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EVALUATION OF EXPERIMENTAL MAIZE HYBRIDS TESTED IN MULTI-LOCATION TRIALS USING AMMI AND GGE BIPLOT ANALYSES

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ABSTRACT

Multi-environment yield trials are essential in estimation of genotype by environment (GE) interaction and identification of superior genotypes in the final selection cycles. The objective of this study was to evaluate stability and adaptability of grain yield of experimental maize hybrids by GGE (Genotype and Genotype by Environment Interaction) biplot and AMMI (Additive Main Effects and Multiplicative Interaction) analyses. This study comprised 19 experimental NS (Novi Sad) maize hybrids tested across twelve environments in northern Serbia in a randomized complete block design. The ANOVA (Analysis Of Variance) indicated significant effects of genotypes (G), environments (E) and their interaction (GE). On the same time, the highest percentage of variation was explained by E (77.83%) while G and GE effects together explained the rest of variation (<30%). Joint effects of genotype and interaction (G+GE) was partitioned using GGE biplot analysis where the first two components were significant, explaining 62.40% (44.34% PC1 and 18.06% PC2) of the GGE sum of squares. The two principal components in AMMI analysis were significant, explaining 53.99% (30.87% PC1 and 23.12% PC2) of interaction variation. Stability of analysed genotypes was similar in both methods. It is concluded that there is no large difference between the AMMI and GGE biplot analyses in evaluation of experimental maize hybrids in different climatic conditions and that both methods can be used equally successfully.

Key words: breeding, GE interaction, hybrids, grain yield, maize (*Zea mays* L.)

INTRODUCTION

The Institute of Field and Vegetable Crops has nearly 75-year old tradition of maize breeding. This complex and time-consuming process consists of several phases and results in the development of new maize hybrids which perform better than existing ones in terms of yield and other agronomical traits. In Serbia, maize is being produced on 1.2 million hectares across the country. Thus, production conditions vary from region to region. Considering the natural (environmental) differences of amongst growing regions, after the hybrid registration (authorisation) a network of post-commission trails is formed in order to test the hybrids reaction in different agro-ecological conditions and to determine which hybrid is suitable for growing in a particular region. Results of those trails indicate those hybrids that meet the requirements for high and stable yield, which are going to be marketed afterwards. Despite the substantial financial resources and time invested, characteristics of only a few hybrids from each breeding cycle meet the producer's requirements in specific growing conditions and are accepted in the production (Stojaković et al., 2010).

Selection based on yield only, may not always be adequate when genotype by environment interaction is significant (Kang, 1991). The presence of genotype by environment interaction (GEI) frequently changes the hybrid ranks in different environments due to cross interaction making their proper selection difficult. Therefore, it is essential that the genotype by environment interaction is taken into account, properly understood and analysed. There are a few methods for GEI analysis which all aim at identification of genotypes suitable for certain growing regions. Ilker et al. (2009) finds that, until now, many statistical models have been developed to explain the interaction. For instance, type B correlation (Yamada, 1962), joint regression (Finlay and Wilkinson, 1963; Eberhart and Russel, 1966; Perkins and Jinks, 1968). Yet, interactions are usually explained in more complex methods based on analysis of variance, regression analysis, principal component analysis (PCA), cluster analysis and other methods of multivariate analysis such as AMMI model (Additive Main Effects and Multiplicative Interaction, Gauch and Zobel, 1996) and GGE biplot analysis (Genotype and Genotype by Environment interaction, Yan et al., 2000).

Analysis of variance as an additive model explains only main effects and informs whether or not the GE interaction is a significant source of variation. It does not, however, provide the insight into the individual genotypes and localities which are the components of the interaction (Samonte et al., 2005).

AMMI analysis combines ANOVA and principal component analysis (PCA) where the sources of variability in the genotype by environment interaction are partitioned by PCA. The interpretation of results obtained from AMMI analysis is performed with a biplot that relates genotypic means to the first or some of the principal interaction components.

GGE biplot analysis enables visual (graphical) presentation of interaction estimate. This method also combines ANOVA and PCA by partitioning together sums of squares of genotypes and sums of squares of GEI (which are relevant in genotype evaluation) using PCA method. The biplot technique is used for the presentation and estimation of genotypes in different environments (Gabriel, 1971). GGE biplot shows the first two principal components (PC1 and PC2) which are obtained by decomposition of singular values of multi-location trials yield data. GGE analysis enables the identification of the genotypes with the highest yields in different environments, comparison of their performances in different environments, identification of so-called “ideal“ genotype, as well as „mega-environments“ (model of regional distribution or target environment).

The aim of this paper is to examine the yield and yield stability of new hybrids in several different agro-ecological environments in Serbia using AMMI and GGE biplot method. Additionally, this study aims to determine the more suitable model for estimation of new hybrid performances and agronomical traits and to obtain sufficient data for hybrid recommendation for every growing region taking into account the specificities of hybrids and growing conditions of the regions.

MATERIALS AND METHODS

Field trials

Multi-location field trails are conducted in five locations during 2007 and in seven locations during 2008 in Serbia: Rimski Šančevi (RS), Srbobran (SR), Pančevo (PA), Sombor (SO), Sremska Mitrovica (SM), Kikinda (KI) and Aleksinac (AL). Climatic characteristics of test locations are given in Table 1. Combination of years (2007, 2008) and locations (RS, SR, PA, SO, SM, KI, AL) were treated as 12 environments (RS07, RS08, SR07, SR08, PA07, PA08, SO07, SO08, SM07, SM08, KI08 and AL08). The trails were arranged in a randomized complete block design with three replications. Nineteen maize hybrids FAO maturity groups 600-700 which were developed in the Institute of Field and Vegetable Crops in Novi Sad are chosen for the trails. Seventeen newly developed hybrids registered in 2005 and 2006 and two commercial check hybrids (G12 and G17) are used for the trails. The experimental plot size for each hybrid was 9.75 m^2 (two rows, each 6.5m long). The distance between rows was 0.75 m, and 0.22 m within rows, with density 60 606 plants/ha. The planting and the harvest were performed by machine. Each hybrid was planted in four rows, but only the two middle rows were harvested and measured. The standard agro-technical practices were applied according to local agro-ecological conditions. The grain yield in t/ha with 14% moisture was measured.

Table 1. Climatic characteristics of test locations

Locations	Geographic position			Seasonal rainfall (mm)		Soil type
	Latitude	Longitude	Altitude(m)	2007	2008	
Rimski Šančevi (RS)	45°20′N	19°51′E	82	367.7	333.2	Non-carbonate chernozem
Sombor (SO)	45°46′N	19°06′E	87	312.8	389.6	Carbonate chernozem
Sr. Mitrovica (SM)	44°58′N	19°36′E	100	358.8	313.3	Chernozem
Srbobran (SR)	45°32′N	19°44′E	79	319.8	252.1	Chernozem
Pančevo (PA)	44°52′N	20°39′E	82	364.5	305.9	Carbonate chernozem
Aleksinac (AL)	43°34′N	21°43′E	177	272.8	350.6	Alluvial soils and pseudogley
Kikinda (KI)	45°49′N	20°27′E	82	383.9	286.8	Carbonate meadow soil

Statistical analysis

Analysis of variance is calculated using the model:

$$Y_{ij} = \mu + G_i + E_j + GE_{ij}$$

where Y_{ij} is the corresponding variable of the i-th genotype in j-th environment (location), μ is the total mean, G_i is the main effect of i-th genotype, E_j is the main effect of j-th environment, GE_{ij} is the effect of genotype x environment interaction.

AMMI model can be presented with the following formula:

$$Y_{ger} = \mu + \alpha_g + \beta_e + \sum_{n=1}^{N} \lambda_n \gamma_{gn} \delta_{en} + \rho_{ge} + \varepsilon_{ger}$$

where Y_{ger} is the observed yield for the genotype g in the environment e the replication r, μ is total mean estimated with $\overline{Y}$, α_g is genotypic deviation from the total mean estimated from the difference $\overline{Y}_g - \overline{Y}$, β_e is the deviation of a mean from the total mean estimated from the difference $\overline{Y}_e - \overline{Y}$, N is the number of interaction principal component axis (IPCA), λ_n is a singular value for n interaction principal component axis, γ_{gn} is genotypic eigenvector for IPCA axis n, δ_{en} is eigenvector of environment for IPCA axis n, ρ_{ge} is a residue when not all PCA axis are included and ε_{ger} is an error.

In ANOVA non-additive residue is GE interaction. The multiplicative part in AMMI model uses PCA analysis to decompose the interaction into several principal components (PCA axis) from 1 to N and residue ρ_{ge} which remains if not all possible PCA axis are included. G-1 and E-1 are the possible numbers of axis, but usually only the first few are of interest. The degrees of freedom are determined using Gollob method (Gauch, 1992).

GGE biplot analysis can be presented with the formula:

$$Y_{ij} - \mu - E_j = \lambda_1 \varepsilon_{i1} \eta_{1j} + \lambda_2 \varepsilon_{i2} \eta_{2j} + e_{ij}$$

where *Yij* is the corresponding variable of the *i*-th genotype in *j*-th environment (location), μ is the total mean, E_j is the main effect of *j*-th environment, λ_1 and λ_2 are singular values of principal components PC1 and PC2; ε_{i1} and ε_{i2} are eigenvectors in *j*-th environment (location) for PC1 and PC2 of *i* genotype in *j* environment.

This method groups the effect of genotype, which in AMMI analysis represents additive component, and the effect of the interaction. Then it analyses these effects with PCA method (Balestre et al., 2009). In this paper, GGE biplots representing mean vs. stability and "ideal" genotype were constructed with genotype focus scaling and graphical view of "which won where" with symmetrical scaling. Both analyses were performed using program Excel Biplot Macros (Lipkovich and Smith, 2002).

RESULTS AND DISCUSSION

Analysis of variance for yield at 12 environments indicate that the effects of genotype, environment and their interaction on yield were significant, with the proportion of the total treatment variation of 9.17% for genotype, 77.83% for the environment and 13% for interaction (Table 2). Similar results on maize obtained Fan et al. (2007). In their research, the effects of environment and genotype explained 69% and 8.5% of total treatment variance respectively, whereas the interaction explained 16% of the total treatment variance. According to Gauch and Zobel (1996), in standard multi-location trails, 80% of the total sum of treatments is environment effect and 10% effect of genotype and interaction.

Table 2. Analysis of variance of maize hybrids grain yield in 2007 and 2008

Source of variation	df	SS	MS	F	%SS
G[1]	18	330.5	18.36	14.6**	9.17
E[2]	11	2805.2	255.02	142.2**	77.83
GxE	198	468.4	2.37	1.88**	13
PC 1	28	144.6	5.16	4.11**	
PC 2	26	108.3	4.17	3.31**	
PC 3	24	65.1	2.71	2.16	
PC 4	22	38.6	1.75	1.39	
PC 5	20	33.6	1.68	1.34	
Residuals	78	78.1	1	0.8	
Block	24	43	1.79	1.43	
Error	432	543.4	1.26	-	
Total	683	4190.6	-	-	100

[1] genotype, [2] environment p<0.01

A large yield variation explained by environments indicated that the environments were diverse, with large differences among environmental means causing most of the variation in grain yield. Environment grain yield ranged from 5.44 t/ha in Pančevo 08 to 12.59 t/ha in Rimski Šančevi 08. Genotype grain yield ranged from 8.73 t/ha (G16) to 11.28 t/ha (G2) (Table 3). GE interaction was a crossover type with different yield ranking of genotypes across environments. With further decomposition of GEI using AMMI analysis, two significant principal components were separated explaining 53.99% of variance interaction (30.87% PC1 and 23.12% PC2).

The AMMI biplot (Figure 1) clearly shows that certain genotypes in one environment have higher yield than in

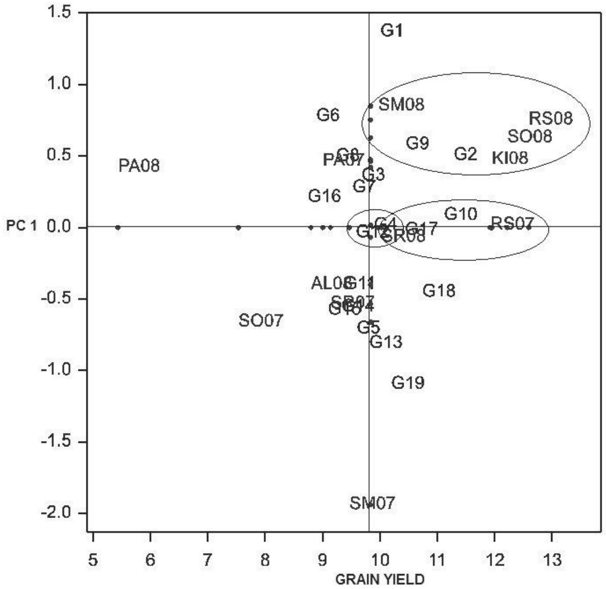

Figure 1. AMMI biplot of 20 maize hybrids across 12 environments

Table 3. Average grain yield of maize hybrids in 2007 and 2008

	RS07	PA07	SM07	SO07	SR07	RS08	PA08	SM08	SO08	SR08	KI08	AL08	Mean
G1	12.56	10.76	6.59	7.27	8.52	13.16	6.11	10.95	13.37	10.98	11.97	7.82	**10.00**
G2	13.74	9.97	9.13	9.67	10.12	13.94	6.68	11.79	13.60	11.53	14.53	10.71	**11.28**
G3	12.84	7.65	7.67	9.06	8.32	12.56	4.31	10.02	12.28	10.07	12.92	8.43	**9.68**
G4	12.43	8.82	9.85	7.12	8.59	12.52	5.48	10.84	12.08	10.40	11.62	8.97	**9.89**
G5	12.08	9.15	10.25	6.93	10.37	9.63	6.03	9.68	11.59	8.67	11.15	9.58	**9.59**
G6	11.12	8.48	7.17	5.97	8.06	12.70	5.55	8.46	12.70	7.27	11.51	7.69	**8.89**
G7	11.48	9.78	8.56	7.18	8.71	12.05	5.93	9.29	12.11	8.66	11.94	8.54	**9.52**
G8	11.11	8.18	7.13	7.25	9.28	12.13	4.63	10.49	11.23	9.82	11.47	8.08	**9.23**
G9	11.76	9.48	9.84	6.79	9.10	13.90	6.22	12.50	12.49	11.14	13.11	9.02	**10.45**
G10	12.37	10.39	11.24	6.81	9.85	14.57	6.71	11.01	13.98	12.42	12.78	11.24	**11.11**
G11	12.17	8.53	9.62	8.69	8.42	11.51	4.10	8.88	12.03	9.76	11.60	7.08	**9.36**
G12	11.92	7.93	10.29	7.19	6.89	12.23	5.46	10.57	12.32	9.40	12.02	8.74	**9.58**
G13	11.78	7.94	11.51	7.80	8.98	12.87	5.14	9.08	11.71	10.69	11.64	8.57	**9.81**
G14	11.33	9.17	9.54	7.71	9.85	11.75	5.11	9.42	9.90	8.88	10.48	8.75	**9.32**
G15	10.51	7.47	8.76	8.99	8.82	10.33	4.70	8.50	11.51	10.10	11.15	8.22	**9.09**
G16	9.59	8.38	8.87	4.67	9.37	12.77	4.12	9.16	11.39	8.01	11.34	7.15	**8.73**
G17	12.13	9.80	10.65	6.71	10.32	14.24	6.93	9.72	12.84	10.94	11.91	9.02	**10.43**
G18	13.33	10.49	11.29	8.54	10.14	13.62	5.75	10.32	12.89	11.08	11.48	9.91	**10.74**
G19	12.30	8.67	11.90	8.78	9.94	12.75	4.34	8.79	12.15	10.66	12.45	9.58	**10.19**
Mean	**11.92**	**9.00**	**9.47**	**7.53**	**9.14**	**12.59**	**5.44**	**9.97**	**12.22**	**10.03**	**11.95**	**8.79**	**9.84**

other, i.e. there is a specific interaction between genotypes and environments. Genotypes with PC values near zero demonstrate broader adaptability, and genotypes with higher PC1 value are more suitable for localities with PC1 values of the same sign. For example, the genotypes G17 and G10 are suitable for SR08 and SR07 environments, whereas genotypes G2 and G9 correspond to environments SM08, RS08, SO08 and KI08. Assessment of individual genotype performances can be based on their positions relative to the X and Y axis. The best genotypes are considered to be those that have high yield with stable performance in most localities. Of the six high yielding genotypes (G2, G10, G18, G17, G9, G19), G10 prove to be the most desirable. Being a high yielding, it is the best genotype for all the environments. Genotype G1 showed the lowest stability, whereas G4, G17, G12 prove to be stable genotypes.

GGE biplot analysis also enables visual assessment of adaptability and yield stability. GGE biplot is presented with two principal components explaining a total of 62.40% GGE variation (PC1 44.34%, PC2 18.06%, Figure 2). The first principal component is represented on the x axis and across its value is estimated yield, i.e. genotypes that have higher PC1 values are considered be more productive. The second principal component is represented on the y axis and presents the stability of genotypes. Estimation of yield and stability of genotypes was done by using so-called AEC (average coordinates of the environment) method (Yan, 2001; Yan and Hunt, 2001). The average environment is defined by the average values of PC1 and PC2 for the all

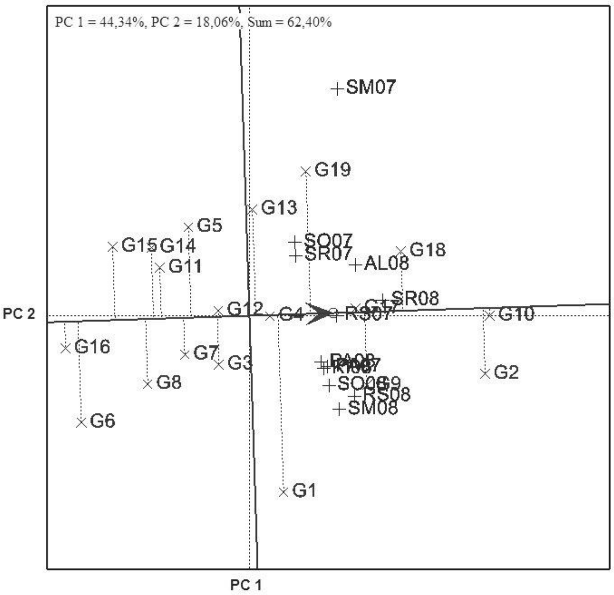

Figure 2. GGE biplot showing “mean vs. stability” of 20 maize hybrids across 12 environments

environments and is presented with a circle. The line which passes through the circle and the origin of the plot is called the average environment axis (AEA). The line, which is perpendicular to the AEA line and passes through the origin, is called the average ordinate environment (AOE). This line divides the genotypes into those with higher yield than average (to the right of it) and into those with lower yield than average (on its left side). By projecting the genotypes on AEA axis, the genotypes are ranked by yield, where the yield increases in the direction of the arrow. In this study, the highest nominal yield had genotypes G10, G2, G18, G9, G17, G19, and the lowers had G16, G6 and G15. Stability of the genotypes depends on their distances from the AE abscissa. Genotypes closer to abscissa are more stable than others. In this study, the greatest stability in the high yielding group had genotypes G10 and G17, while the most stable of all was G4.

The same genotype ranking is shown on the graph of so-called "ideal" genotype (Figure 3). An "ideal" genotype is defined as one that is the highest yielding across test environments and is absolutely stabile in performance (that ranks the highest in all test environments) (Yan and Kang, 2003). Although such an "ideal" genotype may not exist in reality, it can be used as a reference for genotype evaluation. A genotype is more desirable if it is located closer to the "ideal" genotype (Kaya et al., 2006). The closest to the "ideal" genotype was G10.

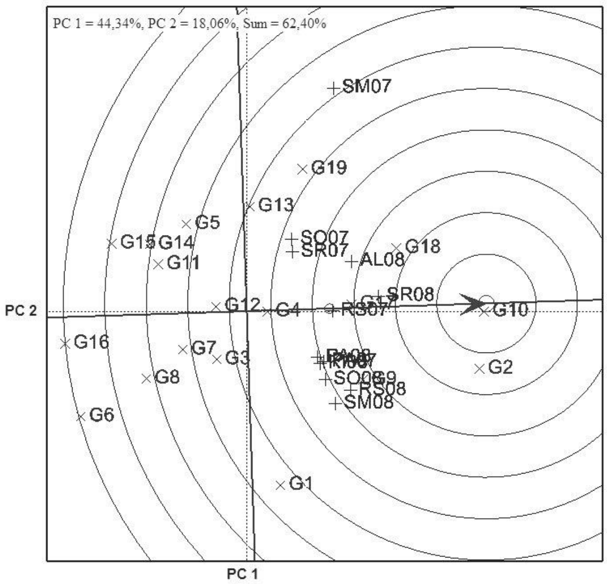

Figure 3. GGE biplot showing the "ideal"genotype

The graph "which won where" enables the allocation (identification) of potential mega-environments (Yan et al., 2000 and Yan and Hunt, 2001). The genotypes that are furthest from the origin are connected with a straight line forming a polygon. The lines starting from the origin are normal to the polygon sides and divide the polygon into several sectors. The locations within one sector are the ones where the certain genotype had the best yield and can be considered as mega-environments for that genotype. In the Figure 4, three groups of environments can be distinguished. One group contains SR08, AL08, RS07 environments and G10, G17, G18 and G4 hybrids. The second one encompasses SM08, RS08, SO08, PA08, PA07 and KI08 environments and G2 and G9 hybrids. The third group includes SM07, SO07 and SR07 environments and G19 and G13 hybrids. Very similar distribution of environments is shown on the AMMI graph with a larger group that contains the same environments as the GGE biplot analysis (SM08, RS08, SO08, KI08 and PA07). The other smaller group contains environments SR08 and RS07 while the third group consist of SR07, AL08 and SO07 environments. The environments PA08 and SM07 are excluded since they don't belong to none of the distinguished groups.

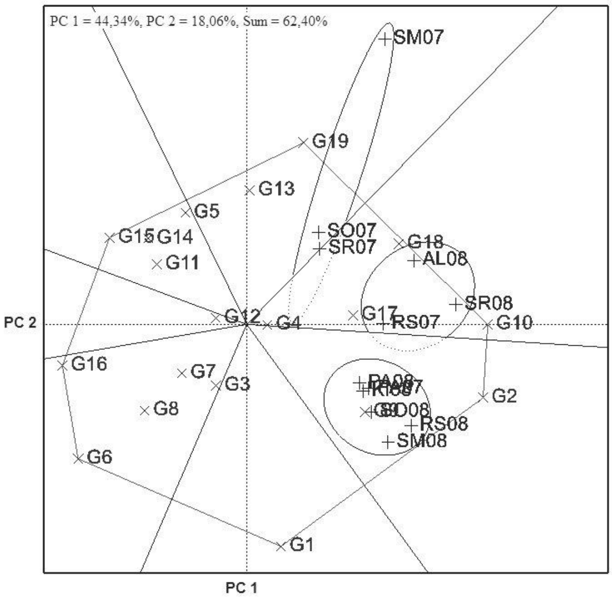

Figure 4. GE biplot showing "which won where"or which is best for what

When the results of the both models are compared, the same six genotypes had the best performances. Also, in terms of stability and mega-environment analysis, there are similarities between the two models. However, there are conflicted opinions about the reliability of both models and which one more credibly represents genotypes, locations and their interactions. According to Yan et al. (2007), AMMI biplot can not be accurately presented, especially when performances of individual genotypes in certain environments are observed, and are necessary to use AMMI2 charts for assessment of a so called "which won where" model. In addition, AMMI biplot always explains less G+GE variation than the GGE biplot and its shape is completely subjective because the axes are in different units (original unit for the abscissa and square root of the original unit for the ordinate). The AMMI1 biplot also presents the environment main effects of the test environments or E, which is irrelevant to cultivar and test-environment evaluation (Yan and Kang, 2003). A disadvantage of the AMMI model according to Setimela et al. (2007) is its insensitivity to the most important part of the crossover GE. Since there is no clear biological separation between the two terms G and GE, the AMMI model does not offer any advantage to the breeder for genotypic and location evaluation when analyzing METs data (Setimela et al., 2007)

In contrast, Gauch et al. (2008) argue that a shortcoming of GGE biplot method for evaluation of the genotypes is in its impossibility to separate G from GE, which is not the case in AMMI. The abscissa of an AMMI1 biplot captures 100% of G. Unlike PCA part of GGE, the ANOVA part of AMMI can separate G from GE. This is very important because the large GE relative to G could drive a large portion of G into the third and higher components that a GGE2 biplot misses. Also, more advantages of AMMI biplot over the GGE according to Gauch et al. (2008) are that AMMI biplot can be simpler constructed and interpreted because its axes are used directly and there is no need to be rotated. In addition, for selection of mega-environments, AMMI2 biplot can also be used successfully by relating the first two principal components (PC1 and PC2).

In our research both models prove to be very useful in assessing the performance of genotypes and showed no differences in selection of best genotypes. Stojaković et al, 2010 used both models to analyse set of 15 commercial hybrids in 30 environments across Serbia and also concluded that, besides methodological, there is no large difference in the results obtained by both models. GGE biplot model provide many possibilities such as “which won where” separation of potential mega-environments (Yan and Rajcan, 2002), genotype evaluation within mega-environments, identifying so-called “ideal” environments, most discriminatory for genotypes and the most representative, etc. Those visual interpretations which are not always possible to obtain trough AMMI model makes the GGE biplot model superior for visualisation of GE interaction. GGE biplot is also more logical and biological for practice than AMMI in terms of explanation of PC1 score, which represents genotypic effect rather than additive main effect (Yan, 2000).

According to the both analyses, hybrid G10 had the highest yield. This hybrid is characterised also by high stability and is expected to have the greatest commercially success. Hybrids which should also be included in further testing are G18, G9, G19, G4 and G13. In terms of the regional distribution it should be emphasized that it has a predictive character and requires continued multi-year testing in both early and later stages of selection. In doing so, multivariate (and other) mathematical models can be of great benefit.

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