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GENETIC ANALYSIS OF SPIKE LENGTH IN WHEAT

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The permanent need for efficient plant breeding comes from the increment of human population, which is projected to reach 9.7 million by 2050. Novel approaches could be used to reach these goals more rapidly, raising the question of efficiency, as well. Spike length is one of the important components of grain yield formation in wheat. The influence of individual plan traits is getting more important to grain yield formation per area unit in stressful growing conditions, which are increasingly present due to global climate changes. The objectives of the present research were three-fold: (i) to determine the influence of a genotype, environment and their interaction on spike length and to evaluate stability of the trait; (ii) to present cause-causing links on a graphical example; (iii) to transform a number of possibly correlated variables into a smaller number of uncorrelated variables called principal components. Samples were obtained from 96 winter wheat cultivars grown in 2011/12 and 2012/13 on two locations. The wheat genotype population was profiled with 28 microsatellites. The ANOVA of the total phenotypic variation of the experiment shows that genotypes took the largest portion, followed by the influence of the GE interaction. Additional analysis of the GE interaction using the PCA analysis shows a statistical significance of the first two main components. In the conducted research, the dispersion of the points represents two subpopulations, but

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the geographical origin could not explain the grouping of genotypes within the same, so the division into two groups was done on the basis of their lines of descent.

Keywords: AMMI, interaction, microsatellites, spike length, wheat

INTRODUCTION

The constant need for efficient plant breeding comes from the increment of human population, which is projected to reach 9.7 million by 2050. Beside this, new environmental challenges, including climate change, energy efficiency and sustainability also require from breeding science to set specific goals. Novel approaches can be used to reach these goals more rapidly, but the question of efficiency is raised, as well. One of the main objectives of conventional breeding is lowering the variability within the genotype population, by diminishing genotype by environment interaction for the traits of relevance which should be used in future crosses. Moreover, the influence of individual plan traits is getting more important to grain yield formation per area unit in stressful growing conditios, which are increasingly present due to global climate changes. Spike length is one of the important components of yield via grain yield per plant, and this is the source of assimilates closer to the caryopses. Spike structure has more benefits of utilizing illumination than the other parts of the plant and it will also stay green and functional for a longer time together with the awns. Because of these features, it contributes, on the average, 40-50% of the dry matter accumulated in the kernels (SHARMA, 2003). Plant height in wheat is a complex trait; its components include spike length and internode lengths (CUI *et al*., 2011). These traits are agronomically important for morphogenesis and grain yield formation in wheat.

The general task is to identify all sources of phenotypic variance (ELEKHDAR *et al*., 2017; KHAN *et al*., 2017). In the process of variation source identification, two different components need to be singled out, the additive component and the multivariate component. Having in mind a well-known fact that the total phenotypic variance can roughly be analysed into four sources of variation – the influence of the genotype, the influence of the environment, their mutual interaction, and error (agronomically unexplainable, hence not important, variation), it is necessary to analyse these sources of variation (KNAPP *et al*., 2017; KHAN *et al*., 2017). The additive effect of this variance consists of the first two variation sources (the genotype portion and the environment portion), while the multivariate part depends on their mutual interaction. ANOVA for spike length in this case explains the additive part of the total phenotypic variability, being an additive model, while the multivariate part remains shown in its entirety, only. GAUCH and ZOBEL (1996) have introduced AMMI Analysis (Additive Main Effects and Multiplicative Interaction), in order to additionally analyse the multivariate component. AMMI model contains ANOVA, but also disassembles and analyses GE interaction (MLADENOV *et al*., 2016). This additional GEI analysis could be done by Principle Component Analysis (PCA) (ANNICCHIARICO, 1997; SINGH *et al*., 2009; BANJAC *et al*., 2014; SOLONECHNYI *et al*., 2016; MOHAMMADI *et al*., 2017).

The method based on the analysis of the multivariation of the phenotypic part of the experiment can also be transmitted to the part of the experiment carried out in the laboratory, especially when the molecular experiments are concerned. The PCoA method is designed to explore and visualize similarities or dissimilarities of data. It starts with a similarity matrix or dissimilarity matrix and assigns a location in a low-dimensional space to each item, e.g. as a 3D graphics.

The objectives of the present research were three-fold: (i) to determine the influence of a genotype, environment and their interaction on spike length and to evaluate stability of the trait; (ii) to present cause-causing links on a graphical example; (iii) to transform a number of possibly correlated variables into a smaller number of uncorrelated variables called principal components.

MATERIALS AND METHODS

Plant material and field experiment

The grain samples were obtained from 96 winter wheat cultivars grown in 2011/12 and 2012/13 on two locations: Novi Sad and Sremska Mitrovica (E1- Novi Sad 2012, E2 - Novi Sad 2013, E3 - Sremska Mitrovica 2012, E4 - Sremska Mitrovica 2013). The wheat cultivars were planted in a randomized complete block design with four replications and all the cultivars are agronomically suitable for production in these locations (Tab. 1). Pedigrees and more information about used genotypes can be found in Mladenov et al., 2018. The sowing in both growing seasons was completed by the end of October, while the harvest was completed in the last ten days of June. The spike length was measured on each cultivar for each replication from the base of the spike to the tip, excluding awns.

Determination the causal relationship between plant height, spike length, the number of grains per spike, 1000 kernel weight and the grain yield has been done and for this reason those traits will be introduced later in the manuscript, not in the sense of phenotypic analysis, but as a tool for indirect correlations and fulfilling broader image.

Table 1. Used genotypes of winter wheat in two seasons (2011/2012 and 2012/2013) across two locations (Novi Sad and Sremska Mitrovica)

No.	*Genotype*	*Y.r.*	*No.*	*Genotype*	*Y.r.*	*No.*	*Genotype*	*Y.r.*
G1	Pesma	1995	G36	NS40 S	2006	G71	NS3-7289	Ком
G2	Renesansa	1994	G37	Teodora	2006	G72	NS Pudarka	2013
G3	Obrij	1983	G38	Etida	2006	G73	NS3-6767/2	Ком
G4	NS rana 5	1991	G39	Isidora	2007	G74	Sava	1970
G5	Pobeda	1990	G40	Gordana	2008	G75	Partizanka	1973
G6	Evropa 90	1990	G41	Gora	2009	G76	NS rana 2	1975
G7	Ljiljana	2000	G42	Biljana	2009	G77	Balkan	1979
G8	Sonata	2000	G43	Natalija	2009	G78	Posavka 2	1979
G9	Vila	2001	G44	NS Desetka	2010	G79	Jugoslavija	1980
G10	Kantata	2001	G45	NS Nena	2010	G80	Lasta	1987
G11	Cipovka	2002	G46	NS Dika	2010	G81	Rodna	1988
G12	Dragana	2002	G47	NS Arabeska	2010	G82	Tamiš	1988
G13	Jefimija	2003	G48	NS Artemida	2010	G83	Danica	1990
G14	Balada	2003	G49	NS Emina	2010	G84	Proteinka	1990
G15	Rapsodija	2003	G50	NS Avangarda	2010	G85	Rana niska	1990
G16	Arija	2003	G51	NS Futura	2010	G86	Milica	1992
G17	Simfonija	2003	G52	NS Ilina	2010	G87	Hejs 2	19
G18	Simonida	2003	G53	NS Enigma	2010	G88	Divna	1994
G19	Balerina	2003	G54	NS Tavita	2011	G89	Prima	1995

G20	Diva	2003	G55	NS 91/04	Kom	G90	Tera	1995
G21	Astra	2003	G56	NS 50/07	Kom	G91	Tiha	1995
G22	Helena	2004	G57	NS 269/08	Kom	G92	Prva	1997
G23	Oda	2004	G58	NS 48/08	Kom	**G93**	Zlatka	1997
G24	Milijana	2004	G59	NS3-6954	Kom	**G94**	Mina	1997
G25	Nirvana	2004	G60	NS3-6741	Kom	**G95**	Delta	1998
G26	Bambi	2004	G61	NS3-6926	Kom	**G96**	Sonja	1998
G27	Lana	2005	G62	NS 36/10	Kom			
G28	Zvezdana	2005	G63	NS 168/10	Kom			
G29	Janja	2005	G64	NS 55/10	Kom			
G30	Fundulea 4	1987	G65	NS 176/10	Kom			
G31	Bastijana	2005	G66	NS 128/10	Kom			
G32	Dama	2006	G67	NS 151/10	Kom			
G33	Srna	2006	G68	NS3-7106	Kom			
G34	Angelina	2006	G69	NS3-6706/2	Kom			
G35	Barbara	2006	G70	NS3-6939	Kom			

†No. number of genotype, Y.r. -year of realest, Kom. -genotypes that still are in the National Commission for cultivar recognition

Molecular experiment

The genomic DNA from all genotypes was isolated from fresh leaves using the CTAB protocol by DOYLE and DOYLE (1990). The wheat genotype population was profiled with 28 microsatellites (data not shown). The additional cultivar called *Chinese Spring* was used as a positive control and it was placed on 87 spots, instead of the cultivar *Heys 2*. The microsatellites were positioned along almost all three genomes. Detail information about DNA manipulation can be found in MLADENOV *et al*., 2018.

Statistical analyses

The genotype-by-environment (GE) interaction was tested using AMMI (Additive Main Effects and Multiplicative Interaction) analysis by ZOBEL *et al.* (1998). Data processing was performed in GenStat 9th Edition (trial *ver*.) VSN International Ltd. (www.vsn-intl.com). After the standardization of the data, the analyses of path coefficients were done in *Microsoft Excel* by AKINTUNDE (2012). The determination of the internal genetic structure was done by an additional analysis through the principal coordinate analysis (PCoA) by using an *Excel* add-in called *GenAlex* software (PEAKALL and SMOUSE, 2012).

The marker trait associations were analyzed in the Tassel software, version 2.1. (Bradbury et al., 2007) using two models: GLM and MLM (YU *et al*., 2006). The Q matrix for further association analysis was determined based on the average value of three iterations of log probability of data obtained by the Structure software (PRITCHARD *et al*., 2000).

RESULTS AND DISCUSSION

The ANOVA for spike length of the total phenotypic variation of the experiment shows that genotypes took the largest portion of 45.49%, followed by the influence of the GE interaction (20.05%). The smallest portion in the overall variability of the spike length was taken

by agroecological environments (2.43%). All sources of variation showed high statistical significance in the variation of the experiment (Tab. 2). The uniformity of the investigated agroecological environments in terms of meteorological parameters (data not shown) and the basic soil properties contributed to this source of variation taking the smallest portion in relation to the other two observed sources.

Table 2. The AMMI analysis of the variance in spike length for 94 genotypes of winter wheat (Triticum vulgare L.) and one genotype T. spelta L. (Nirvana) and T. compactum Host (Bambi) grown in two years (2011/2012 and 2012/2013) on two locations (Novi Sad and Sremska Mitrovica)

Share of variation	*Degrees of freedom*	*Sum of square*	*Middle of square*	*F value*	*F table*		*Share in total variation %*
					0,05	*0,01*	
Total	1535	3244.1	2.113	-	-	-	100
Treatmans	383	2205.3	5.758	6.41**	1,00	1,00	67.98
Genotypes	95	1475.9	15.536	17.31**	1,00	1,00	45.49
Environments	3	78.8	26.28	20.28**	2.60	3.78	2.43
Blocks	12	15.6	1.296	1.44	1.75	2.18	0.48
Interaction	285	650.5	2.283	2.54**	1,00	1,00	20.05
$IPCA_1$	97	301.7	3.11	3.47**	1,00	1,00	46.38
$IPCA_2$	95	219.6	2.311	2.58**	1,00	1,00	33.76
Residue	93	129.2	1.389	1.55**	1,00	1,00	-
Error	1140	1023.3	0.898	-	-	-	-

** $p<0,01$

Additional analysis of the GE interaction using the PCA analysis shows a statistical significance of the first two main components, $IPCA_1$ and $IPCA_2$, which participated in the GE variation with 46.38% and 33.76% respectively, with both axes having a statistically significant effect on the GE interaction variation, which is in agreement with BRBAKLIĆ (2015) and AKRAM (2008). The first two main components jointly explained more than 80% of the variation of the genotype by environment interaction. The statistical significance of the remainder is a consequence of an agronomically explicable variation, expressed as $IPCA_3$, which due to its small effect on the GE variation is not particularly identified and singled out. In order to identify the source of the GE interaction variation, the AMMI biplot is shown and is based on the number of main components which can be extracted from the sum of the squares of the genotype and environment interaction (ZOBEL *et al*., 1988).

According to biplot, and in terms of average values, it could be noticed that all agroecological environments are at the level of the experimental overall average. According to the arrangement of E3 and E4 points, it can be concluded that the cultivars achieved higher average values of spike length in these environments compared to E1 and E2 points. However, this result does not favour the E3 and E4 agroecological environments for obtaining bigger spike lengths, given the high values of interaction, which indicates the poor stability of this trait. Based on the graphic representation, the interaction of genotypes and agroecological environments

shows that there was a difference in the multiplicative effects. Similar results were also reported by SHARMA *et al*. (2003). The additive effects had greater influence on the variation of the genotypes than the multivariate effects, whose influence, even though smaller, was statistically significant according to AMMI ANOVA. This means that the differences between the genotypes in the sample were the basis of the variation, which is in accordance with CUI *et al*. (2011). These differences can also be observed in the change of the rank of genotypes for spike length in the examined agroecological environments. All genotypes were divided into seven groups according to the average values achieved. The average value for the whole experiment was $\bar{\bar{x}}$=8cm. The first, second, third and seventh groups consisted made up of one genotype (*Bambi* $\bar{\bar{x}}$=3.1cm, *NS Artemida* $\bar{\bar{x}}$=6.7cm, *NS Rana* 2 $\bar{\bar{x}}$=8.9cm and *NS Arabeska* $\bar{\bar{x}}$=8.5cm, respectively). Observing these four groups, the most stable reaction, expressed by a low GE interaction value, had the *Bambi* cultivar (G26). The fourth, fifth and sixth groups are composed of the largest number of examined varieties, with genotypes grouped into the group 5 according to the average value of the experiment for the examined trait. The *NS 168/10* line achieved the highest values for the examined trait ($\bar{\bar{x}}$ =10.8cm) and was singled out by a high stable reaction for the examined trait. *NS Arabeska* (G47), *NS Rana 2* (G76) and *NS Artemida* (G48) cultivars have achieved the greatest GE interaction in different agroecological conditions, making them the least stable in the selected material (Fig. 1). The genotypes with the least interaction with agroecological environments were grouped into the groups 4 and 5, with the cultivars from the group 5 being closer to the average value of the experiment for spike lenght. The lines and cultivars that have been singled out for their stable reaction are: *Renesansa, Obrij, NS Rana 5, Pobeda, Ljiljana, Cipovka, Dragana, Simfonija, Simonida, Helena, Zvezdana, Janja, Srna, Angelina, Barbara, NS Desetka, NS Futura, NS Ilina, NS 91/04, NS3-6939, Sava, Danica, Milica, Prima, Prva* and *Delta*.

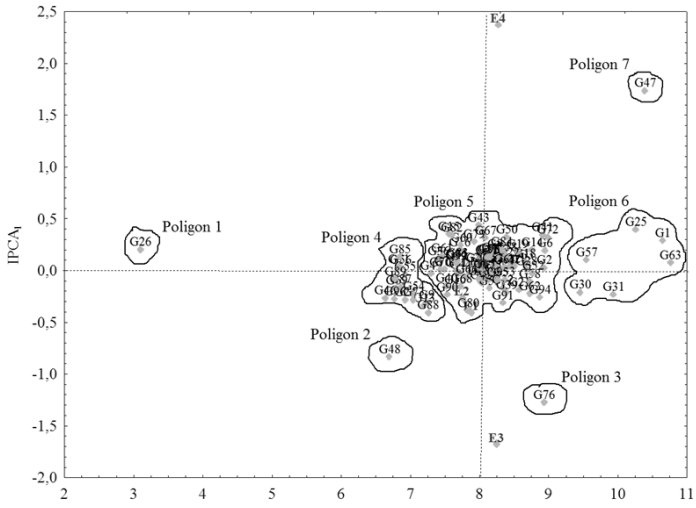

Figure 1. The AMMI1 biplot of 94 genotypes of wheat (*Triticum vulgare* L.) and one genotype of *T. spelta* L. (Nirvana) and *T. compactum* Host (Bambi) grown in two years (2011/2012 and 2012/2013) on two locations (Novi Sad and Sremska Mitrovica) for spike length.

Indirect correlations (*Path coefficient*) were used to determine the causal relationship between plant height, spike length, the number of grains per spike, 1000 kernel weight and the dependent variable (Y) which represents the wheat grain yield. Compared to simple correlations, they give a more detailed picture of the relationship between the examined variables, because they do not only show the relationships between independent and dependent variables, but also the relationships between independent variables in their joint influence on the dependent variable or indirect influence of the examined traits on the trait which is singled out as Y (WRIGHT, 1921; LI, 1975; KANG *et al*., 1983). This allows for the isolation of those phenotypic traits that mostly affect the formation of the wheat grain yield. In this way, their interconnectedness is noticed better and the strength of influence of certain yield components on the end result is determined.

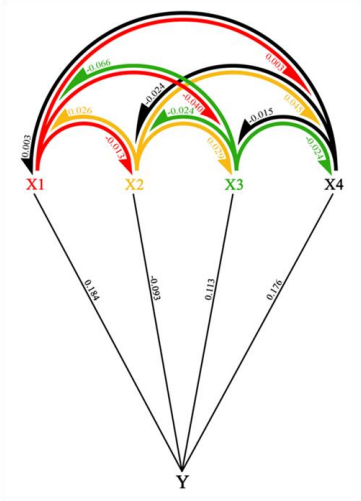

Figure 2. PATH analysis of 94 genotypes of wheat (*Triticum vulgare* L.) and one genotype of *T. spelta* L. (Nirvana) and *T. compactum* Host (Bambi) grown in two years (2011/2012 and 2012/2013) on two locations (Novi Sad and Sremska Mitrovica). X1- plant height, X2- spike length, X3- the number of grains per spike, X4- 1000 kernel weight, Y- yield.

In addition to the path coefficient (p), the direct correlation coefficients (r) between the examined traits are also shown. The results of the direct coefficients analysis show that the strongest and statistically highly significant link is established between the plant height (X1) and yield (Y), r = 0.184**, which is in accordance with CHATURVEDI and GUPTA (1995) and KHAN *et al*. (1999). Moreover, a positive and statistically significant correlation was obtained between the 1000 kernel weight (X4) and yield (r = 0.176*), which is in agreement with the studies of other authors (MONDAL and KHAJURIA, 2001; SARKAR *et al*., 2002). Although the values of the indirect coefficients are low, regularity has been noticed in the influence of the spike length, over all other traits, on the yield. The greatest influence on forming the yield is the spike length over the 1000 kernel weight (r_{H2H4}=0.045), then over the number of grains per spike (r_{H2H3}=0.029) and finally over the plant height (r_{H2H1}=0.026), Fig. 2.

The order of the material used in the experiment was also presented by an analysis of the main coordinates, using PCoA (Principal Coordinates Analysis).

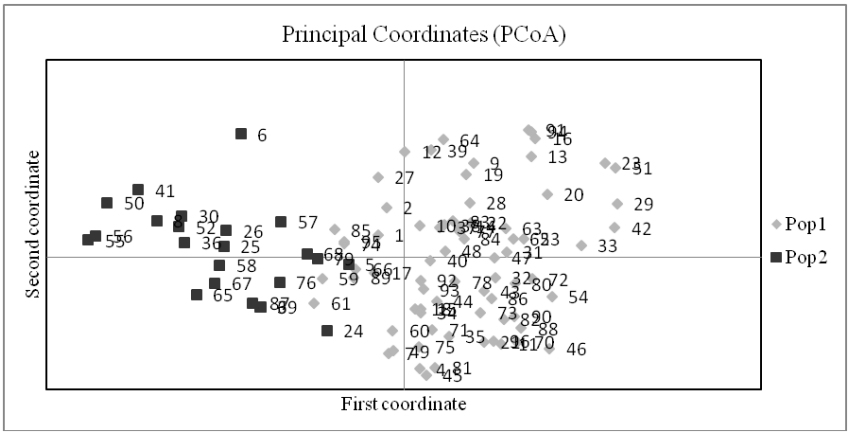

Figure 3. The distribution of 94 wheat genotypes (*Triticum vulgare* L.) and one genotype *T. spelta* L. (Nirvana) and *T. compactum* Host (Bambi) in two subpopulations across a two dimensional system. Pop1=Q1 – light colour; Pop2=Q2 – dark colour

PCoA is a scaling or ordination method that starts with a matrix of similarities or dissimilarities between a set of individuals and aims to produce a low-dimensional graphical plot of the data in such a way that the distances between points in the plot are close to the original dissimilarities. Thus, the starting point matrix of similarities or dissimilarities for PCoA is different from that of PCA, which starts with the initial data matrix (e.g. presence versus absence of alleles in molecular marker data). When there are relatively few characters and no missing data, the output of PCA and PCoA will be similar. However, ROHLF (1972) found that in PCoA, the treatment of the missing data is more satisfactory than that in PCA. PCoA is recommended over PCA when there are lots of missing data and when there are fewer individuals than characters. An analysis of the main components for genotype variability explained over 18% of the total variability using the first three components. The first distinguished coordinate explained 7.76% of the total variation, the second coordinate carried out 6.07% of the variation, while the third component explained 4.68% of the variation. Cumulatively, the first two coordinates explained 13.83% of the variation (Fig. 3). The dispersion of the points represents two subpopulations, which have also been determined in the previous graphic representation. According to the literature, the division into subpopulations should have been done on the basis of the cultivars found in genealogies or on the basis of their geographical origin (HAO *et al*., 2011; NEUMANN *et al*., 2011; CHEN *et al*., 2012; DODIG *et al*., 2012; NIELSEN *et al*., 2014; BRBAKLIĆ *et al.*, 2015). However, in the conducted research, the geographical origin could not explain the grouping of genotypes within the same subpopulations, due to the narrow genetic variability of the examined material. The division into two groups was done on the basis of their lines of descent (PRADHAN *et al*., 2016). The second group (Q2) consists of all the foreign cultivars that were examined in this research (*Obrij*, *Fundulea 4* and *Chineese Spring*, as well as

the oldest cultivars from the *NS rana 2* and *Yugoslavia* experiments, together with eight lines which are in the process of recognition and the rest of the new cultivars). The first group (Q1) is composed of the remaining cultivars examined, as well as ten lines that are in the process of cultivar recognition.

Table 3. Relation marker-trait among 28 microsatellites and wheat yield of 94 genotypes of winter wheat (Triticum vulgare L.) and by one genotype of T. spelta L. (Nirvana) and T. compactum Host (Bambi) using GLM (General Linear Model) and *MLM (Mixed Linear Model) across four environments (E1 – Novi Sad 2011/12.; E2 – Novi Sad 2012/13.; E3 – Sremska Mitrovica 2011/12.; E4 – Sremska Mitrovica 2012/13.)*

Chromosome	Microsatellite	Environment	*GLM*	*MLM*
			p marker	*p* marker
1A	gwм357	E1	-	2.42E-05
		E2	1.15E-06	1.15E-06
		E3	1.81E-05	1.81E-05
		E4	0.0019	0.0019
2A	gwм636	E2	1.34E-05	1.34E-05
		E3	6.13E-08	6.13E-08
		E4	0.0011	0.0011
2A	barc5	E3	0.0057	0.0057
3A	barc12	E1	0.0069	0.0069
		E2	0.0072	0.0072
		E3	0.0379	0.0379
6A	cfa2114	E2	0.0011	1.95E-05
		E3	0.0465	0.0011
7A	gwm631	E3	0.0027	0.0027
5B	barc110	E3	0.0044	0.0044
6B	gwm680	E3	0.0032	0.0032
2D	wmc18	E3	0.0179	0.0179
4D	wmc457	E1	0.0109	0.0109
		E2	0.0126	0.0126
		E4	0.0011	0.0011
5D	gwm190	E1	0.007	0.0465
		E3	2.42E-05	0.007

Using GLM model 21 positive relationships among traceability and molecular markers were recorded, while using MLM model, this number was 22 (Tab. 3). Microsatellites that exibited a relation with yield in both models were: *gwm357*, *gwm636*, *cfa2114*, *gwm631*,

gwm190, *barc5*, *barc12*, *barc110*, *gwm680*, *wmc457*, which is in partial accordance with ALSALEH *et al*., (2015); ZHANG *et al*., (2015); BRBAKLIĆ *et al*., (2015).

CONCLUSION

The purpose of use of statistical models is their agronomic interpretation. Using different statistical tools will lead to different presentations of results, but by comparing the results and correctly interpreting them, an accurate picture of what happens in field experiments can be obtained and nothing can replace that in agronomy. Regardless of the size of the examined population, if the selection criteria are the same or at least approximately similar, the variation in the spike length trait within the population will be small in the case of cultivars envisaged for intensive use of wheat. In path analysis regularity has been noticed in the influence of the spike length, over all other traits, on the yield. The greatest influence on forming the yield is the spike length over the 1000 kernel weight (r_{H2H4}=0.045). Principal coordinate analysis (PCoA) assigned 96 genotypes to two groups in the conducted research. The geographical origin could not explain the grouping of genotypes within the same subpopulations, due to the narrow genetic variability of the examined material. The division into two groups was done on the basis of their lines of descent.

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GENETIČKA ANALIZA DUŽINE KLASA KOD PŠENICE

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Izvod

Nove tehnike u oplemenjivanju biljaka se koriste da bi se podigao stepen efikasnosti u proizvodnji hrane, ne bi li se zadovoljile potrebe za sve bržom rastućom ljudskom populacijom. Dužina klasa je jedna od najvažnijih osobina kada se formira prinos zrna pšenice. Ciljevi ovog rada su bili trojaki: da se odredi uticaj genotipa, spoljašnje sredine i interakcije, ne bi li se procenila stabilnost spomenute osobine; prikazati uzročno posledične veze između dužine klasa i prinosa zrna; pretvoriti broj verovatnih varijabli u manji broj, nazvanim glavne komponente. Ogled je postavljen na dve lokacije tokom 2011/12 i 2012/13 godine i činilo ga je 96 genotipa pšenice. Udeo genotipa bio je najveći, dok je udeo interakcije bio drugi po delu u ukupnoj fenotipskoj varijansi. Ispitivana populacija podelila se u dve grupe, pri čemu to nije objašnjeno poreklom određenih sorti, već predačkom linijom.

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