

# EVALUATION OF WHEAT GENOTYPES FOR SALINITY TOLERANCE UNDER REAL SALINE CONDITIONS – IN SITU

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## Abstract

Salinity is one of the major abiotic stress factors that limit the productivity of crops, including wheat, in many regions of the world. Therefore, the priority in wheat breeding, to ensure global food security, is developing varieties that are adapted to saline environments. In situ, evaluation of wheat genotypes can provide valuable information on the performance of different genotypes under natural saline conditions and can help to identify the most salt-tolerant genotypes. To ensure an accurate evaluation of the performance of twenty-seven wheat genotypes under different environments, the trial was conducted on two different soil types (solonetz and chernozem) in two growing seasons. AMMI analysis shows that the environmental factor had the largest share (55.15%) in the variation of grain yield, where soil type had a dominant effect. Genotypes Renesansa, Harmonija, and Bankut 1205 achieved a high grain yield on both soil types. However, among the mentioned genotypes, the genotype Harmonija showed the highest tolerance to salinity. A significant proportion of the genotype and environment interaction (GEI; 25.89%) shows that there is a change in the ranking of genotypes across environments. According to the AMMI<sub>1</sub> biplot, the genotypes Renesansa and Harmonija were distinguished by high grain yield and high stability. The environment Chernozem 2015/2016 had the greatest contribution to the GEI and the highest grain yield, while Solonetz 2017/2018 was characterized by the highest stability and the lowest grain yield. According to the AMMI<sub>2</sub> biplot, genotype Harmonija achieved high stability in unfavourable environmental conditions that characterized the environment Solonetz 2017/2018.

**Key words:** additive effects, multivariate effects, GEI, AMMI, salinity stress, stability

## Introduction

Wheat is one of the most widely grown and consumed cereals in the world, and it provides around 20% of the calories and protein consumed by humans globally, making it an important source of nutrition for millions of people (Shiferaw et al., 2013; Ernstein et al., 2022). Given the widespread consumption of wheat-based foods around the world, any disruptions

to wheat production can have significant impacts on food availability, prices, and nutrition. Therefore, ensuring the sustainability and resilience of wheat production is critical for meeting the food needs of a growing global population (Lopes et al., 2018). However, wheat production faces challenges such as extreme temperatures, drought, soil salinity, and soil degradation (Sha-

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hid et al., 2018; Raimondo et al., 2020; Saddiq et al., 2021). Soil salinity is one of the major abiotic stresses that negatively impact plant growth and development (Dimitrijević et al., 2012; Farooq et al., 2022). Different wheat genotypes can exhibit varying levels of tolerance to salinity stress, which can affect their ability to produce acceptable grain yields under such conditions (Mansour et al., 2020; Banjac et al., 2022). Evaluation of wheat genotypes under real field salinity conditions—in situ—is crucial to identify salt-tolerant and sensitive ones, and this information can be useful for the regionalization of realized varieties and for the development more effective breeding strategies for improved wheat productivity in saline environments (El-Hendawy et al., 2017; Mansour et al., 2020; Moustafa et al., 2021).

In order to identify wheat genotypes that are well adapted to salinity stress conditions, it is necessary to conduct experiments in a variety of environmental conditions. Many researchers used AMMI analysis with the aim of assessing the stability of genotype performance across multiple environments (Petrović et al., 2010; Dimitrijević et al., 2011; Mladenov et

al., 2019; Verma and Singh, 2021; Banjac et al., 2022; Perišić et al., 2022; Ahakpaz et al., 2023). This method combines ANOVA (Analysis of Variance) with principal component analysis (PCA) to separate genotype and environment effects and their interaction (GEI) (Gauch and Zobel, 1996). In the context of wheat salinity tolerance, AMMI analysis can be used to evaluate the yield stability performance of wheat genotypes under salinity stress and identify those that are more tolerant (Petrović et al., 2010; Pour-Aboughadareh et al., 2021; Banjac et al., 2022; Kumar et al., 2022; Farokhzadeh et al., 2022). This information can be used to improve field crop productivity and sustainability in saline environments.

The aim of this research is to: (I) establish the share of factors of genotype, environment, and their interaction in the total variation of grain yield; (II) evaluate the stability performance of the wider wheat germplasm across different environments; (III) identify genotypes that exhibit satisfactory grain yield and high stability; (IV) single out genotypes that exhibit specific adaptability to salinity stress conditions.

## Material and methods

### *Plant material and Experimental Design*

Twenty-seven divergent wheat genotypes were used as material for the research, including: two local populations (Banatka and Grbljanka), one old Hungarian variety (Bankut 1205), 20 old and newly varieties created at the Center for Small Grains in Kragujevac (Šumadija, Kosmajka, Gružanka, Morava, Zastava, KG-56, Orašanka, KG-58, KG-78, Lepenica, Oplenka, Ljubičevka, Srbijanka, Šumadinka, Aleksandra, Perfekta, Harmonija, Rujna, and Premija) and 4 varieties (Jugoslavija, NSR-5, Renesansa, and Pesma) released by the Institute for Field and Vegetable Crops in Novi Sad.

A trifactorial wheat trial was established, according to a completely randomized block design, in three replicates on two localities: Rimski Šančevi, near Novi Sad (Bačka) and Kumane (Banat), during two growing seasons

(2015/2016 and 2017/2018). Sowing was carried out mechanically with a distance of 10 cm between rows, where the elementary plot size was 2m<sup>2</sup>. During experimental research, the standard cropping practices for wheat production were applied. The basic soil preparation was carried out in the autumn, with plowing of crop residues and mineral NPK fertilizer using a moldboard plow at 30 cm. Top dressing with 250 kg ha<sup>-1</sup> AN (27% N) was performed in the spring. Chemical crop protection measures to control pests, diseases, and weeds during wheat vegetation were performed only when necessary. In both growing seasons, harvesting was done in the phenophase of full maturity, when grain moisture dropped below 14%. The grain yield per plot was measured and calculated to grain yield per hectare with 13 percentage of moisture.

### Soil conditions

Solonetz soil type, which characterizes the location Kumane, is characterized by a high content of sodium and clay, which results in a distinctive structure and chemical properties. The high sodium content of solonetz soils can cause soil particles to bind tightly together, resulting in a hard, dense soil structure that can be difficult for plant roots to penetrate. This can lead to reduced water and nutrient uptake, as well as decreased plant growth and yield (Belić et al., 2012).

### Meteorological conditions

Meteorological data were obtained from the Republic Hydrometeorological Service of Serbia's website (<https://www.hidmet.gov.rs>). The winter of 2015/2016 in the analyzed localities was relatively mild, with temperatures above the long-term average. February, March, and April were characterized by warm weather, with temperatures and the sum of precipitation

The soil type at the location Rimski Sancevi is chernozem. It is a highly fertile and productive soil type that is well-suited for agriculture, rich in organic matter, nutrients, and minerals. Chernozem soil is formed from the accumulation of organic matter over long periods of time, which creates a thick, black layer of topsoil. Chernozem soils also have a unique physical structure, with a crumbly texture that allows for good drainage and aeration (Hadžić et al., 2002).

around the long-term average, which had a positive effect on the growth and development of wheat. The very high amount of rainfall in June allowed for a good filling of the grain. However, in this period there was a wheat lodging that was particularly related to old varieties and local landraces, which are characterized by a higher stem height.

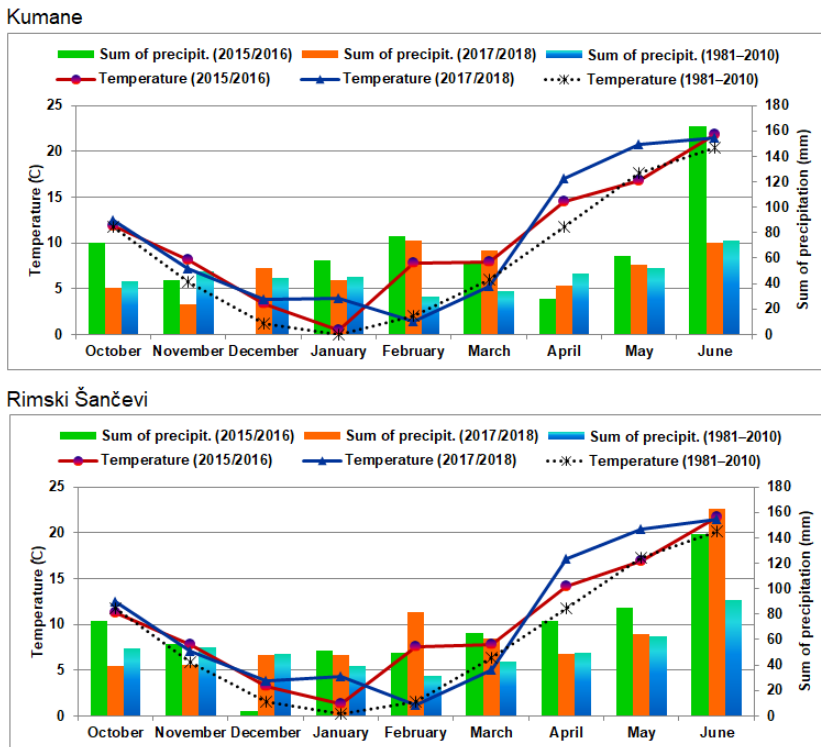


Figure 1. Meteorological conditions during the experiment

Grafiikon 1. Meteorološki uslovi tokom izvođenja eksperimenta

The October, November, and December 2017/2018 growing seasons were marked by higher temperatures compared to the multi-year average, with significantly lower amounts of precipitation than average in both localities. February and the first decade of March were characterized by significantly lower temperatures than the multi-year average and a formed snow cover. At the end of March, there was a temperature rise, which favoured the accelerated development of wheat. Very high mean temperatures in April and May contributed to the drying of the surface layer of the soil and a

water deficit for the plants. June was characterized by temperatures within the average range, with a higher amount of precipitation in the Rimski Šančevi locality. Dry weather and high temperatures in the second half of the month accelerated the ripening of wheat in both localities. In general, the 2017/2018 growing season was characterized by lower rainfall and significantly higher average temperatures in April and May compared to the 2015/2016 growing season in both localities (Figure 1).

### *Statistical analysis*

In order to analyze the influence of the analyzed factors and their interaction on the variation of grain yield, an AMMI (Additive Main Effects and Multiplicative Interaction) analysis was carried out using the GenStat, Trial Version 18.1.0.17005 (<https://www.vsni.co.uk>). AMMI analysis combines elements of both ANOVA (Analysis of Variance) and PCA (Principal Component Analysis) to decompose the genotype (G)

and environment (E) effects and their interaction (GEI) into additive main and multiplicative interaction effects (Gauch and Zobel, 1996). The obtained PCA scores (IPCA<sub>1</sub> and IPCA<sub>2</sub>) were used to generate AMMI<sub>1</sub> (IPCA<sub>1</sub> vs. grain yield) and AMMI<sub>2</sub> (IPCA<sub>1</sub> vs. IPCA<sub>2</sub>) biplots, which are useful graphical representations of the genotype and environment effects.

## **Results and discussion**

The AMMI model is considered an efficient method for analyzing GEI data in agricultural research because it allows for the unique separation of main and interaction effects, which is essential for identifying stable genotypes and making informed breeding decisions (Gauch, 2006). The AMMI analysis of grain yield shows that there is a highly significant ( $p < 0.01$ ) influence of additive and multivariate sources of variation. When considering additive effects, the environment contributed the most to grain yield variation (55.15%), while the genetic factor's part (Genotype) was significantly lower (11.16%) (Table 1). Also, a greater share of environmental factors on wheat grain yield was established by Verma and Singh (2021), and

Pour-Aboughadareh et al. (2021). The analysis of environmental factors showed that the soil type had a greater influence on grain yield than the factor of vegetation season (Figure 2). Therefore, conditions of soil salinity stress significantly reduced grain yield. This is following results established by Dimitrijević et al. (2012), who stated that the reduction in grain yield was up to 40% in alkaline soil. Also, Nadeem et al. (2020), Mansour et al. (2020), and Elfناه et al. (2023) found that growing wheat on treatments with an applied high salt concentration significantly reduces grain yield, compared to the grain yield achieved on control and treatments with a low salt concentration.

The least decrease in grain yield under the influence of salinity, was found in the Šumadija and Rujna genotypes. However, although the most tolerant, the mentioned genotypes achieved very low grain yields on both soil types. However, these genotypes should not be immediately rejected as genetic material, but modern laboratory analyses should be used to check whether they possess genes for salinity tolerance and whether they can serve as a genetic resource in breeding for salinity tolerance. Genotypes Renesansa, Harmonija, and Bankut 1205 achieved a high grain yield on both soil types and are considered desirable genotypes for cultivation, both in favourable and unfavourable environmental conditions. However, among the mentioned genotypes, Harmonija showed the highest tolerance to salinity, i.e., the least decrease in grain yield under the influence of stress factors, which is why it is considered

particularly adapted to unfavourable salinity conditions. On the other hand, the Zastava and NSR-5 genotypes, which had a large difference in grain yield under the influence of salinity, achieved an above-average yield on chernozem. Therefore, the mentioned genotypes are suitable for cultivation under favourable environmental conditions, which will allow them to reach their full genetic potential. When it comes to the factor of the growing season, genotypes Šumadija, Premija, Gružanka, Harmonija, NSR-5, and Oplenka showed the least variation in grain yield. Among the mentioned genotypes, the genotype Harmonija stands out as the most productive. The highest difference in grain yield between growing seasons was recorded in the local landraces Banatka and Grbljanka and in the old variety Bankut 1205, which can be successfully grown under favourable environmental conditions (Figure 2).

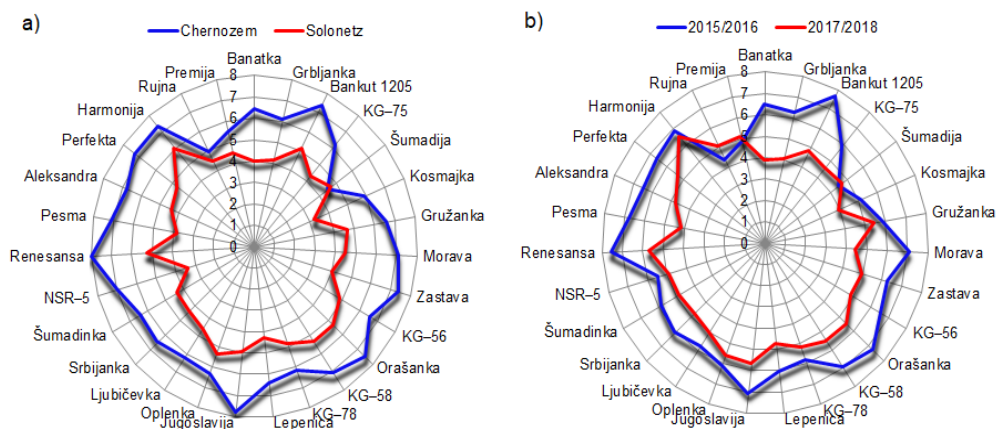


Figure 2. Grain yield ( $t\ ha^{-1}$ ) variation under the influence of soil type (a) and vegetation season (b) in the analyzed wheat genotypes

Grafikon 2. Varijacija prinosa zrna ( $t\ ha^{-1}$ ) uslovljena tipom zemljišta (a) i vegetacionom sezonom (b) kod analiziranih genotipova pšenice

The share of GEI in the phenotypic expression of grain yield was significant (25.89%), which means there was a change in the ranking of genotypes across environments due to the large differences in the analyzed environments. Similar results were established by Mohammadi

et al. (2018), Wardof et al. (2019), and Verma and Singh (2021). The interaction is decomposed into its interaction components, where the first two main components ( $IPCA_1$  and  $IPCA_2$ ) explain 90.27% of the interaction (Table 1).

Table 1. AMMI-ANOVA for grain yield of examined wheat genotypes grown on solonetz and chernozem  
 Tabela 1. AMMI-ANOVA prinosa zrna ispitivanih genotipova pšenice, gajenih na solonjecu i černozeumu

Source of variation	Df	SS	MS	F – value	The share of total variation (%) <sup>1</sup>
Total	323	913.20	2.83	-	100.00
Treatments	107	805.50	7.53	18.63**	88.21
Genotypes	26	102.00	3.92	9.71**	11.16
Environments	3	467.10	155.71	52.75**	51.15
Block	8	23.60	2.95	7.30**	2.58
Interactions	78	236.40	3.03	7.50**	25.89
IPCA <sub>1</sub>	28	146.90	5.24	12.98**	62.14
IPCA <sub>2</sub>	26	66.50	2.56	6.33**	28.13
IPCA <sub>3</sub>	24	23.00	0.96	2.37**	9.73
Residuals	0	0.00	-	-	-
Error	208	84.1	0.40	-	-

<sup>1</sup> The share of interaction principal components (IPCA<sub>1</sub>, IPCA<sub>2</sub>, and IPCA<sub>3</sub>) is expressed in relation to the sum of squares of interactions (100%).

The AMMI<sub>1</sub> biplot (IPCA<sub>1</sub> vs grain yield) shows that the influence of additive and multivariate variation on grain yield expression is almost equal (Figure 3). Genotypes or environments that are plotted on the same vertical line of the AMMI<sub>1</sub> biplot do not differ in yields, as they have similar additive effects. Similarly, genotypes or environments that are positioned on the same horizontal line have similar interaction patterns and do not differ in the multivariate part of the variation (Gupta et al., 2022; Matković Stojšin et al., 2022). The environment Chernozem 2015/2016, where the highest grain yield was achieved, had the greatest contribution to the GEI. Agroecological environments that are characterized by less favourable soil (solonetz) or less favourable meteorological conditions (Chernozem 2017/2018) are located below the abscissa, where the environments of Solonetz 2015/2016 and Chernozem 2017/2018

differ from each other in the additive but not in the multivariate part of the variation. The highest stability, but also the lowest grain yield, was found in the environment of Solonetz 2017/2018. In this case, the high stability resulted from the genotypes' reduced capacity to express their genetic potential for grain yield in unfavourable environmental conditions. The genotypes NSR-5, Srbijanka, Lepenica, Ljubičevka, Šumadinka, KG-56, Zastava, Gružanka, Morava, KG-58, Orašanka, Renesansa, and Harmonija showed high stability, i.e., low values of the first interaction component. A genotype with both a high yield and a high degree of stability is considered more desirable, as it has the potential to consistently perform well across different environments (Kumar et al., 2022; Ahakpaz et al., 2023). Genotypes Harmonija, Orašanka, Renesansa, KG-58, and Bankut 1205, in addition to their high stability, are also

characterized by a high average grain yield. Regardless of the high stability, the genotype Kosmajka was characterized by low grain yield values in both localities. Moderate stability was established in the genotypes Jugoslavija, Pesma, Banatka, KG-78, Premija, and Šumadija, among which the genotype Jugoslavija stood out due to its high grain yield. The genotype Rujna, in addition to the low value of the grain yield, was distinguished by its pronounced instability (Figure 3).

By creating an AMMI<sub>2</sub> biplot, an additional 28.13% of the interaction was explained (Figure 4). Considering the fact that the AMMI<sub>2</sub> biplot accounts for the majority of the interaction, Khan et al. (2020) find that it is superior at estimating the complex GEI pattern. Environments and genotypes that are positioned near the origin have less influence, whereas those that are dis-

tant from the origin have a stronger impact on GEI (Yan et al., 1998). In this study, genotypes that had low values of interaction components, i.e., high grain yield stability across different environments, are KG-58, Renesansa, KG-56, Srbijanka, Lepenica, Ljubičevka, Šumadinka, Kosmajka, Grbljanka, NSR-5, and Orašanka. The environments Chernozeem 2017/2018 and Solonetz 2015/2016 had the smallest contribution to GEI. The mentioned environments did not differ from each other in the multivariate part of the variation; their vectors have the same orientation and give the same ranking of genotypes. Accordingly, chernozeem in a less favourable vegetation season (2017/2018) caused the same behaviour of wheat genotypes as solonetz (soil with increased alkalinity) in a favourable vegetation season (2015/2016), which illustrates the importance of the climatic factors that interact

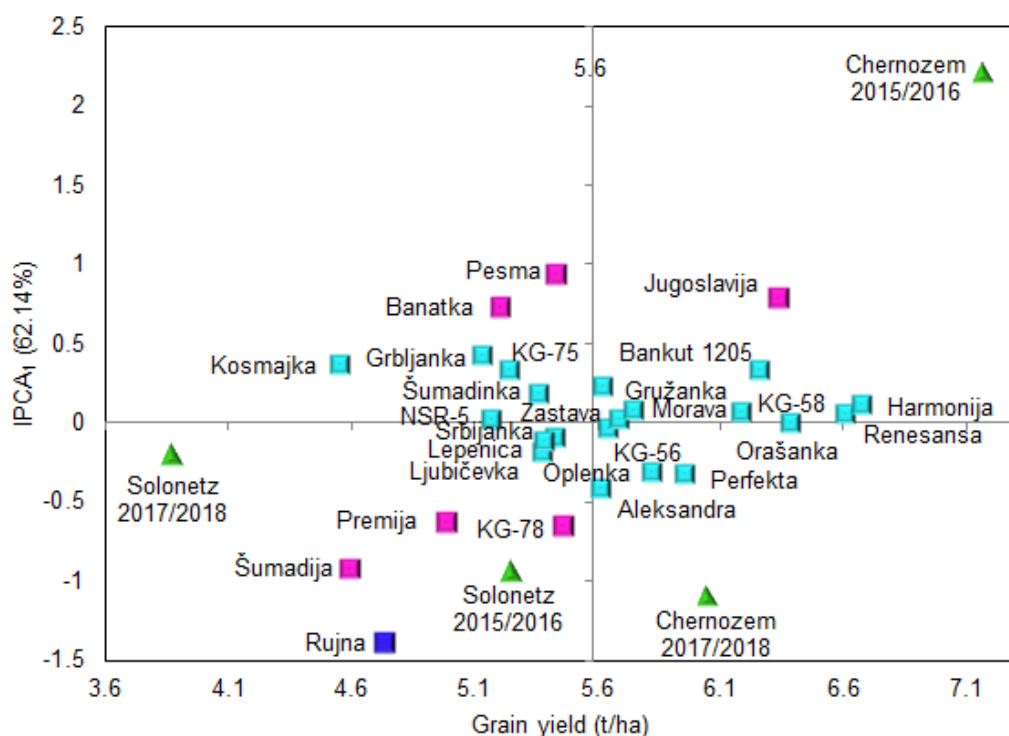


Figure 3. AMMI1 biplot (IPCA1 vs grain yield) for grain yield stability assessment of examined wheat genotypes

Grafikon 3. AMMI1 biplot (IPCA1 vs prinos zrna) za procenu stabilnosti prinosa ispitivanih genotipova pšenice

synergistically with the soil to influence plant growth in real field conditions – in situ.

On the other hand, the environments Chernozem 2015/2016 and Solonetz 2017/2018, positioned in different quadrants of the biplot, had a high effect on GEL. The importance of the growing season in achieving genotype stability is reflected in the fact that the chernozem soil type in different growing seasons showed a drastic difference in the multivariate part of the variation.

Genotypes with a higher IPCA score are more adapted to certain environments but less consistent across different environments (Khan et al., 2020; Farokhazadeh et al., 2022; Ahakpaz et al., 2023). In this research, the genotypes Rujna, Šumadija, Premija, Harmonija, Pesma,

Morava, Perfekta, and Aleksandra had high values of one or both interaction components. However, some of the mentioned genotypes had positive interactions with certain environments. The genotype Harmonija achieved specific adaptability to unfavourable environmental conditions that characterized Solonetz 2017/2018 (Figure 4). Also, this genotype achieved the highest grain yield in saline soil conditions in both analyzed growing seasons (Figure 2). Genotypes Aleksandra, Morava, and Perfekta expressed stable reactions in the Solonetz 2015/2016 environment. The genotype Pesma, together with the old variety Bankut 1205 and local landrace Banatka, expressed specific stability in favourable conditions of the environment Chernozem 2015/2016 (Figure 4).

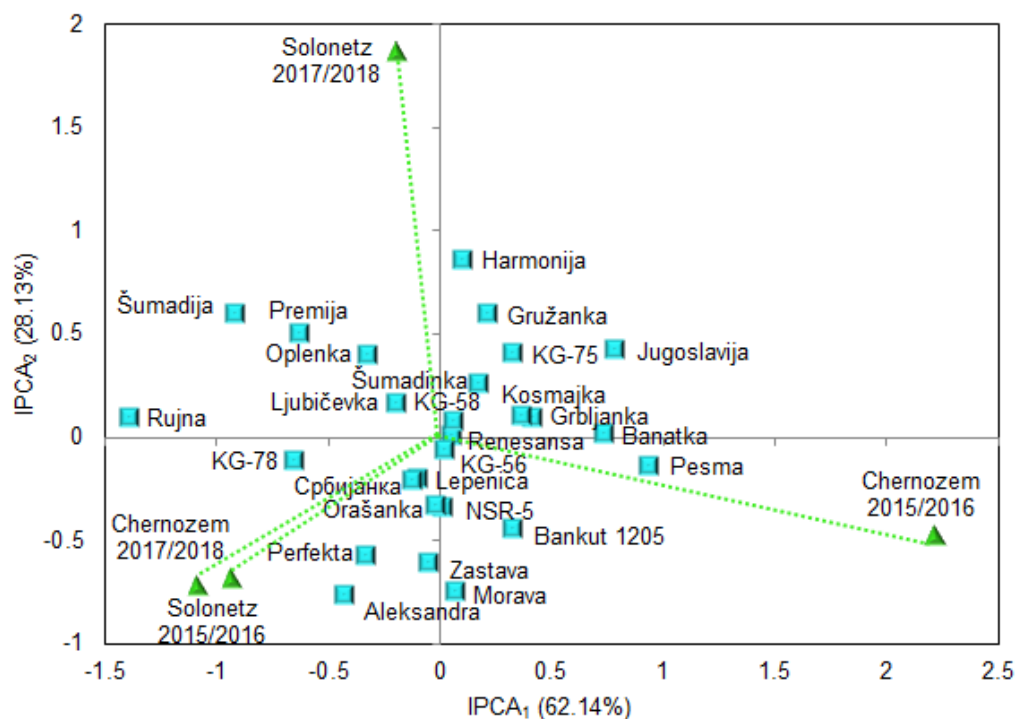


Figure 4. AMMI2 biplot (IPCA1 vs IPCA2) for assessing the stability of wheat genotypes grown in different agroecological environments

Grafikon 4. AMMI2 biplot (IPCA1 vs IPCA2) za procenu stabilnosti genotipova pšenice gajenih u različitim sredinama



## Conclusion

The AMMI model can be a valuable tool for crop breeding and agricultural research, helping to identify stable and the best genotypes for specific environments. The results showed significant influences of genotype, environment (soil type and vegetation season) as well as GEI on the phenotypic variation of wheat grain yield. Regardless of low grain yields, genotypes Rujna and Šumadija did not react to salinity stress conditions by reducing grain yield. Genotypes Renesansa, Harmonija, and Bankut 1205 achieved high grain yields on both types of soil, where the smallest decrease in stress conditions was recorded in the genotype Harmonija. The favourable conditions of the Chernozem 2015/2016 environment allowed the geno-

types to express their genetic potential, which resulted in high yield instability in the given environment. On the other hand, the highest stability and the lowest grain yield were found in the environment of Solonetz 2017/2018, where genotypes could not express their genetic potential for grain yield. Genotypes Renesansa, Harmonija, KG-58 and Orašanka expressed high above-ground grain yield and stability and were considered suitable for growth across a range of environments. According to the AMMI<sub>2</sub> biplot, genotype Harmonija performed specifically well under stressful conditions in Solonetz in the 2017/2018 vegetation season, and therefore it can be rated as a salinity-tolerant genotype.

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## PROCENA TOLERANTNOSTI GENOTIPOVA PŠENICE NA SALINITET U REALNIM USLOVIMA SALINITETA – *IN SITU*

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### Sažetak

Zaslanjenost spada u najvažnije faktore abiotičkog stresa koji ograničavaju produktivnost useva, uključujući pšenicu, u mnogim regionima sveta. Stoga je razvoj sorti pšenice koje su prilagođene uslovima zaslanjenosti prioritet za globalnu sigurnost hrane. Procena genotipova u realnim uslovima – *in situ* može pružiti odgovarajuće informacije o performansama različitih genotipova u realnim uslovima i pomoći u identifikaciji tolerantnih genotipova na salinitet. Da bi se obezbedila tačna procena, dvadeset sedam genotipova pšenice ispitivano je u različitim agroekološkim sredinama. Ogljed je zasnovan na dva različita tipa zemljišta (solonjec i černoze) tokom dve vegetacione sezone. AMMI analiza pokazuje da je faktor spoljašnje sredine imao najveće učešće (55,15%) u varijaciji prinosa zrna, gde je dominantan uticaj imao tip zemljišta. Genotipovi Renesansa, Harmonija i Bankut 1205, ostvarili su visok prinos zrna na oba tipa zemljišta. Među navedenim genotipovima najveću toleranciju na salinitet ispoljio je genotip Harmonija. Značajan udeo interakcije genotipa i sredine (GEI; 25,89%) u ukupnom variranju pokazuje da postoji promena u rangiranju genotipova u različitim sredinama. Prema AMMI<sub>1</sub> biplotu, genotipovi Renesansa i Harmonija su se odlikovali visokim prinosom zrna i visokom stabilnošću. U agroekološkoj sredini Černoze 2015/2016, koja je imala najveći doprinos interakciji, postignut je najveći prinos zrna, dok je u agroekološkoj sredini Solonjec 2017/2018 ostvarena najveća stabilnost, ali i najniži prinos zrna. Prema AMMI<sub>2</sub> biplotu, genotip Harmonija je postigao visoku stabilnost u nepovoljnim uslovima sredine Solonjec 2017/2018.

**Ključne reči:** aditivni efekti, AMMI, GEI, multivarijacioni efekti, stabilnost, stres saliniteta

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