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## Identification of Favourable Testing Locations for Barley Breeding in South Pannonian Plain

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### ABSTRACT

The aim of this study was to identify desirable, and also non-informative or highly correlated locations using GGE biplot. In this study, ten barley genotypes were tested across five locations for two growing seasons in official state trials performed by the Ministry of Agriculture, Forestry, and Water Management of the Republic of Serbia. In both growing seasons, environment had the highest influence on barley yield, explaining 77.70% in 2010/11 and 86.41% in 2011/12 growing season of the total variation. A significant grain yield variation explained by environmental effects indicated that the environments tested in our study were highly diverse. Together, PC1 and PC2 amounted 86.03% and 66.91% of the genotype and genotype × environment interaction sum of squares, in 2010/11 and 2011/12, respectively. The results indicate that Rimski šančevi was most favorable location and should be used for further multi-location trials while location Sremska Mitrovica was the least informative and it can be excluded from further trials. Excluding one of two similar environments could save resources with minimal risk to lose important information about genotypes performance. According to the results of our study, it can be concluded that GGE biplot is useful method for environment evaluation.

Keywords: Environment; GGE biplot; Interaction; Ideal environment

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## 1. Introduction

The main objective of barley (*Hordeum vulgare* L.) breeding is a creation of new high-yielding cultivars characterized by good quality and other beneficial agronomical traits, such as optimal thousand grain weight, plant height, resistance to lodging (Dogan et al 2016; Mirosavljević et al 2016). In order to estimate the performance of promising genotypes in advanced generations, pre-registration trials are

conducted for several years at different locations (Stojaković et al 2012). After the selection of superior genotypes as potential new cultivars, they are tested in official multi-location trials for two seasons conducted by the Ministry of Agriculture, Forestry, and Water Management of the Republic of Serbia. Official trials are carried out in target regions that represent the major agro-climatic conditions of the area for which the cultivars are to be realized. These trials are more detailed than breeders' pre-

registration trials, and provide more accurate information about cultivar performance.

Apart from quality and grain yield results, data collected from these trials, should also provide information about the adaptability and stability of tested genotypes (Stanislavljević et al 2013). The evaluation of tested genotypes is influenced by a genotype × environment interaction ( $G \times E$  interaction), which represents differential response of genotypes to different environmental conditions. In order to identify the optimal environmental conditions for assessing and selecting promising genotypes, it is necessary to properly understand the effect of the  $G \times E$  interaction (Rakshit et al 2012; Sayar & Han 2016). Nonparametric, regression and multivariate approaches have been used to understand the  $G \times E$  interaction pattern. However, relatively small number of studies was conducted in order to determine desirability and representativeness of testing locations. Meng et al (2016) showed that genotype plus genotype × environment interaction (GGE) biplot enables the identification and evaluation of favorable locations. GGE biplot is widely applied and accepted by plant breeders for interpretation of the  $G \times E$  interaction. The GGE biplot analysis combines two concepts-the GGE concept (Yan et al 2010) and biplot concept (Gabriel 1971). This analysis is a data visualization tool constructed by plotting two principal components, derived by the singular value decomposition of the environment-centred  $G \times E$  table. The GGE analysis was previously used for a graphic analysis of multi-environmental trial data. Kaya et al (2006) tested the efficiency of the GGE

model to investigate the association among nine rain-fed environments in bread wheat breeding and suggested that multi-locations trials should be carried out in a number of groups of locations sampled from the target region. It was also used by Tonk et al (2011) in order to identify discriminative locations for cultivar selection in Turkey. Kendal & Aktas (2016) examined associations among 7 testing locations across two growing seasons for barley breeding in Turkey using the GGE biplot method.

Considering that genotypes evaluation from multi-location trials is resources and time consuming process, the goal of this study is to find locations that provide desirable information about genotype characteristics. The locations that prove to be highly correlated or offer unreliable information about genotype performance could be eliminated from further cultivar evaluation trials.

## 2. Material and Methods

Data for this study were obtained from official registration trials conducted by the Ministry of Agriculture, Forestry, and Water Management of the Republic of Serbia. These trials were carried out across two growing seasons (2010/11 and 2011/12) at five locations: Kikinda (KI), Pančevo (PA), Rimski šančevi (RS) Sremska Mitrovica (SM) and Sombor (SO). Climatic characteristics of test locations are given in Table 1. According to Table 1, KI had the lowest rainfall level in both growing seasons and long term average. PA and RS are characterized by highest average long term rainfall. In 2010/11 and

**Table 1- Characteristics of test locations**

| Locations              | Geographic position |           |              | Seasonal rainfall (mm) |         |         | Soil type               |
|------------------------|---------------------|-----------|--------------|------------------------|---------|---------|-------------------------|
|                        | Latitude            | Longitude | Altitude (m) | Long term (1981-2011)  | 2010/11 | 2011/12 |                         |
| Rimski šančevi (RS)    | 45° 20' N           | 19° 51' E | 82           | 472                    | 386     | 361     | Non-carbonate chernozem |
| Sremska Mitrovica (SM) | 44° 58' N           | 19° 36' E | 100          | 419                    | 398     | 408     | Chernozem               |
| Pančevo (PA)           | 44° 52' N           | 20° 39' E | 82           | 486                    | 398     | 408     | Carbonate chernozem     |
| Sombor (SO)            | 45° 46' N           | 19° 06' E | 87           | 439                    | 360     | 319     | Carbonate chernozem     |
| Kikinda (KI)           | 45° 49' N           | 20° 27' E | 82           | 397                    | 355     | 305     | Carbonate meadow soil   |

The model for a GGE biplot (Yan & Holland 2010) based on the singular value decomposition (SVD) of the first two principal components is shown in Equation 1.

2011/12 growing season, highest level of rainfall was recorded in SM and PA. Next to KI, SO could be singled out as location with lower level of rainfall. Ten two-rowed winter barley genotypes were used in these trials; a standard variety marked as G1 and nine advanced lines marked with symbol G2-G10.

The trials, with four replications, were arranged in a randomized complete block design in plots of 5 m<sup>2</sup>. Plant density was adjusted to the recommended density proposed by seed companies. The standard agro-technical practices were applied in accordance to local agro-ecological conditions.

$$Y_{ij} - \mu - \beta_j = \lambda_1 \xi_{i1} \eta_{j1} + \lambda_2 \xi_{i2} \eta_{j2} + \varepsilon_{ij} \quad (1)$$

Where;  $Y_{ij}$ , observed yield of genotype  $i$  in environment  $j$ ;  $\mu$ , grand mean;  $\beta_j$ , main effect of environment  $j$ ;  $\mu + \beta_j$ , mean yield across all genotypes in environment  $j$ ;  $\lambda_1$  and  $\lambda_2$ , singular values (SV) for the first and second principal component (PC1 and PC2), respectively;  $\xi_{i1}$  and  $\xi_{i2}$ , eigenvectors of genotype  $i$  for PC1 and PC2, respectively;  $\eta_{j1}$  and  $\eta_{j2}$ , eigenvectors of environment  $j$  for PC1 and PC2, respectively;  $\varepsilon_{ij}$ , residual associated with genotype  $i$  in environment  $j$ .

Data processing was performed using Gea-R (R program) and the means were compared using Tukey's test.

### 3. Results and Discussion

The analysis of yield variance (Table 2) in the multi-environment trials indicated that the influences of the environment (E) and G × E interaction were

significant ( $P < 0.01$ ). The influence of genotype (G) was highly significant ( $P < 0.01$ ) in the growing season 2010/11, while in the growing season 2011/12 was significant ( $P < 0.05$ ). In both growing seasons, the highest effect of E was observed on barley yield, and this factor affected 77.70% in 2010/11 and 86.41% in 2011/12 of the total treatments variation. Based on the ANOVA data, it is evident that percentages of the G × E interaction in the total treatment variation were 15.84% and 10.48%, in 2010/11 and 2011/12 growing season, respectively. The contribution of genotype to the total treatment variation was 6.46% in 2010/11 and 3.10% in 2011/12. According to Pržulj & Momčilović (2012), the variation in barley grain yield was mostly under control of the growing season and the genotype × year interaction. Similarly, Pržulj et al (2015) reported that in yield trials the effect of the environment affected 80-90% of the treatment variation, and the variation due the G × E interaction was higher than the genotypic variation. Further, Kendal & Dogan (2015) and Kendal & Tekdal (2016) stated had the major contributions to treatment sum of squares were environments, G × E and G, respectively. Mortazavian et al (2014) also reported that the environment constitutes the highest percent of the total yield variation, while the influence of the G and G × E interaction is usually smaller.

A remarkable grain yield variation explained by environmental effects, indicated that the environments tested in our study were highly diverse. The average grain yield ranged from 6.99 t ha<sup>-1</sup> in KI to 10.42 t ha<sup>-1</sup> in RS in 2010/11 and from 7.35 t ha<sup>-1</sup> in KI to 10.75 t ha<sup>-1</sup> in RS in 2011/12 (Table

**Table 2- Analysis of variance of barley genotype yields in the growing seasons 2010/11 and 2011/12**

| Growing season | Source of variations | Degrees of freedom | Sum of squares | Means of square | F values | % Sum of squares |
|----------------|----------------------|--------------------|----------------|-----------------|----------|------------------|
| 2010/11        | Genotype             | 9                  | 28.4           | 3.16            | 6.87**   | 6.46             |
|                | Environment          | 4                  | 341.5          | 85.37           | 63.49**  | 77.70            |
|                | G × E                | 36                 | 69.6           | 1.93            | 4.21**   | 15.84            |
| 2011/12        | Genotype             | 9                  | 11.9           | 1.32            | 2.30*    | 3.10             |
|                | Environment          | 4                  | 331.3          | 82.83           | 59.45**  | 86.41            |
|                | G × E                | 36                 | 40.2           | 1.12            | 1.95**   | 10.48            |

\* and \*\*, significant at the 0.05 and 0.01 level of probability, respectively

3 and 4). The average genotype yield varied from 8.36 to 9.70 t ha<sup>-1</sup> in the 2010/11 growing season, and from 8.46 to 9.28 t ha<sup>-1</sup> in the 2011/12 growing season. In the 2010/11 growing season genotype G10, followed by G5, were the highest yielding genotypes, with an average grain yield of 9.59 t ha<sup>-1</sup>. In the next growing season, the genotype with the overall highest grain yield was G1, followed by G9. On average, the higher grain yield was recorded in 2010/11 in relation to 2011/12.

The first two principal components (PC1 and PC2) were obtained by partitioning the G and G × E interaction through the GGE biplot analysis (Figure 1 and 3). PC1 accounted to 60.40% in 2010/11 and

43.09% in 2011/12 while PC2 amounted to 25.63% in 2010/11 and 23.82% in 2011/12 of the G and G × E interaction. Together, they accounted to 86.03% and 66.91% of the G and G × E interaction sum of squares, in 2010/11 and 2011/12, respectively.

According to Ding et al (2008), GGE biplot is an effective tool for: 1) analysis of mega-environment and specific genotypes can be recommended to specific mega-environments, 2) evaluation of genotype, and 3) evaluation of environment (the power to discriminate among genotypes in target environments). The selection of relevant testing locations provides the adequate information is necessary for superior genotypes

**Table 3- Grain yield (t ha<sup>-1</sup>) of the tested barley advanced lines in the 2010/2011 growing seasons**

| Genotype | KI                   | PA                  | RS                   | SM                   | SO                   | Average             |
|----------|----------------------|---------------------|----------------------|----------------------|----------------------|---------------------|
| G1       | 6.94 <sup>n-q*</sup> | 8.03 <sup>i-q</sup> | 10.29 <sup>a-g</sup> | 9.87 <sup>a-j</sup>  | 9.83 <sup>a-k</sup>  | 8.99 <sup>BC</sup>  |
| G2       | 6.79 <sup>o-q</sup>  | 8.96 <sup>c-n</sup> | 8.73 <sup>f-o</sup>  | 10.20 <sup>a-h</sup> | 10.55 <sup>a-f</sup> | 9.04 <sup>ABC</sup> |
| G3       | 6.68 <sup>o-q</sup>  | 8.02 <sup>i-q</sup> | 7.78 <sup>k-q</sup>  | 9.94 <sup>a-i</sup>  | 9.39 <sup>b-l</sup>  | 8.36 <sup>C</sup>   |
| G4       | 6.64 <sup>p-q</sup>  | 7.50 <sup>m-q</sup> | 10.56 <sup>a-f</sup> | 9.75 <sup>a-k</sup>  | 9.35 <sup>c-l</sup>  | 8.76 <sup>BC</sup>  |
| G5       | 8.21 <sup>h-q</sup>  | 8.11 <sup>i-q</sup> | 11.68 <sup>a</sup>   | 9.94 <sup>a-i</sup>  | 10.00 <sup>a-i</sup> | 9.59 <sup>A</sup>   |
| G6       | 6.39 <sup>q</sup>    | 7.86 <sup>j-q</sup> | 10.85 <sup>a-c</sup> | 10.68 <sup>a-f</sup> | 9.22 <sup>d-m</sup>  | 9.00 <sup>ABC</sup> |
| G7       | 6.62 <sup>p-q</sup>  | 8.04 <sup>i-q</sup> | 11.19 <sup>a-d</sup> | 10.06 <sup>a-i</sup> | 8.95 <sup>c-n</sup>  | 8.97 <sup>ABC</sup> |
| G8       | 7.26 <sup>m-q</sup>  | 8.30 <sup>g-q</sup> | 10.28 <sup>a-g</sup> | 9.77 <sup>a-k</sup>  | 10.96 <sup>a-c</sup> | 9.31 <sup>AB</sup>  |
| G9       | 7.35 <sup>l-q</sup>  | 8.63 <sup>f-p</sup> | 11.44 <sup>ab</sup>  | 10.37 <sup>a-g</sup> | 9.09 <sup>e-m</sup>  | 9.38 <sup>AB</sup>  |
| G10      | 7.01 <sup>n-q</sup>  | 8.66 <sup>f-p</sup> | 11.41 <sup>abc</sup> | 10.45 <sup>a-f</sup> | 10.99 <sup>a-c</sup> | 9.70 <sup>A</sup>   |
| Average  | 6.99 <sup>D</sup>    | 8.21 <sup>C</sup>   | 10.42 <sup>A</sup>   | 10.10 <sup>AB</sup>  | 9.83 <sup>B</sup>    | 9.11                |

\*, different letters indicate significant difference at the 0.05 level of probability

**Table 4- Grain yield (t ha<sup>-1</sup>) of the tested barley advanced lines in 2011/12 growing season**

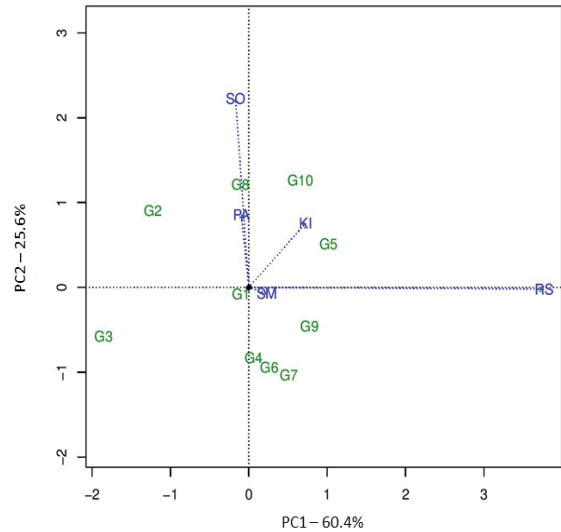
| Genotype | KI                   | PA                  | RS                   | SM                   | SO                   | Average            |
|----------|----------------------|---------------------|----------------------|----------------------|----------------------|--------------------|
| G1       | 7.94 <sup>f-o*</sup> | 8.24 <sup>c-o</sup> | 10.55 <sup>a-d</sup> | 9.72 <sup>a-i</sup>  | 9.95 <sup>a-h</sup>  | 9.28 <sup>A</sup>  |
| G2       | 7.25 <sup>k-o</sup>  | 8.12 <sup>f-o</sup> | 10.59 <sup>a-d</sup> | 9.53 <sup>a-k</sup>  | 8.88 <sup>c-o</sup>  | 8.88 <sup>AB</sup> |
| G3       | 7.25 <sup>k-o</sup>  | 7.24 <sup>l-o</sup> | 10.91 <sup>a-c</sup> | 9.34 <sup>b-l</sup>  | 9.93 <sup>a-h</sup>  | 8.93 <sup>AB</sup> |
| G4       | 7.03 <sup>m-o</sup>  | 8.00 <sup>f-o</sup> | 10.63 <sup>a-d</sup> | 7.40 <sup>j-o</sup>  | 10.62 <sup>a-d</sup> | 8.74 <sup>AB</sup> |
| G5       | 7.70 <sup>h-o</sup>  | 7.82 <sup>g-o</sup> | 11.02 <sup>a-c</sup> | 9.25 <sup>b-m</sup>  | 10.42 <sup>a-c</sup> | 9.24 <sup>AB</sup> |
| G6       | 6.87 <sup>o</sup>    | 7.53 <sup>i-o</sup> | 10.67 <sup>a-d</sup> | 10.05 <sup>a-g</sup> | 10.19 <sup>a-f</sup> | 9.06 <sup>AB</sup> |
| G7       | 6.94 <sup>n-o</sup>  | 6.98 <sup>m-o</sup> | 10.09 <sup>a-g</sup> | 9.10 <sup>b-o</sup>  | 9.18 <sup>b-n</sup>  | 8.46 <sup>B</sup>  |
| G8       | 8.07 <sup>f-o</sup>  | 7.16 <sup>l-o</sup> | 10.09 <sup>a-g</sup> | 9.19 <sup>b-n</sup>  | 9.84 <sup>a-h</sup>  | 8.87 <sup>AB</sup> |
| G9       | 7.30 <sup>k-o</sup>  | 8.13 <sup>f-o</sup> | 11.33 <sup>ab</sup>  | 9.66 <sup>a-j</sup>  | 9.85 <sup>a-h</sup>  | 9.25 <sup>AB</sup> |
| G10      | 7.12 <sup>l-o</sup>  | 7.75 <sup>h-o</sup> | 11.67 <sup>a</sup>   | 8.57 <sup>d-o</sup>  | 9.78 <sup>a-i</sup>  | 8.98 <sup>AB</sup> |
| Average  | 7.35 <sup>A</sup>    | 7.70 <sup>D</sup>   | 10.75 <sup>A</sup>   | 9.18 <sup>C</sup>    | 9.86 <sup>B</sup>    | 8.97               |

\*, different letters indicate significant difference at the 0.05 level of probability

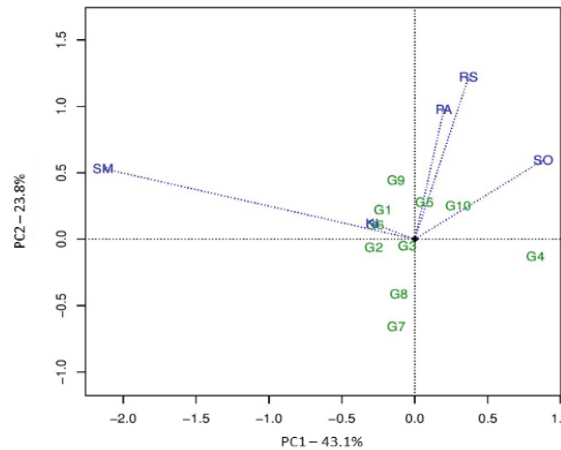
identification. A desirable testing location should be highly discriminative and also representative. According to Blanche & Myers (2006), a highly discriminative location is one that maximizes the observed genotypic differences between genotypes, while representative or key locations are sites that differentiate genotypes in a similar way.

On a GGE biplot, lines that connect coordinates of environment with the biplot origin are termed vectors. The angle between the vectors shows mutual relations among testing environments. If the angle between environments is obtuse, environments are negatively correlated. If angle is acute, environments are positively correlated, while environments are not associated when the angle is 90°. Since the angles of environment vectors SO with PA, and SM with RS were acute, it was concluded that they were closely correlated in the 2010/11 growing season (Figure 1). The angle between SM and SO, RS and PA, SM and PA, RS and SO was slightly obtuse or near to 90°, it means that these environments were negatively or not associated. In the 2011/12 growing season, highly correlated environments were KI with SM, and PA and RS (Figure 2). Moreover, SO was positively correlated with the environments RS and PA. Close associations between testing environments, suggest that same information about cultivar characteristics could be available from fewer testing environments, reducing the test cost (Yan et al 2015). The angle between the vectors for the environment SO, and the environments SM and KI was higher than 90°, indicating that SO was negatively correlated with SM and KI. Moreover, the presence of an obtuse angle among environments is an indicator of a strong crossover of the  $G \times E$  interaction (Yan & Tinker 2006). It practically means that the selection at the location SO is irrelevant or even contra productive to the selection at SM and KI in the season 2011/12.

Some environmental factors, such as soil type and management practices are predictable, i.e. they are not different from year to year. On the other hand, the year-dependent factors, such as precipitation, temperature and disease attack, cause a high year-to-year variability. These random environmental



**Figure 1- GGE biplot of the association among the tested environments in discriminating the genotypes in 2010/11**



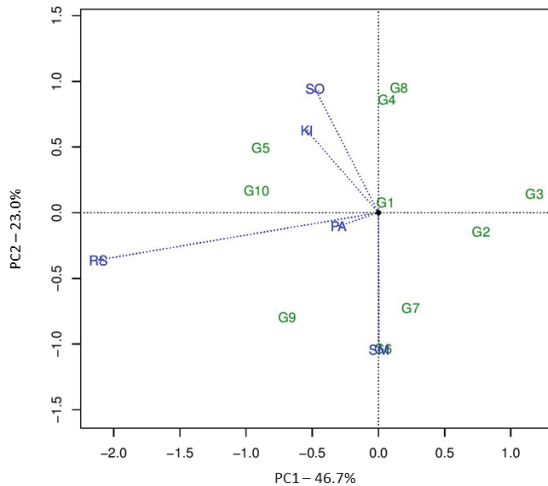
**Figure 2- GGE biplot of the association among the tested environments in discriminating the genotypes in 2011/12**

factors are highly variable and have a strong influence on the  $G \times E$  interaction. As a result of the influence of these unpredictable environmental factors, for both years the pattern of environment groupings was not repeatable in terms of locations that were grouped together. For example, in the

season 2010/11 the location RS was not correlated with location PA, while in the season 2011/12 these two locations had a close association.

GGE biplot enables the evaluation of discriminative ability of a location. Environment points with greater vector length are more discriminative (Yan et al 2011). Environment RS had the longest vector length in the 2010/11 growing season and were the most discriminative location. In 2010/11 SM was represented by shortest vector, and had least discriminative ability. Since non-discriminative environments provide small amount of information about genotype performance, they are not desirable for genotype evaluation. Among all testing locations examined in 2011/12, the RS and SM environments were most discriminative, while KI was the least discriminative environment.

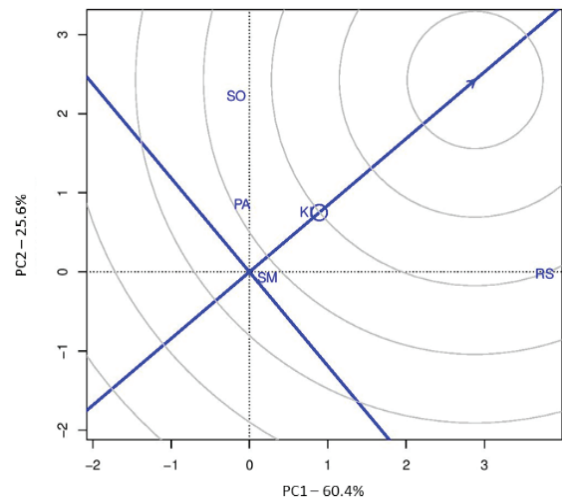
According to Figure 3 (biplot showing combined data from both growing seasons), it can be concluded that PA was the least discriminative test location in average. Further, SO and KI, and RS and PA were positively associated environments.



**Figure 3- GGE biplot of the association among the tested environments in discriminating the genotypes according to combined data of 2010/11 and 2011/12**

Another important measure that GGE biplot enables is the evaluation of environments compared to the “ideal” environment. On Figure 4 and 5, the open

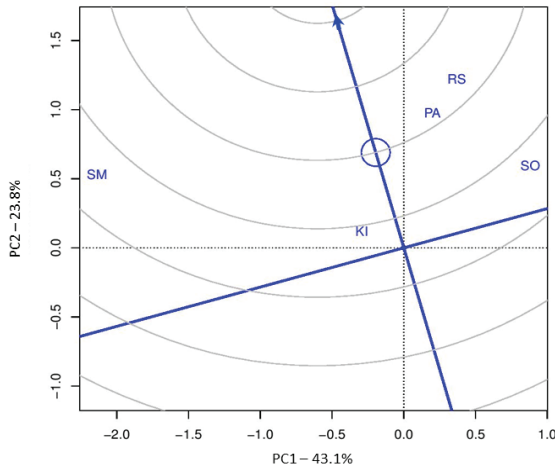
circle represents the average environment, defined as the average PC1 and PC2 scores of all environments. The line that passes through the average environment coordinate and the biplot origin is termed an “average environment axis” and the arrow placed on that axis represents the “ideal” environment. The “ideal” environment should be the most representative and discriminative location for conducting trials and can be used for the evaluation of other tested environments (Mitrović et al 2012). The tested environments located closer to the “ideal” environment, are more desirable, i.e. more representative and discriminative. The angle between the environment and the average environment axis shows the representativeness of the environment. The larger the angle between the axis and the environments the less representative the environment is.



**Figure 4- GGE biplot of comparison of the tested location with the ideal location in 2010/11**

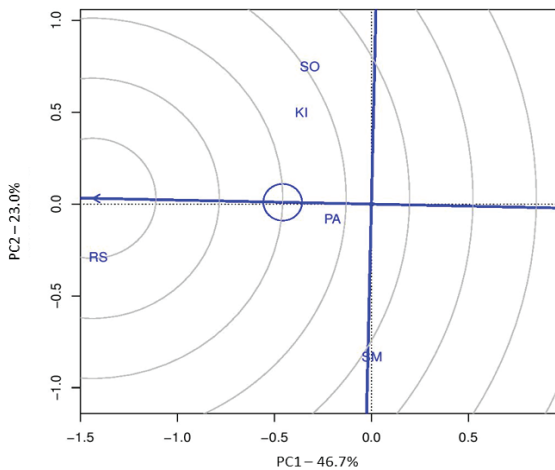
In the growing seasons 2010/11 and 2011/12, RS was placed closest to the “ideal” environment in relation to the other tested environments (Figure 4 and 5). Therefore, RS could be identified as the most desirable and effective location for cultivar evaluation in both growing seasons. In contrast, SM in 2010/11 and 2011/12 were the most distant from the “ideal” environment, and the least information could be acquired from this location. The most

representative environments were the location KI in the season 2010/11 and the locations KI and PA in the season 2011/12.



**Figure 5- GGE biplot of comparison of the tested location with the ideal location in 2011/12**

Based on biplot showing combined data from 2010/11 and 2011/12 growing season, RS were closest to the “ideal” environment, while SM was placed farthest from “ideal” environment (Figure 6). Moreover, PA and RS were most representative environments.



**Figure 6- GGE biplot of comparison of the tested location with the ideal location according to combined data of 2010/11 and 2011/12**

Although the crop yield is a result of E, G and  $G \times E$  interaction effects, only G and  $G \times E$  are relevant for cultivar and mega-environment identification (Yan et al 2010). GGE biplot is a data visualization tool that allows the visual interpretation of the  $G \times E$  interaction, including environmental evaluation. According to Yan et al (2007), due to the discriminative ability and representativeness of GGE view, the biplot was an effective tool for environment evaluation, which was not possible with the AMMI model.

Limited seed and other resources cause that plant breeders use few locations for selection. Therefore, the identification of discriminative and representative locations is highly desirable in order to optimize genotype selection. Excluding one of two similar environments could save resources with minimal risk to lose important information about genotypes performance (Rakshit et al 2012). If the location is not similar to other locations and has a high discriminative ability and representativeness, then this location could be relevant for development of new barley cultivars because it could provide significant information about genotype traits.

#### 4. Conclusions

According to the results of this study, it can be concluded that GGE biplot is useful method for environment evaluation. In both years, RS was placed closest to the “ideal” environment. This location was also the most discriminative in the 2010/11 growing season, and second most discriminative in 2011/12. On combined biplot, based on data from two growing season, RS was also marked as the most discriminative one. This indicates that this location should be used for further multi-location trials. The location SM was the least informative environment and placed farthest from the “ideal” environment in both growing seasons, and on biplot from combined data. These results suggest that the location SM could be replaced in further trials with another trial location from another production region in Serbia. Major difference in the characteristics of the studied environments could

be result of variation in agro-ecological conditions between two growing seasons.

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