SECTION 5: GENETICS AND PLANT BREEDING

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GENETIC VARIABILITY OF SOME MORPHOLOGICAL AND PRODUCTIVE TRAITS OF LOCAL BEAN POPULATIONS (PHASEOLUS VULGARIS L.)

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Abstract
Diversity and genetic variability of ten local bean populatios (*Phaseolus vulgaris* L.) from Kichevo region, for several morphological and productive traits have been analysed in two-years experiment. The population Tetovski grav (Tetovo bean) was used as standard for comparation with the other populations. Regarding the obtained results, populations are divergent for nearly all of the investigated traits: leaf color, shape and persistence, color of flower wings and bracteole, color of immature and dry pods, pod shape, shape of pod cross-section, pod wall fibre, position of pods, position and orientation of pod beak, number of pods per plant, number of seeds per pod, seed pattern, color, brilliance and shape, 100 grains mass and seed yield per plant. Population's yield per plant varied from 118.8 g to 181.8 g, except for the Trkalezen bel grav (45.3 g). Highest yield, and the only one higher than the standard, had Splesnat tetovec, therefore we recommend it for wide production in Kichevo region. The variability within the populations for all traits was low. Among the investigated productive characters, in average of all populations, least variable trait was the number of seeds per pod (9.74% CV), while most variable was grain mass per plant (12.72% CV). In average of all of the productive traits, the population Bel cincar had highest variability (20%). The populations Siten cincar, Splesnat tetovec, Trkalezen bel grav and Splesnat tetovec were most uniformed (approximately 15% CV). Compared to the standard Tetovski grav, other populations differ significantly for large number of the traits.
Key words: *Phaseolus vulgaris* L., bean local populations, diversity, variability, morphological traits, productive traits.

Introduction

Genus *Phaseolus* originates from America, or more specificaly two centres of origin: Mesoamerican and Southamerican are recognized by many researchers (Toro *et al*. 1990, Gepts *et al*. 1986, Voysest *et al*. 1994, Angioi *et al*., 2009; Kwak *et al*., 2009). Approximately 70 *Phaseolus* wild species are more important for the development of a humankind (Freytag and Debouck, 2002), out of which five are domesticated and cultivted in the past. Among them, *Phaseolus vulgaris* L. or common bean is the most widely cultivated, taking 90% of the world bean production. In Europe bean was primarily grown as decorative plant, and later as a food crop. During the Turkish rule this crop spreads in Macedonia and Serbia with noumerous populations characterized primarily with large seeds. Still today some landraces are locally named "turkish seed". Balkan Peninsula is

considered as secondary center of origin due to the high bean diversity present in this region (Krasteva, 2002).

R. Macedonia has long tradition of bean cultivation. Farmers in the rural areas maintain various populations within several decades (Ivanovska and Popsimonova, 2006). In Kichevo region populations with white compressed or kidney shaped grains are predominant, which local name is most often variation of the well known Tetovski grav (Tetovo bean). However, populations with patterned and colored grains are grown as well. Due to absence of bean breeding program this rich diversity of local poulations was not determined till present. Main breeding objective in all programs is yield improvement (Ghobary and Abd-Allah, 2010). Seed yield is a complex trait affected by many factors and success of its improvement relay on use of characters directly associated with yield as selection criteria (Karasu and Oz, 2010, Chitra and Rajamani, 2010, Cabral *et al*. 2011). Therefore, the objective of present study was to estimate the genetic variability of some morhological and productive characters of ten populations collected from Kichevo region. The information obtained will be used as value indicators for conservation of this material in the gene bank, and for establishment of breeding collection in the future program.

Material and methods

The diversity and genetic variability of ten local bean populations (*Phaseolus vulgaris* L.), maintained by farmers from Kichevo region for a long period have been analized. Populations with their local name and origin are listed in Table 1. Landrace Tetovski grav, cultivated on larger areas in RM, was used as a standard for comparation with the other populations. The experiment was conducted in two years (2006 and 2007) at the village Crvivci, Kichevo, on 640 m above sea level, by use of RCBD with three replications. Planting was performed manually, in May 2006 and April 2007, in nests with 50x50cm distance, with 4-5 seeds in each nest. After the planting, standard agrotechnical measures were applied. The plants were irrigated 3 times in 2006 and five times in 2007. For determination of populations diversity, plant characters were recorded according to Descriptor list for *Phaseolus vulgaris* (IPGRI, 1982).

During the vegetation and after the harvest qualitatuive characters of leaves (color, shape and persistence), flowers (color of wings and bracteoli), pods (color of immature and dry pods, shape, curvature, position on the plant, cross-section shape, pod wall fiber, beak position and orientation) and seed (coat pattern, coat darker and lighter color, brilliance and shape) were analyzed. Productive characters: number of seeds per pod (average of 10 pods/plant), number of pods per plant, mass of 100 seeds (g) and seed yield per plant (g) were evaluated.

In IPGRI and UPOV descriptors seed shape is visuelly clasified in 4 groups. Genchev (1989) suggests classification based on the variation among the three seed dimensions. Five shapes (Tab. 2) are grouped according to the relations length : width and height : width of seeds (Dekaprelevic, 1925; citation by Tudzarov, 1981). This classification is similar to the botanical and it is used in many experiments (Vasić, 1986). Seed dimensions were measured from 10 seeds per each plant.

The obtained data were analyzed for variance using the statistical package SPSS. For determination of genotypes variability basic statistical parameters for the characters were estimated for each repetition and year. The differences between means were compared by LSD test. Statistical significance was considered at $P<0.01$ and $P<0.05$ level.

Table 1. Local name and origin of the investigated populations

No	Original local name	Translation of local name	Origin (village)
1	Tetovski grav	Tetovo bean	v. Forino
2	Bel cincar	White cincar	v. Bigor Dolenci
3	Sharen trkalezen grav	Paterned round bean	v. Tuin
4	Siten cincar	Small cincar	v. Bachishta
5	Splesnat tetovec	Compressed tetovo bean	v. Zajas
6	Sharen cincar	Paterned cincar	v. Bigor Dolenci
7	Trkalezen bel grav	Round white bean	v. Rechani
8	Splesnat grav	Compressed bean	v. Tuin
9	Kafen i crn cincar	Brawn and black cincar	v. Bigor Dolenci
10	Splesnat kichevski	Compressed kichevo bean	Kichevo

Table 2. Classification of bean seed shape based on the relations among the seed sizes

Subspecies	Seed shape	Length : width	Height : width
Sphaericus (Savi)	Sphaerical	1,0-1,3	0,66-1,0
Ellipticus (Mart.)	Eliptical	1,3-1,7	0,71-1,0
Oblongus (Savi)	Oval	1,7-2,3	0,71-1,0
Subcompressus	Semicompressed	1,3-1,7	0,36-0,7
Compressus (D.C.)	Compressed	1,7-2,3	0,36-0,7

Results and discussion

The variability of populations regarding their qualitative characters is presented in Fig. 1. All characters are listed in the legend along with the relation among alternative forms of the characters. Populations having the given form enclosed in the parenthesis are listed with the numbers specified in Table 1. Highest diversity among the genotypes with the largest number (4) of alternative trait forms was determined for the color of flower wings, pod position, darker color of the seed coat and shape of the seed. All plants were uniformed only for two characters: bracteole color (green) and position of the pod beak (marginal). Low diversity (with two alternative trait forms) was registered for leaf color and persistence, pod wall fiber, pod beak orientation and seed coat pattern. Bean seed has high variations regarding the size, shape and color, depending on the genotype. This phenotype variation is important for commercial purposes, influencing on cookability, processing, taste and texture of the grains (Adams and Bedford, 1993, Kelly *et al.*, 1998). Varieties with white seeds are predominant on the Balkan Peninsula (Mitranov, 1981a; Vasić *et al.*, 1993). In the collection of 129 genotypes maintained by the Institute of Field and Vegetable Crops in Novi Sad, Vasić (2004) also determined high diversity regarding the seed shape and color, out of which most seeds were white, greeny-yellow, pale green, gold, patterned, brawn, pink and black. Populations with colored seeds are also maintained by the farmers, mainly for their own needs. In the collections of South America, bean with black, red and patterns of different colors is predominant (Antunes *et al*., 1981; Voysest *et al*., 1994). Breeding directions are often determined by the seed shape and color (Acquaah *et al*., 1992; Brothers and Kelly, 1993; Gvozdanović *et al*., 1996).

Number of seeds per pod

The population Siten cincar had highest number of seeds per pod in both years (5.83 in 2006, 4.56 in 2007). Compared to the standard Tetovski grav, only 3 populations had statistically significant diferent average values. The values were higher in 2006 in almost all of the populations (Tab.3). Similar results for the number of seeds per pod obtained by analyses of different varieties and populations are reported by Mitranov (1981a, 1981b) with average values of 3.8-5 seeds; Vasić *et al*. (1996) with 3.22-4.05 seeds, Stoilova and Kiryakov (2000) with 4.2 seeds, Vasić *et al*. (2002) with 2.8-4.4 seeds, Vasić (2004), with 2.4-4.4 seeds, Duran *et al*. (2005) with 2.8-4.2 seeds, Kazemi *et al*. (2012) with 3.2-3.9 and Salehin and Rahman (2012) with 4.5-6.1 seeds per pod. Highest variablity among the plants was recorded in the population Tetovski grav in 2006 (14,44%), and in general all populations were more variable in this year (Tab. 3). In average the variability of this trait was low (9.74%), which is in accordance with the results obtained by other autors (Adams, 1967, 1982; Mitranov, 1981a, Vasić *et al*., 1996, Duran *et al*., 2005). On the contrary, Stoilova and Kiryakov (2000) have determined higher variability (29%) in 40 bean samples maintained by the genebank in Sadovo.

Number of pods per plant

Highest pod number in the two years was determined in the population Splesnat grav (81.63 in 2006, 59.42 in 2007), and the smallest mean value was recorded in Bel cincar (49.17 in 2006, 33.52 in 2007). The only exception was found in Trkalezen bel grav, which also have three times smaller plant height and belongs to other growth group (Tab.3). The values of all populations were higher in 2006, while only Splesnat grav had significantly higher value (70.53) than the standard (65.57). Vasić (1994) also determined 17-74.9 pods per plant, as well as Kazemi *et al*. (2012) – 25.6-50.5. Lower average values are reported by Mitranov (1981b), 11-54; Stoilova and Kiryakov (2000), 14.3; Vasić *et al*. (2002), 4.4-14.6, Vasić (2004), 3.1-18.9, Salehin and Rahman (2012), 7.1-8 and Hossein *et al.* (2012), 19.64-30.15, due to various growth groups of the examined material. For the same reason much larger range of average values are published by Bozoclu and Sozen (2007), 1-163 pods, and Yongzhong (1994), 17.2-100 pods. The uniformity of the plants within the populations was much smaller in two populations in 2007 (21.42% in Bel cincar and 22.74% in Splesnat kichevski), although all populations expressed high variability in this year (Tab. 3). Most stable in 2007 was Tetovski grav (12.89%) and in 2006 Splesnat kichevski (9.50%). Compared to the relatively low variability of this trait determined in this research, other autors refer higher variability in their investigations: 30-44% (Vasić, 1994), 38.1% (Stoilova and Kiryakov, 2000), 33.6-40% (Vasić, 2004) and 28.6% (Duran *et al.*, 2005), also becouse of the different growth groups of analyzed material.

Mass of 100 grains

The grain mass along with the number of grains per pod and number of pods per plant are directly contributing to the yield. Whatever the breeding directions are, the primary goal is creation of high yielding varieties with different grain size (Ninhuis and Singh, 1986, Karasu and Oz, 2010). The values for this character are obtained by measurement of 100 air-dried seeds from each plant. In average for the two years, highest values expressed Kafen and crn cincar (67g) and Bel cincar (66g). Values over 60g were recorded in Splesnat tetovec, Sharen cincar and Splesnat grav as well (Tab. 3). During the analysis of 1000 grains mass in various bean genotypes, Vasić registered lower average values: 322-448g (Vasić *et al*., 1995), 161.8-443.8g (Vasić *et al*., 2002) and 161.8-483.1g (Vasić, 2004). Similar results are published by Duran *et al*. (2005) with average 100 grains mass of

23.3-42.7g, Stoilova and Kiryakov (2000) with 46.7g, Salehin and Rahman (2012) with 39.6-44.5g and Hossein *et al*. (2012) with 26.7-44.6. Higher values (16.2-80g), in accordance with the results obtained in this investigation are registered by Bozoglu and Sozen (2007) in the collection of 292 local bean populations in Turkey. The populations were less uniformed for 100 grains mass in 2006 (14.43%-22.75%), while in 2007 the variablity was lower and ranged from 7,57% to 15,15% (Tab. 3). Similarly, Duran *et al*. (2005) determined 10.6% variability, but also lower uniformity (42.2%) is reported by Stoilova and Kiryakov (2000) and (27.2%) by Vasić (2004). Zizumbo-Villareal *et al*. (2005) analyzed wild, weedy and cultivated bean populations. They registered average mass of 100 grains 6g, 20g, 39g and variability of 12-50%, 19-21%, 37-42%, consequently.

Seed yield per plant

The values for the seed yield are obtained indirectly through the values of the previously described characters. Yield of all genotypes was much smaller in 2007 (38.35-164.20g), than in 2006 (52.32-211.95g), with highly significant diferences among the years. In average for the two years highest seed yield per plant was recorded in Splesnat grav (181.8g) and Splesnat tetovec (177.8g). Only these populations have overcome the standard yield (156.8g). Interpopulation variability was higher in 2006 (25-41%) compared to 2007 (19-32%) in almost all of the genotypes (Tab. 3).

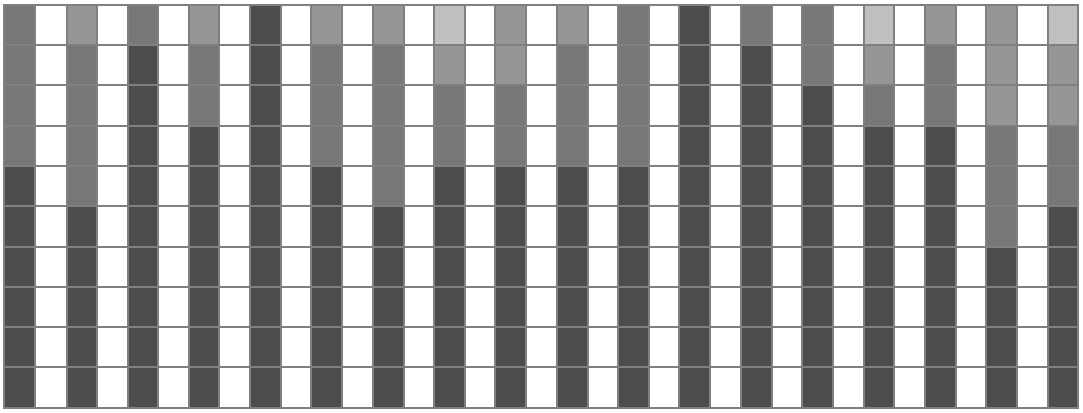

Figure 1. Variability of qualitative characters of the investigated bean populations

Legend:

1. Leaf color: medium green 6 (1, 2, 4, 5, 8, 10), dark green 4 (3, 6, 7, 9);
2. Leaf shape: quadrangular 5 (4, 5, 6, 7, 9), triangular 4 (1, 2, 3, 8), oval 1 (10);
3. Leaf persistence: intermediate 9 (1, 2, 3, 4, 5, 6, 8, 9, 10), all leaves persistent 1 (7);
4. Flower wings color: white 7 (1, 2, 4, 5, 7, 8), pale lilac 2 (6, 9), lilac 1 (3);
5. Calyx / bracteole color: green 10;
6. Immature pod color: shiny green 6 (1, 2, 4, 6, 7, 9), green 3 (5, 8, 10); dull green to silver grey 1 (3);
7. Dry pod color: pale yellow to white 5 (2, 4, 6, 7, 10), deep yellow 4 (1, 5, 8, 9), carmine red 1 (3);
8. Position of pods: combined 6 (1, 2, 3, 4, 7, 8, 9), centre 2 (10), base 1 (5), top 1 (6);
9. Pod cross-section: pear-shaped 6 (1, 2, 4, 7, 8, 10), roundelliptic 2 (3, 5), very flat 2 (6, 9);
10. Pod curvature: straight 6 (1, 2, 3, 6, 7, 9), slightly curved 3 (5, 8, 10), curved 1 (4).
11. Pod wall fiber: strongly contracting 6 (3, 4, 6, 7, 9, 10), leathery podded 4 (1, 2, 5, 8);

12. Pod beak position: marginal 10 (all genotypes);
13. Pod beak orientation: downward 9 (1, 2, 3, 4, 5, 6, 7, 9, 10), straight 1 (8);
14. Seed coat patterns: absent 8 (1, 2, 4, 5, 7, 8, 9, 10), rhomboid spotted 1 (3), striped 1 (6);
15. Seed coat darker color: white 7 (1, 2, 4, 5, 7, 8, 10), purple 1 (3), brown and black 1 (9), black 1 (6);
16. Seed coat lighter color: white 7 (1, 2, 4, 5, 7, 8, 10), brown 2 (3, 6), brown and black 1 (9);
17. Brilliance of seed: medium 4 (2, 4, 7, 8), shiny 3 (1, 5, 10), matte 3 (3, 6, 9);
18. Seed shape: kidney shaped 5 (1, 2, 5, 6, 9), elliptic 2 (3, 6), semi-compressed 2 (8, 10), oval 1 (4).

Vasić *et al*. (1996) determined lower yield per plant (29.8-147.5 g) by analysis of 2 lines and 3 varieties of bean. Among them, the line selected from the population Tetovac had highest yield, which is in accordance with our results, having in mind that Tetovac originate from Tetovski grav. They also reported variability of 37.7-42.7% for these genotypes, which together with the variability of 15-30% determined by Vasić (2004) by analysis of 129 genotypes is in accordance with our results. Higher range of mean values (1-99g) is reported by Bozoglu and Sozen (2007) in 292 local populations from Turkie, while much lower mean values (8.4-17.7g) and variability (29,8%) are published by Duran *et al*. (2005) obtained by investigation of 65 bean samples from Carebean, due to the determinant growt type of the genotypes.

Table 3. Mean values and variability of the populations for the productive characters

Genotypes	Year	Number of seeds per pod		Number of pods per plant		Mass of 100 seeds (g)		Seed yield per plant (g)	
		$\bar{X}$	CV(%)	$\bar{X}$	CV(%)	$\bar{X}$	CV(%)	$\bar{X}$	CV(%)
Tetovski grav	2006	4.64	14.44	77.50	10.93	58.26	22.75	211.74	33.52
	2007	3.56	9.06	53.64	12.89	52.81	14.90	101.86	25.86
	Avrg	4.10		65.57		55.54		156.80	
Bel cincar	2006	4.57	12.53	49.17	12.59	72.94	20.94	167.13	33.01
	2007	3.53	12.36	33.52	21.42	59.06	15.15	70.49	32.04
	Avrg	4.05		41.34		66.00		118.81	
Sharen trkalezen grav	2006	4.54	10.83	67.82	19.76	56.64	22.64	178.35	41.41
	2007	4.52	7.35	45.27	19.45	56.36	10.59	115.60	24.21
	Avrg	4.53		56.54		56.50		146.98	
Siten cincar	2006	5.83	11.86	74.28	10.59	40.34	14.43	176.07	25.66
	2007	4.56	7.08	49.94	14.90	30.66	13.46	70.09	23.26
	Avrg	5.19		62.11		35.50		123.08	
Splesnat tetovec	2006	4.42	11.88	76.08	12.30	62.04	17.90	211.95	30.05
	2007	4.39	6.67	49.52	14.98	65.96	7.57	143.69	19.46
	Avrg	4.40		62.80		64.00		177.82	
Sharen cincar	2006	4.27	11.35	55.16	13.18	61.17	17.34	146.82	31.91
	2007	4.13	7.04	34.68	17.17	63.83	13.45	90.96	20.86
	Avrg	4.20		44.92		62.50		118.89	
Trkalezen bel grav	2006	3.98	7.17	23.56	17.63	55.62	15.27	52.32	25.54
	2007	3.86	6.99	16.62	15.50	59.38	10.18	38.35	23.51
	Avrg	3.92		20.09		57.50		45.33	
Splesnat grav	2006	4.23	9.57	81.63	9.59	57.43	18.83	199.40	25.41
	2007	4.24	7.83	59.42	15.42	64.57	10.96	164.20	24.58
	Avrg	4.23		70.53		61.00		181.80	
Kafen i crn cincar	2006	4.36	10.08	61.61	12.57	72.72	16.73	197.25	28.08
	2007	3.77	8.28	37.46	16.43	61.28	13.22	87.67	27.51
	Avrg	4.06		49.53		67.00		142.46	
Splesnat kichevski	2006	4.28	12.08	71.59	9.50	48.98	18.31	151.22	27.70
	2007	4.38	10.44	45.14	22.74	60.02	12.78	119.23	30.86
	Avrg	4.33		58.37		54.50		135.23	
Average variability			9.74		14.98		15.37		27.72
$LSD_{0.05}$	For	0.239		3.253		5.177		25.43	
$LSD_{0.01}$	genotypes	0.321		4.357		6.854		34.06	
$LSD_{0.05}$	For years	0.107		1.455		2.288		11.37	
$LSD_{0.01}$		0.144		1.949		3.065		15.23	

Conclusions

The examined bean populations from Kichevo region showed high diversity regarding most of the characters. Highest diversity of the plants for the qualitative characters was determined for the flower wings color, pod position, seed coat darker color and seed shape. All plants had green bracteole color and marginal pod beak position. Low diversity was registered for leaf color and persistency, pod wall fibre, pod beak orientation and seed coat patterns. Concerning the productive characters, in general the mean values were higher in 2006. Two populations, Splesnat tetovec and Splesnat grav, had highest average values in the two years and were the only ones with higher yield than the standard Tetovski grav. Intrapopulation variability was lowest for seed number per pod and highest for seed yield per plant. Nearly all traits were more variable in 2006, except for the number of pods per plant that expressed higher variability in 2007.

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ГЕНЕТСКА ВАРИЈАБИЛНОСТ НА НЕКОИ МОРФОЛОШКИ И ПРОДУКТИВНИ СВОЈСТВА КАЈ ЛОКАЛНИ ПОПУЛАЦИИ ГРАВ (*PHASEOLUS VULGARIS* L.)

Ивановска Соња, Јанкуловска Мирјана, Ајрули Гафур, Попсимонова Гордана, Кратовалиева Сузана, Агич Рукие

Апстракт

Анализирани се дивергентноста и генетската варијабилност на десет локални популации грав (*Phaseolus vulgaris* L.) од кичевско, во текот на двегодишен експеримент за повеќе морфолошки и продуктивни својства. Популацијата тетовски грав се користеше како контрола за споредба со другите популации. Според добиените резултати од анализата, популациите се дивергентни речиси за сите анализирани својства: боја и форма на листови, опаѓање на листовите при берба, боја на цветни крилца и на брактеоли, боја на мешунки во техничка и во потполна зрелост, форма на мешунка и форма на напречен пресек на мешунката, присуство на целулозни конци во мешунката, позиција на мешунките на растението, поставеност и ориентација на клунчето на мешунката, број на мешунки на растение, број на семки во мешунка, боја, сјајност и облик на семе, форма на шарата на семето, маса на 100 семки и принос на семе од растение. Приносот на растение вариираше од 118,8 g до 181,8 g, со исклучок на приносот на тркалезен бел грав (45,3 g). Највисок и единствено повисок принос од стандардот имаше сплеснат тетовец и затоа оваа популација се препорачува за широко производство во кичевско. Варијабилноста на единките за сите својства беше ниска. Од анализираните продуктивни својства, просечно за сите популации најмалку варијабилно својство беше бројот на зрна во мешунка (9,74%), а најваријабилна беше масата на зрна од растение (27,7%). Врз основа на сите продуктивни својства, популацијата бел цинцар беше најваријабилна (20%), а популациите ситен цинцар, сплеснат тетовец, тркалезен бел грав и сплеснат грав беа најуниформни со варијабилност од приближно 15%. Во однос на стандардот тетовски грав, останатите популации се разликуваа значајно за најголем број својства.

Клучни зборови: *Phaseolus vulgaris* L., грав, локални популации, дивергентност, варијабилност, морфолошки својства, продуктивни својства.

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Original scientific paper

MULTIVARIATE ANALYSIS OF QUANTITATIVE TRAITS IN MAIZE HYBRIDS

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Abstract
Eight maize hybrids (Zea mays) have been evaluated for 12 quantitative traits in Macedonia during 2009 year, under rainfed and irrigated conditions. The characters included in this study were plant height, plant height to the first ear, number of ears per plant, ear length, ear diameter, number of rows per ear, kernel length, kernel weight per plant, 1000 kernel weight, hectoliter weight, days between anthesis and silking and vegetative growth duration. All the traits were analyzed using multivariate analysis techniques (two-way cluster and principal component analyses). The first three PCs with eigenvalues >1 contributed to 87.68% of the variability amongst analyzed hybrids. The two-way cluster analysis categorized the hybrids in two clusters. The quantitative traits were classified in three main clusters, each composed of different number of subclusters. Both methods revealed that the hybrids under investigation displayed a wide range of variation for most of the traits that could be further on exploited in a breeding programme. Further work on the selected plant material will be continued for additional characterization of the maize genotypes and direct use in breeding and production.
Key words: principal component analysis, two-way cluster analysis, quantitative traits, maize.

Introduction

Maize (Zea mays L.) is one of the most important cereal crops in the world. On a global level, maize has gained tremendous importance due to rising demand from diversified sectors like food, feed and ethanol production. To address global needs which are mainly due to continuing growth of the world population and energy insufficiencies, improvement of maize productivity and quality through breeding is vital (Tester and Langridge, 2010).

Information on the variability of the breeding material and classification of the available germplasm into genetically divergent groups is imperative for deciding which method will be applied in breeding programmes (Babic et al., 2008). At the same time, a precise description of the starting breeding material is essential for the final result of the breeding process. For genetic improvement of maize, identification or creation of genotypes that are high yielding and adapted to specific environmental conditions is very important. Among various abiotic and biotic stress factors, drought makes a significant contribution to the maize genotype (Löffler et al., 2005, Setimela et al., 2005). As a result, one of the main objectives in maize breeding programs is the selection of genotypes which will withstand drought stress (Golbashy et al., 2009, Richards et al., 2002). Multivariate data analysis is one of the techniques that facilitate a graphic display of the underlying latent factors and as such, it provides an interface between the individual samples and the variables under study

(Nielsen and Munck, 2003). If the dissimilarity between two genotypes is defined on a multivariate criterion, it is useful to be able to determine which plant characteristics cause the dissimilarity as well as the relative contributions of the various characteristics to the total variability in the germplasm (Ariyo 1993). The classification of diversity among the genotypes into groups with similar characteristics can be utilized to produce superior hybrids for enhanced crop production (Ariyo, 1993; Aydin et al., 2007). In the current study, a set of data comprising agronomic traits of 8 commercial maize hybrids were subjected to multivariate data analysis, namely, PCA and two-way cluster analysis. The main objectives of this study were (1) to explore the extent and pattern of phenotypic variability in maize hybrids based on their agronomic data, (2) to classify the germplasm into similar groups and (3) to identify the main traits contributing to the overall variability.

Material and methods

Eight maize hybrids from diverse backgrounds and maturity groups were used in this study. The experiment was established as a nested design with three replications, and two treatments: with and without irrgigation at Gradsko, Macedonia during 2009 growing seasons. Distance between the rows was 0.7 m with hills spaced according to the maturity group. Plots were overplanted and thinned, obtaining a final density of approximately 68027 plants/ha for the hybrids ZP360 and OS378 which belong to FAO 300, 62111 plants/ha for the hybrids ZP480 and OS499 (FAO 400), 57143 plants/ha for hybrids ZP599 and OS552 (FAO 500) and 52910 plants/ha for the hybrids ZP677 and OS602 (FAO 600). Plant height (cm), plant height to the first ear (cm), number of ears per plant, ear length (cm), ear diameter (cm), number of rows per ear, kernel length (cm), kernel weight per plant (g), 1000 kernel weight (g) and hectoliter weight (g) were estimated on a sample of 10 plants from each plot. Days between anthesis and silking and vegetative growth duration were determined on a plot basis. Two-way cluster analysis and PC analysis were performed using R statistical package.

Results and discussion

Effective application of multivariate analysis techniques on agronomic characters can result in meaningful grouping of studied hybrids (Mostafavi et al., 2011). On the basis of their genetic diversity and with regard to analyzed quantitative traits, the maize hybrids were grouped into two major clusters by the two-way cluster analysis. Both clusters comprised four hybrids and were further on divided on two subclusters. The quantitative traits were divided in three groups, each containing different number of subgroups (Fig. 1).

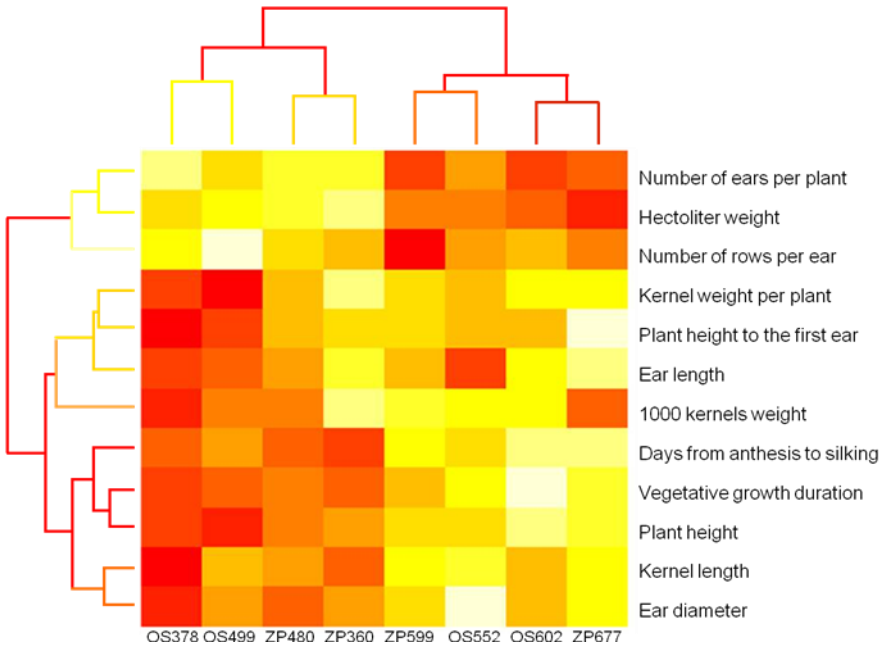

Figure1. Two-way clustering of quantitative traits in maize hybrids

Number of ears per plant, hectoliter weight and number of rows per ear were classified into the third cluster. The second cluster comprised kernel weight per plant, plant height to the first ear, ear length and 1000 kernel weight. The remaining traits were included into the first cluster. The principal component analysis gave remarkably similar results as the two-way cluster analysis. The highest portion of the total variability of studied hybrids was encompassed in the first three PCs. The first PC explained 40.84% of the total variability, the second 27.24% and the third 19.59%, which sums 87.68% of the total variability (Table 1). The highest correlation with the first PC showed plant height (0.687), vegetative growth duration (0.807) and days between anthesis and silking (0.939). The same traits were positioned in the first subcluster of the first cluster on the heatmap. Kernel length and ear diameter, which were included in the second subcluster of the first cluster, had high correlation with both PC3 (0.672 and 0.774, respectively) and PC1 (0.551 and 0.609, respectively). Ear length, plant height to the first ear and kernel weight per plant, which comprised the second cluster, had highest positive associations with the PC2. In addition, 1000 kernel weigh was separated from them and had highest positive correlation with the PC3 (0.863). Analyzed characters that were negatively correlated with the all three PCs (number of ears per plant, hectoliter weight and number of rows per ear), belonged to the third cluster. As a result, it can be observed that plant height, vegetative growth duration, days between anthesis and silking, kernel length and ear diameter were characters that mostly contributed to the variability of the analyzed maize hybrids. The factor loadings for the PC1 and PC2 are presented on Figure 2.

Table 1. Principal component loadings of quantitative traits in maize

Rotated Component Matrix	Components		
	PC1	PC2	PC3
Plant height	.687	.628	.235
Plant height to the first ear	.342	.816	.315
Vegetative growth duration	.807	.408	.281
Days between anthesis and silking	.939	.234	.153
Kernel weight per plant	.581	.759	.258
Number of ears per plant	-.812	-.320	-.353
Ear length	.077	.943	.046
Ear diameter	.551	.053	.774
Kernel length	.609	.133	.672
Number of rows per ear	-.418	-.490	-.322
1000 kernel weight	-.069	.357	.863
Hectoliter weight	-.960	-.176	-.031
Eigen vector	7.861	1.467	1.193
% of Variance	40.841	27.243	19.592
% of Cumulative Variance	40.841	68.084	87.676

As noted by Cui et al. (2001), although many clustering algorithms are available for exploring genetic diversity in crops, it is rarely obvious which one would be the best for a particular data set. Similarly, it is hardly known *a priori* which trait or group of traits will be best to use in clustering. Recently, PCA has been used by various authors for the reduction of multivariate data into a few artificial varieties which can be further used for classifying of materials. This approach is especially valuable for the screening of a large number of genetic resources by a large number of descriptor variables (Cartea et al., 2002; Granati et al., 2003; Kamara et al., 2003; Marticorena et al., 2010; Beiragi et al., 2012). Based on the studied phenotypic traits, Wietholter et al. (2008) concluded that the traits that most contributed to the classification of Brazilian corn landraces were plant height, ear insertion, female flowering, male flowering and kernel row number per ear. Similar results were presented by Aydin et al. (2007). Though quantitative traits could be better used for grouping of maize genotypes, highly heritable qualitative and quantitative traits were preferred. Based on this, Abu-Alrub et al. (2006) used kernel traits as best descriptors for classifying Peruvian highland maize germplasm, followed by ear traits. Tassel traits were found to be less reliable descriptors for classifying the germplasm. This was more evident for the traits *viz*. ear height, number of kernels/row and 100 seed weight (Ranatunga et al., 2009).

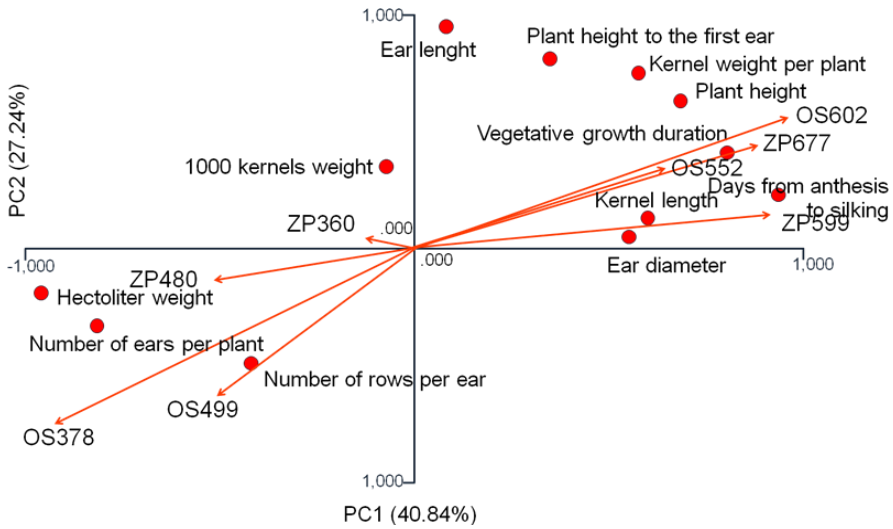

Figure 2. Principal component analysis of quantitative traits in maize

Conclusions

The two-way cluster analysis and principal component analysis provided useful analytic and graphic tools to study and characterize germplasm, in this study maize hybrids, especially when the characterization is based on the study of phenotypic, morphological and agronomic descriptors where the influence of environmental factors is possible.

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МУЛТИВАРИЈАЦИСКА АНАЛИЗА НА КВАНИТИТАТИВНИТЕ СВОЈСТВА КАЈ ХИБРИДИ ПЧЕНКА

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Апстракт

Во рамките на истражувањето беа евалуирани 12 квантитативни својства кај 8 хибриди пченка (Zea mays), во услови со и без наводнување. Беа анализирани својствата: висина на растение, висина до првиот кочан, број на кочани од растение, должина на кочан, дијаметар на кочан, број на редови на кочан, должина на зрно, маса на зрна од растение, маса на 1000 зрна, хектолитарска маса, денови од метличење до свилење и должина на вегетација. Сите својства беа анализирани со помош на мултиваријациски техники двонасочна кластер-анализа и анализа на главни компоненти). Првите три PC со eigen вредности>1со 87,42% учествуваа во варијабилноста помеѓу хибридите. Двонасочната кластер-анализа ги раздвои анализираните хибриди во две групи. Квантитативните својства беа групирани во три кластери, согласно првите три PC. Двата метода покажаа дека анализираните хибриди се многу варијабилни во однос на повеќето својства, што може да биде искористено во селекциските програми. Одбрани хибриди пченка ќе бидат искористени за дополнителни анализи, како и вклучување во селекциска програма или директно производство.

Клучни зборови: двонасочна кластер-анализа, анализа на главни компоненти, квантитативни својства, пченка, хибриди.

UDC:635.64:575.22
Original Scientific PAper

OPTIMIZATION OF PCR CONDITIONS TO AMPLIFY DNA MICROSATELLITES IN TOMATO (LYCOPERSICON ESCULENTUM MILL.)

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Abstract
DNA microsatellites are important molecular tools in determination of genetic variability among organisms. The aim of this study was to optimize PCR conditions for amplification of twelve microsatellite loci (LESSF, LECH13, LE21085, LEMDDNa, LEEF1Aa, LELEUZIP, LEWIPIG, LELE25, LE20592, TMS9, LE2A11 and LECHSOD) using tomato (*Lycopersicon esculentum* Mill.) DNA samples. Tomato DNA was isolated using Promega's Wizard ® Genomic DNA purification kit and CTAB method slightly modified during previous study. Six morphologically different tomato varieties (var. *grandifolium* (with red fruits), var. *cerasiforme* (with yellow fruits), var. *pruniforme*, var. *pyriform*e and var. *racemigerum*) were included in the research. In this study, PCR conditions for amplification of selected microsatellite loci were optimized using appropriate primer pairs. Amplification conditions were modified by altering the number of cycles and concentration of DNA. DNA concentration of 15 ng and 30 cycles gave the best amplification for nine of twelve microsatellite loci (LECH13, LE21085, LEMDDNa, LEEF1Aa, LELEUZIP, LE20592, TMS9, LE2A11 and LECHSOD). The optimal PCR conditions were the same for these microsatellite loci, except annealing temperature for LE21085 locus (50 °C). Attempts to optimize the PCR conditions for LESSF locus, LEWIPIG locus and LELE25 locus were unsuccessful. The optimization of PCR conditions requires individual approach regarding the different genotypes and variable laboratory conditions. Obtained PCR products could be used in molecular characterization of tomato varieties.
Key words: DNA microsatellites, PCR, optimization, *Lycopersicon esculentum*.

Introduction

The microsatellites known as simple sequence repeats (SSRs) are new class of DNA markers which are used for detecting a higher level of genetic variability. The term microsatellite was coined by Litt and Luty (Joshi *et al.*, 1999). They are short tandem repeats (2-10 bp), middle repetitive, tandemly arranged, hypervariable DNA sequences dispersed throughout fungal, plant, animal and human genomes (Kahl, 2001). SSRs have been used in population genetics, parentage testing, individual identification and for shortening breeding programs (Ben-Meir *et al,*. 1996). Microsatellites are present in both coding and noncoding regions and are usually characterized by a high degree of length polymorphisms (Zane *et al.*, 2002).

With the PCR discovery in late 1980s, microsatellites became the most powerful Mendelian markers ever found (Jarne and Lagoda, 1996). SSR-containing fragments are flanked by conserved DNA. These sequences could be used as templates for designing appropriate primers for amplifying

the DNA section with microsatelites. Most primers generated distinct amplification products, resulting in fingerprint-like banding patterns after agarose gel electrophoresis and ethidium bromide staining. These fingerprints allowed distinction among different plant taxa at an interspecific as well as intraspecific level (Weising *et al.*, 1995). An important limitation, however, regarding the use of microsatellites for polymorphisms or genetic diversity studies is the prior need for optimization of the PCR conditions for each SSR marker (Doğrar and Akkaya, 2011). This process includes different factors that can affect PCR reaction such as: Mg++ concentration, DNA quantity, number of cycles, annealing temperature etc. This study is dealing with optimization of the PCR amplification conditions for 12 regions that contain DNA microsatellites in tomato genome.

Material and methods

Plant material

The six different tomato varieties used in this research were obtained from GenBank of Agricultural Institution in Skopje. According to Brezhnev, those tomato varieties belong to three subspecies of *Lycopersicon esculentum* Mill. (var. *grandifolium* from subsp. *cultum* Brezh.; var. *cerasiforme* (with red fruits), var. *cerasiforme* (with yellow fruits), var. *pruniforme* and var. *pyriforme* from subsp. *subspontaneum* Brezh. and var. *racemigerum* from subsp. *spontaneum* Brezh.).

DNA extraction

DNA was extracted from few fresh leaves of 10 individual plants per each variety using Promega's Wizard ® Genomic DNA purification kit. DNA was also extracted from pooled seeds of each variety using slightly modified CTAB method (Doyle & Doyle, 1987 and Cullings, 1992, Miskoska-Milevska *et al.*, 2011). The sample intake was 50 mg of tomato leaves i.e. seeds. DNA quality was checked in 0.8 % agarose gel, stained with ethidium bromide.

PCR conditions

All PCR amplifications were performed in a thermal cycler (Techne TC-512). The optimization of PCR conditions for amplification of microsatellite loci (LESSF, LECH13, LE21085, LEMDDNa, LEEF1Aa, LELEUZIP, LEWIPIG, LELE25, LE20592, TMS9, LE2A11 and LECHSOD) was carried out using appropriate primers, produced by Operon, Huntsville, AL. They were labeled using the method of tailed primer because later on the PCR products were included in fragment analysis by DNA analyzer. The fluorescent labeled M13-primer was designed by Applied Biosystems, USA. PCR amplification products were analyzed by 2 % agarose gel electrophoresis, stained with ethidium bromide and photographed under ultraviolet light by using G-Box system (Sygene).

The DNA extraction and optimization of the PCR conditions were done 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.

Results and discussion

The microsatellite primers have been already efficiently used for studies of genetic diversity, mapping and variety identification in different crops. They are specific for each individual genome or specie. The appropriate selection and combination of microsatellites loci are important in receiving of specific profiles that could be used to assist genetic characterization. The selection of twelve microsatellites loci was based on the number of alleles and diversity index from previous studies (Arens *at al.*, 1995; Smulders *et al.*, 1997; Bredemeijer *et al.*, 1998; Areshchenkova and

Ganal, 1999, Vosman *et al.*, 2001, Alvarez *et al.*, 2001, Bredemeijer *et al.*, 2002. He *et al.*, 2003; Villalta *et al.*, 2005; Garcia-Martinez *et al.*, 2006).

The primers selection is extremely important for effective amplification. In this study, PCR conditions for amplification of selected microsatellite loci were optimized using appropriate specific primers. Also, fluorescent labeled M13 primer was included in the research. The primers were labeled with the method of tailed primer. It includes three primers: forward primer with M13 universal tail at 5' end, reverse primer without a tail and fluorescent labeled M13 primer. Namely, the tail of forward primer has the same sequence as fluorescent labeled M13 primer. M13 primer consists of 19 bases (5'-CACGACGTTGTAAAACGAC-3') and was fluorescent labeled at 5' end. 6-FAM (blue dye) was used for fluorescent dyeing. In this way fluorescent labeled M13 sequence was incorporated in PCR products and at same time enables their detection by DNA analyzer. Some general information for selected microsatellites and appropriate primer pairs are shown in Table 1.

The study showed that there was no single set of conditions that could be applied to all PCR amplifications. Each specific microsatellite locus was efficiently amplified after precise adjustment of its component concentrations, its optimal time and its optimal annealing temperature.

Table 1. General information for microsatellite loci and primers used in this study

Locus	Repeat motif	Primer sequences (5'-3')	Fragment size (bp)
LESSF	$(CCCCA)_4$	F: M13-tac gct ctc aag tac cgt aag R:cct aca ttg aca tga cca aat	216-320
LECH13	$(TA)_{6-1}(GA)_4$	F: M13-taa caa tca aaa gaa ctt cgc R:atc ccc tta ttg att aca tcc	124-132
LE21085	$(TA)_2(TAT)_{9-1}$	F: M13-cat ttt atc att tat ttg tgt ctt g R: aca aaa aaa ggt gac gat aca	90-132
LEMDDNa	$(TA)_9$	F: M13-att caa gga act ttt agc tcc R: tgc att aag gtt cat aaa tga	204-420
LEEF1Aa	$(TA)_8(ATA)_9$	F: M13-aaa taa tta gct tgc caa ttg R: ctg aaa gca gca aca gta ttt	131-220
LELEUZIP	$(AAG)_{6-1}TT(GAT)_7$	F: M13-ggt gat aat ttg gga ggt tac R: cgt aac agg atg tgc tat agg	96-110
LEWIPIG	$(CT)_{8-1}(AT)_4$	F: M13-gag tca aag ttt gct cac atc R: ctc ttc tga act tgc ttt gag	247-269
LELE25	$(TA)_{13-1}$	F: M13-ttc ttc cgt atg agt gag t R: ctc tat tac tta tta tat tcg	211-250
LE20592	$(TAT)_{15-1}(TGT)_4$	F: M13-ctg ttt act tca aga agg ctg R: act tta act tta tta ttg cca cg	150-175
TMS9	$(GATA)_{26}$	F: M13-ttg gta att tat gtt cgg ga R: ttg agc caa ttg att aat aag tt	337-354
LE2A11	$(ATCT)_{5-1}$	F: M13-aat ttt gta agg aga aga cgg R: tca tat tct tca cac caa agg	143-176
LECHSOD	$(CTT)_6$	F: M13-tta tca att cat cat tgt ggc R: agg ggt agt gac agc ata aag	195-207

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

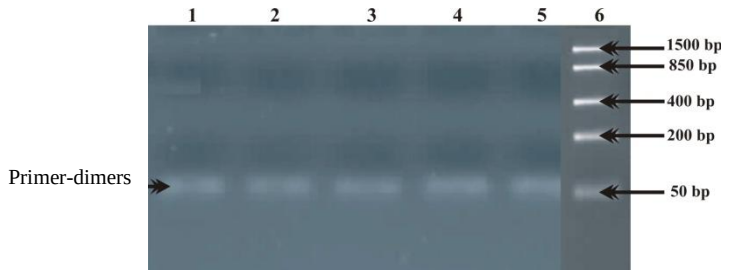

Figure 1. Agarose gel electrophoreses (20 g/L): Lanes 1-5 - illustration of failure to amplify LESSF locus; lane 6 - 50 bp DNA ladder

In practice, many reasons can cause failure of PCR amplification. Some of the often encountered problems during PCR optimization include: no detectable PCR products or a low yield of the desired product, the presence of nonspecific bands, the formation of "primer-dimers" or "primer-oligomer" (Figure 1).

Different amplification conditions of selected microsatellites were found in previous studies (Arens *at al.*, 1995; Smulders *et al.*, 1997; Vosman *et al.*, 2001, Bredemeijer *et al.*, 2002). In our study, PCR did not work under the conditions described in published literature. This happened probably due to the different protocol of DNA extraction from tomato and different laboratory conditions. Therefore, it was necessary to make some modification in PCR protocols for each microsatellite in order to produce readable results. Namely, according to Doğrar and Akkaya (1999), when amplifying the SSR markers, it might be necessary to optimize PCR cycling conditions for each marker, since the reported conditions are tuned for particular thermocycler, particular types of PCR tubes and brands of components used in those reactions. The different brands of reagents or thermocyclers, also even the minor differences between the wall thicknesses of the PCR tubes, could be a critical point and might result in inadequate PCR amplification (Doğrar and Akkaya, 1999).

Selected microsatellites required PCR optimization for each pair of primers. For nine microsatellites, amplification conditions were modified by altering the number of cycles and concentration of DNA. In published PCR protocols were presented various values for number of cycles, 45 cycles Vosman *et al.*, 2001, Bredemeijer *et al.*, 2002) and 30 cycles (Arens *at al.*, 1995; Smulders *et al.*, 1997). Obtained results showed that 30 cycles were optimal for nine of the analyzed microsatellite loci.

The annealing temperature was specified for each primer in the relevant reference. The number of cycles and annealing temperature for each microsatellites locus are listed in Table 2.

The used amplification procedure was the one already presented by Arens *et al.* (1995) but with some modifications. The DNA concentration was increased in the reaction mixture (15 ng). Instead of 50 mM KCl and 20 mM Tris-HCL (pH 8.4) was used 1 x PCR buffer (Promega). As mentioned before, fluorescent labeled M13 primer (0.032 μM) was added to the PCR mix, in order to continue later with fragment analysis. Figure 2 illustrates obtained results from PCR amplification of LEMDDNa locus for different DNA concentrations.

Table 2. Number of cycles and annealing temperatures for analyzed primers

Locus	Number of cycles	Annealing temperature
LECH13*	30	55 ºC
LE21085*	30	50 ºC
LEMDDNa*	30	55 ºC
LEEF1Aa*	30	55 ºC
LELEUZIP*	30	55 ºC
LE20592*	30	55 ºC
TMS9**	30	55 ºC
LE2A11*	30	55 ºC
LECHSOD*	30	55 ºC

* Smulders *et al.*, 1997

**Areshchenkova and Ganal, 1999

The amplification reaction was carried out in a total volume of 25 μL, containing 15 ng DNA, 1 x PCR buffer without $MgCl_2$, 1.5 mM $MgCl_2$, 0.1 mM dNTP, 0.5 U of *Taq Gold* polymerase, 0.32 μM of forward primer, 0.32 μM of reverse primer and 0.032 μM of fluorescent labeled universal M13 primer. The optimal PCR conditions were the same for the microsatellites, except annealing temperature for LE21085 locus (50°C).

Also, optimization of PCR conditions included minimizing the duration of denaturing, annealing and extension steps during the amplification cycles (per 45 seconds). The time of final extension was altered to 4 minutes. Basically, PCR reactions for nine of the analyzed primers were performed in the following conditions: one cycle of 94 ºC for 3 minutes; 30 cycles of 94 ºC for 45 seconds, 50 ºC or 55 ºC for 45 seconds and 72 ºC for 45 seconds. After the final cycle, elongation step of 72 ºC for 4 minutes was added.

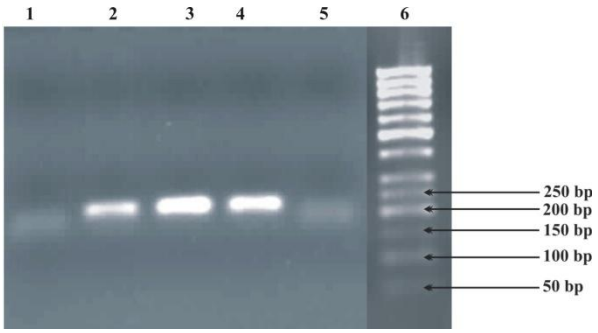

Figure 2. Illustration of PCR amplification of LEMDDNa locus for different DNA concentrations in agarose gel (20 g/L): Lane 1- negative PCR control (no DNA); lanes 2, 3 and 4 - PCR products; lane 5- failure to amplify LEMDDNa locus; lane 6 – 50 bp DNA ladder

Successful amplification of LE20592 locus in different tomato DNA is shown in Figure 3.

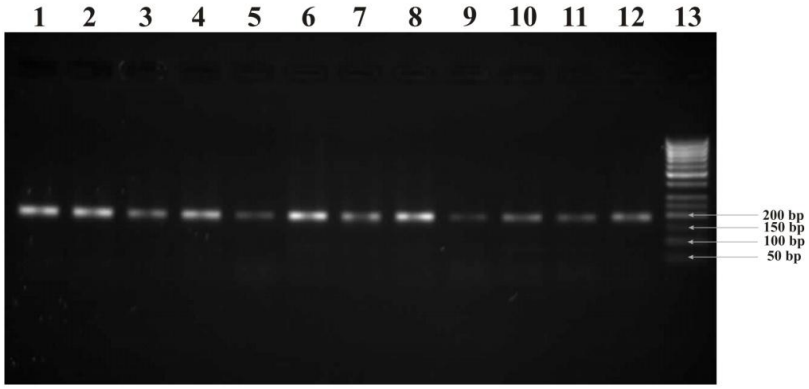

Figure 3. Agarose gel electrophoreses (20 g/L) of PCR products for LE20592 locus in different tomato DNA samples: lanes 1 - 12 - PCR products; lane 13- 50 bp DNA ladder

The quality and quantity of template DNA obtained with different DNA extraction protocols may also affect the PCR results (Narina *et al.* 2011). In this research, two different DNA isolation protocols were used and they gave the same results in PCR amplification for selected microsatellite loci.

Optimization of conditions for nine of the DNA microsatellites (Table 2) was done successfully. With these microsatellite loci, PCR amplification was observed in all six varieties. Three microsatellites did not generate amplification in research tomato varieties. Namely, PCR products were not observed for LESSF locus, LEWIPIG locus and LELE25 locus (Figure 1). This problem could be directly related to different genotypes and variable laboratory conditions. Also, these results are in accordance with the results published by Smulders *et al.* (1997). According to Smulders *et al.* (1997) the lack of amplification of an allele in certain cultivars or accession could be a result of divergence in the primer sequence flanking the microsatellite, creating a null-allele. It can also appear by the production of an undetectable amount of PCR product (Lavi *et al*., 1994). The effect of mismatches on amplification may vary with primer length, sequence context and reaction conditions (Devos *et al.*, 1995). According to Bell and Ecker (1994), the optimal PCR conditions for the amplification of a fragment will differ between genotypes. Based on these facts, it is obvious that optimization of PCR conditions would be necessary for individual cultivars or species from which no fragments have yet been amplified (Smulders *et al.*, 1997). According to Narina *et al.* (2011), the genomic DNA sequence variation between two different species might cause the variation in cross amplification success as the primers might identify a different region in the species of interest from the expected region.

Conclusions

In this study, PCR conditions for nine of twelve microsatellite loci (LECH13, LE21085, LEMDDNa, LEEF1Aa, LELEUZIP, LE20592, TMS9, LE2A11 and LECHSOD) were optimized. Therefore, only these nine pairs of primers were used for molecular characterization of corresponding tomato varieties. These microsatellites gave amplification across six tomato varieties. Attempts to optimize PCR conditions for the rest three microsatellites loci (LESSF, LEWIPIG and LELE25) were unsuccsessful and they were not part of the other research studies.

Also, it is important to point out that during the optimization of PCR conditons it is necessary to have individual approach regarding the different genotypes and variable laboratory conditions.

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ОПТИМИЗАЦИЈА НА PCR-УСЛОВИТЕ ЗА АМПЛИФИКАЦИЈА НА ДНК-МИКРОСАТЕЛИТИ ВО ДОМАТ (*LYCOPERSICON ESCULENTUM* MILL.)

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Апстракт

ДНК-микросателитите се важна молекуларна алатка во одредувањето на генетската варијабилност кај организмите. Целта на ова истражување беше оптимизација на PCR-условите за амплификација на дванаесет микросателитски локуси (LESSF, LECH13, LE21085, LEMDDNa, LEEF1Aa, LELEUZIP, LEWIPIG, LELE25, LE20592, TMS9, LE2A11 и LECHSOD) со користење на ДНК примероци од домат (*Lycopersicon esculentum* Mill.). Изолацијата на ДНК беше реализирана со користење на комерцијален кит на Promega и CTAB-методот со мали измени во текот на претходното истражување. Во истражувањето беа вклучени шест морфолошки различни вариетети домати (var. *grandifolium* (со црвени плодови), var. *cerasiforme* (со жолти плодови), var. *pruniforme*, var. *pyriform*e и var. *racemigerum*). Оптимизацијата на PCR-условите за амплификација на избраните микросателитски локуси беше остварена со користење на содветни прајмери. Условите за амплификација беа изменети во однос на бројот на циклуси и концентрацијата на ДНК. Амплификацијата на деветте микросателитски локуси (LECH13, LE21085, LEMDDNa, LEEF1Aa, LELEUZIP, LE20592, TMS9, LE2A11 и LECHSOD) беше најдобра при конценрација на ДНК од 15 ng и 30 циклуси. Оптималните PCR-услови беа исти за овие микросателитски локуси, со иклучок на температурата на анилирање за LE21085 локусот (50 °C). Неуспешно завршија обидите за оптимизација на PCR-условите за LESSF-локусот, LEWIPIG-локусот и LELE25-локусот. Оптимизацијата на PCR-условите бара индивидуален пристап во однос на различни генотипови и варијабилни лабораториски услови. Добиените PCR-ампликони може да се користат во молекуларна карактеризација на различни вариетети домати.

Клучни зборови: ДНК-микросателити, PCR, оптимизација, *Lycopersicon esculentum*.

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RESULTS OF THE INVESTIGATIONS OF MORPHO-BIOLOGICAL AND PRODUCTIONAL PROPERTIES OF SOME DOMESTIC AND FOREIGN VARIETIES OF ORIENTAL TOBACCO

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Abstract
For investigation of some morpho-biological and productional characteristics of certain varieties of oriental tobacco, four domestic and three foreign varieties were set up in 2007 and 2008 in the field of Scientific Tobacco Institute – Prilep. Domestic variety P 12-2/1 was used as a check. Investigations were made on diluvial soil, using randomized block design with 4 replications and the results obtained were statistically processed by the method of analysis of variance. From the results obtained, the following conclusions can be drawn: The results show that: the highest average height of the stalk with inflorescence was observed in the variety Gumus Hadzhikej (104.47 cm) and the lowest in P 12-2/1 (42.88 cm); the highest leaf number per stalk (55.6) was obtained in P-76/85, and the lowest in the variety Greek Basma (29.2); the largest leaf was measured in Greek Basma variety (26.50 cm length x 12.37 cm width), and the smallest in the Izmir variety (17.12 cm length x 7.15 cm width); the highest yields (25 g/stalk and 3673 kg/ ha) were obtained in the variety P-76/85 and the lowest in Izmir variety (8.5 g/stalk and 1847 kg/ha).
Key words: tobacco, oriental varieties, yield.

Introduction

The structure of tobacco production in R. Macedonia is dominated with over 95% by the oriental tobacco types Prilep, Yaka and Djebel. Apart from these three types, in the last few years the production of the type Basmak was also registered, accounting for 8.54% of the total yield of oriental tobacco in the country. Scientific Tobacco Institute-Prilep is constantly creating new varieties, interesting for their characteristics both to farmers and to manufacturers. Due to different influences of soil and climatic conditions on the properties of analyzed varieties, two year investigations were necessary to obtain more reliable results. Varieties that are included in investigations are typical representatives of the type. For some of them (Greek Basma, Izmir, Gumus Hadzhikej) such investigations were needed in order to study their growth and development in our agroecological conditions and to assess their yield and quality.

Considering the fact that breeding is a long-term process based on phenotypic and genotypic studies of the available plant material, the most frequent investigations are those of the characters related to yield. The aim of this paper is to present the results on morphobiological and productional characters of the varieties investigated and to give directions for further selection work in creation of new varieties of oriental tobacco.

Material and methods

Field trials were carried out in Tobacco Institite (TI) - Prilep during 2007 and 2008, with seven recognized varieties of oriental tobacco, such as: Prilep 12 - 2/1 (check), Prilep 76/86, PNS-72 and P-23 (R. Macedonia), Basma (Greece) and Izmir and Gumus Hadzhikej (Turkey). Tobacco seed was provided from the collection of Tobacco Institute-Prilep and its Gene bank. The experimental design of the plot was randomized block system with four replications.

Macedonian varieties were transplanted at 45 x 15 cm spacing, with 34 plants in each row and 102 plants in a plot. Introduced varieties (Izmir, Greek Basma, Gumus Hadzhikej) were transplanted at 45 x 12 cm spacing, with 42 plants in a row and 126 plants per plot. Morphological measurements were made on 20 plants from each replication. Due to their morphological characters (habitus, leaf length, etc.), the varieties Greek Basma, Izmir and Gumus Hadzhikej can be transplanted at lower spacing, just as in the countries of their origin (Turkey, Greece). The estimation of yields per stalk and hectare, due to different spacing of tobacco plants, was made in accordance with the useful plot area. The results of morphological measurements were processed by variational-statistical method and differences between the mean values were tested by LSD test. The arithmetic mean (x), the error of the arithmetic mean (sx), standard deviation (δ) and coefficient of variation (CV%) were calculated according to the methods specified by Najceska (2002) and Filiposki (2011).

Meteorological data for air temperature (°C) and precipitation (mm) during the vegetation of tobacco in the examined period (May to September) are shown in Table 1.

Table 1. Meteorological data for the period May – September 2007/2008, Tobacco institute - Prilep

Meteorological data	Year	Monts					**X** /∑
		V	VI	VII	VIII	IX	
Mean monthly air temperature ˚C	2007	16.9	21.6	25.3	23.7	16.9	20.9
	2008	16.7	19.9	22.3	23.6	15.8	19.7
Total precipitations mm	2007	74.3	79.5	5.3	54.2	16.6	229.9
	2008	41.3	10.0	11.0	11.0	110.0	183.3
Days with precipitations	2007	14	11	1	2	5	33
	2008	8	5	4	2	10	29

Results and discussion

Morpho-biological and productional characteristics

Karajankov et al. (2007) reported that tobacco plant height is a type and varietal character. According to the data presented in Table 2, the highest stalk height with inflorescence was measured in Turkish variety Gumus Hadzhikej (104.47 cm). This variety had a mean value of 103.72 ± 0.79 cm in 2007, and 105.22 ± 0.76 cm in 2008. The check variety Q 12-2/1 had the lowest average height (42.88 cm), with a mean value of 43.65 ± 0.68 cm in 2007 and 42.10 ± 0.89 cm in 2008. According to the above, P 12-2/1, PNS-72 and P-23 are classified as short height varieties and P-76/85 Greek Basma, Izmir and Gumus Hadzhikej as varieties with medium growth.

Table 2. Stalk height with inflorescence, cm

Variety	2007			2008			Average	Difference	
	$\bar{x} \pm c\bar{x}$	σ	cV%	$\bar{x} \pm c\bar{x}$	σ	cV%		Absolute cm	Relative %
P 12-2/1 Ø	43.65±0.68	1.41	3.23	42.10±0,89	2.34	5.66	42.88	/	100.00
P-76/86	88.90±0.77 $^{++}$	3.72	4.18	79.40±0.96 $^{++}$	5.22	6.57	84.15	+41.27	196.27
PNS-72	57.38±0.98 $^{++}$	3.87	6.80	58.82±0.87 $^{++}$	3.15	5.35	58.10	+15.22	135.51
P-23	47.39±0.67	1.52	3.20	48.88±0.79^{+}	2.15	4.39	48.14	+5.26	112.27
Greek Basma	82.50±0.85 $^{++}$	4.25	5.15	86.85±0.78^{++}	3.77	4.34	84.68	+41.80	197.49
Izmir	83.67±0.80 $^{++}$	4.57	3.82	107.75±1.02^{++}	7.87	7.30	95.71	+52.83	223.23
Gumus Hadzhikej	103.72±0.79 $^{++}$	4.61	4.45	105.22±0.76^{++}	4.34	4.13	104.47	+61.59	243.66

	2007	2008
LSD 5%=	4.15 cm stalk height with inflorescence $^{+}$	6.62 cm stalk height with inflorescence $^{+}$
1%=	5.69 cm stalk height with inflorescence $^{++}$	9.08 cm stalk height with inflorescence $^{++}$

Error of the mean value was the highest in Izmir ± 1.02 cm in 2008, and the same year nonuniformity was recorded in this variety. The lowest error of the mean value was recorded in P-23 ± 0.67 cm, which shows that it is considerably stable and uniform variety. In 2007, the lowest standard deviation of 1.41 cm was estimated in variety P 12-2/1, and the lowest variational coefficient of 3.20% in variety P-23. In 2008, the largest standard deviation of 7.87 cm and variational coefficient of 7.30% was recorded in Izmir variety. The investigated varieties are characterized by relatively low values of these two parameters, which is a sign of stability and uniformity of the stalks. With LSD test, statistical significance of 1% in the two years of investigation compared to the check was estimated in P-76/86, PNS-72, Greek Basma, Izmir and Gumus Hadzhikej compared to the check (P 12-2/1). Number of leaves in investigated varieties is shown in Table 3.

The highest average number of leaves (55.6) was recorded in variety P-76/86, with mean values ranging 53.0 ± 0.80 in 2007 and 58.2 ± 1.02 in 2008. The lowest average of number of leaves was counted in Greek Basma (29.2), with mean values of 31.4 ± 0.71 in 2007 and 27 ± 0.92 in 2008. The standard deviation and vartiation coefficient are low, which is an indication of indicator for stable varieties. LSD test showed that the varieties were highly significant, except for the variety Gumus Hadzhikej. Karajankov et al. (2007) divided all tobacco types and varieties into three basic groups according to their leaf size. Korubin Aleksoska (2005) reports the highest length of middle belt leaves in oriental variety Dz-291, with mean and mean error values of 25,67 cm $\pm$ 0,37 cm ($\bar{x} \pm c\bar{x}$**).**

Table 3. Number of leaves

Variety	2007			2008			Average	Difference	
	$\bar{x} \pm c\bar{x}$	σ	cV%	$\bar{x} \pm c\bar{x}$	σ	cV%		Absolute	Relative,%
P 12-2/1 Ø	37.9±0.77	1.60	4.23	32.6±0.67	1.02	3.13	35.2	/	100.00
P-76/86	53.0±0.80 ++	2.42	4.56	58.2±1.02 ++	4.24	7.29	55.6	+20.35	157.73
PHC-72	42.8±0.93 ++	2.59	6.06	40.0±0.65 ++	1.18	2.95	41.4	+6.15	117.45
P-23	47.2±0.73 ++	1.76	3.74	47.5±0.64 ++	1.39	2.93	47.4	+12.1	134.33
Greek Basma	31.4±0.71	1.13	3.61	27.0±0.92	1.61	5.99	29.2	-6.05	82.84
Izmir	41.6±0.60 ++	1.06	2.54	41.2±1.08 ++	3.42	8.30	41.4	+6.15	117.45
Gumus Hadzhikej	42.5±0.96 ++	2.78	6.54	42.2±0.90 ++	2.40	5.70	42.4	+7.1	120.14

	2007	2008
LSD 5%	= 1.79 number of leaves +	2.38 number of leaves +
1%	= 2.46 number of leaves ++	3.27 number of leaves ++

Table 4. The largest leaf length , cm

Variety	2007			2008			Average	Difference	
	$\bar{x} \pm c\bar{x}$	σ	cV%	$\bar{x} \pm c\bar{x}$	σ	cV%		Absolute cm	Relative %
P 12-2/1 Ø	25.03±0.77	1.11	4.42	25.43±0.89	1.42	5.57	25.23	/	100.00
P-76/85	22.04±1.00	1.98	8.98	26.55±1.01	1.93	7.27	24.30	-0.94	96.29
PNS-72	22.70±1.12	1.56	6.87	22.52±0.96	1.47	6.54	22.61	-2.62	89.62
P-23	20.98±0.79	1.05	5.02	21.33±0.69	0.72	3.36	21.16	-4.08	83.85
Greek Basma	25.49±0.90	0.91	3.59	27.50±0.82	1.31	4.78	26.50	+1.27	105.01
Izmir	17.50±0.82	1.49	8.53	16.74±1.09	1.41	8.39	17.12	-8.11	67.86
Gumus Hadzhikej	19.32±0.83	1.86	9.62	19.84±1.04	1.52	7.65	19.58	-5.65	77.61

According to the data presented in Table 4, the average length of the largest leaf on the stalk ranges from 26.50 cm in variety Greek Basma to 17.12 cm in Turkish variety Izmir. In 2007, the highest values for standard deviation (1.98 cm) and variation coefficient (8.98%) were obtained in variety P-76/86. In 2008, the lowest standard deviation of 0.72 cm and variation coefficient of 3.36% was recorded in variety P-23. It can be concluded that the highest variation coefficient of 9.62% for this character was recorded in variety Gumus Hadzhikej, which is probably due to low adaptibility of this variety to agro-ecological conditions and the applied cultural practices, which were the same for all varieties. According to the length of the largest leaf on the stalk, Turkish varieties are characterized by smaller size of the middle belt leaves. Such a positive characteristic allows them to participate with in higher classes in purchase random. The largest leaf width in investigated varieties is shown in Table 5.

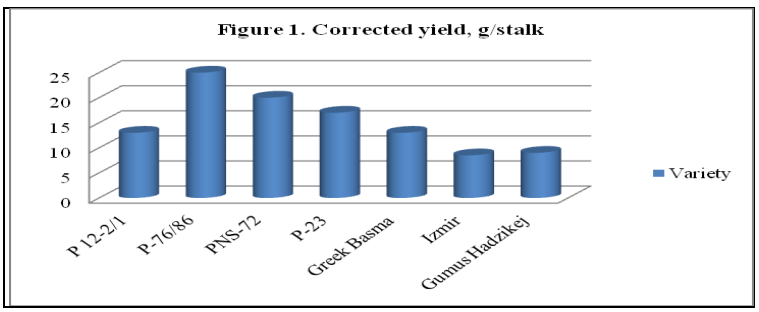
Table 5. The largest leaf width, cm

Variety	2007			2008			Average	Difference	
	$\bar{x} \pm c\bar{x}$	σ	cV%	$\bar{x} \pm c\bar{x}$	σ	cV%		Absolute, cm	Relative %
P 12-2/1 Ø	10.72±0.79	0.78	7.30	11.97±1.09	1.00	8.37	11.35	/	100.00
P-76/85	11.20±1.11	0.98	8.72	13.45±1.13	1.22	9.09	12.33	0.98	108.64
PNS-72	11.15±1.08	0.93	8.31	11.48±0.94	0.71	6.19	11.32	-0.03	99.74
P-23	9.95±0.91	0.59	5.91	10.40±0.94	0.64	6.19	10.18	-1.17	89.69
Greek Basma	11.88±0.73	0.45	3.79	12.85±1.02	0.94	7.29	12.37	1.02	108.99
Izmir	6.74±1.09	0.56	8.23	7.55±1.14	0.70	9.21	7.15	-4.2	62.98
Gumus Hadzhikej	8.88±1.28	1.03	11.68	9.00±1.15	0.85	9.46	8.94	-2.41	78.80

Table 6. Corrected yield, g/stalk

Variety	Year	g/stalk	Average	Difference	
				Absolute, g	Relative, %
P 12-2/1 Ø	2007	12.00	13.00	/	100.00
	2008	14.00			
P-76/86	2007	25.00^{++}	25.00	+12	192.31
	2008	25.00^{++}			
PNS-72	2007	20.00^{++}	20.00	+7	153.85
	2008	20.00^{++}			
P-23	2007	17.00^{++}	17.00	+4	130.77
	2008	17.00^{++}			
Greek Basma	2007	12.00	13.00	0	100.00
	2008	14.00			
Izmir	2007	8.00	8.5	-4.5	65.38
	2008	9.00			
Gumus Hadzhikej	2007	9.00	9.0	-4	77.61
	2008	9.00			

		2007	2008
LSD	5%	1.93 g/stalk $^{+}$	1.91 g/stalk $^{+}$
	1%	2.64 g/stalk $^{++}$	2.62 g/stalk $^{++}$



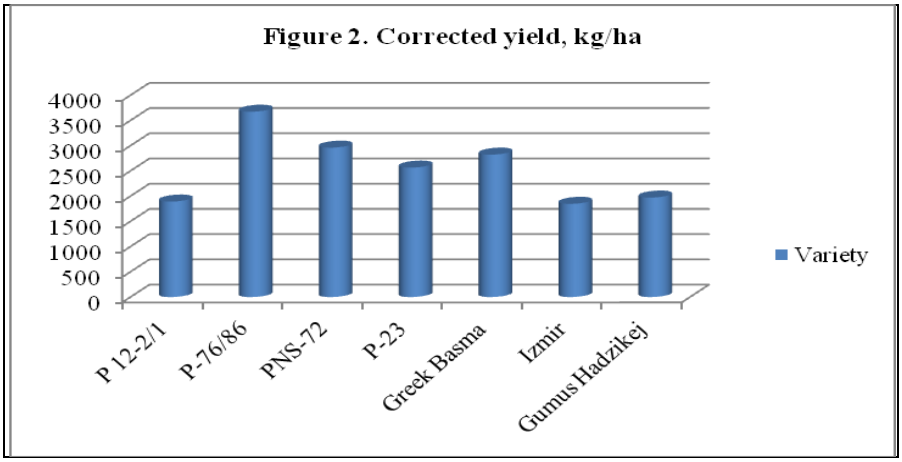
Data in Table 5 show the highest average width of the largest leaf on stalk in variety Greek Basma (12.37 cm) and the least in variety Izmir (7.15 cm). In 2007, the highest standard deviation of 1.03 cm and variation coefficient of 11.68% was recorded in Turkish variety Gumus Hadzhikej, and the lowest variations in Greek Basma. In 2008 the highest standard deviation of 1.22 cm and variation coefficient of 9.09 was registered in variety P-76/86. The highest variations in this year were recorded in the variety P-23.

Table 7. Corrected yield, кg/ha

Variety	Year	kg/ha	Average yield	Difference	
				Absolute, kg	Relative, %
P 12-2/1 Ø	2007	1708	1892	/	100.00
	2008	2075			
P-76/86	2007	3657	3673	+1781	194.16
	2008	3688			
PNS-72	2007	2922	2961	+1070	156.54
	2008	3000			
P-23	2007	2543	2565	+674	135.60
	2008	2587			
Greek Basma	2007	2543	2822	+930	149.17
	2008	3100			
Izmir	2007	1759	1847	-45	97.62
	2008	1934			
Gumus Hadzhikej	2007	2035	1971	+80	104.23
	2008	1908			

		2007	2008
LSD	5%	313 kg/ha $^{+}$	265 kg/ha $^{+}$
	1%	429 kg/ha $^{++}$	364kg/ha $^{++}$

According to the data presented in Tables 6 and Table 7 (Figure 1 and Figure 2), the highest average yield per stalk (25 g) and per hectare (3673 kg) were obtained in variety P-76/85, while the lowest one in the variety Izmir (8.5 g per stalk and 1847 kg per hectare). Statistically significant differences of 1% for this character (g/stalk and kg/ha) were recorded in varieties P-76/86, PNS-72 and P-23 in both years of investigation. Statistically significant difference of 1% for the character kg / ha was estimated in Greek Basma in both years, and in variety Izmir in 2007 (LSD=5%). Compared to the introduced varieties, the local varieties of the type Prilep gave higher yield.



Conclusions

The highest stalk with inflorescence was observed in Gumus Hadzhikej variety (104.47 cm), with mean values of 103.72 ± 0.79 cm in 2007 and 105.22 ± 0.76 cm. in 2008. The check variety P 12-2/1 showed the lowest average height (42.88 cm), with mean values of 43.65 ± 0.68 cm in 2007 and 42.10 ± 0.89 cm in 2008. According to variational and statistical data for this character, we concluded that these varieties are stable. Varieties P 12-2/1, PNS-72 and P-23 are classified as varieties with small height, while varieties P-76/86, Greek Basma, Izmir and Gumus Hadzhikej are classified as varieties with medium height. The highest number of leaves was recorded in variety P-76/86 (55.6), with mean values of 53.0 ± 0.80 in 2007 and 58.2 ± 1.02 in 2008. The lowest average number of leaves was found in Greek Basma (29.2), having a mean value of 31,4 ± 0.71 in 2007 and 27 ± 0.92 in 2008. Standard deviation and variation coefficient were low, which is an indication of stable varieties.

The average length of the largest leaf ranges from 26.50 cm in Greek Basma to 17.12 cm in Izmir variety. The highest average width of the largest leaf was recorded in Greek Basma (12.37 cm), while the lowest was in Izmir variety (7.15 cm).

The highest average yield (25 g/stalk and 3673 kg/ha) was registered in P-76/86, while the lowest (8.5 g/stalk and 1847 kg/ha) in Izmir variety.

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РЕЗУЛТАТИ ОД ПРОУЧУВАЊЕТО НА МОРФО-БИОЛОШКИТЕ И ПРОИЗВОДНИТЕ СВОЈСТВА НА ДОМАШНИ И СТРАНСКИ ОРИЕНТАЛСКИ СОРТИ ТУТУН

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Апстракт
Со цел да се проучат некои морфо-биолошки и производни својства кај некои домашни и странски ориенталски сорти тутун, во текот на 2007 и 2008 година во кругот на Научниот Институт за тутун - Прилеп беше поставен опит со 4 домашни и 3 странски ориенталски сорти тутун, каде како контролна варијанта беше користена домашната сорта П 12-2/1. Испитувањата беа изведени на делувијален тип на почва во 4 повторувања по методот случаен блок систем, а добиените резултати беа варијационо статистички обработени по методот на анализа на варијанса. Добиените резултати покажаа дека: во просек, со најголема височина на стракот со соцветие се одликува сортата Ќумус Хаџикеј (104.47 cm) а со најмала (42.88 cm) контролната сорта П 12-2/1. Со најголем број на листови по страк (55.6) се одликува сортата П-76/85, а со најмал (29.2) сортата Грчка басма. Сортата Грчка басма се одликува со најголема должина на најголемиот лист од стракот (26.50 cm) и широчина(12.37 cm), а со најмала должина (17.12 cm) и широчина (7.15 cm) се одликува сортата Измир. Највисок принос по страк (25 g) и хектар (3673 kg) регистриран е кај сортата П-76/85, а со најнизок принос по страк (8.5 g) и хектар (1847 kg) се одликува сортата Измир.
Клучни зборови: тутун, ориенталски, сорти, принос.

UDC:633.71(497.775)
Expert paper

RESULTS OF BROADLEAF TOBACCO BREEDING IN SCIENTIFIC TOBACCO INSTITUTE - PRILEP

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Abstract
Scientific Tobacco Institute - Prilep possesses a rich collection of varieties and lines of Virginia and Burley tobaccos, which has been well maintained and regularly enriched. As a result of intensive breeding, many varieties and lines of these types have been created, distinguished by their high yields and quality. The aim of this investigation is to present the basic characteristics of both registered varieties and the newly created perspective lines. The presented varieties and lines were obtained by intervarietal hybridization, all of them in male-sterile form. Later, they were included in varietal trials. The results of Burley tobacco breeding show that, starting from the first registered Macedonian Burley B-96/85 CMS F_1, each following variety (line) were increased in size, number of leaves and yields (e.g., from 3000 kg/ha in B-96/85 CMS F_1 to 4350 kg/ha in Pelagonec CMS F_1). The quality and number of dry leaves were typical for Burley tobacco. The results for Virginia show that, starting from the first registered variety of Macedonian Virginia MV-1, all subsequent lines had larger dimensions, higher leaf number and increased yields (e.g., from 2500 kg/ha in MV-1 to 3591 kg/ha in the line V-88/09 CMS F_1. The color of dry leaves in most of these lines was golden yellow, which is typical for this type of tobacco.
Key words: tobacco, varieties, lines, Virginia, Burley.

Introduction

In parallel with selection of oriental and semioriental tobaccos, Scientific Tobacco Institute works on selection of broad-leaf varieties of the types Virginia and Burley. The share of raw material of these two types in composition of cigarettes is over 75% and it is the main carrier of taste and strength of tobacco smoke. Great attention during the selection process is paid to three main objectives: increase of yield, higher resistance to diseases and improvement of some characters which contribute to achieve higher quality of the raw material. The main goal of the selectionists of these two tobacco types is to obtain primarily male-sterile varieties that will be better in one or more characters than the already created. Fertile and male-sterile varieties and lines of domestic and foreign origin are used as genetic basis for hybridization. As a result of these activities, a number of Virginia and Burley varieties and lines which give satisfactory yields have been created, with raw material bearing all characteristics of the corresponding type. From the multiannual trials of Virginia and Burley varieties and lines it can be seen that most of the characters within the corresponding type are different, but they are still characteristic for the type to which they belong.

Material and methods

Data from previous investigations carried out in the Experimental field of Scientific Tobacco Institute - Prilep with five Burley and 6 Virginia varieties and lines were used as material for presentation of the results. Soil on which the trials were set up was of alluvial and colluvial type. From transplanting to the end of curing, all cultural practices typical for corresponding tobacco type were applied. Subject of analyses for the type Burley were sublimated results of the varieties B-96/85 CMS F1, Burley 1 CMS F_1, B-2/93 CMS F_1, Pelagonec CMS F_1 and line B-98/ N CMS F_8. For the type Virginia, analyses were made on variety MV-1 and lines V-78/07 CMS F_1, V-82/07 CMS F_1, V-63/04 CMS F_1, V-53 CMS F_1 and V-88/09 CMS F_1. Analyzes were made on the length and width of the largest leaf, leaf number, stalk height, yield per hectare and the color of dry leaves (raw material).

Results and discussion

Results for both types of tobacco presented in the tables are product of multiannual measurements and observations of certain characteristics of the varieties, and as such they can be taken as basic. Although these properties are strictly genetically controlled, they are closely related to soil and climate conditions in the region of growing, applied agrotechnics, conditions in which tobacco was cured, etc.

Characteristics of Burley tobaccos

Some of the characters in investigated varieties and lines of burley tobacco obtained by intervarietal hybridization are presented in Table 1.

Table 1. Characteristics of Burley varieties and lines

Characters (average values)	Varieties/Lines				
	B 96/85 CMS F_1	Burley-1 CMS F_1	B-2/93 CMS F_1	Pelagonec CMS F_1	B-98 N CMS F_8
Length/Width of the largest leaf, in cm	58 × 36	60 × 37	63 × 39	70 × 42	65 × 38
Leaf number per stalk	30	30	30	34	32
Stalk height, in cm	195	185	200	200	188
Yield/ha	3000	3500	4000	4350	3690
Cured leaves color	light brown	brown	light brown to brown	light brown to brown	brown

As can be seen from the data in Table 1, the highest length (70 cm) and width (42 cm) of the largest analyzed leaf was recorded in hybrid variety Pelagonec CMS F_1, the last recognized creation of the Scientific Tobacco Institute - Prilep. The lowest length (58 cm) and width (36 cm) of the largest leaf was recorded in the first recognized Burley variety B-96/85 CMS F_1. In other varieties and lines the length of the largest leaf ranges from 60 cm in variety Burley 1 CMS F_1 to 65 cm in line B-98 / N CMS F_8, and the width ranges from 37 cm in Burley 1 CMS F_1 to 39 cm in B-2/93 CMS F_1.

The lowest number of leaves per stalk (30) was found in varieties B-96/85 F_1, Burley 1 CMS F_1 and F_1 B-2/93 CMS 1, and the highest (34) in variety Pelagonec CMS F_1. In line B-98 / N CMS F_8 a

total of 32 leaves was counted. According to Djugerski (2009), in creation of new Burley varieties, the number of technically suitable leaves on the stalk should range from 26 to 32.

The highest stalk (200 cm) was measured in varieties B-2/93 CMS F_1 and F_1 Pelagonec CMS F_1, and the lowest (185 cm) in variety Burley 1 CMS F_1. Risteski et al. (2012) in 2010 and 2011, in the Experimental field of Tobacco Institute- Prilep made investigations on 6 Burley varieties and came to conclusion that the average stalk height ranged between 150,5 cm in variety B-21 to 191,5 cm in variety Pelagonec CMS F_1. The same author (2011), describing the basic characteristics of Pelagonec CMS F_1 states that stalk height in this variety can be expected to range from 180 to 220 cm.

The highest yield per hectare (4350 kg) was found in variety Pelagonec CMS F_1, and the lowest (3000 kg) in variety B-96/85 CMS F_1. Line B-98 / N F8 CMS reached a yield of 3690 kg / ha. Korubin - Aleksoska Ana (2004) reports that the yield of dry mass in variety Burley 1 CMS F_1 ranges from 3500 to 4000 kg/ha and in B-2/93 CMS F_1 from 3500 to 4500 kg / ha.

Figure 1. B-96/85 CMS F_1

Figure 2. Burley-1 CMS F_1

Figure 3. B-2/93 CMS F_1

Figure 4. Pelagonec CMS F1

Figure 5. B-98/N CMS F8

Through the color of cured leaves of Burley tobacco, organoleptic assessment of the quality of raw material can be made. According to the applicable Standards for qualitative assessment of leaf tobacco of the type Burley, tobaccos in all shades of light brown color are classified in I grade tobaccos, and all other colors go to the lower grades. The color of cured leaves in varieties described in Table 1 refers to cured leaves from the middle belt, which are also carriers of the character stalk yield. From the description of leaf color it can be concluded that the most typical color for the type Burley (light brown to brown) is found in varieties Pelagonec CMS F_1 and F1 B-2/93 CMS F_1, due to which this raw material is mainly classified in upper grades. Predominantly brown color is observed in raw material of the variety Burley 1 CMS F_1 and line B-98 / N CMS F_8, but it has somewhat lower quality compared to the raw of previously mentioned varieties. Light brown raw material was observed in variety B-96/85 CMS F_1, i.e. compared to other varieties investigated, this variety gives somewhat lower quality.

Figure 6. MV-1 CMS F_1

Figure 7. V-53 CMS F_1

Figure 8. V-78/07 CMS F_1

Figure 9. V-82/02 CMS F1

Figure 10. V-63/04 CMS F1

Figure 11. V- 88/09 CMS F_1`

Characteristics of Virginia tobacco

Basic characteristics of some varieties and lines of Virginia tobacco obtained in intervariety hybridization are presented in Table 2.

Table 2. Characteristics of Virginia varieties and lines

Characters (average values)	VARIETIES/LINES					
	MV-1	V-53 CMS F_1	V-78/07 CMS F_1	V-82/02 CMS F_1	V-63/04 CMS F_1	V-88/09 CMS F_1
Length/Width of the largest leaf, in cm	55 × 35	58 × 36	63 × 38	62 × 37	61 × 36	66 × 41
Leaf number per stalk	26	33	30	30	30	33
Stalk height, in cm	195	197	182	183	185	186
Yield/ha	2500	3549	2634	2896	2998	3591
Cured leaves color	Lemonish yellow	Golden yellow	Golden yellow	Golden yellow	Golden yellow	Golden yellow

According to the presented data, the biggest length (66 cm) and width (41 cm) of the largest leaf were observed in line V-88/09 CMS F_1 and the smallest (55 cm length and 35 cm width) in variety MV-1. In other tobacco lines, the length of the largest leaf ranges from 58 cm in F1 V-53 CMS F_1 to 63 cm in V-78/07 CMS F_1, and the width from 36 cm in lines V-53 CMS F_1 and V-63/04 CMS F_1 to 38 cm in V-78/07 CMS F_1. The lowest number of leaves per stalk (26) were registered in the variety MV-1 and the highest (33) in varieties V-53 CMS F_1 and V-88/09 CMS F_1. In all other lines the number of leaves was 30. A number of authors (Cavkaroski D. et al., 1992, Ristski I. 2000, Haws. S.N.J. Ir (1978) reported the occurrence of the so-called mammoth properties in Virginia tobacco, when unusually large number of leaves are formed on the stalk and plants begin to bloom when a daylight decrease. According to the presented results, the maximum stalk height was recorded in line V-53 CMS F_1 (197 cm) and the minimum height in line V-78/07 CMS F_1 (182 cm). In other varieties and lines this character ranged from 183 cm to 195 cm in varieties V-82/07 CMS F_1 and MV-1, respectively.

The highest yield per hectare was recorded in line V-88/09 CMS F_1 (3591 kg) and the lowest in variety MV-1 (2500 kg). In other investigated lines, the yield per hectare ranges from 2634 kg in V-78/07 CMS F_1 to 3549 kg in line V-53 CMS F_1.

Kocoska K. (2008) and Risteski I. (2011), in their investigations conducted in Tobacco Institute-Prilep during 2003 and 2004 reported that the average yield per hectare of the American fertile variety Sp. G-58 was 2828 kg. The same authors made investigations with other Virginia varieties in 2008 and 2009 on the same location and revealed that the American fertile variety K-326 achieved an average yield of 2684 kg/ha. From this it can be concluded that, in view of this character, the newly created Virginia varieties in Tobacco Institute -Prilep are not far behind some well-known American varieties.

Organoleptic assessment of quality of this tobacco type, among other indicators, takes into consideration the color of cured leaves. According to the operative Standards for qualitative assessment, I class consists of leaves from the middle belt with lemonish yellow, yellow, golden yellow and orange color, bright and uniform. The raw material of the variety MV-1 is characterized by a lemonish yellow color, while all other lines are golden yellow. Tobacco raw in all other colors and shades is classified in lower grades. It can be concluded again that, with regard to the color of cured leaves, the new varieties created in Tobacco Institute provide a good quality raw typical for Virginia tobacco.

The objectives and aims of Tobacco Institute-Prilep will continue to be directed toward creating male-sterile hybrid varieties of the types Virginia and Burley, because they displayed a series of advantages compared to fertile varieties. In many cases they appeared to be more vigorous and achieve better yields compared to their parents, which is a kind of transgression. It was also noted that these varieties have a faster root growth and a more uniform development, with better adjustment to environmental stress, higher resistance to diseases, etc.

Conclusions

According to the leaf size, all of the investigated Virginia and Burley varieties and lines can be classified in the first grade tobacco.

According to the character number of leaves per stalk, except for the variety MV-1, which has 26 leaves, all other varieties and lines in both tobacco types are characterized by higher number of leaves.

According to the character stalk height, all of the investigated varieties and lines belong to the group of high tobaccos.

As a result of somewhat bigger leaf size and higher number of leaves per stalk, Burley tobacco varieties give higher yields/ha compared to those of the type Virginia, but both types are achieving yields close to the world average.

All varieties and lines maintained the characteristic color of the type to which they belong.

A common conclusion can be drawn that Tobacco Institute has made an undisputed progress in breeding of these two types, which is confirmed by the fact that each newly created variety or line is characterized by improved properties compared to the previously created.

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РЕЗУЛТАТИ ОД ОПЛЕМЕНУВАЊЕТО НА КРУПНОЛИСНИТЕ ТУТУНИ ВО НАУЧНИОТ ИНСТИТУТ ЗА ТУТУН- ПРИЛЕП

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Апстракт
Научниот Институт за тутун – Прилеп располага со богата колекција на сорти и оплеменувачки линии од типовите вирџинија и берлеј кои редовно се збогатуваат и одржуваат. Како резултат на интензивното оплеменување на овие два типа тутун, создадени се повеќе сорти и линии кои се одликуваат со многу добри приноси и квалитет. Целта на испитувањето е да се прикажат основните карактеристики на веќе признатите сорти и перспективните линии. Сите сорти и линии кои се предмет на презентација добиени се по пат на меѓусортово вкрстување, и сите се во машкостерилна форма. Подоцна истите беа вклучени во сортови опити. Резултатите од оплеменувањето на типот берлеј покажуваат дека почнувајќи од првата призната македонска сорта Б-96/85 ЦМС F_1 кај секоја наредна сорта (линија), димензиите, бројот на листовите и приносите се зголемуваат (пример од 3000 kg/ha кај Б-96/85 ЦМС F_1, до 4350 kg/ha кај сортата Пелагонец ЦМС F_1).Квалитетот и бројот на сувите листови се типични за типот берлеј. Резултатите од оплеменувањето на типот вирџинија покажуваат дека почнувајќи од нашата прва призната македонска сорта МВ-1 сите последователни линии се одликуваат со поголеми димензии и број на листови, а приносите се зголемуваат (пример од 2500 kg/ha кај МВ-1, до 3591 kg/ha кај линијата V-88/09 ЦМС F_1). Бојата на сувите листови кај повеќето линии е златно жолта карактеристична за овој тип на тутун.
Клучни зборови: тутун, сорти, линии, вирџинија, берлеј.