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Research Article

Stability of the expression of the maize productivity parameters by AMMI models and GGE-biplot analysis

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Abstract

The objective of this study was to estimate genotype by locality, by year, by treatments $(G \times LxYxT)$ interaction using AMMI model, to identify maize genotypes with stable number of rows of grains performance in different growing seasons. The trials conducted with seven maize lines/genotypes, four treatments, two years and at the two locations. The results showed that the influence of genotype (G), year (Y), locality (L), and $G \times L$, $G \times T$, $G \times T \times T$, $G \times Y \times T$ interaction on maize number of rows of grains were significant (p < 0.01). The genotype share in the total phenotypic variance for the grains number rows of was 53.50%, and the interaction was 21.15%. The results also show that the sums of the squares of the first and second major components (PC1 and PC2) constitute 100% of the sum of the squares of the interaction $G \times L$. The first PC1 axis belongs to all 100%, which points to the significance of the genotype in the total variation and significance of the genotype for overall interaction with other observed sources of variability. The highest stability in terms of expression of the grains number of rows had the genotype L - 6, followed by the genotypes L - 4, L - 5 and L - 3. The lowest stability was demonstrated by the genotypes L - 2 and L - 1, which confirmed that these genotypes are not important for further selection in terms of this trait.

Keywords: G×Y×L×T interaction; number of rows of grains; PCA1 and PCA2; Zea mays

Introduction

Maize (*Zea mays* L.) (2*n*=20), belongs to the family Poaceae. Diversified uses of maize worldwide include: grain, starch products, maize oil and forage for animals. Maize in the world sowed of 185 million

hectares, average yield was 5.62 t ha⁻¹ while total production was over 1.04 billion tonnes. Maize cultivars are grown in approximately 1.10 million hectares annually in Serbia and national average yield was 7.52 t ha⁻¹ (Ikanović *et al.*, 2018). Hybrid seeds demanded by maize growers are provided by mostly national or international seed companies in Serbia.

For a long period, maize breeding has been focused on increased and stabile yield (Babić *et al.*, 2018). Maize breeding programs depend on the understanding and knowledge of genetic diversity and relationship among inbred lines and breeding material. That is especially fundamental in assigning inbreds to heterotic groups and planning outstanding hybrid crosses (Srdić *et al.*, 2007). Maize, according to the sowing area of crops, is on the third place in the world, and on the first place in Serbia. According to the harvested areas, Serbia is the fourth in Europe (Popović, 2010; Ikanović *et al.*, 2018). That is due to the fact that maize has such a versatile usage from the unprocessed product for livestock feed, to that it is processed in many different industries such as food, pharmaceutical and more and more nowadays maize is row material for production of energy. The main goal in production of this crop is achieving high and stabile yields, and recently more and more, higher biomass. Grain yield beside the genetic potential is highly influenced by many factors such as: applied crop practices, soil fertility, and level of ground water, altitude, amount and distribution of precipitation, i.e. conditions of the environment (Branković-Radojčić *et al.*, 2017). Filipović *et al.* (2015), emphasized that maize breeders are due to the global climatic changes, challenged to create highly adaptable genotypes, which are capable to produce high and stabile grain yields in different environments.

Genotype stability in different environments is the consequence of its genetic structure, but there is a few information about genetic components that determine genotype stability, and how the selection and breeding have the influence on them (Lee et al., 2003). Factors that influence grain yield and thus the economic aspect of maize production are associated with polygene action, but are also under great influence of the environment. In the research of Pavlov and Crevar (2014), it was confirmed that beside the hybrid combination as the major factor, very important influence expressed environmental factors such as years and locations on the parameters of seed production. Therefore, trials in maize breeding process that are focused on grain yield are performed on the larger number of locations and in several successive years. These experiments usually observe the relative success of genotype performances in different environments (Kandus et al., 2010). The aspect of the G×E interaction is very important in breeding programs and as well in the commercial introduction of new hybrids. Deitos et al. (2006) indicate that G×E interaction is important in breeding process because it influences the genetic gain, as well as the recommendation and choosing the varieties with high adaptability. Petrović et al. (2009) concluded that breeding for the targeted environment highly depends on the identification of the major sources of phenotypic variation in that region. In order to develop a variety or a hybrid which possess lower G×E interaction, for the dominant sources of variation, variety should have the balanced proportion between stabile and high yield (Boakyewaa, 2012).

The considerable variation in soil and climate has resulted in large variation in yield performance of maize hybrids annually; thus GEI (genotype x environment interaction) is an important circumstance for plant breeders and agronomists. Evaluation of genotypic performance in a number of environments provides useful information to identify their adaptation and stability (Crossa, 1990). Multi-environment yield trials are used commonly to release superior genotypes for target sites in plant breeding programs. GEI is universal phenomenon when different genotypes are tested in a number of environments. The large GEI variation usually impairs the accuracy of yield estimation and reduces the relationship between genotypic and phenotypic values. GEI due to different responses of genotypes in diverse environments makes choosing the superior genotypes difficult in plant breeding programs (Ilker *et al.*, 2009). Numerous studies on various plant species indicate that the genotype has a contribution about 50% in realization of yield (Filipović et al., 2015; Živanović *et al.*, 2017; Ikanović et al., 2018).

By using statistical methods, it is possible to exclude the consequences of interaction of genotype and external environment and to isolate stable and high yield genotypes. AMMI model combines the analysis of variance of genotypes and the environment main effects with principal component analysis of the GEI into a

unified approach (Gauch and Zobel, 1996). However, GGE biplot method, which is always close to the best AMMI models in most cases (Ma *et al.*, 2004), was recently developed to use some of the functions of these methods jointly. It allows visual examination of the relationships among the test environments, genotypes and the genotype by environment interactions (Ding *et al.*, 2007). The differences of the two methods, GGE biplot analysis is based on environment-centred PCA, whereas AMMI analysis is referred to double centred PCA (Ding *et al.*, 2007). AMMI stands for the additive main effect and multiplicative interaction (Gauch, 1992) and GGE biplot stands for genotype main effect plus G×E interaction (Ma *et al.*, 2004).

The aims of this study were to identify superior experimental hybrids with number of rows of grains as well as to select the best location for testing hybrids, year, locations and treatments (with sulfonylurea), developed in the maize breeding program of the domestic company of Serbia by using and comparing AMMI and GGE biplot methods.

Materials and Methods

Plant material

The research was carried out at two sites: in Zemun Polje ($44^{\circ}52^{'}N$, $20^{\circ}19^{'}E$, 81m asl) and in Pančevo ($44^{\circ}52^{'}14^{''}N$ $20^{\circ}38^{'}25^{''}E$, 77m asl) during 2010 and 2011 and the basic plant materials consists of six lines of maize (L-1, L-2, L-3, L-4, L-5 and L-6). The types of soil in Pančevo and Zemun Polje is chernozem. Examines were based on a completely random block system, in three reps, with 20 plants in each repetition. Each genotype was planted in one row with ten houses with two plants each, so that the size of the elementary plot was $2.8~\text{m}^2$ ($0.7m \times 0.4m$). The density of crops was 74.280 plants per hectare. Sowing and harvesting were done manually and, in the experiment, standard maize cultivation technology was applied except for variants with treatment (Božović, 2018; Božović *et al.*, 2018).

Treatments

The experiments were four sub-treatments with Sulfonylurea herbicides: $(1.) T_1$ -Treatment 1; Control was without herbicide treatment was done only with water; $(2.) T_2$ - Treatment 2; active substance Nicosulfuron, and the Motivell preparation in the amount of 1.25 l ha⁻¹ (6.3 ml per treatment); $(3.) T_3$ - Treatment 3; active substance Rimsulfuron, and the Tarot preparation in the amount of 60 g ha⁻¹ (0.3 g per treatment); $(4.) T_4$ - Treatment 4; Active substance Forasulfuron, and the Ekvip preparation in the amount of 2.5 l ha⁻¹ (12.6 mL per treatment).

Herbicides were applied when corn was in the phase of 9-10 developed leaves (15-16 per BBCH scale) with a Solo spiral type with Tee Jet KSR11003, with a water consumption of 250 l ha⁻¹. At the time of the technological maturity, at both localities, from each tested genotype, 10 plants from three reps were taken in order to obtain data on the following the number of rows of grains. Biometric data processing is based on repetition for tested trait.

Statistical analysis

The AMMI model (The Additive Main effects and Multiplicative Interaction) was used to assess the $G \times E$ interaction, and it can be represented by the following formula (Gauch and Zobel, 1996): $\mathbf{Y}_{ger} = \mu + \alpha_g + \beta_e + \Sigma_n \lambda_n \gamma_{gn} \delta_{en} + \rho_{ge} + \epsilon_{ger}$

Where: Y_{ger} is the yield for the genotype g in the environment e the replication r. The additive parameters are: m – the grand mean, α_g – a/the genotypic mean deviation from the grand mean, β_e – the environmental mean deviation. The multiplicative parameters are: λ_n – a singular value for n interaction principal component axis n, γ_{gn} – the genotypic eigenvector for IPCA axis n, δ_{en} – the eigenvector of the environment for IPCA axis n, ρ_{ge} – a residue when not all PCA axis are included and e_{ger} - the error. Statistical data analysis was performed

using the GenStat 12th computer statistical program (GenStat, 2009). AMMI analyses were performed in Excel Biplot Macros (Johnson and Bhattacharyya, 2010).

Meteorological data

Environmental factors have great influence on qualitative and quantitative traits. The crucial impact on the maize productivity has the amount and the distribution of precipitation (Šarčević-Todosijević *et al.*, 2014; Popović *et al.*, 2016). Maize responds very stressfully to the drought, especially in certain development phases (Popović, 2010; Maksimović *et al.*, 2018). Meteorological data (monthly precipitation and average temperature) were collected from the weather station located near the experimental fields, in Pančevo and Zemun Polje, Serbia. In first year in Pančevo average monthly temperature was 17.44 °C and in second year 19.53 °C. In Zemun Polje in first year average monthly temperature was 18.51 °C and in second year 19.57 °C, Figure 1A.

In first year in Pančevo total amount of precipitation during crop growth cycle was 480 mm and 497 mm in Zemun Polje and in second year 368 mm in Pančevo and 496.60 mm 328 mm in Zemun Polje, Figure 1B.

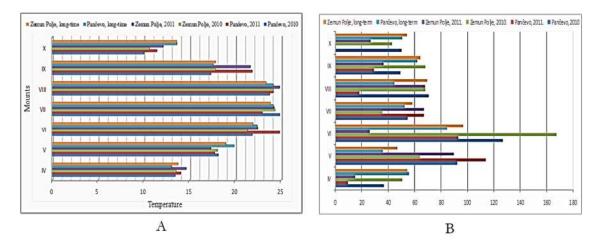


Figure 1. This is a figure present meteorological data of tested year (A) Average temperature, °C. (B) Total precipitation, mm in Pančevo and Zemun Polje, Serbia

Results

AMMI model for the grains number of rows

Table 1 shows the analysis of the variance of the AMMI model for the grains number of rows in the investigated maize lines. The degradation of the total sum of the squares to the additive (genetic) and non-additive (ecological) component by analysing the variance for the number of rows of grains indicates a statistically very significant influence of genotype, year, locality, and statistically significant influence of interactions $G \times L$, $G \times T$, $G \times T$, $G \times T$, and $G \times T$ (Table 1).

The sums of squares for localities was more than twenty times lower, while sums of squares for years and treatments had more than 100 times lower value than the sum of squares of genotypes. The effect of treatment in total variation is not statistically significant. The genotype share in the total phenotypic variance for number of grain rows was 53.50%, and the interaction was 21.15%. There was a significant difference between the reaction of genotypes to different ecological factors, which results from a large sum of squares of individual interactions, as well as a relatively high share of interactions in the overall variation of this trait. Considering the existence of a significant proportion of the genotype interaction with other sources of variation (years,

localities and treatments), AMMI analysis of its main components IPCA1 and IPCA2 was done. The first major component, IPCA1, comprised 100% of the sum of the squared interaction G×L and showed a statistically significant effect, while the share of the second component was 0.00% and did not show statistical significance. Also, the large sum of the genotype squares indicates a great divergence between the observed genotypes for the observed trait.

Table 1. The additive main effects and multiplicative interactions analysis of variance for number of grains rows for tested maize lines

Common Commission	DE	Number of rows of maize grains		
Sources of variation	DF	SS	SS (%)	MS
Genotype (G)	5	590.27	53.50	118.05**
Year (Y)	1	5.28	0.48	5.28**
Locality (L)	1	25.09	2.27	25.09**
Treatment (T)	3	3.51	0.32	1.17 ^{ns}
GxY	5	2.91	0.26	0.58 ns
GxL	5	20.27	1.84	4.05**
IPCA1 (100%)	5	20.3	100.00	4.05 ns
IPCA2 (0%)	3	0.0	0.00	0.00 ns
GxT	15	28.47	2.58	1.90*
Y x L	1	2.17	0.20	2.17 ns
ΥxΤ	3	19.73	1.79	6.58 ns
LxT	3	3.20	0.29	1.07 ns
GxYxL	5	1.27	0.12	0.25 ns
GxYxT	15	65.66	5.95	4.38**
GxLxT	15	56.02	5.08	3.73**
YxLxT	3	3.51	0.32	1.17 ns
GxYxLxT	15	33.97	3.08	2.26*
Error	192	242.00	21.92	1.26 ns
Total	287	1103.33	100.00	-

ns - Non significant; *, ** - significant at 0.05 and 0.01

The results also show that the sums of the squares of the first and second major components (PC1 and PC2) constitute 100% of the sum of the squares of the interaction $G \times L$. Also, the first PC1 axis belongs to all 100%, which points to the significance of the genotype in the total variation and significance of the genotype for overall interaction with other observed sources of variability, Table 1.

Genotype by Environment interactions $(G \times E)$ effects on maize grains number of rows is usually significant due to the diverse environmental conditions at growing sites. The combined ANOVA showed differences among environments (E) and genotypes (G) to be significant indicating that they were diverse. However, genotype by environment interactions for maize grains number of rows was not significant.

Table 2 gives values of IPCAg components of the interaction, that is, the AMMI stability value for the number of rows of grains for tested lines of maize. Lines, L-5, and L-3 are distinguished by minimum values or values that weigh zero for the first main component, IPCAg1, versus the L-6, L-4 and L-2 lines whose values are away from zero. The second IPCAg2 component was zero for all lines, since it was zero in the analysis of the variance.

Stability of maize genotypes

Stability of the number of grain rows in tested maize lines, observed by location is shown in Figure 2. The highest stability in terms of expression of the number of rows of grains was expressed by the genotype L-3, whose average value for the tested trait almost coincides with the total average for all the genotypes involved in the study. The genotype L-5, which has the best expression of this feature, also features high stability. L-6 and L-4 genotypes have been much less stable, while genotypes L-2 and L-1 are not significant for the process of further refinement in terms of the trait as they have under average expression of the number of rows of grains (Figure 2A).

Table 2. IPCA component of interaction and AMMI value of stability for number of rows of maize grain	ıs
at examined maize lines	

	Number of rows of maize grains				
Genotype	A	Genotype x Locality			
	Average	IPCAg1	IPCAg2		
L-1	10.50	0.190	0.000		
L-2	10.08	-0.425	0.000		
L-3	12.10	0.036	0.000		
L-4	13.00	-0.487	0.000		
L-5	13.88	0.005	0.000		
L-6	13.42	0.681	0.000		

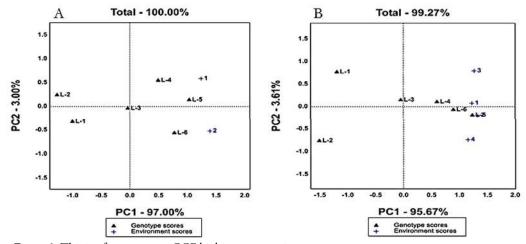


Figure 2. This is a figure, present GGE biplot representation
(A) GGE biplot representation of stability of expression for the number of rows of grain of lines of maize based on locality and (B) based on the treatment

Figure 2B shows stability of the expression of the number of rows of grain at observed lines according to the treatments. It can be concluded that the highest stability in terms of expression of the number of rows of grains had the genotype L-6, followed by the genotypes L-4, L-5 and L-3. The lowest stability was demonstrated by the genotypes L-2 and L-1, which again confirmed that these genotypes are not important for further selection in terms of the trait. It can be concluded that the constant stability of expression of the number of rows of grains has been demonstrated by the genotype L-5, both in terms of the examined locations and the applied treatments. A similar conclusion can be drawn for the genotype L-3, for which can be said that does not represents something new in selection, because its average values are at the average level.

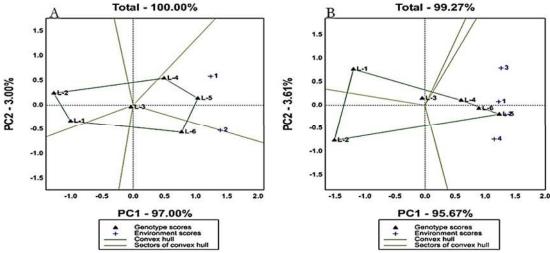


Figure 3. This is (A) figure present GGE-biplot

GGE-biplot view for the number of rows of grain of lines of maize, according to the "which-won-where" model based on the locality and (B) based on the treatment

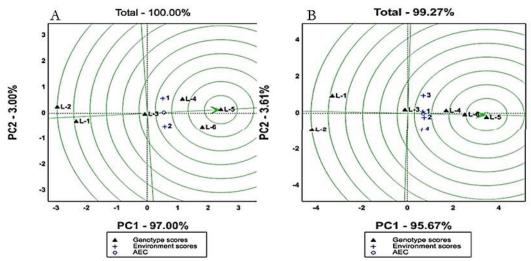


Figure 4. This is (A) figure present GGE-biplot, GGE biplot view for the grain number of rows of maize lines according to the ideal genotype model based on the locality and (B) based on the treatment

In Figure 3A it can be seen that the axes projected from the coordinate start, splits a biplot on 5 sectors. The best expression in locality 1 had genotypes L-5 and L-4. As the value of the locality 2 is at the very border of the two sectors, the phenotypic expression of the mentioned genotypes was high in this locality. The genotype L-6 had good expression only in locality 2. The other three genotypes did not show a good result in any of the observed localities.

The field of the coordinate system in Figure 3B is divided into four sectors. All treatments and four genotypes are located in one and the largest sector. Based on the phenotypic expression shown for the number of rows of grains, the genotypes L-4, L-5 and L-6 occupy the same sector on biplot. The mentioned genotypes showed the best values in all examined treatments, which, according to their average values, are also located in the same sector. Genotypes L-1, L-2 and L-3 did not show a good result in the treatment.

Figure 4 shows a comparison of the tested lines with an ideal genotype for expressing the number of rows of grains by locations and treatments. In Figure 4A and 4B biplot shows that the value of phenotypic expression

and the stability of the genotype L-5 coincide with the ideal genotype, followed by L-6, L-4, etc. Even in the applied treatments, the genotype L-5 is the closest to the ideal genotype, both in terms of stability and in terms of shown expression. Genotypes L-6 and L-4 had satisfactory stability but lower expression rates for the observed trait.

Discussion

The grain number of rows, number of grains per year, mass 100 grains and grain yield belong to the group quantitative properties. Quantitative properties are conditioned by the larger number of genes with bad individual effect i.e. minor genes, for which it is characteristic continuous variability and great ecological variability, which means that the yield does not depend solely of genetic factors but also from factors external environment (Borojević, 1965).

The combined ANOVA showed differences among environments (E) and genotypes (G) to be significant indicating that they were diverse. The proportions of the total variance in grain yield attributable to the environments were the highest (87.27%) while genotypes and G×E contributed 5.45% and 0.84%, respectively (Adu *et al.*, 2013).

The nonsignificant $G \times E$ interaction effects for grain yield suggests that a promising genotype selected in one of these locations will also be suitable for production in the other locations in the same agro-ecological zone. Environments were found to contribute greatly to the variations in performance of genotypes. This indicates that, unpredictable environmental conditions are one of the major constraints to selecting superior and widely adapted maize varieties. The use of GGE biplot analyses provided clear bases for determining stability and performance of the 100 extra-early maize genotypes (Adu *et al.*, 2013).

Genotypes that in different ecological conditions have the value of the first component close to zero are considered stable. According to Sabaghniaa *et al.* (2006) genotypes with a minimum variance, as well as a minimum value of IPCg1 axis or when this value of weighting zero in different ecological conditions are considered stable.

Modern plant breeding has been historically oriented toward high agronomic yield rather than the nutritional quality (Morris and Sands, 2006). Such trend indicates that it is necessary to balance between yield and quality in breeding process. Nevertheless, obtained results indicate that OPVs maintained in gene bank, as well as OPVs continuously grown on fields represent potential source for development of good initial material, thus providing grain quality enhancement in white maize breeding collections. The increased incidence of intolerance and allergies to gluten and gluten-containing products has opened up new possibilities for the use of maize flour and maize-based products (Padalino *et al.*, 2011). If adequate measures are applied during cultivation, undesirable effects on OPVs -Open Pollinated Varieties, can be prevented and efficient coexistence of different types of agriculture can be provided (Lorenzana and Bernardo, 2008; Urechean and Bonea, 2017).

AMMI model, GGE model and principal component analysis (PCA) are singular value decomposition (SVD) based statistical analyses often applied to yield-trial data (Gauch, 2006). According to the results of the AMMI and GGE biplot analysis obtained the similar findings from our multi-environment trials data, both of statistical methods can be used reliably by the plant breeders to evaluate maize experimental hybrids and to identify proper test environments.

Maize production is inconceivable without herbicide application, and certainly depends on crop susceptibility. Some injuries could be induced by herbicides, what could result in yield losses. This is especially prominent in maize seed production, due to the line's susceptibility to various stressful conditions, including herbicides. Growing season had significant influence on susceptibility. All applied herbicides from sulfonylurea increased grain yield in 2014, but in 2015 nicosulfuron expressed the lowest selectivity, by decreasing grain yield and soluble proteins up to the 21th day after herbicide application, when compared to control (Dragićević *et al.*, 2017). Genotypes of interest in production are genotypes with high values of the PC1 component when it

comes to the properties in which the plus variants, i.e. the higher the average value of the tested property and the lower the value of the PC2 component, i.e. close to zero. In the properties in which the minus variants are preferred in the selection process (for example, the height of the stem, etc.), genotypes with a lower PC1 value and a PC2 value tend to be zero, are desirable. Low-grade genotypes PC2 are characterized by broad adaptability, as opposed to specific adaptive genotypes that are located far from the coordinative start. Therefore, the high value of PC2 indicates that the best expression of the trait (Božović *et al.*, 2018) is in specific agro-ecological conditions.

Conclusions

Results from this study have suggested that AMMI analysis is very applicable for the analysis of maize lines and different years, locality, treatment and their interaction.

The genotype share in the total phenotypic variance for the number of rows of grains was 53.50%, and the interaction was 21.15%. The results also show that the sums of the squares of the first and second major components (PC1 and PC2) constitute 100% of the sum of the squares of the interaction G×L. Also, the first PC1 axis belongs to all 100%, which points to the significance of the genotype in the total variation and significance of the genotype for overall interaction with other observed sources of variability.

Value of phenotypic expression and the stability of the genotype L-5 coincide with the ideal genotype, followed by L-6, L-4, etc. Even in the applied treatments, the genotype L-5 is the closest to the ideal genotype, both in terms of stability and in terms of shown expression. Genotypes L-6 and L-4 had satisfactory stability but lower expression rates for the observed trait.

Authors' Contributions

In this research, all authors contributed effectively. VR wrote the manuscript; VP designed the experiments; MK, LJK and DB carried out the experiments; VF and VU analysed the data, VS writing-review & editing; VP supervised the project and revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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