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EVALUATION OF NEW NS MAIZE HYBRIDS USING BIPLOT ANALYSIS

Milisav STOJAKOVIĆ, Mile IVANOVIĆ, Goran BEKAVAC, Aleksandra NASTASIĆ, Božana PURAR, Bojan MITROVIĆ, Dušan STANISAVLJEVIĆ

Institute of Field and Vegetable Crops, Novi Sad, Serbia

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The study analyzed two-year results of a testing of 20 new maize hybrids from FAO MG 600 as compared to a standard. Data on the hybrids NS6683, NS6686, NS281633, and NS396432 are discussed in the paper in greater detail. In order to study grain yield, grain moisture, root and stalk lodging, and resistance to pests and diseases, field trials using a RCB design with four replicates were conducted in six locations in 2009 and five locations in 2010. The results were presented in the form of GGE biplots in order to rank hybrids relative to the standard while taking into account the genotype x environment interaction and to identify the highest-yielding genotypes in different environments. It was determined that the new NS hybrids had higher grain yield than the standard by 0.883 to 1.720 tha^{-1},

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Corresponding author Milisav Stojaković, Institute of Field and Vegetable Crops, Novi Sad, Serbia, M. Gorkog 30, email: milisav.stojakovic@ifvcns.ns.ac.rs, phone: 021 4898-286, 064 8205-764

lower grain moisture by 0.85 to 2.54%, better tolerance to root and stalk lodging, and pest and disease resistance on a par to the standard. The study identified so-called ideal locations for particular hybrids, which may be of use when determining which areas the hybrids are best suited for.

Key words: genotype x environment interaction, GGE biplot analysis. Grain yield, maize hybrids

INTRODUCTION

The hybrid maize industry began to develop in the U.S. in the first half of the 20th century. Today, hybrid maize predominates in the commercial seed sales worldwide. Hybrid maize rests on the phenomenon of heterosis. The concept of heterosis, or hybrid vigor, has been introduced in order to explain the superior performance of F_1 progeny relative to the parents. Research carried out by SHULL (1908, 1909) resulted in the development of the concept of hybrid maize, which has been in use in an unchanged form ever since. As part of his research on self pollination in open-pollinated populations, SHULL (1908) described the method of inbreeding/hybridization as a new way of producing maize seeds.

After the discovery of heterosis, it took a full 60-70 years for the notion of a heterotic group and the real significance of this concept in hybrid maize breeding to be fully defined. Based on geographic location (temperate continental, tropical, and subtropical climates and transition areas between them), a large number of heterotic groups have been described in the literature so far, and new groups are being added to the lot all the time (TROYER, 2000). Most maize hybrids being grown in Europe today belong to either the heterotic pair Reid x Lancaster or European flint x American dent (GARAY, *et al*., 1996; MALVAR *et al*., 2005). In southeastern Europe, a heterotic pair has been found between the races and local populations of maize grown in the Balkans (RADOVIĆ and JELOVAC, 1995; STOJAKOVIĆ *et al*., 2000).

The grain yield of a maize hybrid results from the combined effects of its genetic yield potential, i.e. genotype (G), environment (E), and genotype x environment (GE) interaction. The correct evaluation of a hybrid's performance in a given environment is made more difficult by the presence of genotype x environment interaction. This kind of interaction represents the response of a genotype (variety or hybrid) to different environmental conditions (KANG, 1998). The choice of hybrids for a given area is especially hindered by cross interactions. Such interactions are reflected in the different ranking of hybrids from one location to another, which makes it more difficult to draw any reliable conclusions. One suitable method for the visual analysis of multi-environment trials is GGE biplot analysis (YAN, 1999; 2001; YAN *et al*., 2000; MITROVIĆ *et al*., 2011).

The objective of this study was to evaluate the traits of some new NS maize hybrids from FAO Maturity Group 600 relative to the standard and to determine the effects of the growing environment on their grain yields.

MATERIALS AND METHODS

In a joint effort between Institute of Field and Vegetable Crops, Novi Sad, and Ministry of Agriculture, Forestry, and Water Management of the Republic of Serbia, several new NS hybrids of maize were tested for the purposes of registration. These hybrids, NS6686, NS6683, NS396432, and NS281633, belong to FAO Maturity Group 600 and have standard quality grain. In order to determine the value for cultivation and use of the new cultivars, they were tested for the following essential traits: grain yield, grain moisture percentage at harvesting, resistance to root and stalk lodging, and resistance or tolerance to diseases and pests.

Trials were carried out in 2009 and 2010 using a randomized complete block (RCB) design with four replications. They were conducted in six locations in 2009 (Novi Sad (NS), Pančevo (PA), Sremska Mitrovica (SM), Sombor (SO), Vrbas (VB), and Zemun Polje (ZP)) and five in 2010 (Pančevo (PA), Sremska Mitrovica (SM), Sombor (SO), Vrbas (VB), and Zemun Polje (ZP)). Twenty hybrids were tested in total (19 new ones plus the standard ST). The hybrids of the Institute of Field and Vegetable Crops were marked with the letters NS, while the rest were given the numbers 2-16. Experimental units were $10.5m^2$ in size and consisted of two rows, seven meter long each. Row-to-row and plant-to-plant distance was 0.75 x o,22m, which produced 64,000 plants per hectare. The plots were machine planted to the final density (no thinning), while harvest was performed using the two-row plot combine HEGE 180, which automatically records grain weight and grain moisture content. Grain yield (tha^{-1} at 14% moisture) was calculated from the following formula: sample weight (kg/plot) x (100-sample moisture percentage/86) x (10,000/plot area). Stalk firmness and resistance to the causal agent of corn smut, the fungus *Ustilago maydis*, were expressed as percentage. Resistance to the pathogen *Exherohilum maydis* was estimated by the UPOV method using a scale of 0.5 to 5, where 0.5 represented the lowest and 5 the highest level of infection. Resistance to *Ostrnia nubilalis*, which was also determined according to the UPOV method, was expressed on a scale of 1.0 to 10.0, with 1.0 representing the lowest and 10.0 the highest level of plant damage.

The trials were set up on plots where wheat had been the preceding crop and deep plowing was implemented the previous autumn. Prior to sowing, the soil was analyzed for the presence of soil pests, soil insecticides were applied where needed, and soil agrochemical analysis was performed. Based on the agrochemical analysis, a mineral fertilizer recommendation was made for each site separately. The amounts of mineral fertilizer used and the timing and method of their incorporation were suited to maize hybrid requirements necessary for the expression of high genetic potential for yield.

The ranking of the hybrids relative to the standard taking into account genotype by environment interactions was carried out using GGE biplot analysis (YAN, 1999; 2001; YAN *et al.*, 2000). This method is based on two concepts. Firstly, when evaluating genotypes, only the effects of the genotype and genotype by environment interaction are important, so they are taken into consideration simultaneously (G+GE), and secondly, the genotypes are represented and evaluated in different environments using the biplot technique (GABRIEL, 1971). A GGE biplot shows the first two principal components (PC1 and PC2) obtained by the decomposition of individual data values. Besides enabling comparisons among the performances of different hybrids, GGE biplot analysis also makes it possible to identify the highest-yielding genotypes in different environments, the so-called ideal genotype for a particular environment, and the so-called megaenvironments (the delineation model). In the present study, the graph in which the hybrids are compared with the standard has been constructed using genotype focused scaling, while the graph in which different locations are grouped into megaenvironments has been made by using symmetric scaling.

RESULTS AND DISCUSSION

Genetic affiliation of parent lines: Based on the percentage of contribution of particular types of germplasm in their pedigrees, the inbred lines were grouped into clusters (Fig.1). The eight inbreds that are the parents of the NS maize hybrids were divided into two clusters. The first cluster is predominantly comprised of BSSS-type lines originating from crosses between the line B73 and some unrelated exotic germplasm (lines 1, 3, and 5) and pure BSSS-type lines (line 7, a reselection of B73). At a higher level, this group is joined by line 8, a product of a cross between BSSS and Iodent. The second cluster also consists of two subclusters. The first subcluster incorporates lines 2 and 6, derived by pedigree selection from crosses between the Lancaster line Mo17 and lines originating from local dent populations and a US-type dent line originating from the Krug population. These lines are then joined by line 4, which is predominantly of the Lancaster type (Mo17) but is also one third Reid Yellow Dent.

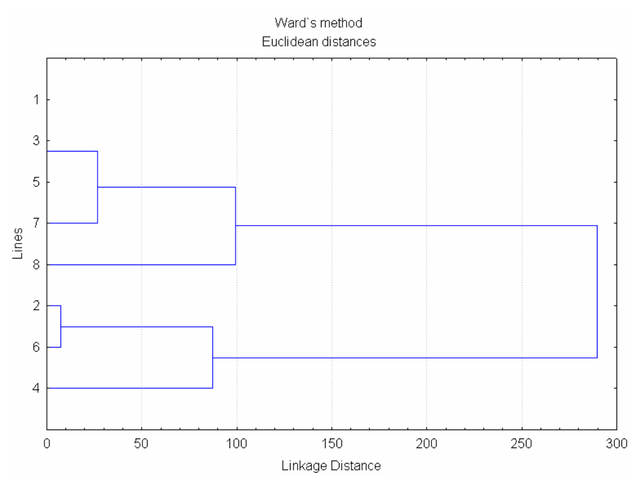

Fig.1. Dendrogram of 8 inbred lines based upon their genetic background

Characteristics of the hybrids: For the purposes of comparison with the standard, the experimental hybrids NS6686, NS6683, NS281633, and NS396432 were tested for grain moisture content at harvesting, resistance to root and stalk lodging, resistance to pests and diseases, and grain yield. Overall, the new hybrids outperformed the standard with respect to the studied traits in nearly every case (Tab.1). Compere to standard, new hybrids had less grain moisture at harvest by 0.85 to 2.54% (NS396432 and NS6686, respectively), lower root and stalk lodging percentage (NS6686 2,23%; NS281633 3,81%, while ST 5,77%). The percentage of plants infected by *Ustilago maydis* and *Exherohilum turcicum* was lower in all cases except that of NS6686 being infected by *Exherohilum turcicum.* The percentage of plants damaged by *Ostrinia nubilalis* was either on a par to the standard or within the acceptable deviation range. All of the new hybrids outyielded the standard by a highly significant margin, as their grain yields were higher than those of the standard by 0.884 tha^{-1} (NS396432) to 1.720 tha^{-1} (NS281633) (Tab.1).

Tab.1. Grain moisture content, root and stalk lodging, resistance to pests and diseases, and grain yield

Hybrid	Grain moisture (%)	Stalk lodging (%)	Ustilago maydis (%)	Exherohilum turcicum (0.5-5)	Ostrinia nubilalis (1-10)	Grain yield (tha^{-1})
ST	24.20	5.77	0.28	2.30	2.70	10.098
2	23.04	2.64	0.34	1.25	2.50	10.417
3	21.46	2.28	0.21	1.15	2.60	10.567*
4	21.91	2.98	0.00	2.00	2.40	10.582*
6	23.27	3.10	0.11	2.75	2.15	10.881**
7	19.81	2.80	0.05	1.40	2.10	11.312**
8	21.68	1.25	0.36	3.15	2.10	11.524**
9	19.77	2.38	0.00	1.80	2.20	11.661**
10	22.07	1.81	0.32	1.50	2.50	10.606*
11	22.41	2.68	0.06	1.90	2.30	11.295**
12	25.05	1.58	0.33	1.50	2.75	11.791**
13	21.30	2.64	0.00	2.15	2.75	12.010**
14	19.38	2.24	0.32	1.55	2.60	10.828**
15	22.36	1.82	0.11	2.90	2.85	11.778**
16	22.62	2.32	0.11	1.90	2.25	10.218
NS396432	23.35	3.08	0.05	1.65	2.50	10.982**
NS6683	22.78	2.88	0.00	1.50	2.30	11.377**
NS6686	21.66	2.23	0.00	2.40	3.35	11.083**
NS281633	22.70	3.81	0.10	1.90	3.10	11.818**

* significant at the 5% level, ** significant at the 1% level

Threshold values (according to the Serbian Law on the Registration of New Varieties of Agricultural Crops): grain moisture percentage - 25.20 (moisture in the standard + 1);

stalk firmness - up to 5% with moisture level of less than 25%, up to 3% with moisture level of more than 25%.

Hybrid ranking for grain yield relative to standard: Analysis of yield variance in multi-environment trials showed a significant influence of the environment (E), genotype (G), and genotype x environment (GxE) interaction. In both years, environment effects contributed the most to total yield variance - 60.73% in 2009 and 61.06% in 2010. The respective contributions of genotype and G x E effects were 14.35 and 10.58% in 2009 and 6.46 and 10.12% in 2010 (Tab.2). After the joint effect of the genotype and G x E interaction was broken down by GGE biplot analysis, it was found that the PC1 and PC2 values were significant and explained 77.04% (PC1 59.6%, PC2 17.04%) of the GGE sum of squares in 2009 and 76.33% (PC1 44.46%, PC2 31.87%) in 2010.

Tab.2. GGE analysis of grain yield variance

Year	Source of variation	df	SS	MS	F	%SS
2009	G[1]	19	268.76	14.15	20.10**	14.35
	E[2]	5	1137.11	227.42	323.18**	60.73
	GxE	95	198.19	2.09	2.96**	10.58
	residual	360	268.45	6.45	-	14.34
	total	479	1872.51	-	-	100.00
2010	G[1]	19	153.30	8.07	4.54**	6.46
	E[2]	4	1448.33	362.08	203.60**	61.06
	GxE	76	240.13	3.16	1.78**	10.12
	residual	300	530.18	2.44	-	22.36
	total	399	2371.93	-	-	100.00

1 genotype ** p<0.01
2 environment
** significant at the 1% level

A comparison can be made between the genotypes and the standard using the axis that connects the position of the standard on the biplot and the origin of the coordinate system (YAN, 2001). In 2009, all of the NS maize hybrids had a higher average grain yield than the standard in all locations (Fig. 2). Compared with the standard, the highest average yield was produced by the hybrid NS281633, followed by NS6686, NS6683, and NS396432. The hybrids NS281633, NS6686, and NS6683 produced the highest individual yields in the ZP09 location, followed by NS09, PA09, SM09, SO09 and VB09. The same hybrids performed better at ZP, NS and PA than at SM, SO, and VB. The hybrid NS296432 gave the highest and lowest grain yields at NS and VB, respectively. At ZP the hybrid's yield was above the trial average, whereas in the other locations it was below the same mean.

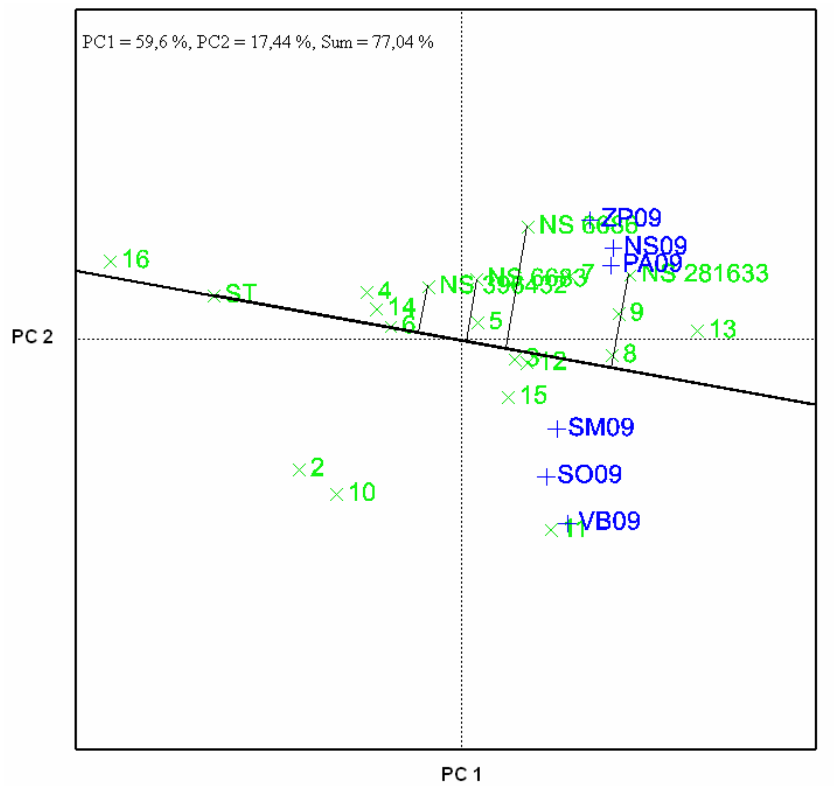

Fig. 2. GGE biplot of 20 maize hybrids tested in 6 locations in 2009

In 2010, all of the NS maize hybrids outyielded the standard in all the location in the following order: NS6686, NS6683, NS281633, and NS396432 (Fig. 3). The hybrid NS6686 had the highest grain yield at VB, followed by SM, PA, ZP, and SO. The hybrid's yields were higher than the trial average at SM and VB and lower than it in all the other locations. In the case of NS6683 and NS281633, the locations were ranked similarly according to how the two hybrids performed in terms of yield levels (SM, VB, PA, ZP and SO). The SM and VB locations suited these two genotypes better than PA, ZP, and SO did. At SM, PA, and VB NS396432 produced yields that were above the trial-average, whereas at PZ and SO the hybrid's yields were lower than the trial mean.

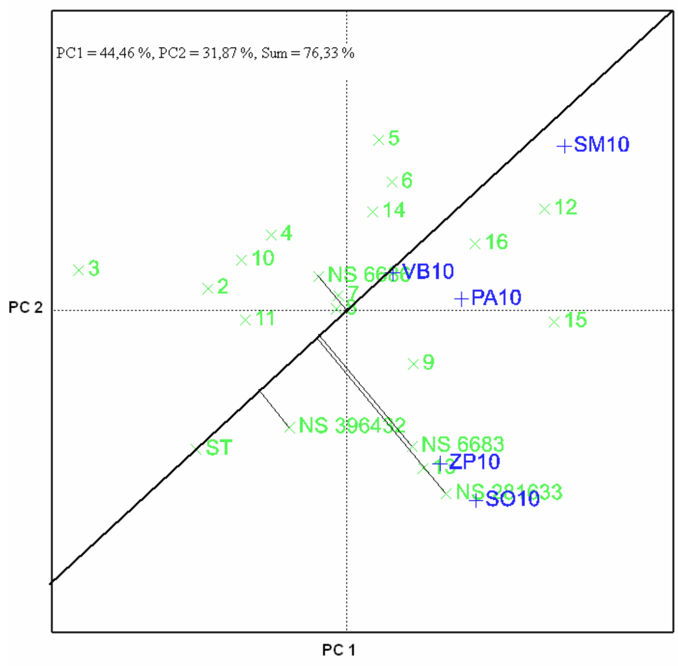

Fig. 3. GGE biplot of 20 maize hybrids tested in 5 locations in 2010.

In the depiction of megaenvironments, the environments were represented through a combination of the study year and the location in which the testing was carried out. In 2009, the trials were conducted in six locations, while in 2010 five locations were used, which makes for a total of 11 locations. GGE biplot analysis showed the significance of the first two principal components, which accounted for 36.66% (PC1) and 20.56% (PC2) of the variance.

The graphic representation of multi-environment trials makes it possible to group the locations into megaenvironments and to identify the best hybrids in each location (YAN *et al*. 2000). Genotypes that are farthest away from the origin of the coordinate system are connected by a line and thus form a polygon. Lines radiating from the origin of the biplot that intersect the sides of the polygon perpendicularly divide the polygon into sectors. Environments located within a given sector are those that the vertex hybrid in that sector is best suited for. The 20 maize hybrids studied in a total of 11 locations in 2009 and 2010 form a polygon incorporating six sectors (Fig. 4). Environments in which the hybrids were tested are located in three sectors. In the largest sector, which includes seven environments (ZP10, ZP09, PA09, NS09, VB09, SM09, and SO09), hybrid no. 13 was the highest-yielding. The second sector incorporates the PA10 and SO10 environments and has hybrid no. 12 as the leading genotype. The VB10 environment is in a sector of its own and has hybrid no. 16 as the best performer. The NS hybrids NS281633, NS6683, and NS6686 are in the first and largest sector, while NS396432 is not in any of the sectors, indicating that there was no environment in which it produced the highest grain yield.

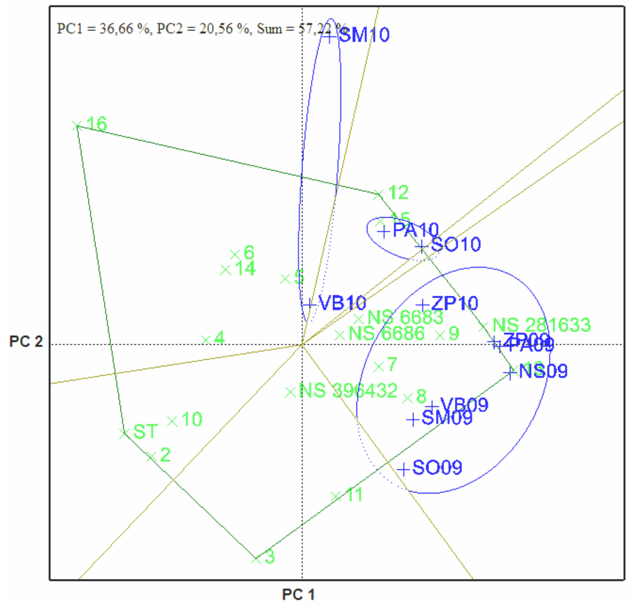

Fig. 4. GGE biplot showing the megaenvironments in 2009 and 2010.

Serbia has favorable natural conditions for maize growing, which is why maize is the most commonly cultivated crop in the country, where it is planted on more than 40% of the total plowland every year. Maize has been grown in this part of the world for more than 400 years. In the course of that time, a broad range of maize variability has been created locally that has been preserved until the present in the form of local dent and flint populations and transitional forms between them, which, after evaluation, have been divided into 16 genetically distinct groups. Through spontaneous and planned hybridization within this germplasm and between it and the introduced dents from the US maize belt, a unique heterotic source of dent maize has been developed (TRIFUNOVIĆ 1986). The first inbred lines derived from the local populations combined well with the inbreds of the Lancaster (C103, Mo17) and BSSS (B14, B37, A632, B73) type. As a result, domestic inbreds, along with the US lines of the Lancaster and Reid Yellow Dent type (C 103, WF9, N6, 38-11, and others), took part in the development of double cross hybrids that were grown locally in the 1970s. Domestic inbreds are still being used in maize breeding programs, both in the development of inbred lines and in the creation of commercial hybrids. In the hybrids from the latest cycle of selection, which were tested in the present study, domestic inbred lines contributed to the germplasms forming part of the hybrids' parental components (data not shown). Because they have retained genetic distance relative to both sources from the heterotic pair BSSS x Lancaster, domestic inbreds are still being used in the process of reselection to improve inbreds of both the BSSS and Lancaster type (STOJAKOVIĆ *et al*., 2005; 2007). Percentage contributions of domestic germplasm to the pedigrees of lines used, in this investigation ranged from 12.5% in the reselected lines of the B73 type (lines 1,3, and 5) to 31.25% (line 6, Lancaster type) and 37.50% (line 2, Lancaster type). Inbred lines originating from a total of seven heterotic groups participated in the pedigrees of the parental lines, with the BSSS group and its line B73 being the most common (47.17%). In modern maize breeding, Reid Yellow Dent germplasm and its two largest subgroups, BSSS and Iodent, account for 50% of all germplasm (TROYER 1999).

As the standard for the registration of new maize hybrids, a hybrid was used that had to meet several criteria, namely it had to be a new generation hybrid (this one was released in 2000) that has a high yield potential and good agronomic traits and is common enough in commercial production. All of the new NS maize hybrids from the latest cycle of selection outperformed the standard with respect to all the major traits. Grain yield was increased by 0.884 tha^{-1} (NS396432) to 1.720 tha^{-1} (NS281633) (Tab.1). Given that one selection cycle lasts about 10 years, this means that the genetic potential for grain yield has increased by more than 100 kg of dry grain/hectare/year, which is in agreement with our previous findings (IVANOVIĆ *et al*., 2002; 2006), as well as TROYER and ROCHEFORD (2002), but in disagreement with our recent investigation (STOJAKOVIĆ *et al*., 2010).

CONCLUSION

All the NS hybrids that were the subject of this study (NS6683, NS6686, NS281633, and NS396432) outperformed the standard with regard to all of the traits investigated: grain yield, grain moisture content at harvesting, root and stalk lodging, and resistance to pests and diseases. The grain yield potential of the new hybrids has been increased by 0.884 to 1.720 tha^{-1}, while grain moisture at harvesting has been reduced by 0.85 to 2.54%. The study identified the so-called ideal locations for particular hybrids, which may be of use when determining which areas the hybrids are best suited for.

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PROCENA VREDNOSTI NOVIH NS HIBRIDA KUKURUZA PRIMENOM BIPLOT ANALIZE

Milisav STOJAKOVIĆ, Mile IVANOVIĆ, Goran BEKAVAC, Aleksandra NASTASIĆ, Bojan MITROVIĆ, Dušan STANISAVLJEVIĆ

Institut za ratarstvo i povrtarstvo, Novi Sad, Srbija

Analizirani su dvogodišnji rezultati ispitivanja važnijih osobina 20 novih hibrida kukuruza FAO 600 grupe zrenja u poređenju sa standardom. Detaljnije su komentarisani podatci koji se odnose na hibride NS6683, NS6686, NS281633 i NS396432. Za ispitivanje prinosa zrna, sadržaja vlage u zrnu u berbi, čvrstoće stabla i otpornosti na bolesti i štetočine postavljen je ogled u polju po RCB dizajnu u 4 ponavljanja, na 6 lokacija u 2009. i na 5 lokacija u 2010. godini. Rezultati su predstavljeni preko GGE biplotova u cilju: rangiranja hibrida u odnosu na standard uzimajući u obzir prisustvo interakcije genotip-sredina, i identifikacije najprinosnijih genotipova u različitim sredinama. Kod novih NS hibrida kukuruza je ustanovljen viši prinos zrna od standarda za 0,883 do 1.720 tha^{-1}, niži sadržaj vlage u zrnu za 0,85 do 2,54%, veća čvrstoća stabla i otpornost prema bolestima i insektima na nivou standarda. Identifikovani su tzv. idealni lokaliteti za pojedine hibride što može biti od koristi prilikom njihove rejonizacije.

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