Science, 8, 157-160.
13. Stancheva I., Iv. Mitova, E. Atanasova, R. Toncheva. 2004. Influence of sources and norms of nitrogen fertilization on yield and quality of lettuce. Ecology and industry. 6. N- (1, 82-83).
14. Stoimenov, G., V. Tsenova, Y. Kirkova. 2009. "Irrigation effect on different hybrids of maize yield". Climate, экология, сельскоехозяйствоевразии, Irkutsk, p. 353-361.
15. Stoycheva, D., M. Kercheva, V. Kolev, Ts. Simeonov, 2007. Assessment of the impact of land use on the chemical composition of the soil solution. Soil Science - Sustainable Agriculture and Environmental Protection, International Conference, 13-17. May, Sofia p.210
16. Thomas B. Reed, Agua Das, and Paul S. Anderson, 2004, Gasification: A process common to all biomass stoves A presentation at the 2004 ETHOS meeting.
17. Vernon, L. P., 1960. Spectrophotometer determination of chlorophylls and pheophytins in plant extracts, Anal. Chem. 32, 1144-1150, 1.

INFLUENCE OF COMPOST MIXTURE ON THE LETTUCE YIELD AND QUALITY FORMATION

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Abstract
In Bulgaria there are many natural materials that present an interest for human activity in relation of their use and properties. Five natural products were tested. In base of their quantity and quality characteristics was offered different mixture in various combination of vermiculites, ash from straw biomass, wood biomass and pig manure. The aim of the study is based on the characterization and evaluation of the proposed natural products. Different ratio of mixtures was tested as a soil improver and their impact on yield and chemical characteristic of plant production. Pot experiment with lettuce is set out in the following versions: control, 2, 5, 7, and 11% by soil weight. The highest yield is obtained in variants with 11% of all compost mixture. In mixtures (2, 3 and 5) with large manure content has been observed highest increase of yield as a result of more imported nutrients by organic manure. Chemical characteristics of lettuce crop production, with the participation of all variants of compost mixtures show that the main nutrients - nitrogen, phosphorus and potassium are normal for plant species. The level of tested heavy metal is below toxic concentration. The results of total sugars content and photosynthetic activity correspond with data obtained in biomass harvesting in pot experiment. In all variants with 7-11% compost mixture was observed the highest level of photo-synthetically active chlorophyll "a".

Keywords: vermiculite, greensand, straw ash, pig manure, pot experiment.

Introduction
In order to ensure the positive balance of nutrients and organic matter in the soil, it is necessary to use high quality fertilizers. Due to shortage of organic fertilizers in our country, in order to increase fertility, yield and quality of agricultural production, it is necessary to seek new organic reserves. In our country there are many natural materials that represent an interest to human activity associated with the use of their properties in agricultural practices. Several studies have shown that there are natural products which, under certain conditions alone or in combination, can improve the physical and chemical properties of soils and crop yields. Such natural products are vermiculite, greensand, plant ash, wood biomass, etc. which improve the properties of soils (Marinova et al. 2012, Mitova and Marinova 2012).Many studies (Pestnikov, 1990, Vasiliev, 2009) found that greensand is not only a source of microelements (K_2O from 3.7% to 4.4%, P_2O_5 from 0.41% to 0.92%) as plant nutrition and also a material how improves soil structure and soil moisture storage.Plant ash has alkaline properties, removes the acidity of the soil and helps to mineralize nitrogen from humus and in practice is not depleting resources (Nikolova et al. 2010). Biomass of wood is composed of an organic substance synthesized in the process of development of microorganisms, plants and animals. In practice, it is used as a raw material for bioenergy and in the preparation of compost mixes for plant breeding (Brezin et al. 2013).In order to enhance their effect and the content of the main nutrients, it is necessary to compose them with organic products, rich with macro and microelements and this kind of product is the organic manure produced by different species and groups of animals. Along with the macro elements - nitrogen, phosphorus, potassium, large amounts of organic matter and billions of microorganisms are introduced through it, which increase the soil biogenicity and improve the nutrition of the plants (Lampkin, 2002, Marinova, 2013, Sutton et al.

1999). The aim of the study is on the base of the characteristic and the evaluation of the prepared compost mixtures from natural products, to show their impact over the soil properties in pot experiments.

Material and methods

Several natural products - vermiculite, greensand, straw ash, wood biomass and pig manure - are presented for study and evaluation. Based on their qualitative and quantitative characteristics were offered different combinations for use in agricultural practice. After tests of chemical and agrochemical characterization of the starting materials, several compost samples were prepared by mixing in the following ratio:

Table 1. Compost mixture in different ratio

Compost №	Vermiculite	Greensand	Ash from straw	Wood biomass	Pig manure
1.	1	1	1	1	1
2.	1	2	1	1	2
3.	1	1	2	1	2
4.	1	1	1	2	1
5.	1	2	2	1	3

A pot experiment was conducted with prepared mixtures on alluvial meadow soil, using 3 kg pots in three replicates. The alluvium meadow soil is low acid with pH in water 5,8; with low humus content and with worse agro-chemical characteristics compared to leached Smolnitsa. It is low reserved with common nitrogen – about 0,1%, middle reserved with moveable potassium 15,5 mg K_2O/100g and good reserved with moveable phosphorus - 21,9mg/100g. By this kind of soil the content of determined heavy metal is under the limited allowed concentrations (in accordance with № 3 Regulation). The pot experiments are made by the following control variants, soil and addition of 2, 5, 7 and 11% of different mixture, added to the weight of the soil. Test crops by the pot experiments are maize silage and lettuce variety Djentli, because the leafy vegetable crops are particularly suitable as indicators for testing certain factors (Mengel and Kirkby 1982). In the plants tissues, total nitrogen was determined by the Keldahl method - decomposition with concentrated H_2SO_4 and 30% H_2O_2.Macro and micro elements are identified by the "dry" burned in a muffle furnace and subsequent dissolution in 20% HCl, followed by measuring on Atomic Absorption Spectrophotometer. In the study, the chlorophyll was determined in fresh mass (mg %), spectrophotometrically in 80% acetone leaching by the Vernon method, 1960. The total sugars content was determined refractometrically (%) (Digital Refractometer – 32145).

Results and discussion

The determination of the chemical and agrochemical characteristics of the compost mixtures is an important point in determining of the impact on yield and crop production. The yield of lettuce on alluvial-meadow soil with different compost mixtures and different ratio is presented in table2.In general, the difference in lettuce yield for each variant is not significant, because there aren't found significant differences in the chemical characteristics of the individual compost mixtures. It was found that the yield increase in all studied variants in compared to the control. Variation of yield values for individual replicates is noted. In the mixtures III and V with greater input of manure and wood biomass, which is a main product that carrying out the essential nutrients the higher increase of yield was observed. The trend observed in all variants is a gradual increase in yield with an increase of percentage of composite mixtures. The highest yield was obtained in the 11% variant, but the data at the 7% lower rate also showed satisfactory results (table. 2).

Table 2. Yield of lettuce grown on alluvial meadow soil in g/per pot

Lattuce	I replication	II replication	III replication	Average
Controlee clean soil	8,0	5,7	4,5	6,0
First mix in ratio 1:1:1:1:1				
2%	6,7	10,7	5,8	7,5
5%	9,0	12,9	12,3	11,4
7%	11,4	20,1	15,0	15,5
11%	13,9	13,8	20,0	15,9
Second mix in ratio 1:2:1:1:2				
2%	10,0	16,3	14,4	13,5
5%	9,7	11,8	15,3	12,3
7%	11,8	16,9	16,3	15,0
11%	12,3	13,5	15,3	13,7
Third mix in ratio 1:1:2:1:2				
2%	7,4	17,5	8,1	11,0
5%	19,3	12,1	18,9	16,7
7%	9,3	18,3	18,0	15,2
11%	10,6	26,1	12,5	16,4
Fourth mix in ratio 1:1:1:2:1				
2%	7,2	9,8	10,8	9,3
5%	8,2	13,7	16,0	12,6
7%	4,8	24,4	12,4	13,8
11%	11,3	21,7	13,4	15,4
Fifth mix in ratio 1:2:2:1:3				
2%	7,9	11,2	10,2	9,8
5%	13,1	11,0	19,5	14,5
7%	11,5	13,5	12,2	15,4
11%	18,8	18,0	21,1	19,6

Table 3. Chemical characteristics of the lettuce plant production of the alluvial meadow soil experiment

Variant	N %	P %	K %	Ca %	Mg %	Zn mg/kg	Cu mg/kg	Mn mg/kg
Control clean soil	0,41	0,33	3,7	1,17	0,36	31	4	87
Imix 2%	0,30	0,21	3,4	1,00	0,30	27	6	88
5%	0,38	0,20	3,2	0,91	0,28	25	5	66
7%	0,42	0,18	3,0	0,88	0,28	25	5	42
11%	0,46	0,16	3,0	0,46	0,16	10	4	27
II mix 2%	0,30	0,30	4,5	0,84	0,26	35	5	68
5%	0,45	0,31	4,9	0,85	0,26	40	6	51
7%	0,53	0,31	5,3	0,85	0,30	40	6	51
11%	0,60	0,45	7,5	0,90	0,35	34	6	51
IIImix 2%	0,48	0,33	4,8	0,99	0,27	32	7	49
5%	0,50	0,33	5,0	0,98	0,25	35	6	45
7%	0,58	0,37	5,6	0,95	0,25	40	6	45
11%	0,68	0,40	7,3	0,85	0,25	44	5	41
IV mix 2%	0,47	0,37	6,0	0,69	0,25	31	3	65
5%	0,55	0,33	6,5	0,70	0,25	31	3	60
7%	0,62	0,30	6,2	0,82	0,25	30	4	43
11%	0,84	0,26	5,8	0,92	0,25	29	5	28
Vmix 2%	0,50	0,33	5,5	0,60	0,25	0,30	4	25
5%	0,51	0,35	5,8	0,62	0,25	0,30	4	25
7%	0,55	0,35	6,6	0,68	0,26	0,30	5	26
11%	0,59	0,45	7,2	0,75	0,28	30	4	28

From the lettuce plant analysis, it appears that as the mixture rate increases, the total nitrogen, phosphorus and potassium content in the vegetable mass also increases. The P content in plants is from 0.16 to 0.45%. The K content ranges from 3.0 to 7.3%. There is no clearly expressed pattern for content of heavy metals in plant tissues compared to the control. Lead and cadmium have the same values for all variants <0.1. Heavy metals in lettuce in all variants are below the allowable concentration. The content of sugar in aqueous solution (Brix%) is an important indicator as it can determine the growth of plants. When we measure the sugar levels in plants it directly corresponds to how much sugar production has taken place in the plant. The measured sugar levels in lettuce from the vegetation experiment directly correspond to the plant development.

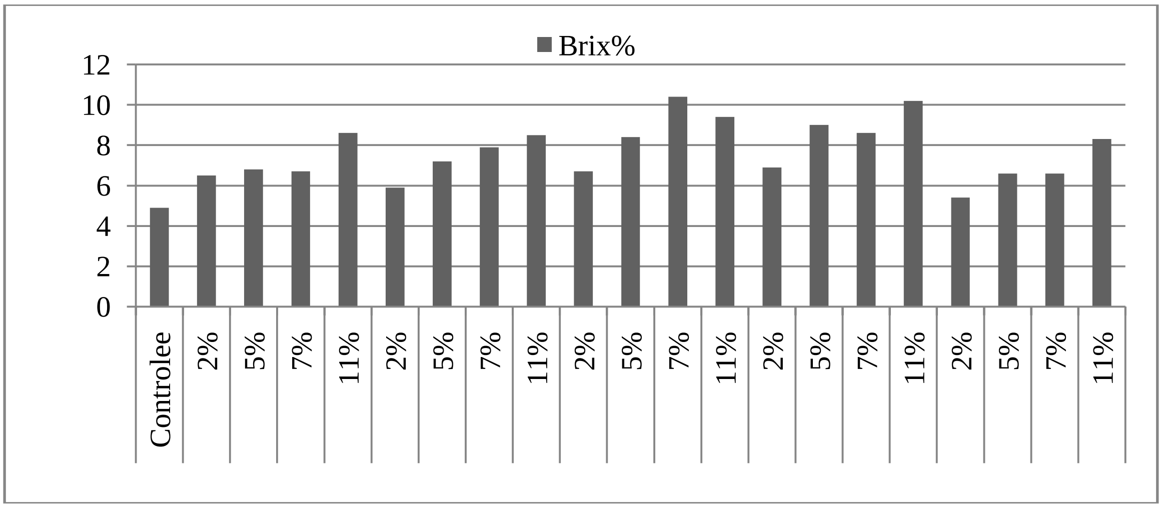

Figure 1.Total sugars content in plant tissue from pot experiment with lettuce grown on alluvial meadow soil

In all variants was observed increasing of the total sugars content in compared to control variant, ranging from 4,9 to 10,4%.There is a trend for increase the total sugar content with increasing the percent proportions of the composite mixture. In the case of a mixture №III with higher pig manure content, the highest sugar content was reported at a 7% ratio per soil weight. Photosynthesis is a physiological process inherent in photoautotrophic green plants. It is a system of reactions in which chlorophyll-containing organisms use light energy and from simple inorganic substances they synthesize organic compounds and emit oxygen. Plastid pigments are organic compounds that absorb light energy with a wavelength of 390-1100 nm. The pigment system consists of several types of pigments: chlorophylls, carotenoids and others. The content of plastid pigments is an important factor determining the growth and development of plants. As an indirect indicator, it can be related to the absorption of nutrients and, above all, nitrogen. Therefore, the determination of the content of chlorophyll "a", "b" and carotenoids is important for monitoring the response of plants to fertilization with different compost mixtures.

The measures carried out for plastid pigments content in lettuce leaves growing on alluvial meadow soil varied between 5,65 to 13,92 mg / l of chlorophyll "a" and between 4,01 and 9,65 mg / l of chlorophyll "b". For all variants of the compost mixtures, the ratio 2: 1 for chlorophyll "a" and "b" is retained. The lettuce reaction clearly shows an increase in the levels of plastid pigments relative to the control. Corresponding with sugar content and yield, the highest levels were recorded in 7-11% variants of the compost mix over the weight of the soil. Higher content of chlorophylls has been reported for compost 5, due to the high nitrogen content incorporate in soil with the pig's manure, which is the largest amount of soil weight. Due to the presence of different nutrient content in individual mixtures, the increase is particularly related with pig manure from livestock farms.

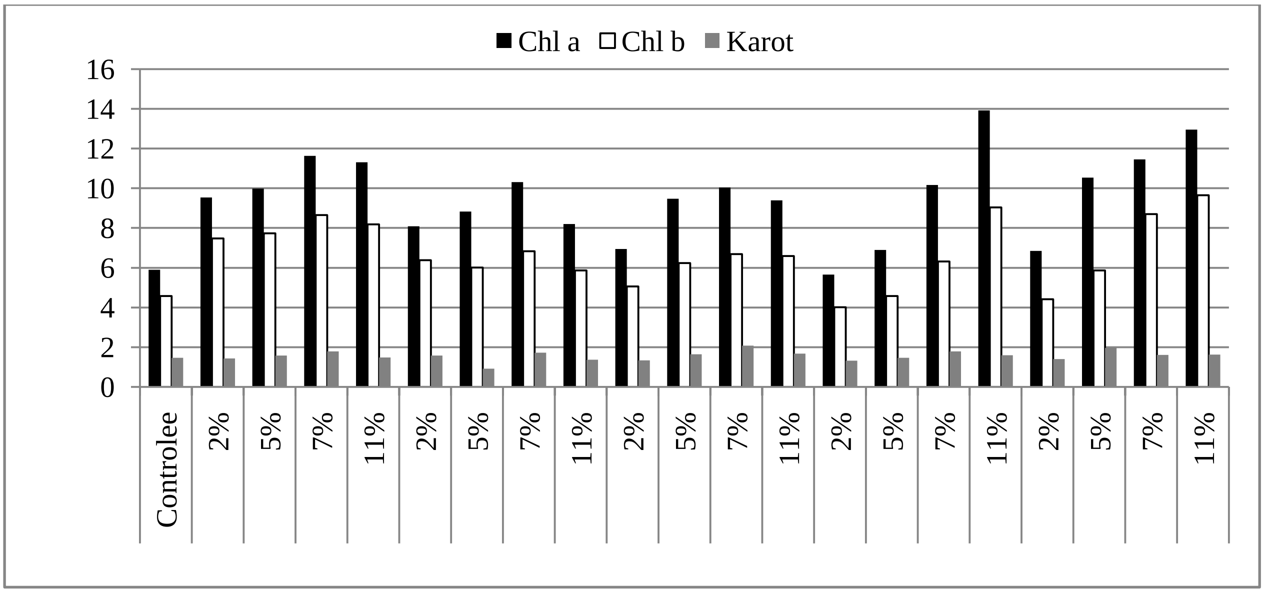

Figure 2.Content of plastid pigments in pot experiment with lettuce grown on alluvial meadow soil

Conclusions

The reported lettuce yield grown on alluvial meadow soil, shows that there is a gradual increase in yield, depending on the ratio of the mixtures in compared to the control. The highest yield was obtained in the 11% variant, but the data at the 7% lower rate also showed satisfactory results. In the variants of mixtures with a larger share of manure - II, III and V there is a general tendency of yield increase as a result of more nutrients input with the manure. The chemical characterization of plant production on alluvial meadow soil involving all compost mixtures and variants shows that there are no significant differences in the values of macroelements and heavy metals relative to the control. The results obtained for total sugars content and photosynthetic activity corresponds to the data obtained for the lettuce yield. The data confirm that photosynthetic potential is closely related to nitrogen feed and yield. The high levels of chlorophylls reported at an earlier stage of plant growth are the guarantor of higher and sustainable yields. In all composite mixtures, the best results for the photosynthetic active chlorophyll content "a" are showing at 7-11% of the blends.

References

1. Brezin, V., P. Antonov, A. Kovacheva. 2013. Plant source biomass for the production of biogenic fuels.Educational house at UF Sofia, p.48.
2. Lampkin, N., 2002. Organic farming. Old Pond Publishing, United Kingdom, p.65.
3. Marinova, Sv., R. Toncheva, E. Zlatareva, Hr. Pchelarova. 2012. Characteristics of vermiculite and its influence on the yield of lettuce in pot experiments. Int. conf. *BALWOIS-12, Conf. on Water, Climate and Environment 28.05-2.06. 2012, Ohrid.*
4. Mitovova, I., S. Marinova 2012. Influence of enriched vermiculus on yielding and quality in lettuces. Psychological science agrochemistry and ecology, yy.HL51, 2.21-27
5. National Ordinance No. 3, SG 38 CCPS 1979.
6. Nautical Journal, КубГАУ, No82 (08), UDC 631.812.11
7. Nikolova, S., B. Hristov, N. Dimitrova, A. Zhivkova.2010. Methods in the Field of Organic Farming, Handbook, 180 pp.
8. Pestnikov, A.V., 1990. Use zeolite in plant health. Agrochemistry, 7, 113-125
9. Sutton, A. L., D. Jones, B. Joern, D. Huber, 1999. Animal Manure AS and Plant
10. Vasiliev, A. A., 2009. Glauconite is an effective natural mineral fertilizer of potatoes. Agrarian herald of the Urals No. 6/60 /, 35-37.
11. Yakovlev, Elena, A. Bakalov, 2012. Glauconit as a potential new local fertilizer a Kuban region.

IMPORTANCE OF *CRAMBE SP.* IN MEDICAL AND COSMETIC INDUSTRIES

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Abstract
Crambe sp. are an annual and perennial plants with shrub form and belonging to Brassiceae family. Sterols in seed oil uses at margarine as additive for reduce to blood cholesterol. Likewise; similiar to *Simmondsia chinensis* oil and whale seminal oil, it uses at production of liquid wax. İnstead of materials, which ones proven carcinogenic effect, Erukamid ,obtained with transform of erucic acid, uses at personal care products (such as perfume, lotion and dye for cosmetics), dyes (for polissage), medical equipments have importance for healthy generation. Nitryl and isothiocyanate in the *Crambe* leaves have potential uses against to cancer. It is a natural alternative to harmful mineral oils and silicon with omega-9 content. Otherwise *Crambe* oil protect stem cell, decelerate skin aging and fight with free radicals by stimulate to collogen production.

Keywords: *Crambe sp*., seed oil, cosmetic, medicinal use.

Introduction
Crambe is a member of the Brassicaceae family and is an annual and perennial oil seed plant of which seeds can be consumed. The species complex extends throughout the Mediterranean region, Ethiopia and East Africa. *Crambe* has representation in the Mac-aronesian, Euro-Siberian, Mediterranean, Sindico-Saharan, Irano-Turkishand Sudan-Zambezian regions (Leppikand White, 1975). *Crambe* is not a well known species in Turkey although it is present in the country's flora. Renewables, like plant derived oils, are a sustainable means of supplying the essential products needed by world. Besides, global oilseed production is of the order of 450 Mt/year, with that figure predicted to scale up to 500 Mt by 2020 (OECD-FAO). Plant oils are already major agricultural merchandises with around 20% by value used for non-food applications in industry. Erucic and lauric acid are two plant-derived fatty acids and have been in fighting with petroleum alternatives for many years. Priorly cost has been major problem for development of new plant-derived oils. But now increasing crude oil cost and people concern about finite supply. Because of this reasons there is need to develop renewable products from plant oils (Stymne and Dyer, 2006). *Crambe* is one of the most hopeful "new" plants. *Crambe* oil extracted from *Crambe* seed. High in erucic acid (%57 of %35-50 oil), the seed oil is important in industrial applications inculding use as erucamide, coating, lubricants an nylon (Hirsinger, 1989; Cooke and Konstant, 1991; Lazzeri et al., 1994; Capelle and Tittonel, 1999). *Crambe* oil has very high capacity about removing warmth and high quality as well as some advantage about commercialization and storage (Comlekcioglu *et al*., 2008; Razavi and Nejad-Ebrahimi, 2009). *Crambe* has a potential use in industry such as biodiesel, oil industry and machine oil. Furthermore, seed cake after oil extraction can be used as an important feed for livestock. The industrial improvements and a wide range of people needs in developed countries led researchers seeking alternative products. *Crambe* is a preferable alternative agro-industrial material because of high erucic acid content in its seeds. (Seyis et al., 2012).

Usage areas of *crambe*
Crambe seeds have high glucosinolates content. It unmodified or after enzymatic hydrolysis by myrosinase, can negatively affect the nutritional quality of defatted proteinic meal in feeding. For these compounds and their degradation products, there are some potential interesting applications

after meal detoxification by glucosinolate removal and isolation. Non-edible uses include addition to mineral lubricants, in the manufacture of greases, and as a mouldlubricant in steel casting. Fully hydrogenated crambe oil is a white solid that has potential value as a component of wax compositions. However, a greater potential use exists in the industrial field for erucic acid products. Crude erucic acid is obtained by saponification of *Crambe* oil. This erucic acid yields two primary products on ozonolysis, the 13-carbon atom dibasic brassylic acid and the 9-carbon atom monobasic pelargonic acid (Lazzeri et al., 1994). Brassylic acid is used in the manufacture of polyester, plasticizers, alkydresins, lubricants, rubber additives, surface-active agents, new types of nylon, and other polymers (Cornelius and Simmons, 1969; Vargas-Lopez et al., 1999) . Pelargonic acid finds use in the field of plasticizers, alkydresins, vinylstabilisers, salts, pharmaceuticals and synthetic flavours (Cornelius and Simmons,1969). Otherwise, the primary market for high-erucic acid oils is erucamide, a slip agent critical to the manufacture and use of polyolefin films. Films such as polyethylene are produced commercially for familiar products such as bread wrappers, shopping and garbage bags, shrink wraps, and plastic sheeting (Vargas-Loperz et al., 1999). *Crambe* oil has biological degradation ability. This is significant for environment. The oils, rich in erucic acid, are used in various areas of the industry such as anti-blocking agent in polyethylene films, adhesive in printing, as anticorrosive material, in steel sheet metal industry, textile, detergent, perfume and various other industries (Tansı et al., 2003; Erickson et al., 1990). *Crambe* seed oil is an emollient that provides the skin with a perceivable softness without greasiness. The oil is high in omega-9 essential fatty acid making it ideal for skin nutrition and boosting skin hydration. For color cosmetics, it improves pigment dispersion. It is ideal for use in creams, lotions & body butters, lip products, hair care, sun care, body cleansers and baby care (Anonymous, 2017.).

Conclusions

Crambe has potential uses with high erucic acid content. It is an alternative product for production of biodiesel. *Crambe* has an important potential around the world for reduce the threat of global warming and eliminate air pollution which arising from fuel oils. Besides erucic acid content, *Crambe* seed oil have wide usage area such as medical and cosmetic industry with its different ingredient. Considering all of these, *Crambe* has remarked in the world and increased in demand on industrial oil market in recent.

References

1. Anonymous,2017. http://www.cosmeticingredients.co.uk/ingredient/lipovol%C2%AE-crambe
2. Capelle A. and Tittonel E.D. 1999. *Crambe*, a potential non-food oil crop. I. Production. Agio Food Industry High Tech. 10: 22–27.
3. Comlekcioglu N., Karaman S., &Ilcim A. (2008). Oil composition and some morphological characters of *Crambe orientalis* var. *orientalis* and *Crambe tataria* var. *tataria* fromTurkey. *Natural Product Research*, 22(6):525-532.
4. Cooke L. and Konstant D.A. 1991. What's new in oilseeds? Checkout *Crambe*! Agric. Res. USDA Res. Serv. 39: 16–17.
5. Cornelius J.A., Simmons E.A. (1969) *Crambe abyssinica*—a new commercial oilseed. Trop Sci11:17–22.
6. Erickson, D.B., Bassin, P., 1990. Rapeseed and *Crambe*: Alternative Crops with Potential Industrial Uses, Agricultural Experiment Station, Kansas State University, Manhattan, Bulletin 656.
7. Hirsinger F. 1989. New annual oil crops. In: Robbelen G.,Downey R.K. and Ashri A. (eds), Oil Crops of the World: Their Breeding and Utilization. McGraw-Hill Publishing Co.,pp. 518–532.
8. Lazzeri L., Leoni O., Conte L.S. and Palmieri S. 1994. Some technological characteristics and potential uses of *Crambe abyssinica* products. Ind. Crops Prod. 3: 103–112.

9. Leppik, E., White, G. (1975). Preliminary assessment of *Crambe* germplasm resources. *Euphytica*, 24:681-689.
10. Razavi, S.M., Nejad-Ebrahimi, S. (2009). Chemical composition, allelopatic and cytotoxic effects of essential oils of flowering tops and leaves of *Crambe orientalis* L. from Iran. *Nat. Prod. Res*., 23 (16): 1492-1498.
11. Seyis F., Aydın, E., Çopur, M. (2013). Alternatif yağ bitkisi: *Crambe* (*Crambe abyssinica* Hochst. Ex R.E.Fries). *Anadolu J. Agr. Sci.,*28 (2):108-114.
12. Stymne, S.,&Dyer J. (2006). Production of waxesters in *Crambe*outputsfromthe EPOBIO projectNovember 2006.
13. Tansı, S.,Yaniv, Z., Kahraman, S. 2003. Çukurova Koşullarında *Crambe spp*'nin Kültürü Olanakları ile Kalitesinin Belirlenmesi Üzerine Bir Araştırma. Adana Tarım Orman Turkish Journal of Agricultural and Natural Sciences Special Issue: 1, 2014 1042 ve Gıda Teknolojileri Araştırma Grubu Tübitak,Proje No:Togtag-2665.
14. Vargas-Lopez J.M.,Wiesenborn D., Tostenson K., Cihacek L. (1999). Processing of *Crambe* for oil and isolation of erucic acid. J Am Oils Chem Soc76:801–809.

S-GENOTYPING OF SOME SWEET CHERRY CULTIVARS RELEASED WITHIN BREEDING PROGRAMMES IN THE BALKAN REGION

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Abstract
Sweet cherry cultivars generally exhibit *S*-ribonuclease (*S-RNase*)-based gametophytic self-incompatibility and require pollination with pollen of compatible genotypes, which are indispensable to stable fruit production. Therefore, the determination of *S*-genotype provides relevant information for sweet cherry breeders and growers. The aim of this study was to identify the *S*-allelic constitution and incompatibility group in eight sweet cherry cultivars which were named and released at Fruit Research Institute, Čačak, Republic of Serbia ('Asenova Rana' and 'Čarna'), Research Station for Fruit Growing, Iasi, Romania ('Alexus', 'Bucium' and 'Margonia') and Fruit Growing Institute, Plovdiv, Republic of Bulgaria ('Kossara', 'Rosalina' and 'Rosita'). The use of the polymerase chain reaction (PCR) method with consensus primers for the second introns of *S-RNase*, as well as primers specific for S_1–S_7 and S_9 alleles enabled determination of the following *S*-genotypes in the assessed cultivars: S_1S_2 ('Alexus'), S_1S_4 ('Čarna'), S_2S_9 ('Kossara' and 'Rosita'), S_3S_6 ('Bucium'), S_3S_9 ('Asenova Rana' and 'Rosalina') and S_5S_6 ('Margonia'). In addition, the *S*-genotypes of two parental cultivars were reported in this manuscript for the first time – 'Boambe de Cotnari' (S_2S_7) and 'Ranna Tcherna' (S_1S_2). Based on the obtained *S*-allelic constitutions, the assessed cultivars were assigned to the following incompatibility groups: I, II, VI, IX, XI, XIV, XV, XVI and XLIII. The results generated in this study provide a valuable resource for cross design in developing new cultivars and for orchard management in the efficient high-yielding fruit production.

Keywords: *Prunus avium*, *S-RNase*, incompatibility group.

Introduction

Sweet cherry (*Prunus avium* L.) is an economically important fruit species in the Balkan countries. According to Food and Agriculture Organization of the United Nations, the average annual sweet cherry production (2010–2014) were 77,192 t, 29,196 t and 22,316 t in the Romania, Republic of Bulgaria and Republic of Serbia, respectively. Sweet cherry breeding programmes in these countries are paid particular attention to self-fertility, compact habitus, early, heavy and regular cropping, early or late ripening time, large and attractive fruits, resistance to major causal agents of economically important diseases, pests, frost and fruit cracking (Budan et al. 2013, Corneanu et al. 2016, Radičević et al. 2016). Among the most typically used conventional breeding methods are planned hybridisation, open pollination and clonal selection. The sweet cherry breeding work at Fruit Research Institute, Čačak, Republic of Serbia (FRI – Čačak) has resulted in the release of two sweet cherry cultivars (Radičević et al. 2016). Corneanu et al. (2016) reported that twenty-eight new sweet cherry cultivars were obtained and approved during 1994–2015 at Research Station for Fruit Growing, Iasi, Romania (RSFG – Iasi). The first achievements of sweet cherry breeding programme at Fruit Growing Institute, Plovdiv, Republic of Bulgaria (FGI – Plovdiv) have accomplished through releasing of four cultivars (Malchev and Zhivondov 2016). Most sweet cherries are self-incompatible, therefore cross-compatible cultivars that flower simultaneously are planted together to allow successful cross pollination and satisfactory cropping. Gametophytic self-incompatibility mechanism in sweet cherry is governed by the multiallelic *S*-locus with *S-RNase* (Bošković and Tobutt 1996) and *SFB* genes (Yamane et al. 2003). The use of the consensus and allele-specific PCR-based methods in sweet cherry has enabled the identification of 25 *S*-alleles (Vaughan et al. 2008) and 47 incompatibility groups, a group of '0' of unique *S*-genotypes and group of self-compatible cultivars (Schuster 2012). Due to high polymorphism, the *S*-locus has also been used as a genetic marker for identification of domestic and foreign sweet cherry cultivars at FRI – Čačak (Marić and Radičević 2014, Marić et al. 2015, Radičević et al. 2013, 2015). This study was aimed to determine the *S*-genotypes of sweet cherry cultivars developed within breeding programmes of three institutions (FRI – Čačak, RSFG – Iasi and FGI – Plovdiv) in the Balkan region, in order to assign the cultivars to accurate incompatibility groups for planning parental combinations in further breeding work and for orchards management by choosing the suitable pollenizer. Furthermore, these results were enabled to check the reported parental pairs of the released sweet cherry cultivars.

Material and methods

Plant material and DNA extraction

Fifteen sweet cherry cultivars, including parents ('Bigareau Burlat', 'Bigarreau de Schrecken', 'Boambe de Cotnari' 'Drogans Gelbe', 'Majova Rana', 'Ranna Tcherna' and 'Van') and derivatives ('Alexus', 'Asenova Rana', 'Bucium', 'Čarna', 'Kossara', 'Margonia', 'Rosalina' and 'Rosita'), were sampled from the collections FRI – Čačak, RSFG – Iasi and FGI – Plovdiv (Table 1 and 2). Young leaves of the assessed cultivars were collected, frozen in liquid nitrogen and stored at –80°C prior to DNA extraction. Frozen samples were ground in Mixer Mill MM 400 (Retsch GmbH, Haan, Germany) and genomic DNA was then extracted according to the method reported by Doyle and Doyle (1987), with the modification of the extraction buffer which included addition of β-mercaptoethanol (1%) and polyvinylpyrrolidone (2% PVP 40). DNA samples in TE buffer (10 mM Tris pH 8.0 and 1 mM EDTA) were kept at –20°C until use.

Consensus and allele-specific PCR analysis of the S-RNase

S-genotyping of assessed sweet cherry cultivars was based on the methods of Sonneveld et al. (2001, 2003). In order to identify the *S-RNase* alleles, the PCRs were performed with the consensus primer pairs specific for the second intron (PaConsII-F + -R) and primers specific for alleles S_1 to S_7, as well as for S_9 allele (Sonneveld et al., 2001, 2003). For the aforementioned alleles, the following annealing temperatures were used: 64°C for S_1, 60°C for S_2, 66°C for S_3, 63°C for S_4, 52°C for S_5, 65°C for S_6, 59°C for S_7 and 61°C for S_9. Sweet cherry cultivars with known *S*-genotypes were used as standards.

Detection and visualization of the PCR products

PCR products of *S-RNase* gene obtained with the consensus and allele-specific primers were separated by electrophoresis in 2% (70 V for 4 h) and 1.5% (70 V for 2–3 h) agarose gels, respectively. The gels were stained with ethidium bromide, and obtained DNA fragments were visualized using BIO-PRINT-1500/26M (Vilber Lourmat) imaging system and sized by comparison with a 1 Kb plus DNA ladder (Invitrogen, Groningen, the Netherlands).

Results and discussion

S-allele detection with consensus and allele-specific PCR assays

The use of consensus primers for the second introns and allele-specific primers allowed the identification of *S-RNase* alleles in the released and parental sweet cherry cultivars.

The amplification of the second intron of *S-RNase* with PaConsII-F + -R primers resulted in two PCR products, which corresponded to *S*-alleles of the assessed sweet cherry cultivars, except for parental cultivars 'Bigarreau Schrecken', 'Boambe de Cotnari' and 'Van'. The size of PCR product for the the second intron ranged from ~570 (S_6 allele) to ~2,380 bp (S_7 allele) (Figure 1). Amplification of the second intron disabled the identification of the S_1/S_3 alleles and S_2/S_7, therefore discrimination of these alleles required additional analysis. As reported earlier (Sonneveld et al. 2003, Schuster et al. 2007, Ipek et al. 2011), small size differences were found when using consensus primers for the amplification of the second intron of the S_1 and S_3, as well as S_2 and S_7 alleles. Confirmation of *S-RNase* alleles of the assessed sweet cherry cultivars was conducted using the specific primers for the S_1 to S_7, as well as S_9 allele. The PCR product of ~820 bp corresponded to allele S_1 and was identified in two released cultivars – 'Alexus' and 'Čarna' (Figure 2a), and five parental cultivars – 'Bigarreau de Schrecken', 'Drogans Gelbe', 'Majova Rana', 'Ranna Tcherna' and 'Van'. In three released cultivars – 'Alexus', 'Kossara' and 'Rosita' (Figure 2b), and parental cultivars – 'Boambe de Cotnari' and 'Ranna Tcherna', the PCR product of ~640 bp corresponding to S_2 allele was obtained. Use of S_3 allele-specific primers enabled amplification of fragment of ~960 bp in three released cultivars – 'Asenova Rana', 'Bucium' and 'Rosalina' (Figure 2c), and three parental cultivars – 'Bigareau Burlat', 'Bigarreau de Schrecken' and 'Van'. In 'Čarna' (Figure 2d) and 'Majova Rana', the PCR product of ~820 bp corresponding to S_4 allele was detected. The DNA fragment of ~300 bp corresponding to S_5 allele was identified in 'Margonia' (Figure 2e) and 'Drogans Gelbe', whereas a fragment of ~470 bp corresponding to S_6 allele was identified in two released cultivars – 'Bucium' and 'Margonia' (Figure 2f). In parental cultivar 'Boambe de Cotnari', the PCR product of ~580 bp corresponding to S_7 allele was identified. Use of S_9 allele-specific primers enabled amplification of fragment of ~500 bp in four released cultivars – 'Asenova Rana', 'Kossara', 'Rosalina' and 'Rosita' (Figure 2g), and 'Bigareau Burlat' as parental cultivar. The size of PCR products for aforementioned *S*-alleles were in agreement with results stated by Sonneveld et al. (2001, 2003), who reported that DNA fragment of 820, 640, 960, 820, 300, 470, 584 and 495 bp corresponded to S_1, S_2, S_3, S_4, S_5, S_6, S_7 and S_9 alleles, respectively.

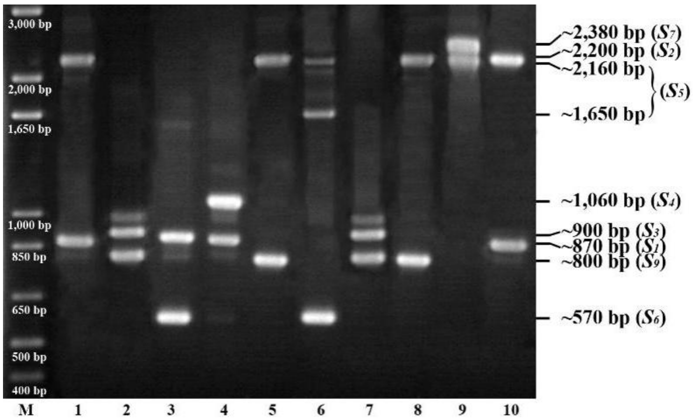

Figure 1. PCR products of the *S-RNase* amplified fragment obtained with consensus primers for the second intron of the assessed sweet cherry cultivars: 1 – 'Alexus', 2 – 'Asenova Rana', 3 – 'Bucium', 4 – 'Čarna', 5 – 'Kossara', 6 – Margonia', 7 – 'Rosalina', 8 – 'Rosita', 9 – 'Boambe de Cotnari', 10 – 'Ranna Tcherna'; 1Kb plus DNA ladder (M).

S-genotypes, incompatibility groups and assessment of parentage

The *S*-genotype of each released and parental cultivar was determined after combining the results obtained upon amplification with the consensus and the allele-specific primers (Table 1 and 2). Out of fifteen sweet cherry cultivars, the *S*-genotypes for six released cultivars – 'Alexus' (S_1S_2), 'Bucium' (S_3S_6), 'Kossara' (S_2S_9), 'Margonia' (S_5S_6), 'Rosalina' (S_3S_9) and 'Rosita' (S_2S_9), and two parental cultivars – 'Boambe de Cotnari' (S_2S_7) and 'Ranna Tcherna' (S_1S_2) are published in this paper for the first time. The following *S*-allelic constitutions were confirmed: S_1S_3 ('Bigarreau de Schrecken' and 'Van'), S_1S_4 ('Čarna' and 'Majova Rana'), S_1S_5 ('Drogans Gelbe') and S_3S_9 ('Asenova Rana' and 'Bigareau Burlat'), and these results were in agreement with the findings reported by Schuster (2012), Marić and Radičević (2014) and Marić et al. (2017). According to the genotypes detected, all assessed cultivars were assigned to their coresponding incompatibility groups (IGs), which were previously described by Schuster (2012). Therefore, the nine following IGs were determined: I (‘Alexus’ and ‘Ranna Tcherna’), II (‘Bigarreau de Schrecken’ and ‘Van’), VI (‘Bucium’), IX (‘Čarna’ and ‘Majova Rana’), XI (‘Boambe de Cotnari’), XIV (‘Drogans Gelbe’), XV (‘Margonia’), XVI (‘Asenova Rana’, ‘Rosalina’ and ‘Bigareau Burlat’) and XLIII (‘Kossara’ and ‘Rosita’). This study revealed that ‘Kossara’ and ‘Rosita’ sharing the same *S*-genotype and belong to the same IG, which was consistent with results reported by Zhivondov et al. (2011), who stated that ‘Rosita’ was not suitable pollenizer for ‘Kossara’.

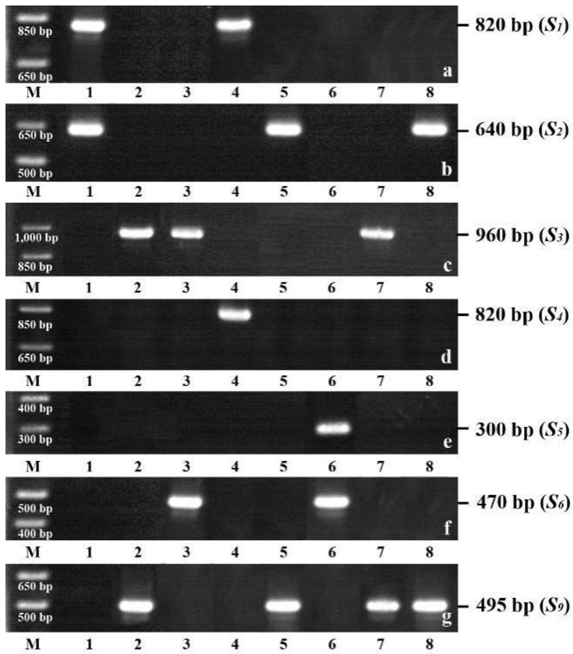

Figure 2. PCR products of the *S-RNase* amplified fragment obtained with primers specific for alleles S_1 (a), S_2 (b), S_3 (c), S_4 (d), S_5 (e), S_6 (f) and S_9 (g) in released sweet cherry cultivars: 1 – ‘Alexus’, 2 – ‘Asenova Rana’, 3 – ‘Bucium’, 4 – ‘Čarna’, 5 – ‘Kossara’, 6 – Margonia’, 7 – ‘Rosalina’, 8 – ‘Rosita’; 1Kb plus DNA ladder (M).

The pedigree of three cultivars from the Republic of Bulgaria (‘Kossara’, ‘Rosalina’ and ‘Rosita’) and one cultivar from the Romania (‘Alexus’) was confirmed by their *S*-genotyping. ‘Kossara’ was inherited one *S*-allele from each parent (S_2 from ‘Ranna Tcherna’ and S_9 from ‘Bigareau Burlat’). For the open-pollinated cultivars ‘Alexus’, ‘Rosalina’ and ‘Rosita’, the *S*-alleles inherited from female parents were confirmed (S_1 from ‘Lijana’, S_3 from ‘Van’ and S_9 from ‘Bigareau Burlat’, respectively). *S*-genotypes of two cultivars from the Republic of Serbia (‘Asenova Rana’ and ‘Čarna’) and two cultivars from the Romania (‘Bucium’ and ‘Margonia’) suggested incorrectly reported parents for these cultivars. ‘Asenova Rana’ (S_3S_9) and ‘Margonia’ (S_5S_6) revealed *S*-genotypes that could not be explained by proposed parentage [‘Drogans Gelbe’ (S_1S_5) × ‘Majova Rana’ (S_1S_4) and ‘Van’ (S_1S_3) O.P., respectively]. Marić et al. (2017) reported that these discrepancies could be resulted from pollen contamination, information presented by the breeders or technical errors in the processing of seedlings. Based on *S*-allelic constitutions of ‘Bucium’ (S_3S_6) and ‘Čarna’ (S_1S_4), the possible female parents may be ‘Van’ (S_1S_3) and ‘Majova Rana’ (S_1S_4), respectively. Since ‘Bucium’ and ‘Čarna’ had not inherited any *S*-allele of their respective male parent, ‘Boambe de Cotnari’ (S_2S_7) and ‘Bigarreau de Schrecken’ (S_1S_3) could not be in their pedigrees.

This study reports the first part of comprehensive *S*-genotype screening of sweet cherry genetic resources developed in the Balkan countries, which will be useful for the cross design in the further breeding programmes and for orchard management of these cultivars. For some of the assessed cultivars, no information on their *S*-genotypes was available in the literature. Hence, the crucial genetic compatibility information for released cultivars 'Alexus', 'Bucium', 'Kossara', 'Margonia', 'Rosalina' and 'Rosita', as well as parental cultivars 'Boambe de Cotnari' and 'Ranna Tcherna', was provided in this manuscript.

Table 1. *S*-genotypes and incompatibility groups of sweet cherry cultivars released in the Balkan countries

Origin*	Cultivar	Parentage	*S*-genotype	IG**
RO	'Alexus'	'Lijana' O.P.***	S_1S_2	I
RS	'Asenova Rana'	'Drogans Gelbe' × 'Majova Rana'	S_3S_9	XVI
RO	'Bucium'	'Van' × 'Boambe de Cotnari'	S_3S_6	VI
RS	'Čarna'	'Majova Rana' × 'Bigarreau de Schrecken'	S_1S_4	IX
BG	'Kossara'	'Ranna Tcherna' × 'Bigareau Burlat'	S_2S_9	XLIII
RO	'Margonia'	'Van' O.P.	S_5S_6	XV
BG	'Rosalina'	'Van' O.P.	S_3S_9	XVI
BG	'Rosita'	'Bigareau Burlat' O.P.	S_2S_9	XLIII

*Country according ISO 3166 code list.
**Incompatibility group, according to Schuster (2012).
***By open-pollination.

Table 2. *S*-genotypes and incompatibility groups of parental sweet cherry cultivars

Cultivar	*S*-genotype	IG	Reference for *S*-genotype
'Bigareau Burlat'	S_3S_9	XVI	Schuster (2012); confirmed in this study
'Bigarreau de Schrecken'	S_1S_3	II	Schuster (2012); confirmed in this study
'Boambe de Cotnari'	S_2S_7	XI	This study
'Drogans Gelbe'	S_1S_5	XIV	Schuster (2012); confirmed in this study
'Lijana'	S_1S_6	XX	Schuster (2012)
'Majova Rana'	S_1S_4	IX	Marić and Radičević (2014);confirmed in this study
'Ranna Tcherna'	S_1S_2	I	This study
'Van'	S_1S_3	II	Schuster (2012); confirmed in this study

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References
1. Bošković, R. and Tobutt, K.R. (1996). Correlation of stylar ribonuclease zymograms with incompatibility alleles in sweet cherry. Euphytica, 90: 245–250.
2. Budan, S., Zhivondov, A. and Radičević, S. (2013). Recent achievements in cherries breeding in some Balkan countries. Acta Horticulturae, 981 (1): 83–90.
3. Corneanu, G., Iurea, E., Sîrbu, S. and Corneanu, M. (2016). New sweet cherry genotypes from Research Station for Fruit Growing Iaşi, Romania. Acta Horticulturae, 1139: 85–90.
4. Doyle, J.J. and Doyle, J.L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin, 19: 11–15.
5. Ipek A., Gulen H., Akcay, M.E., Ipek, M., Ergin, S. and Eris, A. (2011). Determination of self-incompatibility groups of sweet cherry genotypes from Turkey. Genetics and Molecular Research, 10 (1): 253–260.
6. Malchev, S. and Zhivondov, A. (2016). Breeding programme for developing new sweet cherry cultivars in the Fruit Growing Institute, Plovdiv, Bulgaria. Agricultural Science and Technology, 8 (4): 289–291.

7. Marić, S. and Radičević, S. (2014). Primena PCR metode u određivanju *S*-genotipa trešnje (*Prunus avium* L.) u Institutu za voćarstvo u Čačku. Voćarstvo, 48 (185/186): 29–37.
8. Marić, S., Radičević, S., Cerović, R. and Vranić, D. (2015). Determinacija *S*-alelne konstitucije nekih sorti trešnje (*Prunus avium* L.). Voćarstvo, 49 (189/190): 43–48.
9. Marić, S., Radičević, S., Lukić, M., Cerović, R. and Paunović, S.A. (2017). Determination of *S*-genotype in apple and sweet cherry cultivars released at Fruit Research Institute, Čačak. Genetika, 49 (1): 127–138.
10. Radičević, S., Marić, S., Cerović, R. and Đorđević, M. (2013). Assessment of self-(in) compatibility in some sweet cherry (*Prunus avium* L.) genotypes. Genetika, 45 (3): 939–952.
11. Radičević, S., Marić, S. and Cerović, R. (2015). *S*-allele constitution and flowering time synchronization – preconditions for effective fertilization in sweet cherry (*Prunus avium* L.) orchards. Romanian Biotechnological Letters, 20 (6): 10997–11006.
12. Radičević, S., Cerović, R., Marić, S. and Đorđević, M. (2016). Cherry breeding work at Fruit Research Institute – Čačak (Republic of Serbia). Book of Proceedings of VII International Scientific Agriculture Symposium 'Agrosym 2016'. Jahorina, Republic of Srpska, Bosnia and Herzegovina: 472–478.
13. Schuster, M. (2012). Incompatible (*S*-) genotypes of sweet cherry cultivars (*Prunus avium* L.). Scientia Horticulturae, 148: 59–73.
14. Schuster, M., Flachowsky, H. and Köhler, D. (2007). Determination of self-incompatible genotypes in sweet cherry (*Prunus avium* L.) accessions and cultivars of the German Fruit Gene Bank and from private collections. Plant Breeding, 126: 533–540.
15. Sonneveld, T., Robbins, T.P., Bošković, R. and Tobutt, K.R. (2001). Cloning of six cherry self-incompatibility alleles and development of allele-specific PCR detection. Theoretical and Applied Genetics, 102: 1046–1055.
16. Sonnenveld, T., Tobutt, K.R. and Robbins, T.P. (2003). Allele-specific PCR detection of sweet cherry self-incompatibiliy (*S*) alleles S_1 to S_{16} using consensus and allele-specific primers. Theoretical and Applied Genetics, 107: 1059–1070.
17. Vaughan, S.P., Bošković, R.I., Gisbert-Climent, A., Russell, K. and Tobutt, K.R. (2008). Characterisation of novel *S*-alleles from cherry (*Prunus avium* L.). Tree Genetic & Genomes, 4: 531–541.
18. Yamane, H., Ikeda, K., Ushijama, K., Sassa, H. and Tao, R. (2003). A pollen-expressed gene for a novel protein with an F-box motif that is very tightly linked to a gene for *S*-RNase in two species of cherry, *Prunus cerasus* and *P. avium*. *Plant* and *Cell Physiology*, 44 (7): 764–769.
19. Zhivondov, A., Malchev, S. and Gercheva, P. (2011). Suitable pollinators of the new sweet cherry cultivar Kossara. Rastenievudni nauki, 48 (1): 31–33.

THE EFFECT OF HARVESTING TIMES ON OIL AND FATTY ACID COMPOSITION OF PEANUT VARIETIES GROWN IN MAIN CROPPED CONDITION IN CUKUROVA REGION *(MEDITERRANEAN AREA)* IN TURKEY

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Abstract
This study was conducted at the experimental area of the Department of Field Crops, Faculty of Agriculture, Cukurova University as a main crop in 2015. The objective of this study was to determinate the effect of harvesting dates on oil and fatty acid composition of peanut (*Arachis hypogaea L.) varieties grown in main cropped condition in Cukurova region.* The experimental design was a split plot with three replications. The Halisbey, Sultan, Arioglu-2003, Osmaniye-2005, NC-7, Batem-5025, Flower 22, Flower-32, Flower-36, Brantley and Wilson peanut varieties (Virginia market type) were used as a plant material in this research. The plants were harvested at 149, 156, 163 and 170 days after sowing (DAS). Oil and fatty acids (palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, arachidic acid, behenic acid and lignoceric acid) content and Oleic acid to Linoleic acid ratio (O/L) of peanut varieties were investigated. As a result, the oil percentage of peanut varieties was increased from 47.8% to 50.3% when the harvesting delayed from 149 DAS to 170 DAS. While the palmitic and linoleic acid percentage was decreasing, the stearic and oleic acid percentage was increased when the harvesting delayed from 149 DAS to 170 DAS in peanut varieties. The others fatty acids were not affected by the harvesting time.

Keywords: Peanut, main crop, harvesting time, oil content, fatty acid.

Introduction

Peanut (*Arachis hypogaea* L.) is the fourth major oilseeds crop of the world next to soybean, rapeseed and cotton. Peanut contributes 8.7% of the total oil seeds production (45 million ton) in the world in 2015 (FAO, 2015). About two-thirds of total peanut production is crushed for oil and the remaining one-third is used in confectionery products in the world (Dwivedi et al. 1996). Peanut oil accounted for 3.0% of the world's vegetable oil production in 2015. For this reason, peanut is an important oilseed crop for vegetable oil production. Peanut seeds contain 35-56% oil and 25-30% protein and 9.5-19.0% carbohydrate on a dry seed basis. In addition, they are a good source of mineral (P, Ca, Mg and K) and vitamins (E, K and B group). For this reason, it is an important source of edible oil and protein for human nutrition. Peanuts are also a cheap source of protein, a good source of essential vitamins and minerals, and a component of many food products (Ingale and Shrivastava, 2011; Chamberlin et al. 2014 and Chowdhury et al. 2015). Ishag (2000) and Kaba et al. (2014) reported that peanut has indeterminate growth habit. For this reason, flowering and pod formation continue long time during the growing period. The peanut plants produced many flowers (18-142 flowers $plant^{-1}$) but, only 15-20% of flowers produce mature pods (Lim and Hamdan, 1984). Young et al. (1982) reported that total pod production continually increased with growth period, but that harvested yield reached a peak and then decline due to increased field losses at the longer period. Oil content is an important quality characteristic in peanut seed. The oil content of peanut seed influences by genotypic variation, growing conditions and maturity. Court et al. (1984), Sattayarak (1997), Lu et al. (1997) and Canavar and Kaynak (2013) reported that oil content was increased by delaying the harvesting times. Andersen and Gorbet (2002) and Chowdhury et al.

(2015) reported that the nutritional and storage quality of peanut are determined by its fatty acids composition. Young and Worthington (1974), Dwivedi et al. (1996) and Isleib et al. (2008) reported that fatty acid composition of peanut seed oil influenced by varietal and seasonal variation, genotypic variation, air and soil temperature, planting date, soil nutrient, growing conditions and maturity. The amount of saturated and unsaturated fatty acids in peanut oil varies from 10.92% to 17.47% and from 81.13% to 94.81% respectively. The major fatty acids components are oleic acid, linoleic acid and palmitic acid in peanut oil. Peanut oil is rich in oleic and linoleic acids. Oleic acid content in peanut genotypes can vary from 21 to 85% and linoleic acid from 2 to 43%. Andersen and Gorbet (2002) and Gulluoglu et al. (2016a) reported that, seed maturity can also influence the fatty acid composition of peanut. In general, oleic acid increases and linoleic acid decrease with seed maturity. The increase in oleic acid with seed maturity is normally accompanied by a decrease in palmitic and linoleic acid. Bovi (1982) Raheja et al. (1987) and Önemli (2012) reported that there was a negative correlation between oleic acid and linoleic acid. Holaday and Pearson (1974) found that higher temperatures during the last 4 weeks before harvest resulted in higher oil and oleic acid content and correspondingly higher O/L ratios. Oil content and fatty acid composition of peanut have been studied in different cultivars and different environments and it has been reported that the oil content of peanut cultivars varied between 37.9-56.3%, oleic acid (C18:1) 37.7-82.2%, linoleic acid (C18:2) 2.9-41.5%, palmitic acid (C16:0) 9.6-13.2%, stearic acid (C18:0) 1.6-3.7%, arachidic acid (C20:0) 1.2-1.7% and behenic acid (C22:0) 1.2-3.5% (Dwivedi et al. 1996; Yav et al. 2008; Önemli, 2012; Chaiyadee et al. 2013; Mzimbiri et al. 2014; Chowdhury et al. 2015; Gölükcü et al. 2016 and Gulluoglu et al. 2016b). Peanut has been grown as a main and double cropped after a small grain harvest in the Cukurova region in Turkey. Fatty acid composition of peanut is not constant. The fatty acid composition of peanut oil varies depending on varieties, growing conditions and maturity. The objective of the study is to investigate the effect of harvesting times on oil content and fatty acids composition of peanut varieties grown as main crop in Mediterranean region in Turkey.

Material and methods

This experiment was conducted in 2015 at Research Farm of Cukurova University (41°04´N, 36°71´E, and 36 m) as a main crop. Halisbey, Sultan, Arioglu-2003, Osmaniye-2005, NC-7, Batem-5025, Flower-22, Flower-32, Flower-36, Brantley and Wilson peanut varieties belonging to Virginia market type were used as a plant material in this research. The soil texture was clay loam. The soil tests indicated that pH of 7.5 with high concentrations of K_2O and low concentrations of P_2O_5. In addition, the organic matter and nitrogen content of the soil were very low. The lime content was 20.5% in the upper layers with increased levels in lower layers. Mediterranean climate prevails in this region. Winters are warm and rainy, whereas summers are dry and hot. The average monthly air temperature and precipitation during the research period (April-September) was varied between 16.9-30.1°C and 71.9 mm and 205.4 mm, respectively. The average relative humidity was between 47.9% and 69.0%. The differences between the year and long term for the climatic data were not significant (Anonymous, 2015). The experiment was designed at split plot design (harvesting times as main plots and varieties as subplots) and replicated three times. The experimental site was cultivated deeply by the moldboard following the harvest of the previous crop in the autumn and then the soil was prepared by using disked-harrowed the day of planting. 250 kg ha^{-1} of Di-ammonium phosphate (45 kg ha^{-1} N, 115 kg ha^{-1} P_2O_5) fertilizer was applied and incorporated to soil before planting. Ammonium nitrate (33%N) at the rates of 200 kg ha^{-1} was applied two times; before first (beginning of flowering) and second (pod formation) irrigation. Plots consisted of 4 rows 5.0 m long and 70 cm apart. The seeds were sown in line manually by hand on 5 April 2015 and with 70 x 15 cm distance. During the growing period, recommended pesticides and fungicides were applied to control insects and diseases. During the growing period, other standard cultural practices were done. The plants were harvested by hand at four different times with one week intervals (149 DAS, 156 DAS, 163 DAS and 170 DAS) at the beginning of September.

Data collection and analysis

Determination of oil content: Seeds of each variety were harvested separately at maturity stage when the seed moisture was reduced to 12% or less at both growing seasons. Once harvested, seeds were cleaned and dried to approximately 7% moisture. Seed oil content in three samples from each genotype was determined by Soxhlet extractor according to AOCS (2010) using petroleum ether (40-60°C) as a solvent. Determination of fatty acids content: fatty acid profile was measured as *fatty acid methyl esters* using Gas Chromatograph (GC) according to AOCS (2010). O/L was calculated by the oleic acid content (%) to linoleic acid content (%) ratio (Gulluoglu et al. 2016b). The collected data on different parameters were statistically analyzed to obtain the level of significance using JUMP 8.1.0 package program with split plot design. The means differences were compared with the Least Significant Differences (LSD, 5%) Test.

Results and discussion

Fatty acids composition

The average data belonging to saturated and unsaturated fatty acids content, oil content and O/L values of peanut varieties at different harvesting times has been presented in Table 1 and 2. The major fatty acids components are oleic acid, linoleic acid and palmitic acid in peanut oil. Peanut oil is rich in oleic and linoleic acids. The amount of saturated and unsaturated fatty acids in peanut oil varies between 10.92% and 17.47% and between 81.13% and 94.81%, respectively.

Table 1. The saturated fatty acids content of peanut varieties at different harvesting times in main crop growing season

Treatments	Palmitic acid (%)	Stearic acid (%)	Arachidic acid (%)	Behenic acid (%)	Lignoceric acid (%)
Harvesting Times (A)					
149 DAS	10.16	3.23	1.22	2.80	1.64
156 DAS	9.81	3.34	1.19	2.76	1.59
163 DAS	9.46	3.50	1.21	2.83	1.57
170 DAS	9.15	3.73	1.21	2.86	1.55
Varieties (B)					
Halisbey	9.76	3.28	1.21	3.11	1.84
Sultan	10.01	3.47	1.17	2.94	1.64
Arioglu-2003	10.14	3.12	1.15	2.69	1.79
Osmaniye-2005	10.30	2.80	1.25	2.99	1.82
NC-7	8.77	3.32	1.18	2.82	1.42
Batem-5025	8.42	3.50	1.24	2.98	1.51
Flower-22	12.16	3.89	0.93	2.54	1.47
Flower-32	11.57	4.04	0.93	2.59	1.48
Flower-36	11.52	4.14	0.90	2.35	1.43
Brantley	5.55	3.28	1.54	2.95	1.62
Wilson	7.92	3.11	1.77	2.98	1.591.59
LSD ($\%5_A$)	0.651	0.039	NS	0.019	NS
LSD ($\%5_B$)	0.220	0.059	0.046	0.059	0.068
LSD ($\%5_{AxB}$)	0.441	0.119	NS	0.118	NS

As it can be seen from Table 1, the differences between the harvesting times were statistically significant for the palmitic acid, stearic acid and behenic acid percentage, but it was not significant for the arachidic acid and lignoceric acid percentage. The palmitic acid, stearic acid, behenic acid, arachidic acid and lignoceric acid percentages varied between 9.15-10.16%, 3.23-3.73%, 2.76-2.86%, 1.19-1.22% and 1.55-1.64%, respectively at the harvesting times (Table 1). The stearic acid percentage was increased when the harvesting date was delayed from 149 DAS to 170 DAS, but palmitic and lignoceric acid content was decreased in peanut varieties. The average palmitic, stearic, arachidic, behenic and lignoceric acids percentage of peanut varieties varied between 5.55-11.57%, 2.80-4.14%, 0.90-1.77%, 2.35-3.11% and 1.42-1.84%, respectively. The differences between the peanut varieties for the saturated fatty acids were statistically significant. The interaction between the palmitic acid percentage x harvesting time, stearic acid percentage x harvesting time and behenic acid percentage x harvesting time were significant. The fatty acid composition of peanut oil varies depending on the genotype, seed maturity, climate conditions, growth location, and interaction between these factors (Carrin and Carelli, 2010). The fatty acids composition of Virginia type peanut varieties varies between 9.0-9.1% palmitic, 2.2-2.4% stearic, 56.4-60.3% oleic, 24.2-26.8% linoleic, 1.1-1.8% arachidic, 1.0-1.1% eicosenoic and 1.8-2.4% behenic acids (Brown et al. 1975). The palmitic acid percentage of the peanut varieties was found the highest among the saturated fatty acids. The saturated fatty acids content in peanut oil were strongly influenced by genotype (Isleib et al. 2008). Young et al. (1972) grew eight different peanut cultivars in Oklahoma for one year to assess effects of digging date on oil quality. They dug at five different digging dates, starting with 113 DAP for early maturing and 120 DAP for later maturing genotypes. Digging delays tended to give peanut oil with higher stearic and oleic acid and less linoleic acid. The results are corresponded well with the findings of Knauft et al. (1986), Dwivedi et al. (1996), Andersen and Gorbet (2002), Yav et al. (2008), Önemli (2012), Chaiyadee et al. (2013), Mzimbiri et al. (2014), Chowdhury et al. (2015), Gölükcü et al. (2016) and Gulluoglu et al. (2016a).

Table 2. The oil content, unsaturated fatty acids content and O/L value of peanut varieties at different harvesting times in main crop growing season

Treatments	Oil content (%)	Oleic acid (%)	Linoleic acid (%)	Linolenic acid (%)	O/L value*
Harvesting Times (A)					
149 DAS	47.8	52.83	24.44	1.77	2.162
156 DAS	48.6	53.97	23.42	1.78	2.304
163 DAS	49.4	55.08	22.65	1.79	2.432
170 DAS	50.3	56.05	21.94	1.78	2.555
Varieties (B)					
Halisbey	49.4	53.69	23.66	1.80	2.269
Sultan	49.2	53.71	24.62	1.80	2.182
Arioglu-2003	50.7	52.41	25.09	1.74	2.089
Osmaniye-2005	51.0	51.53	26.69	1.69	1.931
NC-7	47.3	59.77	17.79	1.75	3.360
Batem-5025	50.4	59.79	17.29	1.86	3.458
Flower-22	46.6	46.12	29.48	1.82	1.564
Flower-32	46.5	43.55	32.06	1.84	1.358
Flower-36	49.2	46.10	30.00	1.76	1.537
Brantley	47.8	79.21	2.19	1.77	36.169
Wilson	51.1	53.43	25.39	1.75	2.104
LSD ($\%5_A$)	0.70	0.399	0.215	NS	-
LSD ($\%5_B$)	1.17	0.341	0.381	0.022	-
LSD ($\%5_{AxB}$)	2.34	0.682	0.762	0.043	-

*O/L value: Oleic acid (%)/Linoleic acid (%)

Peanut oil is rich in oleic and linoleic acids. The oleic acid, linoleic acid and linolenic acid percentage varied between 52.05-56.05%, 21.94-24.44% and 1.77-1.79% respectively at the harvesting times (Table 2). The differences between the harvesting times were significant for the oleic and linoleic acids percentage. While the oleic acid percentage was 52.83% at the harvesting 149 DAS, it increased up to 56.05% at the harvesting 170 DAS. As the harvesting time was delayed, the oleic acid percentage increased. However, the linoleic acid percentage was decreased from 24.44% to 21.94% when the harvesting time was delayed from 149 DAS to 170 DAS (Table 2). As the harvesting time was delayed, the linoleic acid percentage decreased substantially. Statistically significant differences were found among the peanut varieties for oleic and linoleic acids percentage. The average oleic acid percentage ranged from 43-55 to 79.21% (Table 2). The highest oleic acid percentage was recorded in Brantley (79.21%) and the lowest in Flower-32 (43.55%). Brantley was a high oleic type genotype and for this reason, its oleic acid percentage was found high. The linoleic acid percentage of peanut varieties varied between 2.19-32.06%. The highest linoleic acid percentage was found in Flower-32 (32.06%) and the lowest was in Brantley (2.19%). Andersen and Gorbet (2002) reported that oleic acid content in peanut genotypes varied from 21 to 85% and linoleic acid from 2 to 43%. Brown et al. (1975) reported that, oleic and linoleic acid accounted for 75-80% of the total fatty acids in peanut oil. Escobedo et al. (2015) reported that the peanut oil was rich in oleic and linoleic acids. Peanut oil composition is influenced by several groups of factors including environmental factors, genetic factors and interaction between environmental and genetic factors (Andersen and Gorbet, 2002; Isleib et al. 2008 and Chaiyadee et al. 2013). Andersen and Gorbet (2002) reported that seed maturity can also influence the fatty acid composition of peanut. In general, oleic acid increases and linoleic acid decrease with seed maturity. Young et al. (1972) found that digging delays tended to give peanut oil with higher stearic and oleic acid and less linoleic acid. Knauft et al. (1988) pointed out that digging delays tended to give peanut oil with higher stearic and oleic acid and less linoleic acid. Bovi (1982) Raheja et al. (1987) and Önemli (2012) reported that there was a negative correlation between oleic acid and linoleic acid. These results are in agreement with the findings of Young et al. (1972), Young and Worthington (1974), Bovi (1982), Raheja et al. (1987), Knauft et al. (1986), Hinds (1995), Dwivedi et al. (1996), Andersen and Gorbet (2002), Isleib et al. (2008), Önemli (2012), Chowdhury et al. (2015) and Gulluoglu et al. (2016a).

Oil content

As it can be seen from Table 2, the differences between the harvesting times were statistically significant for oil content. The oil content varied between 47.8-50.3%. The oil percentage was increased when the harvesting time was delayed. The oil content was 47.8% at first harvesting date (149 DAS) while it was increased to 50.3% at forth harvesting date (170 DAS). Court et al. (1984) utilized five successive digging dates in Ontario ranging from 113 DAP over two years to examine differences in two genotypes. They found that delayed digging, increased oil content. Singh and Oswalt (1995), Lu et al. (1997) and Canavar and Kaynak (2013) reported that oil content was increased by delaying the harvesting time. The oil content of peanut varieties varied between 46.5-51.1% on based of dry weight and the highest oil content (51.1%) was in Wilson and the lowest (46.5%) was in Flower-32 varieties. The differences between the peanut varieties were statistically significant. Holaday and Pearson (1974), Brown et al. (1975), Raheja et al. (1987), Hassan et al. (2005), Yav et al. (2008) and Isleib et al. (2008) reported that the oil percentage of peanut seed varies between 35 to 56% depending on genotype and growing conditions, and the oil content of peanut varieties influence by genotype, seed maturity, climatic conditions, geographical location and growing conditions. Similar result were reported by some other researchers (Özcan and Seven, 2003; Yav et al. 2008; Hassan and Ahmed, 2012; Önemli, 2012; Chowdhury et al. 2015 and Gölcüklü et al. 2016).

Oleic acid to Linoleic acid ratio (O/L)

Oleic acid to Linoleic acid ratio (O/L) and iodine value determines the quality, storability and shelf-life of peanut oil and its products. High-oleic peanut has longer self-life than low-oleic peanut and it has better flavor quality or stability than low-oleic peanut (Brown et al.1975; Yav et al. 2008 and Chaiyadee et al. 2013). The O/L ratio of peanut varieties varied between 2.162-2.555 at the harvesting times (Table 2). The O/L value was increased from 2.162 to 2.555 when the harvesting delayed to 170 DAS from 149 DAS. These results showed that early harvesting is not suitable for the oil quality and processing. The O/L value of the peanut varieties (excluded Brantley variety) varied in between 1.358-3.458 (Table 2). The O/L ratio was higher in Batem-5025 and NC-7 and was lower in Flower-32, Flower-36 and Flower 22 varieties. Escobedo et al. (2015) reported that O/L value of peanut varieties varied in between 1.8-2.1 grown in Mexico. The O/L ratio was found very high in Brantley (36.169). The O/L is very high in oleic type peanut varieties due to high oleic acid percentage. Holaday and Pearson (1974) found that higher temperatures during the last 4 weeks before harvest resulted in higher oil and oleic acid content and correspondingly higher O/L ratios. The authors postulated that this temperature -O/L relationship may provide at least partial explanation for observed problems with oxidative stability in peanuts grown in colder climates or with colder temperatures during the later weeks of the growing season. These findings were supported by some other researchers (Young and Worthington, 1974; How and Young, 1983; Dwivedi et al.1996; Hashim et al. 1993; Andersen and Gorbet, 2002; Chaiyadee et al. 2013 and Gulluoglu et al. 2016a).

Conclusions

Oil content is an important quality characteristic in peanut seed and the oil content influences by genotypic variation, growing conditions and maturity. The oil content increases by delaying the harvesting times. The nutritional and storage qualities of peanut are determined by its fatty acids composition. The fatty acid composition of peanut seed oil influenced by genotypic variation, air and soil temperature, planting date, soil nutrient, growing conditions and maturity. The seed maturity can also influence the fatty acid composition of peanut. In general, oleic acid increases and linoleic acid decrease with seed maturity. The increase in oleic acid with seed maturity is normally accompanied by a decrease in palmitic and linoleic acid. There was a negative correlation between oleic acid and linoleic acid. Higher temperatures before harvest resulted in higher oil and oleic acid content and correspondingly higher O/L ratios. As a result; the oil content, oleic acid and stearic acid percentages of the peanut varieties was increased when the harvesting times delayed. The oil and fatty acids content of peanut varieties were found differ at the harvesting times.

References

1. Andersen, P.C. and Gorbet, D.W. 2002. Influence of year and planting date on fatty acid chemistry of high oleic acid and normal peanut genotypes. J. Agric. Food Chem. 50:1298-1305
2. Anonymous, 2015. The meteorological data for Adana. The Turkish State Meteorological Service Adana Regional Directorship, 2013 and 2014
3. AOCS. 2010. Official and recommended methods. American oil Chemists' Society Press. Champaing, IL, USA
4. Bovi, M.L.A. 1982. genotypic and environmental effect on fatty acid composition, iodine value and oil content of peanut (*Arachis hypogaea* L.) Ph.D. Dissertation. University of Florida, p. 119.
5. Brown, D.F., Cater, C.M., Mattil, K.F. and Darroch, J.G. 1975. Effect of variety, growing location and their interaction on the fatty acid composition of peanuts. J. of Food Science 5:1055-1060.
6. Canavar, Ö and Kaynak, M.A. 2013. Determination of yield and yield components and seed quality of peanuts (*Arachis hypogaea* l.) at different harvest times. Int. J. of Agronomy and Plant Production 4(S):3791-3803
7. Carrin, M.E. and Carelli, A.A. (2010). Peanut oil: Compositional data. Eur. J. Lipid Sci. Technol., 112:967-707
8. Chaiyadee, S., Jogloy, S., Songsri, P., Singkham, N., Vorasoot, N., Sawatsitang, P., Holbrook and Patanothai, A. 2013. Soil moisture affects fatty acids and oil quality parameters in peanut Int. J. of Plant Production 7(1):81-96.
9. Chamberlin, K.D., Barkley, N.A, Tillman, B.L., Dillwith, J.W., Madden, R., Payton, M.E and Bennett, R.S. 2014. A comparison of methods used to determine the oleic/linoleic acid ratio in cultivated peanut (*Arachis hypogaea* L.) Agricultral Science 5(3):227-237.

10. Chowdhury, F.N., Hossain, D., Hosen, M. and Rahman S. 2015. Comparative study on chemical composition of five varieties of groundnut (*Arachis hypogaea* L.). World J. of Agricultural Science 11(5)247-254
11. Court W.A., Roy, R.C and Hendel, J.G. 1984. Effect of harvest date on agronomic and chemical characteristics of Ontario peanuts. Canadian J. Plant Science 64:521-528
12. Dwivedi, S. L., Nigam, S.N., Jambunathan, R., Sahrawat, K.L., Nagabhushanam, G.V.S and K. Raghunath. 1996. Effect of genotypes and environments on oil and oil quality parameters and their correlation in peanut (*Arachis hypogaea* L.). Peanut Science 20:84-89
13. Escobedo, R.V., Luna, P.H., Torres, I.C.J., Mopreno, A.O. and Ramirez, M.C.R. 2015. Physicochemical properties and fatty acid profile of eight peanut varieties grown in Mexico. J. of Food 13(2):300-30
14. FAO. 2015. Production Year Book (www.fao.org).
15. Gölükcü, M., Toker, R., Tokgöz, H. and Kadiroğlu, A. 2016. Antalya koşullarında yetiştirilen bazı yerfıstığı (*Arachis hypogaea* L.) çeşitlerinin yağ içerikleri ve yağ asidi bileşimleri. Gıda 41(1):31-36.
16. Gulluoglu, L., Bakal, H., Onat, B., Kurt, C and Arioglu, H. 2016a. the effect of harvesting dates on yield and some agronomic and quality characteristics of peanut grown in Mediterranean Region (Turkey) Turkish Journal of Field Crops. 21(2): 224-232
17. Gulluoglu, L., Bakal, H., Onat, B., El Sabagh, A and Arioglu, H. 2016b. Characterization of peanut (A*rachis hypogaea* L.) seed oil and fatty acids composition under different growing season under Mediterranean Environment, Journal of Experimental Biology and Agricultural Sciences. DOI:http://dx.doi.org/10.18006/2016, 4(5S):564-571
18. Hashim, J.B., Koehler, P.E., Eitenmiller, R.R. and Kvien, C.K.1993. Fatty acid composition and tocopherol content of drought stressed florunner peanuts. Peanut Science 20:21-24.
19. Hassan, F. and Ahmed, M. 2012. Oil and fatty acid composition of peanut cultivars grown in Pakistan. Pakistan J. of Botany 44(2):627-630
20. Hassan, F., Manaf, A. and Ejaz, M. 2005. Determinants of oil and fatty acid accumulation in peanut. Int. J. of Agriculture and Biology 7(6):895-899
21. Hinds, M.J. 1995. Fatty acid composition of caribbean-grown peanuts (*Arachis hypogaea* L.) at three maturity stages. Food Chemistry 53:7-14
22. Holaday, C.E. and Pearson, L. 1974. Effects of genotype and production area on the fatty acid composition, total oil and protein in peanuts. Journal of Food Science 39(6):1206-1209.
23. How, J.S.L. and Young, C.T. 1983. Comparision of fatty acid content of imported peanuts. JAOCS 6(5)945-947.
24. Ingale, S. and Shrivastava, S.K. 2011. Nutritional study of new variety of groundnut (*Arachis hypogaea* L.) JL-24 seeds. African J. of Food Science 5(8):490-498
25. Ishag, H.M. 2000. Phenotypic and yield response of irrigated groundnut cultivars in a hot environment. Exp. Agric. 36: 303-312.
26. Isleib, T.G., Tilman, B.L., Patte, H.E., Sanders, T.H., Hendrix, K.W and Dean, L.O. 2008. Genotype-by-environment interaction for seed composition traits of breeding lines in the uniform peanut performance test. Peanut Science 35:130-138
27. Kaba, J.S., Ofori, K and Kumaga, F.K. 2014. Inter-relationships of yield and components of yield at different stages of maturity in three groundnuts (*Arachis hypogaea* L.) varieties. International J. of Life Science Research 2(1):43-48
28. Knauft, A., A.J. Norden and D.W. Gorbet. 1986. The Effect of Three Digging Dates on Oil Quality, Yield, and Grade of Five Peanut Genotypes Grown without Leaf spot Control. Peanut Science 13: 86-89
29. Lim, E.S and Hamdan, O. 1984. The reproductive character of four varieties of groundnuts (*Arachis hypogaea* L.). Pertanica 7:25-31
30. Lu, K.C., Yeh, M.S., Yang, K.H and Lu, H.S. 1997. Effects of genotype, population density and harvest date on yield and quality of peanut. J. of Agric. Res. China 46(2):116-131

31. Mzimbiri, R., Shi, A.M., Liu, H and Wang, Q. 2014. A Review: peanut fatty acids determination using hyper spectroscopy imagine and its significance on food quality and safety. Food Science and Quality Management 28:90-97.
32. Önemli, F. 2012. Impact of climate change on oil fatty acid composition of peanut (*Arachis hypogaea* L.) in three market classes. Chilean J. Agri. Res. 72(4):383-488
33. Özcan, M. and Seven, S. 2003. Physical and chemical analysis and fatty acid composition of peanut, peanut oil and peanut butter from ÇOM and NC-7 cultivars. Grasas y Aceites 54(1):12-18.
34. Raheja, R.K., Battai, S.K., Ahuja, K.L., Labana, K.S and Singh, M. 1987. Comparison of oil content and fatty acid composition of peanut genotypes differing in growth habit. Plant Foods for Human Nutrition 37:103-108
35. Sattayarak, S. 1997. Effects of harvesting dates on different characters and seed quality of peanut grown in Southern Thailand. http://agris.fao.org/agris-search/.do?recordID= TH2000000950
36. Singh, F. and Oswalt, D.L. 1995. Groundnut Production Practices. ICRISAT. SAT, e-journal 9:29
37. Yav, A.S., Richard, A., Osei, A.K., Kofi, A.D.H., Seth, O.D and Adelaide, A. 2008. Chemical composition of groundnut, *Arachis hypogaea* (L.) landraces. African J. of Biotechnology 7(13):2203-2208.
38. Young, C. T., Manson, M.E., Matlock, R.S and Waller, G.R. 1972. Effect of maturity on the fatty acid composition of eight varieties of peanut grown at Parkins, Oklahoma in 1968. JAOCS 49:314-317
39. Young, C.Y and Worthington, R.E. 1974. Fatty acid composition of spanish peanut oil as influenced by planting location, soil moisture conditions, variety, and season. AOCS 51:312-315
40. Young, J. H., Person, N.K., Donald, J.O and Mayfield, W.D. 1982. Harvesting, Curing and Energy Utilization, pp.458-485. In H.E. Pattee and C. T. Young (eds). Peanut Science and Technology, Amer. Peanut Res. Educ. Society Inc., Yoakum. TX, USA.

THE DETERMINATION OF SOME AGRONOMIC AND OIL QUALITY CHARACTERISTICS OF PEANUT BREEDING LINES

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Abstract

This study was conducted as a main crop in University of Cukurova, Faculty of Agriculture Field Crops Department in 2016. The objective of this research was to determine some agronomic and quality characteristics of peanut breeding lines. In this study, 22 peanut lines (F6) belonging to Brantley (High oleic) x Halisbey (High yield) crossing were used as a plant material. The experimental design was a Randomized Complete Block with three replications. Pod number and pod weight per plant, 100-seed weight, shelling percentage, oil and protein content, saturated (Palmitic, stearic, arachidic and lignoceric acid) and unsaturated (Oleic, linoleic and linolenic acid) fatty acids percentage and pod yield per hectare values of lines were investigated. As a result, the pod number per plant of breeding lines was between 24.9-34.9 pod plant^{-1}, pod weight was 73.3-91.2 g plant^{-1}, 100-seed weight 118.0-148.6 g, shelling percentage was 59.5-66.9% and pod yield was 6064-9232 kg ha^{-1}. The oil content and oleic acid percentage of breeding lines were varied between 41.20-55.95% and 51.97-80.00%, respectively.

Keywords: Peanut, breeding lines, agronomic characteristic, oleic acid and pod yield.

Introduction

Peanuts are grown worldwide in the tropics and temperate zones primarily as an oilseed crop. The world annual peanut production is around 45 million tons (Carrin and Carelli, 2010 and FAO, 2015). About two-thirds of total peanut production is crushed for oil and the remaining one-third is used in confectionery products in the world (Dwivedi et al. 1996). For this reason, peanut (*Arachis hypogaea* L.) is an important oilseed crop for vegetable oil production in the world (peanut oil accounted for 3.0% of the world's vegetable oil production) (FAO, 2015). Peanut seeds contain 44-56% oil and 22-30% protein and 15.0-18.0% carbohydrate. In addition, they are a good source of mineral (P, Ca, Mg and K) and vitamins (E, K and B group). For this reason, it is an important source of edible oil and protein for human nutrition in the world. Peanuts are also a cheap source of protein, a good source of essential vitamins and minerals, and a component of many food products (Savage and Keenan 1994 and Gulluoglu et al. 2016a). Yield of peanut is a complex phenomenon, a function of genetic factor as influenced by climate and managements. Variety selection is one of the main factors that play an important role on yield and quality of peanut. Cox (1979), Ketring (1984) and Caliskan et al. (2008), indicated that management practices such as variety selection, time of sowing and growing period of varieties may influence the growth, yield and seed quality of peanut. Arioglu et al. (2016) found that the pod yield was varied between 3830-8790 kg ha^{-1} and the highest pod yield was obtained from Sultan (8790 kg ha^{-1}) and Halisbey (7792 kg ha^{-1}) varieties. The oil and protein content of varieties were varied between 47-51% and 24-28%, respectively.The fatty acid composition of peanut is becoming increasingly important diet for health living. The nutritional qualities of peanut depend on the relative proportion of saturated and unsaturated fatty acids in the oil. A high proportion of polyunsaturated fatty acid is desirable because it lowers plasma cholesterol and low-density lipoprotein (LDL) content, which may reduce the risk of coronary heart disease (Dwivedi et al. 1996 and Mzimbiri et al. 2014). The oil content of Virginia type peanut cultivars varied between 45.0-58.6% (Carrin and Carelli, 2010). The fatty acid composition of peanut oil varies depending on the genotype, seed maturity, climate conditions, growth location, and interaction between these factors (Young, 1996). The major fatty acids present as acylglycerols in peanut oil are palmitic (C16:0), oleic (C18:1), and linoleic (C18:2) acids. Normally, stearic (C18:0), arachidic (C20:0), eicosenoic (C20:1), behenic (C22:0), and lignoceric (C24:0) acid occur in minor proportions, while a trace of linolenic fatty acid (C18:3) can take place (Carrin and Carelli, 2010). The fatty acids composition of Virginia type peanut varieties varies between 9.0-9.1% palmitic, 2.2-2.4% stearic, 56.4-60.3% oleic, 24.2-26.8% linoleic, 1.1-1.8% arachidic1.0-1.1% eicosenoic and 1.8-2.4% behenic acids (Brown et al. 1975). The annual peanut production is around 148.000 tons from the 38.000 ha harvested area in Turkey and the average pod yield is 3.9 ton ha^{-1}. Peanut can be grown at different seasons such as main and double crop for the suitable climate in Mediterranean region in Turkey. Main crop peanut production in the Mediterranean region accounts for 60% of the country's total production (TUIK, 2015). NC-7 peanut variety was commonly grown in the Mediterranean region. This variety has lower yield and susceptible to iron chlorosis. Other side, it has lower oleic acid content. It is necessary to store as a long period of peanut seed, the oleic acid content has to high. For these reasons, the farmers need to high yielding, tolerant to iron chlorosis and high oleic acid content peanut varieties. The agronomic and quality characteristic of peanut varies depending on growing conditions and varieties. The objective of the study was to determine some agronomic and quality characteristics of peanut breeding lines (Brantley x Halisbey) grown as a main crop in Turkey.

Material and methods

This experiment was conducted in 2016 at Research Farm of Cukurova University (Southern Turkey, 36^o59^I N, 35^o18^1 E; 23 elevation) as a main crop. Halisbey and Brantley varieties (st) and 22 advanced (F6) breeding lines (Brantley x Halisbey) were used as a plant material in this research. The soil texture was clay loam. The soil tests indicated that pH of 7.5 with high concentrations of K_2O and low concentrations of P_2O_5. In addition, the organic matter and nitrogen content of the soil were very low. The lime content was 20.5% in the upper layers with increased levels in lower layers. Mediterranean climate prevails in this region. Winters are warm and rainy, whereas summers are dry and hot. The average monthly air temperature and precipitation during the research period (April-September) was varied between 16.9-30.0^{o}C and 0.4 mm and 130.0 mm, respectively. The average relative humidity was between 61.2% and 70.0%. The differences between the year and long term for the climatic data were not significant (Anonymous, 2016). The experiment was designed at Randomized Complete Block with three times. The experimental site was cultivated deeply by the moldboard following the harvest of the previous crop in the autumn and then the soil was prepared by using disked-harrowed the day of planting. 250 kg ha^{-1} of Di-ammonium phosphate (45 kg ha^{-1} N, 115 kg ha^{-1} P_2O_5) fertilizer was applied and incorporated to soil before planting. Ammonium nitrate (33%N) at the rates of 200 kg ha^{-1} was applied two times; before first (beginning of flowering) and second (pod formation) irrigation. Plots consisted of 4 rows 5.0 m long and 70 cm apart. The seeds were sown in line manually by hand on 11 April 2016 and with 70 x 15 cm distance. During the growing period, recommended pesticides and fungicides were applied to control insects and diseases. During the growing period, other standard cultural practices were done. The plants were harvested by hand at the 20th of September 2016.

Data collection and analysis

Per plant data were measured from the 20 plants that were randomly selected from the central rows of each plot and then harvested by hand. Average pod number (pods $plant^{-1}$) and pod weight (g $plant^{-1}$) were calculated as the quotient of their respective values and the number of sampled plants. Pod yield was determined on the center two rows of each plot. Samples were dried to uniform 10% moisture, cleaned, and weighted. Pod yield per hectare, shelling percentage (%), 100-seed weight (g), protein and oil content (%) data were obtained after harvest (Gulluoglu, 2011). Determination of oil and protein percentage was analyzed and estimated according to Association of Official Analytical

chemists (AOCS, 2010). Fatty acid content was measured as *fatty acid methyl esters* using Gas Chromatograph (GC) according to AOCS (2010).
The collected data on different parameters were statistically analyzed to obtain the level of significance using JUMP 8.1.0 package program with split plot design. The means differences were compared with the Least Significant Differences (LSD, 5%) Test.

Results and discussion
The data belonging to pod number and pod weight per plant, shelling percentage, 100-seed weight, pod yield per hectare, oil content, protein content and fatty acids composition of peanut breeding lines has been presented in Table 1 and 2.

Table 1. The data belonging to pod number and pod weight per plant, shelling percentage, 100-seed weight, oil content, protein content and pod yield per hectare of peanut breeding lines

Breeding lines	Pod number (pods plant^{-1})	Pod weight (g plant^{-1})	Shelling percentage (%)	100-seed weight (g)	Protein content (%)	Oil content (%)	Pod yield (kg ha^{-1})
Halisbey	31.0	80.8	60.8	131.6	24.53	45.27	8197
Brantley	27.8	62.5	67.0	124.9	24.46	46.10	6287
YF-1	31.1	73.3	61.9	124.8	27.05	45.25	7147
YF-2	30.0	77.8	62.8	130.8	25.02	45.89	8643
YF-3	27.4	80.3	65.5	147.2	26.13	42.50	8531
YF-4	31.6	79.8	60.6	118.4	25.69	45.45	8627
YF-5	28.5	81.5	65.6	141.7	26.45	45.00	9025
YF-6	31.3	91.2	60.3	130.5	26.42	45.46	8165
YF-7	31.0	81.8	61.2	146.8	27.19	48.46	8277
YF-8	31.6	82.0	66.9	122.0	25.64	43.38	7943
YF-9	30.9	85.3	64.1	130.7	25.87	43.15	8420
YF-10	27.7	83.5	59.1	148.0	26.08	44.84	7608
YF-11	30.3	85.2	61.0	143.0	25.13	43.88	8404
YF-12	31.0	83.3	62.3	139.9	25.41	45.81	9073
YF-13	30.8	82.3	60.2	128.9	25.48	46.72	7592
YF-14	29.4	82.7	64.2	134.8	25.65	49.62	8770
YF-15	33.4	81.7	60.9	148.6	25.76	46.18	9232
YF-16	24.9	73.5	63.4	140.6	25.96	44.68	6064
YF-17	26.9	74.5	63.4	134.5	24.96	41.20	8213
YF-18	30.5	83.7	56.1	132.6	25.63	43.13	8674
YF-19	34.9	87.2	61.6	118.0	27.13	55.95	8834
YF-20	30.2	80.8	61.4	136.9	26.25	42.11	7767
YF-21	29.6	77.7	63.1	133.5	26.06	44.94	7242
YF-22	31.7	85.5	63.3	133.3	25.27	45.08	8643
Average	30.1	80.7	62.4	134.2	25.86	45.42	8141
CV(%)	8.74	7.94	3.35	2.67	1.40	3.40	12.53
LSD (5%)	4.26	10.28	3.44	5.89	0.60	2.54	1675.8

Pod number per plant
It can be seen in Table 1, the differences between the breeding lines were statistically significant for the pod number per plant. The pod number of breeding lines varied between 24.9-34.9 pods plant^{-1}. The average pod number of breeding lines was 30.1 pods plant^{-1}. The highest pod number per plant was obtained from YF-19 (34.9 pods plant^{-1}) and the lowest from YF-16 (24.9 pods plant^{-1}). The genotypic background was effective on pod number of peanut. Pod number per plant in Virginia market type peanut varieties was varied between 16.24-40.47 pods plant^{-1} depending on variety and

management (Gulluoglu, 2011, Arioglu et al. 2013; Gulluoglu et al. 2016b; Arioglu et al. 2016 and Kurt et al. 2017).

Pod weight per plant

The pod weight per plant of breeding lines varied between 62.5 - 91.2 g plant^{-1}. The differences between the breeding lines were statistically significant for the pod weight per plant. The average pod weight was 80.7 g plant^{-1}. The pod weight was 62.5 and 91.2 g plant^{-1} in parent of line it was increased to 91.2 g plant^{-1} in some breeding lines (YF-6). The pod weight of the lines except YF-6 was found higher than Brantley variety (Table 1). Pod weight per plant is an important yield component in peanut production. Pod weight was the higher in YF-15, YF-5, YF-19 and YF-12 breeding lines than the others lines. Virginia market type peanut varieties had larger pods and seeds compared to other market types. The yield and some agronomic characteristics of peanut are influenced by genotype and environmental conditions. Pod weight per plant in Virginia market type peanut varieties was varied between 37.28-93.67 g plant^{-1} depending on variety and management (Gulluoglu, 2011; Arioglu et al. 2013; Gulluoglu et al. 2016b; Arioglu et al. 2016 and Kurt et al. 2017).

Shelling percentage

The differences between the breeding lines were statistically significant for the shelling percentage. The shelling percentage of breeding lines varied between 56.1-66.9%. The shelling percentage was the highest in YF-8 (66.9%) and the lowest in YF-18 (56.1%) breeding lines. The average shelling percentage of breeding lines was found 62.4% (Table 1). The shelling percentage was higher in YF-3 (65.5%), YF-5 (65.6%), YF-9 (64.1%) and YF-14 (64.2%) than the others breeding lines. The shelling percentage is an important characteristic for the pod quality in peanut. It varies between 63.63-68.83% depending to variety and management in peanut. Some agronomic characteristics of peanut are influenced by several groups of factors including environmental factors, genetic factors and interaction of these factors (Isleib et al. 2008). These results are in agreement with the findings of Gulluoglu (2011), Arioglu et al. (2013), Gulluoglu et al. (2016b), Arioglu et al. (2016) and Kurt et al. (2017).

100-seed weight

The differences between the lines for the 100-seeds weight were statistically significant. The 100-seeds weight of the breeding lines varied between 118.0-148.6 g and the average 100-seed weight was 134.2 g. While the 100-seed weight of the parent was 124.9 g and 131.6 g, it was increased to 148.6 g in YF-15 and 148.0 g in YF-10 (Table 1). Virginia market type peanut varieties had larger pods and seeds compared to other market types. The yield and some agronomic characteristics such as pod number, pod weight, 100-seed weight and shelling percentage of peanut are influenced by genotype and environmental conditions. The 100-seed weight of the peanut varieties varies from 112.52 to 138.05 g (Gulluoglu, 2011; Arioglu et al. 2013; Gulluoglu et al. 2016b; Arioglu et al. 2016 and Kurt et al. 2017).

Protein and oil content

It can be seen in Table 1, the protein and oil content of the breeding lines varied between 24.46-27.18% and 41.20-55.95%, respectively. The differences between the lines for the protein and oil content were statistically significant. The oil content was the highest in YF-19 (55.95%) and the lowest in YF-17 (41.20%) lines. The average oil content was 45.42% in breeding lines. Brown et al. (1975) and Holaday and Pearson (1974) reported that genotypic differences for oil content were highly influenced by location, seasons, and growing conditions in peanut. Oil content of peanut has been studied in different cultivars and different environments and it has been reported that the oil content of Virginia type peanut cultivars varied between 45.0-58.6% (Carrin and Carelli, 2010). The highest protein percentage was obtained from YF-4 (27.18%) and the lowest from YF-20 (24.46%).

The average protein content was 25.87% in breeding lines (Table 1). Protein content of peanut varieties genetically controlled. The protein content of the peanut varieties varies between 24.15-28.50% (Arioglu et al. 2013; Gulluoglu et al, 2016b and Kurt et al. 2017).

Pod yield

The pod yield of the breeding lines varied between 6064 kg ha^{-1} and 9232 kg ha^{-1}. The differences between the lines were statistically significant for the pod yield. The average pod yield was 8141 kg ha^{-1}. The highest pod yield was obtained from YF-15 (9232 kg ha^{-1}) and the lowest from YF-16 (6064 kg ha^{-1}) (Table 1). The yield and some agronomic characteristics of peanut are influenced by genotype and environmental conditions during the growing season. Cox (1979), Ketring (1984) and Caliskan et al. (2008), indicated that management practices such as variety selection, time of sowing and growing period of varieties may influence the growth, yield and seed quality of peanut. Canavar and Kaynak (2008) indicated that planting date had a statistically significant effect on pod yield per plant and pod yield per hectare. Arioglu et al. (2016) found that the pod yield was varied between 3830-8790 kg ha^{-1} and the highest pod yield was obtained from Sultan (8790 kg ha^{-1}) and Halisbey (7792 kg ha^{-1}) varieties. Gulluoglu (2011), Gulluoglu et al. (2016b) and Kurt et al. (2017) reported that the pod yield of Virginia type varieties varies from 6108.5 to 9025.0 kg ha^{-1} in main crop growing season. Canavar and Kaynak (2010) reported that three physiological processes best explain the variation in peanut yields. These are partitioning of assimilate between the reproductive and vegetative structures, the length of the pod filling period and the rate of the pod establishment.

Fatty acids composition

The nutritional and storage qualities of peanut are determined by its fatty acids composition. Peanut oil contains saturated and unsaturated fatty acids. The fatty acid composition of peanut is becoming increasingly important diet for healthy living. The nutritional qualities of peanut depend on the relative proportion of saturated and unsaturated fatty acids in the oil. It has been seen in Table 2, the amount of saturated fatty acids such as palmitic (C16:0), stearic (C18:0), arachidic (C20:0) and lignoceric (C24:0) acids in peanut breeding lines varied between 3.69-10.28%, 2.84-33.92%, 0.92-1.36% and 2.35-2.78%, respectively. The unsaturated fatty acids such as oleic (C18:1), linoleic (C18:2) and linolenic (C18:3), acids content in breeding lines varied between 51.97-80.00%, 2.71-28.42% and 1.40-1.69%, respectively. The fatty acid composition of peanut oil varies depending on the genotype, seed maturity, climate conditions, growth location, and interaction between these factors (Carrin and Carelli, 2010). The fatty acids composition of Virginia type peanut varieties varies between 9.0-9.1% palmitic, 2.2-2.4% stearic, 56.4-60.3% oleic, 24.2-26.8% linoleic, 1.1-1.8% arachidic1.0-1.1% eicosenoic and 1.8-2.4% behenic acids (Brown et al. 1975).Peanut oil contains saturated and unsaturated fatty acids. The amount of saturated and unsaturated fatty acids in peanut oil varies from 10.92 to 17.47% and from 81.13 to 94.81%, respectively. The major fatty acids components are oleic, linoleic and palmitic acids in peanut oil (Chowdhury et al. 2015). Peanut oil is rich in oleic and linoleic acids. The oleic acid content varied between 51.97-80.00% in breeding lines. The oleic acid content was the highest in YF-8 (80.00%).The oleic acid content was 51.64% and 80.03% in Halisbey and Brantley (parents), respectively. The oleic acid content of YF-8 (80.00%), YF-18 (73.04%), YF-1 (70.80%), YF-21 (67.46%) and YF-14 (64.40%) lines were higher than Halisbey. The linoleic acid content varied between 2.71-28.42% in breeding lines. The linoleic acid content was the lowest in YF-8 (2.71%) line. The linoleic acid content of YF-8 (2.71%), YF-18 (9.29%), YF-21 (14.53%) and YF-14 (17.32%) lines were lower than 20% (Table 2). Oleic acid content in peanut genotypes can vary from 21 to 85% and linoleic acid from 2 to 43% (Andersen and Gorbet, 2002). Oleic (a monounsaturated fatty acid) and linoleic (a polyunsaturated fatty acid) account for 75-80% of the total fatty acids in peanut oil (Brown et al. 1975). The results are corresponded well with the findings of Gulluoglu et al. (2016a).

Table 2. Fatty acids compositions of peanut breeding lines

Breeding lines	Palmitic Acid (%)	Stearic Acid (%)	Oleic Acid (%)	Linoleic Acid (%)	Linolenic Acid (%)	Arachidic Acid (%)	Lignoceric Acid (%)
Halisbey	7.89	2.92	51.64	27.86	1.38	1.28	2.26
Brantley	5.91	3.95	80.03	3.17	1.73	1.25	2.52
YF-1	7.53	2.88	70.80	12.07	1.40	1.36	2.51
YF-2	10.10	3.12	53.64	26.55	1.49	1.01	2.57
YF-3	9.78	3.58	55.03	25.05	1.60	0.92	2.56
YF-4	10.18	2.84	51.97	28.42	1.42	1.04	2.60
YF-5	9.97	3.28	53.99	26.33	1.51	0.99	2.50
YF-6	9.95	3.29	53.86	26.25	1.58	1.01	2.59
YF-7	3.69	3.69	58.78	26.91	1.69	1.04	2.67
YF-8	6.07	3.92	80.00	2.71	1.84	1.26	2.78
YF-9	10.16	3.04	53.47	26.77	1.48	1.04	2.56
YF-10	9.92	3.66	55.19	24.60	1.63	0.98	2.57
YF-11	9.82	3.46	55.91	24.23	1.58	1.00	2.55
YF-12	9.63	3.40	55.84	24.33	1.61	1.02	2.64
YF-13	10.28	2.96	52.88	27.39	1.45	1.00	2.54
YF-14	8.50	3.11	64.40	17.32	1.49	1.15	2.55
YF-15	9.65	3.44	56.58	23.69	1.61	1.00	2.57
YF-16	10.20	2.90	52.99	27.20	1.48	1.02	2.68
YF-17	10.24	3.06	53.45	26.56	1.49	1.06	2.63
YF-18	7.39	3.72	73.04	9.29	1.59	1.23	2.35
YF-19	9.61	3.48	56.92	23.27	1.64	0.99	2.64
YF-20	9.91	3.11	54.70	25.59	1.54	0.98	2.67
YF-21	8.03	3.15	67.46	14.53	1.50	1.24	2.56
YF-22	9.85	3.33	54.89	25.15	1.61	0.97	2.66

Conclusions

The yield and some agronomic characteristics of peanut are influenced by genotype and environmental conditions during the growing season. The data belonging to pod yield and some agronomic characteristics of breeding lines (F6) were found higher than parents (Brantley and Halisbey). The pod number per plant of breeding lines was between 24.9-34.9 pod plant^{-1}, pod weight was 73.3-91.2 g plant^{-1}, 100-seed weight was 118.0-148.6 g, shelling percentage was 59.5-66.9% and pod yield was 6064-9232 kg ha^{-1}. The oil content and oleic acid percentage of breeding lines were varied between 41.20-55.95% and 51.97-80.00%, respectively. As a result; YF-1, YF-8, YF-14, YF-18 and YF-21 lines can be register as new variety.

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References

1. Andersen, P.C. and Gorbet, D.W. (2002). Influence of year and planting date on fatty acid chemistryof high oleic acid and normal peanut genotypes. J. Agric. Food Chem. 50:1298-1305
2. Anonymous. (2016). The meteorological data for Adana. The Turkish State Meteorological Service Adana Regional Directorship, 2016
3. AOAC. (2010). Official methods of analysis of the association of analytical chemists. 18th Edition, Washington, D.C. USA

4. Arıoglu, H., Kurt, C., Bakal, H., Onat, B., *Gulluoglu, L.* and Sinan, N.S. (2013). The effects of pix (*mepiquat-chloride*) application in different gowing stages on pod yield and some agonomic characters of peanut. Turkish Journal of Field Crops, 18(2):260-267
5. Arıoglu, H., Bakal, H., *Gulluoglu, L., Kurt, C. and Onat, B.* (2016). Ana ürün koşullarında yetiştirilen bazı yerfıstığı çeşitlerinin önemli agronomik ve kalite özelliklerinin belirlenmesi. Tarla Bitkileri Merkez Araştırma Enstitüsü Dergisi, 25 (Özel sayı-2): 24-29
6. Brown, D.F., Carl, M.C., Karl, F.M. and James, G.D. (1975). Effect of variety, growing location and their interaction on the fatty acid composition of peanut. J. Food Sci.40:1055-1060
7. Caliskan, S., Caliskan, M.E., Arslan, M. and Arioglu, H. (2008). Effects of sowing date and growth duration on growth and yield of groundnut in a Mediterranean-type environment in Turkey. Field Crops Res. 105:131-140
8. Canavar, Ö. and Kaynak, M.A. (2010). Growing degree day and sunshine radiation effects on peanut pod yield and growth. African J. of Biotech, 9(15):293-301
9. Carrin, M.E. and Carelli, A.A. (2010). Peanut Oil: Compositional data. Eur. J. Lipid Sci. Technol., 112:967-707
10. Chowdhury, F.N., Hossain, D., Hosen, M. and Rahman, S. (2015). Comperative study on chemical composition of five varieties of groundnut (*Arachis hypogaea* L.). World j. of Agricultural Science 11(5)247-254
11. Cox, F.R. (1979). Effects of temperature on peanut vegetative and fruit growth. Peanut Science 6:14-17
12. Dwivedi, S.L., Nigam, S.N., Jambunathan, R., Sahrawat, K.L., Nagabhushanam, G.V.S. and Raghunath, K. (1996). Effect of genotypes and environments on oil and oil quality parameters and their cprrelation in peanut (*Arachis hypogaea* L.). Peanut Science 20:84-89
13. FAO. (2015). Production Year Book (www.fao.org).
14. Gulluoglu, L. (2011). Effects of growth regulator applications on pod yield and some agronomic characters of peanut in Mediterranean region. Turkish Journal of Field Crops. 16(2):210-214
15. Gulluoglu, L., Bakal, H., Onat, B., *El Sabagh, A. and* Arioglu, H. (2016a). Characterization of peanut (*Arachis hypogaea* L.) seed oil and fatty acids composition under different growing season under Mediterranean environment. Journal of Experimental Biology and Agricultural Sciences. DOI:http://dx.doi.org/10.18006/2016, 4(5S):564-571
16. Gulluoglu, L., Bakal, H., Onat, B., Kurt, C. and Arioglu, H. (2016b). The effect of harvesting dates on yield and some agronomic and quality characteristics of peanut grown in Mediterranean region (Turkey) Turkish Journal of Field Crops. 21(2): 224-232 (DOI:10.17557/tifc.20186)
17. Isleib, T.G., Tilman, B.L., Patte, H.E., Sanders, T.H., Hendrix, K.W. and Dean, L.O. (2008). Genotype-by-environment interaction for seed composition traits of breeding lines in the uniform peanut performance test. Peanut Science 35:130-138
18. Ketring, D.L. (1984). Temperature effects on vegetative and reproductive development of peanut. Crop Sci. 24:877-881
19. Kurt, C., Bakal, H., *Gulluoglu, L.* and Arioglu, H. (2017). The effect of twin row planting pattern and plant population on yield and yield components of peanut (*Arachis hypogaea* L.) at main crop planting in Cukurova region of Turkey. Turkish Journal of Field Crops, 22 (1):24-31DOI: 10.17557/tjfc.301768
20. Mzimbiri, R., Shi, A.E., Liu, H. and Wang, Q. (2014). A Review: peanut fatty acids determination using hyper spectroscopy imagine and its significance on food quality and safety. Food Science and Quality Management 28:90-97.
21. Savage , G.P. and Keenan, J.I. (1994). The composition and nutritive value of groundnut kernel. In Smart, J. (ed) The Groundnut Crop: A scientific basis for improvement. Chapman and Hall, London, pp 173-213
22. TUIK, (2015). Turkish Production Year Book.

BREEDING OF CEREAL CROPS AT DOBRUDZHA AGRICULTURAL INSTITUTE – GENERAL TOSHEVO, BULGARIA

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Abstract
The climate of Bulgaria is very diverse in spite of its small territory. The soil and climatic conditions in the region where Dobrudzha Agricultural Institute is situated are suitable for obtaining high and stable yields from all winter cereals. The breeding program of the institute is aimed at developing high-yielding cultivars of common and durum wheat, triticale, malting and feed barley adaptable to growing under variable soil and climatic conditions. The aim of this investigation is to present the major directions, problems and achievements of the breeding work on the winter cereals at Dobrudzha Agricultural Institute.The results were summarized on several levels:Evaluation of the risk factors for the development, the yield formation and the quality indices;Developing and study on a gene pool of the best world and Bulgarian accessions;Developing of own initial material by using the methods of intervarietal hybridization, experimental mutagenesis and other biotechnology approaches;Developing of a more efficient methodology for field and laboratory evaluation of the breeding materials;Testing of new varieties and production of certified planting material.The portfolio of the institute is quite variable. From the cultivars developed here, 36 genotypes of common wheat and 5 genotypes of durum wheat, 11 triticale cultivars and 6 winter barley varieties have been included in the National Vareital List of Bulgaria.

Keywords: breeding program, wheat, barley, triticale, stress factors.

Introduction

In growing of cereals, the choice of a suitable cultivar is an important factor for the efficiency of production. The frequent stress factors during the comparatively long vegetative growth determine the high importance of this choice. The breeding of cereal crops in Republic of Bulgaria is carried out at several centers, which have at their disposal rich initial breeding materials and apply specific approaches for evaluation. The genetic potential of the developed materials is tested under conditions of permanent stress. In this way the most promising genotypes are sorted out; they are then subjected to testing within the system of the national Executive Agency of Variety Testing, Field Inspection and Seed Control, and further introduced in practice. Dobrudzha Agricultural Institute (DAI) is the largest breeding center for cereals in Bulgaria. It is located in a region where the conditions are suitable not only for obtaining of high and stable yields from these crops but also allow testing of the breeding materials under different types of stress. The portfolio of DAI is highly diverse. Out of the cultivars developed here, 36 genotypes of common wheat, 5 genotypes of durum wheat, 11 cultivars of triticale and 6 varieties of winter barley are included in the Varietal List of Bulgaria. The aim of this investigation was to outline the main trends, problems and achievements of the breeding work with the winter cereals at Dobrudzha Agricultural Institute.

Material and methods

The historical overview is based on reports and statements published in the research communication volumes of DAI. Data are presented on the cereals included in the national varietal list (Table 1). The observations have been made within competitive varietal trials designed in 15 m^2 plots in five replicates. The sowing norm was 420 germinating seeds (g.s.) per 1 m^2 for two-rowed barley, 450 g.s. for the feed barley forms and 550 g.s. for wheat and triticale. The previous crop was grain pea. At the beginning of February, nutrition with 4 $t.ha^{-1}$ active matter of nitrogen was done. The agronomy practices not subject to this investigation were in accordance with the technology approved for growing of the respective crop. The region of Dobrudzha, where the Institute is located, has soil and climatic conditions favorable for the development of the cereals. In the winter months, the low temperatures may be critical without snow cover. The absolute minimum temperature measured in this region is -29.4°C, and the absolute maximum +41.1°C. Due to the frequent flows of ground-level cooling air currents, the spring comes here with 10-15 days later. The summer is cool and the autumn is long, with gradually decreasing temperatures. Winds are frequent, with predominant northern component. Especially damaging is the hot air mass transport during the grain filling stage. There are two well expressed periods of drought during March – April and July – August. The mean annual precipitation sum is 510 mm. The predominant soils in the region are the leached chernozems. Due to the heavy soil composition, the values of the hydrological indices are comparatively high.

Table 1. Registered cereal varieties of DAI included in the Varietal List of Republic of Bulgaria (2017)

Winter wheat
Aglika, GTP Albena, Antonovka, Bozhana, Boliarka, Galatea, Goritsa, Demetra, GTP Dragana, Enola, Iveta, GTP Kalina, GTP Kami, Karat, GTP Karina, GTP Katarzhina, GTP Kiara, GTP Korona, Kosara, GTP Kristal, Kristalina, GTP Kristi, Lazarka, GTP Laska, Merilin, GTP Milena, GTP Neda , Nikodim, Pchelina, GTP Rada, Sladuna, Stoyana, GTP Tina, Todora, Fani, Zhana
Durum wheat
Melina, Mirabel, Mirela, Saturn 1, Severina.
Triticale
Akord, Atilla, Borislav, Blagovest, Bumerang, Dobrudzhanets, Doni 52, Irnik, Kolorit, Lovchanets, Respekt
Winter malting barley
GTE Ahat, Kaskadyor 3, Oniks, GTE Yaspis
Winter feed barley
Pagane, Tangra

Results and discussion

Wheat breeding in Bulgaria has a history of more than 110 years. After the approval of the first breeding program in 1964, a series of cultivars were developed at DAI which possess high productivity, quality grain and comparatively short stem (Panayotov and Rachinski 2002). The attempts to develop triticale started at the same time. With the release of the first hexaploid triticale, Bulgaria became the seventh country in the world to synthesize this crop. The main goals were to combine the high productivity of wheat with the low requirements of rye to the soil fertility and the nutrition regime, and with its disease resistance. The research programs on the breeding of durum wheat and barley started much later. They were focused on the improvement of the level of frost resistance, which is a problem in this crop. Cereal crops with a typical winter type of development are grown in the region of Dobrudzha. To be able to express all their positive qualities and realize their production potential, the plants have to over winter without significant damages. When vegetative growth resumes in spring, frost damages are observed most frequently. The Dobrudzha plateau is open to the north – north-west, which makes it vulnerable to the influx of cold air masses. When this is combined with strong winds and lack of snow cover, the plants are subjected to critical low temperatures. A peculiarity of the breeding at DAI is the high level of frost and winter resistance, which is decisive under the changeable conditions of Bulgaria. The criteria for evaluation are high and are similar to the Russian and Ukrainian breeding requirements. Apart from being tested under field conditions, the breeding materials are also tested in low-temperature chambers with the aim to differentiate them well. The rainfalls in the region of Dobrudzha are unevenly distributed and frequently are a limiting factor for production. The autumn droughts influence mostly the preparation of the sowing areas and sowing itself. Often the emergence of the plants is not uniform, and they sometimes enter the winter months at an unsuitable stage of development. Especially unfavorable are the summer droughts, which are usually soil and atmospheric ones (Petrova 2013). They are characterized with high temperatures, low air humidity, and soil moisture less than 70 % from the maximum field water capacity. Their effect on the nutrition of grains is negative, and the crops ripen prematurely. The peculiarities of the cultivars developed at DAI are the following: 1) faster resumption of vegetative growth in the spring months allowing faster rate of biomass accumulation by utilization of the autumn and winter moisture reserves; 2) earlier date to heading allowing flowering, pollination and fertilization to occur also under more favorable conditions; 3) expressed dynamic relationship between duration and rate of grain filling. As a result from the combined use of different laboratory techniques and multiple individual selection in the hybrid populations, the developed wheat cultivars possess high productivity and resistance to low temperatures and drought (Tsenov et al. 2012a; Tsenov et al. 2012b):

Cultivars with high level of frost resistance – Aglika, GTP Albena, Bozhana, Bolyarka, Demetra, Iveta, Lazarka, GTP Laska, Merilin, GTP Milena, Todora, Fani.

Cultivars tolerant to drought – GTP Albena, Galateya, GTP Dragana, Enola, Iveta, GTP Kristal, GTP Kristi, Lazarka, GTP Laska, GTP Rada, Todora.

The cultivars released during the last decade represent a step forward in the breeding of this crop. An indication for their adaptability potential is the high yields realized, which are stable over years under changeable soil and climatic conditions. An unequivocal success are cultivars Akord and Respekt, which by their level of frost resistance correspond to the standards Bezostaya 1 and Mironovskaya 808. The durum wheat cultivars Severina, Mirabel, Melina and Mirela developed at DAI have no alternative in the European varietal list with regard to this type of stress. Progress was made in the breeding of barley, too. Cultivars GTE Yaspis и Pagane are with a high level of frost and winter resistance. The two-rowed barley GTE Ahat is tolerant to drought. The resistance to diseases of the cultivars introduced in the practice is at a sufficiently high level. Due to the long-term systematic breeding work they do not differ from the accessions developed under conditions of high pressure from various pathogens. Analysis and evaluation for resistance of the developed initial breeding material are done at the laboratory of plant pathology, and the lines and new cultivars are tested against infection field background. The changes in the race composition of the pathogen casual agents of brown rust and powdery mildew are investigated (Ivanova 2014; Stanoeva and Iliev 2014). The resistance to them and its stability and durability are clarified. When investigating the physiological specialization of powdery mildew on wheat, genes *Pm 3c*, *Pm 7* and *Pm 3b* demonstrated highest efficiency to the studied populations. Genes *Pm 4в*, *Pm 5* and *Pm 3d* had low efficiency. Completely inefficient were genes *Pm 6*, *Pm 8* and *Pm 2+6*. With regard to brown rust, genes *Lr* 9, *Lr* 19, *Lr* 40, *Lr* 41, *Lr* 42, *Lr* 43 and *Lr* 51 were with absolute efficiency. Genes *Lr* 24, *Lr 25, Lr* 29, *Lr* 35, *Lr 36, Lr* 47, *Lr* 50 and *Lr* 52 were highly efficient, while *Lr* 3ка, *Lr 11, Lr 15, Lr 18, Lr 26* and *Lr* 30 were absolutely inefficient. In the recent years, net blotch is becoming an economically important disease on barley. The causal agent (*Drechlera teres* Ito.) is aggressive and under suitable conditions its fast development defoliates the plants at the most critical vegetative stages. Purposeful research work on this pathogen has not been carried out at DAI but the field investigations showed high resistance of two of the new registered cultivars - GTE Ahat and Tangra. The phytosanitary situation in Bulgaria is currently complicated due to various reasons. High attacking rates of infection caused by diseases with previous sporadic occurrence are becoming more frequent. This imposes the necessity to reconsider the main directions of research work, to quickly identify sources of resistance and to include them in the breeding process. The improvement of quality has always been a priority of the breeding programs at DAI. Among the wheat cultivars, of which seed production is carried out, 24 % belong to the group of the strong wheats (Aglika, GTP Albena, GTP Laska, GTP Milena, Demetra, Iveta, Lazarka, Merilin, Pchelina), and 43 % are medium with increased strength (Bozhana, Bolyarka, Enola, Galatea, Goritsa, GTP Dragana, GTP Kami, GTP Katarzhina, GTP Kristi, GTP Neda, GTP Rada, Kosara, Kristalina, Sladuna, Stoyana). Although there are no preferential prices for quality, the vision of the research team is to introduce in practice cultivars which allow the production of foods without improvers. Triticale bread is very suitable for people with gluten intolerance. Therefore, the Breadmaking Laboratory is searching for possibilities to make flour mixtures of triticale with common wheat types (Tsvetkov and Stoeva 2003). This type of bread has pleasant taste, aroma and durability. The developed durum wheat cultivars carry gene γ 45, which is an indicator of high quality. According to the reports of independent experts, some of them possess exceptional gluten characteristics and represent a unique type especially suitable for culinary use corresponding to the international standards (Petrova et al. 2013; Petrova et al. 2015). In 2017, a new winter malting barley cultivar Oniks was registered. The results from the technological analysis proved the high level of Oniks as a malting barley variety. The mean malt extract is above 80 % and reaching 81.5 % at one of the testing locations. These characteristics of the cultivar entirely meet the high criteria of the European Brewery Convention. After adopting and improving a number of methodologies for fractionation of storage proteins, the Laboratory of Biochemistry solves numerous tasks assisting the breeding process (Todorov 2006): 1) identification of the allelic composition of storage proteins; 2) applying express methods for determining the quality potential; 3) planning hybrid combinations based on the fraction composition of the parental forms; 4) using bio chemical markers in the variety maintenance and preserving the homogeneity in the reproduction process, etc. The yield is a resultant trait and the efforts for its enhancement relate to a number of theoretical and applied researches on certain qualities and properties. Morphological traits have been improved, which are related to the more efficient utilization of the environmental factors, including also the nutrition regime. Plant height was significantly reduced (Tsenov et al. 2009). Until recently, quite different genes were involved in Bulgarian breeding for reduction of stem height (Tsenov *et al.* 2009) in comparison to the West European breeding. The reasons for this are numerous, mainly the higher susceptibility to abiotic stress (primarily drought), the later date to heading, and a significant negative pleiotropic effect on the yield (Panayotov, 2013). At this stage, thanks to the efforts of our research team, the correlation between plant height and resistance to lodging was successfully broken. The fact that a part of the new triticale cultivars are medium high but their stem is strong and flexible is indicative. This problem is most serious in barley. In connection with the breeding for resistance to lodging, the phenomenon was investigated from a mechanical point of view (Mihailov et al. 2005). The physical and mechanical characteristics of the barley plant were determined, as well as the dynamics of their physiological change over stages of development (Mihailov et al. 2005). A model of the plant was developed in condition of tension and deformation at stages critical for the occurrence of lodging (Mihailov et al. 2006). The effects of the nitrogen norm and the previous crop on the degree of lodging was investigated (Tonev et al. 2006). The correlations between the main traits connected to lodging were investigated in systematic barley groups, which are easily applicable criteria for selection at various stages of the breeding process. As a result, the new cultivars are with a significantly higher resistance to lodging (Mihova et al. 2014). The investigations focused on the duration of the vegetative growth are mainly within the context of the strategies for avoidance of a certain type of stress and formation of the qualitative indices (Mihova 2012). During the individual periods, they have been updated but always remain specific for each crop. The most recent investigations on the effect of the environment show that there is no significant difference between the early and the late wheat cultivars with regard to yield (Mihova 2012). The accent is on the formation of a good dynamic relationship between duration and rate of grain filling.

In barley, the earlier date to heading is not a priority, the most suitable time being 1st – 5th May. The late spring frosts are typical for the region of Dobrudzha; they cause serious damages and high sterility rate of the spikes (Mihova 2013). All new barley cultivars have their dates to heading within the above period and the variation over years is low. The durum wheat cultivars have a date to heading close to the national standard Saturn 1 (20th – 2nd May). Concerning the other crops, the following cultivars have earlier date to heading: 1) common wheat – GTP Albena, Bolyarka, Galateya, Enola, Iveta, GTP Kalina, GTP Karina, GTP Kristal; 2) triticale Akord, Kolorit. It is a disputable question if "a plateau" has been reached in breeding and what is the way to increase the production potential. Most often, this is related to improving the biology and physiology of the crops (Tsenov et al. 2009). An evidence for a progress in this respect is the fact that the recently introduced wheat cultivars exceed by yield the varieties developed a decade ago with 12 – 18 %. As a result from the serious research work carried out on triticale, the progress is even greater (15 – 25 %). In the process of data accumulation, the structure of yield also changes. Until recently, the higher spike productivity was at the basis of the breeding strategy in wheat, primarily through higher number of florets and grains formed per spikelet at the expense of lower number of productive tillers. The contemporary high level of breeding and the demands of the market impose the necessity to search for new approaches to increase productivity. One of the options to do this is to simultaneously increase the number of productive tillers (Panayotov 2013). Some of the *Rht* genes used in the breeding centers abroad are especially interesting. They ensure a favorable stem/spike ratio allowing the use of intensive production technologies. In the breeding program of barley, there is no such discussion; the goal has always been a balance between the components of yield. The main reason for this is the biology of the crop (Mihova et al. 2017). In barley, each spikelet has only one floret. After the segmentation of the spike and the setting of the spikelets, this crop does not have the potential to react by forming higher grain number even under favorable conditions. Thousand kernel weight is a comparatively conservative trait which varies within a narrow range, especially in the poly-rowed forms. The data collected show that the focus should be on keeping the tillering coefficient but also on the equal contribution to yield of the productive tillers formed (Mihova and Dimova 2012). Among the wheat varieties developed at DAI, these have the highest tillering potential: Demetra, GTP Karina, GTP Kristal, GTP Milena and Todora. The initial seed production of the developed cereal varieties is the linking element between science and practice. It includes 36 genotypes of common wheat and 5 genotypes of durum wheat, 11 triticale cultivars and 6 winter barley varieties (Figures 1 and 2). The use of authentic sowing material is a component of the production, which cannot be compensated for by other measures. The scheme of seed production and variety maintenance is scientifically sound and goes through several main stages: 1) selection of spikes authentic for the cultivar; 2) two-year testing of the progenies; 3) preliminary propagation; 4) pre-basic seeds; 5) basic seeds. Apart from the cultivars, which Dobrudzha Agricultural Instittute offers, the Cereals Breeding Department has signed by now contracts for granting exclusive licenses for reproduction and distribution of 8 common winter wheat cultivars in Bulgaria. After having been registered, the wheat cultivars Aglika, Galatea, Enola, Lazarka, Merilin and GTP Milena were included in the varietal list of Republic of Turkey. With the assistance of our partners from Tekirdag, Konya and Kirkaleli, common wheat varieties Bozhana, Goritsa, GTP Dragana, GTP Kalina, GTP Kami, GTP Kiara, Pchelina and GTP Rada, durum wheat Melina and Mirabel, and triticale Akord and Respekt are being tested at the moment. Our team is also actively working on the international programs of CIMMYT and ICARDA, and on joint projects with breeding centers in Krasnodar – Russia, Fundulea – Romania, Beltsi – Moldova and Odessa – Ukraine. They allow the possibility not only to exchange genetic materials and share ideas, but also to correctly investigate the new breeding lines and cultivars.

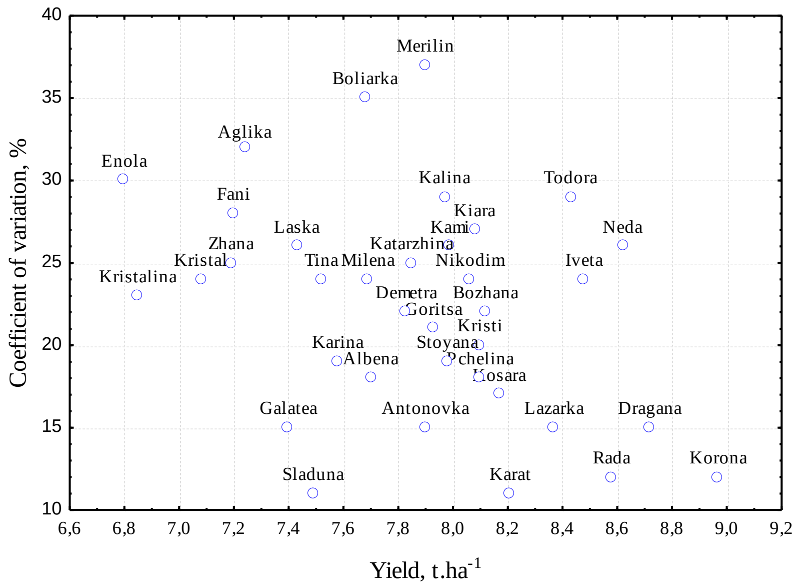
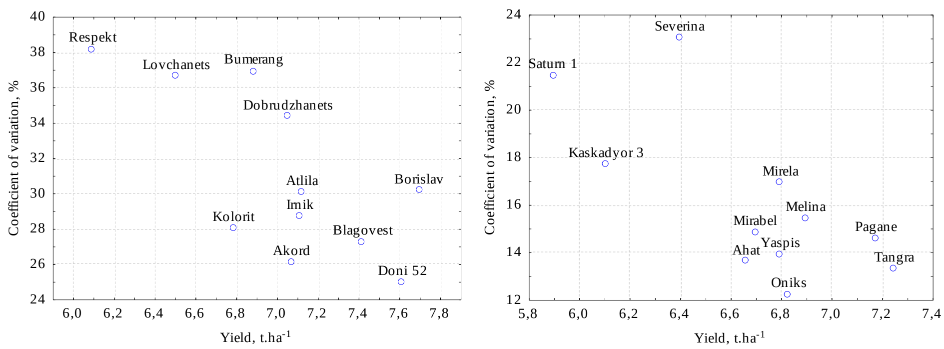


Figure 1. Average yield (2012-2016) from common wheat cultivars to be introduced in practice from which DAI provides seeds.



A) B)

Figure 2. Productivity of new and already introduced in production cultivars of triticale (A), durum wheat, feed and malting barley (B), (average 2012-2016).

Conclusions

A total of 102 common wheat varieties and 7 durum wheat cultivars, 13 triticale forms and 6 cultivars of winter barley have been developed at DAI. Over 35 % of the wheat areas in Bulgaria are sown with cultivars, which are the scientific product of our research institute. In triticale, there is practically no successfully introduced foreign breeding. The level of frost resistance of the durum wheat forms has no alternative. An indisputable advantage of the new barley varieties is their resistance to lodging in combination with tolerance to stress. The rich and diverse varietal list of DAI allows the producers to choose the cultivars, which are best for them and to develop their appropriate varietal structure. The cultivars are with confirmed adaptability and possess good balance between productivity potential and stability of its realization under unfavorable growing conditions. Under the increasing market competitiveness, a new vision for the breeding program of the cereal crops is outlined. The strategy for improvement of a complex of traits has been reconsidered, including the structure of yield. In compliance with the traditions, however, the accent is still on the high efficiency in production and on introduction of products for quality foods.

References

1. Ivanova, V. (2014). Race and Virulence Dynamics of *Puccinia triticina* and Effectivness of *Lr* genes in Bulgaria during 2005-2009. Turkish Journal of Agriculture and Natural Sciences, Special issue 2, 709-720.
2. Mihailov, R., Mihova, G., Tonev, T., Demirev, Vl. (2005). Investigation the Lodging Resistance of Barley Variety Aheloy 2 by mechanics Point of View. Journal of Mechanics of Machines 64: 139-142.
3. Mihailov, R., Mihova, G., Dishev, D., Tonev, T. (2006). Pressure Status and Deformation Status Modelling of the Barley Plant. Field Crops Studies, 3 (3): 341-350.
4. Mihailov R., G. Mihova, T. Tonev, Vl. Demirev, 2005. Physical characteristics of barley variety Aheloy 2. 10th Jubilee National Congress on Theoretical and Applied Mechanics, 13-16 September 2005, Varna, 288-292.
5. Mihova G., R Mihailov, T. Tonev, V. Demirev, 2006. Correlations between traits related to lodging resistance in barley. Field Crops Studies, 3 (1): 37-43.
6. Mihova, G. (2012). Phenological specificity of winter barley under the conditions of north-east Bulgaria. Scientific Works of the Institute of Agriculture – Karnobat, 1: 17-32.
7. Mihova, G. and Dimova, D. (2012). Yield components characterization of various feed barley forms. Field Crops Studies, 8 (1): 23-36.
8. Mihova, G. (2013). Winter barley breeding at Dobrudzha Agricultural Institute – General Toshevo. Scientific Works of the Institute of Agriculture – Karnobat, 2 (1): 23-38.
9. Mihova, G., Ivanova, A., Doneva, S., Petrova, T. (2014). Economic characterization of new winter two-row barley cultivars developed at Dobrudzha Agricultural Institute. Scientific Works of the Institute of Agriculture – Karnobat, 3 (2): 125-134.
10. Mihova, G., Baychev, V., Chamurliyski, P., Stoyanov, H. (2017). Yield Formation in Winter Cereals under Contrasting Conditions of the Environment. 2nd International Balkan Agriculture Congress, 16-18 May 2017, Tekirdağ, Turkey.
11. Petrova, I., Aleksandrova, E., Marinova, G., Batchvarov, V. (2015). Trends in the Variety Development of Durum Wheat in Bulgaria. Scientific Works of University of Food Technologies, Vol. LXII: 91-95.
12. Petrova, I., Ivanova, S., Mihalkova, N., Markov, P. (2013). A Technological Assessment of a New Winter Durum Wheat Variety Mirela. Plant Science 5: 12-16.
13. Petrova, T. (2013). Risk factors with abiotic character for winter wheat production in South Dobroudja. Integrated system for precise and sustainable management of the agricultural production risks specific for Dobroudja area, http://www.ysys.ro.
14. Stanoeva, Y. and Iliev, I. (2014). Dynamics of distribution of the cause agent of powdery mildew *Blumeria graminis tritici* on wheat during 2005-2009. Turkish Journal of Agricultural and Natural Sciences, Special Issue 2: 1863-1869.
15. Todorov, I. (2006) Study on grain storage proteins and their use as genetic markers in wheat breeding. DSc. Thesis, Dobrudzha Agricultural Institute, 398.
16. Tonev, T., Mihova, G., Mihailov, R., Penchev, P. 2006. Study on stem morphological differences in winter barley genotypes according to predecessor and nitrogen rate, and in relation to stem lodging. Field Crops Studies, 3 (2): 269-281.
17. Tsenov, N., Kostov, K., Todorov, I., Panayotov, I.., Stoeva, I., Atanassova, D., Mankovsky, I., Chamurliyski, P. (2009). Problems, achievements and prospects in breeding for grain productivity of winter wheat. Field Crops Studies 5 (2): 261-273.
18. Tsenov, N., Chamurliyski, P., Petrova, T., Penchev, E. (2012a). Breeding of cold tolerance the common winter wheat (*Triticum aestivum* L.) at Dobrudzha Agricultural Institute. Field Crops Studies 8 (1): 53-64.
19. Tsenov, N., Ivanova, A., Atanasova, D., Petrova, T., Tsenova, E. (2012b). Breeding indices for assessment of drought tolerance of winter bread wheat. Field Crops Studies 8 (1): 65-74.

20. Tsenov, N, Gubatov, T., Tsenova, E. (2015). Gene action in the inheritance of date to ear emergence and time to physiological maturity in bread wheat crosses (*Triticum aestivum* L.). Agricultural Science and Technology 7(1): 11-18.
21. Panayotov, I., and Rachinski, T. (2002). Wheat Breeding as a Basis of Grain Production in Bulgaria. Jubilee Session on the 50th Anniversary of Dobroudja Agricultural Institute "Breeding and Agrotechnics of Field Crops", Vol. I, 21-37.
22. Panayotov, I. (2013). Etude on a new design for productivity in wheat, *Triticum aestivum* L. In: Wheat – genetic and breeding studies, "Abagar", V. Tarnovo, 724-772.
23. Tsvetkov, S., Stoeva, I. (2003). Bread Making Quality of Winter Hexaploid Triticale (*X. Triticosecale* Wittmack) in Bulgaria. Bulg. J. Agric. Sci., 9: 203-208.

PREFERENCE MANAGEMENT ACTIVITIES BASED ON GOOD AGRICULTURAL PRACTICES IN TOBACCO PRODUCTION

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Abstract
Modern management requires from professional and scientific workers in the field of biotechnical, natural and social sciences to contribute to the policy and strategy for improving the development of agricultural production through multidisciplinary approach. In this regard, it is particularly necessary to identify the priorities, limitations, problems and objectives related to the development of tobacco production. The concept of good agricultural practices (GAP) may give a special contribution to economically justified and good quality tobacco production. The average production of tobacco in the Republic of Macedonia in the last fifty years was 30,000 tons and application of GAP is of particular importance for its improvement. This concept has been developed in recent years as a result of rapid changes and globalization in agricultural production. The recommendations and information provided by GAP refer to the environment, economic and social security of production. The use of the widely accepted principles of GAP, general indicators and practical application help the national policy in preparation of strategies to ensure that all manufacturers, processors, consumers, merchants, etc. participate and use the benefits of its application. In this paper we present some principles of the GAP concept in tobacco production as well as the interdependence between tobacco production and tobacco growing areas.

Keywords: management-concept, behavior, tobacco production, agricultural strategy, good agricultural practices.

Introduction

In the Republic of Macedonia, tobacco is traditionally grown agricultural crop with high economic and social significance not only for the population engaged in its production but also for the whole country. Although no one denies the economic and social aspect of tobacco, nowadays there is a strong and loud anti-tobacco campaign on the harmful effects of tobacco smoke on human health as well as the risk of water pollution caused by leaching of nitrogen from the applied fertilizers and uncontrolled use of agrochemicals, as emphasized by environmental associations. This obliges us to participate in the raising public awareness for gradual redirection to high-quality tobacco varieties and to other crops, accepted by the EU (previously considered by appropriate scientific research and state institutions). Tobacco growers should develop proper management procedures based on good agricultural practices in order not only to enable high quality production of tobacco and other crops, but also to raise environmental concerns and continuous training of farmers for their activities, starting from breeding process and ending with sale of baled tobacco and all other activities related to the human factor. This paper emphasizes the need for the use of GAP in tobacco production, which would increase the average yield per hectare, as indicated by the impact of unidentified 46% of the impact factors according to the econometric model of correlation used in the study.

Results and discussion

Good agricultural practice (GAP)

The promotion, use and further development of tobacco production techniques and strategies that satisfy the requirements of buyers, the improvement of farmers' success, their continuous training and care for the environment are priorities for modern agricultural production. They can be defined as agricultural practices which contribute to the achievement of higher yields and quality, enabling at the same time the preservation and improvement of the environment, with special attention and care for soil, water and air pollution and survival and undisturbed development of flora and fauna. Nowadays, the attention is focused on farmers' skills to influence the production by applying appropriate methods and tools for achieving higher yields, with constant care for preservation of the environment. The system of measures and methods used in the processes of production should be adapted to the numerous sensitive environmental problems, genetic diversity, wild animals and their habitats, and, in some cases, to the social structure of agricultural communities.[1]Also, manufacturers must take into account the demands (preferences, taste, indications) of consumers all over the world, because today they are better informed and more critical than in the past and want to know how the products are grown and which materials are used during their production.

The key areas which should be targeted by management activities are the following:[2]

- Soil management and irrigation
- Integrity diversity / Selection
- Yield management
- Integrated pest management
- Agrochemicals management
- Forest management
- Management of proper manipulation and storage of tobacco
- Farmer's storage of tobacco
- Non- tobacco related material / foreign material
- Farmers training
- Socio-economic issues

The above mentioned management activities need to be continuously improved with new elements based on acquired scientific knowledge and good agricultural practices.

Management of soils and irrigation

The selected tobacco-producing system must be adaptable to specific situations. Land and water should be managed carefully, taking into account their physical, chemical and biological characteristics. Each specific situation requires adaptation of the previous practice of land and water management and adoption of new techniques. The control of land erosion can significantly reduce the possibilities of surface water and groundwater pollution and preserve the quality of land and its fertility. Therefore, land and water management is a key factor for efficient and environmentally sustainable tobacco production.

Land is natural, dynamic and sensitive resource.

When determining the selection criteria, the following items should be considered:[3]

[1] Drucker P. (2007). Innovation and Entrepreneurship, Foreword by Christopher Bones

[2] http://www.fao.org/docrep/013/i1645e/i1645e00.pdf

[3] Porter, E. M.(1985). Competitive Advantage: Creating and sustaining superior performance, New York:Free Press, London

• Soil type, depth and fertility
• Slope of the land
• History of crop rotation
• Drainage
• Tobacco cultivation on a steep land, unless it is terraced, should be avoided; such areas could be more convenient for growing fruit trees
• It is necessary to apply contour planting of tobacco and to adopt new experiences and techniques, such as terraced farming, and linear planting, which are used to prevent erosion, help vertical water infiltration and retention of land, reduce land movement and erosion, etc.
• Cover crops are cultivated to protect and improve the soil without intention of harvesting. Land should be planted (covered) as long as possible with living plants (herbs) or crop residues, in order to prevent erosion losses and the harmful effects of extreme temperatures. Where feasible, soil should be covered with straw and, if possible, minimum or no ploughing should be applied.

Water Management
Water is natural resource of limited quantities and its maintenance is of particular importance for plants, animals and humans.
- It is not allowed to apply or mix the agrochemicals near water sources
- Fertilizers or agrochemicals must not be poured into water sources
- It is necessary to protect water sources for irrigation
- Avoid irrational consumption and use of water
- Prevent the contamination of water sources by the water-fertilizer mixture used in the seed plots or other plantations
- Water pollution (with fertilizers, agrochemicals, fossil fuels, oils, etc.) must be reduced to a minimum

Avoid the following:
• Agricultural chemicals and fertilizers which are very likely to leach
• Excessive use of agrochemicals and fertilizers
• Re-using irrigation water
It is recommended to make protective belts between arable land and environmentally sensitive areas. Strategically placed protective belts can effectively reduce the leaching of sediments, nutrients and agrochemicals inside and outside arable land. Protective belts allow the farmers to achieve a significant degree of economic and environmental delicacy in their operations, increasing the habitats for the wildlife and protecting the diversity of species. Farmers should moderately use chemicals to protect plants from various diseases and pests. After their use, the chemicals from plants and soils are washed by precipitations and the unclean water with toxic chemicals is carried to rivers, lakes and seas. This way they destroy the aquatic animals but they also contaminate the soil and the aquifers. It is neither good for the coasts visited by tourists (river, lake and sea beaches). Man can contaminate the groundwaters by making stables for domestic cattle or throwing the garbage near the sources of drinking water. Water is an integral part of human life but it is the most sensitive to pollution and all efforts should be made to protect it from contamination. The same is also valid for the air: the human health depends on clean air, which contains more oxygen.

Diversity of integration and selection
The diversity of integration and selection is the basis for successful harvest in terms of agricultural and environmental quality and it provides good economic perspective.
Guiding principles and methods:
• Methods and procedures should be established for the accepted species to test their agronomic (agricultural) convenience and market acceptability

• Certification and seed production programs should guarantee genetic purity of various types of tobacco
• Only certified, registered or approved species can be grown, the origin of which can be checked in accordance with the rights of the producers of new species (seeds), protection of the species and other intellectual property rights
• Only certified, registered or recommended species resistant to certain types of diseases typical for the area and in accordance with the needs of the market can be grown
• For conventional use only conventionally developed and cultivated species can be planted
• Practical measures of bio-protection and the preservation of product identity must be strictly enforced to prevent interference with GM (genetically modified) products.

Production Management (Crop/Harvest Management)
Production management denotes the establishment of a framework (with rules and procedures) for tobacco production which requires agricultural and ecologically acceptable good practice starting from planning of the production to post-harvest activities in order to obtain the requested type of tobacco and expected shape of the leaf.
<u>Guiding principles and methods</u>
• Tobacco seedling should be :
-produced in environmentally friendly system, without use of harmful fumigants such as methyl bromide
-from certified seed of registered and recommended species
- uniform
- healthy and resistant
• In treatment and preparation of soil, care should be taken to the following:
- make as few environmental disorders as possible
- maintain the fertility and soil structure
- apply crop rotation and soil conservation, increase the fertility and minimize the erosion
- weed control, improvement of the process of growing and productivity of tobacco
•Fertilization should be:
- adapted to the nutrition status of soil, crop requirements and local conditions
- applied at the right time and in agreement with owners of the private plantations
- performed with fertilizers that are approved and recommended for tobacco
- agrochemicals should be used only when really necessary and in accordance with IPM (see IPM Section)
• Optimization of the production per unit area shall be obtained by:
- adjustment to the most productive and most resistant practice suitable for each type of tobacco and local conditions, keeping the required quality
-achieving uniformity and complete compatibility
- minimizing losses in production due to physical damage to the leaf, overmaturation or excessive coloration (of leaves)
• Harvest, protection and preparation for the market:
-harvest should take place at optimum time for each tobacco priming (hand)
-protection should be carried out in most appropriate manner to obtain optimum quality
-leaves should be sorted according to their size
-bales must not be pressed too strong
- tobacco should be stored correctly, in accordance with recommended conditions, in order to keep its quality
- local research for further development of the concepts and details of GAP based on the acquired knowledge on local development as a feedback between local practical experience and innovation.

Integrated pest management

Integrated Pest Management (IPM) is systematic approach to crop protection that uses relevant information to improve protection decisions, with emphasis on integrity of the methods used. IPM does not mean complete elimination of agrochemicals but their adequate application as a defense from pests and diseases, which producers cannot maintain to an acceptable level using alternative measures. The application of agrochemicals should be safe for the product and in accordance with legal regulations.

Guiding principles and methods

•Fundamentals of the Integrated Pest Management:

-rotation of tobacco with other crops that reduce tobacco pests and ensure a pest-free period for tobacco

- providing constant yields for all crops; supplying necessary information on the history of the planted areas, soil analysis, pest species, weed and diseases
- survey and inspection of areas to be planted with tobacco in relation to the possible occurrence of diseases, weed problems, experiences from the previous production and data on yields of the crops grown before tobacco
- sampling of soil and roots from plants to determine the presence of nematodes
- use of approved resistant tobacco species
- use of certified seed that is not infected by disease causing agents
- use of seedlings free of diseases or pests, uniform and healthy
- planting at a time that is least suitable for development of pests
- use of traps for protection from animals that could destroy tobacco
- promoting the multiplication of natural enemies of pests and destroying alternative parasite areas
- avoiding possible transmission of infection from tobacco products, waste tobacco materials or infected land by maintaining good hygiene in the period of planting as well as in the early stages of plant development
- profound cleaning of the tools and instruments used for planting
- destroying the remaining seedlings and residues of tobacco at the end of production cycles as soon as possible
- destroying all waste from tobacco at the end of each production cycle

Management of agrochemicals

Management of agrochemicals encompasses a large sphere of activities, including the following:[4]

➢ agrochemicals should be used only when absolutely necessary and in accordance with IPM (Integrated Pest Management);

➢ *use only of those agrochemicals* registered and *approved for* the particular tobacco type

➢ follow all instructions and warnings labeled on the agrochemicals

➢ using personal protective equipment (PPE) during handling, mixing and application of agrochemicals

➢ using methods to ensure safety of people, animals and the environment in general

➢ regular rotation of tobacco with cereal plants in the interval between the harvests (keeping the principle: tobacco - cereal plants - tobacco)

➢ caution about the negative effect of agrochemicals residues on the use value of the harvest (yield)

➢ proper storage of agrochemicals, i.e. proper stock management of agrochemicals

➢ proper removing and deposition of agrochemicals and their residues (sediments) in the package or in the sprayer

[4] Stošić, B. (2007). Menadžment inovacija - Ekspertni Sistemi, Modeli i Metodi. Beograd, FON

- relevant legislation (legal regulations) in case of hazardous use of agrochemicals, protection of the users against safety risks at work, i.e. legally regulated protection in the use of agrochemicals, instructions in the case of accident and acting in accordance with them, monitoring the health of the users of protective chemicals and the living organisms around them and regulations on the hazardous chemicals storage
- prohibition of eating, drinking and smoking while preparing and performing the agrochemicals protection
- prohibition of mixing or dissolving agrochemicals in the vicinity of water (riverc, wells, fountains, etc.). Sprayer must not be directed toward water or plants in the vicinity while treating with agrochemicals

Agrochemicals selection management

Special attention should be given to the selection of agrochemicals. Their choice should be based on the purpose of use, the manner and place of use. When selecting the agrochemicals, the most important is:[5]

- to select certified product with lowest toxicity and shortest decomposition period, safe for humans, wild animals and environment in general and at the same time efficient in the control of pests, weeds and diseases
- to apply selected chemicals adequate for each situation and not harmful to predators that feed on dead pests and/or residues;

The way of storing agrochemicals is a very important factor for safety not only of agrochemicals but also of people, animal and plant life and environment in general. Therefore, the users of agrochemicals should pay high attention to the following:

- each user is obliged to own and use adequate personal protective equipment;
- agrochemicals stocks should be stored in suitable place designated only for this purpose, well protected, packed (wrapped) in adequate materials;
- warehouses for storing the agrochemicals should be away from water sources (away from ponds, streams and drinking water wells);
- all agrochemical stocks should be stored in accordance with the requirements for safe storage of materials, clearly marked on a panel and labeled with basic storage instructions. Proper handling of the stocks includes: good arrangement of agrochemicals and easy access to all of them, good visibility, maximum safety with regulated temperature and humidity, isolation of agrochemicals with special anti-corrosive substances;
- identification of all flammable materials (by name) and their separation from other products, with clear identification of all hazards, specific precautions, instructions on how to react in the case of danger inside and outside;
- safe storage of stocks, i.e. their arrangement in appropriate order, with a distance between the lines, out of the reach of unskilled persons, especially children, and also protected from the reach of wild animals. In order to prevent people from touching the agrochemicals and to avoid accidents, labels and warning sign for the toxic effects of agrochemicals are required;
- providing a stable construction resistant to water, snow and other atmospheric disorders (storm, wind, hail, etc.), secured floor and walls
- impermeability of floors and walls against possible spread of agrochemicals and their uncontrolled leakage and contamination of water sources;
- use of adequate washing agents on the floors sprayed with agrochemicals or washing the containers (e.g. bins) used for dissolving agrochemicals and spraying of the crops. Proper removal of agrochemical residues in special secured places, inaccessible to animals and children;

[5] OECD, Eurostat. Oslo Manual - Guidelines for Collecting and Interpreting Innovation Data, 3rd Edition

- storage of agrochemicals in original packages with original factory labels. After use, package should be tightly closed and returned to the appropriate safe place;
- agrochemicals should be kept away from flammable materials and the the risk of fire;
- securing good accessibility to all agrochemicals in case of necessary action or unpredictable situations during spraying, falling, dispersing, fire or flood in the room where agrochemicals are stored;
- maintenance of stocks, with visible labels providing instructions on how to handle and store the *chemical*;
- keeping minimum reserves of the most necessary agrochemicals;

Tobacco production and its correlation with planted areas

Tobacco production in the Republic of Macedonia in the last seventy years ranges about 25,000 tons, i.e. 12,5 kg per inhabitant.

The highest production was reached in 1986 (35,020 t) and the lowest in 1961 (8,040 t).

The average production of tobacco per hectare is gradually increasing. Thus, compared to the 1084 kg/ha in the last seventy years, it has grown to 1,281 kg/ha in the last thirty years and 1,394 kg / ha in the last ten years.

Data on tobacco production and planted areas over the past thirty years are presented in Table 1 and Fig. 1.

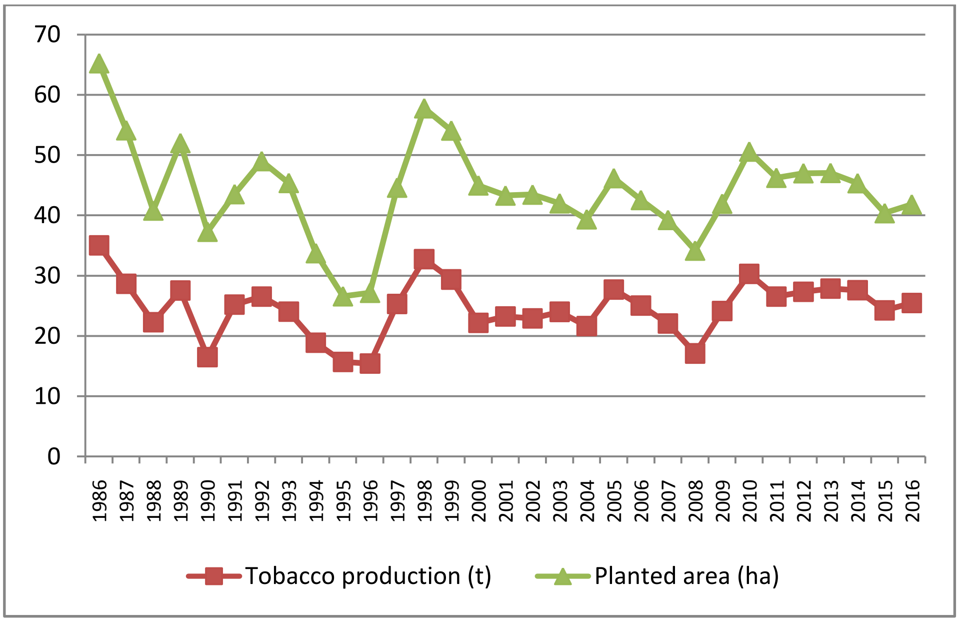

Figure 1. Tobacco production in the Republic of Macedonia, 1986-2016

The coefficient of correlation between tobacco production and area planted with tobacco was 0.733 high (calculated by the formula $Y=\beta_1 + \beta_2 X_1 + \varepsilon_1$), whereas the coefficient of determination was 54%. This means that tobacco production is determined by the planted tobacco hectares with 54%, while the remaining 46% are determined by other factors, among which is the impact of good agricultural practices (GAP).

Table 1. Tobacco production in the Republic of Macedonia[2], 1986-2016

Year	*Tobacco production (in tons)*	Planted area, in hectares
1986	35.020	30.216
1987	28.648	25.465
1988	22.259	18.534
1989	27.537	24.456
1990	16.452	20.825
1991	25.195	18.324
1992	26.502	22.497
1993	24.002	21.373
1994	18.862	14.864
1995	15.683	10.891
1996	15.412	11.738
1997	25.308	19.290
1998	32.746	25.016
1999	29.368	24.700
2000	22.175	22.790
2001	23.217	20.067
2002	22.911	20.530
2003	23.986	18.008
2004	21.630	17.715
2005	27.691	18.485
2006	25.036	17.507
2007	22.056	17.183
2008	17.087	17.064
2009	24.122	17.809
2010	30.280	20.300
2011	26.537	19.693
2012	27.333	19.656
2013	27.859	19.178
2014	27.578	17.758
2015	24.237	16.126
2016	25.443	16.373
31	762.172	604.429

Farmers Training

Training on the farm (field) is one of the most important elements of each GAP program. It gives opportunity to the farmers to update their knowledge with the latest technological achievements and advantages and points out to the significance of GAP. Farmers' training is carried out throughout the world and various methods and techniques are used for this purpose. Farmers are included in GAP trainings organized by agriculture companies, industrial groups, institutions and universities, government agencies and private consultants. Farmers' trainings include frequent individual visits, group meetings, days spent in the field, radio and TV advertisements, printed brochures and posters, and other educational devices.

Guiding principles and methods:
• to provide continuous training and education in all GAP elements so that each participant is aware of its importance
• to treat the following issues: industry expectations, safe working conditions, prevention of the use of child labour, proper handling of agrochemicals, application, care and storage management, distribution, integration of diversities, land and water management, crop management, IPM, NTRM, forestry, handling of tobacco on the farm, classification and storage.
• to include formal training plans for all employees, contractors and farmers
• to organize initial training/education sessions in each area, followed by new courses each season in appropriate period
• to organize group meetings using visual techniques (posters), maps and multimedia, as well as practical group demonstrations as an integral part of the individual visits to farmers.
• to keep records of the courses and visitors (certified by signature of the instructor and the coach)
• to comprise guidelines, recommendations, manuals etc. which are in compliance with good management and guiding principles of GAP
• to cover the relevant legislation, dangers and risks from the application of agrochemicals, safe working methods, necessary actions, health control, etc.
• to introduce integrated pest management plans in order to reduce the use of agrochemicals

Conclusions

In today's modern economy, including tobacco production as its constituent part, no progress can be imagined without the application of management activities based on good agricultural practices. Tobacco industry encompasses a long chain of interconnected subjects such as farmers (tobacco growers), people employed in all tobacco producing organizations, traders (purchasers) of leaf tobacco, manufacturers and other subjects related to the basic industry. They all have a special role to promote the tobacco business through preference of proper management activities in all operations and processes of production, treatment and processing of tobacco. Tobacco is grown in many different regions of the world under different agrarian systems and levels of development. For that reason, there is no single pattern which would strictly state the activities of the agribusiness management, with special reference to tobacco production as a basic activity. It is necessary to apply correct management activities based on the principles of good agricultural practices, which would represent a common model, highly flexible and adaptable to different socio-economic and farming conditions of the regions or countries. This model will emphasize the principles that will be adapted and modified to specific farming situations, as well as their continuous improvement for production of good quality tobacco, with simultaneous concern for preservation of the environment. Thus, if certain unification still exists, it consists of constant adaptation and improvement of management activities in different fields and processes of tobacco production.

References

1. Porter, E. M.(1985). Competitive Advantage: Creating and sustaining superior performance, New York:Free Press, London
2. Langdon M. (2009). The innovation master plan: The CEO's guide to innovation, Chapter I-Why Innovate, 14
3. Drucker P. (2007). Innovation and Entrepreneurship, Foreword by Christopher Bones
4. Robins, S. P., Kolter M. (2005). Menadžment, 8. izdanje. Beograd, Data Status,
5. Schumpeter, J. A. (1939). McGraw Hill, Business Cycles. New York,
6. Trott, P. (2005). Innovation Management and New Product Development. London, Prentice Hall,
7. European Commission. (1995) Green Paper on Innovation. European Commission, December
8. Stošić, B. (2007). Menadžment inovacija - Ekspertni Sistemi, Modeli i Metodi. Beograd, FON.
9. Lajović D., Vulić V. (2010). Tehnologija i Inovacije, Ekonomski Fakultet, Podgorica.

10. Мицески Т. (2009) “Здравствена статистика”, Универзитет “Гоце Делчев”, Економски факултет, Штип.
11. Davila, T., Epstein M. J., and Shelton R. (2006). Making Innovation Work - How to Manage It, Measure It, and Profit from It. Wharton School Publishing,
12. OECD, Eurostat. Oslo Manual - Guidelines for Collecting and Interpreting Innovation Data, 3rd Edition.

EFFECT OF HUMIC ACID SEED TREATMENT ON YIELD AND SOME YIELD CHARACTERISTIC OF CORN PLANT (*Zea mays* L. *indentata*)

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Abstract

This study was aimed to determination of seed treated humic acid effects to yield and some yield characteristics of corn plant (*Zea mays* L. *indentata*) as grown second crop conditions. Study was set up according to randomized complete blocks experimental design with 3 replicates under Harran Plain conditions in 2012, Sanliurfa, Turkey. Motril hybrid corn variety was used as a plant material. Humic acid seed treatments were 0 (control), %2.5, %5, %7.5, %10, and %12 humic acid concentrations. Humic acid concentrations were applied to corn seed before sowing. Some yield and yield characteristic of corn plant such as tassel flowering duration, plant height, leaf number per plant, grain weight of ear, thousand kernel weights and grain yield were evaluated in the study. As a result of research; statistical significant differences were seen among humic acid seed treatments at tassel flowering duration and leaf number per plant ($P \leq 0.05$). Also, humic acid seed treatments were significant at grain weight of ear, thousand kernel weights and grain yield ($P \leq 0.01$). Tassel flowering duration decreased with humic acid seed treatment whereas leaf number per plant, grain weight of ear, thousand kernel weights and grain yield values increased with humic acid seed treatment. The highest values were found in % 7.5 humic acid seed treatment.

Keywords: Corn, humic acid, seed treatment, Harran Plain.

Introduction

Corn is used as an animal feed and industry raw material as well as directly and indirectly in human nutrition. Corn grain has a very important place in the world for human and animal feeding. Corn is cultivated in 184 800 969 hectares in the world, producing amount is 1 037 791 518 tons and average yield is 5615.7 kg ha^{-1} (Anonymous, 2017a). Corn ranks first in terms of production quantity, but in the third place after wheat and rice in terms of planting area in the world. In Turkey, grain corn cultivation area is 680 000 hectares, production is 6 400 000 tons and yield is 9420 kg ha^{-1} (Anonymous, 2017b). Also corn is an important plant for Turkey's industry. Corn plant consumes more nutrients from soil and produces too much dry matter in the unit area. The intake of the plant's nutrients depends on the amount and condition of these substances in the soil, the climate and soil factors, the growing season and the developmental period of the plant. In order to obtain high yields from the corn plant, which is in need of nutrients in high amounts, the soil fertility must also be high. Due to the chemical fertilizers used in agriculture, the chemical, physical and biological properties of the soil have been degraded and especially the level of organic matter has fallen below 1% (Gok et al. 1995). One of the most economical and rapid solutions to the problem of organic matter in modern agriculture is direct application of humic acid to the soil or plant. Humic acid improves soil's physical structure. Application of humic acid increases soil aeration and water holding capacity of the soil. Humic acid maximizes the cation exchange capacity of the soil and regulates soil pH. It will also help to lower the pH of the soil to a more neutral level and will flush high levels of salts out of the root zone, all of which will help to promote better plant health and growth. Humic acid is considered to increase the permeability of plant membranes and enhance the uptake of nutrients. Moreover, humic acid increases the availability of nutrients in our fertilizers and in those already existing in our soil (Piccolo et al. 1997). Humic acid increases the uptake of nitrogen, phosphorus, potassium, iron, zinc and trace elements in the soil by the plant (Kacar and Katkat 2011). Humic acid increases seed germination and accelerates the formation and development of fringe root. It is an excellent root stimulator. It allows the plant to grow faster and stronger. It has a stimulating effect on the development and multiplication of useful soil microorganisms. Humic acid increases the amount and activity of microorganisms in the soil. Humic acid also increases the tolerance of the plant against to stress conditions of plants such as cold, hot and physical effects and resistance to disease (Kacar and Katkat 2011). Many researchers reported that humic acid application has positive effects on plant growth and yield characteristics (Bohme et al. 2001, Hopkins and Stark 2003, Bozoglu et al. 2004, Karakaya and Paksoy 2008, Day et al. 2011, Gursoy et al. 2016). Sharif et al. (2002) found that addition of 0.5-1.0 kg ha^{-1} humic acid resulted in increased wheat grains yield by 25-69% over control. Selcuk and Tufenkci (2009) stated that application of humic acid (0, 2, 4 L HA/da) to corn plant resulted in a significant increase in the kernel number of ear, length of ear, height of plant and thousand kernel weight. It is stated by Oren and Basal (2005) that humic acid application affects earliness, seed weight and yield in a positive way. Cimrin et al. (2001) reported that application of humic acid increased the yield and quality. Many researchers (Chain and Aviad 1990, Padem and Ocal 1998, Kaya et al. 2005) reported that humic acids have an impact on plant growth and development, and that when applied in low quantities they affect development positively. Nevertheless, they stated that when applied in excessive amounts, they have ineffective or negative effects on development. It has been reported that humic acid application promotes germination by increasing enzyme activities in seeds and increases germination rate, root and shoot growth (Rauthan and Schnitzer 1981). In this study, it was aimed to determine the effect of different humic acid concentrations applied to seed on yield and some yield components of corn plant.

Material and methods

This study was conducted in 2012, Sanliurfa, Turkey. The experimental field is located in Harran Plain (altitude: 465 m; 37^0 08' N and 38^0 46' E) where the climate varies from arid to semi-arid. Table 1 provides the climatic data obtained at the Sanliurfa City Meteorological Station. As can be seen from Table 1 that the weather is hot and dry in the months of June, July and August where maximum temperatures were all above 40 ^oC while the relative humidity was below 50%. Rainfall was very low from June to August in 2012.

Table 1. Monthly some climatic data during 2012 corn growth period in Sanliurfa†.

Meteorological observations	Months					
	June	July	August	September	October	November
Min. Temp. ^0C	17.6	20.0	22.4	15.6	11.6	7.1
Max. Temp. ^0C	42.2	44.2	42.1	39.9	37.0	26.9
Av. Temp. ^0C	30.6	33.3	32.3	28.4	21.0	14.9
Av. Humidity (%)	21.2	18.8	29.0	28.0	48.5	65.6
Rainfall (mm)	5.8	0.2	0.2	2.0	35.2	68.4
Sunshine (hour)	11.9	12.0	10.8	9.6	6.1	4.2

†Data collected from the Sanliurfa Meteorological Station (Anonymous, 2012).

The soil of the research field was clay, slightly alkaline, high in lime and very low in salt contents. Field capacity of the soil was 33.8% on dry basis, permanent wilting point was 22.6% and bulk density was 1.41 g cm^{-3}. Some physical and chemical properties of research soil were given in Table 2. Motril hybrid single cross dent corn variety (*Zea mays* L. *indentata*) was used as crop material. Humic acid seed treatments were 0 (control), %2.5, %5, %7.5, %10, and %12 humic acid concentrations. Solutions were prepared and sprayed onto the seeds with a small hand sprayer. In control applications, the seeds were just sprayed with water. After application of humic acid to the seeds were dried for 24 hours at room temperature. Land was ploughed and cultivated then prepared for planting with a single pass of a disk-harrow. The experiment was laid out in a randomize block design with three replications. Each plot area was 14 m^2 (5 m x 2.8 m) and consisted of four rows of 5 m in length. The plants were grown 70 cm apart between the rows with 18 cm spacing in each row. The seeds were sown in second part of June at a 50-60 mm depth. At sowing, 80 kg ha^{-1} of pure N, P and K, as a 15-15-15 composed fertilizer, was applied to each plot; this was followed by 160 kg ha^{-1} of N as urea when the plants reached 30-40 cm in height. Irrigation water was first applied to all the plots using a sprinkler irrigation system. After the emergence of plants, plots were irrigated equally by the furrow irrigation system. All tested characteristics were measured on randomly selected 20 plants in the center of each plot. An analysis-of-variance (ANOVA) was performed to evaluate statistically differences between results. Means of the data obtained from research were compared using least significant difference (LSD) at $P \leq 0.05$.

Table 2. Some physical and chemical properties of research soil

Deep (cm)	Organic Matter (%)	Total Salt (%)	pH	$CaCo_3$ (%)	P_2O_5 kg/da	K_2O kg/da	Texture (%)		
							Sand	Clay	Silt
0-20	0.81	0.098	7.7	25.4	3.6	99.3	24.16	53.84	22.0

Results and discussion

Tassel flowering duration, plant height, leaf number per plant, grain weight of ear, thousand kernel weights and grain yield values and LSD groups were given in Table 3.

Tassel Flowering Duration (day)

According to variance analyses, humic acid concentrations were significant ($P \leq 0.05$) at tassel flowering duration. Tassel flowering duration ranged from 53.66 to 56.00 day (Table 3). The longest tassel flowering duration value was seen at control application whereas the shortest tassel flowering duration value was found at %7.5 humic acid concentration. Flowering period decreased with humic acid application. Similar findings were reported by Dogan (2002) and Day et al. (2011). Oren and Basal (2005) reported that humic acid applications provide earliness.

Plant Height (cm)

Variance analyses results show that humic acid concentrations were not significant at plant height. The longest plant height value was found at %7.5 humic acid concentration as 229.33 cm whereas the shortest plant height was 227.33 cm at control application (Table 3). Similar results were obtained by Padem (1998) and Selcuk and Tufenkci (2009). These authors observed that humic acid application increase plant height.

Leaf Number per Plant (number $plant^{-1}$)

Humic acid concentrations for leaf number per plant were significant ($P \leq 0.05$) according to variance analyses. Leaf number per plant values varied from 11.13 to 13.70 number $plant^{-1}$ (Table 3). The highest leaf number per plant value was seen at %7.5 humic acid concentration. Also the lowest leaf number per plant value was found at control application. Some researchers' emphases that humic acid effects positively yield characteristics of plant (Bozoglu et al. 2004, Karakaya and Paksoy 2008, Day et al. 2011).

Grain Weight of Ear (g)

Humic acid concentrations were significant ($P \leq 0.01$) at grain weight of ear. Grain weight of ear values varied between 131.33 and 167.66 g. The highest grain weight of ear value was found at %7.5 humic acid concentration as 167.66 g whereas the lowest grain weight of ear value was 131.33 g at control application (Table 3). Our results were supported by Gursoy et al. (2016). It was stated that humic acid increased grain weight in plant (Oren and Basal 2005).

Table 3. Tassel flowering duration, plant height, leaf number per plant, grain weight of ear, thousand kernel weights and grain yield values and LSD groups

Humic acid (%)	Tassel flowering duration (day)	Plant height (cm)	Leaf number (number plant^{-1})	Grain weight of ear (g)	Thousand kernel weights (g)	Grain yield (kg da^{-1})
% 0	56.00 a†	227.33	11.13 c†	131.33 c†	364.66 e†	940.14 c†
% 2.5	54.66 b	228.66	12.46 b	160.66 b	374.00 d	1146.00 ab
% 5	54.00 bc	229.33	13.50 ab	163.00 ab	392.33 b	1162.64 ab
% 7.5	53.66 c	229.66	13.70 a	167.66 a	400.00 a	1186.64 a
% 10	54.33 bc	229.00	13.23 ab	162.00 ab	394.33 ab	1158.66 ab
% 12	54.66 b	229.00	13.20 ab	158.33 b	383.00 c	1131.71 b
Average	54.555	228.83	12.872	157.16	384.722	1120.968
LSD	0.917	-	1.222	6.8956	6.012	54.50
Significance	*	ns	*	**	**	**

†There is no statistical difference among values annotated with the same letter at 0.05 according to the LSD test

*, ** and ns denotes P≤0.05, P≤0.01 and no significant, respectively

Thousand Kernel Weights (g)

Variance analyses results show that humic acid concentrations at thousand kernel weights were significant (P≤0.01). Thousand kernel weights values were between 364.66 g and 400.0 g. The highest thousand kernel weights value was found at %7.5 humic acid concentration as 400.0 g whereas the lowest thousand kernel weights value was 364.66 g at control application (Table 3). These results are in accord with the findings of some researchers (Chain and Aviad 1990, Padem and Ocal 1998, Kaya et al. 2005). Selcuk and Tufenkci (2009) and Oren and Basal (2005) stated that humic acid effects positively thousand kernel weights.

Grain Yield (kg da^{-1})

Humic acid concentrations were significant (P≤0.01) at grain yield. Grain yield varied from 940.14 kg da^{-1} to 1186.64 kg da^{-1} (Table 3). The highest grain yield value was found at %7.5 humic acid concentration as 1186.64 kg da^{-1} whereas the lowest grain weight of ear value was 940.14 kg da^{-1} at control application (Figure 1). Humic acid effected positively grain yield. Similar research results reported by some other researchers (Chain and Aviad 1990, Padem and Ocal 1998, Kaya et al. 2005). It was stated that significant positive effects of humic acid on the grain yield (Dogan 2002, Bozoglu et al. 2004, Cimrin et al. 2001, Day et al. 2011, Gursoy et al. 2016). Sharif et al. (2002) found that addition of 0.5-1.0 kg ha^{-1} humic acid resulted an increase wheat grain yield by 25-69% over control. It was stated that humic acid is created a synergetic effect during uptake of nutrients from soil (Lee and Bartlett 1976, David et al. 1994). Also it could be the effect of humic acid substances in manner similar to plant growth substances (Casenave de Sanfilippo et al. 1990). Increase in grain yield may be due to these effects.

Conclusions

Based on the results of research it may be concluded that statistical significant differences were seen among humic acid seed treatments at tassel flowering duration and leaf number per plant (P≤0.05). Also, humic acid seed treatments were significant at grain weight of ear, thousand kernel weights and grain yield (P≤0.01). Tassel flowering duration decreased with humic acid seed treatment whereas leaf number per plant, grain weight of ear, thousand kernel weights and grain yield values increased with humic acid seed treatment. The highest values at tested characteristics were found in % 7.5 humic acid seed treatment.

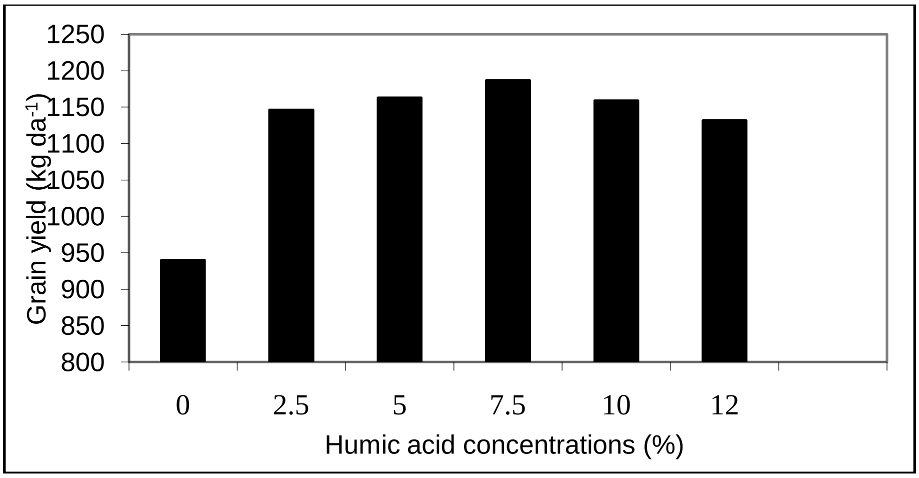

Figure 1. The grain yield values obtained from the different seed application of humic acid

References

1. Anonymous, 2012. https://www.mgm.gov.tr/sanliurfa.aspx. 30.12.2012.
2. Anonymous, 2017a, http://www.fao.org. 23.07.2017
3. Anonymous, 2017b http://www.tuik.gov.tr/VeriBilgi.do?alt_id=45. 23.07.2017
4. Bohme, M., Hoang, L.T. and Vorverk, R. (2001). Effect of different substrates and mineral as well as organic nutrition on the growth of cucumber in closed substrate systems. Acta Horticulturae, 548: 165-172.
5. Bozoglu, H., Pesken, E. and Gulumser, A. (2004). Sira araligi ve potasyum humat uygulamasının bezelyenin verim ve bazı özelliklerine etkisi. A.U. Tarim Bilimleri Dergisi, 10 (1): 53-58.
6. Casenave de Sanfillipo, E., Arguello, J.A., Abdala G. and Orioli, G.A. (1990). Content of auxin–inhibitor and gibberellin like substances in humic acids. Biol. Pl., 32: 346-351.
7. Cimrin, K.M., Karaca, S. and Bozkurt, M.A. (2001). Misir bitkisinin gelisimi ve beslenmesi uzerine humik asit ve NPK uygulamalarinin etkisi. Tarim Bilimleri Dergisi, 7 (2): 95-100.
8. Chen Y, Aviad T (1990). Effects of humic substances on plant growth. In MacCarthy, P., Clapp, C. E., Malcolm, R.L. and Bloom, P.R. (eds), Humic Substances in Soil and Crop Science: Selected Readings, Madison, WI: SSSA and ASA, 161– 186.
9. David, P.P., Nelson, P.V. and Sanders, D.C. (1994). A humic acid improves growth of tomato seedling in solution culture. J. Pl. Nutr., 17: 173–84.
10. Day, S., Kolsarici, O. and Kaya, M. (2011). Humik asit uygulamalari zamani ve dozlarinin ayciceginde (*Helianthus annuus*) verim, verim ogeleri ve yag oranina etkisi. Akdeniz Universitesi Ziraat Fakultesi Dergisi, 24 (1): 33-37.
11. Dogan, E. (2002). Sera kosullarinda humik asit katkili kati ortam kulturuyle yetistirilen domatesin gelisim, verim ve meyve ozelliklerinin belirlenmesi. A.U. Fen Bilimleri Enstitusu Bahce Bitkileri Anabilim Dali Yuksek Lisans Tezi. 2002. Ankara.
12. Gok, M., Anlarsal, A.E., Ulger, A.C., Yucel, C. and Onac, I. (1995). Bazi baklagil yesil gubre bitkilerinde N_2 fiksasyonu ve biyomas verimi. Ilhan Akalan Toprak ve Cevre Sempozyumu, January 1-2, 1995, Ankara, Turkey, p: 207-216.
13. Gursoy, M., Nofouzi, F. and Basalma, D. (2016). Effects humic acid application at different stages of growth on yield and yield components of winter rapeseed crops. Journal of field Crops Central Research Institute, 25 (2): 131-136.
14. Hopkins, B., and Stark, J. (2003). Humic acid effects on potato response to phosphorus. Idaho Potato Conference, January 22-23, 2003, Idaho, USA, p:87-92.
15. Kacar, B. and Katkat, V. (2011). Bitki Besleme. Nobel Yayinevi, p: 678, Ankara, Turkey.

16. Karakaya, Z. and Paksoy, M. (2008). Yaz sezonunda yetistirilen brokkoli'de (*Brassica oleracea* L. *var. italica*) bazı organik maddelerin bitki gelisimi, verim ve kaliteye etkileri. Selçuk Univ. Ziraat Fak. Dergisi, 22 (44): 1-6.
17. Kaya, M., Ata, M., Khawar, KM,, Çitfçi, CY. and Ozcan S. (2005). Effect of pre-sowing seed treatment with zinc and foliar spray of humic acids on yield of common bean (Phaseolus vulgaris L.). International Journal of Agriculture & Biology, 6: 875-878.
18. Lee, Y.S. and Bartlett, R.J. (1976). Stimulation of plant growth by humic substances. Soil Sci. Soc. Am. J., 40: 876-9.
19. Oren, Y. and Basal, H. (2005). Humik asit ve cinko (Zn) uygulamalarinin pamukta (*Gossypium Hirsutum* L.) verim, verim komponentleri ve lif kalite ozelliklerine etkisi. ADU Ziraat Fakultesi Dergisi, 3 (2): 77-83.
20. Padem, H. (1998). Humik asit katkili yaprak gubresi uygulamasinin hiyar'da (*Cucumis sativus* L.) fide kalitesi ve besin elementi icerigine etkisi. S.D.Ü. Fen Bilimleri Enstitusu Dergisi, 3 (1): 27-41.
21. Padem H, Ocal A (1998). Effect of humic acid added foliar fertilizer on some nutrient content of eggplant and pepper seedlings. XXV th International Horticulture Congress, Benelux, Brussels, 17 August 1998, Abstract Book, 180 pp.
22. Piccolo, A., G. Pietramellara and Mbagwu, J.S.C. (1997). Reduction in soil loss from erosion–susceptible soils amended with humic substances from oxidized coal. Soil Technology, 10: 235-45.
23. Rauthan BS, Schnitzer M (1981). Effects of a soil fulvic acid on the growth and nutrient content of cucumber (Cucumis sativus) plants. Plant and Soil 63: 491-495.
24. Selcuk, R. and Tufenkci, S. (2009). Artan dozlarda cinko ve humik asit uygulamalarinin misirin verim ve besin icerigine etkisi. Yuzuncu Yıl Univ. Fen Bil. Enst. Yuksek Lisans Tezi, pp:54, Van.
25. Sharif, M., R.A. Khattak and Sarir, M.S. (2002). Wheat and nutrients accumulation as affected by humic acid and chemical fertilizers. Sarhad J. Agric., 18: 323–329.

EFFECT OF NITROGEN AND FOLIAR APPLICATION OF PHENOLIC COMPOUNDS ON FLAG LEAF PIGMENTS AND GRAIN YIELD OF SPRING WHEAT (*Triticum aestivum* L.) GENOTYPES

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Abstract

Application of phenolic compounds such as proline, glycinebetaine, silicon, cytokinins, methanol etc. are getting important consideration in modern agricultural research for managing plants to get maximum yield under different environmental conditions. However there is a lack of knowledge about the effects of these phenolic compounds on grain yield, yield components and physiological properties such as leaf photosynthetic pigments. In the context the the present study was conducted during 2015-2016 growing season at the experimental field in the faculty of agriculture, University of Cukurova, Adana, Turkey to evaluated the effects of some phenolic compounds such as proline, glycinebetaine, silicon, cytokinins, methanol etc on chlorophyll (Chl) as well as total carotenoid contents of four bread wheat genotypes ('Adana-99', 'FSD-2008', 'Basribey-98' and 'Seher-06') under two nitrogen levels *viz.,* low nitrogen level, LN: 60 kg N ha^{-1} and high nitrogen level, HN: 180 kg N ha^{-1} under well-watered condition. The results of the LN showed that the grain yield was only significantly correlated with $Chl_{a/b}$ (r=-0.751**) of wheat genotypes. While, under HN, Chl_a, Chl_b, carotenoid and $Chl_{a/b}$ were strongly correlated with grain yield (r=0.422*, r=0.635**, r=0.444* and r=-0.590**) respectively. Among the phenolic compounds, proline highly sitimulated the relationships between grain yield and Chl_b, carotenoids and $Chl_{a/b}$ (r=0.795*, r=0.795* and r=-0.811*), respectively. Similary, methanol effects were strongly correlated with grain yield and Chl_b and $Chl_{a/b}$ (r=0.844** and r=-0.772*), respectively. Therefore it is concluded that foliar application of methanol and proline were significantly increased the flag leaf Chl_b concentration which ultimately lead to increase the grain yield of wheat genotypes.

Keywords: wheat, nitrogen, phenolic compounds, pigments.

Introduction

Wheat (*Triticum aestivum* L.) is the most important cereal crops in Turkey. The arable agricultural land of Turkey is only 24 million hectares, where wheat is cultivated between 7.5-9.8 million ha land (TUIK, 2016). Among them, only 5 million hectares of agricultural land can be irrigated in Turkey, almost all of wheat cultivated depends on rainfall. For this reason annual wheat production fluctuates range from 17 to 19 million tonnes. The annual wheat requirement of the country with a population of 80 million is about 19 million tons. While annual population growth rate in Turkey is 1.35% (TUIK, 2016), the average rate of yield increase only 0.64% per year (Kuşçu, 2002). It is anticipated that the situation will be continue, due to the growing population and declining farmland that ultimately threat to food security of increasing population of the country. In addition earlier studies on genetic improvement of wheat in Turkey is still insufficient (Sener et al. 2009; Kuşçu, 2006; Avcin et al. 1997). Therefore it is very essential to increase the research on genetic improvement of wheat in combination with modern agricultural management practices and technological for sustainable wheat production under stressful environments. Recently, phenolic compounds application of different phenolic compounds such as proline, glycinebetaine, silicon, cytokinins, methanol etc. are getting important consideration in modern agricultural research (Bruulsema et al. 2008; Chien et al. 2009). But knowledge about the effects of these phenolic compounds on physiological properties such as leaf photosynthetic pigments is still in intial stage of the country. These pigments mainly consist of chlorophyll a, chlorophyll b and carotenoids. Chl_a can act as a light harvesting pigment and the reaction centre for leaf photosynthesis (Raven et al 2005). Chl_b can act as an accessory light-harvesting pigments and helps Chl_a to perform leaf photosynthesis (Ishikita et al 2006). Carotenoids serve two major recognized functions in photosynthesis i.e., first, as an accessory light-harvesting pigment and, the other, as a triplet quencher to provide protection against photo-oxidative damage (Young 1991). Photosynthetic pigments and their ratio to each other can shows wide variation due to the variation of plant species, genetic progress (Siddique et al. 1989), stress factors (XinWen et al. 2008; Manivannan et al. 2007), growth stage (Kura-Hotta et al. 1987), plant nutrient deficiency etc. For instance Kitajima and Hogan. (2003) reported that $Chl_{a/b}$ ratio increased while Chlorophyll content decreased in response to N limitation in photosynthetic cotyledons and leaves of seedlings of four tropical woody species. Considering the above issues the present study was under taken to evaluated the effects of some phenolic compounds such as proline, glycinebetaine, silicon, cytokinins, methanol etc on chlorophyll (Chl) as well as total carotenoid contents of four bread wheat genotypes under two nitrogen levels *viz.,* low nitrogen level, LN: 60 kg N ha^{-1} and high nitrogen level, HN: 180 kg N ha^{-1}under well-watered condition.

Material and methods

An experiment was conducted during the 2015-16 wheat growing seasons at the experimental field of the faculty of agriculture, University of Cukurova, Adana (37°00′ N, 35°21′ E, 29 m above sea level), Turkey. The soil type is a fine loamy, montmorillonitic typic xerofluvent, low in organic matter and slightly alkaline (pH 7.1–7.6). Treatments were with a similar developmental pattern of four spring bread wheat genotypes *viz.,* 'Adana-99', 'FSD-2008', 'Basribey-95' and 'Seher-06' ; two nitrogen levels *viz.,* low nitrogen level, LN: 60 kg N ha^{-1} and high nitrogen level, HN: 180 kg N ha^{-1} and foliar application of five phenolic compounds *viz.,* Cytokinins, 40µM,; Silicon, 6 mM; Glycinebetaine, 100mM; Proline, 100mM; Methanol, 20% (v/v)). The experiment was conducted with split-split plot design with three replications. Nitrogen levels were assigned in main plots, phenolic compounds were in sub plots and genotypes were arranged in sub-sub plots. Phenolic compounds tratments were applied in three times at Zadok's growth stages (ZGS) 20, 30 and 60 (Zadoks et al. 1974). The sowing density was 500 viable seeds m^{-2}. Every plots consisted of 6 m long 8 rows with a row spacing of 0.15 m.

Extraction Processes

Chlorophylls (Chla and Chlb) and carotenoid was determined in two leaves disks of 8 mm diameter taken from an identical position of the same leaves (flag leaves collected at antesis period). After the disks were weighed, pigments were thoroughly extracted in 80% (v/v) acetone using a glass mortar, and the homogenate was then filtered. Absorbance was measured at the relevant wavelength with a spectrophotometer (Shimadzu, UV-1208, Kyoto, Japan). Chlorophylls and carotenoids as mg/l was calculated by using the following formulae by Arnon‟s (1949) method. Carotenoid mg/g = 7.6 (OD 480) – 1.49 (OD 510) x (v/1000) x (w). Chlorophyll a (mg/gm) = 12.7(OD 663) – 2.69(OD 645) x (v/1000) x (w).Chlorophyll b (mg/gm) = 22.9 (OD 645) – 4.68 (OD 663) x (v/1000) x (w). Total chlorophyll (mg/gm) = OD 652 x (1000/34.5) x (v/1000) x (w). Carotenoid mg/g = 7.6 (OD 480) – 1.49 (OD 510) x (v/1000) x (w).Where OD = Optical density, V = Final vol. of 80% acetone (25ml), W = Wt. of sample taken (0.25g).

Statistical analysis

Data were subjected to analysis of variance (ANOVA), and means were compared using Least Significant Difference (LSD) values were calculated at the 0.05 level of probability. All calculations were performed with MSTAT-C and SPSS statistical software.

Results and discussion

Effects of nitrogen on grain yield of wheat genotypes

Increased nitrogen level was significantly increased the average grain yield of cultivars from 688 kg da^{-1} to 730 kg da^{-1} (Fig. 1). At the non drought area, over the low limits of nitrogen application was generally positively affected the grain yield (Khan et al. 2000; Jan et al. 2003; Amjed et al. 2008). In this study, genotypes had shown Nitrogen x Genotype interaction, except, Adana-99 and Baribey-97, increases of nitrogen amount was increased grain yield in FSD-2008 and Seher cultivars (Fig.1).

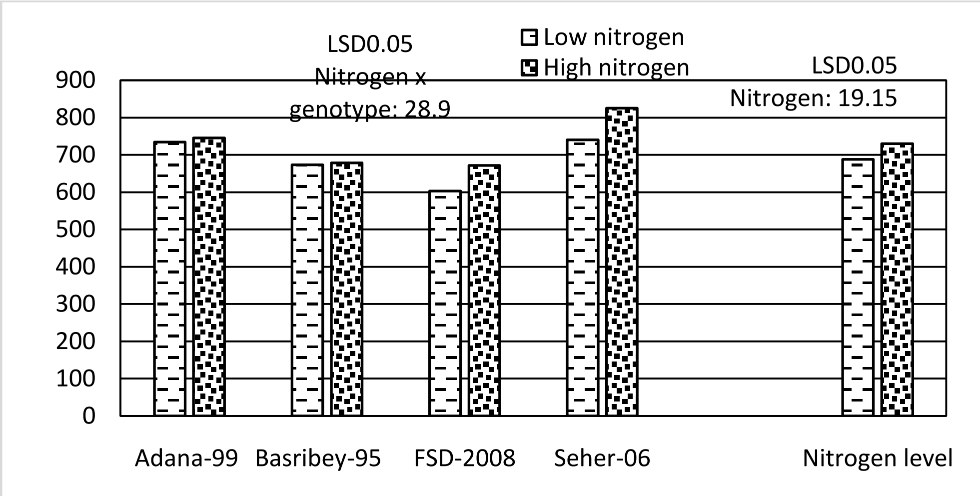

Figure 1. Effect of nitrogen levels on grain yield.

Effects of phenolic compounds on grain yield of wheat genotypes

Exogenous application was affected grain yield (P= 0.102) only at the low nitrogen treatment (Fig. 2). Methanol and cytokinin were improved the grain yield (8%). Under non-stress condition (High level of nitrogen), exogenous application didn't affect on grain yield.

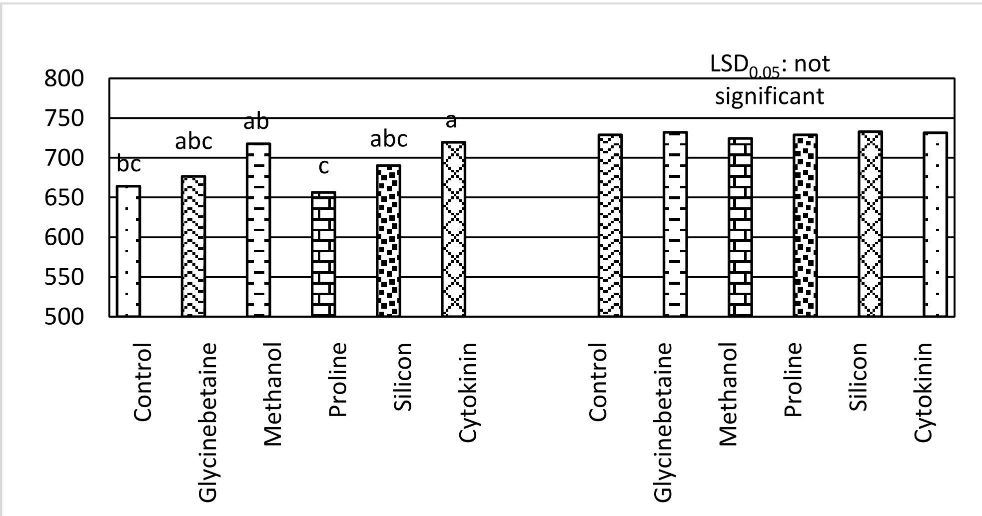

Figure 2. Effects of phenolic compounds on grain yield at the level of low and high nitrogen.

Relationships between grain yield and flag leaf pigments

If, both nitrogen levels and $Chl_{a/b}$ ratio were negatively correlated with grain yield, but all of the pigment content showed the positive correlation. However, Chl_b, Chl_{a+b} and $Chl_{a/b}$ were most effective for grain yield than of Chl_a and carotenoid. In a similar study Kitajima and Hogan, (2003) reported that when $Chl_{a/b}$ ratio was increased, chlorophyll content was decreased due to nitrogen limitation in photosynthetic cotyledons and seedlings leaves of tropical woody species.

Table 1. Correlation coefficient between grain yield and flag leaf pigments under low and high levels of nitrogen.

Pigment	Low level of nitrogen	High level of nitrogen
Chl_a	-0.393	0.422*
Chl_b	0.256	0.634**
Chl_{a+b}	-0.230	0.533**
$Chl_{a/b}$	-0.751**	-0.590**
Carotene	0.098	0.445*

Relationships between grain yield and flag leaf pigments under application of different phenolic compounds

In the present study, there was no significant relationship was found between grain yield and flag leaf pigment contain under control conditions, but application of different phenolic compounds and pigment types were significantly correlated with grain yield (Table 2). In the study, proline was most effective phenolic compound to improve the relationships between grain yield and Chl_a, Chl_b and carotene. However, silicon and cytokinins were not influenced that grain yield of all wheat genotypes. Earlier study reported by Maghsoudi et al (2016) noticed that silicon improved Chl_a and Chl_b in all cultivars under water deficit conditions.

Table 2. Correlation coefficient between grain yield and flag leaf pigments under application of different phenolic compounds

	Control	Glisin betaine	Methanol	Proline	Silicon	Cytokinins
Chl_a	0.213	0.410	0.536	0.662	0.003	0.049
Chl_b	0.536	0.600	0.845**	0.794*	0.371	0.347
Chl_{a+b}	0.325	0.501	0.664	0.722*	0.147	0.158
$Chl_{a/b}$	-0.608	-0.762*	-0.773*	-0.811*	-0.669	-0.626
Carotene	0.294	0.519	0.636	0.795*	0.159	0.467

Conclusions

The results and discussion of the present study it can be concluded that grain yield and $Chl_{a/b}$ were negatively correlated. Application of low level of nitrogen was significantly correlated with grain yield, but under high level of nitrogen, Chl_a, Chl_b, carotenoid and $Chl_{a/b}$ were strongly correlated with grain yield. Among the phenolic compounds, proline highly sitimulated the relationships between grain yield and Chl_b, carotenoids and $Chl_{a/b}$, respectively. Similary, methanol effects were also strongly correlated with grain yield and Chl_b and $Chl_{a/b}$, respectively. Therefore it is concluded that foliar application of methanol and proline were significantly increased the flag leaf Chl_b concentration which ultimately help to increased the grain yield of wheat genotypes.

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References

1. Amjed A,, A.Ahmad., W.H.Syed., T. Khaliq., M. Asif., M.Aziz and M. Mubeen. 2008. Effects of nıtrogen on growth and yıeld components of wheat. Sci.Int. (Lahore), 23(4), 331-332, 2011.

2. Arnon, D.I., 1949. Copper enzymes in isolated chloroplasts: polyphenoloxidase in Beta vulgari L. Plant Physiol., 24: 1-15. Erdtman, 1952.
3. Asghar A, M. A. Choudhry, M. A. Malik, R. Ahmad and Saifullah, 2000. Effect of Various Doses of Nitrogen on the Growth and Yield of Two Wheat (Triticum aestivum L.) Cultivars. Pakistan Journal of Biological Sciences, 3: 1004-1005.
4. Avcin, A., M. Avci, O. Donmez, 1997. Genetic gains in yields of durum wheat (Triticum durum L.) cultivars under Central Anatolian Conditions, J. Field Crops Cent. Res. Inst. 6: 1–12.
5. Bruulsema, T., C. Witt and F. Garcia, 2008. A global framework for fertilizer BMPs. *Better Crops*, 92: 13-15.
6. Chien, S. H., L. I. Prochnow and H. Cantarella, 2009. Recent developments of fertilizer production and use to improve nutrient efficiency and minimize environmental impacts. *Advances in Agronomy,* 102: 267-321.
7. Hameed, E., W.A. Shah, A.A. Shad, J. Bakht and T. Muhammad. 2003. Effect of different planting dates, seed rate and nitrogen levels on wheat. Asian J. Plant Sci., 2(6): 467-474.
8. Ishikita, H., W. Saenger, J. Biesiadka, 2006. How photosynthetic reaction centers control oxidation power in chlorophyll pairs P680, P700, and P870. P Nati. Acad. Sci. USA, 103, 9855–60.
9. Jan, M.T., M. Shah and S. Khan, 2002. Type of Nfertilizer rate and timing effect on wheat production. Sarhad J. Agri., 18(4): 405-410.
10. Khan, S., N.D. Shah and M.T. Sheikh, 2000. Effect and economics of different levels of nitrogen fertilizer on the yield of zargoon wheat variety under irrigated conditions of Baluchistan. Sarhad J. Agri., 16(6): 581- 585.
11. Kitajima K, K.P. Hogan, 2003. Increases of chlorophyll a/b ratios during acclimation of tropical woody seedlings to nitrogen limitation and high light. Plant Cell Environ. 26:857–865.
12. Kura-Hotta M, K. Satoh, S. Katoh, 1987. Relationship between photosynthesis and chlorophyll content during leaf senescence of rice seedlings. Plant Cell Physiol. 28:1321–1329.
13. Kuşcu, A., 2006. Grain yield progress and associated physiological and agronomical traits in spring wheat (Triticum aestivum L.) in Çukurova during the last quarter of the 20th century, PhD Dissertation of Çukurova University, Institute of Natural and Applied Sciences.
14. Maghsoudi K., Y. Emam, M. Ashraf, 2015. Influence of foliar application of silicon on chlorophyll fluorescence, photosynthetic pigments, and growth in water-stressed wheat cultivars differing in drought tolerance. Turk J Bot (2015) 39: 625-634.
15. Manivannan P., C. Abdul Jaleel, B. Sankar, A. Kishorekumar, R. Somasundaram, G.M.A. Lakshmanan, R. Panneerselvam, 2007. Growth, biochemical modifications and prolinemetabolism in *Helianthus annuus* L. as induced by droughtstress. Colloids and Surfaces B: Biointerfaces 59: 141–149.
16. Raven, P. H., R.F. Evert, S.E. Eichhorn, 2005. Biology of Plants 7th ed (7th ed.) 119–127 (W. H. Freeman and Co. 2005).
17. Sener, O., M. Arslan, Y. Soysal, M. Erayman, 2009. Estimates of relative yield potential and genetic improvement of wheat cultivars in the Mediterranean region, J. Agric. Sci. 147: 323–332.
18. Siddique, K. H. M., R.K. Belford, M.W. Perry, D. Tennant, 1989. Growth, development and light interception of old and modern wheat cultivars in a Mediterranean-type environment. Aust. J. Agric. Res., 40, 473-487.
19. Slafer, G.A. F.H. Andrade, 1989. Genetic improvement in bread wheat (Triticum aestivum) yield in Argentina. Field Crops Research 21, 289–296.
20. TUIK, 2016. Turkish Statistical Institute. Turkish Production Year Book.
21. Xinwen X., X. Hailiang, W. Yangling, W. Xiaojing, Q. Yongzhi, X. Bo, 2008. The effect of salt stress on the chlorophyll level of the main sand – binding plants in the shelterbelt along the Tarim Desert Highway, Chinese Science Bulletin, 53: 109-111.

22. Yang, D., Y. Li, Y. Shi, Z. Cui, Y. Luo, M. Zheng, J. Chen, Y. Li, Y. Yin, Wang. 2016. Phenolic compounds cytokinins increase grain yield of winter wheat cultivars by ımproving stay- gren characteristics under heat stres. PLOS ONE. 11(5). p.e0155437.
23. Young A.J., 1991. The photoprotective role of carotenoids in higher plants. Physiologia Plantarum. 83(4):702-8.
24. Zadoks, J.C., T.T. Chang, C.F. Konzak, 1974. A decimal code for the growth stages of cereals. Weed Res. 14: 415–421.

SEED CHARACTERISTICS OF SOME ORIENTAL TOBACCO VARIETIES DEPENDING OF THE TRANSPLANTING DENSITY

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Abstract
The transplanting density is a very important factor when it comes to the acquisition of the planned yield of seed with appropriate quality taken from the certified tobacco seed plots. The main goal of this research was to establish tobacco seed yield and quality given by the tobacco planted with various transplanting density. The experiment was set in the Experimental Field of Scientific Tobacco Institute - Prilep. Further analyses of seed quality characteristics were performed in the accredited Laboratory for seed quality control of agricultural plants. The analyses were performed on two varieties of oriental tobacco type Prilep: P-66-9/7 and NS-72 in different transplanting variants: 50x15cm, 45x15 cm and 40x15cm. The area was prepared with the usual agro technical measures for tobacco seed plots for certification. The transplanting was performed manually in 5 repetitions. Morphological measures were performed on 10 plants of each tobacco varieties and variant of density of transplanting. The following morphological characteristics were analyzed: height and width of the stalk, height and width of fruit, and height and width of capsule. Also the weight of the fruit was analyzed together with the number of capsules in the fruit and the seed amount in the fruit of every stalk. After seed harvest and processing, the morphological measurement and laboratory analyses for seed quality were performed concerning the seed germination expressed in percentage and the apsolute weight expressed in grams. We can conclude that the seed given by a stalk which has growth on larger vegetation area produces better values concerning the yields and quality. The tobacco variety P-66-9/7 has average value of 13.68 g, NS-72 variety has 15.51 g. Which means that the stalks which growth on smaller vegetation for example P-66-9/7 has average value of 5.36 g and NS-72 has 6.76 g. Insignificant number of the results have shown a digression but it can be concluded that the seed obtained by the plants grown in larger vegetation area produce seed with better characteristics considering the quality and quantity of the seed.

Keywords: tobacco seed, vegetation area, transplanting density, variety P-66-9/7, variety NS-72.

Introduction

During the process of production of tobacco seed great influence have the conditions in which the stalks have grown because from them the seed material is obtained. As one of the most important conditions for successful production is the transplanting density. According to Uzunoski (5) the distance from one to another plant is very important factor for tobacco production due to the fact that the biological area of every plant is being set in this way. Once we have defined the goal of our research, the influence of the vegetative area (transplanting density) on some of the characteristics of the tobacco seed. The transplanting density is very important factor for planning a yield of tobacco seed which has adequate quality and it's given by tobacco seed plots for certification. The main goal of this research was to establish the tobacco seed yield and the quality of the seed material given by tobacco which has variable transplanting density.

Material and methods

The experiment was set in the Experimental Field of the Scientific Tobacco Institute- Prilep on soil which is usual for oriental tobacco type production. The basic soil processing was performed by one plowing in autumn and two plowing in spring. Before the spring plowings the soil was fertilized with 300 kg/ha NPK (8:22:20). The seedling of the tobacco varieties which was examined was produced in cold beds, covered with polyethylene with usual agro technique, protection and care. The analyses were performed on two varieties of oriental tobacco type Prilep: P-66-9/7 and NS-72 in different transplanting condition. The transplanting was performed manually in 5 repetitions in accordance to the *random block system,* in which three variations of each tobacco variety: First variant (40x15cm), Second variant (45x15cm) and Third variant (50x15cm). During vegetation period two hoeing were performed. Also the experimental trial was treated for disease protection and pest protection. When required they were watered in order to prevail with preferable conditions for good tobacco seed quality and quantity. During vegetation period morphological measurements were performed on each variant on 10 stalks in each repetition. After the harvest the seed was sent for further processing. Seed harvest was performed once the seed has reached full ripeness, as recommended by Drazic (2) the harvest should be performed once the fruit, the capsule and the seed are dehydrated and have darker coloration. After seed has finished with further processing, each variety is left in separate spaces, according to Aceska (1) her analysis of the influence of tobacco seed ripeness towards the quality characteristics, have shown that good seed germination is given by leaving the seed to rest for several months. During this period the tobacco seed is subdued to complex biochemical processes which are established by Mladenoski (3) during his researches.

Results and discussion

The results from the analysis and morphological measurements are presented in the following table:

Table 1. Dimensions of stalk, the fruit and capsule in tobacco variety P-66-9/7

Variant	Research conditions (vegetative area)	Stalk		Fruit		Capsule	
		Height (cm)	Weight (cm)	Height (cm)	Weight (cm)	Height (cm)	Weight (cm)
1	Density 40x15 cm	70.0	39.5	12.6	7.8	1.6	1.1
2	Density 45x15 cm	75.0	40.0	17.9	8.8	1.7	1.2
3	Density 50x15 cm	79.0	40.0	18.7	9.4	1.6	1.1

From the table we can conclude that the vegetative area has positive influence on the tobacco variety P-66-9/7 dimensions.

Table 2. Dimensions of stalk, the fruit and capsule in tobacco variety NS-72

Variant	Research conditions (vegetative area)	Stalk		Fruit		Capsule	
		Height (cm)	Weight (cm)	Height (cm)	Weight (cm)	Height (cm)	Weight (cm)
1	Density 40x15 cm	48.5	38.5	10.2	9.6	1.8	1.3
2	Density 45x15 cm	51.0	41.5	14,8	16.9	1.8	1.4
3	Density 50x15 cm	55.0	45.0	13.8	14.0	1.8	1.3

The vegetative area has positive influence on each part of the plant we have analyzed from tobacco variety NS-72. From the two tables we can conclude that the vegetative area has great influence on P-66-9/7.

Table 3. Characteristics of the fruit in tobacco variety P 66-9/7

Variant	Research conditions (vegetative area)	Weight of the fruit (g)	Number of capsule in the fruit	Weight of seeds in the fruit (g)
1	Density 40x15 cm	12.60	48	5.36
2	Density 45x15 cm	15.10	54	6.85
3	Density 50x15 cm	24.60	108	13.68

From the table we can see that the vegetative area has great influence on the cluster weight, number of capsule and the seed quantity seed in the fruit.

Table 4. Characteristics of the inflorescence in tobacco variety NS-72

Variant	Research conditions (vegetative area)	Weight of the fruit (g)	Number of capsule in the fruit	Weight of seeds in the fruit (g)
1	Density 40x15 cm	13.55	63	6.76
2	Density 45x15 cm	18.91	79	14.89
3	Density 50x15 cm	29.41	119	15.51

Tobacco seed variety NS-72 has great influenced from the vegetative area on the characteristics which are presented in the table. If we compare the two tobacco varieties we can see that the larger vegetative area has greater influence on the tobacco variety NS-72, especially the third variant (50x15cm).

Table 5.Tobacco seed characteristics in tobacco variety P 66-9/7

Variant	Research conditions (vegetative area)	Absolute seed weight g	Germination energy %	Total germination %
1	Density 40x15 cm	0.0848	87	87
2	Density 45x15 cm	0.0864	93	93
3	Density 50x15 cm	0.0892	91	91

The absolute seed weight, germination energy and the total germination were analyzed in the laboratory with methods which are in line with the Book of rules. When it comes to the temperature of the tobacco seed germination Prespanoski (4) established that the optimal tobacco seed germination temperature is in the range of 20 to 24 ˚C. The data for this characteristic presented in the table show variable values which depend of the variant. If we compare the values, we can conclude that tobacco seed absolute weight is higher in the seed which is given from stalks which were grown on larger vegetative area.

Table 6.Tobacco seed characteristics in tobacco variety NS-72

Variant	Research conditions (vegetative area)	Absolute seed weight g	Germination energy %	Total germination %
1	Density 40x15 cm	0.0968	90	98
2	Density 45x15 cm	0.0988	92	98
3	Density 50x15 cm	0.0995	91	97

From the data presented we can conclude that parameter absolute weight in tobacco variety NS-72 has minimal variations in the value which depends on the variant. The variant which was grown on larger vegetative area has no significant varieties in tobacco seed absolute weight, which means that the larger vegetative area has almost no influence on the tobacco seed absolute weight. When it comes to the energy and tobacco seed germination we can conclude that the size of vegetative area (transplanting density) has no influence on the two tobacco varieties which were analyzed in different variants.

Conclusions

The analyzed tobacco plants which were grown on larger vegetative area have shown better results when it come to the tobacco varieties and their characteristics. Tobacco plants grown on larger vegetative area have higher values concerning the height and width of the stalk, height and width of cluster of capsule, and height and width of capsule. The number of capsules in cluster of capsules (119) has highest value in tobacco variety NS-72. Tobacco variety NS-72 has biggest ammount of tobacco seed in one capsule cluster (29.41g).Tobacco seed variety NS-72 has highest absolute weight (0.0988g). Tobacco seed variety NS-72 has highest total germination (98%).

References

1. Аческа, Н., 1987, Влијанието на зрелоста на семето врз дормантноста, енергијата на ртење и вкупната ртливост на тутунското семе од типот Прилеп и Вирџинија (магистерски труд) Земјоделски факултет-Скопје. Стр.120.
2. Dražić, S., 1980, Proućavanje veze izmedju vremena sakupljanja ploda (ćaura), njegovog položaja u cvasti I semenskih kvaliteta duvana (magistarski rad) Poljoprivredni fakultet u Zemunu. Стр. 85.
3. Младеноски,Т., 1996, Биологија на семето, Земјоделски институт-Скопје. Стр.137-138.
4. Преспаноски, Љ., 1997, Ртливост на тутунското семе од сортите МВ-1 и П-23 при различни температурни услови и фитопериодизам (дипломска работа), Природно математички факултет- Интитут за биологија- Скопје. Стр.102.
5. Узуноски, М., 1985, Производство на тутун. Стопански весник. стр.107-110. Скопје

EFFECTS OF DIFFERENT PLANTING SYSTEMS ON SOME PRODUCTIVE AND QUALITY TRAITS OF RICE

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Abstract
The traditional planting system of rice in the Republic of Macedonia is wet direct seeding (hand broadcasting of seeds into pre-standing water in the fields). In this paper, the effects of different planting methods of rice (wet seeding as well as dry seeding by using seed drills) on productivity and quality of rice are presented. Field trials were set up at the Experimental Station of the Institute of Agriculture – Skopje, locality Mishjak, Kocani region, with two varieties (San Andrea and Onice) included. The following planting systems were explored: 1. wet direct seeding, with seeding rate of 250 kg seeds per ha (control); 2. dry seeding, 150 kg seeds per ha; 3. dry seeding, 200 kg seeds per ha. During the harvesting, the number of tillers per m^2, biological yield, paddy rice yield, head rice yield and white rice yield were analyzed. The highest number of tillers per m^2 (San Andrea 383.00 and Onice 731.33) and the highest average paddy yield (San Andrea 11,780.00 kg ha^{-1} and Onice 10,113.33 kg ha^{-1}) were achieved in the control. The highest biological yield in Onice (21,493.33 kg ha^{-1}) was found in the control, while in San Andrea (20,650.00 kg ha^{-1}) in the treatment dry seeding, 150 kg seeds per ha. For the head rice yield, the highest percentage was determined in the control (San Andrea, 54.57%) and in the treatment dry seeding, 150 kg seeds per ha (Onice, 66.72 %). Both varieties (San Andrea with 6,428.35 kg ha^{-1} and Onice with 6,143.83 kg ha^{-1}) reached the highest white rice yield in the control. In general, in the treatment dry seeding with 200 kg seeds per ha, the higher values of number of tillers per m^2, paddy yield and white rice yield were assessed, compared to the treatment dry seeding with 150 kg seeds per ha, in both varieties.

Keywords: variety, seeding rate, tillers, yield, paddy rice, head rice yield.

Introduction

The rice production region in Republic of Macedonia is located along the Bregalnica river, mainly in the eastern part of the country, with 90 % of the area located in the territory of Kochani Valley. Average production area of 4,926.36 ha, average paddy yield of 4,692.67 kg ha^{-1} and 23,145.12 t average yearly production of paddy for the 1939- 2014 period have been reported (Andreevska and Andov 2015). The area under rice varies depending on the quantity of accumulated water in the irrigation system used for rice production. The rice crop is grown in flooded paddy fields with constant water bed during the whole vegetation, from seeding to a month before harvest, when the water supply is suspended in order to drain and prepare the rice fields for harvest. During vegetation, the fields are drained only on specific occasions, such as during herbicide application and fertilization. So far, the standard (traditional) seeding method in our country is wet seeding by broadcast (hand dispersal in water bed with seed presoaked in water for 24 hours). This production operation is tedious because it is performed in water and needs large labor force, thus increasing the production costs. The broadcast is often done with poor quality, because of the lack of trained workers. This results in variable and inadequate crop density, leading to low yields. The aim of this study was to examine the possibility of mechanized seeding of the rice crop in dry conditions with a seeding machine. Two sowing rates (seeding material per area) were studied- 150 kg/ha and 200 kg/ha, compared to the traditional seeding way (broadcast in water bed) in two introduced Italian rice varieties (*San Andrea* and *Onice*.).

Material and methods

The field trial was set up in the experimental station of the Institute of Agriculture Skopje (Rice Research Unit in Kochani), in Mishjak area in Kochani. The following three variants were studied in two rice varieties (*San Andrea* and *Onice*) and two sowing rates:

1. mechanical seeding in dry conditions, sowing rate 150 kg ha^{-1};
2. mechanical seeding in dry conditions, sowing rate 200 kg ha^{-1} and
3. traditional seeding method, sowing rate 250 kg ha^{-1} (control).

Kochani Valley, where the trial site is located, belongs to the temperate continental-sub-mediterranean region of the Republic of Macedonia (Filipovski et al. 1996).

In Table 1, the chemical characteristics of the soils from the studied localities are presented. According to some previous surveys (Petkovski et al. 1997), the soil type in the locality where the field trial was set up is alluvial. The pH of the soil is slight acid in water solution (Filipovski, 1984) while in N KCl solution is acid (Ubavić et al. 2001). According to the used Al method, the easily available Phosphorus was at high level, while the easily available Potassium is at medium level (Džamić et al.1996).

Table 1. Chemical characteristics of the soil from the trial site

Depth (cm)	$CaCO_3$ %	pH		Easily available Phosphorus and Potassium (mg/100 g soil)	
		H_2O	N KCl	P_2O_5	K_2O
0 – 30	-	5.6	4.7	18.87	19.80

At the end of vegetation, during harvest, average samples (rice sheaf from 1 m^2 crop area) were taken in each replication, on the basis of which the number of productive tillers/ m^2, biological yield (straw + paddy), and paddy rice yield were determined. The hygroscopic moisture of paddy rice (in time of harvest) and the milling fractions were determined under the laboratory conditions. Milling fractions (head rice yield, brokens, total milled rice, chalky grains, rice bran and hull) were assessed by milling average sample of 100 g paddy of each replication on laboratory milling machine during 1.40 min. The white rice yield was determined on the basis of the obtained paddy rice and head rice yield data. The average value for each sub-variant was calculated from the data of the replications. Results were analyzed by two-way ANOVA and LSD test at 0.05 and 0.01 probability level.

Results and discussion

The results for the number of productive tillers m^{-2} are shown in Table 2. There was significant difference in number of tillers between the three seeding variants in both varieties. The highest average number of productive tillers was achieved in the control-traditional seeding method (*San Andrea*-383.00*, Onice*-731.33). This is understandable as the control had the highest seeding rate of 250 kg ha^{-1}. In the variants with mechanical seeding, the results were lower. Accordingly, in variant No. 1 where the lowest rate was applied (150 kg ha^{-1}) the lowest number of productive tillers m^{-2} was produced (*San Andrea*-270.33, *Onice*-395.50). Aside from the seeding method, the results significantly differed on varietal level, as *Onice* produced significantly higher number of tillers compared to *San Andrea*. The highest average biological yield in *Onice* (straw + paddy) was achieved in the control (21,493.33 kg ha^{-1}, Table 3), while the lowest in variant No. 1 (17,225.00 kg ha^{-1}). Statistically significant differences were found between variant No. 1 and the control ($P \leq 0.01$) and variant No. 1 and variant No. 2 ($P \leq 0.05$). The highest average biological yield in *San Andrea* was determined in variant No 1. (20,650.00 kg ha^{-1}**)**, while the lowest in variant No. 2 (18,545.00 kg ha^{-1}**)**. Significant difference was found only between variant No.1 and No. 2 ($P \leq 0.05$).

Table 2. Number of productive tillers m^{-2}

Variant	Variety		Average
	San Andrea	*Onice*	
No. 1 (150 kg ha^{-1})	270.33	395.50	332.92
No. 2 (200 kg ha^{-1})	329.00	463.17	396.09
Control	383.00	731.33	557.17
Average	327.44	530.00	428.72

Statistical analysis

Source	F test sign.	LSD test- pairwise comparisons on varietal level		*San Andrea*	*Onice*
Seeding	P $\leq$0.01	44.97 ($LSD_{0.05}$)	Var. No. 1 Control Var. No. 2	-112.67 ** -58.67*	- 335.83** -67.67**
Variety	P $\leq$0.01		Var. No. 2 Control Var. No. 1	-54.00* 58.67*	- 268.16** 67.67**
Seeding x variety	P $\leq$0.01	63.96 ($LSD_{0.01}$)	Control Var. No. 1 Var. No. 2	112.67** 54.00*	335.83** 268.16**
		* significant difference at 0.05 level of probability ** sign. difference at 0.01 level of probability			

Table 3. Biological yield (kg ha^{-1})

Variant	Variety		Average
	San Andrea	*Onice*	
No. 1 (150 kg ha^{-1})	20,650.00	17,225.00	18,937.5
No. 2 (200 kg ha^{-1})	18,545.00	19,805.00	19,175.00
Control	20,300.00	21,493.33	20,300.00
Average	19,831.67	18,515.00	19,173.33

Statistical analysis

Source	F test sign.	LSD test- pairwise comparisons on varietal level		*San Andrea*	*Onice*
Seeding	P $\leq$0.05	2,040.49 ($LSD_{0.05}$)	Var. No. 1 Control Var. No. 2	350.00 ns 2,105.00*	- 4,268.33** - 2,580.00 *
Variety	P >0.05 (n.s.)		Var. No. 2 Control Var. No. 1	-1,755.00 ns -2,105.00*	- 1,688.33 ns 2,580.00*
Seeding x variety	P $\leq$0.01	2,902.30 ($LSD_{0.01}$)	Control Var. No. 1 Var. No. 2	- 350,00 ns 1,755.00 ns	4,268.33** 1,688.33 ns
		* significant difference at 0.05 level of probability; ** significant difference at 0.01 level of probability; ns- not significant;			

The results for the paddy rice yield at 14 % grain moisture are presented in Table 4. Significant difference in results on both seeding and varietal level were obtained. The highest average was obtained in the control (traditional seeding way)- 10,946.67 kg ha^{-1}, (*San Andrea*- 11,780.00 kg ha^{-1}, *Onice*-10,113.33 kg ha^{-1}). The control had the highest seeding rate of 250 kg ha^{-1} and the highest crop density. In variant No. 2- mechanical seeding with 200 kg ha^{-1} rate the average yield dropped for 1,526.67 kg ha^{-1}. In variant No. 1, lowering the rate to 150 kg ha^{-1} resulted in additional yield drop of 375.00 kg ha^{-1} and a total of 1,901.67 kg ha^{-1} compared to the control. This variant had the lowest yield- 9,045.00 kg ha^{-1} average (*San Andrea*-9,385.00 kg ha^{-1}, *Onice*-8,705.00 kg ha^{-1}). Significant difference was found between the control and variant No.1 (P $\leq$ 0.05). On varietal level, *San Andrea* gave higher average paddy yield (10,313.33 kg ha^{-1}) compared to *Onice* (9,301.11 kg ha^{-1}) at 0.05 level of probability (P $\leq$0.05).

Table 4. Paddy rice yield at 14 % grain moisture (kg ha^{-1})

Variant	Variety		Average
	San Andrea	*Onice*	
No. 1 (150 kg ha^{-1})	9,385.00	8,705.00	9,045.00
No. 2 (200 kg ha^{-1})	9,755.00	9,085.00	9,420.00
Control	11,780.00	10,113.33	10,946.67
Average	10,313.33	9,301.11	9,807.22

Statistical analysis

Source	F test sign.	LSD test- pairwise comparisons		
Seeding	$P \leq 0.01$	1,608.76 ($LSD_{0.05}$) 2,288.22 ($LSD_{0.01}$)	Var. No. 1 Control Var. No. 2	-1,901,67 * -375.00
Variety	$P \leq 0.05$		Var. No. 2 Control Var. No. 1	-1,526,67 375.00
Seeding x variety	P>0.05 n.s.		Control Var. No. 1 Var. No. 2	1,901,67* 1,526.67
		* significant difference at 0.05 level of probability; ** sign. difference at 0.01 level of probability; ns- non significant;		

The results for the milling fractions, including the head rice yield are shown in Table 5 and Table 6. In *Onice,* the highest head rice yield was obtained in variant No. 1 (66.72%). Similar results were found in variant No. 2 (66.67 %). Both variants with mechanical seeding resulted in statistically higher head rice yield compared to the control ($P \leq 0.01$). The lowest value was determined in the standard seeding variant (the control)- 60.75 %. In *San Andrea*, the mechanical seeding resulted in lower head rice yield compared to the control, where the highest value was obtained (54.57 %). The lowest head rice yield was found in variant No. 1 (47.87 %). Significant differences were obtained between the control and variant No. 1 ($P \leq 0.01$) and variant No. 1 and variant No. 2 ($P \leq 0.05$). *Onice* variety showed higher head rice yield (64.71 % average) compared to San *Andrea* (51.43%), regardless of the seeding technique, with significant difference at both level of probability (0.05 and 0.01). The seeding significantly affected the white rice yield. The highest white rice yield was determined in the control (6,286.09 kg ha^{-1} average, *San Andrea*- 6428.35 kg ha^{-1} and *Onice*- 6,143.83 kg ha^{-1}). The seeding variant No. 1 and No.2 where lower seeding rate was applied produced lower white rice yield. The lowest yield was obtained in variant No.1 (5,150.29 kg ha^{-1} average, *San Andrea*-4,492.60 kg ha^{-1}*, Onice*-5,807.98 kg ha^{-1}, Table 7). Significant differences were found between the control and variant No. 1 ($P \leq 0.05$). On varietal level, *Onice* variety produced significantly higher ($P \leq 0.05$) white rice yield (6,002.93 kg ha^{-1}) compared to *San Andrea* (5,326.31 kg ha^{-1}). In the rice producing conditions of Republic of Macedonia, the effect of sowing method was previously studied by Andov et al. (2011) on variety *San Andrea*. During 2009 and 2010, three sowing methods were investigated: manual seed broadcasting onto watered soil (standard method of sowing), manual seed broadcasting onto dry soil surface and sowing with seed drills on dry soil. The highest average number of tillers m^{-2} (428), the highest average biological yield (16,860.00 kg ha^{-1}), and paddy rice yield (7,612.00 kg ha^{-1}) were obtained when sowing by seeding machine on dry soil. According to Basnayake et al. 2006, the increase in seeding rate from 50 kg ha^{-1} to 200 kg ha^{-1} in conditions when the rice crop was affected by weed increased the grain yield in both broadcast and row seeded rice for 26% and 29% respectively. The reason for the increase in yield was the increased plant density, which resulted in decline in weed competition thereby contributing to the higher grain yield. Similar results were reported by Ahmed at al. 2014, who studied the effect of seeding rates (20, 40, 60, 80 and 100 kg ha^{-1}) and weed infestation (partially-weedy and weed-free conditions) in dry seeded rice. Under weed-free conditions, higher crop yields were obtained at the seeding rate of 40 kg ha^{-1} and thereafter, yield decreased slightly beyond 40 kg seed ha^{-1}. Under partially-weedy conditions the increase in seeding rate from 20 to 100 kg/ha resulted in increased yield by 30 to 33%, while weed biomass decreased by 41–60% at 35 days after sowing and 54–56% at crop anthesis. The authors concluded that increasing seeding rates in dry seeded rice can suppress weed growth and reduce grain yield losses from weed competition.

Table 5. Milling fractions (whole grains, broken grains, total milled rice, chalky grains, bran and hulls) obtained in the studied seeding variants and varieties [%]

Variety	Whole grains %	Broken grains %	Total milled rice %	chalky grains %	Bran %	Hulls %
150 kg ha^{-1}						
San Andrea	47.87	15.30	63.17	0.33	10.70	25.80
Onice	66.72	4.00	70.72	0.98	6.57	21.73
200 kg ha^{-1}						
San Andrea	51.85	12.58	64.43	0.27	10.50	24.80
Onice	66,67	3.90	70.57	1,04	6.57	21.82
Control						
San Andrea	54.57	10.80	65.37	0,63	10.50	23.50
Onice	60.75	10.00	70.75	7.00	0.35	21.90

Table 6. The effect of the method and the seeding rate on head rice yield in the examined rice varieties [%]

Variant	Variety		Average
	San Andrea	*Onice*	
No. 1 (150 kg ha^{-1})	47.87	66.72	**57.30**
No. 2 (200 kg ha^{-1})	51.85	66.67	**59.26**
Control	54.57	60.75	**57.66**
Average	**51.43**	**64.71**	**58.07**

Statistical analysis

Source	F test sign.	LSD test- pairwise comparisons on varietal level		*San Andrea*	*Onice*
Seeding	$P > 0.05$ ns	3.41 ($LSD_{0.05}$)	Var. No. 1 Control	-6.70 **	5.97**
			Var. No. 2	-3.98*	0.05ns
Variety	$P \leq 0.01$		Var. No. 2 Control	-2.72 ns	5.92**
		4.86 ($LSD_{0.01}$)	Var. No. 1	3.98*	-0.05 ns
Seeding x variety	$P \leq 0.01$		Control Var. No. 1	6.70**	-5.97**
			Var. No. 2	2.72 ns	-5.92**
		* significant difference at 0.05 level of probability; ** sign. difference at 0.01 level of probability; ns- non significant;			

Table 7. White rice yield (kg ha^{-1})

Variant	Variety		Average
	San Andrea	*Onice*	
No. 1 (150 kg ha^{-1})	4,492.60	5,807.98	5,150.29
No. 2 (200 kg ha^{-1})	5,057.97	6,056.97	5,557.47
Control	6,428.35	6,143.83	6,286.09
Average	5,326.31	6,002.93	5,664.62

Statistical analysis

Source	F test sign.	LSD test- pairwise comparisons		
Seeding	$P \leq 0.05$	985.98 ($LSD_{0.05}$)	Var. No. 1 Control	-1135.80 *
			Var. No. 2	- 407.18 ns
Variety	$P \leq 0.05$		Var. No. 2 Control	-728.62 ns
		1402.41 ($LSD_{0.01}$)	Var. No. 1	407.18 ns
Seeding x variety	$P > 0.05$ not sign.		Control Var. No. 1	1135.80*
			Var. No. 2	728.62 ns
		* significant difference at 0.05 level of probability; ** sign. difference at 0.01 level of probability; ns- non significant;		

Conclusions

The seeding methods significantly affected the examined productive parameters in rice. The control, where the standard seeding method was applied (hand broadcasting in water bed and highest seeding rate of 250 kg ha^{-1}) resulted in the highest crop density, the highest paddy rice yield and the highest white rice yield. The lowest values were obtained in the mechanical seeding variant No.1, where the lowest seeding rate of 150 kg ha^{-1} was applied. On the basis of the obtained results it may be argued that the difference in results stem from the different seeding rate, rather than the method used for seeding (mechanical dry seeding versus traditional seeding in water bed).

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References

1. Ahmed, S., Salim, M., and Chauhan, B. S. (2014). Effect of weed management and seed rate on crop growth under direct dry seeded rice systems in Bangladesh. PLoS ONE, 9 (7): e101919. http://doi.org/10.1371/journal.pone.0101919
2. Andov, D., Andreevska, D., Simeonovska, E. and Jankulovski, Lj. (2011). Vlijanie na načinot na seidba kaj orizot (*Oryza sativa L.*) vrz prinosot i potrošuvačkata na voda. Fakultet za zemjodelski nauki i hrana- Skopje, 56 (7):15.
3. Andreevska, D. and Andov, D. (2015). Značenje na orizot vo zemjodelskoto proizvodstvo na R. Makedonija. In Dimitrovski, R., Andreevska, D., Andov, D., Atanasova, I., Ajtovska, Lj., Petrov – Fafa, V. and Ilieva, V. (eds), Kočanskiot oriz. Kochani: Opština Kočani, 33- 36.
4. Basnayake, J., Fukai, S., Sipaseuth, Schiller, J.M. and Monthathip C. (2006). Advances in agronomic research in the lowland rice environments of Laos. In Schiller, J.M., Chanphengsay, M.B., Linquist, B. and Appa Rao, S. (eds), Rice in Laos. Los Baños, Philippines: International Rice Research Institute, 349–369.
5. Džamić, R., Stevanović, D. and Jakovljević, M. (1996). Praktikum iz agrohemije. 162 pp. Beograd.
6. Filipovski, G. (1984). Pedologija. Treto izdanie. 599 pp. Univerzitet „Sv. Kiril i Metodij“. Skopje.
7. Petkovski D., Mukaetov, D. and Andreevski, M. (1997). Sodrzina na nekoi lesnorastvorlivi teski metali vo aluvijalnite pocvi od Kocansko Pole. Makedonska zemjodelska revija, 44 (1-2): 1- 5.
8. State Statistical Office, Republic of Macedonia. Statistical Yearbooks of the Republic of Macedonia (2011-2014).
9. Ubavić, M., Kastori, R., Oljača, R. and Marković, M. (2001). Ishrana voćaka. 150 p. Banjaluka.

INFLUENCE OF HARVESTING METHODS ON YIELD AND QUALITY OF RICE IN THE REPUBLIC OF MACEDONIA

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Abstract
In this study, the influence of two different paddy harvesting methods (manual harvesting and combine harvesting) on paddy yield, head rice yield and white rice yield was investigated. Field trials were set up in three different locations in the rice producing regions Kocani, Cesinovo-Oblesevo and Stip under the standard production technology, adopted in the entire Macedonian rice production. Two Italian introduced varieties (San Andrea and Onice) were included in the trials. Samples were taken from the treatments with different methods of harvesting (manual and combine harvesting) from which the paddy rice yield data were collected directly. In order to determine the head rice yield, laboratory milling of paddy grains was performed on a paddy quality testing machine (three average samples of 100 g per treatment, duration of milling 1,40 min.). On the base of the data for paddy rice yield and head rice yield, the white rice yield was estimated. According to the results obtained, the highest paddy rice yield (11,925.67 kg ha^{-1}) as well as the highest white rice yield (6,710.57 kg ha^{-1}) were achieved in the treatment with manual harvesting in Kocani region (San Andrea variety). The highest head rice yield (65.80 %) was determined in the treatment with manual harvesting in Stip region (Onice variety), while the lowest head rice yield (43.90 %) was in the treatment with combine harvesting of the variety Onice in Kocani region. In general, the method of manual harvesting resulted in higher values of paddy yield, head rice yield and white rice yield compared to the method of mechanized, combine harvesting in all the examined treatments (two varieties, three locations).

Keywords: varieties, paddy rice, white rice, head rice yield.

Introduction

The implemented methods and time of harvesting are some of the conditions influencing the crop yield and grain quality. During the rice harvesting, inappropriate procedures might cause the loss of the paddy rice yield, followed by the grain quality deterioration. Unlike the other cereals (where the milling process transforms the grains into flour), milling paddy rice results in obtaining whole white grains. Therefore, any mechanical damages of the paddy grain, potentially caused by the different parts of the combine harvester are unwanted. But, the use of inadequate harvesting machinery certainly results in mechanical damages of the paddy grain, such as breakages, cracks, excessive peeling etc. The damaged paddy grains are problematic for storage, since they are highly prone to quality deterioration. The outcome of the post-harvest procedures (milling) on damaged paddy grains is always lower head rice yield and consequently lower yield of white, milled rice per unit of harvested area. Moreover, as the result of milling low quality paddy rice, the grain discoloration often appear – non-typical color for the variety, such as black to brown grains, reddish, brown, yellowish, amber etc. Also, the grain spoilage from fungal growth or molds might happen. The rice harvesting in the Republic of Macedonia is mostly performed by using combine harvesters that are also used for harvesting other small cereals (wheat, barley). Actually, very few of the used combine harvesters in our country are designed for rice only. The most of the harvesters that are in use in the

Republic of Macedonia are very old and produced by the "Zmaj "company. The adjustment of the combines' working parts of the specialized rice harvesters and especially adaptation of the universal harvesters from one to another mode of operation (depending of the crop – rice or other cereal) are very often irregularly done and followed by mistakes. All this can cause grain damage and quality deterioration of the paddy rice. The milling fractions including the head rice yield and white rice yield of different rice varieties grown in the environmental conditions of Republic of Macedonia were previously studied by Andreevska et al., 2015; 2014; Andov et al., 2014 and Ilieva et al., 2009. The aim of this study was to determine the influence of the harvesting methods on yield and quality of rice in three rice producing regions in the Republic of Macedonia.

Material and methods

The study was carried out by setting up field macro-trials in three rice producing regions (Sredorek-Kocani, Ularci-Cesinovo-Oblesevo and Tarinci-Stip). The standard production technology was applied. Two varieties were included in the investigation – San Andrea, the predominant rice variety in the Macedonian rice production and Onice, Italian introduced variety. The paddy yield was assessed on two types of samples – the first was taken out from manually harvested rice and the second – sampled from combine harvested rice bulk. The head rice yield was determined by laboratory milling of paddy grains on a paddy quality testing machine (three average samples of 100 g per treatment, duration of milling 1,40 min.). The white rice yield was estimated on the base of the paddy yield and head rice yield. The obtained data were statistically performed (ANOVA) and differences tested with LSD test (probability levels 0.05 and 0.01). In Table 1, the chemical characteristics of the soils from the studied localities are presented. According to some previous investigations (Petkovski et al., 1997), the soil type in Sredorek-Kocani locality is alluvial. The pH of the soil is slight acid in water solution (Filipovski, 1984) while in N KCl solution is acid (Ubavić et al. 2001). According to the used Al method, the easily available Phosphorus was at high level, while the easily available Potassium is at medium level (Džamić et al.1996). Regarding the soil of the locality Ularci-Cesinovo-Oblesevo, the pH in water solution is slightly alkaline while pH in nKCl is neutral and the soil is slightly carbonated (according to American classification). The content of the easily available Phosphorus is at very high level and the content of the easily available Potassium is at high level. In the locality Tarinci-Stip, the soil pH in water solution is slightly alkaline, while in nKCl it is alkaline. The content of the easily available Phosphorus is at low level. The content of the easily available Potassium is at optimal level.

Table 1. Chemical characteristics of the soils from the investigated localities

Depth (cm)	$CaCO_3$ %	pH		Easily available Phosphorus and Potassium (mg/100 g soil)	
		H_2O	N KCl	P_2O_5	K_2O
Locality Sredorek-Kocani					
0 – 30	-	5.6	4.7	18.87	19.80
Locality Ularci-Cesinovo-Oblesevo					
0 – 30	1.07	7.6	6.6	25.58	34.97
Locality Tarinci-Stip					
0 – 30	5.78	8.0	7.30	6.62	25.75

Results and discussion

The results for the paddy rice yield obtained by manual harvesting as well as by combine harvesting are presented in Table 2. The highest average paddy rice yield was achieved in the locality Sredorek-Kocani through manual harvesting for both varieties (*San Andrea*- 11,925.67 kg ha^{-1}, *Onice*-11,223.33 kg ha^{-1}), while the lowest in the locality Tarinci-Stip through combine harvesting (*San Andrea*-3,100.00 kg ha^{-1}, *Onice*-4,900.00 kg ha^{-1}). For the variety San Andrea, the differences between the mean values of paddy rice yield in two treatments (manual and combine harvesting) were significant. In the variety Onice, there was significant difference between the means of paddy rice yield in two treatments (manual and combine harvesting) in the localities Sredorek-Kocani and Tarinci –Stip for both levels of probability, while in the locality Ularci-Cesinovo-Oblesevo the significance was at 0.05 level of probability.

Table 2. Paddy rice yield [kg ha^{-1}]

San Andrea			
	Harvesting method		
Locality:	Manual harvesting	Combine harvesting	Average
Sredorek-Kocani	11,925.67	7,916.52	9,921.10
Tarinci-Stip	5,373.33	3,100.00	4,236.67
Ularci-Cesinovo-Oblesevo	9,416.67	6,834.00	8,125.33
Tarinci-Stip	8,905.22	5,950.17	
Onice			
	Harvesting method		
Locality:	Manual harvesting	Combine harvesting	Average
Sredorek-Kocani	11,223.33	5,230.70	8,227.02
Tarinci-Stip	7,256.67	4,900.00	6,078.33
Ularci-Cesinovo-Oblesevo	10,806.67	9,484.80	10,145.73
Average	9,762.22	6,538.50	
LSD 0,05	1,098.94		
LSD 0,01	1,493.29		

Table 3. Head rice yield [%]

San Andrea			
	Harvesting method		
Locality:	Manual harvesting	Combine harvesting	Average
Sredorek-Kocani	56.27	51.69	53.98
Tarinci-Stip	64.57	56.00	60.28
Ularci-Cesinovo-Oblesevo	59.67	52.90	56.28
Average	60.17	53.53	
Onice			
	Harvesting method		
Locality:	Manual harvesting	Combine harvesting	Manual harvesting
Sredorek-Kocani	57.60	43.90	50.75
Tarinci-Stip	65.80	57.00	61.40
Ularci-Cesinovo-Oblesevo	61.57	46.80	54.18
Average	61.66	49.23	
LSD 0,05	3.25		
LSD 0,01	4.42		

The results presented in Table 3. show the highest average head rice yield that was achieved through manual harvesting in the locality Tarinci-Stip in both varieties (*San Andrea*- 64.57% and *Onice*- 65.80%), while the lowest head rice yield was obtained through combine harvesting in the

locality Sredorek-Kocani (*San Andrea*- 51.69% and *Onice*- 43.90%). Significant differences (for both levels of probability) were found between head rice yield's mean values of the two treatments (manual harvesting and combine harvesting), for both varieties, in all three localities in this study.
In Table 3, the results for the white rice yield, obtained through different methods of harvesting are presented, for the two varieties in three localities. There it is stated that the highest white rice yield was estimated for the treatment with manual harvesting, in the localities Sredorek-Kocani (San Andrea variety, 6,704.13 kg ha^{-1}) and Ularci-Cesinovo-Oblesevo (Onice variety, 6,634.23 kg ha^{-1}). The lowest white rice yield was gained for the treatment with combine harvesting in the locality Tarinci-Stip (*San Andrea*- 1,734.60 kg ha^{-1}) and in the locality Sredorek-Kocani (*Onice*- 2,296.85 kg ha^{-1}). For both the investigated varieties San Andrea and Onice, significant differences between the means of the white rice yield were found, for both levels of probability, in all three localities.

Table 4. White rice yield [kg ha^{-1}]

San Andrea			
	Harvesting method		
Locality:	Manual harvesting	Manual harvesting	Average
Sredorek-Kocani	6,704.13	4,092.61	5,398.37
Tarinci-Stip	3,471.81	1,734.60	2,603.21
Ularci-Cesinovo-Oblesevo	5,606.17	3,615.98	4,611.07
Average	5,260.70	3,147.73	
Onice			
	Harvesting method		
Locality:	Manual harvesting	Manual harvesting	Average
Sredorek-Kocani	6,477.66	2,296.85	4,387.26
Tarinci-Stip	4,771.12	2,793.77	3,782.44
Ularci-Cesinovo-Oblesevo	6,634.23	4,438.10	5,536.16
Average	5,961.00	3,176.24	
LSD 0,05	583.59		
LSD 0,01	793.21		

Conclusions

On the base of the results of the study on different methods of harvesting (manual and combine), conducted in three regions on two rice varieties (San Andrea and Onice), the following conclusions on their influence to rice yield and quality could be done:
The highest paddy rice yield (11,925.67 kg ha^{-1}) as well as white rice yield (6,710.57 kg ha^{-1}) were achieved in Sredorek-Kocani region in the variety San Andrea, by using manual harvesting.
The highest head rice yield (percent of whole grains of white rice) of 65.80% was gained in the variety Onice, locality Tarinci-Stip by using manual harvesting. The same variety had the lowest head rice yield of 43.90% in the locality Sredorek-Kocani, when combine harvesting was applied.
In both examined varieties and in three regions, significantly better results for paddy yield, head rice yield and white rice yield were achieved when manual harvesting was applied, compared to combine harvesting.
Mechanized harvesting might have negative effects on yield and quality of harvested rice in case of inappropriate use of the equipment. Therefore, serious attention has to be paid to the right choice of proper combine harvesters and especially to careful adjustments of their working parts for different modes of operation.

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References

1. Danica Andreevska**,** Dobre Andov, Emilija Simeonovska (2014). Dressing percentage and yield of white rice in *Bianca* and *Galileo* – two newly introduced rice varieties (*Oryza sativa* L.), grown under agro-ecological conditions of Macedonia. 4th Congress of Ecologists of the Republic of Macedonia with international participation.Ohrid, 12-15 October 2012. Book of Proceedings p.212-217.
2. Dobre Andov, Danica Andreevska, Emilija Simeonovska (2014). Yield of white rice and byproducts of rice milling in some newly introduced Italian rice varieties grown under agro-ecological conditions of Macedonia 4th Congress of Ecologists of the Republic of Macedonia with international participation.Ohrid, 12-15 October 2012. Book of Proceedings p.218-223.
3. Danica Andreevska**,** Dobre Andov, Mirjana Menkovska, Emilija Simeonovska, Trajče Dimitrovski (2015): Head rice yield of some domestic and foreign rice varieties (*Oryza sativa* L.) in the Republic of Macedonia. Macedonian Journal of Animal Science, Vol. 5, No. 2, pp. 89–94. On line ISSN 1857 – 7709.
4. Džamić Ružica, Stevanović D., Jakovljević M. (1996). Praktikum iz agrohemije. 1-162. Beograd.
5. Filipovski, Ğ. (1984). Pedologija. Treto izdanie. 599 pp. Univerzitet „Sv. Kiril i Metodij". Skopje.
6. Ilieva Verica, Andreevska Danica, Andov D., Markova Natalija, Jankulovska Mirjana (2009). Dressing percentage of white rice in corelation with the harvest time of the rice. Yearbook of the Faculty of Agricultural sciences and food, Skopje, vol. 54, 19-27.
7. Petkovski D., Mukaetov D., Andreevski M. (1997). Sodrzina na nekoi lesnorastvorlivi teski metali vo aluvijalnite pocvi od Kocansko Pole. Makedonska zemjodelska revija, 44 (1-2), 1- 5.
8. Ubavić M., Kastori R., Oljača R., Marković M. (2001). Ishrana voćaka. 1-150. Banjaluka.

CORRELATION AND PATH COEFFICIENT ANALYSIS FOR SOME EAR YIELD RELATED TRAITS IN POPCORN (*ZEA MAYS* VAR. EVERTA)

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Abstract
The aim of this study is firstly to determine the suitable observations in popcorn breeding researches. And secondly aim is to identify all the possible interaction between grain yield and yield component in popcorn with correlation and path coefficient analysis. The experiments were designed in a randomized block design with 3 replicates. These experiments were conducted in Samsun, Turkey in 2014 and 2015. The results unveiled a significant and positive correlation between grain yield and the all component. The greatest positive effect on grain yield are yield per ear, grain yield per ear and grain moisture according to the path analysis, respectively. The presented results have demonstrated the potential of privileged of observation yield per ear, grain yield per ear and grain moisture thus increasing yield in popcorn.

Keywords: Popcorn, correlation, path analysis, yield, yield component.

Introduction
Corn (*Zea mays* L.) is one of the important cereal crops in the world after wheat and rice. Corn has become an indispensable product in cereals due to its use in human and animal feeding and industrial use. Popcorn (*Zea mays* everta) can be easily distinguished by plant and seed characteristics in other varieties groups. Corn is a product that can be preferred in terms of nutrition due to its rich nutritional content, vitamins and minerals it contains. Also, corn is a good dietary product with stomach acidic absorption properties, low calorie and whole grain corn intake by reducing the feeling of hunger and a good body weight control can be a good choice, unlike snack foods corn has low calorie and fat ratio, high carbohydrate ratio due to it has a special place among corn variety groups (Hansen, 2012; Ozkan, 2007; Ulger, 1998; Lilburn et al. 1994). The ability of the corn to explode and be used as a snack is the most important feature that distinguishes itself from other corn types. The production area and amount are increased in order to supply the increasing need of popcorn. Because popular culture behaviours increase the cinema and shopping mall in Turkey. Popcorn agriculture is made with contract in Turkey, as similar in the world. The popcorn cultivation area is approximately 10 thousand ha in Turkey. In recent years, corn cultivation area located in Elbistan, Cukurova (Adana-Mersin), Aydın, Denizli, Kayseri, Konya, Karaman and Kırşehir provinces (Ozturk, 2017). There are a few popcorn varieties registered and produced in Turkey. The varieties has a low yield potentials. Popcorn has an economic potential for farmers with the high yield varieties because of popcorn contracted production in Turkey. Studies are continued to develop high yields hybrid varieties resistant to disease and damages, which can be adapted to various ecological conditions in the modern corn breeding. Grain yield, which is the most interesting feature of plant breeding research, is a highly complex feature, which is the result of mutual interactions of the physiological and morphological characters in successive different phenological periods during vegetation of plants. The breeding of high yield genotypes depends on knowing how these characters are affecting yield and on identifying the causes of variation in grain yield for a given environment (Ozturk et al. 1999). It is not always possible to determine the interaction

between grain yield and yield factors in corn breeding studies only according to the correlation analysis. Since the interaction between any two variables may sometimes depend on a third variable, it may not be sufficient to explain the causal interaction between the yield and the yield components with the correlation coefficient (Okut et al. 1993). Independent of each other change in other one from two properties in a positive or negative way have on impact on yield causes decreasing or increasing changes in other property. There is opportunity determination like these indirect effects with correlation coefficient. For this reason, path coefficient analysis separates to direct and indirect effects correlation coefficient between yield and yield components. It supplies direct and indirect effect each of properties on yield, proportionality. Interaction of characters becomes more understandable and useable consequently more effective selection can occur. Studies conducted is different genotype and environment conditions for high productive of genotypes breeding in corn, response of identification a large number of properties interaction with grain yield, according to path analysis. It was determined that direct and indirect effects of number of grain, grain weight, yield per ear, weight yield per ear, was high and significant (Wright et al. 1934; Steynberg et al.1983; Sade, 1994). Also in many studies it was determined that grain moisture. Selection method is a widely used and successful method in plant breeding. Response to selection depends on many factors such as the interaction of the characters. Plant breeders work with some yield components related to yield in the selection programs. It is very important to determine relative importance of such characters contributing to grain yield directly or indirectly. Correlation and path coefficient analyses may assist to determine certain characters to be used in the improvement of the complex character such as yield. Information about correlative characters in popcorn has been still very limited. The direct and indirect effects of specific yield components might be precisely identified and applied in breeding programs of popcorn by determining of interaction among grain yield and yield components. Aim of this study is to determine yield components through correlation and path coefficient analyses. Thus results might be utilized by the breeders to develop new high yielding popcorn varieties.

Material and methods

The trial was arranged in the randomized completely block design with three replicates. It has 8 popcorn varieties under drop irrigated conditions during the 2014 and the 2015 main growing seasons. The experiment conducted on the location where Karadeniz Agriculture Institute in Carşamba, Samsun (Latitude 41°13' and Longitude 36 °40') is in the north part of Turkey near Black Sea with altitude of 3 m and dominated by the Blacksea climatically conditions. The experimental area has a heavy soil structure with clay-silt soil at 0-20 cm depth and clay-loamy structure at 20-40 cm depth. Standard agronomical practices were applied in both years. Each plot had three rows 5 m length with spacing 70 cm between rows and 18 cm between plants (Anonymous 2010). Two seeds were sowed in each hill and then thinned to one plant to have a final plant density of 71420 plants ha^{-1}. Seed harvest date was month in October. Observations and measurements including seven characters which tasselling, plant height, first ear height, weight per ear, grain weight per ear, grain moisture, grain/ ear ratio. Analysis of variance was performed for each character and LSD (least significant difference) test was applied to compare the differences (Steel and Torrie, 1980). Since genotype x year interaction was not significant for grain/ear ratio, mean values were obtained over two years. Phenotypic correlations were calculated and considering grain yield as a dependent variable, path coefficient analyses were carried out according to the procedures given by Dewey and Lu (1959).

Results and discussion

The analysis of variance revealed significant differences in all 8 quantitative characters and varieties (Table 1). This indicated the existence of sufficient variability among genotypes for all the characters studied except for grain/ear ratio. The chosen parents have diverse with a different genetic background. The hybrid varieties were significant differences for measured traits in the p≤ 0.01 and 0.05 statistical level. Correlation among the traits may be the result of the genetic association among the characters. Interaction is very important which is between grain yield and its component traits for the breeders.

Table 1. Mean values of some agronomic characters of the hybrid popcorn varieties combined over two years

Varieties	Grain yield (kg/da)	Tasseling (day)	Plant length (cm)	First Ear height (cm)	Yield per ear (g)	Grain Yield Per ear (g)	Grain moisture (%)	Grain/ ear ratio (%)
Koçcin (st)	538,4 a	70,6 b	290 ab	111,6 b	132,8 ab	108,4	19,9 b	81,6
TCM 2012-2	521,2 a	71 b	270 b	146,6 a	124,4 b	102,9	21,6 a	82,7
TCM 2012-3	504,7 ab	73,3 a	315,6 a	146,6 a	127,4 b	106,1	20,3 b	83,2
TCM 2012-5	477,6b	69,3 b	275 b	101,6b	108,3 b	88,8	19 b	82,0
TCM 2012-4	437,0 bc	71,3 a	280 b	115 b	92,5 c	77,7	18,7 b	84,0
Antcin (st)	422,6 c	70,0 b	282,5 ab	133,3 a	106,8 b	90,0	21,3 a	84,3
TCM 2012-1	376,8 d	71,5 ab	225 d	118,3b	148,8 a	124,4	21,4 a	83,6
TCM 2012-6	372,9 d	72,0 ab	310 a	127,5 ab	154,2 a	127,5	20,2 b	82,7
VK (0.05)	14,7	2,91	6,21	6,42	5,52	5,87	3,24	7,45
LSD (%5)	50,2	3,54	29,1	22,2	12,8	10,4	2,48	--
Prob.	**	*	**	*	*	*	*	N.S.

Table 2. Correlation coefficients among the traits of eight popcorn varieties and profanities

	Grain weight	Tasseling	Plant length	First Ear height	Yield per ear (g)	Grain Yield Per ear (g)	Grain moisture	Grain/ ear ratio
Grain yield	1,00	0,067	0,272	0,218	0,565*	0,382	0,072	-0,254
Tasseling		1,00	0,013	-0,113	-0,030	0,003	-0,010	0,241
Plant height			1,00	0,724**	0,140	0,066	0,266	-0,336
First Ear height				1,00	0,279	0,229	0,174	-0,130
Ear weight					1,00	0,986**	-0,220	0,322
Ear grain weight						1,00	-0,224	0,473*
Grain moisture							1,00	-0,053
Grain/ ear ratio								1,00

Table 2 indicates that the correlation coefficients among traits. The most important correlation was between weight per ear and grain weight per ear (r = 0.986**). The r values indicated that ear weight and had the highest positive correlation (r=0.565*) with grain yield. Phenotypic and genotypic correlations were worked out on yield and yield contributing characters and are presented in Table 2. Genotypic correlations has a higher value than the corresponding phenotypic values. It was estimated that there was strong inherent interactions between studied characters. Its expression was reduced due to the influence of environment. Plant length was found that positively correlated with first ear height. Hence selection for these characters would improve the yield. Similar results were reported by Sridhar (2016), Natarajet al. (2014), Vijayabharathi et al. (2009), Kumar and Sathyanarayana (2001) Sharma and Kumar (1987), Hua et al . (2004) and Kumar et al. (2007). Grain yield, which is accepted as a major economic character in corn and due to its complex nature depends on all other yield components. Change in anyone of the components may ultimately affect the yields. Hence, these correlated traits have to be analysed for direct and indirect effects over other yield components on the grain yield (Kumar et al., 2011). Therefore, the total correlations were analysed how partitioned in to the direct and the indirect effects. Path analysis were used for grain yield per plant as dependent variable. Path analysis revealed that grain yield has an interaction all independent variables. This analysis also allows separate between direct effect and their indirect effects. This partitioned correlation might help to selection criteria for popcorn breeders. The results are presented as amount and percentage in Table 3, respectively.

Table 3. Direct and indirect effects of different traits on grain yield of popcorn path coefficient (amount)

Traits	Direc effect	Tasseling	Plant height	First Ear height	Yield per ear	Grain yield per ear	Grain moisture	Grain/ ear ratio
Traits	0,184		-0,0005	0,006	0,089	-0,007	-0,002	-0,024
Tasselling	-0,035	0,002		-0,039	0,422	-0,163	0,051	0,033
Plant height	-0,054	-0,020	-0,026		0,841	-0,569	0,033	0,131
First ear height	3,011	-0,005	-0,005	-0,153		-2,445	-0,043	-0,032
Yield per ear	-2,480	0,0005	-0,024	-0,012	2,968		-0,043	-0,047
Grain yield per ear	0,195	-0,001	-0,009	-0,009	-0,66	0,555		0,0054
Grain moisture	-0,101	0,044	0,0120	0,0071	0,968	-1,174	-0,010	
Grain/ ear ratio								

Table 4. Direct and indirect effects of different traits on grain yield of popcorn path percentage (%)

Traits	Direct Effect	Tasseling	Plant height	First Ear height	Yield per ear	Grain yield per ear	Grain moisture	Grain/ ear ratio
Tasselling	58,66		0,14	1,98	28,62	2,24	0,60	7,74
Plant height	4,78	0,313		5,30	56,39	21,76	6,92	4,52
First ear height	3,51	1,33	1,66		53,96	36,49	2,17	0,84
Yield per ear	54,18	0,09	0,009	0,27		43,99	0,77	0,58
Grain yield per ear	44,64	0,00	0,042	0,22	53,42		0,78	0,86
Grain moisture	13,54	0,12	0,66	0,66	46,06	38,56		0,37
Grain/ ear ratio	4,35	1,91	0,51	0,30	41,78	50,66	0,44	

Path analysis revealed that highest positive contribution on grain yield are tasselling (58,7%), weight per ear (54,2%), grain weight per ear (44,6%) and grain moisture (13,5%). In addition that the other traits give low positive direct effect grain yield. Thus these traits could be used more confidently as the selection criteria in the grain yield of popcorn. The present results can be comparable with the results of Sridhar (2016) and (Kumar, et al 2007).

Conclusions

It was revealed that grain yield is positively correlated with ear length, yield per ear, grain moisture, tasselling, plant length, but negative associated with grain/ear ratio. Path coefficient analysis revealed that ear length is the largest positive direct effect on grain yield per plant followed by yield per ear, grain yield per and grain moisture. In conclusion, yield per ear might be used as a selection criteria due to its highly positive direct effect as well as indirect effects on all other characters on grain yield. Also grain yield per ear and ear moisture may be considered as yield component as selection criteria in popcorn breeding. Hence direct selection for these traits might be effective.

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References

1. Bhatt, G. M., 1973. Significance of path coefficient analysis in determining the nature of character association. Euphytica 22: 338-343, 1973.

2. Dewey, D.R., K.H. Lu, 1959. A correlation and path analysis of components of crested wheat grass seed production. Agron . J. 51: 515-518.
3. Dofing, S.M., Croz-Mason, N.D. and Compton, M. A. T. (1991). Inheritance of expansion volume and yield in two popcorn x dent corn crosses. Crop Science. 31, 715-718. 4.
4. Farhatullah. (1990). Correlated response of maize grain yield with yield contributing traits. Sarhad Journal of Agriculture, Pakistan. 6, 445-457. 5.
5. Gravois, K. A., Helms, R. S., 1992. Path analysis of rice yield and yield components as affected by seeding rate, Agron. J., 84, 1-4, 1992.
6. Hansen, R., 2012. Popcorn Profili, a national information resource for value-added agriculture.2012.
7. Hua, T., Yiqin, H., Yas-ajianbing and Yonglian, Z. (2004). Genetic analysis of yield traits with elite maize hybrid - Yuyu 22. Acta Agronomica-Sinica. 30, 922-926. 6.
8. Jibouri, H.A., Miller, P.A. & Robinson, H.F. (1958). Genotypic and environment variances in an upland cotton crops of interspecific origin. Agronomy Journal. 50, 633-634. 2.
9. Joshi, B.K, 2005. Correlation, regression and path coefficient analyses for some yield components in common and Tartary buckwheat in Nepal. Fagopyrum 22: 77-82.
10. Kumar, J., Sundaram, T., Prabu, D.A and Rajan, A.R.R. (2007). Correlation studies in maize evaluated for grain yield and other yield attributes. International Journal of Agricultural Sciences. 3, 57-60. 7.
11. Lilburn, M. S., 1994. Research Note: The Use of Popcorn in Dietsfor Growing, Turkeys. Maize Abstracts, January 1994, Volume: 10, No: 1, p.83.1994.
12. Nguyen, V., Cooper, L., Lowndes, J., Melanson, K., Angelopulos, T.J., Rippe, J.M. and Reimers, K., 2012. Popcorn is more satiating than potato chips in normal-weight adults, Nutrition Journal, 11-71, 1-6.2012.
13. 0kut, H., Orhan, H., 1993. Korelasyon katsayısı ve path analizi, I. Ulusal Ekonometri ve istatistik Sempozyumu, 11 12 Kasım 1993, Ege Un., İzmir, 1993.
14. Özkan A. 2007. Çukurova koşullarında değişik azot dozu uygulamalarının iki cin mısırı (z*ea mays everta* Sturt.) çeşidinde tane verimi, tarımsal özellikler ve bazı kalite özelliklerine etkisi, Ç.Ü Fen Bilimleri Enstitüsü, Doktora Tezi..
15. Öztürk, A., Akten, 1999. Kışlık buğdayda bazı morfolojik karakterler ve tane verimine etkileri, Türk Tarım ve Ormancılık Dergisi, 23 (Ek Say1 2), 409-422, 1999.
16. Öztürk. A., Erdal, Ş., Pamukçu, M., ve Özata, E., 2017. Türkiye’de Özel Mısır Tiplerinin Kullanımı ve Geleceği Intanrantional conference on Agriculture, Forest, Food, Science Tecnologies, 15-17 May 2017. Nevşehir, Turkey
17. Patil, S.J., Hayavadan, P.V., Mahadevappa, M. (1969). Interrelationship between grain yield, ear height and internode characters in Zea mays (L.). The Mysore Journal of Agricultural Sciences. 3, 272-276. 8.
18. Pordesimo, L. O., Anantheswaran, R. C., Fleishmann, A. M., Lin, Y. E., Hanna, M A. (1990). Physical properties as indicators of popping characteristics of microwave popcorn. Journal of Food Science. 55, 1352-1355. 9.
19. Reddy, V. S., Chandra Mohan, Y., Rao, N.V., Krishna, L., (2003). Character association and path analysis in popcorn. (Zea mays var. everta). Crop Research. 25(2), 297-300
20. Sade, B., 1994. Melez mısır çeşitlerinde (Zea mays L. indentata) Tane verimi ve bazı komponentlerinin korelasyonu ve path analizi, Selcuk U. Z. F. Dergisi, 7, 28-39, 1994.
21. Selvaraj, C.I., Nagarajan, P. (2011). Interrelationship and path-coefficient studies for qualitative traits, grain yield and other yield attributes among maize (Zea mays L.). International Journal of Plant Breeding and Genetics. 5(3), 209-223. 11.
22. Sharma, R.K., Kumar, S. (1987). Association analysis for grain yield and some quantitative traits in popcorn. Crop improvement. 14(2), 201-204. 12.

23. Song, A., Eckhoff, S.R., Paulsen, M., Litchfield, J .B., 1991. Effects of kernel size and genotype on popcorn popping volume and number of unpoped kernels. Cereal Chemistry. 68(5), 464-467. 13.
24. Srıdhar M., Krıshna K. Muralı, Sultana Razıa and Bhave M.H.V. 2016. Character assocıatıon and path analysıs for graın and yıeld component traıts ın popcorn. International Journal of Agrıculturel Science and Research ISSN (E):2321-0087 Vol 6 Issue 6 December 2016 p 145-150.
25. Steynberg, R. E., Nel, P. C., Hammer, P. S., 1983. The influence of fertilization on certain yield components of maize in long- term fertilizer experiment, Maize Abstract, 1, 27, 1983.
26. Ülger AC. 1998. Farklı azot dozu ve sıra üzeri mesafelerinin patlak mısırda (*Zea mays everta* Sturt.) tane verimi ve bazı tarımsal özelliklere etkisi; Çukurova Üniversitesi Ziraat Fakültesi Dergisi, 13 (1): 155-164.1998
27. Wright, S., 1934. The method of path coefficients, Ann. Math. Stats., 5, 614-617, 1934
28. Wright, S., 1921. Correlation and Causation. Journal of Agricultural Research. 20, 557-585
29. Vijayabharathi, A., Anandakumar, C.R., Gnanamalar, R.P., 2009. Analysis of correlations and path effects for popping expansion in popcorn. (Zea mays var. everta Sturt.). Electronic journal of plant breeding. 1, 60-64

STATUS AND MEASURE FOR IMPROVE PASTURE CONDITIONS IN THE EASTERN PLANNING REGION OF MACEDONIA

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Abstract
The Eastern Planning Region occupies an area of 3548,7 km^2 or 14,2% of the territory of the Republic of Macedonia. The region has 188.387 ha agricultural land. By that, the grasslands covers 119.504 ha, of which 110.640 ha under pastures and 8.864 ha under meadows, representing a significant source in the production of animal feed, especially in the mountainous areas of the region. On the other hand, on livestock unit comes 2,23 ha pasture area which shows that in this region livestock is poorly developed. As a consequence of this situation which from year to year deteriorates, pastures as a natural resource for providing feed degrade, reducing the quality of grass production and their economic value. In the absence of human factor as a corrector of the specific environmental conditions, hay production is relatively small, ranging from 300-600 kg^{-1}ha^{-1}. Considering the current situation, it is necessary to take certain agro-technical measures, such as introduction of methods of systematic grazing, overseeding, fertilization, weeds protection, etc., butalso introduced a system of organizational measures,as well a certain investments for larger agro and hydro technical operations how this status will be improved and agriculture but particularly livestock production become an important branch in economic development of this part of the country.

Keywords: pastures, yield, protection, overseeding, fertilization.

Introduction
The grasslands (meadows and pastures) are important land areas covered with annual, biennial and multi-annual plant species that are used for production of fodder – one part for hay and the remainder for grazing of the cattle. These areas are permanently or over long years overgrown with plants which are used as food for the domestic animals. According to the recent statistics data 1.264.000 thousand ha i.e. around 50% of the total land of the country is categorized as agricultural land (State Statistical Office of the Republic of Macedonia, 2016). Around 64% of the agricultural land 809.823 ha belongs to the category of 'pastures' (750.359 ha) and 'meadows' (59.464 ha), and the remainder is arable land. In the same period, the areas under grasslandsin the Eastern Planning Region are 119.504 ha – 110.640 ha under pastures, and 8864 ha under meadows (14,7% of the total grasslandsin Macedonia), which emphasizes their importance in fodder production, especially in the mountainous-hilly parts of the region.The reason for such very small productivity, on one side, is the fact that only land areas that are not good or not capable for production of other agricultural crops are left under meadows (such as uneven land, slopes, shallow or rocky ground, flooded areas, etc.); and on the other side very little attention was traditionally paid to the grasslands(with rare exceptions). It is an undeniable fact that the production potential of the grasslandsin the Eastern Planning Region is significantly higher than the current production, but it is also a fact that the grasslands were continuously exploited and never replenished in terms of nutrients, hence the small average yield as a result of the extensive production, minor or zero investments, incorrect exploitation of the greenswards, etc. (Аждерски, 1998). In accordance with the above, in this paper we tried to show data and propose measures that will assist the Eastern Planning Region and the relevant national and regional institutions in the future planning of the development of the forage

production in the region and also help the improvement of the regional and local development in sustainable manner. The economic, social and environmental protection (including protection of the biodiversity) shall be satisfied, by defining the manners in which the biodiversity can be used in a sustainable manner.

Material and methods

The main objective of this research is to provide a clear image, data and informations on the current status of the pastures in the Eastern Planning Region and in those parts of the municipalities that gravitate towards the Bregalnica River basin. The aim is to:

- preserve their territory and increase their value,
- ensure the largest maximum accretion of the grass in accordance with the natural conditions,
- identify the possibility for their renewal, development, measures for cultivation, protection, improvement, etc.

The assessment of the Region in this paper is based on the overall understanding of the advantages and challenges in relation to realization of the following three functions: 1) protection; 2) development and 3) logistical support. The investigations should provide framework for actions and future efficient management, including decisions aimed towards all sectors involved and their potential and challenges regarding protection and improvement of the situation with the pastures as nature resources. This will, in turn, help to advance the animal husbandry in the region and improve the living standard of the local population.

Results and discussion

The Eastern Planning Region occupies 3548.7 km^2 i.e. 14.2% of the territory of the Republic of Macedonia (State Statistical Office, 2015). The Region includes 11 municipalities: Berovo, Pehchevo, Delchevo, Shtip, Vinica, Zrnovci, Karbinci, Makedonska Kamenica, Kochani, Cheshinovo-Obleshevo and Probishtip. Altogether they comprise 74.12% of the Bregalnica River basin. The rest of the territory belongs to the municipalities in the neighboring planning regions: Sveti Nikole, Konche, Lozovo, Radovish and Kratovo. Furthermore, the municipalities of Gradsko, Kriva Palanka, Kumanovo and Veles include significant portions of the Bregalnica River basin (between 13 and 52 km^2).

Agriculture and use of agricultural land

According to State Statistical Office (2016), the Eastern Planning Region has 188,387 ha of agricultural land, of which 77,718 ha (41.2%) is arable land and the rest are pastures.In terms of land under pastures, most of this land is under hill pastures. Carriers of cheap food for the grazing animals are the municipalities of Berovo, Stip and Kochani. The pastures in these municipalities are 58,858 ha i.e. 64.9% of the total land under pastures in the Eastern Planning Region. The farming capacities for production of hay mainly exist in Berovo, followed by Delchevo and Pehchevo. Together they have over 77% of the total meadows in the Region. As a result of the migrations from village into cities, that started in the second half of 20th Century and is still present (State Statistical office, 2012), the intensity of use of the land under pastures has been significantly reduced and continues to decline (Jovanovska & Melovski 2013). For example, the neglecting of the animal husbandry gradually leads towards abandoning of the land that was used as pastures, which in turn leads towards nature taking over that land with bushes and other vegetation. The traditional animal husbandry practices have special relevancy for cherishing of the antropogenous habitats which are relevant for preservation of the biodiversity. Hence, the implementation of activities and making od development and enabling policies in the context of revitalization of the animal husbandry practices will be necessary, in order to sustain this traditional manner of land use in the future.

Pasture types in the Eastern Planning Region

Depending on the season in which the pastures are used, they are divided into:

- Summer pastures,
- Winter pastures.

The *summer pastures* in the Eastern Planning Region so far were only exploited without any significant improvements. The strip grazing is not used, and there is no fertilizing, cleaning of rocks, weeds and other agrotechnical measures. Greater care is paid on water supply to the pastures – water cisterns are established at Ograzhden as well as watering places on Golak, Vlaina, Obozhna, Plachkovica, Osogovo, Maleshevo mountains and others, and they were also cleaned from spruces. Very little was done, though, in terms of roads. Most of the pastures are difficult to access and far away from the main roads.

Winter pastures include: low-land pastures, hill pastures, and forest pastures.

Low-land (valley) pastures: These areas mainly feature meadows but there are pastures as well. The mixing of the continental and the Mediterranean climate and the local mountain climate, results in occurrence of specific grass vegetation. The production yield on these areas is not satisfactory – it is up to 2.0 t/ha of hay.The floral (grass) communities feature many annual species, most of which are leguminosae (*Fabaceae*), which is especially relevant for these areas.

Hill pastures: This type of pastures is mainly present in the Northern and northwestern part of the surveyed area (Mangovica, Kuchukov, Bogoslovec and Slan Dol). Large areas under pastures can be also seen in the southern and (partially) western slopes of Osogovo, Maleshevo and the northern mountains of Plachkovica. They spread up to the altitude of 800 meters.Species that are present are low-growing, and they also thrive in the winter period, although with reduced rhythm. In the warm summer months (July, August), the top soil cover dries off and in autumn, when the temperature drops and the first rain occurs, the grass vegetation regenerates again. Although the grazing on these pastures is long-term, the production is low and varies between 300 and 800 kg/ha of hay.

Forest pastures: These pastures are located in the zone with permanent forests. In the Eastern Planning Region and in the areas that gravitate towards the Bregalnica River basin, they spread above the hill pastures, all to way to the upper zone of the forests i.e. the high-mountain pastures (800 – 1600 and up to 2000 meters altitude) and they are defined as forest enclaves. They are used as natural resource in late summer, when the cattle migrates towards the high mountain pastures; in summer when the cover mass on the high mountain pastures dries out; and in autumn, when the cattle migrates again now in opposite direction.

Capacity of the pastures in the Eastern Planning region

The information on production of hay at the pastures in the Eastern Planning Region and in the parts that gravitate towards the Bregalnica River basin for 2015 show that their average production is consistent with the national level production, which means that their economic value is within the productivity of other pastures in Macedonia. The average production at these pastures is 440 kg/ha of hay, which corresponds to the manner and dynamics of the animal husbandry practiced by the rural population in the East Planning Region (Table 1).

The productivity of the pastures along the Bregalnica River basin is from 320 kg/ha of hay in Probishtip, to 680 kg/ha in Berovo. This is low productivity by it has potential to be higher. In addition to the human factor, the absence of meliorative and agro technical measures, one of the main reasons for this weak production i.e. economic value of the pastures, is the rapid decline of the number of cattle and the declining tendencies in the animal husbandry as main activity. We need to emphasize here that the yield from the pastures cannot be the only indicator and basis for determination of their capacity, but should be used only for orientation. During our field research we take sample at several square meters of land, in relation to the entire pasture area, and they show only the average mean value for a particular vegetation period (when the yield was measured).

Table 1. Areas under pastures (ha) and production from the pastures (kg/ha of hay) in the Eastern Planning Region and in the parts that gravitate towards the Bregalnica River basin in the period 2011 – 2015

Municipalities	Botanic species at the pastures	% representation at the pastures	Land under pastures (ha)	Grass production of the pastures (kg/ha of hay)
Berovo	Poaceae Fabaceae Various grass	55 18 27	14924	630
Delchevo	Poaceae Fabaceae Various grass	60 20 20	7123	520
Vinica	Poaceae Fabaceae Various grass	45 35 20	10306	490
Makedonska Kamenica	Poaceae Fabaceae Various grass	50 35 15	26	380
Pehchevo	Poaceae Fabaceae Various grass	35 55 10	20602	570
Zrnovci	Poaceae Fabaceae Various grass	40 40 20	85	440
Kochani	Poaceae Fabaceae Various grass	40 45 15	13368	415
Karbinci	Poaceae Fabaceae Various grass	50 30 20	38	390
Probishtip	Poaceae Fabaceae Various grass	45 48 7	15359	320
Cesinovo-Oblesevo	Poaceae Fabaceae Various grass	38 44 18	14	365
Stip	Poaceae Fabaceae Various grass	53 37 10	22205	430
Sv. Nikole	Poaceae Fabaceae Various grass	65 22 13	14675	370
Lozovo	Poaceae Fabaceae Various grass	66 21 13	?	355
Konce	Poaceae Fabaceae Various grass	59 25 16	2300	430
Kratovo	Poaceae Fabaceae Various grass	47 38 15	12200	445
Total			110640	440 (average)

Source: Markova et al. 2011; Prentovic, T. (own research)

For more correct results, the sampling should be continuous and the yield should be measured every year, in a course of several decades. Only that average yield would be the solid basis for determination of the capacity of such heterogeneous floral circumstances as they exist with the grass vegetation at the pastures in the Eastern Planning Region.

Measures for pastures protection

Several measures can be implement in terms that would maintain or improve pasture status from quantitative and qualitative aspect. In this paper our focus are agrotechnical measures, especially those which should be applied every year or every second year, such as: surface cultivation, fertilization and reseeding.

The *surface cultivation* includes a number of agro technical operations implemented due to the regular natural changes in the phytocenoses and in the soil and, due to the climate influence, changes in the plant cover and in the manner of use of the greenswards. The actual cultivation includes measures in order to create ploughed soil layer that will allow reseedings of the pastures.

Fertilizing. The use of the pastures for grazing takes away large quantities of mineral nutrients from the soil, the soil becomes poorer i.e. its fertility is reduced. One average natural pasture that includes a number of natural grass species, and less other plant communities, needs the following quantities of nutrients in order to produce 100 kg of hay (Table 2).

Table 2. NPK quantities required for production of 100 kg of hay, depending on the development phase

Mineral nutrition	Utilization in the phase of stem elongation – before blooming/kg	Utilization in the phase of blooming of the grass /kg
Nitrogen (N)	1.8 – 2.0	4.6 – 5.4
Phosphorus (P_2O_5)	0.7 – 1.0	0.4 – 0.5
Potassium (K_2O)	1.8 – 2.2	0.7 – 0.8

Source: Ivanovski, 2000

The mineral fertilizers on the pastures in the Eastern Planning Region never been used. It is expensive agrotechnical measure and to be used it has to be financially supported by the relevant institutions. In our conditions, the most favorable NPK ratios could be: 1-2 : 1 : 1 or 2 : 1,5 : 1, for pastures where the content of phosphorus and potassium are in deficit as well as on land areas with greater humidity. In practice there are three approximate doses used when fertilizing the natural pastures (Table 3).

Table 3. Approximate quantities of nitrogen, phosphorus and potassium (kg/ha) when applying fertilizers to natural pastures

Fertilizing norms	Quantities – kg/ha		
	N	P_2O_5	K_2O
I – small dose	60 – 80	40 – 50	40 – 50
II – middle dose	100 - 120	70 – 80	70 – 80
III – high dose	140 – 160	90 – 100	90 – 100

Source: Ivanovski, 2000

Usually, the small doses are appropriate for the hill-mountain pastures, the middle doses are appropriate for the hill and valley pastures and the higher doses for flat-land, valley and the better-quality hill pastures from which better yield and quality of the feed will be produced.

Fertilizing with the use of organic fertilizers. The organic fertilizers (specifically the manure) improve the fertility of the soil by enriching the microbial activity, which is especially relevant for the mineralization of the organic matters in the soil. They also regulate the pH value, especially at more acidic soils, and reduce the alkalinity of the respective soils, improve the heat and air regime thus reducing the natural phenomenon of anaerobization of the greenswards in the degradation processes. The manure has significant impact on the floral composition of the pastures. Because of the surface use, quantities of 20 t^{-1} ha^{-1} show small effect. It is much more useful to use greater quantities – 40 t^{-1} ha^{-1} – which result in higher yields. However, great care must be paid with the planned quantities because too extensive quantities suppress the development (mainly of the leguminous species) due to the large quantity of nitrogen, and in turn stimulate the development of harmful weeds and grass. The manure should be used in late autumn or early spring, every second or third year, depending on the condition of the pasture. The existing grass vegetation reacts positively, grows quickly, but dye to the specific smell that could be repelling for the cattle, it is better if the first sweeping is used for hay, and the others for grazing. The liquid manure could be used on the pastures in quantities of 20 – 40 t^{-1} ha^{-1}, initially dissolved in water in 1 : 4-5 ratio (liquid manure : water). Sometimes, in a case of summer sweeping, this concentration is 1 : 10. For the sake of better and more complex effect, it is better to use it combination with some phosphorous fertilizer, in a quantity of 50 – 60 kg/ha. It is applied by spraying the surfaces with special cisterns. The organic liquid manure mainly includes nitrogen and potassium so the use of this manure should be in combination with phosphorus, in quantities of 50 – 60 kg/ha. If used every year, if it is not diluted well and if no phosphorus is used as addition, it could case diseases among the domestic animals in a form of diarrhea or bone problems. It is therefore recommended to be used every second year, always in combination with some phosphorus fertilizer.

Reseeding. The reseeding is used for improvement of the natural pastures, specifically those where the vegetation is rare or where low grass with shallow roots started to dominate. The purpose is to increase the yield and improve the quality of the feed, as well as to protect the soil from erosion (on fields subject to intensive erosion processes). This additional sowing is deployed when:

- the grass is rare;
- the grass is destroyed.

The following factors need to be taken into account when selecting the plant species for making of these mixtures: the natural conditions in the environment (the needs of the particular species in terms of soil reaction, climate conditions, nutrition regime, air regime and other agro-ecological conditions); the agronomic characteristics of particular species (yield, nutrition value, resistance to diseases and pests, dynamics of development during the vegetation period, etc.); the biological characteristics of the species (the duration of the lifecycle, the ability to replenish, multiply, etc.); the relations between different species (low vs high grass, etc.); the duration of use of the pastures (problems related to the persistence of particular species in the mixture and maintaining of productivity in situation of different lifespan of the pastures); the economic purpose of the pastures (use of mixtures intended for grazing, use of mixtures for production of hay, combined use of the pasture), etc. Considering the above parameters, one of the mixtures for advancing of the pastures could include the following plant species:

- Red clover *(Trifolium pretense):* 6 – 8 kg/ha;
- Timothy-grass *(Phleum pretense):* 5 – 6 kg/ha;
- Meadow fescue *(Festuca pratensis):* 6 – 7 kg/ha;
- Smooth brome *(Bromus inermis):* 8 – 10 kg/ha

If reseeding is used on terrains for remedying of the erosion, the following combinations of grass-clover mixtures that have significant positive impact in regulation of this process of degradation of the soil are recommended:

I. Leguminous based mixtures:

1. Onobrychis sativa	100%			
2. Onobrychis sativa	70%	+	Agropyron cristatum	30%
3. Onobrychis sativa	80%	+	Bromus inermis	20%

4. Onobrychis sativa	70%	+	Dactylis glomerata	30%
5. Lotus corniculatus	60%	+	Dactylis glomerata	10%
			Agropyron cristatum	20%
			Festuca rubra	10%
6. Lotus corniculatus	30%	+	Trifolium repens	30%
			Dactylis glomerata	20%
			Agropyron repens	10%
			Festuca sulcata	10%

II. Grass based mixtures

7. Dactylis glomerata	30%	+	Agropyron crystatum	30%
			Lolium perenne	20%
			Lotus corniculatus	10%
			Trifolium incarnatum	10%
8. Dactylis glomerata	10%	+	Festuca pratensis	10%
			Agropyron cristatum	10%
			Festuca arundinaceae	20%
			Bromus sp.	10%
			Trisetum flavescens	10%
			Lolium perenne	20%
			Festuca sulcata	10%

a) Mixtures for skeletal-carbonate soils

1. Onobrychis sativa	100%			
2. Onobrychis sativa	70%	+	Dactylis glomerata	30%

The reseeding of pastures located on terrains with mild inclination must be accompanied with intensive fertilization. The areas that were additionally sown must be carefully used in the first year, otherwise very serious thinning out could occur.

Conclusions

The Eastern Planning Region occupies 3,548.7 km^2. The region has 119,504 ha under grasslands, from which 110,640 ha is under pastures and 8,864 ha is under meadows, representing important source of production of feed, especially in the hilly-mountain areas of the region. As a result of the environmental condition (arid climate, and shallow, skeletal and poor with nutrients soils), the pastures in the Region have low productivity. In the absence of humans as correctors of the specific environmental conditions, they contribute with small hay production, in average from 300 – 600 kg/ha. In order to increase the yield and to improve the quality of the forage, it is necessary to apply measures that improve the state of the pastures, such as: surface cultivation, fertilization, reseeding, protection from weeds, additional sowing and some other measures for care. The contemporary use of the pastures must also include activities for melioration that play great role in the protection of the endangered terrains from erosion and in the regulation of the water regime (arranging of springs; arranging/making of watering places) as well as creating conditions for watering of the livestock via catchments and reservoirs with rainwater.

References

1. Jovanovska, D., Melovski, Lj. (2013). Land cover succession as a result of changing land use practices in northeast Macedonia. Proceedings of the 4th Congress of Ecologists of Macedonia

with international Participation, Ohrid. Macedonian Ecologist Society, Special issue 28, Skopje, 185-197.

2. Markova, N., Hristova, E., Zlatkovski, V., Ilieva, V., Mitrev, S. (2011). Pasture utilization in the Eastern planning region in Republic of Macedonia and the necessity for determination of their grass composition. 1st National Agriculture Congress andExposition on behalf ofAli Numan Kıraç withInternational Participation, Turkey, 2657-2664.
3. State Statistical Office of the Republic of Macedonia. (2015). From: http://www.stat.gov.mk/.
4. State Statistical Office of the Republic of Macedonia. (2016). From: http://www.stat.gov.mk/.
5. Аждерски, Ј. (1998). Агоеколошки ефекти при користење на тревници за зголемување на овчарското производтво во Малеш и Пијанец. Докторска дисертација, Земјоделски факултет – Скопје.
6. Ивановски, П. (2000). Фуражно производство, Универзитет „Св. Кирил и Методиј“ Скопје

THE IMPACT OF USING COMBINATIONS OF N, S AND B IN OILSEED RAPE - BRASSICA NAPUS L. ON QUANTITATIVE PROPERTIES OF SEED

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Abstract

As a result of the use of the following elements - nitrogen (N), sulphur (S) and boron (B) - quantitative properties of two genotypes of oilseed rape - Brassica napus L., Zorica (variety) and Rohan (hybrid) were determined. The main purpose of the study was determination of the production mode, set in both genotypes of oilseed rape versus managing various nutrients. An experiment was set in the Skopje region, on total experimental area of $650m^2$. For this purpose, the following combinations of fertilizers were used: N_1 with 110 kg/ha nitrogen, N_2 with 150 kg/ha nitrogen, S_1 with 30 kg/ha sulphur, S_2 with 70 kg/ha sulphur, B_1 with 1.0 kg/ha boron, and B_2 with 2.0 kg/ha boron (at spring time, foliar application) versus standard variant: N:P:K in the ratio 10:20:30 (N 50kg/ha, 90 kg/ha P_2O_5 and 180 kg/ha K_2O) used in autumn. During the experiment the following parameters were monitored: height of plants (cm), number of branches per plant, number of pods per plant, length of pod (cm), number of seeds in the silique, and seed yield (t/ha). From the combinations of nutrients and variations that have been set in terms of genotypes, the results of yield showed statistical significance at level of 0.05 from variants N_2PK, $N_2PK+S_2+B_2$ in variety Zorica and $N_1PK+S_1+B_1$ in the hybrid Rohan.

Keywords: *Brassica napus L.*, nitrogen, sulphur, boron, foliar application, yield.

Introduction

During the 20th century, agricultural production has developed a strategy to improve the efficiency of the use of fertilizers and to obtain higher yields through low-cost investments. Today, science has the challenge and opportunity to develop efficiency from the use of certain nutrient element and to present best management practices. In modern agriculture, the application of this strategy should prioritize the development of effective genotypes and perceive the importance of the use of nutrients in increasing yield (Fageria, et al., 2008). Properly used nitrogen levels positively affect the vegetative and reproductive development of the rape, but at the same time increase the utilization of other nutrients such as sulfur (S) and boron (B) (Barker and Bryson, 2006). On the other hand, the use of nitrogen more than necessary can cause the falling down of the stem, increase the content of chlorophyll in the seeds and have a negative impact on the environment (Brennan et al., 2000; Karamanos et al., 2003 and 2007; Et al., 2005). Concerning obtaining the yield of 2000 kg / ha, the needs of nitrogen range from 124-150 kg / ha (Ukrainetz et al., 1975). Sulfur is the fourth most important element in the agricultural production system. The sulfur deficit always reduces the yield of oilseed rape (Malhi and Gill, 2007). Sulfur not only affects the yield increase, but at the same time improves the utilization of nitrogen by plants (Karamanos et al., 2007). The balanced relationship between N and S is crucial in achieving the maximum yield of oilseed rape in sulfur-free conditions (Mahli and Gill, 2007). The requirements of sulfur in oilseed rape are 3-10 times higher compared to barley (Mahli and Gill, 2002). On the other hand, the larger amounts of sulfur cause adverse effects on the quality, increasing the content of glucosinolates - a non-feedable component of oilseed rape (Falk et al., 2007). From microelements, boron (B) plays a leading role in the yield of oilseed rape (Malhi et al., 2003). The lack of boron (B) is a global problem in the production of oilseed rape, with its susceptibility far more pronounced compared with cereals (Grant and Bailey, 1993; Shorrocks,

1997. As a high-demand crop, the demand for oilseed rape for boron are 2 kg / ha, in contrast to corn and wheat where needs for boron are less than 1 kg / ha (Malhi, 2001; Gupta, 2007). The correct use of boron can affect positively the height of plants, number of branches / plants, number of fruits, number of seeds in the silique, oil content, etc. and the seed yield (Stevenson and Cole, 1999), leading to a yield increase of up to 7% (Porter, 1993), or from 7 to 11% (Troeh and Thompson, 2005). The negative effects of boron application are also registered as on the yield of seed (Karamanos et al., 2003). The main purpose of the research is to determine the production regime in the oilseed rape as opposed to the management of various nutrients. The specific objectives are to determine the optimum amount and time of use of nitrogen (N), sulfur (S) and boron (B), on the development, yield components and yield of seed in the examined genotypes.

Material and methods

The experiment was placed in the Skopje region, at the site Hippodrome (41 ° 99'42.57 "N; 21 ° 52'21.07" E), on a total experimental area of 650 m^2, by applying standard agrotechnical measures. The sowing was carried out on October 1 with two genotypes of oilseed rape: Rohan (hybrid) and Zorica (variety), with sowing rate of 8.0 kg / ha (with spinning, if necessary in the spring months). The trial was set in 4 replications, with the dimensions of the basic parcel of 6,6 m^2 (1,10 m x 6,0 m), with five rows in the parcel at the interim distance of 25 cm. The distance between the variants was 0.5 m, and between replications of 1 m. The combinations in the treatment by variants were in the following order: Control Ø, N_1PK, N_2PK, $N_1PK + S_1 + B_1$, $N_2PK + S_2 + B_2$. The choice of dosing was also the amount of application arranged in the following way:

- N_1 - with 100 kg / ha (50 kg pre-season and 50 kg by spring harvesting, at the end of the stem elongation stage and the stage of beginning of flowering, (3rd and 4th stage according to BBCH - Biologische Bundesanstalt, Bundessortenamt and Chemical Industry) in order to create the potential for the formation of fertile branches and complement the loss of wintering;
- N_2 - with 150 kg / ha (50 kg pre-seed and 100 kg by feeding in the spring, at the end of the stretching stage and the stage of beginning of flowering);
- S_1 - with application of sulfur 30 kg / ha of spring soil (beginning of vegetation),
- S_2 - with application of sulfur 70 kg / ha of spring soil (beginning of vegetation),
- B_1 - with the application of pine 1.0 kg / ha (whole quantity at spring time, in stage budding), which is equivalent to 150 g / ha foliar application.
- B_2 - with application of the boron 2.0 kg / ha (on two occasions): 1.0 kg / ha at vegetation start + 1.0 kg / ha in the butonization phase, which is equivalent to 300 g /ha foliar application.
- The quantities of P and K were standard in all variants: 90 kg / ha P_2O_5 and 180 kg / ha K_2O, used from autumn.

The fertilizers used in this research were regulary available on the market: Ammonium nitrate (34%) (17% ammonia and 17% nitrate form), Ammonium sulphate (24%) and boron 8% (Agrosal). The data analysis was perceived through the arithmetic mean, the ratio for the calculation and yield representation in t / ha for each of the variants. The results of the mean values for all parameters were statistically processed by ANOVA single factor analysis and the LSD test was calculated, with the M.Office Excel and SPSS program (PSAW 18).

Results and disscusion

The overwintering of the oilseed rape began in the phase of the foliage (BBCH 3, 13, 19, 21), that was when more than 3 and up to 9 leaves were formed, and the beginning of the forming and development of first side shoots. The plants that have well developed leaf rosette, short and thick hypocotyl and well developed but less elongated epicotyl in autumn successfully withstand low temperatures. In this period, the winter genotypes oilseed rape successfully crossed both development stages – jarization and light stage, when even after severe winter damages caused by low temperatures, they had exceptional regeneration capacity.

Table 1. Height of plants (cm), number of branches / plant, length of silique (cm)

VARANTS	±	Genotype		±	Genotype		±	Genotype	
		Zorica	Rohan		Zorica	Rohan		Zorica	Rohan
ø Contol	193.8	187.5	188.8	7.9	7.2	8.1	6.6	6.7	6.5
N_1PK	194.8	188.8	200.8	8.5	8.0	9.0	6.7	6.8	6.6
N_2PK	193.9	192.5	195.3	8.1	7.3	8.8	6.8	6.9	6.8
$N_1PK+S_1+B_1$	200.3	194.5*	206.0	8.3	7.8	8.3	7.0	7.3	6.8
$N_2PK+S_2+B_2$	196.1*	196.0	196.3	8.3	8.0	8.4	7.1*	7.1	7.2
LSD 0.05	5.04	16.0	16.9	1.10	1.92	2.36	0.54	1.05	1.27
0.01	6.68	21.4	22.5	1.45	2.57	3.10	0.71	1.40	1.70

* Significant at the level of probability P=0.05

Plant height in oilseed rape represents a genetic hereditary characteristic, changes according to the phenological phase in which they are located and is right-oriented with the uptake of genotypes in the later stages of development. Thus, an average of the inflorescence emergence (BBCH 53, 55), until maturing (BBCH 79 - almost all siliques reached the final size), and full ripeness (89 - nearly all siliques ripe, seeds dark and hard), the plants increased their height almost three times, which was the result of intense growth and development in this period of vegetation. The highest plants were recorded in variant $N_1PK+S_1+B_1$ (200.3 cm), while the lowest growth was recorded by plants from the control variant. Statistical significance for the plant height parameter was determined in the variant $N_2PK+S_2+B_2$ (196.1cm) at the level of 0.05 (Table 1). Regarding the two genotypes, the applied doses of fertilizers showed a statistically significant effect in the Zorica genotype at a level of 0.05 in variants $N_1PK+S_1+B_1$ (194.5 cm). The number of branches of the plant in oilseed rape depends on the time of sowing and the application of other agrotechnical measures during the vegetation. In the specific trials, the number of branches / plants was determined on two occasions: 1. flowering stage and 2. full-maturation stage (89). The data in Table 2 shows that the average number of branches in all examined variants ranges from 7.9 to 8.3 in the variant $N_2PK+S_2+B_2$. Among the genotypes there is no statistical difference, and the difference in the number of branches per plant ranges from 7.2 in the control variant to 8.0 in variants N_1PK and $N_2PK + S_2 + B_2$ for Zorica genotype and 8.1 to 9 , 0 for Rohan genotype. With respect to the length of the siliques, the values range from 6.6 cm in the control variant to 7.1 cm in the variant $N_2PK+S_2+B_2$. In terms of statistical feasibility, the values of this characteristic showed the significance of the variant $N_2PK+S_2+B_2$ at level of 0.05.

Table 2. Number of siliques / plant and numper of seeds / silique

VARIANTS	±	Genotype		±	Genotype	
		Zorica	Rohan		Zorica	Rohan
ø Control	281.1	257.3	305.0	25.0	24.5	25.5
N_1PK	332.6	297.0	368.3	27.4*	24.8	28.5*
N_2PK	330.6	295.8	365.5	27.0*	26.5	27.3
$N_1PK+S_1+B_1$	352.5*	348.5*	356.5	29.0**	27.3**	29.5**
$N_2PK+S_2+B_2$	363.8**	338.0*	389.5*	29.3**	28.2**	28.8*
LSD 0.05*	59.5	69.12	87.81	1.90	2.79	2.64
0.01**	78.7	92.48	117.49	2.52	3.73	3.54

*;** Significant at the level of probability P=0.05 and P=0.01

In the study, the number of siliques / plants ranged from 288.1 in the control variant to 363.8 in the variant $N_2PK+S_2+B_2$ with a difference of 82.7 siliques (Table 2). Regarding the genotypes, the Rohan hybrid had a minimum of 305 siliques in the control variant up to maximum of 389.5 siliques in the variant $N_2PK+S_2+B_2$ and was characterized by the formation of a greater number of siliques of the plant in almost all other variants of the experiment. In the Zorica genotype, the lowest number of siliques was found in control whey, whereas the highest number of fruits (348.5) were determined with the applied quantities of the variant $N_1PK+S_1+B_1$. For the number of siliques per plant, the smallest significant difference at the level of 0.05 showed the variant $N_1PK+S_1+B_1$, and at the level of 0.01% quantities of the variant $N_2PK+S_2+B_2$ (363.8). The highest number of seeds per silique was obtained in the variants $N_1PK + S_1 +B_1$ (29.0) and in the variant $N_2PK+S_2+B_2$ (29.3), the control variant formed siliques with the lowest number of seeds, on average 25.0. The genotype Rohan had a higher number of seeds / pod in all other variants in relation to the genotype Zorica. Statistically significant difference was appeared in all variants: the variants and the level of 0.05, the variants N_1PK (27.4), and N_2PK (27.0), while the level of 0.01 in variants $N_1PK+S_1+B_1$ and $N_2PK+S_2+B_2$. In terms of genotype, genotype Zorica showed significance only at the level of 0.01 in variants $N_1PK+S_1+B_1$ and $N_2PK+S_2+B_2$, while genotype Rohan determining a significance level 0.05 in variants N_1PK and $N_2PK+S_2+B_2$, and level of 0.01 in the variant with the smaller applied quantities of all used elements $N_1PK+S_1+B_1$.

Table 3. Yield (t/ha)

VARIANTS		±	Genotype		STDEV (ρ)
			Zorica	Rohan	
Ø Control		3.96	3.33	4.54	0.84
N_1PK		4.29	3.83	4.75	1.00
N_2PK		4.63	4.83*	4.42	0.82
$N_1PK+S_1+ B_1$		5.21*	4.42	6.00*	1.01
$N_2PK+ S_2 +B_2$		5.08*	4.92*	5.25	0.87
LSD	0.05 *	1.02	1.27	1.15	
	0.01**	1.35	1.69	1.54	

* Significant at the level of probability P=0.05

In terms of yield (Table 3), statistical significance at the level of 0.05 showed variants $N_1PK+S_1+B_1$ and the variant $N_2PK+S_2+B_2$ with realized yields of 5.21 t / ha and 5.08 t / ha. Regarding the genotypes, the statistical significance at the level of 0.05 showed genotype Zorica with variants N_2PK (4.83 t / ha) and $N_2PK+S_2+B_2$ (4.92 t / ha), while genotype Rohan achieved the highest yield in the variant $N_1PK+S_1+B_1$ (6.00 t / ha).

Conclusion

Methodologically set and used combinations of nutrients that have a higher importance for oilseed rape achieved a higher yield in variants $N_1PK+S_1+B_1$ and variant $N_2PK+S_2+B_2$. In relation to the examined genotypes, significant results for specific quantitative properties were obtained from variants N_2PK, $N_2PK+S_2+B_2$ in variety Zorica and $N_1PK+S_1+B_1$ in the hybrid Rohan (yield of 6.00 t / ha). Our research will expanding over the next years to monitor the impact of these nutritional elements in given combinations and in different meteorological conditions. Thus, we could recommend an adequate and economical combination for high yield and high quality rapeseed on the territory of Macedonia.

References

1. Barker A. V., Bryson G. M. (2006). Handbook of plant nutrition. CRC Press, Boca Raton, USA 2149.
2. Brennan R., Mason M., Walton G. (2000). Effect of nitrogen fertilizer on the concentrations of oil and protein in canola (Brassica napus) seed. Journal of Plant Nutrition, 23(3), 339-348.
3. Falk K., Tokuhisa J., Gershenzon J. (2007). The effect of sulfur nutrition on plant glucosinolate content: physiology and molecular mechanisms. *Plant Biology, 9* (5), 573-58.

4. Fageria N. K., Baligar V. C., LiY. C.(2000).The Role of Nutrient Efficient Plants in Improving Crop Yields in the Twenty First Century, Journal of Plant Nutrition, Volume 31, pages 1121-1157.
5. Grant C., Bailey L. (1993). Fertility management in canola production. *Canadian Journal of Plant Science, 73*(3), 651-670.
6. Gupta U. (2007). Boron, in *A.V. Barker, D.J. Pilbeam (eds.) Handbook of plant nutrition.* CRC Press, Boca Raton, USA 241-277.
7. Karamanos R., Goh T., Flaten D. (2007). Nitrogen and sulphur fertilizer management for growing canola on sulphur sufficient soils. *Canadian Journal of Plant Science, 87*(2), 201-210.
8. Karamanos R., Goh T., Stonehouse T. (2003). Canola response to boron in Canadian prairie soils. *Canadian Journal of Plant Science, 83*(2), 249-259.
9. Malhi S. S. (2001). Improving Canola Yields with Balanced and Efficient Nutrition. Final report prepared for canola council of Canada [Online] www.saskcanola.com/ quadrant/System/research/reports/report-Malhi-balancednutrition-long.pdf
10. Malhi S., Gill K. (2002). Effectiveness of sulphate-S fertilization at different growth stages for yield, seed quality and S uptake of canola. *Canadian Journal of Plant Science, 82*(4), 665-674.
11. Malhi S., Raza M., Schoenau J., Mermut A., Kutcher R., Johnston A., Gill, K. (2003). Feasibility of boron fertilization for yield, seed quality and B uptake of canola in northeastern Saskatchewan. *Canadian Journal of Soil Science, 83*(1), 99-108.
12. Nadian H., Najarzadegan R., Saeid K. A., Gharineh M., Siadat A. (2010). Effects of Boron and Sulfur Application on Yield and Yield Components of Brassica napus L. in a Calcareous Soil. *World Applied Sciences Journal, 11*(1), 89-95.
13. Porter, P. (1993). Canola response to boron and nitrogen grown on the southeastern coastal plain 1. Journal of Plant Nutrition, 16(12), 2371-2381.
14. Rathke G.W., Christen O., Diepenbrock W. (2005). Effects of nitrogen source and rate on productivity and quality of winter oilseed rape (*Brassica napus L.*) grown in different crop rotations. *Field Crops Research, 94* (2), 103-113.
15. Shorrocks V. M. (1997). The occurrence and correction of boron deficiency. Plant and Soil, 193 (1-2), 121-148.
16. Stevenson F. J., Cole M. A. (1999). Cycles of soils: carbon, nitrogen, phosphorus, sulfur, micronutrients: Wiley.com
17. Troeh F. R., Thompson L. M. (2005). Soils and soil fertility: Blackwell Ames 211-231, 269-305.
18. Ukrainetz H., Soper R. J., Nyborg M. (1975). Plant nutrient requirements of oilseed and pulse crop, in J. T. Harapiack, ed. Oilseed and pulse crops in western Canada - A symposium. Western Cooperative Fertilizers Ltd.Calgary, AB 325-374.
19. Weber E., BleiholderH. 1990. Erläuterungen zu den BBCH-Dezimal-Codes für die Entwicklungsstadien von Mais, Raps, Faba-Bohne, Sonnenblume und Erbse – mit Abbildungen. Gesunde Pflanzen 42, 308-321.

EFFECT OF BIOMASS AT DIFFERENT GROWTH STAGE ON GRAIN YIELD AND QUALITY IN BREAD WHEAT (*Triticum aestivum* L.) GENOTYPES

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Abstract
The aim of this research was to determine the effects of location, genotypes and the interaction of location x genotypes on biomass, canopy temperature, yield and some of the quality traits of the bread wheat genotypes under field conditions. Thus, it was established with 25 genotypes in randomized completely blocks design with 4 replications at 3 locations in Trakya Region, Turkey, in 2013-2014 growing season. Grain yield, biomass, canopy temperature, plant height, 1000-kernel weight, test weight, and relationship among these characters were investigated. For determining biomass of the genotypes, data was taken at three plant growth stages; tillering, shooting and heading. Combined analysis of variance across three locations revealed highly significant variation among wheat genotypes for grain yield, biomass of stem elongation, heading stage and canopy temperature. The mean yield of the genotypes was 723.0 kg da^{-1}, and the highest yields were obtained from Entry 22 with 826.3 kg da^{-1}. The highest biomass was scaled in Entry-9 during three-plant growth stages. Additionally, a positive correlation was observed between grain yield and biomass in tillering, shooting, and heading growth stages of the genotypes. These results indicated that higher biomass at early plant growth stage was more significant for yield potential. There was detected slightly negative relationship between canopy temperature and biomass, and grain yield. It could be that the canopy temperatures of genotypes were measured lower during the increasing of biomass in plant development. During tillering stage, higher biomass promoted to plant height and positively affected protein ratio, values of gluten and sedimentation. In shooting phase of genotypes, biomass positively affected and increased in 1000-kernel weight, protein ratio, gluten value and sedimentation value, as well. But increasing in biomass during heading stage, negatively affected and decreased in canopy temperature, 1000-kernel weight, test weight, protein ratio, gluten index and sedimentation value.

Keywords: Bread wheat, genotypes, yield, biomass, quality parameters.

Introduction

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. The static stability is defined as the lack of response to environmental variations while the dynamic stability is defined as the average response (Pfeiffer and Braun, 1989). Improvement of grain quality is a major objective of wheat (*Triticum aestivum* L.) breeding programs. Beside for enhancement of biological and nutritive value of end-use products, the quality components of grain play an important role in the economic value of new cultivars determining (Mangova and Rachovska, 2004). Canopy temperature depression (CTD) measured with an infrared thermometer was significantly positively correlated with performance at the international sites when measured between 1200 and 1600 hours, after full canopy establishment. The correlation of CTD with yield was not affected by the irrigation status of the crop under well-watered conditions. The possible use of these traits in selection for yield under hot conditions is discussed (Reynolds et al., 1994). Biomass assessment is essential not only for studies monitoring crop growth, but also in cereal breeding programs as a complementary selection tool (Araus et al., 2009). The normalized difference vegetation index (NDVI) is widely used at ground level, and from low, high and satellite altitudes to measure vegetative greenness and canopy photosynthetic size (Reynolds et al., 2001). Trakya region of Turkey has a diversion climate conditions with distinct agro-ecological zone. Although the average annual rainfall is 560 mm in the region this precipitation ranges between 350 mm to 850 mm. During the growing season (October-June) the distribution of this rainfall is not regular. Due to this fluctuation of rainfall in some growing year drought problem occurs and decrease grain yield. The main objective of this research was to assess and effect of the biomass on yield and some quality parameters of some genotypes.

Material and methods

This experiment was conducted at three locations of Trakya region, Turkey for 2013-2014 growing seasons. Twenty five winter wheat genotypes, 5 of them were local check and 20 advanced lines, were examined under field condition. A randomized complete block design with four replications was used at each location. Each plot was sown into 6 rows, and plot sizes were 6 m^2 at harvesting. In the experiment 500 seeds m^2 was used at planting and sowings were performed by using a plot drill.

Table 1. The climatic value of the 2013-2014 growing year in Edirne and Tekirdağ location

Months	Edirne Location			Tekirdağ Location		
	Rainfall (mm)	Humidity (%)	Temperature (^oC)	Rainfall (mm)	Humidity (%)	Temperature (^oC)
October	30.7	77.5	12.8	96.4	76.2	14.3
November	73.9	86.7	11.0	36.6	79.0	12.9
December	2.3	82.2	2.7	2.4	74.1	6.2
January	74.9	87.4	5.5	44.0	85.0	8.0
February	3.8	86.0	7.6	6.0	83.2	8.7
March	124.5	81.4	10.1	65.2	81.6	9.9
April	36.8	81.6	13.6	41.2	83.3	13.4
May	61.7	76.6	18.6	65.2	80.3	17.5
June	68.8	73.8	22.9	60.0	76.2	21.8
Mean/Total	477.4	81.5	11.6	417.0	79.9	12.5

Data recorded for grain yield, biomass, canopy temperature (Jackson et al., 1981; Fisher, 2001; Reynolds et al., 2001; Guttierrez-Rodriguez et al., 2004; Babar et al., 2006; Morgounov et al., 2014), and plant height between these characters were investigated. For quality parameters, thousand kernel weight, test weight, protein ratio, gluten value, gluten index, hardness and sedimentation (Blakeney et al., 2009; Köksel et al., 2000; Marti et al., 2007) and relationship between these characters were investigated. Biomass (NDVI) of the genotypes was recorded at three times; tillering (GS25), stem elongation (GS35), and heading stage (GS55) of the plant growth. The canopy temperature was measured when the plants were in the heading period (GS57-61). Physiological parameters were taken in Edirne location. Also, regression graphs are used to predict adaptability and relationship of the characters of genotypes. The data were assessed by analysis of variance and the differences among the means were compared with LSD (Least Significant Differences) at a 5% significant level (Gomez and Gomez, 1984; Kalaycı, 2005). Correlation coefficients among all quality traits were evaluated based on the means of all genotypes in the individual environment.

Results and discussion

Combined analysis of variance across three locations revealed highly significant variation among wheat genotypes for grain yield, biomass at stem elongation and heading stage, and canopy temperature (Table 2). The results of study are presented in Table 2. Main effect of locations and genotypes interaction was also highly significant for investigated parameters. The mean grain yield of the genotypes was 723.0 kg da^{-1}, and the highest yields were obtained in Entry 22 and 24 lines with 826.3 kg da^{-1}, and 823.5 kg da^{-1}, respectively. The highest biomass was scaled in OK81306/STAR'S'4-9 and TCI011322-22 at three plant growth stage. Biomass in genotypes increased with plant growth development and the mean biomass in GS25, GS35 and GS55 was 0.50, 0.71 and 0.77, respectively. There was no significant difference among genotypes for canopy temperature due to experiment carried out normal field condition. The mean canopy temperature was 25.6 ^{o}C, minimum 24.55 ^{o}C and maximum 26.80 ^{o}C. Plant height for lodging resistant is one of the important characters for Trakya region. In this experiment there was significant difference among genotypes for plant height, ranked from 91.3 cm to 115.3 cm and mean was 102.5 cm.

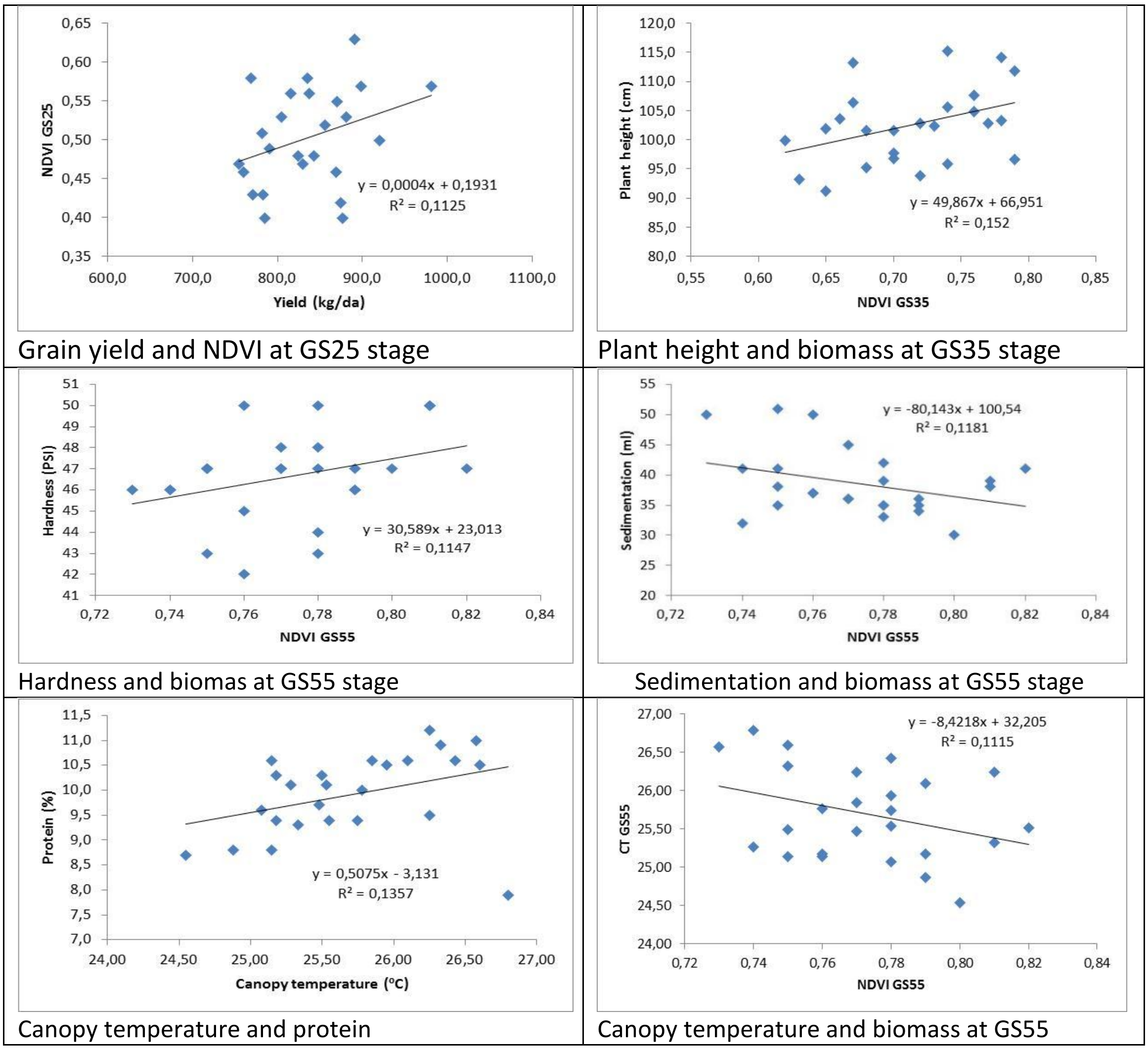
Table 2. The mean grain yield and physiological parameters of the genotypes

No	Genotypes	GY	NDVI (GS25)	NDVI (GS35)	NDVI (GS55)	CT (GS57-61)	PH
1	Aldane	601.2 j	0.46 b-e	0.78 ab	0.75 fgh	26.33 a-d	103.5 cde
2	TE5843-2	748.9 b-e	0.53 a-e	0.70 a-f	0.76 d-g	25.15 b-e	101.8 d-g
3	TCI-01-590	722.8 def	0.55 a-d	0.76 abc	0.77 c-f	26.25 a-d	105.0 cde
4	TE 5793-4	676.0 gh	0.43 cde	0.62 f	0.77 d-g	25.85 a-e	100.0 e-h
5	Selimiye	709.0 efg	0.52 a-e	0.77 ab	0.74 gh	25.28 b-e	103.0 c-f
6	TE 5793-6	681.4 fgh	0.40 e	0.65 def	0.78 b-e	25.75 a-e	102.0 d-g
7	CMSW1WM331S-7	786.9 ab	0.47 b-e	0.67 c-f	0.81 ab	25.33 b-e	113.3 a
8	OK81306/STAR-3-8	766.2 bcd	0.50 a-e	0.67 c-f	0.79 bcd	24.88 de	106.5 cd
9	OK81306/STAR-4-9	777.2 b	0.63 a	0.79 a	0.82 a	25.53 a-e	112.0 ab
10	Bereket	621.9 ıj	0.51 a-e	0.74 a-d	0.75 fgh	25.15 b-e	115.3 a
11	TE6038-11	728.8 cde	0.49 b-e	0.72 a-f	0.76 d-g	25.78 a-e	103.0 c-f
12	TE6217-12	729.1 cde	0.46 b-e	0.70 a-f	0.75 fgh	26.60 ab	97.0 ghı
13	TE6217-13	729.2 cde	0.42 de	0.63 ef	0.77 c-g	25.48 a-e	93.3 ıj
14	TE6217-14	718.0 d-g	0.48 b-e	0.70 a-f	0.78 b-e	25.55 a-e	97.8 f-ı
15	Pehlivan	647.5 hı	0.53 a-e	0.78 ab	0.75 fgh	25.50 a-e	114.3 a
16	BBVD-16	772.6 bc	0.56 abc	0.74 a-d	0.79 bc	26.10 a-d	96.0 hıj
17	BBVD-17	749.0 b-e	0.47 b-e	0.68 b-f	0.73 h	26.58 ab	95.3 hıj
18	BBVD-18	745.1 b-e	0.48 b-e	0.68 b-f	0.81 ab	26.25 a-d	101.8 d-g
19	BBVD-19	641.4 hıj	0.58 ab	0.72 a-f	0.78 bcd	25.95 a-e	94.0 ıj
20	Gelibolu	744.4 b-e	0.40 e	0.66 c-f	0.74 gh	26.80 a	103.8 cde
21	OCW1S304T-21	705.5 efg	0.58 ab	0.73 a-e	0.78 bcd	25.08 cde	102.5 c-f
22	TCI011322-22	826.3 a	0.57 abc	0.76 abc	0.78 b-e	26.43 abc	107.8 bc
23	BBVD-23	744.8 b-e	0.56 abc	0.74 a-d	0.80 ab	24.55 e	105.8 cd
24	CMSA4Y294S-24	823.5 a	0.57 abc	0.79 a	0.76 e-h	25.18 b-e	96.8 ghı
25	BBVD-25	714.5 efg	0.43 cde	0.65 def	0.79 bc	25.18 b-e	91.3 j
Mean		723.0	0.50	0.71	0.77	25.6	102.5
CV (%)		7.9	20.0	10.3	2.2	4.0	3.8
LSD (0.05)		46.1	0.14	0.10	0.03	1.46	5.5
F		**	ns	**	**	ns	**

Note: Significance at **: P<0.01; *: P<0.05; GY: Grain yield (kg da^{-1}), NDVI: Biomass, CT: Canopy temperature, PH: Plant height (cm), GS: Growth Stage

Biomass assessment is essential not only for studies monitoring crop growth, but also in cereal breeding programs as a complementary selection tool (Araus et al., 2009). Tracking changes in biomass may also be a way to detect and quantify the effect of stresses on the crop, since stress may accelerate the senescence of leaves, affecting leaf expansion and plant growth (Royo et al., 2004; Villegas et al., 2001).

Physiological parameter such as biomass and canopy temperature was affected from environmental fluctuations and it was found various relations among investigated traits. In this research as it expected there was positively relation between biomass with grain yield at GS25 (R^2=0.112), plant height at GS35 (R^2=0.152), and grain hardness at GS55 (R^2=0.114). During grain filling period increasing in canopy temperature positively affected protein value in the genotypes (R^2=0.135). It was found slightly negative relation between canopy temperature and biomass at GS55 plant growth stage. These result showed that genotypes which have higher biomass decreased in canopy temperature (Figure 1).



Grain yield and NDVI at GS25 stage | Plant height and biomass at GS35 stage

Hardness and biomas at GS55 stage | Sedimentation and biomass at GS55 stage

Canopy temperature and protein | Canopy temperature and biomass at GS55

Figure 1. Relationship between yield, some agronomic traits and physiological parameters

Correlation coefficients among yield and quality parameters varied according to location and environment condition. Generally, correlations between grain quality traits at each location and yield were low, although a few were significant (Table 3, 4, 5). A positive correlation was observed between grain yield and test weight at three locations. Grain yield was negatively correlated with protein ration at three locations, and at two locations (Edirne and Lüleburgaz) with gluten value. Grain yield also was negatively correlated with sedimentation at Edirne and Lüleburgaz location, whereas positive correlation was observed at Tekirdağ locations. A moderate positive significant correlation was found between test weight with gluten index in Edirne (r=0.731) and Lüleburgaz (r=0.637**) and slightly correlated in Tekirdağ location. There was significantly positive relation between protein ratio with gluten in Edirne (r=0.851**), and in Lüleburgaz (r=0. 814) location. Protein ratio was positively correlated with sedimentation in Edirne (r=0.620**), and Lüleburgaz (r=0.669**) location. Also, it was found positive relation between gluten index and sedimentation and there was significant correlation in Lüleburgaz (r=0.666**) and Tekirdağ (r=0.584**) location.

Table 3. Correlation coefficients among tested characters of cultivars in Lüleburgaz location

Traits	GY	TKW	TW	PRT	GLT	IND	HARD
TKW	0.188						
TW	0.430*	0.312					
PRT	-0.354	0.105	0.093				
GLT	-0.335	0.087	-0.079	0.814**			
IND	0.083	0.131	0.637**	0.183	-0.205		
HARD	0.291	0.394	0.480*	0.357	0.282	0.230	
SED	-0.199	-0.119	0.390	0.620**	0.403	0.666**	0.112

Table 4. Correlation coefficients among the tested characters of cultivars in Tekirdağ location

Traits	GY	TKW	TW	PRT	GLT	IND	HARD
TKW	-0.102						
TW	0.264	0.526**					
PRT	-0.003	0.095	0.208				
GLT	0.057	0.408*	0.320	0.369			
IND	-0.144	0.028	0.245	-0.041	-0.197		
HARD	0.085	0.233	0.050	0.138	0.208	0.159	
SED	0.244	0.226	0.372	0.117	0.478*	0.584**	0.278

Note: Significance at **: P<0.01; *: P<0.05; GY: Grain yield (kg da^{-1}), TKW: 1000-kernel weight (g), TW: Test weight (kg), PRT: Protein ratio (%), GLT: Gluten (%), IND: Gluten index (%), HARD: Hardness (PSI), SED: Sedimentation (ml).

Protein quality and quantity have received more attention than other quality attributes, partly owing to the significant influence imparted by protein on end-use product quality of both common wheat and durum wheat. Environmental factors, such as nitrogen fertilization, water and temperature, influence protein content (Sissons et al., 2005). In contrast, protein quality is largely under genetic control (Lerner et al., 2006; Rogers et al., 2006). Because of the various environment conditions in Trakya region, protein quality and quantity and other quality characters in wheat vary year by year, location by location. Because of this it was determined varies correlation coefficient among investigated quality parameters by locations. Correlation coefficients among the tested characters varied according to location and environment condition. Generally, correlations between grain quality traits at each location and yield were low, although a few were significant (Table 5). A positive correlation was observed between grain yield and biomass in GS25 (r=0.335), GS35 (r=0.245), and GS55 (r=0.137) growth stages of the genotypes. These results indicated that higher biomass at early plant growth stage was significant for yield potential. There was slightly negative relation between canopy temperature with biomass (r=-0.335), and grain yield (r=-0.197). A moderate negative correlation was found between grain yield with protein ratio (r=-0.283), gluten value (r=-0.278) and sedimentation (r=-0.184). For determining biomass of the genotypes data was taken three plant growth stages, tillering, shooting and heading and there were various relations among characters. During tillering stage higher biomass promoted to plant height so, protein ratio, gluten and sedimentation value was affected positively. Biomass in genotypes during shooting phase of crops positively affected and increased plant height, TKW, protein ratio, gluten value and sedimentation value. Increasing in biomass during heading stage negatively affected and decreased canopy temperature, TKW, TW, protein ratio, gluten index and sedimentation value.

Table 5. Correlation coefficients among the tested characters of cultivars in Edirne location

Traits	GY	NDVI GS25	NDVI GS35	NDVI GS55	CT GS55	PH
NDVI GS25	0.335					
NDVI GS35	0.245	0.769**				
NDVI GS55	0.137	0.297	-0.085			
CT GS55	-0.197	-0.277	-0.074	-0.334		
PH	0.087	0.276	0.390	0.136	-0.210	
TKW	0.045	-0.342	0.109	-0.108	-0.040	0.283
TW	0.125	-0.088	0.086	-0.158	-0.016	-0.028
PRT	-0.283	0.204	0.232	-0.166	0.368	-0.283
GLT	-0.278	0.260	0.260	-0.083	0.123	-0.075
IND	0.180	-0.107	0.074	-0.133	-0.161	-0.025
HARD	0.147	-0.069	-0.137	0.339	-0.008	-0.226
SED	-0.184	0.178	0.307	-0.344	0.238	-0.024
Traits	TKW	TW	PRT	GLT	IND	HARD
TW	0.423*					
PRT	-0.322	-0.205				
GLT	-0.157	-0.335	0.851**			
IND	0.280	0.731**	-0.249	-0.464*		
HARD	0.166	0.122	-0.073	-0.003	0.216	
SED	-0.053	0.184	0.669**	0.578**	0.145	-0.026

Note: Significance at **: P<0.01; *: P<0.05; GY: Grain yield (kg da^{-1}), NDVI: Biomass, CT: Canopy temperature, PH: Plant height (cm), TKW: 1000-kernel weight (g), TW: Test weight (kg), PRT: Protein ratio (%), GLT: Gluten (%), IND: Gluten index (%), HARD: Hardness (PSI), SED: Sedimentation (ml)

Conclusions

Combined analysis of variance across three locations revealed highly significant variation among wheat genotypes for grain yield, biomass of stem elongation, heading stage and canopy temperature. The highest yields were obtained from Entry 22 and 24 lines. The highest biomass was scaled in OK81306/STAR'S'4-9 and TCI011322-22 during three-plant growth stages. The biomass from GS25 up to GS35 and GS55 consistently increased of genotypes except four genotypes. A positive correlation was observed between grain yield and biomass at tillering (GS25), shooting (GS35), and heading (GS55) growth stages of genotypes. These results indicated that higher biomass at early plant growth stage was more significant effect for yield potential. There was detected slightly negative relationship between canopy temperature with biomass, and grain yield. It could be that the lower canopy temperatures of genotypes were measured with increasing in biomass in plant. Also, a moderate negative correlation was found between grain yield and protein ratio, gluten value and sedimentation. During tillering stage, higher biomass promoted to plant height and positively affected protein ratio, gluten and sedimentation. In shooting phase of genotypes, biomass positively affected and increased in 1000-kernel weight, protein ratio, gluten value and sedimentation value, as well. But increasing in biomass during heading stage, negatively affected and decreased in canopy temperature, 1000-kernel weight, test weight, protein ratio, gluten index and sedimentation value. Because of the various environment conditions in Trakya region, protein quality and quantity and other quality characters in wheat vary year by year, location by location. Because of this it was determined varies correlation coefficient among investigated quality parameters by locations.

References

1. Araus, J.L. Casadesús, J. & Bort, J. (2001). Recent tools for the screening of physiological traits determining yield, In: Application of physiology in wheat breeding, M.P. Reynolds, J.I. Ortiz-Monasterio & A. McNab (Eds.), pp. 59-77, CIMMYT, ISBN 970- 648-077-3, Mexico D.F.
2. Babar, M.A., Reynolds, M.P., van Ginkel, M., Klatt, A.R., Raun, W.R., Stone, M.L., (2006). Spectral Reflectance to Estimate Genetic Variation for In-Season Biomass, Leaf Chlorophyll, and Canopy Temperature in Wheat. Crop Breeding and Genetics. Crop Sci 46:1046-1057.
3. Blakeney A.B., Cracknell R.L., Crosbie G.B., Jefferies S.P., Miskelly D.M., O'Brien L., Panozzo J.F., Suter D.A.I., Solah V., Watts T., Westcott T., Williams R.M. (2009). Understanding Wheat Quality. p:8. GRDC, Kingston, Australia.
4. Köksel H, Sivri D, Özboy O, Başman A. ve Karacan H.D. (2000). Hububat Laboratuarı El Kitabı. Hacettepe Üni. Müh. Fak. Yay. No:47, Ankara. (Handbook of the Cereal Laboratory. Hacettepe Uni. Fac. of Eng. No: 47, Ankara, Turkey).
5. Fischer, R.A., (2001). Selektion Traits for Improving Yield Potantial. Application of Physiology in Wheat Breeding. Chapter-13, p. 148-159. International Maize and Wheat Improvement Center, CIMMYT. Mexico.
6. Gomez, K.A. and A.A. Gomez. (1984). Statistical Procedures for Agricultural Research. 2nd Ed. John Willey and Sons, Inc. New York. 641.
7. Gutierrez-Rodriguez, M., Reynolds, M.P., Escalante-Estrada, J.A., and Rodriguez-Gonzalez, M.T., (2004). Association between canopy reflectance indices and yield and physiological traits in bread wheat under drought and well-irrigated conditions. Australian Journal of Agricultural Research 55 (11), 1139–1147.
8. Jackson, R.D., Idso, S.B., Reginato, R.J., Pinter, P.J., (1981). Canopy Temperature as a Crop Water Stress Indicator. Water Resources Research, Vol. 17, No. 4 Page: 1133-1138.
9. Kalaycı, M., (2005). Örneklerle Jump Kullanımı ve Tarımsal Araştırma için Varyans Analiz Modelleri. Anadolu Tarımsal Araştırma Enst. Müd. Yayınları, Yayın No: 21, Eskişehir.
10. Lerner, S.E., Seghezzo, M.L., Molfese, E.R., Ponzio, N.R., Cogliatti, M. & Rogers, W.J. (2006). N- and S- fertilisers effects on grain composition, industrial quality and end-use in durum wheat. Journal of Cereal Science, 44, 2–11.
11. Marti J, Bort J, Slafer G.A, Araus J.L. (2007). Can wheat yield be assessed by early measurements of Normalized Difference Vegetation Index? Ann App Bot 150: 253-257.
12. Mangova M, and G. Rachovska, (2004). Technological characteristics of newly developed mutant common winter wheat lines. Plant Soil Environ. 50: 84-87.
13. Morgounov, A., Gummadov, N., Belen, S., Kaya, Y., Keser, M., Mursalova, J., (2014). Association of digital photo parameters and NDVI with winter wheat grain yield in variable environments. Turk. J. Agric. For. (2014) 38: 624-632
14. Pfeiffer, W.H., Braun H.J., (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.
15. Reynolds M.P, Balota M, Delgado M.I.B, Amani I and Fischer R.A. (1994). Physiological and Morphological Traits Associated With Spring Wheat Yield Under Hot, Irrigated Conditions. Australian Journal of Plant Physiology. 21(6) 717-730
16. Reynolds, M.P., Nagarajan, S., Razzaque, M.A., Ageeb, O.A.A., (2001). Heat Tolerance. Application of Physiology in Wheat Breeding, Chapter 10, p.124-135. International Maize and Wheat Improvement Center, CIMMYT. Mexico.
17. Reynolds, M.P., Ortiz-Monasterio J.I, and McNab A.(eds). (2001). Application of Physiology in Wheat Breeding, Mexico, D.F., CIMMYT.
18. Rogers, W.J., Cogliatti, M., Lerner, S.E. (2006). Effects of nitrogen and sulfur fertilizers on gliadin composition of several cultivars of durum wheat. Cereal Chemistry, 83, 677–683.

19. Royo, C.; Aparicio, N.; Blanco, R. & Villegas, D. (2004). Leaf and green area development of durum wheat genotypes grown under Mediterranean conditions. European Journal of Agronomy, Vol.20, No.4, (April 2004), pp.419-430, ISSN 1161-0301
20. Sissons, M.J., Egan, N.E. & Gianibelli, M.C. (2005). New insights into the role of gluten on durum pasta quality using reconstitution method. Cereal Chemistry, 82, 601–608.
21. Villegas, D.; Aparicio, N.; Blanco, R. & Royo, C. (2001). Biomass accumulation and main stem elongation of durum wheat grown under Mediterranean conditions. Annals of Botany, Vol.88, No.4 (October 2001), pp. 617-627, ISSN 0305-7364
22. Zadoks J, Chang T, and Konzak C. (1974). A decimal code for the growth stages of cereals. Weed research 14:415-421.

DIFFERING IN AGRONOMIC AND QUALITY CHARACTERS IN SOME BACKCROSS–DERIVED LINES IN BREAD WHEAT (*Triticum aestivum* L.) GENOTYPES

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Abstract
The backcrossing method remains an efficient tool for transferring genes into established crop varieties. In this study; we were focused on evaluating the selected some backcross lines for yield, grain-quality traits and some agronomic traits under field condition. The experiment was conducted using 15 genotypes in randomized completely blocks design with four replications in Trakya ARI experimental field, during 2009-2010 and 2010-2011 growing years. Grain yield, days of heading and maturing, plant height and some quality parameters and relationship among these parameters were investigated. According to results, there was statistically difference among genotypes in terms of yield and other investigated characters, except for 1000-kernel weight, test weight. Grain yields decreased in Pehlivan, Aldane, Tekirdağ and Dropia backcross lines. It was determined that TKW increased in the backcross derived lines of the Gelibolu, Dropia and Prostor, gluten value increased in Pehlivan and Tekirdağ cultivars. Backcross line for gluten index of the Pehlivan, Aldane, Gelibolu, Prostor and Dropia cultivars highly increased compared with other components. Sedimentation values of the backcross lines of cultivars decreased in Prostor and increased in Pehlivan, Gelibolu and Dropia. The strongly negative correlations were measured between grain yield and days of maturing (r=-0.689**), plant height (r=-0.655**), and lodging resistant (r=-0.743**). Also, grain yield was negatively correlated with protein ratio (r=-0.608*), gluten value (r=-0.541*), and days of heading (r=-0.607*). The negative correlations were found between grain yield and 1000-kernel weight, test weight, hardness, sedimentation, and winter-kill, as well. The strong positive correlations were measured between protein ratio and gluten, hardness, sedimentation, days of heading and maturing, plant height, and lodging resistant. Grain hardness in genotypes increased with the extension of maturation period of the genotypes. Plant height in genotypes strongly correlated with grain yield, protein ratio, gluten value, gluten index, days of heading and maturing.

Keywords: Bread wheat, backcross lines, genotypes, agronomic characters.

Introduction

Bread wheat is the mainly crops is grown in Trakya region and because of the various environment condition biotic and abiotic stress factors causes its yield and quality. Due to fluctuation of rainfall in some growing year causes decrease in grain yield, yield component and quality in wheat production area (Öztürk and Korkut, 2015). 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). Success of a wheat breeding program depends on the regional adaptability, improved and adaptability of cultivars in the target environments determined by its tolerance to biotic and abiotic stresses. The most important abiotic stress factor is the shortage of rainfall in the region (Altay, 2012). Consistency in yield has always been a problem in crop production due to the strong influence of environmental effects during the various stages of crop growth (Yan and Hunt, 2001; Viana and Cruz, 2002). Improvement of end-use quality in bread wheat depends on a thorough understanding of current wheat quality and the influences of genotype, environment, and genotype by environment interaction on quality traits (Yong et al., 2004). Environmental factors play a main role in the expression of genotype characteristics (Peterson et al., 1998). Protein content is in turn influenced mainly by nitrogen fertilization, while the protein quality is determined primarily by the wheat genotype (Samaan et al., 2006). On the other hand, both the quality and the content of the wheat protein are affected by the climatic conditions during wheat maturation (Johans and Svensson, 1998). The effects of genotype x environment were significant on grain quality. Genotype and environment had more effects on flour protein ratio, gluten content, sedimentation value and 1000-kernel weight (Öztürk et al., 2017). Wheat yield potential in Trakya region changes across region due to various environmental conditions. Temperature and rainfall pattern vary greatly from location to location, from year to year. Because of various environmental conditions there was significant difference among genotypes and locations over studied characters. The objective of backcrossing study is to develop a line as higher yield and agronomic character as possible to the recurrent parents and cultivars is producing in the region.

Material and methods

The experiment was conducted at Trakya Agriculture Research Institute experimental field Edirne, Turkey, during 2009-2010 and 2010-2011 growing year. Fifteen genotypes examined in randomized completely blocks design with four replications. All plots were sown into 6 rows, and plot sizes were 6 m^2 at harvesting. In the experiment 500 seed/m^2 was used at planting. The data for grain yield, 1000-kernel weight, test weight, protein ratio, gluten, gluten index, hardness, sedimentation, days of heading and maturing, plant height and relationship among these parameters were investigated. And all parameters compared with backcross derived line with parents. Pehlivan, Aldane, Tekirdağ, Gelibolu, Prostor, Dropia, Todora and Yantar cultivars was used for recurrent parents and backcross lines of those cultivars. Assessment of the genotypes based on investigated parameters carried out under field condition. Grain yield, thousand kernel weights and test weight, (Blakeney et al., 2009), protein ratio (% NIR AACC 39-10), grain hardness, gluten, gluten index, and sedimentation (Köksel et al., 2000; Anonymous, 2002; Anonymous, 1990) were investigated. The quality analysis of Zeleny sedimentation test and wet gluten content were determined according to ICC standard methods No. 116/1 and 106/2, respectively (Anonymous, 1984). Also, regression graphs are used to predict adaptability of genotypes. Data were analysed statistically for analysis of variance following the method described by Gomez and Gomez (1984). The significance of differences among means was compared by using Least Significant Difference (L.S.D. at a %5) test (Kalaycı, 2005).

Results and discussion

The results of variance analysis are presented in Table 1. It was showed that there was statistically difference among genotypes and backcross lines according to yield and other investigated characters excepted test weight and 1000-kernel weight. As a result, grain yields decreased in Pehlivan, Aldane, Tekirdağ and Dropia backcross line while backcross line of the Gelibolu and Prostor cultivars yield increased. TKW increased in the backcross derived lines of the Gelibolu, Dropia and Prostor, for gluten value increased in Pehlivan and Tekirdağ cultivars backcross lines. Also, backcross line for gluten index of the Pehlivan, Aldane, Gelibolu, Prostor and Dropia cultivars highly increased compared with other components. Sedimentation value of the backcross line of cultivars some decreased and some increased (Pehlivan, Gelibolu and Dropia). The grain yield varied from 587.5 kg da^{-1} for Bezostaja-1 to 780.6 kg da^{-1} for backcross line Bez/Prostor*6. Minimum grain yield of 587.5 kg da^{-1} was produced by a very old wheat cultivar Bezostaja-1, in contrast, maximum grain yield of 780.6 kg da^{-1} was produced by Bez/Prostor*6 backcross line. Averaged across years and cultivars the overall mean grain yield was 712.1 kg da^{-1}. This larger variation in grain yield may be due to diverse genetic and environmental factors and their interaction. Also, Bez/Gelibolu*6 and Bez/Prostor*6 backcross lines had higher yield than their parental cultivar. Plant height is important traits to evaluation genotypes especially for lodging resistance. Lodging resistance is one of the mainly important traits in wheat cultivar in Trakya region. Plant height in genotypes varied from 95.0 cm to 130.0 cm and mean was 108.5 cm.

Bez/Aldane*6 and Bez/Tekirdağ*6 backcross lines had lowest plant height according to their parental cultivar and other genotypes. Earliness is other important characters and there was significant difference among genotypes and heading date varied from 120 to 131 days and mean was 124.4 days. Bez/Prostor*6 backcross lines had than their parental cultivar and other genotypes 1000-grain weight, a very important yield component in wheat, varied from 30.9 g to 39.0 g and mean was 35.0 g and Aldane had the highest 1000-kernel weight. Bez/Prostor*6 and Bez/Dropia*6 backcross lines produced higher yield than their parental cultivar. Test weight is other very important quality component in wheat, varied from 78.2 g to 82.3 g and mean TKW was 79.8 g. Protein quality and quantity have received more attention than other quality attributes, partly owing to the significant influence imparted by protein on end-use product quality of both common wheat and durum wheat. Environmental factors, such as nitrogen fertilization, water and temperature, influence protein content (Sissons et al., 2005). In contrast, protein quality is largely under genetic control (Lerner et al., 2006). In this current study, protein ratio varied between 11.3% and 14.9% and mean protein ratio was 12.6%. The maximum protein ratio of 14.9% was produced by cultivar Bezostaya-1 cultivar. Also, Bez/Pehlivan*6 and Bez/Tekirdağ*6 backcross lines had higher protein ratio than their parental cultivar. Table 1 shows mean and ranges of variation for gluten value and gluten index in all genotypes were evaluated across locations. Gluten ratio of the genotypes varied from 28.1% to 42.1% and mean gluten was 34.3%. Bezostaya had the highest gluten ratio with 42.1%. Gluten index in genotypes varied 61.9% to 97.6%. This larger variation in gluten index may be due to diverse genetic and environmental factors. Bez/Pehlivan*6, Bez/Aldane*6, and Bez/Prostor*6 and Bez/Dropia*6 backcross lines had higher gluten index than their parental cultivars. Sedimentation in genotypes and backcross derived lines varied from 39.5 ml to 66.0 ml and the highest sedimentation was determined in Aldane and Bez/Aldane*6 line (66.0 ml). This variation of sedimentation is due to by environmental conditions, agronomic practice and genotypic traits. The mean sedimentation was 51.8 ml. The ranges of variation for hardness in all genotypes were evaluated at growing cycles. Hardness is affected by environmental conditions, agronomic practice and genotypic traits. Hardness in genotypes varied from 41.5 to 52.0 and mean value was 46.9 g. Bezostaya had the highest hardness. Bez/Aldane*6, and Bez/Gelibolu*6 and Bez/Dropia*6 backcross lines had higher hardness than their parental cultivars (Table 1). Correlations among characters were evaluated for the 15 cultivars included in this study. Correlation coefficients among the tested characters of cultivars in Edirne location were given in Table 2. Protein ratio, gluten, days of heading and maturating, plant height and lodging had negative effect for grain yield. In this study, grain yield was strongly negative correlated with days of maturing (r=-0.689**), plant height (r=-0.655**), and lodging resistant (r=-0.743**). Also, grain yield was negatively correlated with protein ratio (r=-0.608*), gluten value (r=-0.541*), and days of heading (r=-0.607*). It was found negative correlation between grain yield with 1000-kernel weight, test weight, hardness, sedimentation and winter kills. Protein content in the mature grain is largely determined by environmental and farm management factors, with genetics playing a minor role in being either low or high in protein content (Blakeney et al., 2009). Protein ratio was strongly positively correlated with gluten ratio (r=0.778**), grain hardness (r=0.646**), sedimentation (r=0.593*), days of heading (r=0.728**), days of maturing (r=0.700**), plant height (r=0.670**), and lodging resistant (r=0.704**). It was determined that a negative correlation between days of heading with plant height (r=0.690**), lodging resistant (r=0.696**), and between days of maturing with lodging resistant (r=0.805**). Grain hardness in genotypes increased with the extension of maturation period of the genotypes. Plant height in genotypes strongly correlated with grain yield, protein ratio, gluten value, gluten index, days of heading and maturing (Table 2).

Table 1. The mean value of the genotypes on agronomic physiological morphological and quality characters

Entry No	Genotypes	GY	TKW	TW	PRT	GLT	IND	HARD	SED	WK	DH	DM	PH	LOD
1	Bezostaya-1	587.5 g	34.5 ab	82.3 a	14.9 a	42.1 a	83.6 bc	52.0 a	54.0 abc	1.5 d	131.0 a	165.5 a	130.0 a	8.0 a
2	Pehlivan	711.2 b-e	35.9 ab	79.9 abc	13.2 b-e	39.3 ab	74.4 c	48.5 def	52.0 abc	1.5 d	127.5 b	162.5 ab	115.0 bc	6.0 b
3	Bez/Pehl*6	703.4 cde	35.7 ab	79.2 abc	13.6 abc	41.4 a	75.9 c	47.0 f	53.0 abc	1.5 d	128.5 b	163.0 ab	117.5 b	6.0 b
4	Aldane	693.3 de	39.0 a	80.3 abc	13.6 ab	36.9 bcd	94.1 ab	49.0 cde	66.0 a	2.5 b	122.5 de	161.0 b	110.0 b-e	6.0 b
5	Bez/Aldane*6	672.3 ef	37.8 a	80.3 abc	13.5 bcd	34.5 cde	96.5 a	50.0 bcd	66.0 a	2.5 b	123.5 cd	160.0 bc	107.5 cde	5.0 bc
6	Tekirdağ	739.2 a-d	35.4 ab	78.2 c	12.2 def	33.4 de	93.0 ab	47.5 ef	55.5 ab	1.5 d	124.5 c	160.5 b	97.5 fg	3.5 cd
7	Bez/Tekirdağ*6	712.2 b-e	33.9 ab	78.9 abc	12.4 b-f	33.6 de	92.0 ab	47.5 ef	54.0 abc	2.0 c	124.5 c	160.5 b	95.0 g	3.5 cd
8	Gelibolu	743.6 a-d	30.9 b	79.5 abc	12.1 ef	28.3 f	98.3 a	41.5 h	50.5 abc	1.5 d	124.5 c	159.0 bc	102.5 efg	4.0 cd
9	Bez/Gelibolu*6	752.5 abc	31.4 b	79.8 abc	12.1 ef	28.1 f	97.4 a	42.5 gh	51.0 abc	1.5 d	124.5 c	159.0 bc	107.5 cde	4.0 cd
10	Prostor	760.3 ab	35.4 ab	79.6 abc	11.8 f	30.9 ef	90.7 ab	42.0 h	53.5 abc	1.5 d	121.0 ef	156.0 c	102.5 efg	3.0 d
11	Bez/Prostor*6	780.6 a	35.7 ab	79.7 abc	11.6 f	30.9 ef	91.4 ab	42.0 h	39.5 c	1.5 d	120.0 f	156.0 c	105.0 def	3.5 cd
12	Dropia	726.2 bcd	34.0 ab	81.1 abc	12.4 b-f	32.7 de	95.7 a	49.0 cde	45.5 bc	0.5 e	123.0 cd	160.5 b	105.0 def	3.5 cd
13	Bez/Dropia*6	711.3 b-e	35.5 ab	81.6 ab	12.2 c-f	32.0 ef	97.6 a	50.5 abc	54.0 abc	0.5 e	124.0 cd	161.5 ab	107.5 cde	4.0 cd
14	Bez/Todora*6	631.6 fg	34.9 ab	78.8 bc	11.3 f	32.9 de	76.0 c	44.0 g	40.0 bc	3.5 a	123.5 cd	161.0 b	112.5 bcd	6.0 b
15	Bez/Yantar*6	756.5 ab	35.9 ab	78.3 bc	12.3 b-f	38.2 abc	61.9 d	51.5 ab	42.5 bc	2.5 b	124.0 cd	162.5 ab	112.5 bcd	6.0 b
Mean		712.1	35.0	79.8	12.6	34.3	87.8	46.9	51.8	1.7	124.4	160.6	108.5	4.8
C.V (%)		7.1	1.9	7	5	5.7	6.3	1.7	14	10.4	0.7	1.3	3.4	--
LSD (0.05)		50.7	3.3	5.4	1.4	4.2	11.9	1.7	15.4	0.4	1.9	4.4	7.9	1.9
F cultivar		**	ns	ns	**	**	**	**	*	**	**	*	**	**
F year		ns	*	**	**	**	*	**	**	**	**	**	**	**

Note: Significance at **: P<0.01 and *: P<0.05; GY: Grain yield (kg da^{-1}), TKW: 1000-kernel weight (g), TW: Test weight (kg), PRT: Protein ratio (%), GLT: Gluten (%), IND: Gluten index (%), HARD: Hardness (PSI), SED: Sedimentation (ml) WK: Winter kill (0-9), DH: Days of heading, DM: Days of maturing, PH: Plant height (cm), LOD: Lodging resistant

Table 2. The correlation coefficients among yield quality agro-morphological characters in Edirne locations

Traits	GY	TKW	TW	PRT	GLT	IND	HARD	SED	WK	DH	DM	PH
TKW	-0.200											
TW	-0.452	0.047										
PRT	-0.608*	0.340	0.526*									
GLT	-0.541*	0.511	0.160	0.778**								
IND	0.168	-0.223	0.365	-0.114	-0.652**							
HARD	-0.474	0.497	0.375	0.646**	0.703**	-0.289						
SED	-0.251	0.427	0.267	0.593*	0.200	0.421	0.345					
WK	-0.339	0.324	-0.490	-0.053	0.154	-0.423	-0.041	0.008				
DH	-0.607*	-0.152	0.282	0.728**	0.699**	-0.361	0.464	0.169	-0.108			
DM	-0.689**	0.146	0.280	0.700**	0.803**	-0.488	0.778**	0.159	0.066	0.850**		
PH	-0.655**	0.181	0.476	0.670**	0.727**	-0.524*	0.425	-0.029	0.110	0.690**	0.697**	
LOD	-0.743**	0.311	0.253	0.704**	0.812**	-0.610*	0.532*	0.105	0.410	0.696**	0.805**	0.904**

Note: Significance at **: P<0.01 and *: P<0.05; GY: Grain yield (kg da^{-1}), TKW: 1000-kernel weight (g), TW: Test weight (kg), PRT: Protein ratio (%), GLT: Gluten (%), IND: Gluten index (%), HARD: Hardness (PSI), SED: Sedimentation (ml) WK: Winter kill (0-9), DH: Days of heading, DM: Days of maturing, PH: Plant height (cm), LOD: Lodging resistant

Grain yield and investigated quality parameters were assessed and it was found various relationships based on genotypes and environment interaction. As it expected there was negatively relation between grain yield with protein ratio (R^2=0.369), and gluten value (R^2=0.292). Also, it was found slightly and negatively relation between yield and winter kill (R^2=0.115). This result showed that genotypes which have resistant to winter kill had the highest grain yield. There was negative relation between grain yield and test weight (R^2=0.203). There was positively relation between protein ratio with test weight (R^2=0.276) and 1000-kernel weight (R^2=0.115). Gluten value was positively correlated with TKW (R^2=0.260), and negatively correlated with gluten index (R^2=0.425).

Conclusions

According to results, there was statistically difference among genotypes in terms of yield and other investigated characters, except for 1000-kernel weight, test weight. Grain yields decreased in Pehlivan, Aldane, Tekirdağ and Dropia backcross lines. It was determined that TKW increased in the backcross derived lines of the Gelibolu, Dropia and Prostor, gluten value increased in Pehlivan and Tekirdağ cultivars. Backcross line for gluten index of the Pehlivan, Aldane, Gelibolu, Prostor and Dropia cultivars highly increased compared with other components. Sedimentation values of the backcross lines of cultivars decreased in Prostor and increased in Pehlivan, Gelibolu and Dropia. The strongly negative correlations were measured between grain yield and days of maturing, plant height, and lodging resistant. Also, grain yield was negatively correlated with protein ratio, gluten value, and days of heading. The negative correlations were found between grain yield and 1000-kernel weight, test weight, hardness, sedimentation, and winter-kill, as well. The strong positive correlations were measured between protein ratio and gluten ratio, grain hardness, sedimentation, days of heading, days of maturing, plant height, and lodging resistant. It was measured negative correlations between days of heading and plant height, and lodging resistant. Grain hardness in genotypes increased with the extension of maturation period of the genotypes. Plant height in genotypes strongly correlated with grain yield, protein ratio, gluten value, gluten index, days of heading and maturing. Overall, the results of the investigated parameters demonstrated that the environmental conditions such as temperature and rainfall during grain filling period, agronomic practices, and wheat genotypes could affect the grain physical characteristic and hence the grain quality. It was concluded that an increase in some investigated parameters backcross program could be used in breeding program to develop better genotypes.

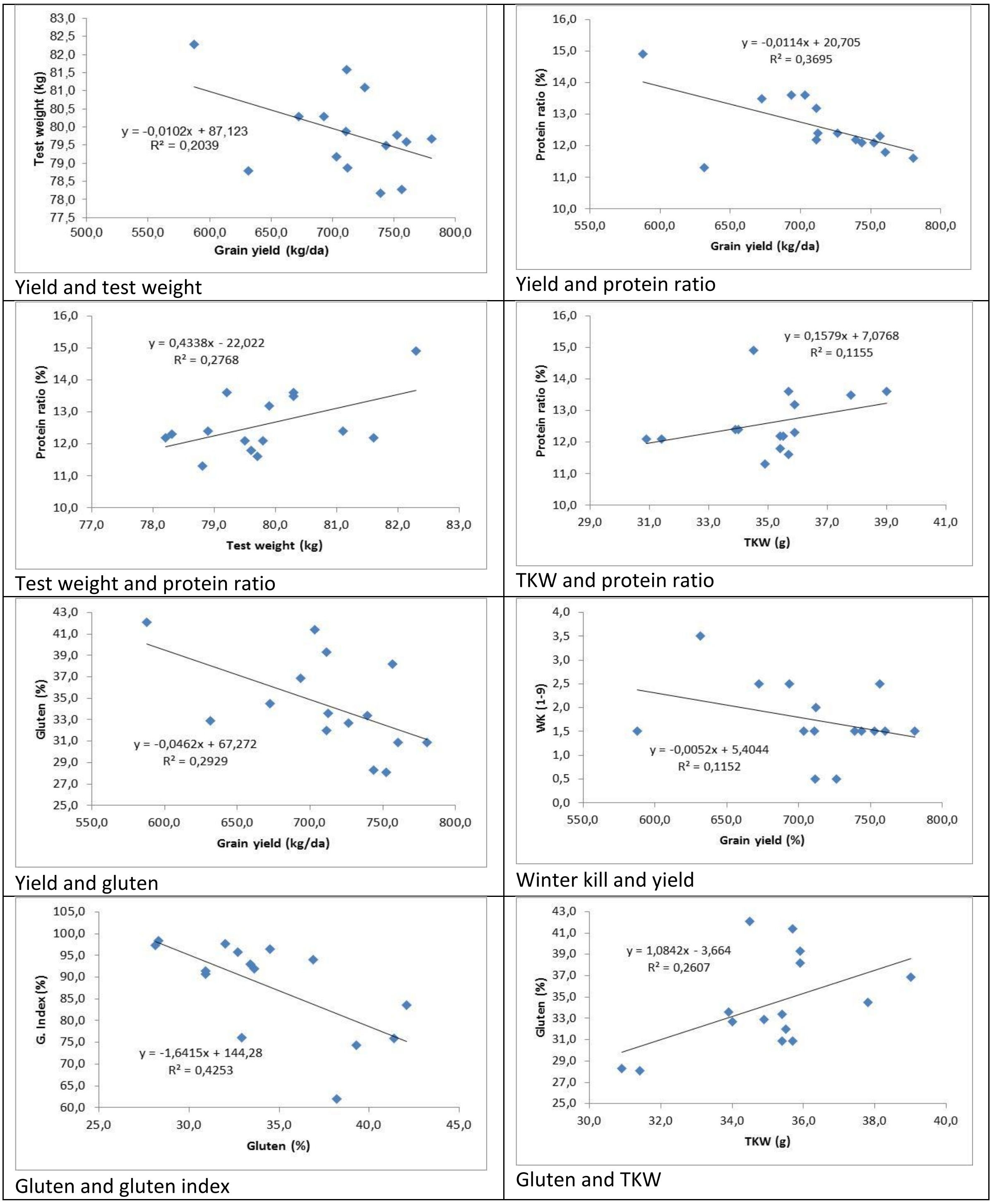

Figure 1. Relation among yield and yield component and quality parameters

References

1. Altay F. (2012). Yield stability of some Turkish winter wheat (*Triticum aestivum* L.) genotypes in the western transitional zone of Turkey. Turkish Journal Of Field Crops, 2012, 17(2): 129-134
2. Anonymous, (1984). ICC.Standard methods of the international association for cereal chemistry (ICC). Methods No. 116/1. Vienna Verlag Moritz Schafer. Detmold, Germany.
3. Anonymous, (1990). AACC Approved Methods of the American Association of Cereal Chemist, USA.
4. Anonymous, (2002). International Association for Cereal Sci. and Technology. (ICC Standart No: 110, Standart No: 105, Standart No: 106, Standart No: 155, Standart No: 116, Standart No: 115).
5. Gomez, K.A. and A.A. Gomez. (1984). Statistical Procedures for Agricultural Research. 2nd Ed. John Willey and Sons, Inc. New York. 641.
6. Samaan J, El-Khayat, G.H, Manthey, F, Fuller, M, Brennan, C.S. (2006). Durum wheat quality: II The relationship of kernel physicochemical composition to semolina quality and end product utilisation. Int. J. Food Sci. Technol., 41, 47–55.
7. Johansson, E, Svensson, G. (1998). Variation in bread making quality: Effect of weather parameters on protein concentration and quality in some Swedish wheat cultivars grown during the period 1975–1996. J. Sci. Food Agric., 78, 109–118.
8. Kalaycı, M., (2005). Örneklerle Jump Kullanımı ve Tarımsal Araştırma için Varyans Analiz Modelleri. Anadolu Tarımsal Araştırma Enst. Müd. Yayınları, Yayın No: 21, Eskişehir.
9. Köksel H, Sivri D, Özboy O, Başman A. ve Karacan H.D. (2000). Hububat Laboratuarı El Kitabı. Hacettepe Üni. Müh. Fak. Yay. No: 47, Ankara. (Handbook of the Cereal Laboratory. Hacettepe Uni. Fac. of Eng. No: 47, Ankara, Turkey).
10. Lerner, S.E., Seghezzo, M.L., Molfese, E.R., Ponzio, N.R., Cogliatti, M. & Rogers, W.J. (2006). N- and S- fertilisers effects on grain composition, industrial quality and end-use in durum wheat. Journal of Cereal Science, 44, 2–11.
11. Öztürk İ, Korkut K.Z. (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.
12. Öztürk İ, Çiftçigil TH, Kahraman T, Avcı R, Seidi M, Girgin VÇ, Tülek A, Tuna B, Akın K. (2017). Relationship Among Biotic Stress Factors And Agronomic Characters In Bread Wheat (*Triticum aestivum* L.) Genotypes under Various Environmental Conditions. Agriculture and Food. Vol: 5, p: 551-561.
13. Peterson C.J., Graybosch R.A., Shelton D.R., and Baenziger P.S. (1998). Baking quality of hard red winter wheat: Response of cultivars to environments in the Great Plains. Euphytica 100 (1-3): 157-162.
14. Sissons, M.J., Egan, N.E. & Gianibelli, M.C. (2005). New insights into the role of gluten on durum pasta quality using reconstitution method. Cereal Chemistry, 82, 601–608.
15. Viana, J.M.S., and Cruz, C.D., (200). Analysis of stability and adaptability through different models of linear regression. Cienc Agrotec Lavras, 26: 455-462.
16. Yan, W., and Hunt, L.A., (2001). Interpretation of genotype x environment interaction for winter wheat yield in Ontario. Crop Sci., 41: 19-25.
17. Yong Z, Zhonghu H, Aimin Z, Van Ginkel M. (2004). Effect of environment and genotype on bread-making quality of spring-sown spring wheat cultivars in China. Euphytica. March 2004, Volume 139, Issue 1, pp 75–83.
18. Zadoks J, Chang T, and Konzak C. (1974). A decimal code for the growth stages of cereals. Weed research 14:415-421.

THE EFFECTS OF USING DIFFERENT SYSTEMS OF PRODUCTION OF TOBACCO SEEDLINGS

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Abstract
Tobacco is an economically important crop in Macedonia. To improve tobacco production is necessary good agricultural practice, which also involves the implementation of new technologies for production of tobacco seedlings. The Floating Trays System (FTS) is an advanced plant growing technology, which is particularly important when it comes to the resistance of tobacco plants and adaptation to a new environment (unavoidable physiological stress after transplantation). It represents an environmentally acceptable technology that allows respectively management chemicals and decreasing the risks of pollution on the humans and environmental management, as well as the establishment of sustainable development at the global level. FTS also provides a high yield and high quality of tobacco. Allows maximum use of dissolved nutrients, so plants form a strong root system and have a rapid development after transplantation. Further development of strong stems enables the formation of an adequate number of leaves even in unfavorable climatic conditions for tobacco, and synthesizes and accumulates more dry mass per plant. The experiment was set in a randomized block system in four repetitions on two oriental tobacco cultivars (*prilep NS 72* and *yaka YV 125/3*), each in three variants: variant 1-control (conventional system of production); variant 2-N and variant 3-P (the soilless system using Floating Trays). Among the systems evaluated, the soilless system is technically the most successful and innovative which represents a technological progress for producing uniform seedlings for their quick formation in the field and to establish more homogeneous plantations. The results obtained in this trial showed significant differences among the tested variants.

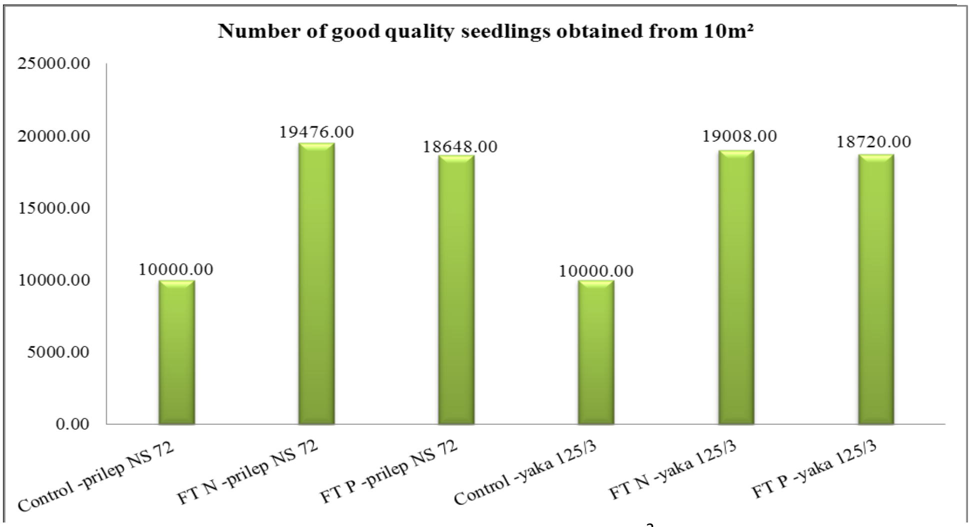
Keywords: Tobacco seedlings quality, floating system versus conventional.

Introduction

The production of oriental tobacco in Macedonia has a very long tradition. Tobacco is one of the most economically important agricultural crops in the country. To produce high-quality tobacco, growers must begin with healthy seedlings. The ideal seedling is disease free, hardy enough to survive transplanting shock, and available for transplanting on time. In general, earlier transplanted seedlings give better yield than late-transplanted tobacco (*Smith et al., 2003*). During the vegetation, a large number of factors have an impact on the tobacco that allow or interfere on the tobacco plant to express its biological and production potentials. Except the biological potential of the varieties, the largest influences have taken scientific farming methods and agro ecological conditions during the growing season. Each type of tobacco, variety, requires separate intervention depending on the intensity of biotic agents, soil and climatic conditions, as well as cultural, traditional practices in one area. Macedonian production is traditionally the production of oriental type of tobacco. The production of oriental tobacco in Macedonia is located almost in all regions, soils with weaker productivity. For successful production, there must be a good quality of tobacco seedlings, to obtain uniformity according to morphological and biological characteristics of tobacco at field. After transplanting in the field tobacco plants for a few days survives a so-called transplantation shock because of unfavorable external conditions. This change is very insignificant in terms of seedlings produced by the classical mode of production, where there is an extremely large imbalance between the root system and the new conditions that often result in extended periods of stress, poor reception and slow plant growth (post transplanted shock). This shock is caused by the loss of most of the root system in the process of uprooting the seedlings from the beds (*Hoyert, 1979*). The production of healthy seedlings is the first step in the production of high quality tobacco. Use of FTS technology, means having more resistant and rapidly recovering plants after transplanting. The percentage of accepted plants is significantly higher in comparison to conventional seedlings. This directly affects crop yield per unit area and reduces labor costs. Improving tobacco production requires good agricultural practice which among other things involves implementation of new production technologies. The aim of this study was to show the differences that are very important between conventional production and the FTS technology that relate to their quantitative properties, and after transplantation to the qualitative properties of tobacco raw material. With the exposed results, we wanted to give contribution to the farmers education improvement of and wide implementation of the FTS production of seedling in Macedonia.

Material and methods

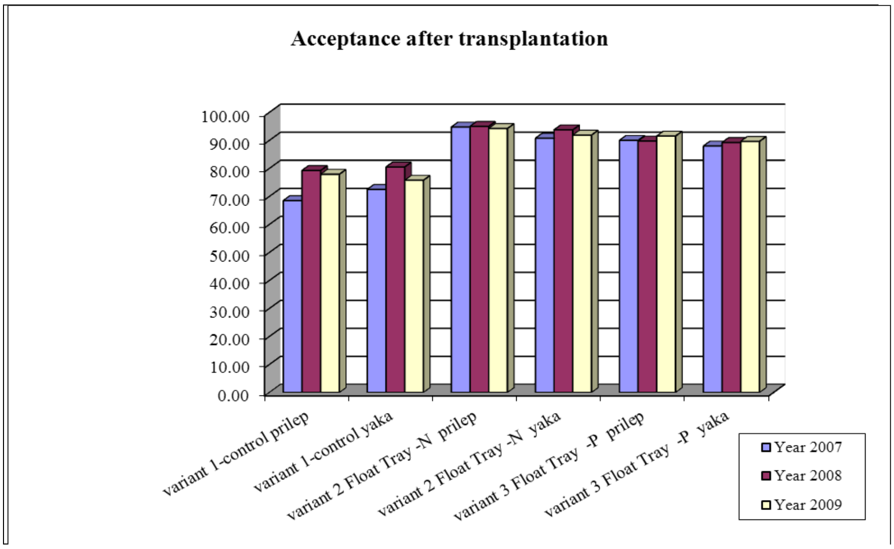
The experiment was conducted in cooperation with the tobacco company Veles Tabak, in Veles, in random block system, four replications on two varieties of oriental tobacco *prilep NS 72* and *yaka JV 125:* Variant 1-control ø (conventional production); Variant 2 - Float Tray (TERRA STAR 22:11:22+2Mg with microelements: Fe-0,0335 %, Cu-0,017 %, Mg-0,1 %, B-0,01 %, Mn-0,017 %, Mo in traces, Zn-0,01 %, Co in traces, +EDTA and Auxin) and Variant 3-Float Tray (CHELAN 11:49:12+2Mg with microelements: Fe-0,0335 %, Cu-0,017 %, Mg-0,1 %, B-0,01 %, Mn-0,017 %, Mo in traces, Zn-0,01 %, Co in traces). The total quantity of fertilizer was added in water beds (0,001 % solution). In the period from 2007 to 2009, the following materials were necessary: the required quantity of certificated tobacco seed (granulated tobacco seed for FT variant), peat with following characteristics: pH ($CaCl_2$) 5,0-6,0; mean structure of particles, organic content: 250-400 mg / L N, 280-450 mg / L P_2O_5 and 320-500 mg / L K_2O % (polystyrene trays with 589 alveolus per tray were filled with peat 50% and perlite 50%); polyethylene for covering water beds; agril (as a protector towards condensation) and protection (10 ppm Ridomil MZ 72 -am. Metalaksil+Mankozeb –against *Pernospora tabacina* Adam, and 10 ppm Fundazol-50 WP -am. Benomyl 50 %), against diseases which damage the seedlings, such as: *Pythium sp*. and *Rhizoctonia solani*. As a prevention, Decis EC 25 (am. Deltametrin) was added in concentration 0.05 % against *Trips tabaci* Lind, *Myzus persicae* Sulz., and other insects. Water conductivity into the pool on FTS was followed regularly (EC is a commonly used indicator of fertilizer salts levels in media and water) for keeping the concentration of fertilizer. Measuring was with conduct meter (DIST WP 4 Conductivity/TDS meter, HANNA instruments, range 0,01-19,99 mS/cm (mmho/cm) with ATC), with reading the conductivity of clean water first, and then conductivity of the solution. Thermoregulation above the pool was conducted with uncovering of the tunnel. Traditional technology of seedlings production needs additional fertilizers to the plants (1 % solution), which follows with application of fungicides and insecticides, and 7 days before transplanting to make vigorous seedlings. All agro technical measures for proper development of the plants were made for both technologies of seedlings production, in order to obtain maximum healthy, usable seedlings per unit area (Graph.1). In this period, suitable agrotechnical measures (watering, nutrition and protection) were applied in order to obtain healthy and well developed seedlings. Before transplanting, the number of usable seedlings was determinate. After transplanting, the number of accepted plants (%) was determinate. Common agro-technical measures for successful production experiment were conducted. The soil preparation for cultivation consisted of basic processing to improve soil structure and to create favorable conditions for normal microbial processes to enhance disposal of water-air-heat regime in the soil, to reduce weeds and etc. Transplantation of tobacco was done manually. Transplanting time was different, applicable for the Veles area and in close connection with the meteorological conditions during the test period. In the three studied years the standard agro–technical measures were conducted. Special attention was given to the choice of surface of the land, the crop rotation where the predecessor was barley (*Hordeum sativum L.*). After transplantation of tobacco seedlings, the number of accepted plants (%), and the crisis period (from transplanting to acceptance) were analyzed, as well as dry mass of tobacco (kg/ha) obtained after harvesting. The results were processed by SPSS for Windows, procedure Sum of squares, Model III.



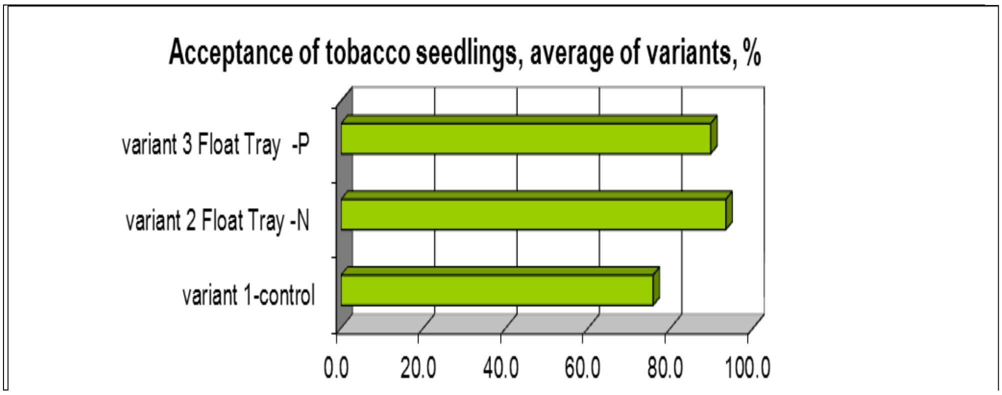
Graph 1. Difference between numbers of seedlings obtained from $10m^2$ from different production technologies, by variants

Results and discussion

Time of transplanting, as an important point of fields stage, primarily depends on the time when the seedlings are ready for transplanting and weather conditions at the time of the year. The period after transplanting is considered crucial for the overall production at the end of vegetation. Therefore, after transplanting, the plants should have enough available moisture well to develop the first leaves. The level of acceptance of tobacco seedlings depends on agro-environmental features at the time of transplanting, agro-technical measures, but mostly from the quality of seedlings. After successful acceptance, the plant develops quickly and steadily by forming leaves from the bottom to the top (*Uzunovski,* 1989). The seedlings derived from the conventional production (variant 1-control ø), have longer critical period after transplantation in both examined varieties, compared with plants from FTS technology. The percentage of acceptance depends very much on the climatic conditions in the year of production in all examined variants Graph 2 shows the acceptance between variants after transplanting. Graph 3 shows that the seedlings produced in a conventional way are less resistant to new conditions and are hardly accepted. The lowest acceptance shows variant 1-control (75.7 %), which proves that accepting tobacco in the field depends on its quality, expressed above all with a well-developed root system and morphological uniformity in terms of its dimensions (*Pearce & Palmer, 2005*). With the conventional production of seedlings it is not possible. Rates of tested nitrogen fertilizer show difference in tobacco seedlings quality, so greater acceptance due to the quality produced seedlings (93.4% acceptance for variant 2-FT, N, and 89.73 % for variant 3-FT, P). This is very important when it comes to the quality of the fertilizer used and recommended in the production of transplants (Graph 3). Because of existence of proper ingredients (food, water, air, etc.) and not existence of mutual competition, seedlings from FTS technology are equal, with balanced growth and development (strong roots, well-developed stem and number of leaves). Plants are morphologically identical during growth and development in terms of height, number of leaves, the size of the leaves that are almost identical (*Turshic, 2000*). After the technological maturity, the tobacco is harvested and dried. Measured dry mass of tobacco (kg/ha) after harvesting show the following results (Table 1).



Graph 2. Acceptance of tobacco seedlings, %



Graph 3. Acceptance after transplanting (average of variants)

Table 1. Average dry mass of tobacco (kg/ha)

PNS 72			*Y V 125/3*		
Harvest	Dry mass, kg/ha	Index	Harvest	Dry mass, kg/ha	Index
Control ø			Control ø		
2007	2139.2	100	2007	1893.0	100
2008	2359.8	100	2008	2548.0	100
2009	2582.1	100	2009	2120.4	100
Average	2360.4	100	Average	2187.1	100
FTS N			FTS N		
2007	2600.9	122	2007	2231.6	118
2008	3060.9	130	2008	2941.2	115
2009	3095.1	120	2009	2599.2	123
Average	2919.0	124	Average	2590.7	118
FTS P			FTS P		
2007	2354.7	110	2007	1923.9	101
2008	2598.9	110	2008	2582.1	101
2009	2889.9	112	2009	2428.2	115
Average	2614.5	111	Average	2311.4	106

The production technology also influences the yield of the dry mass tobacco. The yield is higher for 24 % and 18 % respectively for variants of 2 FTS -N or 11 % and respectively by 6 % for variants of 3 FTS – P, compared with control variant for both varieties. Differences in yield between variants 2 and 3 are result of increased ratios of macro elements in the fertilizer combination. In all tested variants (control, FTS -N and FTS -P), the average yield per dry weight per unit area is lower in the variety *yaka JV 125/3* in comparison to variety *prilep NS 72*, which is a result of the characteristics of the certain variety.

Table 2 Dry mass yield (kg/ha), interaction Harvest/Technology

Harvest	Technology			Average
	Control	FT N	FT P	
2007	2016.1	2416.2	2139.3	2190.5
2008	2453.9	3001.1	2590.5	2681.8
2009	2351.3	2847.2	2659.1	2619.2
Average – technology	2273.7	2754.8	2462.9	

LSD Test

DV	Harvest		Mean value difference	SD	Significance
Yield kg/ha	2007	2008	-491,2667**	143,04326	0,007
	2007	2009	-428,6167*	143,04326	0,015
	2009	2008	-62,6500	143,04326	0,672
DV	**Technology**		**Mean value difference**	**SD**	**Significance**
Yield kg/ha	1	2	-481,0833**	143,04326	0,008
	1	3	-189,2000	143,04326	0,219
	3	2	-291,8833	143,04326	0,072

The average yield values for dry mass show that variant 2 FTS -N contributed with 2754.8 kg/ha or 21% higher yield of variant 1-control. Regarding the harvest, the highest yield was obtained in 2008. From the data presented in the Table 2/LSD, it can be noticed that there is a statistically significant difference between the yield per unit area (kg/ha) in the 2007 harvest compared to the 2008 harvest, with the 2007 harvest relative to the harvest 2009. In terms of production technology, it is noted that there is a statistically significant difference between the yield per unit area (kg/ha) of variant 1 (conventional production) and variant 2 FTS -N. Among the other variants, there is no high statistical difference in relation to the yield per unit area.

Conclusions

FTS technology represents modern and cost-effective tobacco seedling production technology that achieves greater efficiency in work processes and higher results. The used seedlings production area (which will provide an adequate number of plants /area) is almost 2 times smaller than that in conventional way of production. The conditions provided by this technology have a strong impact on the growth and development of the seedlings (more suitable plants for transplantation, on average 90 %), in relation to the classical way of producing tobacco seedlings. Among the systems evaluated, the soilless system (FTS technology) is technically the most successful and innovative which represents a technological progress for producing uniform seedlings. Due to the same dimensions of the plants - the transplant effect is increased, and the basic needs of the labor force is minimal; there is a quick formation in the field with more homogeneous plantations. With high acceptance of the seedlings (variant 2 FTS N - 93.4% was emphasized) plants rapidly continue their growth and development. The effects of using advanced technology versus conventional are multiple. Variant 2 – FTS N, showed higher results in terms of yield and quality. The average yield per dry mass per unit area is the highest in variant 2 of the container N, for example: in the variety *prilep NS 72* – 2919.0 kg ha. FTS is ecologically acceptable technology that enables appropriate chemical management and reduction of risks from human and environmental pollution. It also ensure establishment of sustainable development on a global scale.

References

1. Georgievski K., 1990. Tobacco and tobacco proceedings – with special emphasis on the ecology of tobacco. Economic press, Skopje, 556.
2. Pearce B., Palmer G., 2005. Water Quality Guidelines for Tobacco Float Systems, Using Conductivity Meters for Nitrogen Management in Float Systems. Cooperative Extension Service – University of Kentucky- College of Agriculture, AGR-164.
3. Smith, W. D., Fisher, L. R., Spears, J. F., 2003. Transplant production in the float system. In Flue-Cured Tobacco Information. North Carolina Cooperative Extension Service, Raleigh.
4. Turshic I., 2000. UNIDO project MP/CRO/98/058, Tobacco Institute Zagreb.
5. Hoyert J.H., 1979. Growing and transplanting tobacco seedlings with intact root systems. Md. Agric. Exp. Stn. Coll. Park. Misc. Public. N° 944, 29.
6. Uzunovski M., 1989. Production of tobacco. Economy newspaper, Skopje, Faculty of agriculture, Skopje, 447.

EFFECTS OF DIFFERENT VARIANTS OF STERILE AND FERTILE PLANTS ON MAIZE HYBRID YIELDS

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Abstract
Maize is a very important field crop according to both, its distribution and sown areas. The possibility of different utilisation of maize for food, feed and industrial processing is greatly contribute to high economic significance of this crop. The aim of conducted studies was to determine the effect of various proportions of fertile and sterile plants on the yield by performing trials in a certain location. The three-replication trial was set up according to the randomised complete block design in the location of Zemun Polje under conditions dry land farming. A mixture of different variants of sterile and fertile plants of the commercial hybrid ZPSC 341 was made. Statistical data processing included the analysis of variance according to the randomised complete block design, regression and correlation analyses of grain yield and the percentage of fertile plants in the hybrid ZPSC 341, in order to determine the changes in grain yields in relation to the percentage ratio of sterile to fertile plants. Obtained results indicate that the highest (15.472 t ha^{-1}), i.e. lowest (14.046 t ha^{-1}) average yield was recorded in the hybrid with 80%, i.e. 5% fertility, respectively. The coefficient of correlation points to a weak dependence of the yield and the fertility percentage (rxy=0.101). Based on the coefficient of determination, the percentage dependence between the yield and the percentage of fertile plants was low (R^2=0.010).

Keywords: cytoplasmic male sterility, maize, yield.

Introduction

Maize (*Zea mays* L.), alongside with wheat and rice, is the most important crop in the modern global agricultural production. Based on sown areas and the production, maize ranks third and second, respectively (Glamočlija, 2004). The first description of male sterility was provided by Rhoades (1931). Further researches showed that cytoplasmic factors were responsible for sterility. Cytoplasmic male sterility (CMS) refers to the inability of the plant to produce functional pollen. This trait is conditioned by mutations in the mitochondrial genome, so it is transmitted through the cytoplasm, i.e. it is not transmitted by pollen and is not subjected to the Mendelian inheritance. CMS has found its application in the production of hybrid maize seed, because this production is based on the sterility and therefore it is not necessary to detassel female inbred lines. Maize hybrids developed on the sterile basis are derived by crossing of the female component with a sterile cytoplasm, and the male component with restorer genes for that type of sterility in the nuclear genome, so that male fertility would be restored in the F1 generation, i.e. in the hybrid. Along with the introduction of such a system in the hybrid production, studies on effects of CMS on traits of maize genotypes have been initiated. Many unrelated studies have shown a positive effect of cytoplasmic male sterility on maize grain yield, especially under adverse conditions of drought, deficit of water and nutrients.

Nitrogen requirements of sterile plants are lower by approximately 10-30 kg ha^{-1} than of fertile plants, hence this amount of nutrients instead of being used to form pollen is directed into female reproductive organs, thus resulting in the grain yield increase. The sink strength of maize ears is great and they continuously import N assimilates during grain filling (Hirel et al., 2005). On the other hand, CMS plants may store and redirect nitrogen into the ear so as to contribute to a higher grain yield. Reduced consumption of nitrogen, water and energy for pollen formation in sterile plants during the flowering period may result in a greater number of kernels per ear (Vega et al., 2001). Moreover, the cultivation of commercial crops in this way can prevent contamination by genetically modified (GMO) pollen, in case the sterile hybrid is genetically modified. Considering that the production of sterile hybrids is not more complicated than the production of fertile ones, the proposed production system may be a simple answer to the constant requirements for increasing maize yield without increasing the cultivation areas. The main goal in the commercial maize production is the highest possible grain yield, along with other favourable agronomic traits. Increasingly strong competition in the maize seed market requires studies on the effect of the type of cytoplasm and its interaction with a genotype on yield and some morphological traits for the purpose of the production.

Material and methods

Total of 21 mixtures of 0, 5, 10, …up to 100% fertile plants mixed with the sterile variant of the hybrid ZP 341 were made and used as a basic material. In order to control reliability of the experiment, the original fertile hybrid ZPSC 341 was included three times as a check (ZPSC 341 from manual pollination, ZPSC 341F1 and ZPSC 341 from reciprocal crossing). The ZPSC 341 is a medium early maturity hybrid belonging to the FAO maturity group 300-400 and it has been developed at the Maize Research Institute, Zemun Polje and has been used in the commercial production for years.

Material and methods in performing field trials

The three-replication trial was set up according to the randomised complete block design in the location of Zemun Polje in 2013. The elementary plot size was 5.18 m². The plot consisted of two rows with the inter-row distance of 0.7m, 10 hills with the inter-hill distance of 0.37m and 2 plants per hill. Sowing was done manually on the chernozem type of soil in the first week of May. The common cropping practices were applied: autumn ploughing; seedbed preparation; crop cultivation was done immediately after sowing when herbicides were sprayed (2 L Acetochlor ha^{-1} (a.i. acetochlor)), while corrective spraying was done after emergence at the 3-4-leaf stage (1 L Motivell ha^{-1} (a.i.nicosulfuron) + 0.25 L Callisto ha^{-1} (a.i. mezotrion)). The total number of plants, including a separate number of both, fertile and sterile plants, were recorded for each elementary plot during the pollen season. Harvest was done manually at the stage of physiological maturity. A sample of five ears was taken from each plot to analyse agronomic traits. After harvest, samples were placed in a dryer at 35°C for several days to achieve equilibrium moisture.

Methods of experimental data processing

Statistical data processing encompassed the following: analysis of variance according to randomised complete block design, regression analysis and correlation analysis of grain yield and the percentage of fertile plants in the hybrid ZPSC 341, in order to establish the changes in grain yields related to the sterile to fertile plants ratio (Hadživuković, 1991).

Results and discussion

According to data presented in Table 1, it is obvious that the highest (15.472 t ha^{-1}), i.e. lowest (14.046 t ha^{-1}) average yield was recorded in the hybrid with 80%, i.e. 5% fertility (ZPSC 341F1), respectively. Based on the findings, it was concluded that the soil quality and climatic conditions were of crucial importance in the given location. Similar studies have been performed by

Weingartner et al. (2002b). These authors pointed out that sterility affected the yield increase in maize hybrids, but not significantly. Munsch (2008) has observed 12 hybrids with different types of sterility and determined that the effect of CMS on grain yield varied from - 8% to +8%. Although the type of cytoplasm was not decisive, three hybrids with the C type of cytoplasm expressed a consistent growth of the number of kernels (8.7%, without significance), while one hybrid with the type T of cytoplasm had a significantly lower 1000-kernel weight (-8%, $p<0.05$). On the other hand, Uribelarrea et al. (2002) have compared CMS and detasselled hybrids with their fertile versions and did not determine the yield increase. This is, to a certain extent, explained by modern hybrids that have smaller tassel and higher tolerance to stress (Duvick, 2005).

Table 1. Average yield and its interval variation for the check and different levels of the fertility percentage

Ordinal number	% Fertility	Average yield t ha^{-1}	95% confidence interval for mean yield	
			Lower limit	Upper limit
1	ZP341manual	14.956	11.710	18.202
2	ZP341F1	15.278	13.532	17.024
3	ZP341Rec.	15.178	11.779	18.577
4	0%	14.558	12.819	16.296
5	5%	14.046	10.127	17.965
6	10%	15.087	14.389	15.784
7	15%	14.747	10.666	18.828
8	20%	14.905	12.690	17.119
9	25%	14.771	12.942	16.600
10	30%	14.767	12.578	16.957
11	35%	14.787	13.522	16.053
12	40%	15.148	13.691	16.605
13	45%	14.951	12.145	17.757
14	50%	15.282	15.154	15.409
15	55%	14.932	12.030	17.834
16	60%	15.197	10.769	19.624
17	65%	14.911	14.088	15.734
18	70%	14.688	12.469	16.907
19	75%	14.393	14.037	14.749
20	80%	15.472	12.905	18.038
21	85%	15.197	11.029	19.365
22	90%	14.612	13.003	16.221
23	95%	14.554	13.123	15.984
24	100%	14.519	12.854	16.184

Furthermore, Kaeser (2002) carried out studies on the effects of both, sterile cytoplasm and types of cytoplasm on maize grain yield. Higher grain yields were established in all observed sterile hybrids with all types of cytoplasm in comparison to their fertile counterparts, which was a result of the increased number of kernels per area unit, and of a greater 1000-kernel weight registered in some hybrids. It was also observed that the yield of sterile hybrids were more stable under stress conditions. According to everything stated, it can be concluded that the V generation of ZP hybrids (FAO 300-400) expressed exceptional potential and stability of yield. Moreover, the growing season of these hybrids is shorter and the moisture content at harvest is significantly lower, which is a great advantage due to reduced costs of drying and storing. Results presented in Table 2, indicate that different ratios of sterile to fertile components in the mixtures of seed used for sowing do not significantly affect yield ($r=0.101$).

Table 2. Correlation coefficient of yield and fertility percentage

Location	rxy
Zemun Polje	0.101

In addition, an effect of each independent variable (relative humidity) on a dependable variable (yield) cannot be determined (Table 3). A low effect of various fertile to sterile component ratios is observable over small coefficients of regression (β). Their contribution to the modification of the yield is only 1.0 % (R^2).

Table 3. The values of parameters of quadratic regression model and coefficient of determination

Location	β_0	β_1	β_2	R^2
Zemun Polje	7E-05X^2	0.0082X	11.136	0.010

The coefficient of determination shows the variation of the trait (in this case the yield) and this variation amounted to 0.010. Based on the value of the coefficient of determination it is observable that the percentage dependence is small, which points out that the encompassed variability percentage of factors affecting yield variability was not great. The equation of the estimated quadratic regression is schematically presented in Figure 1, whose abscissa represents the percentage of fertile plants, while its ordinate represents the maize yield. Beside the estimated quadratic regression, Figure 1 shows the value of the coefficient of determination, which shows the extent to which the yield variability of maize is affected by the variation of the percentage of fertile plants. The coefficient of determination in the location of Zemun Polje (0.010) is presented within Figure 1.

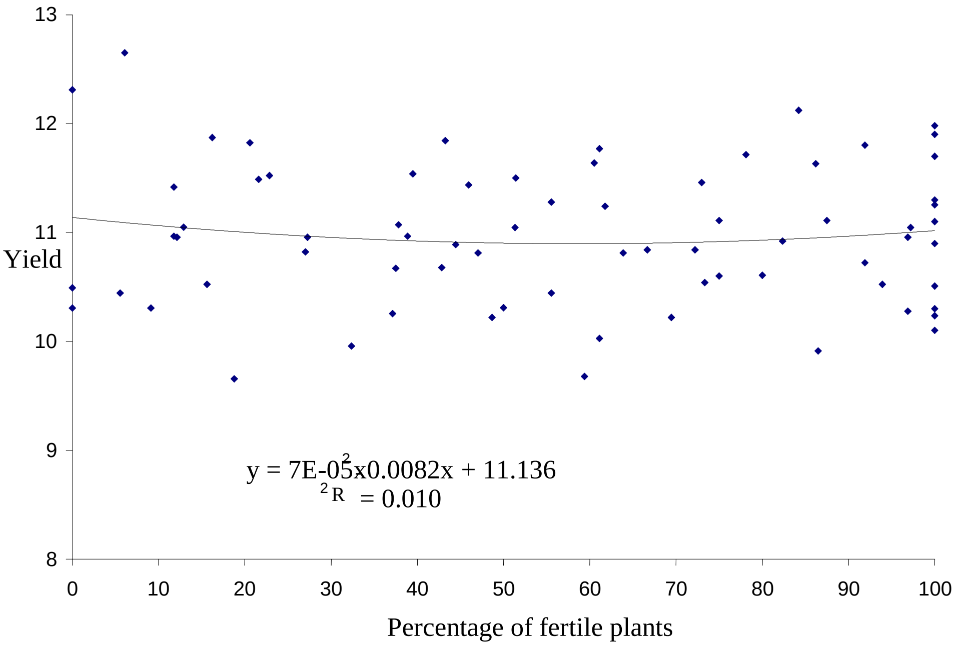

Figure 1. Equation of the estimated quadratic regression for the location of Zemun Polje

The regularity of effects of the percentage of fertility on yield is not observable in Figure 1, which indicates the possibility of their independence.

Conclusions

According to results obtained on the seed production of the commercial hybrid ZPSC 341, i.e. on effects of different percentages of fertile and sterile plants on the yield of the hybrid ZPSC 341, the following may be concluded:

The effect of the location on maize grain yield was significant;

Environmental conditions significantly affected variation of yields over locations;

The highest average yield was 15.472 t ha^{-1};

The lowest average yield was 14.046 t ha^{-1};

80% of fertile plants was the most favourable fertile to sterile variant ratio;

5% fertility was the least favourable ratio;

Coefficients of correlations were positive, but there were no statistical significances of yield and the fertility percentage.

Although obtained results do not provide sufficient information to establish the optimum ratio of sterile to fertile variant of the hybrid ZPSC 341 for the purposes of its commercial production, there are a lot of reasons to assume that the previously applied fertile to sterile variant ratio of 75% to 25% is most likely the optimum ratio for the commercial production of the hybrid ZPSC 341.

References

1. Duvick, D.N. (2005): Genetic progress in yield of united states maize (Z*ea mays* L.). Maydica 50: 193-202.
2. Glamočlija, Đ. (2004): Posebno ratarstvo, Izdavačka kuća Draganić
3. Hadživuković, S. (1991): Statistički metodi s primenom u poljoprivrednim i biološkim istraživanjima. Drugo izdanje. Poljoprivredni fakultet, Novi Sad.
4. Hirel, B., Martin, A., Terce-Laforgue, T., Gonzalez-Moro, M.-B., Estavillo, J-M. (2005): Physiology of maize I: A comprehensive and integrated view of nitrogen metabolism in a C4 plant. Physiologia Plantarum 124: 167–177.
5. Kaeser, O. (2002): Physiological and agronomic traits of cytoplasmic male sterility in maize (Zea mays L) and its molecular discrimination. PhD dissertation. Agronomy and plant breeding, Federal Intsitute of Technology, Zurich, Switzerland.
6. Munsch, M. (2008): Yield potential of modern european plus-hybrids and relevance of genetic diversity for xenia in maize (*Zea mays* L.). PhD dissertation. Agronomy and plant breeding, Federal Intsitute of Technology, Zurich, Switzerland.
7. Rhoades, M. M. (1931): The cytoplasmic inheritance of male sterility in *Zea mays*. J. Genet. 27:71-93.
8. Uribelarrea, M., Carcova, J., Otegui, M.E., Westgate, M.E. (2002): Pollen production, pollination dynamics, and kernel set in maize. Crop Science 42:1910-1918.
9. Vega, C.R.C., Andrade, F.H., Sadras, V.O., Uhart, S.A., Valentinuz, O. (2001): Seed number as a function of growth. A comparative study in soybean, sunflower, and maize. Crop Science 41: 748-754.
10. Weingartner, U., Prest, T.J., Camp, K.-H., Stamp, P. (2002b): The plus-hybrid system: a method to increase grain yield by combined cytoplasmic male sterility and xenia. Maydica 47:127-134.