

**GENOTYPE X ENVIRONMENT INTERACTION AND
AGRONOMIC PERFORMANCE OF WINTER WHEAT
CULTIVARS GROWN IN SEMIARID CONDITIONS**

**GENOTIP X INTERAKCIJA OKOLINE I AGRONOMSKA
PERFORMANCA KULTIVARA OZIME PŠENICE
UZGAJANE U POLUSUŠNIM UVJETIMA**

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ABSTRACT

Multi-environmental trials (METs) are a crucial step in breeding programs for most of major crops around the world. The presence of genotype x environment interaction is universal in multi-environmental trials, it cannot be avoided and has to be clearly explained. The aim of the present study was phenotyping of current germplasm and to assess several agronomic and morphological traits, by multivariate analyses, stability for agronomic traits of high importance for further conventional breeding in wheat genotypes and to survey the shift of key traits. Grain samples were obtained from ten winter wheat cultivars grown in 2009/10, 2010/11, 2011/12 at three locations in the South Pannonian Basin: Novi Sad, Sremska Mitrovica and Pančevo. AMMI analysis of variance for yield showed that the total sum of squares attributed to the impact of environments was 76.50%, GEI was represented with 9.72% and the effect of genotype was 7.00%, while the variation of test weight was mainly determined by the influence of environment (66.41%), GEI (15.63%) and genotype (6.47%). The results of this study indicate the importance of METs and suggest that using larger number of wheat characteristics offers more valid answers on the performance of individual genotypes in semi-arid conditions.

Keywords: AMMI model, multi-environmental trials, stability, semiarid conditions, yield, winter wheat.

SAŽETAK

Pokusi postavljeni u više vanjskih sredina (MET) predstavljaju značajan korak u oplemenjivačkim programima za većinu ratarskih usjeva u svijetu. Ono

što je zajedničko za sve pokuse postavljene u više vanjskih sredina svakako je interakcija genotip x vanjska sredina i upravo je smisao ovog rada objasniti je. Cilj ove studije bio je fenotipizacija aktualne germplazme i ispitivanje nekoliko mofro-agronomskih svojstava, kroz multuvarijacione analize. Radilo se na 10 sorti ozime pšenice u godinama 2009./10., 2010./11., 2011./12. na tri lokacije južno-panonskog bazena: Novi Sad, Sremska Mitrovica i Pančevo. AMMI analiza za prinos zrna pokazala je da je ukupna suma kvadrata, koja pripada utjecaju vanjske sredine iznosila 76.50%, GEI nosi udio 9.72% i efekat genotipa bio je 7.0%. Situacija kod drugog ispitivanog svojstva, hektolitarske mase bila je: udio vanjske sredine 66.41%, GEI 15.63% i udio genotipa 6.47%. Rezultati ovih pokusa govore u prilog tome da je potrebno postavljati pokuse na što više lokacija kroz što više godina, ne bi li se dobila pravilna slika o ponašanju različitih genotipova u više vanjskih sredina, pogotovu u semiaridnim uvjetima.

Ključne riječi: AMMI model, MET, stabilnost, semiaridni uvjeti, prinos, ozima pšenica.

INTRODUCTION

Multi-environmental trials (METs) are a crucial step in breeding programs of all major crops around the world. The presence of genotype x environment interaction (GEI) is universal in METs, it cannot be avoided and has to be clearly explained (Ali et al. 2015.). The main task in wheat breeding is to enhance the features of existing varieties and create new genotypes which will have higher yield per unit of area (Banjac et al. 2014., Ormoli et al. 2015.). On the other hand, along with increasing yield, high quality seed is what presents the key to successful modern agriculture (Banu et al. 2014.). Quality of seed is most often defined as a uniform sum of seed features that after sowing lead to a rapid and uniform germination, forming of healthy seedlings which will give the necessary number of plants in favorable and unfavorable environmental conditions. The seed quality is also reflected in the final growth, maturation of plants, their uniformity and stability of yield (Mladenov et al. 2012., Sharma et al. 2010.). It has long been recognized that wheat productivity and quality of seed vary considerably as a result of genotype, environment and their interaction, which describe most of agronomic traits, their affectedness by the growing environment, as well as by genetic factors (Ahmadi et al. 2012., Doehlert and McMullen 2000., Doehlert et al. 2001.). However, evaluation of genotypes across diverse METs and over several years is needed in order to

identify spatially and temporally stable genotypes that could be recommended for release as new cultivars and/or for use in the breeding programs, especially in semiarid conditions (Sharma et al. 2010.). Understanding the cause of the GEI can help identify superior genotypes based on traits. Usually a number of genotypes are tested over a number of locations/years across METs and it is often difficult to determine the pattern of genotypic performance across environments.

The aim of the present study was to assess accurate phenotyping of current germplasm collection for several agronomic and morphological traits, by multivariate analyses, stability for agronomic traits of high importance for further conventional breeding in wheat genotypes and to survey the shift of key traits.

MATERIAL AND METHODS

Agronomic trials and cultivars

Grain samples were obtained from ten winter wheat cultivars grown in 2009/10, 2010/11, 2011/12 at three locations in South Pannonian Basin: Novi Sad (E1, E2, E3), Sremska Mitrovica (E4, E5, E6), and Pančevo (E7, E8, E9). These locations are characterized by semiarid conditions, with dry, hot spring and summer, neutral autumn and moderately cold winter (Table 1). The ten winter wheat cultivars used in this study were: Evropa 90, NSR-5, Pobeda, Renesansa, Ljiljana, Cipovka, Dragana, Simonida, NS 40 S, and Zvezdana. All cultivars were divided into two groups according to heading time. Heading date was recorded at the day when at least 50% of the spikes in a plot had emerged fully (growth stage 55; Zadoks et al. 1974). Heading time was calculated as the number of days after January 1st to the heading date. First group consisted of cultivars: NSR5, Renesansa, Evropa 90, Simonida, Dragana, and Ljiljana (early maturing ≤ 125 days from January 1st) and the second group consisted of cultivars: Pobeda, NS 40S, Zvezdana, and Cipovka (mid-late maturing ≥ 129 days from January 1st). The wheat cultivars were planted in a randomized complete block design with three replications. Plots of 5 m² with 10 rows spaced 12.5 cm apart were seeded at a rate of ≈ 230 kg·ha⁻¹. Sowing in all three growing seasons was completed by the end of October, and the crops were harvested by early July each year.

Table 1 Mean climatic data for each of three test locations

Tablica 1. Prosječni klimatski podaci za svaki od triju lokaliteta

Location			
	Novi Sad	Sremska Mitrovica	Pančevo
<i>Long term (1981-2010)</i>			
Mean precipitation (mm)			
October-March	44	42	39
April-June	68	61	64
Mean temperature (°C)			
October-March	4,6	4,5	4,9
April-June	16,3	16,2	16,5
	<i>E1 / E2 / E3</i>	<i>E4 / E5 / E6</i>	<i>E7 / E8 / E9</i>
Mean precipitation (mm)			
October-March	59 / 45 / 34	66 / 41 / 29	60 / 34 / 32
April-June	113 / 41 / 55	97 / 55 / 62	67 / 43 / 46
Mean temperature (°C)			
October-March	5,5 / 4,5 / 4,0	5,6 / 4,6 / 4,1	6,3 / 5,2 / 4,3
April-June	16,6 / 17,0 / 17,3	16,7 / 17,0 / 17,3	17,3 / 17,0 / 18,3

Agronomic traits

Yield - YLD ($t \cdot ha^{-1}$) was determined in the field. Test weight - TW ($kg \cdot hl^{-1}$) was determined using a device PM-600 Kett. Thousand grain weight - TGW was calculated from the weight (g) of 4 lots of 100 seeds, multiplied by 10. Randman of seed - RND (%) was determined when 4x100 g of natural seed was sifted through rectangular aperture size 2.2 mm. The remaining seeds on the sieve were measured and expressed as percentage (%). All tests were performed on the harvested seed of each cultivar for each replication.

Statistical analysis

Genotype by environment interaction (GEI) was tested using AMMI (Additive Main Effects and Multiplicative Interaction) analysis according to Zobel *et al.* (1998.). Data processing was performed in GenStat 9th Edition VSN International Ltd (www.vsn-intl.com), trial version.

Since the AMMI model does not make provision for a quantitative stability measure, and such a measure is essential in order to quantify and rank genotypes in terms of yield stability, the following parameter ASV (AMMI Stability Value) was introduced (Purchase *et al.* 2000):

$$ASV = \sqrt{\frac{IPCA1 \text{ Sum of Squares}}{IPCA2 \text{ Sum of Squares}} \times [IPCA1 \text{ score}]^2 + [IPCA2 \text{ score}]^2}$$

RESULTS

AMMI analysis of variance and stability value

AMMI analysis of variance for YLD showed that the total sum of squares attributed to the impact of environments was 76.50%, GEI was represented with 9.72% and 7.00% was the effect of genotype, while the variation of TW was mainly determined by the influence of environment (66.41%), GEI (15.63%) and genotype (6.47%). All sources of variation were statistically significant. Similar to the previous two traits, the distribution of variation share of the influence of environment (50.48%) was higher than GEI (23.83%) and genotype (19.56%), for TGW. AMMI analyses showed that the variation of RND was mainly determined by the influence of environment (82.84%), GEI (9.10%) and genotype (5.63%), as shown in Table 2.

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Table 2 AMMI analysis of variance for 10 wheat cultivars examined across 9 environments
Tablica 2. AMMI analiza varijance za 10 kultivara pšenice ispitanih u 9 područja

Source	df	YLD		TW		TGW		RND	
		F calc.	Total share %	F calc.	Total share %	F calc.	Total share %	F calc.	Total share %
Total	269	-	100	-	100	-	100	-	100
Treatments	89	**	93.23	**	88.51	**	93.87	**	97.58
Genotypes	9	**	7.00	**	6.47	**	19.56	**	5.63
Environments	8	**	76.50	**	66.41	**	50.48	**	82.84
Block	18	ns	2.26	ns	1.41	ns	0.27	**	0.44
Interactions	72	**	9.72	**	15.63	**	23.83	**	9.10
IPCA ₁	16	**	54.08	**	60.00	**	38.50	**	54.58
IPCA ₂	14	**	21.28	**	16.60	**	24.31	**	23.13
IPCA ₃	12	**	11.70	**	8.40	**	16.63	**	8.65
IPCA ₄	10	**	6.56	ns	7.02	**	10.78	**	6.32
IPCA ₅	8	**	-	ns	-	**	5.42	**	4.33
IPCA ₆	6	*	-	-	-	**	2.73	*	2.16
Residuals	6	ns	100	-	-	ns	-	ns	-
Error	162	-	93.23	**	-	-	-	-	-

df: degree of freedom; F calc: F value calculated; YLD: Yield; TW: Test weight; TGW: thousand grain weight; RND: Randman of seed; Level of statistical significance: * P < 0.05; ** P < 0.01; ns: not significant.

Table 3 presents mean value, AMMI model IPCA1 and IPCA2 scores of examined traits for each genotype and the AMMI stability value (ASV). The ASV parameter is used to quantify and classify the genotypes according to the stability of their productivity. In this analysis, ASV is represented by the distance of the varieties from the zero point of the IPCA1 vs. IPCA2 scatter diagram. The varieties with low scores were Renesansa for YLD (0.46) and TW (0.91) and Dragana for TGW (0.52) and RND (0.11), and thus considered more stable according to this criterion.

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Table 3 Mean values for examined traits and ranking order by AMMI stability value (ASV) across 9 environments

Tablica 3. Prosječne vrijednosti i red svrstavanja prema AMMI vrijednosti stabilnosti (ASV) u 9 područja

Genotype	YLD (t·ha ⁻¹)				TW (kg·hl ⁻¹)				TGW (g)				RND (%)			
	Mean value	IPCA ₁	IPCA ₂	ASV	Mean value	IPCA ₁	IPCA ₂	ASV	Mean value	IPCA ₁	IPCA ₂	ASV	Mean value	IPCA ₁	IPCA ₂	ASV
Evropa 90	7.10	0.33	-0.25	0.88	74.71	-3.27	-0.21	11.82	38.74	-0.11	-2.35	2.35	89.43	-2.36	-0.74	5.62
NS rana 5	6.90	0.17	0.36	0.56	76.68	-0.40	-0.61	1.58	42.15	0.76	0.14	1.21	91.24	0.24	0.63	0.85
Pobeda	7.37	0.61	0.20	1.56	77.43	0.59	-0.66	2.22	42.77	0.85	0.16	1.36	93.02	0.88	-1.28	2.44
Renesansa	7.45	-0.04	0.45	0.46	77.75	0.08	-0.85	0.91	41.17	1.97	-0.12	3.13	92.31	-0.88	0.42	2.13
Ljiljana	7.73	0.14	0.31	0.48	78.15	1.23	-0.75	4.52	42.24	1.01	0.87	1.82	93.22	0.23	0.31	0.62
Cipovka	7.29	0.60	0.40	1.58	76.89	0.31	1.95	2.25	38.61	-1.01	-0.67	1.73	91.39	-0.81	0.60	2.00
Dragana	7.46	-0.57	-0.32	1.50	77.75	0.30	0.13	1.09	42.58	-0.10	0.50	0.52	93.59	0.02	0.09	0.11
Simonida	8.31	0.51	-1.10	1.71	77.95	0.64	0.06	2.31	41.20	-0.95	0.01	1.51	91.84	0.64	0.92	1.77
NS 40 S	7.88	-1.16	0.03	2.96	74.62	0.62	-0.16	2.25	38.00	-1.20	1.33	2.32	92.99	0.84	-1.54	2.51
Zvezdana	7.79	-0.59	-0.07	1.49	76.88	-0.10	1.10	1.16	39.76	-1.22	0.14	1.94	92.54	1.20	0.59	2.90

ASV: AMMI Stability Value; YLD: Yield; TW: Test weight; TGW: thousand grain weight; RND: Randman of seed

Biplot analysis

Small distance environmental points from the origin (zero point) indicate that E4, E3 and E9 were assessed as the most favorable to achieve a stable reaction to YLD. Zvezdana had a positive interaction with these environments, which indicates a higher stability for the realization of YLD compared to other genotypes. Environments E1 and E7 expressed the poorest conditions for the development of winter wheat and the achievement of stable yield. However, Simonida achieved positive interaction with E7, while NS40S expressed most benefits in E1 (Fig. 1A).

Environments E4, E2, and E3 had the highest interaction values, which determines them the least suitable for stable TW. Genotypes Dragana, Simonida and NS40S had the shortest distance from the origin (zero point), which makes them more stable than the other genotypes. Evropa 90 and Cipovka showed the highest interaction at the level of the whole experiment (Fig. 1B).

On TGW biplot genotypes achieved the smallest value of interaction and therefore more stable response to E2 and E7. All other environments had higher interaction effects. Although E8 and E9 were farthest from the origin (zero point), Zvezdana, Simonida (E8), and Evropa 90 (E9) had positive interactions with those environments. Dragana, NSR5, and Pobeda showed stable response compared to other genotypes (Fig. 1C).

Length spoke presenting environments and distance from the origin shows that E4 was favored by genotypes for achieving stable RND. On the other hand, E5, E3, E1 represented agro-ecological conditions that were unfavorable for the development of genotypes. Distribution of genotypes points on AMMI 2 biplot shows that genotype Dragana was closest to the starting point, which indicates the minimal interaction of genotype with all environments across the entire experiment. A similar reaction was expressed by the genotype Ljiljana (Fig. 1D).

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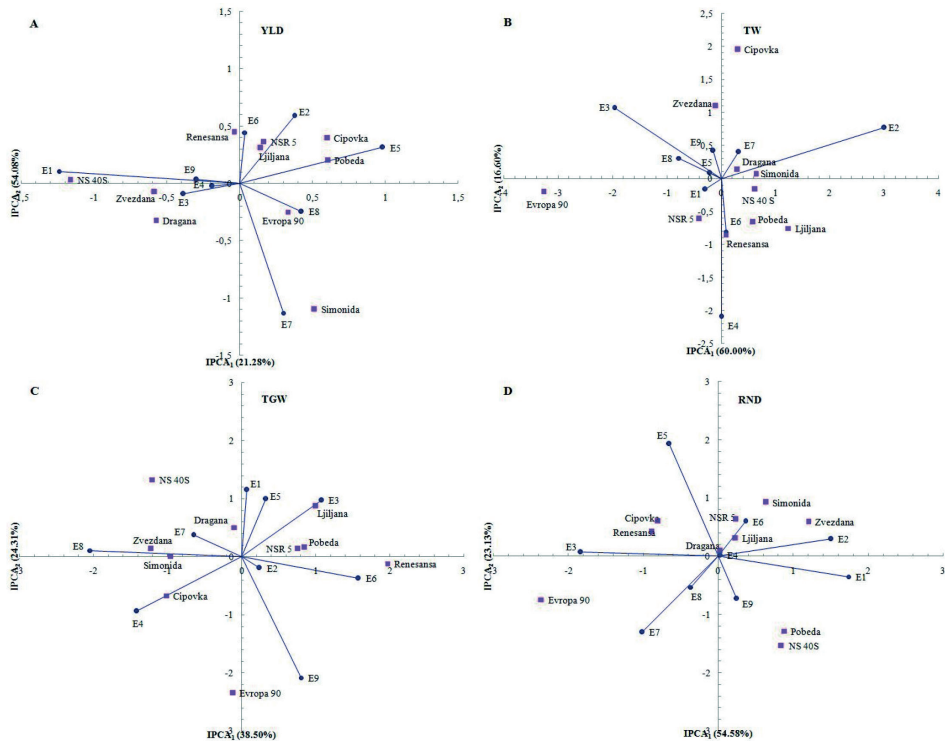


Figure 1: Biplot of the AMMI 2 model for tested traits (YLD, TW, TGW and RND) for 10 examined wheat cultivars grown across 9 environments; A-yield; B-test weight; C-thousand grain weight; D-randman of seed.

DISCUSSION

An ideal genotype is defined as one that is the highest yielding across METs and is absolutely stable in performance. Although such an ideal genotype may not exist in reality, it can be used as a reference for genotype evaluation (Ahmadi *et al.* 2012.). AMMI analysis enables finding and identifying the previously mentioned ideal genotype. Multivariate ordination such as AMMI and Principal Components Analysis (PCA) help in interpreting the genetic control of multiple traits (Ormoli *et al.* 2015.). Purchase *et al.* (2000.) developed a quantitative stability value to rank genotypes through the AMMI model, named the AMMI stability value (ASV). This model was considered to

be the most appropriate single method of describing the stability of genotypes and this parameter was used as an auxiliary criterion to define stable genotypes in other crops, such as wheat (Farshadfar et al. 2011.). In AMMI analysis, the number of environments necessary for this kind of experiment is confusing. According to some authors higher number of environments is more optimal for this kind of analysis (Bhatt and Derera 1975., Gauch and Zobel 1997., Manzoor 2012.), while others (Bassett et al. 1989., Dimitrijević et al. 2011.) stress that influence of the year is more valid. In both cases, what is needed is more environments across different years, basically METs. Agro-ecological conditions of South Pannonian Basin (high temperatures, drought conditions) are often one of the most limiting factors for gene expression linked to the examined traits and achieving high and stable yield. Common characteristics for all METs would be that temperatures were slightly above a 30-year average during the growing period. The precipitation in the first two years was significantly higher, while the third year of the experiment was extremely dry. Due to the global climate change, drought has been frequent in South Pannonian Basin, especially since 1980 (Jovanovic et al. 1996). According to the drought index for July and August, 74% of years in this region are either semiarid or arid. Varying across different METs in these kinds of conditions is the main reason for the existence of GEI. Better agro-ecological conditions were not favorable for achieving a stable reaction yield. However, intensive wheat varieties that were created for growing in favorable agro-ecological conditions had positive interaction with this kind of environments, e.g. Simonida with E7, and NS40S with E1. Unfavorable agro-ecological conditions reduced the difference in yields between the genotypes, resulting in greater stability throughout the whole experiment. Due to the positive interaction of genotypes NSR5 and Ljiljana with E6 (poor agro-ecological conditions) and E2 (good agro-ecological conditions), it can be concluded that these early maturing genotypes are highly adaptable to varying environmental conditions.

Seed quality is defined by a large number of features. Therefore, knowledge of the relationship among the rest three characteristics is of great importance. Interrelationships among TW, TGW and RND depend on the genetic factors and environmental factors. TW and TGW are physical indicators of seed quality, they are varietal feature and can be indicators of the conditions under which wheat grain filling and maturing occurred (Mladenov et al. 2001.). According to AMMI 2 biplot Dragana (early maturing genotype) expressed better stability in terms of TW and TGW than other genotypes, suggesting that

this genotype is highly adaptive to variable METs in this semiarid region of South Pannonian Basin. Moreover, Dragana also had higher means of examined traits. Especially significant is its positive interaction with E7 for TW and TGW, indicating good use of the remaining winter moisture in the soil. RND is one of the additional traits which serves to better understand seed quality. The lowest values of RND interaction were recorded in E4, while Dragana and Ljiljana expressed the greatest stability in terms of this trait.

For all of the examined features AMMI analysis and ASV ranking were in accordance and had similar results. In the presented results all characteristics of wheat changed under the influence of locality – this was reflected through significant GEI. Calculated values of GEI showed that there were differences in the stability of genotype across all four investigated traits. Genotypes were grouped differently according to stability, expressed through interaction scores (AMMI model). Nevertheless, AMMI model is more comprehensive and results of this analysis are considered as plausible. Since AMMI analysis fully explained the sum of squares, it provides a good estimation of genotypes on the observed locations. Genotype Evropa 90 achieved one of the most stable reactions across the whole experiment in terms of YLD. It is well known that Evropa 90 owes the stable yield to fine grain with a large percentage of waste, which indicates low RND (Mladenov et al. 2012.). Minor proportion of genotype indicates that randman is a trait to which the genotype effect is under expression of the morphological properties of grain (seed size), while everything else is under the influence of living conditions through different phenological stages, but mostly during grain filling. Due to its large seed, genotype Dragana does not achieve large TW, as it could, but proportionally to that fact it has large seed, little waste, high RND and stable YLD. By looking at these two features towards stability, Dragana is one of the most stable genotypes, but with the yield on the grand mean for the whole experiment, which can be explained by the fact that cultivars with high TW have better tolerance to high temperature stresses in the phase of grain filling, and because of that they do provide higher yields (Jocković et al. 2014., Mladenov et al. 2011.). The results of this study indicate the importance of METs and suggest that using larger number of wheat characteristics acquires more valid conclusions on the performance of individual genotypes in semi-arid conditions. It can also be concluded that early maturing genotypes were more adaptable for changeable conditions of South Pannonian Basin.

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