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Original scientific article

MITIGATING ABIOTIC STRESSES: A STUDY ON PANNONIAN BASIN WHEAT CULTIVARS FACING DROUGHT, COLD AND HEAT

Velimir MLADENOV[1], Borislav BANJAC[1*], Sofija PETROVIĆ[1], Rada ŠUĆUR[1], Bojan JOCKOVIĆ[2]

[1] University of Novi Sad, Faculty of Agriculture, Department of Field and Vegetable Crops, Novi Sad, Serbia

[2] Institute of Field and Vegetable Crops, National Institute of the Republic of Serbia, Novi Sad, Serbia

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Environmental stresses such as drought, cold and heat in Pannonia Basin significantly endanger the cell activity, plant growth and yields in wheat, which is one of the most strategic cereal grain crops in the world. As science and technology advance, new tools are developed while old ones are refined for use by breeders. Higher agronomical efficiency is possible by combining new and old tools to bridge the abiotic stress issues. Five cultivars of winter wheat (*Simonida*, *Petrija*, *Ljubica*, *Zvezdana* and *NS Mila*), were used in the study carried out at our experimental field (Novi Sad as a center of Pannonia Basin) across three consecutive growing seasons to assess genetic interaction and the level of tolerance and adaptability of different cultivars to abiotic stresses like drought conditions, cold and heat. Four quantitative yield components and grain yield were analiyed to assess expression of adapted genotypes in the region. Among the cultivars, Simonida, which has been in use for the longest period, exhibited the most consistent yield response. Additionally, it demonstrated some degree of partial tolerance to abiotic stress conditions, possibly due to the integration of stress memory into its genetic code, supported by statistical analysis findings.

Keywords: abiotic stress; adaptability; drought; tolerance; wheat

INTRODUCTION

Wheat is the most important cereal grain crop in the world and the principal cereal used for food consumption in most parts of the world. Developing countries account for nearly 50% of the world's wheat production, while it presents more than 20% of the caloric intake for one half of the world's population (KONG *et al.*, 2020). The credit for the widespread use of wheat

Corresponding author: Borislav Banjac, University of Novi Sad, Faculty of Agriculture Novi Sad, Sq. Dositeja Obradovića 8, 21 000 Novi Sad, phone: +381 (21) 485 32 99; e-mail:borislav.banjac@polj.edu.rs

belongs to "*Green revolution"* which brought a quantum leap through improved biomass partitioning as a result of introgression the Rht1 and Rht2 alleles (QUINTERO *et al*., 2018) coupled with increased fertilizer usage and enhanced adaptability of cultivars. Still, nowadays it raise some question regarding narrowing of genetic base which could be used in breeding programs. Plant breeding is a deliberate effort by humans to manipulate nature, with respect to the heredity of plants, to gain advantage. Breeding is hence about manipulating plant attributes, structure and composition, to make them more useful to humans and more tolerant to abiotic stresses (ACQUAAH *et al*., 2012). However, as technology advances, plant breeders can now achieve remarkable plant manipulations, needless to say not without controversy. This includes development and application of biotechnology to plant genetic manipulation, all with one goal – to answer the global climate change. Growing lack of arable land is a fact and one of the solutions for intensification of production is developing cultivars that are more resistant to limiting elements, like drought, cold and heat in region of Pannonia Basin. The plant breeder uses various technologies and methodologies to achieve targeted and directional changes in the nature of plants. New more productive cultivars are increasingly necessary to fulfill humankind's escalating needs for food and fiber. However, genetic gains in yield potential are currently around 1.16% per year by HFFA research (NOLEPPA and CARTSBURG, 2021), and global wheat production would have to increase 2.4% per year to feed the increasing human population (RAY *et al*., 2013). As science and technology advance, new tools are developed while old ones are refined for use by breeders. However, achieving higher agronomical efficiency is possible establishing a synergetic connection between the two to bridge the abiotic stresses issues. Environmental stresses such as drought, cold and heat in Pannonia Basin significantly endanger the cell activity, plant growth and yields in wheat. The environmental signals and stresses that a plant encounters during its life are frequently repeated, despite the common misconception that plants must adapt to constantly changing conditions (KAKOULIDOU *et al*., 2021). To respond to these questiones, it is essential to understand well-known fact that the total phenotypic variance can roughly be analyzed into four sources of variation – the influence of the genotype, the influence of the environment (including stresses); their mutual interaction, and error (unexplainable, and hence not significant, agronomical variation) (BANJAC *et al*., 2022). Aims of this manuscript were twofold (*i*) to evaluate genetic interaction among five wheat cultivars across three stressed vegetation seasons in Pannonia Basin and (*ii*) to assess the level of tolerance and adaptability of different cultivars of wheat to analyzed abiotic stress.

MATERIALS AND METHODS

The study was carried out at the experimental field in the Pannonia Basin, Institute of Field and Vegetable Crops, Novi Sad, Serbia (45°33′N, 19°85′E, 82 m altitude) for three consecutive growing seasons (2015/16, 2016/17 and 2017/18, respectively S1, S2 and S3). The location is characterized by semiarid to arid conditions, with dry, hot spring and summer, neutral to dry autumn and cold winter (Figure 1).

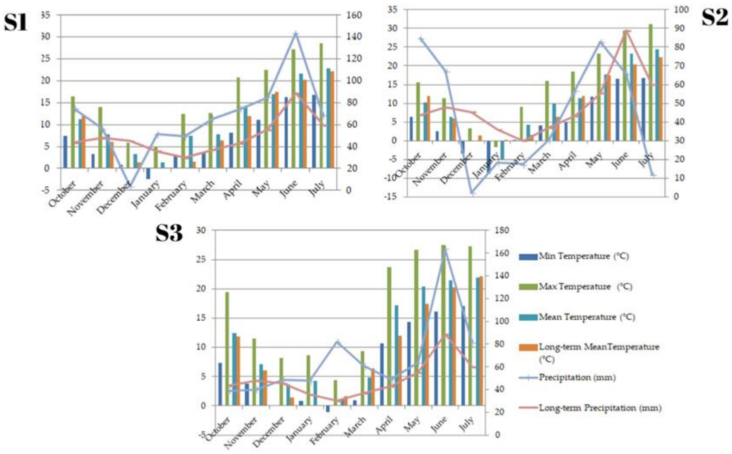

Figure 1. Minimum, maximum, and mean monthly temperatures (°C) and precipitation (mm) values across growing seasons: S1—2015/16, S2—2016/17 and S3—2017/18 in Novi Sad, Pannonia Basin

Extreme temperatures were specially analyzed to estimate the level of abiotic stresses and shown in table 1. Each year with the preceding crop was soybean (*Glycine max* L.). The soil type was Chernozem Chernic (FAO, 1998).

Table 1. Graphical and table design of extreme temperatures and precipitation as a representatives of abiotic stresses for growing vegetation of wheat across three seasons 2015/16, 2016/17 and 2017/18 in Novi Sad, Pannonia Basin. Explanation of used colors is given in the second part of the table, made from detailed statistics by RHMZ (Republic Hydrometeorological Service of Serbia for the period between 1981-2010).

		X	XI	XII	I	II	III	IV	V	VI	VII
S1	T (°C)	11.2	7.8	3.2	1.3	7.5	7.8	14.2	16.9	21.7	22.8
	P (mm)	74.6	56.1	3.6	51.3	49.2	65.5	74.5	85	143.2	68.4
S2	T (°C)	10.2	6.3	-0.1	-4.9	4.2	9.9	11.4	17.6	23.2	24.3
	P (mm)	84.8	67.1	2.2	18.5	17.5	30.5	57	82.9	65.7	12
S3	T (°C)	12.5	7.1	3.7	4.3	1.2	4.9	17.2	20.4	21.4	21.9
	P (mm)	38.9	40.3	48.3	47.7	81.9	60.6	49	63.8	163.2	81.2
Extremely cold	Very cold	Cold	Average	Warm	Very warm	Extremely warm					
Extremely dry	Very dry	Dry	Average	Rainy	Very rainy	Extremely rainy					

Five studied winter wheat cultivars (Simonida, Petrija, Ljubica, Zvezdana and NS Mila) from Pannonia Basin, were planted in a randomized complete block design with six replicates. Each plot consisted of 10 rows, 10 cm apart and 5 m long (harvested area was $5m^2$). Seedling density was considered 500 viable seeds per m^2. All the trails were seeded in middle October (optimum sowing date) and reached maturity in late June or early July. Weeds were controlled manually. Grain yield (Y) was measured by harvesting the all ten rows per plot of each cultivar for each replication (expressed in $t \cdot ha^{-1}$ at 13% moisture). Plant height (PH): the length of the main tillers at maturity were measured in cm from the ground level to the tip of its terminal spikelet excluding awns and the average value was taken. The spike length (SL) was measured on each cultivar for each replication from the base of the spike to the tip, excluding awns. Grain number per spike (GN) was determined by counting kernels on every spike, while spike number (SN) was determined by counting.

Minimum, maximum, mean values and variance were calculated as indicators of trait variability and presented *via* radar plots for visualizing samples. These statistical calculations were done using TIBCO STATISTICA, 2020, version 14.0.0.15 and Excel for Windows. Statistical analysis also was conducted for each parameter by analysis of variance (ANOVA and AMMI) in a factorial design with 3 years, and 5 cultivars. Software R, version 4.2.0 was used for PCA graphical display.

The sustainability index (SI) was estimated by the following formula (BABARMANZOOR *et al*., 2009) and served as the initial stability indication under abiotic stress conditions

$$SI = \left[\frac{Y - \sigma}{Y_{max}}\right] \times 100$$

Since used multivariate PCA model does not include a quantitative stability measure, and as such a measure is essential in order for quantifying and ranking genotypes in terms of yield stability, the following parameter ASV (AMMI Stability Value), introduced by (FARSHADFAR *et al*., 2011), was used:

$$ASV = \sqrt{\left[\frac{IPCA1_{ss}}{IPCA2_{ss}} \times IPCA1_{score}\right]^2 + (IPCA2_{score})^2}$$

RESULTS

One of the most crucial aspects of plant breeding is the examination of phenotypic genetic markers, which is precisely why phenomics as a scientific field is gaining ground daily. Regarding the first examined characteristic SN (spike number), it is clear that abiotic stresses had the largest impact on the traits expression in S3, cultivar NS Mila, when it reached a value of 131. The first season, in which Zvezdana achieved a value of 249, was the most favorable for this trait. The genotypes average SN peaked in the second season (S2; 205), and carry out at its lowest level (48) in S3 (Figure 2, A). Similar to the previous trait, Ljubica achieved the lowest value for plant height (PH) in S2 (59.37cm), while NS Mila reached the highest value (91.05 cm) in S1. The expression of the genes regulating this phenotypic marker was most favorable in S1 when evaluating by season, Conversly, S2 was most affected by abiotic challenges, especially temperature peaks resulting in the lowest values for these cultivars (65.01 cm), as shown in Figure 2, B.

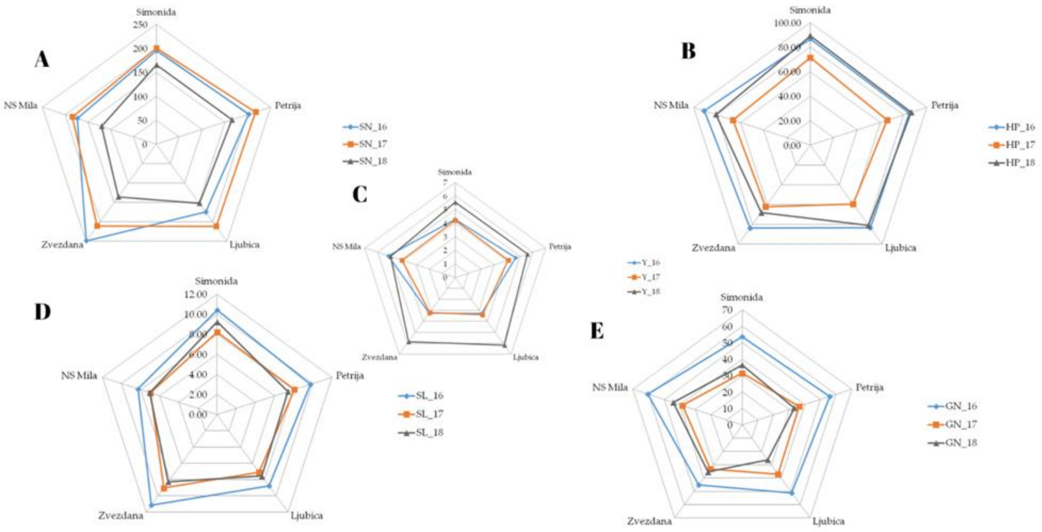

Figure 2. Distribution of parameters in analyzed traits (A—spike number, SN; B—Plant height, PH; C—yield, Y; D—spike length, SL and E—grain number per spike, GN) across growing seasons 2015/16, 2016/17 and 2017/18 in Novi Sad, Pannonia Basin

The minimum value of the most complex trait, determined by the largest number of genes, yield (Y) was achieved by the cultivar Zvezdana in S2, with a yield of 3.2 t·ha^{-1}. In the third growing season (S3), cultivar Ljubica achieved the highest Y (6.6 t·ha^{-1}) achieved the highest yield. The overall trial average level was 4.52 t·ha^{-1}. The limiting factors were most pronounced in S2, which had the lowest Y (3.8 t·ha^{-1}). In contrast, S3 had the highest values at the level of the season average (Figure 2, C). Spike length (SL) is a characteristic often used in wheat as an important yield component. In the first growing season Zvezdana had the longest spike (11.13cm), while NS Mila had the shortest spike in S2. The first growing season was the most advantageous for the manifestation of this trait (9.66cm), while the second growing season produced the lowest values (7.88cm), according to the averages for all genotypes that were studied in the field trials (Figure 2, D). Regarding the trait grain number (GN), Ljubica showed the lowest values in S3 (26), while NS Mila had the highest values in S1 (60). The first growing season, S1, was the best for the GN characteristic to develop, but the S2 and S3 were about equal, as seen in Figure 2, E. Based on the previously assessed results, S2 was the least favorable vegetation season for all traits. Abiotic stresses and pressures were mostly responsible for the decline in trait values, except for SN. With the exception of the Y, which was highest in the S3, the S1 was the most advantageous for all evaluated features. To evaluate the stability of genotypes and the manifestation of those genotypes by traits under abiotic stress, two metrics were used. While ASV is based on the interaction values of the first and second axis, which also represent the majority of the interaction, SI - the first parameter, is based on the highest realized value that the genotype may reach under these stress conditions (Table 2).

Table 2. ASV, IPCAg1, IPCAg2, SI and ASV for used wheat genotypes across seasons 2015/16, 2016/17 and 2017/18 in Novi Sad, Pannonia Basin

	Genotype	IPCAg[1]	IPCAg[2]	SI	ASV
SN	Simonida	2.32	2.75	23.09	16.36
	Petrija	1.90	-0.59	29.24	13.22
	Ljubica	1.96	-2.35	24.15	13.87
	Zvezdana	-4.43	0.12	24.40	30.86
	NS Mila	-1.74	0.07	22.48	12.14
PH	Simonida	1.33	-1.36	74.93	7.55
	Petrija	1.36	-0.10	70.36	7.62
	Ljubica	0.80	1.29	63.86	4.64
	Zvezdana	-2.49	-0.73	62.88	13.92
	NS Mila	-1.00	0.90	69.92	5.68
Y	Simonida	0.16	-0.49	49.62	5.12
	Petrija	0.31	0.06	53.43	9.97
	Ljubica	-0.74	0.05	36.79	23.68
	Zvezdana	-0.60	0.16	36.60	19.09
	NS Mila	0.87	0.22	54.58	27.69
SL	Simonida	-0.57	-0.44	69.74	3.35
	Petrija	0.46	0.13	61.61	2.70
	Ljubica	-0.37	0.07	58.65	2.19
	Zvezdana	0.71	-0.25	68.34	4.14
	NS Mila	-0.23	0.48	56.21	1.41
GN	Simonida	-0.98	0.91	42.65	2.50
	Petrija	0.89	0.72	43.23	2.22
	Ljubica	2.36	-0.40	38.44	5.61
	Zvezdana	-1.07	-2.06	44.39	3.27
	NS Mila	-1.19	0.83	52.44	2.95

SI is the sustainability index that served as the inital metric for evaluation of gene expression in the conditions of abiotic stress. Cultivar Petrija obtained the greatest SI for SN (29.24%), which denotes reduced stability. The SI values from the table, ranging from 22% to 24.4%, confirm that the responses of the other examined genotypes were similar. Since the expression of the major gene influences the expression of the PH, it is logical that all cultivars displayed greater values and are therefore more stable, with Simonida being the most stable (74.93%). Zvezdana and Ljubica belong to the group with low SI parameter values for Y, while Simonida, Petrija, and NS Mila achieved average stability values. Notably, NS Mila showed the highest performance (54.58%). For the SL, all genotypes are categorized as extremely stable, similar to PH.

The ASV represent the distance from the coordinate point to the origin in a two-dimensional space of IPCA1 scores plotted against IPCA2 scores in multivariate models. Since the IPCA1 score contributes more to the genotype x environment interaction sum of square, it is necessary to introduce a weighted value for a more precise evaluation. This weight is calculated for each genotype under abiotic stress conditions (environments) taking into account the relative contribution of IPCA1 and IPCA2. NS Mila was the most stable genotype for SN with a value of 12.14, while Zvezdana showed the least stable reaction, indicating undesirability. Ljubica expressed the desired stability for HP (14.64) while Simonida showed the most consistent reaction for yield (5.12). With a low ASV metric of 1.41, NS Mila obtained the most stable reaction for SL. Petrija was identified as one of the preferred genotypes for the trait GN due to the lowest values (Table 2). Figure 3 shows the statistical significance of the first two major components, according to PCA analysis, which accounted for 76% of the experiment's overall variability. When compared to the contribution of multivariate PCA2 (25.5%), the 50.5% share of PCA1 in the total variance indicates a higher proportion of additive variance.

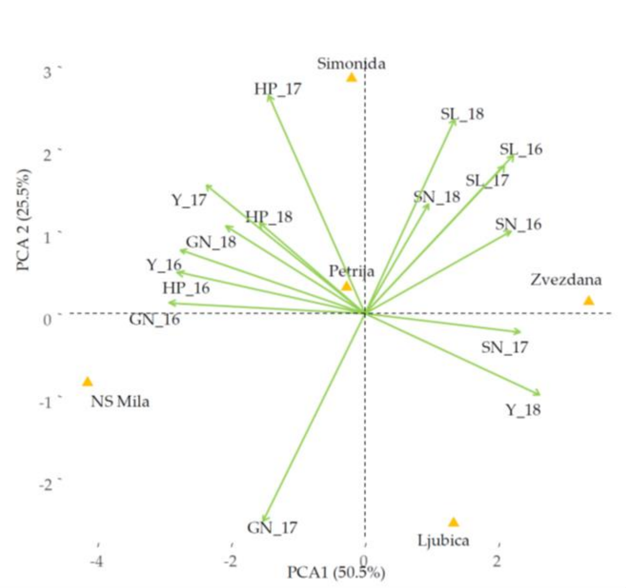

Figure 3. PCA values on the biplot diagram for all tested traits (Seed number, SN; Plant height, PH; Yield, Y; Spike lenght, SL and Grain number per spike, GN) of wheat across growing seasons 2015/16, 2016/17 and 2017/18 in Novi Sad, Pannonia Basin

The analysis, which considers the distribution of points representing genotypes and evaluated attributes, highlights the complexity of wheat's reaction to abiotic stress. This is a result of the diverse genetic constitutions of the studied genotypes and the maner in which their genes are expressed for particular traits. The genotypes were cultivated in diverse and challenging environmental conditions, contributing to a 24% unexplained variability. The occurrence of temperature extremes, as shown in table 2, also influences how wheat responds to abiotic stress situations. Notably, Petrija was shown to be the most stable genotype when considering the entire experiment, including all attributes assessed in all conditions, despite its particulary strong response to GN. In 2017, obtuse angles coincided with the vectors representing qualities under investigation, suggesting weak relationships between the traits. The only exception was the acute angle observed that year between the Y vector and the PH vector, while otherwise, they exibited a negative connection.

DISCUSSION

Precipitation during S1 and S2 of the sowing period, were not sufficient to compensate for soil moisture deficiency and to ensure the normal flow of wheat phenophases. Abiotic drought stress was exacerbated by residual stress from the previous vegetative cycle, which was below average in S3. January, typically one of the coldest months, is characterized by low temperatures posing a challenge for all winter crops unprepared to sustain the cold without snow cover, as was the case in S2. Additionally, the complete absence of days with temperatures above 0°C in 2017 (S2) demonstrates the severity of the temperature stress. Such temperature conditions have introduced the plant into the state of winter dormancy, where its main task is to preserve as long as possible and distribute nutrients during an unfavorable period. This phase has been triggered by such temperature conditions. Phenomena like this was anticipated around this time, but unusually warm February enhanced the metabolic processes in wheat, causing an earlier than anticipated transition into the next stage of wheat development and node development and cell activity was significantly endanger. The plants therefore lacked the necessary time and conducive environment for the regular biological development of the phase they are in. However, ongoing exposure to March and late-April frosts in S2 increased the severity of the shock brought on by abiotic stress, which could be extremely dangerous for the plant, since known fact is that flowering in winter risks frost damage to reproductive structures, and suboptimal radiation levels can reduce yield (DRECCER *et al*., 2018). Although March, April, and May represented the experiment's average monthly rainfall, they were unable to make up for earlier periods' deficiency in moisture. Particularly at the start of the grain filling phase, a plant in the vigorous growth phase needs more moisture. Strong temperature shocks at the most vulnerable stage of wheat development were brought on by high temperatures, which expedited heading and, consequently, the harvest, which, when done under such circumstances, results in increased yield losses (LANNING *et al*., 2010; KRISTENSEN SCHELDE and PLESEN, 2011; BAUM ARCHONTOULIS and LICHT, 2019). Also, as stated before, that kind of abiotic stress, after anthesis can decrease the rate of grain-filling and reduce the grain yield up to 23% (FERRIS *et al*., 1998). Temperatures in June that were above 30°C during the day and 20°C at night had a negative impact on the transition from milky to waxy maturity and decreased the quality of the final phase of wheat maturity. The month of June was exceptionally warm during all three vegetation phases, but in S2 it had notably high including day and night temperatures as well as a high content of precipitation. Plant must establish, build biomass, and flower at a time that corresponds with the best seasonal conditions in order to produce the most seeds of the best size and quantity (FLOHR *et al*., 2017). The highest values were observed for all genotype traits examined during S2, which is characterized from other growing season by the strongest abiotic stress. The exception is the SN, which was highest in the year 2017, and can be attributed to the higher starting soil moisture content. As a result, the plants and environmental factors encoded the gene expression for that phenotypic trait. However, later droughts and high temperatures interrupted and altered this sequence. Also, it is evident that lower plants produced greater yield values, which suggests a particular response to the abiotic stress that predominated in S2, in the accordance with previous research (FISHER *et al*., 2019). This could be explained that under more severe abiotic stress, the plant seeks to produce a larger generative portion, the grain, rather than developing vegetative mass as would be expected, thereby struggling to survive. Based on these results and by understanding the genetic basis for variation in phenology and other adaptive traits can inform crop breeding strategies and contribute to prediction of yield risks, such as drought, frost or heat, and thereby improve crop management (HYLES *et al*., 2020), or explanation could be in environmental adaptation, already described in wheat (LIEBERMAN-LAZAROVICH *et al*., 2022).

In order to identify the genotypes within the wheat species that displayed the most stable reactions through the examined traits, the stability parameters were determined. In order to handle inputs and production calculations more easily, intensive agricultural production by selection prefers stable genotypes that provide a continuous economic yield. Natural selection, on the other hand, promotes genotypes that can adapt to the external environment in order to effectively reproduce and spread the species under abiotic stress in the environment (SZARESKI *et al*., 2021). It is crucial to monitor ecological variables and the genotype's response to them during the modernization of production since human requirements necessitate an unnatural, consistent response from organisms (MLADENOV *et al*., 2019; GUPTA *et al*., 2022). Field experiments show that everything depends on the breeding objective. There are two ways to analyze genotypes. The first level is assessing each evaluated phenotypic marker's stable reaction, and the second level is the estimation of yield stability. Given that the yield is crucial to the species' survival and satisfying consumer demand, it is preferable to adopt the second level in abiotic stress situations. This allows us to specify which genotype, Simonida in this study, is the best under these circumstances. The longest-running variety in use and the fact that it has incorporated some degree of partial tolerance to abiotic stress conditions though stress memory into its genetic code, support this choice and make Simonida cultivar which is the most resistant to limiting elements.

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ANALIZA ABIOTIČKIH STRESOVA U SORTAMA PANONSKE NIZIJE: SUŠA, HLADNOĆA I TOPLOTA

Velimir MLADENOV[1], Borislav BANJAC[1*], Sofija PETROVIĆ[1], Rada ŠUĆUR[1], Bojan JOCKOVIĆ[2]

[1] Univerzitet u Novom Sadu, Poljoprivredni fakultet, Departman za ratarstvo i povrtarstvo, Novi Sad, Srbija

[2] Institut za ratarstvo i povrtarstvo, Institut od nacionalnog značaja za Republiku Srbiju, Novi Sad, Srbija

Izvod

Stres izazvan ekološkim činiocima kao što su suša, niske i visoke temperature u Panonskom basenu značajno ugrožavaju ćelijsku aktivnost, rast biljaka i prinos pšenice, koja predstavlja jednu od najvažnijih strateških žitarica u svetu. Kako nauka i tehnologija napreduju, novi alati se razvijaju, dok se stari usavršavaju i stoje na raspolaganju oplemenjivačima. Ipak, postizanje veće agronomske efikasnosti je moguće ako se napravi zajednička veza između njih, kako bi se premostili problemi nastali pod uticajem abiotičkog stresa. U ovom istraživanju, koje je sprovedeno na našem oglednom polju (Novi Sad kao centar Panonskog basena), je korišćeno pet sorti ozime pšenice (Simonida, Petrija, Ljubica, Zvezdana i NS Mila), u tri uzastopne vegetacione sezone, kako bi se procenila genetička interakcija i nivo tolerancije i prilagodljivosti različitih sorti pšenice na abiotičke stresove kao što su suša, niske i visoke temperature. Prinos i četiri kvantitativne komponente prinosa su analizirane kako bi se procenila ekspresija prilagođenih genotipova u ovom regionu. Rezultati statističke obrade podataka su potvrdili da sorta koja je najduže u upotrebi, Simonida, ima najkonzistentniju reakciju na prinos, i da poseduje određeni stepen delimične tolerancije na uslove abiotičkog stresa, koju je ugradila preko stres memorije u svoj genetički kod.

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