

## Impact of Genotype $\times$ Environment Interactions on the Yield and Stability of Maize Hybrids in Serbia

Nemanja Nišavić<sup>1</sup>, Zoran Čamdžija<sup>1\*</sup>, Tomislav Živanović<sup>2</sup>, Sofija Božinović<sup>1</sup>, Nikola Grčić<sup>1</sup>, Irena Radinović<sup>2</sup>, Dragan Božović<sup>3</sup>

<sup>1</sup>Maize Research Institute “Zemun Polje”, Belgrade, Serbia

<sup>2</sup>Faculty of Agriculture, University of Belgrade, Belgrade, Serbia

<sup>3</sup>Institut “Tamiš”, Pančevo, Serbia

\*Corresponding author. E-mail: zcamdzija@mrizp.rs

### ABSTRACT

Maize grain yield stability becomes one of the most important traits in modern maize breeding due to the variability of climatic elements. The objective of this work is to estimate the productivity of maize hybrids as well as their adaptability and grain yield stability in ten different growing environments across two growing seasons. The average grain yield ranged from 10.39 t ha<sup>-1</sup> to 15.13 t ha<sup>-1</sup>. Hybrids ZP 457 and ZP 5090 performed good in both years. Stability analysis according to Eberhart and Russell method singled out ZP 457 as a stable hybrid, and Zombor and ZP 560 as the adaptable hybrids. According to the  $C_v$ , ZP 457, ZP 427, ZP 5550, and ZP 5090 were identified as hybrids with good performance and stable yield. The “Which-Won-Where” biplot showed that ZP 5090 was the optimal hybrid for most tested environments, while ZP 500 was recommended for one location in 2019. According to the “Mean vs. Stability” biplot, ZP 5090 is the hybrid with the most stable and highest mean yield across the environments, while ZP 3071 had the lowest and least stable. Overall, ZP 5090 is the best performing hybrid in this trial. No environments that are both informative and representative are detected. Nonetheless, PO19 was selected as the ideal environment. Opposite of that, some environments that are non-informative (BT19) can be excluded from future trials. Based on the GGE biplot analyses, the suggestions on the regional distribution of tested hybrids are given. Simultaneously, parental lines for future breeding programs are recommended based on the SNP analysis. The obtained results are important not just for the maize producers and breeders in Serbia, but for the whole “European Corn Belt”, especially under climate change scenarios.

**Keywords:** G  $\times$  E interaction, GGE biplot, maize, stability analysis, yield.

### INTRODUCTION

Due to the variability of climatic elements that occur as a result of global climate changes, the stability of grain yield becomes one of the most important properties of modern maize hybrids and one of the most important goals of maize breeding. For farmers, yield stability is significant because it enables economic predictability and reduces risk (Reckling et al., 2021). Analyses of yield stability are of particular importance because increased climate variability is associated with reduced yield stability (Müller et al., 2018). The available statistical data indicate that climate change is already having a significant negative impact on grain yields worldwide (Buhiniček et al., 2021). Without significant progress in maize breeding,

every 1°C increase in average temperature is predicted to reduce global grain yields by 7.4% (Zhao et al., 2017).

The effects of climate change are also evident in maize cropping systems in Southeast Europe - the European Corn Belt (parts of Bulgaria, Croatia, Hungary, Romania, and Serbia) (Buhiniček et al., 2021). As an example of the effect of drastic droughts that have become more frequent in recent decades, Kravić et al. (2015) report that the grain yields in Serbia decreased by an average of 48% in 2012.

The knowledge of the specificity of regional climate change enables adequate planning of crop production and adaptation of cultivation technology (Petrović et al., 2023). In the case of the so-called European Corn Belt, the effects of climate change are allowing the earlier planting and/or growing

of early-maturity hybrids. These strategies of avoidance are commonly applied since stress can be circumvented by earlier sowing dates or cultivating early maturing hybrids to avoid adverse weather conditions, mostly during flowering (Buhiniček et al., 2021). In drought years, early-maturing maize hybrids complete the grain-filling stage under the conditions of better soil moisture supplies, thus ensuring more stable yields compared to the late-maturing hybrids (Radojčić et al., 2008), even though their genetic potential for grain yield may be lower (Madić et al., 2010).

Another way of achieving grain yield stability is through optimal utilization of genotypes and natural resources via adequate regional distribution, i.e., the recommendation of hybrids for production in a certain area. This is done based on multi-year, micro-, macro-, and demonstration trials. Due to its practical and applicable value, the regional distribution has previously been researched by other authors (Jovanović et al., 2014; Madić et al., 2021). In these trials, the negative consequences of the genotype  $\times$  environment interaction (GEI) can be ruled out by applying reliable statistical methods; thus, the stable and high-yielding genotypes can be selected.

In the last ten years (2015-2024), 815 new maize hybrids have been registered in Serbia (Ministry of Agriculture, Forestry and Water Management of Republic of Serbia, 2024). Furthermore, it is expected that areas under maize in certain areas will decrease up to 7.66% (Tričković et al., 2023). In such a competitive market, from the point of a breeder, it's important to select the most suitable hybrids for specific regions (hybrids with narrow adaptability), as well as those with broader adaptability. The aim of this work is to evaluate the grain yield stability of early maturing maize hybrids (FAO 300-500) for the purpose of recommending commercial ZP hybrids for the most significant production areas in the Republic of Serbia and the region.

## MATERIAL AND METHODS

### Plant Material

The plant material used in this research comprised maize hybrids provided by the

Maize Research Institute "Zemun Polje". A total of 11 maize hybrids, belonging to the maturity groups FAO 300-500, were tested in this experiment (FAO 300: ZP 3714, ZP 3071, Zombor; FAO 400: ZP 427, ZP 457; FAO 500: ZP 500, ZP 5550, ZP 5089, ZP 5090, ZP 555, ZP 560). The hybrids were tested in field trials, and the parental lines were genotyped using SNP markers.

The parental components of these hybrids are 17 inbred lines also from the Maize Research Institute "Zemun Polje". These lines belong to the Lancaster Sure Crop (LSC) or Iowa Stiff Stalk Synthetic (BSSS) heterotic groups and cover maturity groups from FAO 280 to FAO 800.

### Field Trials

The field trials were conducted in four key maize producing regions in Serbia: Srem (Zemun Polje, ZP), Banat (Pančevo, PA), Bačka [Bečej (BC), Bačka Topola (BT)], and Stig (Požarevac, PO). The trials were conducted in the 2019 and 2020 growing seasons in a randomized block design with two replicates. Each plot contained eight rows, 5 m long, with 0.75 m row spacing, resulting in a plot area of 30 m<sup>2</sup>. Plant densities ranged from 66,667 to 72,000 plants ha<sup>-1</sup>, depending on the FAO maturity group (FAO 300-400: 72,000 plants ha<sup>-1</sup>; FAO 500: 66,667 plants ha<sup>-1</sup>). To minimize edge effects, only the four inner rows were examined, giving an effective area of 15 m<sup>2</sup>. Standard agronomic practices for maize cultivation were performed.

### Statistical Analysis

Grain yield data of the hybrids were used to calculate the mean yield and rank the hybrids using a three-factor analysis of variance (ANOVA) and the least significant difference test at the 5% level (LSD 5%). Statistical analysis was performed using the MSTAT-C program (Michigan State University, East Lansing, MI, USA).

To make the results more illuminating the environment was designated as the product of each year and location (Greveniotis et al., 2023). The stability and interaction between hybrids and the environment were examined using GGE biplot analysis and statistical

analysis of the stability parameters of the hybrids.

GGE biplot analysis according to Yan and Kang's model (2003) is:

$$\hat{Y}_{ij} = \mu + \alpha_i + \beta_j + \Phi_{ij} \quad (1)$$

where:  $\hat{Y}_{ij}$  - mean yield of genotype  $i$  in environment  $j$ ;  $\mu$  - grand mean;  $\alpha_i$  - main effect of the genotype  $i$ ;  $\beta_j$  - main effect of the environment  $j$ ; and  $\Phi_{ij}$  - interaction effect between genotype  $i$  and environment  $j$ .

A characteristic of the GGE biplot method is that it does not separate the variability caused by genotype and GEI interaction when considering yield stability, but rather analyzes it jointly:

$$\hat{Y}_{ij} - \mu - \beta_j = g_{i1}e_{1j} + g_{i2}e_{2j} + \varepsilon_{ij} \quad (2)$$

where:  $g_{i1}$  - primary component value for genotype  $i$ ;  $e_{1j}$  - primary component value for environment  $j$ ;  $g_{i2}$  - secondary component value for genotype  $i$ ;  $e_{2j}$  - secondary component value for environment  $j$ ;  $\varepsilon_{ij}$  - standard error for genotype  $i$  in environment  $j$ .

GGE biplot analysis is performed using GEA-R (Genotype × Environment Analysis with R for Windows), Version 4.1 (2017-08-3), developed by CIMMYT's Biometrics and Statistics Unit (Pacheco-Gil et al., 2015). Grain yield stability of hybrids was evaluated via the regression coefficient ( $b_i$ ) and variance of deviation from regression ( $S^2d_i$ ) by Eberhart and Russell (1966), and the coefficient of variation ( $C_V$ ) introduced by Francis and Kannenberg (1978).

### Molecular characteristics

Parental inbred lines were genotyped using the Maize 25K XT Illumina Infinium array, containing 23,908 SNP markers evenly

distributed across the maize genome. This array includes 14,171 markers from the Illumina Infinium 50k Maize array (Illumina, Inc., San Diego, CA, USA) and 8,847 SNPs from the Affymetrix Axiom 600k Maize array (Thermo Fisher Scientific Inc., Waltham, MA, USA). DNA analysis, including extraction using the standard protocol from leaf tissue, was outsourced to and performed by TraitGenetics GmbH (Gatersleben, Germany).

Genotyping data is stored in an Excel spreadsheet (Microsoft, Redmond, WA, USA), with alleles at each marker position coded according to IUPAC nomenclature for each line. Raw genotyping data are filtered using standard scripts in R and TASSEL 5.0 (Bradbury et al., 2007). SNP markers with a miss rate > 5% or a minor allele frequency (MAF) < 5% are discarded, retaining only the most informative markers for further analysis. Genetic distances are calculated via TASSEL 5.0.

## RESULTS AND DISCUSSION

### Regionalization Data

The grain yield results from both years indicate that 2020 was more productive (13.24 t ha<sup>-1</sup>) compared to 2019, which had a mean yield of 12.4 t ha<sup>-1</sup> (Table 1). Analyzing each location individually, Pančevo had the lowest mean grain yield (12.22 t ha<sup>-1</sup>), while Požarevac had the highest mean yield (13.16 t ha<sup>-1</sup>) based on both years. It is worth noting that Požarevac achieved this result due to the high yields in 2020 (13.96 t ha<sup>-1</sup> on average). In contrast, in 2019, Požarevac had one of the lowest mean yields (12.36 t ha<sup>-1</sup>), which is below average for all observed locations in 2019. This marks Požarevac as the location with the highest influence of environmental factors.

Table 1. Mean grain yields across the tested locations in 2019 and 2020

Year	Grain Yield (t ha <sup>-1</sup> )					Average
	Zemun Polje (ZP)	Požarevac (PO)	Bečej (BC)	Bačka Topola (BT)	Pančevo (PA)	
2019	12.47	12.36	12.37	12.58	12.24	12.4
2020	13.16	13.96	13.67	13.23	12.2	13.24
Average	12.81	13.16	13.02	12.9	12.22	12.82

The results obtained at the studied locations align with the regionalization by Jocković (2008). According to this classification, the southern Banat and Pomoravlje regions (locations Pančevo and Požarevac, respectively) belong to the second-tier region, which is more influenced by ecological factors in commercial maize production. In such regions, hybrids of earlier FAO maturity groups tend to achieve higher and more stable grain yields, as confirmed by this study. This is consistent with previous research which highlights better adaptability of early-maturing maize hybrids to more

unstable locations with generally lower mean grain yields (Crevar et al., 2011).

### ANOVA Results

The results of ANOVA are presented in Table 2. All factors had a significant effect on grain yield independently (with the 'year' factor contributing the most), as well as the interaction L × Y, and G × L × Y. Other interactions did not influence the grain yield significantly. This can be attributed to the high adaptability of tested hybrids across different environments.

Table 2. ANOVA table for grain yield of tested maize hybrids across environments

Source of variation	DF	SS	MS	F value
Repetition	1	0.482	0.482	0.633
G	10	41.42	4.142	5.4374**
L	4	27.826	6.957	9.1322**
G × L	40	42.867	1.072	1.4069
Y	1	53.51	53.51	70.2456**
G × Y	10	6.592	0.659	0.8653
L × Y	4	19.949	4.987	6.547**
G × L × Y	40	50.979	1.274	1.6731*
Error	109	83.032	0.762	
Total	219	326.657		

G - genotype; L - location; Y - year; DF - degrees of freedom; SS - sum of squares; MS - mean squares; \* - statistically significant at 0.05 probability level; \*\* - statistically significant at 0.01 probability level.

The mean grain yield of 11 tested hybrids was assessed (Table 3). The mean grain yield ranged from 10.39 t ha<sup>-1</sup> (ZP 3071 in environment BC19) to 15.13 t ha<sup>-1</sup> (ZP 5550 in environment PO20). The top two performing hybrids in both years were hybrids ZP 5090 and ZP 457. Comparing the

two years, it can be concluded that 2019 had less favorable conditions for maize growing. In such conditions, ZP 457 achieved higher grain yield. However, in 2020, which was a more favorable year from the point of maize cultivation, ZP 5090 outperformed ZP 457 which was the 2<sup>nd</sup> highest yielding hybrid.

Table 3. Mean yield (t ha<sup>-1</sup>) of maize hybrids. Different letters indicate significant differences (p < 0.05)

	ZP19	PO19	BC19	BT19	PA19	ZP20	PO20	BC20	BT20	PA20	Mean
ZP 3714	13.47	12.25	12.43	12.65	11.93	12.73	13.54	12.99	11.97	11.44	12.54 <sup>d</sup>
ZP 3071	13.26	12.73	10.39	12.36	12.39	11.61	13.68	13.34	11.99	12.49	12.42 <sup>d</sup>
Zombor	12.86	12.19	11.51	12.91	10.62	12.87	13.9	13.81	13.57	11.77	12.60 <sup>cd</sup>
ZP 427	12.66	12.06	12.96	13.3	11.26	13.2	13.99	13.3	13.14	12.61	12.85 <sup>bcd</sup>
ZP 457	12.48	12.7	13.35	12.75	13.13	14.05	14.35	14.51	13.59	13.11	13.40 <sup>a</sup>
ZP 500	12.15	11.12	10.83	12.49	13.56	12.85	14.65	13.4	12.09	12.03	12.52 <sup>cd</sup>
ZP 5550	12.15	13.1	12.43	13.12	13.44	13.2	15.13	13.6	13.83	12.3	13.23 <sup>ab</sup>
ZP 5089	12.07	12	12.81	11.33	10.68	13.42	12.61	12.57	13.19	12.53	12.32 <sup>d</sup>
ZP 5090	12.05	13.14	13.72	11.57	14.65	13.49	14.51	14.08	14.46	12.94	13.46 <sup>a</sup>
ZP 555	12.02	11.65	12.75	12.38	10.53	13.11	12.75	14.82	14.24	11.06	12.53 <sup>d</sup>
ZP 560	11.99	13.01	12.91	13.47	12.43	14.21	14.41	13.97	13.41	11.92	13.17 <sup>abc</sup>
Mean	12.47	12.36	12.37	12.58	12.24	13.16	13.96	13.67	13.23	12.20	12.82

### Stability Analysis

Based on regression coefficient by Eberhart and Russell (1966), hybrids Zombor, ZP 555, and ZP 560 have  $b_i$  values higher than 1, and are better adapted to favorable conditions (Table 4). Opposite of that, the lowest  $b_i$  values are recorded for hybrids ZP 3071 and ZP 3714. These

genotypes are more adaptable to unfavorable growing conditions, which is generally the case with early maturing hybrids. Finally, with  $b_i$  values close to 1, hybrids ZP 500 and ZP 5550 can be considered genotypes with wide adaptability to all tested environments, and consequently to main maize growing regions in Serbia.

Table 4. Grain yield stability analysis

Genotype	Mean	Sd	$C_v$ (%)	Eberhart and Russell		
				$b_i$	$S^2d_i$	$R^2$
Zombor	12.4775	1.109	8.8882	1.3554	-0.0516	0.764
ZP 3071	12.3025	0.9138	7.428	0.6209	0.3395	0.2361
ZP 3714	12.384	0.6141	4.9591	0.6721	-0.2138	0.6127
ZP 427	12.783	0.8016	6.2708	0.8767	-0.0975	0.6119
ZP 457	13.4215	0.6842	5.0975	0.8649	-0.282	0.8175
ZP 500	12.5495	1.141	9.092	0.9303	0.5884	0.3401
ZP 5089	12.2475	0.8882	7.2518	0.7127	0.2171	0.3294
ZP 5090	13.4695	1.0345	7.6806	0.7986	0.4589	0.3048
ZP 555	12.4065	1.4326	11.5474	1.5857	0.4838	0.6267
ZP 5550	13.23	0.874	6.606	1.039	-0.1401	0.723
ZP 560	12.9775	1.2949	9.9782	1.5437	0.137	0.7269

Genotypes with  $S^2d_i = 0$  are the most stable (Zombor, ZP 427), while higher values indicate reduced yield stability (ZP 500, ZP 5090, ZP 555). Biplot generated based on these two parameters identifies Zombor and ZP 560 as the adaptable hybrids, and ZP 457 as the only stable one (Figure 1a). None was

identified as adaptable and stable. Crevar et al. (2011) used the  $b_i$  and superiority index to recommend stable, high-yield ZP hybrids, even for unfavorable conditions. Hallauer and Carena (2009) and Horhocea et al. (2024) also highlight its simplicity and interpretability of regression coefficients and deviations.

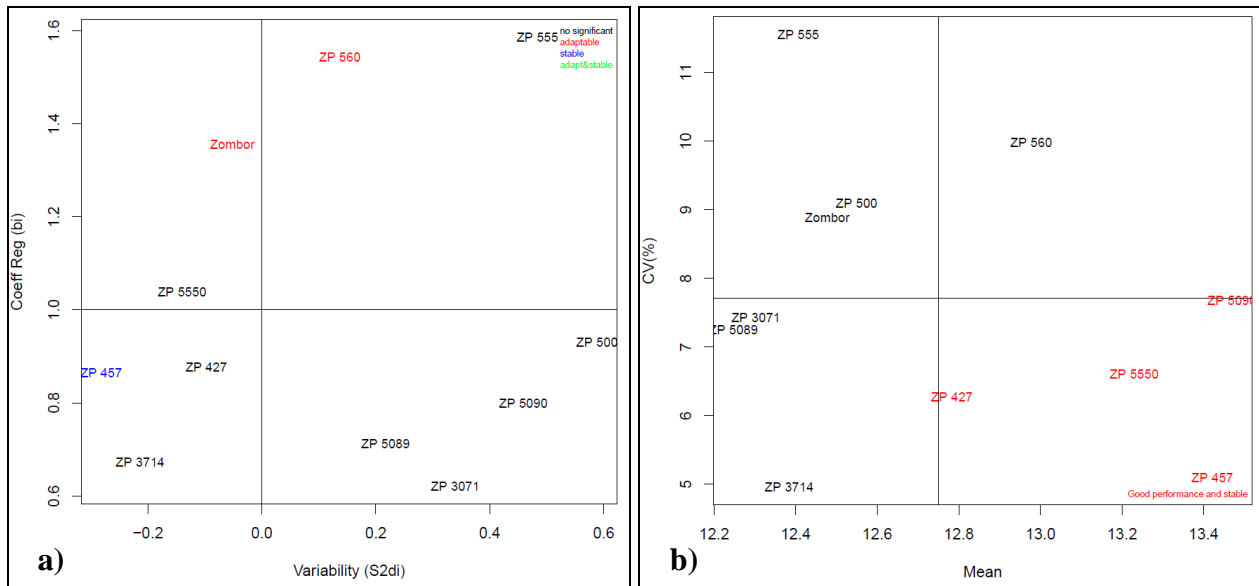


Figure 1. Stability analysis biplot according to the Eberhart and Russell stability parameters (a), and CV parameter by Francis and Kannenberg (b)

According to the coefficient of variation ( $C_V$ ), hybrid ZP 3714 had the lowest value (4.9591), while the highest was recorded for ZP 555 (11.5474) (Table 4). Even though ZP 3714 had low a  $C_V$  value, its mean grain yield was one of the lowest; thus, it cannot be considered as desirable genotype. Based on the graphical representation (Figure 1b), ZP 457, ZP 5550, ZP 5090, and ZP 427 can be considered good-performing and stable hybrids. Their mean yields were significantly higher than ZP 3714 (except for ZP 427), and their  $C_V$  values ranged from 5.0975 (ZP 457) to 7.6806 (ZP 5090).

### GGE Biplot Analysis

On the GGE biplot, each of the 11 tested genotypes (hybrids) is represented in green, while blue letters denote each of the ten

environments studied. The contribution of GGE interaction in grain yield trait in examined maize hybrids is represented by two axes, accounting for 39.42% and 28.91% (Figure 2), totaling 68.33%. A biplot diagram is considered reliable if it encompasses at least 60% (Shojaei et al., 2022), or more than 60% variance if multi-year mean yields are measured (Yang et al., 2009). Environments BC20, ZP20, BT20, and BC19 had the strongest correlation. Therefore, it is suggested they form one megaenvironment, and the other one is formed by PA19, PA20, PO19, PO20, and ZP19. Strong positive correlation between environments implies that the same data can be gathered from a reduced number of environments, thus offering a possibility to reduce expenses.

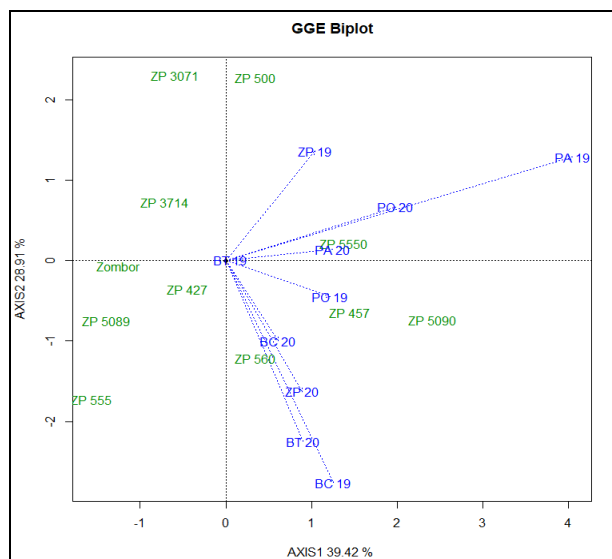


Figure 2. GGE biplot of tested genotypes (green) and environments (blue)

The length of the vector is proportional to the amount of GEI. This means that this interaction was most observable in the environments PA19 and BC19. Considering the environment marker for BT19 is located on the biplot origin, it can be concluded there was no GEI in this environment.

### Genotype Performance

Based on the yield results of FAO 300 hybrids, Zombor stands out with a slightly higher grain yield ( $12.48 \text{ t ha}^{-1}$ ) compared to ZP 3071 ( $12.3 \text{ t ha}^{-1}$ ) and ZP 3714 ( $12.38 \text{ t ha}^{-1}$ ) (Table 3). Additionally, the grain harvest moisture content for Zombor was

very low - 12.85%, which is a great advantage from the point of grain storage (Pavlov et al., 2011). The GGE biplot analysis indicates Zombor is suitable for locations with better growing conditions, as confirmed by its proximity to the BT, BC, and ZP20 environments (Figure 3a). This is consistent with Jocković's regionalization of production regions in Serbia for maize cultivation (Jocković, 2008). Usually, in the Bačka region late maturing hybrids (FAO 600) achieve the highest yields (Ivan et al., 2023), which confirms this is a region with optimal growing conditions for maize cultivation which are also necessary for Zombor.

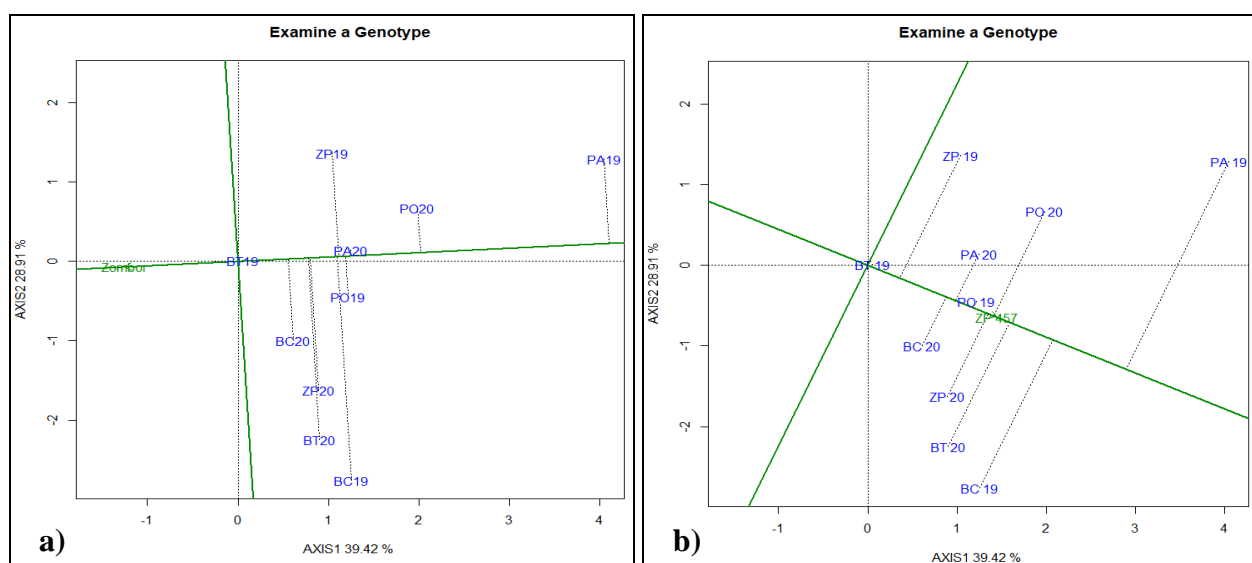


Figure 3. GGE biplot analysis of the best performing hybrid in FAO 300 (Zombor) (a), and FAO 400 maturing group (ZP 457) (b)

As for the FAO 400, hybrid ZP 457 outperformed standard hybrid for this maturity group, ZP 427 - ZP 457 achieved higher grain yields in all environments except ZP19 and BT19. The mean grain yield for ZP 457 was  $13.42 \text{ t ha}^{-1}$ , significantly higher than ZP 427's  $12.78 \text{ t ha}^{-1}$ . Furthermore, ZP 457 achieved above-average grain yields in all tested locations (Table 3). The biplot analysis of the ZP 457 hybrid (Figure 3b) indicates very high yield stability across all tested locations in both years. The least favorable environment for growing this hybrid was BT19, where ZP 457 achieved one of the lowest grain yields. Nonetheless, it

achieved above-average grain yield for that specific location.

In FAO 500 maturity group, ZP 5550 and ZP 5090 achieved significantly higher mean grain yields ( $13.23 \text{ t ha}^{-1}$  and  $13.47 \text{ t ha}^{-1}$ , respectively) compared to ZP 555 ( $12.41 \text{ t ha}^{-1}$ ) and ZP 560 ( $12.98 \text{ t ha}^{-1}$ ) as standard hybrids (Table 3). Grain yield results highlight the superiority of ZP 5090, as this hybrid achieved the highest yield among all the tested hybrids (Table 3). Based on GGE biplot analysis (Figure 4a), ZP 5090 performed exceptionally well across all test environments.

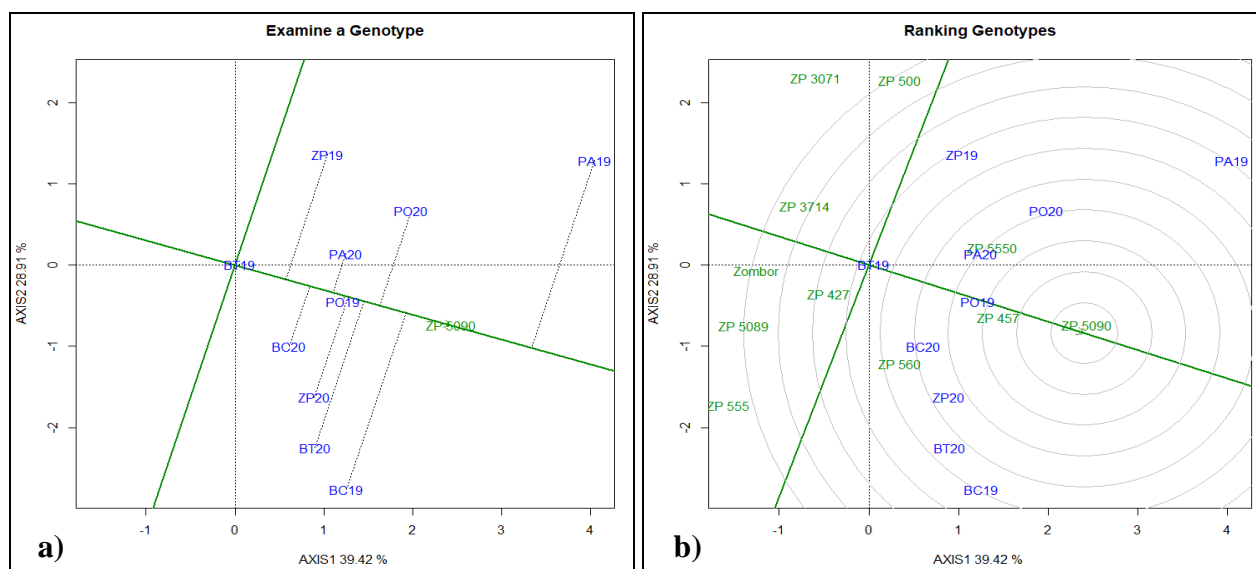


Figure 4. GGE biplot of hybrid ZP 5090 (a); Ranking genotypes relative to the ideal genotype (b)

Besides the good overall performance of ZP 5090, the “Ranking genotypes” biplot, recommends ZP 5090 as the ideal genotype (Figure 4b), due to its proximity to the arrow on the average-environment coordination (AEC). The location of the arrow on the AEC is zero, which indicates absolute stability. Genotypes within a smaller concentric circle with the ideal genotype (ZP 457, ZP 5550, ZP 560) are more desirable than those in a larger circle (e.g., ZP 3071, ZP 555). Furthermore, the smaller the circle containing an environment, the better the performance and stability of the ideal genotype in that environment. Environment PO19 is likely the

most suitable environment for ZP 5090. Since PO19 is one of the locations with the lowest grain yield, this confirms ZP 5090's suitability for growing in less favorable conditions.

Based on the “Mean vs. Stability” biplot (Figure 5a), ZP 5090 had the highest mean grain yield across the environments, while ZP 3071 had the lowest. Not only did ZP 5090 perform the best across the environments - its yield was also the most stable, since it is practically located on the AEC abscissa (AEA). ZP 3071 was one of the most unstable hybrids, after ZP 555 and ZP 500.



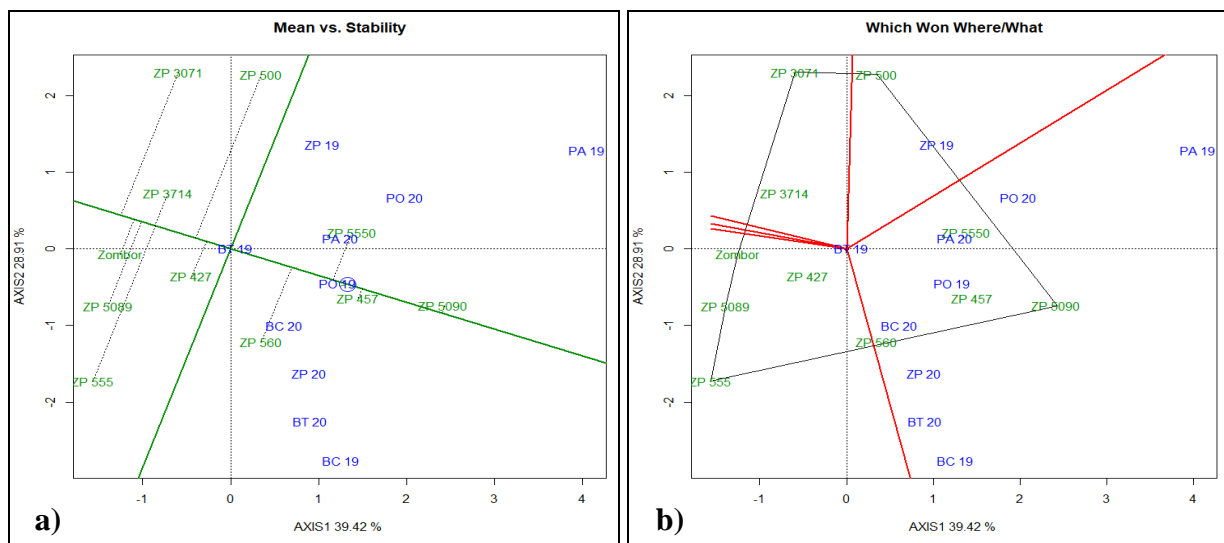


Figure 5. “Mean vs. Stability” (a), and “Which-Won-Where” view of the GGE biplot (b)

According to the ‘which-won-where’ biplot (Figure 5b), ZP 500 is the best choice for the environment ZP 19. Aside from that, ZP 5090 is the best choice for all other environments. In a sector that has ZP 5090 on its vertex, three other hybrids are present: ZP 457, ZP 5550, ZP 560. All of them are ranked as the top performing hybrids (Table 3). Even though ZP 5090 is the best choice for all environments in this sector and has exhibited greater responsiveness (Ma et al., 2024), it is expected that these three hybrids will also perform well. Sectors that had hybrids ZP 555, ZP 5089, Zombor, and ZP 3071 on their vertices contain no environments and are not good choices for any environment.

### Environment Performance

The optimal test environment should be the most representative, but also the most discriminating (informative). In Figure 6a, the ideal environment is located in the center of the concentric circles and is marked with an arrow on the AEA in the positive direction. PO19 is closest to this environment and is the best for selecting genotypes adapted for the whole region, while ZP19 and BT19 were the poorest. The smaller the circle containing an environment, the more attributes it shares with our ideal environment. For example, PO19 shares more attributes with PA19 and PO20, than with BT9.

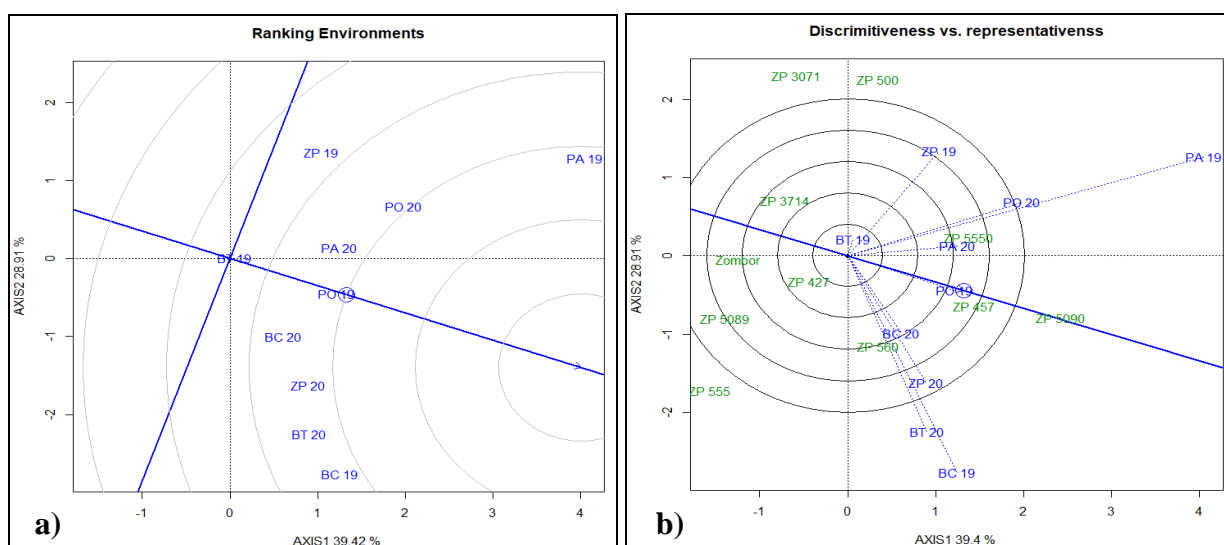


Figure 6. Ranking environments relative to the ideal environment (a); Discriminativness vs. representativeness of test environments (b).

Representativeness and discriminating ability are displayed in Figure 6b. Out of the 10 test environments, PA19 was the most discriminative (informative), followed by BC19 and BT20, while BT19 was the least discriminative. This is confirmed by its near proximity to the biplot origin, which indicates the environment had no significant effect on grain yield.

Environments like BT19, that consistently have low discriminating ability (or are non-

informative) reveal minimal information about the genotype performance. Their usage as testing environments should be avoided, which can also help reduce the testing costs by focusing on more suitable environments.

#### Genetic Distances based on SNP markers

Genetic distances (GD) calculated from SNP markers are in accordance with the pedigree and genetic origin of the parental lines. The values are presented in Table 5.

Table 5. Genetic distances of parental lines of tested maize hybrids based on SNP markers

	L73L024	L73B013	L73B048	ZPL-255/75-5	L76B004	L04BA031	ZPPL301	L74B040	L74B049	L94B034	ZPL-155/18-4/1	L74L065	L 325/75-2	L-335/99	L73B064	L05L061	L05BA040
L73L024	-	0.448	0.443	0.218	0.453	0.458	0.441	0.446	0.445	0.447	0.224	0.305	0.174	0.465	0.443	0.260	0.438
L73B013	0.448	-	0.136	0.460	0.372	0.374	0.229	0.274	0.267	0.343	0.462	0.408	0.463	0.402	0.231	0.449	0.375
L73B048	0.443	0.136	-	0.457	0.390	0.375	0.237	0.241	0.211	0.343	0.457	0.381	0.457	0.421	0.183	0.440	0.376
ZPL-255/75-5	0.218	0.460	0.457	-	0.446	0.460	0.457	0.458	0.456	0.437	0.026	0.217	0.190	0.462	0.458	0.269	0.442
L76B004	0.453	0.372	0.390	0.446	-	0.380	0.397	0.406	0.361	0.369	0.443	0.400	0.450	0.285	0.432	0.446	0.383
L04BA031	0.458	0.374	0.375	0.460	0.380	-	0.383	0.399	0.386	0.364	0.457	0.418	0.460	0.378	0.407	0.426	0.195
ZPPL301	0.441	0.229	0.237	0.457	0.397	0.383	-	0.334	0.296	0.357	0.459	0.392	0.453	0.428	0.223	0.440	0.371
L74B040	0.446	0.274	0.241	0.458	0.406	0.399	0.334	-	0.213	0.358	0.459	0.401	0.454	0.416	0.248	0.437	0.388
L74B049	0.445	0.267	0.211	0.456	0.361	0.386	0.296	0.213	-	0.336	0.456	0.399	0.459	0.402	0.304	0.449	0.409
L94B034	0.447	0.343	0.343	0.437	0.369	0.364	0.357	0.358	0.336	-	0.434	0.404	0.441	0.407	0.358	0.431	0.381
ZPL-155/18-4/1	0.224	0.462	0.457	0.026	0.443	0.457	0.459	0.459	0.456	0.434	-	0.240	0.196	0.460	0.460	0.267	0.441
L74L065	0.305	0.408	0.381	0.217	0.400	0.418	0.392	0.401	0.399	0.404	0.240	-	0.300	0.458	0.378	0.329	0.427
L 325/75-2	0.174	0.463	0.457	0.190	0.450	0.460	0.453	0.454	0.459	0.441	0.196	0.300	-	0.462	0.456	0.164	0.446
L-335/99	0.465	0.402	0.421	0.462	0.285	0.378	0.428	0.416	0.402	0.407	0.460	0.458	0.462	-	0.436	0.459	0.402
L73B064	0.443	0.231	0.183	0.458	0.432	0.407	0.223	0.248	0.304	0.358	0.460	0.378	0.456	0.436	-	0.437	0.364
L05L061	0.260	0.449	0.440	0.269	0.446	0.426	0.440	0.437	0.449	0.431	0.267	0.329	0.164	0.459	0.437	-	0.423
L05BA040	0.438	0.375	0.376	0.442	0.383	0.195	0.371	0.388	0.409	0.381	0.441	0.427	0.446	0.402	0.364	0.423	-

GD values indicate a considerable level of genetic diversity and range from 0.026 to 0.465. The lowest value of 0.026 was recorded between the lines ZPL-255/75-5 and ZPL-155/18-4/1. These two lines belong to the genetic pool of the LSC heterotic group, and their genetic base is very narrow. Genetically the most distant lines were L-335/99 and L73L024 with a GD value of 0.465.

Even though initially it was accepted that crosses of more genetically distant parental lines lead to greater heterosis (Fujimoto et al., 2018), there are records of a negative

correlation between GD and heterosis when the GD was extremely high (Moll et al., 1965). Various authors achieved different results in terms of the correlation between GD of parental lines and grain yield (Grčić et al., 2018; Perić et al., 2021; Čamdžija et al., 2022). Therefore, even though GD values are a good starting point for predicting heterosis and grain yield, they are not a definitive method and multienvironmental trials are a necessity in breeding maize for high and stable yields.

The paternal component of the Zombor hybrid (L73B048) is genetically most similar

(Table 5) to the paternal line of hybrid ZP 427 (L73B013), indicating that they are genetically very close parents (Figure 2). In work by Popović et al. (2020), the highest grain yields of test cross hybrids were achieved in crosses that had L73B013 as the parental line. Additionally, crosses with this line gave good results for 1,000 kernel weight (Popović et al., 2024), a trait that can be considered useful for high-yielding hybrids selection. One notable difference between L73B013 and L73B048 is that the L73B048 line is more suitable for creating hybrids of the early FAO 300 maturity group, i.e., hybrids with rapid moisture release during grain maturation.

ZP 427 and ZP 457 share the same maternal component, while their paternal components show a GD of 0.267 (Table 5), indicating a close genetic basis and common affiliation with the BSSS/ID heterotic group. The efforts of the Maize Research Institute “Zemun Polje” to improve the paternal component of ZP 427 (L73B013) through reselection, resulted in the creation of the paternal component of ZP 457 (L74B049). Given the performance results of ZP 457, the reselection can be considered successful. L74B049 represents a desirable genotype for further use in the breeding program, primarily for traits of high grain yield accompanied by high yield stability and adaptability. Furthermore, this line contributes to lower grain moisture at harvest (Čamdžija et al., 2022).

The parental lines common to ZP 555, ZP 560, and ZP 5090 (L-255/75-5, ZPL-155/18-4/1, and L 325/75-2) belong to the genetic pool of the LSC heterotic group, with a very narrow genetic base and GD values below 0.2. Therefore, the performance differences among these three hybrids should be attributed to other parental components (non-LSC). The non-LSC components of these hybrids (L-335/99, L76B004, and L94B034) are significantly distant from one another, with GD values ranging from 0.285 to 0.407 (Table 5). Based on these results, the inbred line L94B034 (parental component of the ZP 5090 hybrid) represents a significant

improvement of the existing genetics from the non-LSC pool, demonstrated by the adaptability and stability of this hybrid.

## CONCLUSIONS

Results showed the maize hybrids produced in the Maize Research Institute “Zemun Polje” exhibit high variability and have high a potential for successful cultivation. In this research, genotype, location, and year significantly influenced the grain yield of the maize hybrids, as did the interaction between location and year (i.e., environment), and genotype  $\times$  environment. The adaptability and yield stability of tested genotypes varied across environments, which further highlights the importance of targeted genotype selection based on environmental conditions. According to the stability analysis, Zombor and ZP 427 are the most stable hybrids, while ZP 457 is both a stable and high-yielding hybrid. The only differences between hybrids ZP 427 and ZP 457 are their paternal lines, while the maternal lines are the same. The paternal line of ZP 457 (L74B049) is the result of the improvement of paternal line of ZP 427 (L73B013), and the paternal line of Zombor (L74B048) is also closely related. According to GGE biplot analysis, Zombor is a good choice for better growing conditions and those producers who prefer early maturing hybrids, especially in the Bačka region. Based on these results, the improvement of line L73B013 can be considered successful and related lines will be a good basis for future breeding programs.

ZP 5090 proved to be a broadly adaptable hybrid with stable yields across various environments. This is confirmed by multiple results of GGE biplot analysis (including ‘Ranking Genotypes’, ‘Which-Won-Where’, and ‘Mean vs. Stability’). This hybrid can be recommended for cultivation in various conditions, including the least favorable ones (e.g., PO19). Based on the performance of ZP 5090 and its comparison with other hybrids in the same and across other FAO maturing groups, paternal line L94B034 can be considered a significant improvement of the

existing genetics from the non-LSC pool and should be used in future breeding programs. The GD values obtained by SNP analysis are a good starting point for creating maize hybrids as they are positively correlated with yield. However, multi-environmental trials are still a necessity which is why testing across locations and years is important in maize breeding.

Based on the results, two mega-environments are revealed, and specific hybrids are recommended. PO19 was the most representative environment and very good for selecting genotypes adapted for the whole region. Environments PA19, BC19, and BT20 were the most informative, while BT19 provided little discriminating information and should be avoided in future trials.

Given recommendations should optimize maize performance and enhance the resilience of hybrids across different environments, ultimately supporting sustainable agricultural practices and improving food security not only in Serbia, but across the European Corn Belt, Balkans and other regions with similar conditions for maize cultivation.

## REFERENCES

- Bradbury, P.J., Zhang, Z., Kroon, D.E., Casstevens, T.M., Ramdoss, Y., Buckler, E.S., 2007. *TASSEL: software for association mapping of complex traits in diverse samples*. *Bioinformatics*, 23: 2633-2635.
- Buhiniček, I., Kaučić, D., Kozić, Z., Jukić, M., Gunjača, J., Šarčević, H., Štepinac, D., Šimić, D., 2021. *Trends in Maize Grain Yields across Five Maturity Groups in a Long-Term Experiment with Changing Genotypes*. *Agriculture*, 11: 887.
- Čamdžija, Z., Filipović, M., Delić, N., Pavlov, J., Grčić, N., Ristić, D., Kostadinović, M., 2022. *Grain yield predictions of ZP maize hybrids*. *BİSAB, Antalya, Türkiye*: 200.
- Crevar, M., Vančetović, J., Pavlov, M., Stevanović, M., Čamdžija, Z., Božinović, S., Prodanović, S., 2011. *Yield Stability of New ZP Maize Hybrids*. *Selekcija i Semearstvo*, XVII: 49-55.
- Eberhart, S.A., and Russell, W.A., 1966. *Stability Parameters for Comparing Varieties*. *Crop Science*, 6: 36-40.
- Francis, T.R., and Kannenberg, L.W., 1978. *Yield Stability Studies in Short-Season Maize. I. A Descriptive Method for Grouping Genotypes*. *Canadian Journal of Plant Science*, 58: 1029-1034.
- Fujimoto, R., Uezono, K., Ishikura, S., Osabe, K., Peacock, W.J., Dennis, E.S., 2018. *Recent research on the mechanism of heterosis is important for crop and vegetable breeding systems*. *Breeding Science*, 68: 145-158.
- Grčić, N., Delić, N., Stevanović, M., Pavlov, J., Crevar, M., Mladenović, M., Nišavić, N., 2018. *Genetic Distance of Maize Inbred Lines Based on SSR Markers for Prediction of Heterosis and Combining Ability*. *Genetika*, 50: 359-368.
- Greveniotis, V., Bouloumpasi, E., Zotis, S., Korkovelos, A., Kantas, D., Ipsilandis, C.G., 2023. *Stability Dynamics of Main Qualitative Traits in Maize Cultivations across Diverse Environments regarding Soil Characteristics and Climate*. *Agriculture*, 13: 1033.
- Hallauer, A.R., and Carena, M.J., 2009. *Maize*. In: Carena, M.J. (eds.), *Cereals*. Springer, New York, NY, USA: 3-98.
- Horhocea, D., Iordan, H., Petcu, E., Ciontu, C., 2024. *Evaluation of New Maize Genotypes for Seed Yield Potential and Stability*. *Romanian Agricultural Research*, 41: 489-496. <https://doi.org/10.59665/rar4147>
- Ivan, J., Boca, Z., Sabadoš, V., 2023. *The Effects of Agricultural Technology on Maize Yield in Sombor Region in Serbia in 2013-2022 Period*. *Agriculture & Food*, 11: 213-218.
- Jocković, Đ., 2008. *Preporuka NS hibrida kukuruza za setvu*. Available online: <http://www.poljoberza.net/tekstovi/9401/820/preporuka-ns-hibrida-kukuruza-za-setvu> (Accessed on: 25 May 2024).
- Jovanović, Ž., Kresović, B., Tolimir, M., Filipović, M., Dumanović, Z., Lopandić, D., 2014. *Regional distribution of the latest ZP maize hybrids generation by the heat summation method*. *Zbornik Naučnih Radova Instituta PKB Agroekonomik*, 20: 21-25.
- Kravić, N., Babić, V., Ristić, D., Srdić, J., Anđelković, V., 2015. *Grain yield and phenotypic performances of maize inbreds under variable environmental conditions*. *Selekcija i Semearstvo*, 21: 1-10.
- Ma, C., Liu, C., Ye, Z., 2024. *Influence of Genotype × Environment Interaction on Yield Stability of Maize Hybrids with AMMI Model and GGE Biplot*. *Agronomy*, 14: 1000.
- Madić, M., Đurović, D., Stevović, V., Bokan, N., 2010. *Stability Parameters for Grain Yield of Maize Hybrids (Zea mays L.)*. *Genetika*, 42: 137-144.
- Madić, M., Tomić, D., Paunović, A., Stevović, V., Đurović, D., 2021. *Grain Yield of Maize Hybrids of Different FAO Maturity Groups*. In: XXVI Savetovanje o Biotehnologiji sa Međunarodnim Učešćem - Zbornik Radova, Univerzitet u Kragujevcu, Agronomski Fakultet, Čačak, Serbia: 93-100.
- Ministry of Agriculture, Forestry and Water Management, 2024. *Registar priznatih sorti poljoprivrednog bilja*. Ministarstvo poljoprivrede, šumarstva i vodoprivrede Republike Srbije, Beograd, Serbia.
- Moll, R.H., Lonnquist, J.H., Velez Fortuno, J., Johnson, E.C., 1965. *The Relationship of Heterosis and Genetic Divergence in Maize*. *Genetics*, 52: 139-144.

- Müller, C., Elliott, J., Pugh, T.A.M., Ruane, A.C., Ciais, P., Balkovic, J., Deryng, D., Folberth, C., Izaurralde, R.C., Jones, C.D., Khabarov, N., Lawrence, P., Liu, W., Reddy, A.D., Schmid, E., Wang, X., 2018. *Global patterns of crop yield stability under additional nutrient and water inputs*. PLoS ONE, 13: e0198748.
- Pacheco-Gil, R.Á., Vargas, M., Alvarado, G., Rodriguez, F., Crossa, J., Burgueño, J., 2015. *GEA-R (Genotype x Environment Analysis with R for Windows) Version 4.1*. CIMMYT: El Batán, Mexico.
- Pavlov, J., Delić, N., Stevanović, M., Čamdžija, Z., Grčić, N., Crevar, M., 2011. *Grain yield of ZP maize hybrids in the maize growing areas in Serbia*. In: Pospišil, M. (eds.), *Proceedings. 46<sup>th</sup> Croatian and 6<sup>th</sup> International Symposium on Agriculture*, University of Zagreb, Faculty of Agriculture, Zagreb, Croatia: 395-398.
- Perić, S., Stevanović, M., Prodanović, S., Mladenović-Drinić, S., Grčić, N., Kandić, V., Pavlov, J., 2021. *Genetic Distance of Maize Inbreds for Prediction of Heterosis and Combining Ability*. Genetika, 53: 1219-1228.
- Petrović, G., Ivanović, T., Knežević, D., Radosavac, A., Obhodaš, I., Brzaković, T., Golić, Z., Dragičević Radičević, T., 2023. *Assessment of Climate Change Impact on Maize Production in Serbia*. Atmosphere, 14: 110.
- Popović, A., Kravić, N., Babić, M., Prodanović, S., Sečanski, M., Babić, V., 2020. *Breeding potential of maize landraces evaluated by their testcross performance*. Zemdirbyste-Agriculture, 107: 153-160.
- Popović, A., Prodanović, S., Golijan Pantović, J., Grčić, N., Perić, S., Ugrinović, M., Babić, V., 2024. *Effect of landraces and testers on the variability of 1000 seed mass and yield in test cross hybrids of maize*. In: Perišić, V. (eds.), *Biotehnologija i Savremeni Pristup u Gajenju i Oplemenjivanju* Bilja, Institut za povrtarstvo Smederevska Palanka, Smederevska Palanka, Serbia.
- Radojčić, A., Filipović, M., Vančetović, J., Branković Radojčić, D., Pavlov, J., 2008. *Maturing groups and grain yield potential of ZP maize hybrids*. Zbornik Naučnih Radova, 14: 43-47.
- Reckling, M., Ahrends, H., Chen, T.-W., Eugster, W., Hadasch, S., Knapp, S., Laidig, F., Linstädter, A., Macholdt, J., Piepho, H.-P., Schiffers, K., Döring, T.F., 2021. *Methods of yield stability analysis in long-term field experiments. A review*. Agronomy for Sustainable Development, 41: 27.
- Shojaei, S.H., Mostafavi, K., Bihamta, M.R., Omrani, A., Mousavi, S.M.N., Illés, Á., Bojtor, C., Nagy, J., 2022. *Stability on Maize Hybrids Based on GGE Biplot Graphical Technique*. Agronomy, 12: 394.
- Tričković, N., Rončević, V., Živanović, N., Grujić, T., Stefanović, L., Jovanović, N., Zlatić, M., 2023. *Ecological and Economic Effects of Applying the Future Agricultural Production Structure Model (FAPSMS): The Case Study of the Barička River Basin*. Sustainability, 15: 8434.
- Yang, R.-C., Crossa, J., Cornelius, P.L., Burgueño, J., 2009. *Biplot Analysis of Genotype × Environment Interaction: Proceed with Caution*. Crop Science, 49: 1564-1576.
- Yan, W., and Kang, M.S., 2003. *GGE Biplot Analysis: A Graphical Tool for Breeders, Geneticists, and Agronomists*. CRC Press, Boca Raton, FL, USA.
- Zhao, C., Liu, B., Piao, S., Wang, X., Lobell, D.B., Huang, Y., Huang, M., Yao, Y., Bassu, S., Ciais, P., Durand, J.L., Elliott, J., Ewert, F., Janssens, I.A., Li, T., Lin, E., Liu, Q., Martre, P., Muller, C., Peng, S., Penuelas, J., Ruane, A.C., Wallach, D., Wang, T., Wu, D., Liu, Z., Zhu, Y., Zhu, Z., Asseng, S., 2017. *Temperature increase reduces global yields of major crops in four independent estimates*. Proceedings of the National Academy of Sciences, 114: 9326-9331.