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EVALUATION OF WHEAT GENOTYPE STABILITY THROUGH GGE BIPLOT ANALYSIS

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Abstract: Under pronounced climate changes, identifying wheat genotypes with high yield potential and high stability across environments is becoming essential. A multi-environment trial (MET) with 15 wheat genotypes was conducted across three localities (Pančevo, Kragujevac, and Kruševac) during two growing seasons (2022/2023 and 2023/2024). The study evaluated genotype stability and adaptability for the number of grains per spike and assessed the discriminatory power of the test environments. The GGE biplot presented the combined genotype (G) + genotype × environemtnt (GE) effects, with PC1 and PC2 explaining 58.88 and 19.98% of the total variation, respectively. The spelt wheat lines achieved the highest values for the number of grains per spike and were positioned closest to the "ideal genotype". Among the bread wheat genotypes, the line KG-40/1 showed the highest value and was narrowly adapted to the environmental conditions of Pančevo and Kragujevac in 2022/2023 season. The genotypes KG-9/1, KG-43/1, and NS 40S demonstrated high stability and high numbers of grains per spike. Environment Pančevo 2023/2024 exhibited the greatest representativeness and discriminative ability, making it the most suitable for genotype evaluation. These findings provide a practical basis for selecting stable and well-adapted genotypes in future wheat breeding programs.

Keywords: Multi-environment trial (MET), Adaptability, Stability, G × E interaction.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is a fundamental staple food, with global production reaching approximately 799 million tonnes in 2023, obtained from over 220 million hectares of harvested area and an average yield of about 3.6 t ha^{-1} (FAOSTAT, 2025). Wheat, as an important part of the human diet, provides about 18% of global calories and 19% of total protein, highlighting its key role in food security (Erenstein et al., 2022). Global population growth from 7.7 billion to around 9.7 billion by 2050, eventually reaching 10.3 billion in the mid-2080s (UN-DESA, 2024), could increase annual wheat demand by about 132 million tonnes, assuming per capita consumption remains constant (Erenstein et al., 2022). Drought stress is a major abiotic factor affecting wheat growth and yield. Xu et al. (2023) established that drought stress conditions reduced wheat grain yield by over 30%. If such trends in severe drought continue, wheat production may decline further, threatening global food and nutritional security (Mansour et al., 2020). The phenotypic expression of wheat yield components and grain yield depends on the genotype, environment, and the genotype-by-environment interaction (GEI) (Begna, 2020; Bratković et al., 2024). Therefore, understanding GEI is crucial for developing high-yielding wheat varieties that perform consistently across diverse environments (Begna, 2020). To evaluate crop performance under varying environmental conditions, multi-environment trials (METs) are of crucial importance (Taherian et al., 2024; Mullalem et al., 2024). The genotype plus genotype-by-environment (GGE) biplot method is widely applied for analyzing genotype stability and identification of ideal environments for testing genotypes (Omrani et al., 2022).

The aim of this study was to: (I) evaluate the stability and adaptability of the analyzed wheat genotypes for grain number per spike using GGE biplot analysis; and (II) identify the most suitable environment for genotype testing.

MATERIAL AND METHODS

Plant Material and Experimental Design

The study included 15 wheat genotypes: 10 promising KG bread wheat lines (*Triticum aestivum* ssp. *vulgare*) (KG-4/1, KG-9/1, KG-11/1, KG-16/1, KG-21/1, KG-30/1, KG-33/1, KG-40/1, KG-41/1, and KG-43/1) developed at the Centre for Small Grains and Rural Development in Kragujevac, and three bread wheat varieties used as standards: NS 40S, Renesansa, and Pobeda, originating from the Institute of Field and Vegetable Crops in Novi Sad. In addition, two KG spelt wheat lines (*Triticum spelta* ssp.), KG-54-7/3-5 and KG-54-7/3-2, were included to broaden the variability of the analyzed germplasm and to assess their yield potential across different environments. The field experiment was carried out at three localities: Pančevo (Experimental Field of the "Tamiš" Research and Development Institute, 44°56′31″ N, 20°43′18″ E, 80 m), Kragujevac (Experimental Field of the Center for Small Grains and Rural Development, 44°02′47″ N, 20°56′54″ E, 196 m), and Kruševac (Experimental Field of the Institute for Forage Crops Kruševac, 43°34′56″ N, 21°12′16″ E, 148 m), across two growing seasons (2022/2023 and 2023/2024). At all localities, the wheat genotypes were arranged in a randomized complete block design (RCBD) with three replications, and the plot size was 5 m^2. Standard agronomic practices for wheat production were applied. In the first season, sowing was performed on October 26, 2022, in Pančevo, November 4, 2022, in Kruševac, and November 9, 2022, in Kragujevac. In the second season, sowing was performed somewhat later at all localities: November 2, 2023, in Pančevo, November 14, 2023, in Kruševac, and November 20, 2023, in Kragujevac. Harvest was carried out in both seasons when grain moisture fell below 14%. In the first season, the harvest dates were July 13 in Pančevo, July 18 in Kragujevac, and July 12, 2023, in Kruševac. The second season was characterized by an earlier harvest in all localities. Thus, the harvest in Pančevo was carried out on June 26, 2024, in Kragujevac on July 10, 2024, and in Kruševac on July 4, 2024.

At full maturity (BBCH 89), samples were collected for the analysis of grain number per spike. From each plot, 10 plants were randomly selected (30 plants per genotype across three replications), and the number of grains per spike was determined by manually counting all grains on the main spike.

Agro-meteorological Conditions

Meteorological data for Pančevo and Kruševac were obtained from stations located at the experimental fields, while data for Kragujevac came from the nearby station of the Republic Hydrometeorological Service of Serbia (www.hidmet.gov.rs/) (Figure 1).

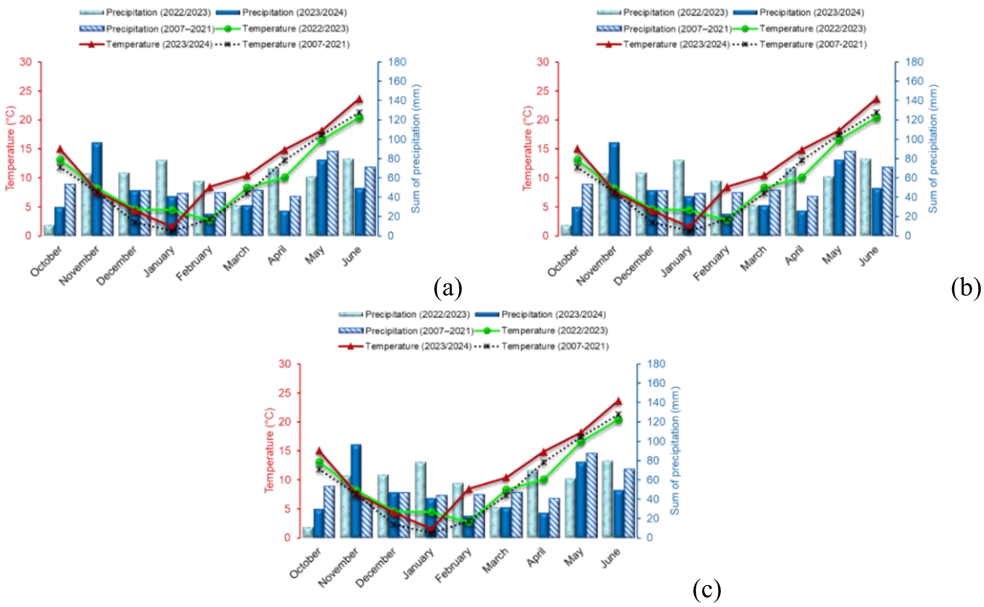

Figure 1. Meteorological conditions during the experiment at three localities: Pančevo (a), Kragujevac (b), and Kruševac (c), during two growing seasons

December-January in both seasons was notably warmer than average. In the first season, precipitation exceeded the average for all localities, whereas in the second season it was below average, prolonging the growth period and delaying dormancy. During the tillering phase (February), temperatures were near average in the first season but extremely high in the second, accompanied by reduced precipitation, particularly in Kragujevac. These conditions accelerated early tillering. During stem elongation and anthesis (March-May) in the first season, Kruševac was characterized by slightly lower temperatures and reduced precipitation, while Pančevo and Kragujevac recorded lower temperatures accompanied by higher precipitation. In April 2024, temperatures were above average at all localities, which contributed to a shortened stem elongation phase. In June of the first growing season, temperatures at all localities were within the multi-year average, while significantly higher precipitation was recorded in Kragujevac and Kruševac (Figure 1).

Statistical Analysis

To identify genotypes with high yield and stable performance across different agroecological environments, as well as to define potential mega-environments, a GGE biplot analysis (Genotype plus Genotype × Environment interaction) was carried out. The first two principal components were used to generate several biplots, including Mean vs. Stability, Which-Won-Where, Discriminativeness vs. Representativeness, and Genotype Ranking. This analysis was conducted using the metan package (Olivoto and Lúcio, 2020) in R version 4.3.2.

RESULTS AND DISCUSSION

To increase wheat yield, it is not sufficient to improve photosynthesis or grain weight, since increasing grain number or sink strength is a key factor for yield improvement (Slafer et al., 2023; Serrago et al., 2025). Positive correlations between grain number per spike and grain yield in wheat were reported by Philipp et al. (2018). Zečević et al. (2018), studying different spelt wheat genotypes, also noted that grain number per spike is an important component of grain yield and contributes to grain yield potential.

From a plant breeding perspective, it is particularly important to investigate GEI, which arises as a result of changes in genotype ranking or fluctuations in absolute differences among genotypes without changes in ranking (Saeidnia et al., 2017). In order to examine the combined effects of genotype and genotype × environment interaction, GGE biplot analysis was applied. The first principal component (PC1) explained 58.88% of the total variation, while the second (PC2) accounted for 19.98%, together explaining 78.86% of the variability in the dataset for grain number per spike (Figure 2a). The high proportion of variance explained by the first two principal components indicates a complex GEI in multi-environment trial (MET) data (Mullalem et al., 2024). In the "Mean vs. Stability" biplot (Figure 2a), the AEC axis represents the average performance of genotypes across environments, while the stability of individual genotypes is determined by their distance from this axis, corresponding to their projection onto the AEC ordinate (Yan et al., 2007; Omrani et al., 2022; Taherian et al., 2024). The genotypes of spelt wheat (G14 and G15) had above-average grain number per spike and moderate stability. Among bread wheat genotypes, KG-40/1 (G8), Renesansa (G12), KG-9/1 (G2), and Pobeda (G13) stand out with above-average grain number per spike. Genotype KG-9/1 (G2) was the most stable among the above-average genotypes (Figure 2a).

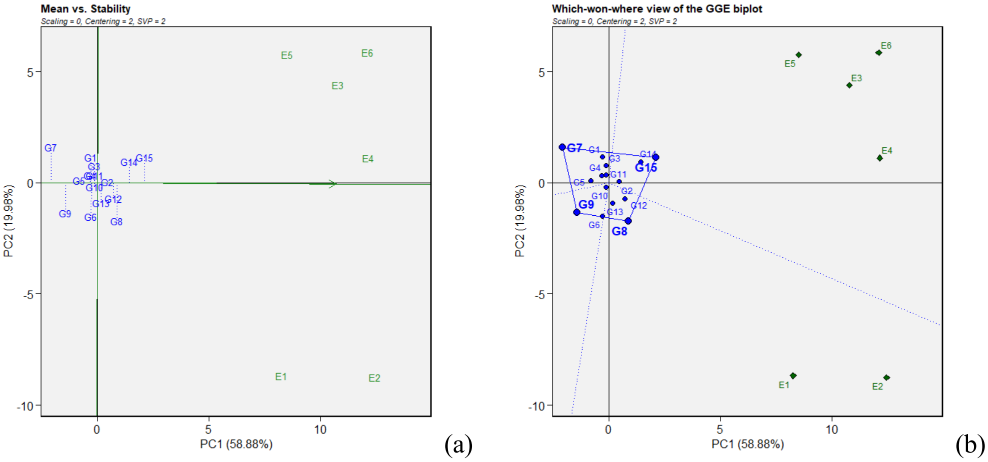

Figure 2. Mean vs. Stability (a) and Which-won-where (b) view of the GGE biplot analysis for number of grains per spike

With the aim of identifying winning genotypes in specific environments, as well as defining mega-environments, a Which-won-where GGE biplot was constructed (Yan et al., 2007), Figure 2b. The genotypes KG-33/1 (G7), KG-41/1 (G9), KG-40/1 (G8), and KG-54-7/3-2 (G15) were positioned farthest from the origin in different directions, forming a polygon. These genotypes are the best or worst in one or more environments (Saeidnia et al., 2023).

A set of perpendicular lines from the origin divided the biplot into sectors. Environments E3 (Kruševac 2022/2023), E4 (Pančevo 2023/2024), E5 (Kragujevac 2023/2024), and E6 (Kruševac 2023/2024) were located within the same sector, forming a mega-environment in which genotype KG-54-7/3-2 (G15) was the winning genotype. Environments E1 (Pančevo 2022/2023) and E2 (Kragujevac 2022/2023) fell into a separate sector, with genotype KG-40/1 (G8) at the vertex, suggesting its specific adaptation to these environments. Genotypes G7 (KG-33/1) and G9 (KG-41/1), positioned at the polygon vertices but within sectors that did not include any environment, were characterized by a low grain number per spike at the trial level. Genotypes KG-9/1 (G2), KG-16/1 (G4), NS 40S (G11) and Pobeda (G13), located closer to the center of the biplot, exhibited stable performance for grain number per spike (Figure 2b).

The "Discriminativeness vs. Representativeness" GGE biplot for grain number per spike identifies Kragujevac 2022/2023 (E2), Kruševac 2023/2024 (E6), Pančevo 2022/2023 (E1), and Pančevo 2023/2024 (E4) as the most discriminative environments, as they have the longest vectors (Figure 3a). Yue et al. (2025) emphasized that highly discriminative environments are suitable for identifying stable genotypes with broad adaptability. In contrast, the environment Kragujevac 2023/2024 (E5) shows the lowest discriminative power. Representativeness is evaluated according to the angle between the environment vector and the AEC axis, where a smaller angle indicates greater representativeness (Saeidnia et al., 2023). Accordingly, the most representative environment is Pančevo 2023/2024 (E4), which forms the smallest angle with the AEC axis. The environments Pančevo 2022/2023 and Kragujevac 2022/2023 form the largest angle with the AEC axis, making them the least representative. Based on the obtained results, we conclude that Pančevo 2023/2024 (E4) is the ideal environment for genotype evaluation, as it is both representative (forming a small angle with the AEC axis) and discriminative (having a long vector) (Figure 3a).

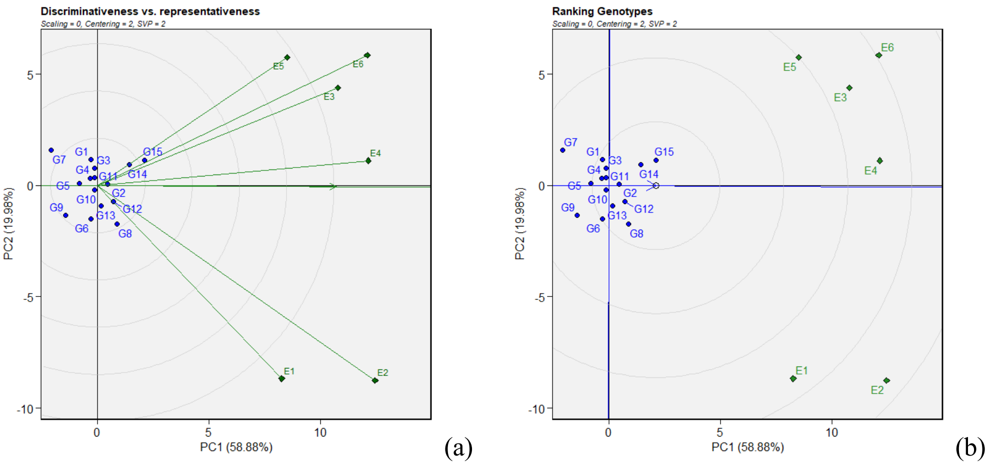

Figure 3. Discriminativeness vs. representativeness (a) and Ranking genotypes (b) view of the GGE biplot analysis for number of grains per spike

A Ranking Genotypes GGE biplot was constructed with the aim of ranking genotypes and identifying the ideal genotype within the evaluated set of genotypes (Figure 3b). The hypothetical "ideal" genotype is represented at the center of the concentric circles, in the direction of the blue arrow. The spelt lines KG-54-7/3-5 (G14) and KG-54-7/3-5 (G15) are positioned closest to the ideal genotype, demonstrated a high value for the examined trait as well as high stability at the trial level. Also located near the ideal genotype the bread wheat genotypes KG-9/1 (G2) and Renesansa (G12). Using this type of GGE biplot, Bishwas et al. (2021) identified an ideal elite wheat line that can be used as a reference for evaluating other lines.

CONCLUSION

Spelt genotypes stood out with the highest values of grain number per spike and moderate stability. Line KG-9/1 showed the highest stability among the above-average genotypes. Two mega-environments were identified, with genotype KG-40/1 winning in the environments of Pančevo and Kragujevac in the 2022/2023 season, while the spelt line KG-54-7/3-2 showed specific adaptability under the conditions of Pančevo and Kragujevac in 2023/2024, and Kruševac in both seasons. Genotypes KG-33/1 and KG-41/1 were characterized by the lowest trait values and pronounced instability. Pančevo 2023/2024 was identified as the ideal environment for genotype evaluation, characterized by high discriminativeness and representativeness.

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