MULTIVARIATE ASSESSMENT OF WHEAT GENOTYPE RESPONSE TO ENVIRONMENTAL VARIATION

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Abstract. This research evaluated the adaptability of diverse wheat genotypes from a broad region under varying agroecological conditions during the 2015/2016 and 2016/2017 growing seasons, in field trial conducted in Novi Bečej, Serbia, using principal component analysis (PCA) and hierarchical heatmap cluster analysis. The PCA results indicated that the first two components explained over 70% of the total variation in both seasons. Plant height loaded positively on PCA1, while yield traits loaded negatively, indicating a negative correlation. Cluster analysis identified four genotype groups, indicating genetic diversity. In the season with a rainfall deficit, all analyzed traits were significantly reduced, with the largest decrease observed in grain weight per plant (44.7%). Genotype Fundulea 4 showed the highest sensitivity to the stress due to lack of rainfall, reaching high values in favorable conditions, but low in conditions of rainfall deficit. Genotypes Marija and Kavkaz showed high plant height but lower yield components, while Pitoma and Skopjanka exhibited high yield traits with lower plant height across both seasons. This study highlights the need for balanced selection to optimize plant height and yield components for better adaptation to stress. Genotype Dunavka stands out as a candidate for improving thousand grain weight, while Skopjanka and Pitoma can increase grain yield in breeding programs.
Keywords: *agroecological conditions, genotype adaptation, yield components, drought stress, PCA analysis, heatmap cluster*

Introduction

Agricultural production is increasingly affected by climate change, which manifests through extreme droughts, high temperatures, and other climate hazards (Chaudhry and Sidhu, 2021; Farhad et al., 2023). Weather conditions, soil quality, and field management are key environmental factors that directly affect crop productivity. Among these factors, weather conditions are the most variable and unpredictable factor affecting crop production, as well as final yield (Prishchepov et al., 2019; Sun et al., 2024).

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Given that wheat is the most cultivated crop in the world and a staple food that provides more than 20% of total protein and calorie intake (Shiferaw et al., 2013; Giraldo et al., 2019), the negative impact of climate change on wheat production poses a serious threat to global food security (Farhad et al., 2023). Drought and high temperatures are abiotic factors that have the greatest impact on wheat production, negatively affecting its growth and development (Tyagi and Pandey, 2022; Sareen et al., 2023). The grain filling phase is the most sensitive period in wheat development, which duration can significantly be shortened by a lack of rainfall, reducing grain size, and ultimately leading to a decrease in yield (Thakur et al., 2023). For this reason, developing wheat varieties that are tolerant to key abiotic factors is one of the biggest challenges for breeders. This challenge is primarily reflected in the fact that efficient selection requires a deep understanding of the nature and extent of variation within the population (Ahmed et al., 2023), knowledge of morphological, physiological, biochemical or yield-related traits that can serve as indicators of tolerance and sensitivity to abiotic stress (Sallam et al., 2019), as well as understanding the relationship between these traits and final grain yield (Banjac et al., 2022; Matković Stojšin et al., 2022).

Morphological traits and yield components are widely used in determining genetic diversity during the breeding process for developing new varieties. Morphological traits, along with phenological traits, significantly contribute to the adaptation of wheat to various abiotic stresses, making their study critically important (Hyles et al., 2020). Many researchers have applied principal component analysis (PCA) and cluster analysis to study genetic diversity and the relationships between yield components and quality in wheat genotypes (Adilova et al., 2020; Luković et al., 2020; Urošević et al., 2023). Principal component analysis (PCA) is a multivariate statistical method that enables the assessment of relationships between variables, transforming a large number of correlated variables into a smaller number of independent variables, called principal components, while retaining the greatest possible part of the variability from the original data set (Adilova et al., 2020; Kumar et al., 2024). Principal component analysis enables researchers to analyze relationships between traits and identify key traits and wheat genotypes for use in breeding programs (Gungor et al., 2022; Urošević et al., 2023). Cluster analysis is suitable for studying genetic variability among genotypes and the possibility of increasing existing and creating new genetic variability through crossing genotypes from different cluster groups (Adilova et al., 2020; Luković et al., 2020; Bendjama and Ramdani, 2022).

This research aims to examine the genetic diversity and adaptability of wheat genotypes, originating from the broader region, in varying agroecological conditions. Additionally, the objective is to identify traits that can serve as markers for the impact of adverse environmental conditions on the plant, or traits that contribute to genotype adaptation to stress. Finally, the goal of the research is to recommend genotypes that could serve as desirable parents in breeding programs for the development of wheat varieties tolerant to specific environmental conditions.

Materials and methods

Site description

The experiment was conducted in field conditions in the growing seasons of 2015/2016 and 2016/2017 in Novi Bečej, Vojvodina region, the northern part of Serbia (45°37’03”N 20°07’53”E, 70 m a.s.l). The main physical and water-physical properties are presented in *Table 1,* and chemical properties in *Table 2*.

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Table 1. *Physical and water properties of the 0 – 30 cm soil layer at the experimental field*

Mechanical composition			Bulk density, g cm^{-3}	Total porosity	FC, %	LCM, %	WP, %	RAW, mm
Sand, %	Silt, %	Clay, %						
15.52	33.04	51.44	1.24	50.41	35	26	22	33

FC – field water capacity (at matric potential of -33 kPa), LCM – lentocapillary moisture (at matric potential of -625 kPa), WP – wilting point (at matric potential of -1500 kPa), RAW – readily available water

The soil at the experimental site belongs to Mollic Gleysol according to the FAO-WRB classification (IUSS Working Group WRB, 2015) and is a heavy clay according to the Tommerup classification. The topsoil is slightly compacted and porous, with typical water properties for heavy soil (*Table 1*). The soil is slightly acidic, medium carbonate, very humus-rich, and well supplied with nitrogen, medium phosphorus content, and very high potassium content (*Table 2*).

Table 2. *Chemical soil properties of the 0–30 cm layer at the experimental field*

pH in water	pH in KCl	$CaCO_3$, %	Organic matter, %	Total N, %	P_2O_5, mg 100 g^{-1}	K_2O, mg 100 g^{-1}
6.28	5.39	4.54	4.22	0.21	13.15	41.02

Plant material and experimental design

In this research, the variability and adaptability of wheat was examined through the analysis of divergent varieties, which were selected on the basis of their origin, agronomic properties and importance in the breeding of old ones, as well as their potential use in the breeding of new varieties. These varieties include a wide range of genetic characteristics, which allows for a detailed analysis of resistance to abiotic stress and, in general, the adaptability of different genotypes to variable growing conditions. The varieties used in this study include 16 wheat genotypes (*Table 3*): Mačvanka 1, Jugoslavija, NS 58-04, Iskra, Jedina, Poljana, Tamiš and Zvezda (Serbia); Dukat, Dunavka, Pitoma and Marija (Croatia), Fundulea 4 and Vali PKA-7114 (Romania); Skopjanka (North Macedonia); and Kavkaz (Russia).

Table 3. *Wheat genotypes used in the experiment and their origin*

No.	Genotype	Origin	No.	Genotype	Origin
1.	Mačvanka 1	Serbia	9.	Dukat	Croatia
2.	Jugoslavija	Serbia	10.	Dunavka	Croatia
3.	NS 58-04	Serbia	11.	Pitoma	Croatia
4.	Iskra	Serbia	12.	Marija	Croatia
5.	Jedina	Serbia	13.	Fundulea 4	Romania
6.	Poljana	Serbia	14.	Vali PKA-7114	Romania
7.	Tamiš	Serbia	15.	Skopjanka	North Macedonia
8.	Zvezda	Serbia	16.	Kavkaz	Russia

The experiment was conducted according to a randomized block design with three replications during two growing seasons (2015/2016 and 2016/2017; *Fig. 1*). The environmental conditions during the experimental period (microclimatic condition of

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area, temperature fluctuations and differences in precipitation, from a season with sufficient precipitation to a dry season) are representative and relevant to the climatic conditions present in this region of Europe (Pannonian Plain, Southeastern Europe). The size of the main plot was 5 m^2.

G5	G14	G10	G1	G7	G13	G2	G11	G8	G16	G4	G15	G9	G3	G6	G12
Block 3															
G2	G13	G16	G11	G8	G4	G3	G15	G9	G12	G6	G5	G10	G7	G1	G14
Block 2															
G15	G3	G12	G6	G9	G14	G1	G5	G7	G10	G13	G11	G2	G8	G2	G4
Block 1															

(a)

(b)

Figure 1. *Experimental plot: trial design (a); wheat at heading phenophase in the 2016/2017 season. G1 – Mačvanka 1; G2 – Jugoslavija; G3 – NS 58-04; G4 – Iskra; G5 – Jedina; G6 – Poljana, G7 – Tamiš, G8 – Zvezda; G9 – Dukat; G10 – Dunavka; G11 – Pitoma; G12 – Marija; G13 – Fundulea 4; G14 – Vali PKA-7114; G15 – Skopjanka; G16 – Kavkaz*

Monoammonium phosphate (MAP) fertilizer was applied before sowing in the amount of 250 kg ha^{-1}. In both growing seasons, sowing was performed with a row spacing of 12 cm and a density of 650 grains m^{-2}. In the first growing season, wheat was sown on October 31, 2015, and in the second season on November 5, 2016. Fertilization was carried out in the last decade of February in both growing seasons, when 250 kg of urea per hectare was applied. Herbicide treatment against broadleaf weeds was applied in February in both growing seasons. Fungicide and insecticide treatments were carried out in April and May in both seasons.

In the 2015/2016 growing season, the harvest was done on July 4, 2016, and in the 2016/2017 season, the harvest was on June 26, 2017. At the full ripening stage of wheat, samples of 30 plants of each variety (10 plants per replicate) were taken for analysis of yield components.

Due to the presence of environmental stress factors (soil conditions combined with the influence of climate factors), the evaluation of genotypes was focused on individual plant

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traits. Individual plant traits play a significant role in the formation of grain yield under stress conditions (Dimitrijević et al., 2013), and allow for a precise assessment of genotype adaptability. As a phenotypic marker of the influence of agro-meteorological conditions on wheat, the following were analyzed: plant height (cm), spike weight (g), number of grains per spike, grain weight per spike (g), grain weight per plant (g), thousand grain weight (g). These measurements were taken at full maturity, when the grain moisture content was below 14%.

Agro-meteorological conditions

Meteorological conditions during the research in the growing seasons of 2015/2016 and 2016/2017 differed significantly, especially in terms of precipitation (*Fig. 2*). To characterise the intensity of the drought, wheat water requirements (ETc) were calculated using the FAO Penman-Monteith equation and crop coefficients (Allen et al., 1998; Jaćimović, 2012) and compared to real evapotranspiration (ETr) calculated using the water balance method (*Fig. 3*).

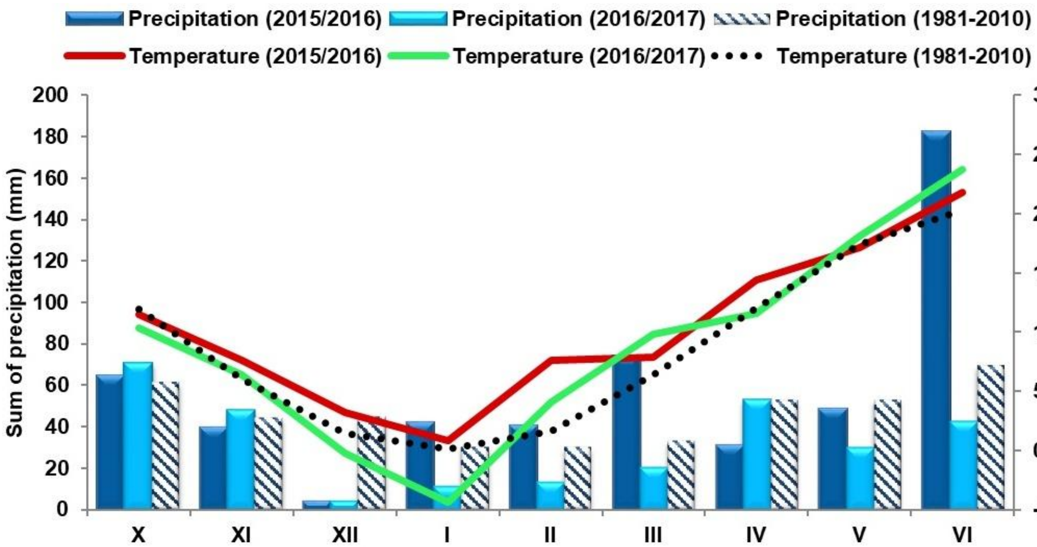

***Figure 2.** Meteorological conditions during the growing seasons 2015/2016 and 2016/2017 (monthly sum of precipitation and average monthly temperatures)*

The first experimental year was more favorable for wheat production. In October, 64.7 mm of precipitation was recorded, which provided optimal conditions for germination and early plant growth. In February, precipitation was 40.4 mm, and in March 74.3 mm, which ensured optimal soil moisture, although only 3.6 mm was recorded in December. This rainfall allowed the plants to develop a healthy root system and achieve adequate growth before the crucial stages of flowering and pollination. The amount of precipitation in this period was sufficient to satisfy the plants' water needs (*Fig. 3a*). A larger water deficit occurred in May, while in June, due to sufficient precipitation, the real evapotranspiration was at the same level of wheat's water needs (ETc). Temperatures during this season were also favorable, with an average of 21.8°C in June, promoting good development and grain filling. In contrast, the second season experienced significantly drier conditions. Although October received a favorable 70.3 mm of precipitation, total rainfall during spring and summer was drastically lower,

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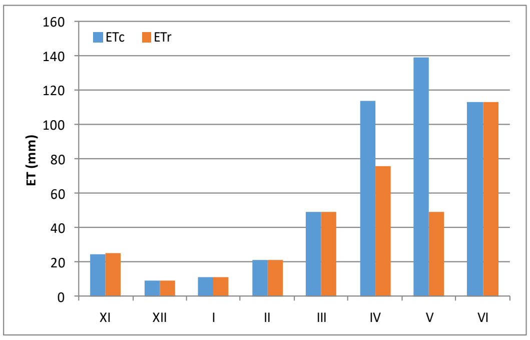
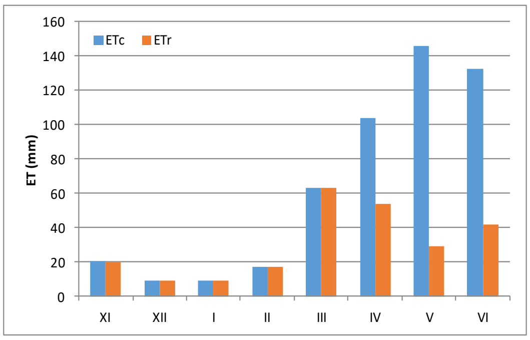
with only 29.4 mm in May and 42.2 mm in June. There was a significant water deficit in May and June (*Fig. 3b*). During the entire spring period, the real evapotranspiration (ETr) was significantly lower than the wheat water needs (ETc), and the 2016/2017 season can be characterized as dry. These drought conditions adversely affected key growth phases, such as flowering, fertilization, and grain filling, leading to stress in the plants. The average temperature increased to 23.7°C in June, accelerating ripening and shortening the wheat growth period, resulting in an earlier harvest on June 26, 2017 (*Fig. 1*). Meteorological data were obtained from the nearest meteorological station located in Zrenjanin, and were accessed via the website of the Republic Hydrometeorological Service of Serbia (http://www.hidmet.gov.rs/; accessed June 20, 2019).



(a)



(b)

Figure 3. *Monthly wheat water requirements in 2015/2016 (a) and 2016/2017 (b) growing season*

Statistical analysis

The influence of analyzed factors and their interactions on phenotypic trait variability was assessed using two-factor ANOVA. Mean comparisons were conducted using the LSD test at 1% and 5% significance levels. Principal Component Analysis (PCA) was applied to reveal patterns between yield components and genotypes, with prior data scaling. The results were visualized through biplots, where the genotypes were represented as points, and the examined traits as vectors. With the aim of grouping wheat genotypes on the basis of similarity in their phenotypic characteristics, a hierarchical cluster heatmap analysis was carried out using Ward's method. The data were first scaled for standardization and then a distance matrix was calculated using Euclidean distance. Clusters were formed for rows (genotypes) and columns (traits) and visualized via heatmaps, with clearly marked cluster groups for rows (genotypes). The selection of the number of clusters into four groups was based on the analysis of the hierarchical clustering results, including dendrogram visualization and consideration of within-group variation. Statistical analyzes were performed using the R Project for Statistical Computing, Version 4.3.2, 2023-10-31ucrt (R Foundation for Statistical Computing, 2023).

Results

Phenotypic variability of analyzed traits during the experiment

Based on the results of the analysis of variance (ANOVA), it was determined that the analyzed factors (genotype and year) and their interaction had the high significant

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($p < 0.01$) influence on the variation of all analyzed traits (*Table 1*). The factor genotype had the greatest contribution in the variation of plant height, spike length and number of grains per spike. The highest value of spike length was observed in genotype Skopjanka (12.07 cm), which had the highest value of grain weight per plant (21.23 g). In average for both years, the highest plant height was determined for the Kavkaz genotype (111.88 cm), which was characterized by the lowest values of spike weight, number of grains per spike, grain weight per spike and grain weight per plant at the trial level. The lowest values of plant height were found in Vali PKA-7114 (80.79 cm) and Mačvanka 1 (81.08 cm), while Skopjanka, Dunavka, and Fundulea 4 also had below-average heights but showed high values for other yield components. Genotype Fundulea 4 had the highest average grain number per spike (60.37), and along with Dunavka, showed the highest grain weight per spike (2.40 g). On the other hand, the year factor had the greatest impact on grain weight per spike, grain weight per plant and thousand grain weight, with the lack of precipitation causing the greatest decrease in grain weight per plant (by 44.7%), followed by grain weight per spike by 41.77% and thousand grain weight by 31.69% In average for both years, the highest value of thousand grain weight was observed in genotype Dunavka (49.37 g; *Table 4*).

Table 4. *Mean values of analyzed traits for sixteen wheat genotypes grown across two growing seasons*

Genotype	Plant height (cm)	Spike length (cm)	Spike weight (g)	Number of grains per spike	Grain weight per spike (g)	Grain weight per plant (g)	Thousand grain weight (g)
Mačvanka 1	81.08^{j}	9.87^{f-i}	2.47^{b-e}	45.32^{de}	1.88^{def}	13.19^{cde}	41.40^{def}
Jugoslavija	89.27^{de}	9.02^{j}	2.56^{b-e}	44.70^{e}	1.93^{c-f}	12.04^{ef}	41.73^{cde}
NS 58-04	89.58^{d}	9.73^{hi}	2.45^{b-e}	49.77^{bc}	1.89^{def}	13.70^{cde}	37.18^{i}
Iskra	94.55^{c}	10.62^{bcd}	2.40^{cde}	42.28^{e}	1.81^{ef}	15.62^{bc}	43.98^{b}
Jedina	83.97^{hi}	9.79^{ghi}	2.39^{de}	45.25^{de}	1.83^{ef}	9.79^{fg}	39.37^{f-i}
Poljana	89.78^{d}	10.04^{e-h}	2.64^{bc}	50.07^{bc}	2.09^{bcd}	13.65^{cde}	42.17^{b-e}
Tamiš	91.12^{d}	9.60^{i}	2.43^{b-e}	46.25^{cde}	1.89^{def}	12.59^{def}	39.45^{fgh}
Zvezda	88.58^{def}	10.18^{efg}	2.36^{e}	42.77^{e}	1.79^{f}	13.77^{cde}	40.20^{e-h}
Dukat	83.25^{ij}	10.32^{b-e}	2.66^{b}	50.98^{b}	2.12^{bc}	14.75^{b-e}	43.85^{bc}
Dunavka	85.77^{ghi}	10.27^{c-f}	3.12^{a}	49.07^{bcd}	2.40^{a}	12.67^{de}	49.37^{a}
Pitoma	84.61^{ghi}	10.72^{b}	2.58^{b-e}	51.57^{b}	2.00^{c-f}	14.44^{cde}	43.10^{bcd}
Marija	100.83^{b}	9.69^{hi}	2.57^{b-e}	42.18^{e}	1.89^{def}	13.07^{cde}	43.97^{b}
Fundulea 4	86.83^{efg}	10.20^{d-g}	3.00^{a}	60.37^{a}	2.40^{a}	14.97^{bcd}	38.07^{hi}
Vali PKA-7114	80.78^{j}	9.67^{hi}	2.63^{bcd}	51.42^{b}	2.03^{b-e}	17.43^{b}	38.37^{ghi}
Skopjanka	86.38^{fgh}	12.07^{a}	3.00^{a}	52.90^{b}	2.23^{ab}	21.23^{a}	40.30^{efg}
Kavkaz	111.88^{a}	10.73^{bc}	1.90^{f}	33.75^{f}	1.35^{g}	8.92^{g}	38.25^{ghi}
Average	89.27	10.16	2.57	47.41	1.97	13.86	41.30
$LSD_{0.05}$	2.684	0.441	0.248	4.363	0.224	2.813	2.199
$LSD_{0.01}$	3.566	0.586	0.329	5.798	0.297	3.739	2.922
2015/2016	91.79	10.32	3.18	51.74	2.49	17.85	49.07
2016/2017	86.74	9.99	1.97	43.09	1.45	9.87	33.52
Reduction (%)	5.50	3.20	38.05	16.72	41.77	44.70	31.69
Source of variation	Sum of squares						
Genotype (G)	5560.152**	41.921**	7.729**	3253.751**	5.816**	727.052**	868.549**
Year (Y)	615.25**	2.624**	35.149**	1802.036**	26.223**	1528.765**	5801.394**
G × Y	773.657**	11.900**	4.659**	1165.035**	4.222**	312.029**	369.660**
Error	346.52	9.352**	2.960**	915.747	2.409**	380.781	232.675
Total	7295.578	65.797	50.497	7136.570	38.670	2948.627	7272.278

Different letters indicate significant differences between genotypes ($p < 0.05$); ** $p < 0.01$

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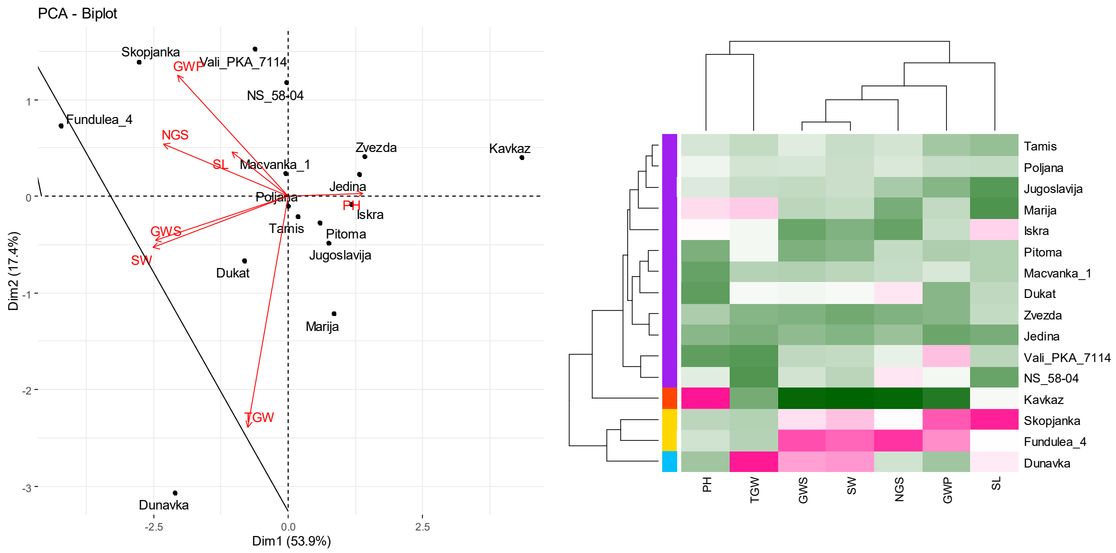
Response of wheat genotypes in 2015/2016 growing season

In the first growing season, PCA1 (Dim 1) and PCA2 (Dim 2) together explain 71.3% of the total variation, indicating that these two components capture most of the variability present in the data (*Fig. 4a*). In the PCA biplot, plant height shows a positive loading on the first axis, while number of grains per spike, spike length, and grain weight per plant, have negative loadings on PCA1. The heatmap chart shows that grain weight per spike, spike weight, number of grains per spike, grain weight per plant, and spike length are grouped together, while thousand grain weight and plant height are separated into distinct clusters (*Fig. 4b*). This pattern is consistent with the PCA biplot and suggests positive correlations between yield components within the same cluster. Yield components were negatively correlated with plant height. The Kavkaz genotype, with the highest PCA1 score, showed high plant height and low values of grain yield components (*Fig. 4a*).



(a) (b)

Figure 4. *PCA biplot (a) and heatmap cluster analysis (b) of analyzed wheat genotypes and phenotypic traits in 2015/2016 growing season. PH – plant height; SL – spike length; SW – spike weight; NGS – number of grains per spike; GWS – grain weight per spike; GWP – grain weight per plant; TGW – thousand grain weight*

Under well-watered conditions, hierarchical clustering revealed four distinct genotype clusters, marked with different colors: violet, red, ochre, and blue. In the heatmap chart, green represents below-average trait values, white indicates average values, and pink shows above-average values (*Fig. 4b*). Genotype Kavkaz is isolated in a separate cluster group, marked in red, reflecting its above-average plant height and low values of grain yield components. On the other hand, the genotypes Fundulea 4 and Skopjanka, which have low scores on the PCA1 axis, exhibiting lower plant height values and higher values of other yield components, form a separate cluster, marked in ochre color (*Fig. 2b*). Thousand-grain weight has the largest contribution to negative values on PCA 2 and forms negative correlations with spike length, number of grains per spike, grain weight per plant, and plant height. Genotype Dunavka, with a high negative score on PCA 2 and a low score on PCA 1, is positioned near the thousand grain weight vector, reflecting a high value for this trait (*Fig. 4a*). It belongs to the cluster group marked in blue, which is

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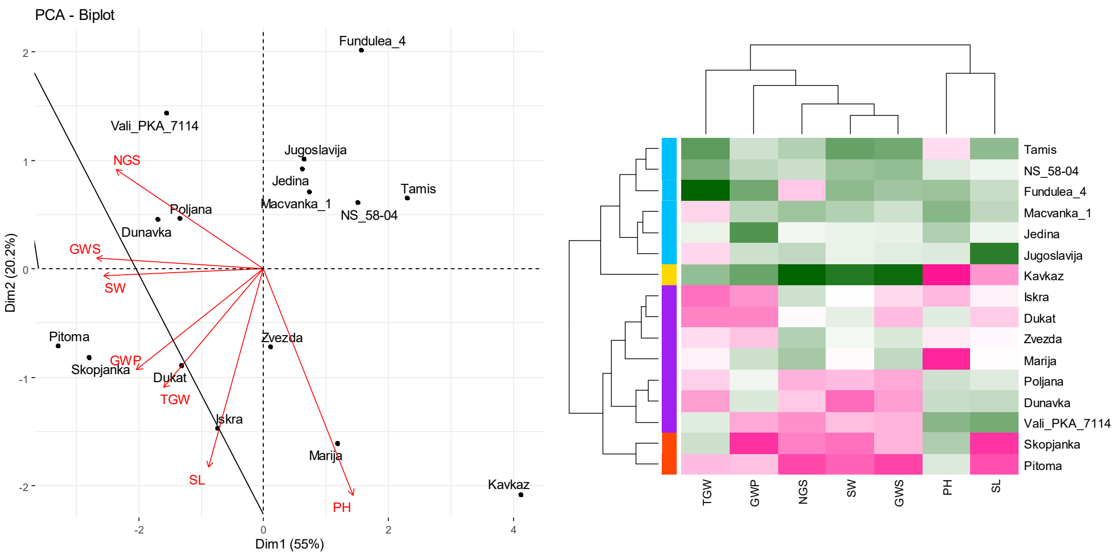
characterized by high values of thousand grain weight, grain weight per cluster, and spike weight, with low values of plant height and grain weight per plant (*Fig. 4b*).

Response of wheat genotypes in 2016/2017 growing season

In the second growing season (rainfall deficit conditions), PCA shows that the first two components explain 75.2% of the total variation (*Fig. 3a*). The plant height vector is positioned in a separate quadrant with a positive value on PC1 and a high negative value on PC2, indicating negative correlations with number of grains per spike, grain weight per spike, and spike weight. Genotypes Marija and Kavkaz, located in the same quadrant with plant height, have high values of this trait, but low values of other yield components, which confirms the negative correlation between plant height and yield traits (*Fig. 5a*). In the heatmap cluster analysis, genotype Kavkaz is isolated in a distinct ochre cluster, showing low values for most yield components (*Fig. 5b*).

Vectors for number of grains per spike and grain weight per spike have positive PCA2 and negative PCA1 scores. The genotypes Vali PKA 7114, Poljana and Dunavka are positioned near the vectors of these traits on the biplot (*Fig. 3a*) and are grouped into a common cluster with Iskra, Dukat, Zvezda and Marija (*Fig. 5b*). Within this group, Poljana, Dunavka and Vali PKA 7114 stand out with above-average values for the number of grains per spike, spike weight and grain weight per spike and lower values for plant height and spike length (*Fig. 5b*). The vectors for spike weight, grain weight per plant, thousand grain weight and spike length have negative values on both axes and are positively correlated with each other. The genotypes Pitoma and Skopjanka are located near these vectors (*Fig. 5a*). They also form a separate cluster group, characterized by a lower plant height value and high values of most yield components. Mačvanka 1, Fundulea 4, Jedina, Jugoslavija, Tamiš and NS 58-04 stand out in a separate cluster (marked in blue), exhibiting below-average values of the analyzed traits in the season with a rainfall deficit (*Fig. 5b*).



(a) (b)

Figure 5. *PCA biplot (a) and heatmap cluster analysis (b) of analyzed wheat genotypes and phenotypic traits in 2016/2017 growing season. PH – plant height; SL – spike length; SW – spike weight; NGS – number of grains per spike; GWS – grain weight per spike; GWP – grain weight per plant; TGW – thousand grain weight*

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Discussion

An effective breeding program relies on the variation present in the genetic pool for traits that enhance yield. Thus, the success of selection depends on sufficient diversity of donor parents, or the variability present in the initial genetic material (Bendjama and Ramdani, 2022). Therefore, the examination of diverse wheat germplasm is crucial for the identification of yield components that can contribute to improved productivity and resistance to abiotic and biotic stresses. Variability of plant height, spike length and number of grains per spike was primarily determined by factor of genotype. This indicates that genotypic differences play a key role in the expression of these traits. Under reduced precipitation (2016/2017), spike length and plant height were the least reduced (3.2% and 5.5%, respectively), which is the result of a more pronounced effect of genotype compared to environmental influences (Matković Stojšin et al., 2018). According to Bendjama and Ramdani (2022), the expression of traits that are more influenced by genotype effect than environment enables selection based on phenotypic values. Similarly, Abdurezake et al. (2024) suggest that the significant impact of the genotype factor is a result of additive gene action, which can facilitate selection for particular quantitative traits.

Protić et al. (2018) reported that an increase in spike length can lead to the development of bigger grains, ultimately resulting in higher grain yield. In line with this observation, the present study found that genotype Skopjanka, which exhibited the longest spikes, also achieved the highest grain weight per plant, indicating a potential link between spike architecture and yield performance.

In our study, genotype Kavkaz recorded the highest average plant height during both growing seasons. However, it showed the lowest values of spike weight, number of grains per spike, grain weight per spike and grain weight per plant at the trial level. This imbalance between vegetative growth and reproductive performance suggests that excessive stem elongation may negatively affect the allocation of resources to yield-related components, possibly due to an unfavorable source–sink relationship. These findings are in line with the suggestion by Wang et al. (2023), who indicate that a semi-dwarf wheat plant type with a relatively longer spike may represent a desirable ideotype for increasing grain yield. Also, Zhou et al. (2025) point out that the introduction of the Rht gene during the Green Revolution enabled the selection of forms with lower plant height, which led to a significant increase in yield due to lodging resistance. Nevertheless, the Kavkaz genotype played a key role in wheat breeding programs around the world, including the former Yugoslavia, together with the varieties Aurora and Skorospelka 35, as a carrier of the wheat-rye translocation (1BL/1RS) (Dimitrijević et al., 2008). This translocation, which transfers genes from the rye chromosome to wheat, has been shown to improve yield, adaptability, and disease and pest resistance (Rabinovich, 1998). In this study, the variety Kavkaz appears in the pedigree of the varieties Mačvanka 1, Poljana, NS 58-04, Tamiš, Zvezda, Skopjanka and Pitoma (Dimitrijević, 1997; Rabinovich, 1998), which indicates its significant contribution to the genetic background of these wheat genotypes.

The number of grains per spike is determined by the dominant influence of the genotype factor, which is in accordance to results obtained by Knežević et al. (2012). Research shows that sink potential during grain filling, which is determined by the number of grains and grain size, is a key limited factor for grain yield (Lo Valvo et al., 2018). Given that the number of grains is more plastic than grain weight (Sadras and Slafer, 2012), the number of grains is a trait that more significantly influences yield improvement (Slafer et al., 2014).

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On the other hand, the factor of environment had a greater effect on grain weight per spike, grain weight per plant and thousand grain weight compared to the genotypic factor, indicating a high sensitivity of these traits to variations in climatic conditions. This dominance of the environmental factor can be attributed to pronounced differences in the amount of precipitation between seasons, whereby the season with a deficit of precipitation resulted in a reduction of grain weight per plant by as much as 44.7%. Popović et al. (2020) found that environmental factors had the greatest contribution to the variation in grain weight per plant in different wheat genotypes. They emphasize that this significant impact is mainly due to variations in climatic conditions and treatments. Additionally, a significant reduction in grain weight per spike (by 41.77%) was found under conditions of insufficient rainfall. Previous studies (Zečević et al., 2010; Knežević et al., 2015; Gungor et al., 2022) highlighted the strong impact of the growing season/environment on grain weight per spike, which forms during early growth stages and reflects the genotype's response to environmental conditions during ontogenetic development (Knežević et al., 2015; Balaban Göçmen et al., 2024). Limited rainfall during the grain filling stage caused a 31.69% reduction in the thousand grain weight, which is consistent with findings by Javed et al. (2022). Although this trait is largely determined by genotype (Urošević et al., 2023), water deficit limited its expression. In average for both years, the highest value of thousand grain weight was observed in genotype Dunavka (49.37 g). Thousand grain weight is an important wheat quality component and represents an indicator of grain size. Therefore, we can single out the Dunavka genotype as a good genetic resource for grain quality improvement.

The significant genotype × year interaction in the expression of the studied traits, which arises from differences in seasons, indicates that it is necessary to study genotypes in different environments. Accordingly, multivariate statistical analyses, such as Principal component analysis (PCA) and hierarchical cluster analysis were applied to analyze the relationship between genotypes and traits in each season separately. These multivariate techniques were applied in similar studies (Luković et al., 2020; Ali et al., 2021; Bendjama and Ramdani, 2022; Urošević et al., 2023). In the both growing season the first two components (PCA1 and PCA2) together explain >70% of the total variation, indicating that these two components capture most of the variability present in the data. Similar results were obtained by Ali et al. (2021) and Urošević et al. (2023).

In both growing seasons, plant height forms negative correlations with most grain yield components. Genotypes Marija and Kavkaz, located in the same quadrant with plant height, have high values of this trait, but low values of other yield components, which confirms the negative correlation between plant height and yield traits. These two genotypes showed reduced efficiency in resource use under conditions of reduced rainfall. These results are in line with the findings of Ullah et al. (2021), who identified a negative correlation between plant height and grain yield traits. According to Philipp et al. (2018), semi-dwarfism in wheat is a desirable trait, not only due to reduced lodging but also because of its efficiency in nutrient utilization, which positively impacts grain yield.

In the well-watered conditions, Fundulea 4 and Skopjanka, exhibiting lower plant height values and high values of other yield components. Thousand-grain weight forms negative correlations with spike length, number of grains per spike, grain weight per plant, and plant height. Philipp et al. (2018) found that thousand grain weight is negatively correlated with the number of grains per spike and is more influenced by grain size than

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grain number. Thus, genotype Dunavka is a suitable candidate for improving wheat grain quality, while Skopjanka and Fundulea 4 could be selected as parents with the aim of increasing grain yield potential in conditions with sufficient rainfall. Luković et al. (2020) suggest that crossing wheat lines from different cluster groups can improve quality and create new genetic variability.

In conditions of rainfall deficit, a different pattern of relationship between traits and genotypes was observed. Namely, the thousand grain weight forms a positive correlation with spike weight, grain weight per plant, and spike length. Similar correlations between thousand grain weight and grain weight per plant were determined by Urošević et al. (2023). Ali et al. (2021) and Zečević et al. (2021) also observed that increased thousand grain weight contributes to higher grain yield. Therefore, in unfavorable conditions, due to the inability of genotypes to express their full genetic potential, there was a significant decrease in the value of all analyzed traits, while positive correlations appeared between traits that formed negative correlations in favorable conditions, which indicates a change in priorities in the redistribution of resources under stress. Skopjanka and Pitoma genotypes stand out with high values of most grain yield components, as desirable genotypes for cultivation in conditions of lack of rainfall. Genotypes Dukat and Iskra are positioned close to the vector of thousand grain weight, exhibiting the highest values of this trait under stress conditions. Under conditions of limited precipitation, the number of grains per spike, grain weight per spike, and spike weight showed a significant positive correlation. Positive correlations between spike weight and grain weight per spike were established by Banjac et al. (2022), while positive correlations between the number of grains per spike and grain weight per spike were reported by Philipp et al. (2018) and Urošević et al. (2023). Also, Zečević et al. (2010) noted that grain weight per spike depends on the number of grains and their chemical composition.

Conclusion

In this study, clear differences in the responses of wheat genotypes to different environmental conditions were established. The genotypes Skopjanka and Pitoma showed the highest adaptive potential, achieving high yields in both favorable and unfavorable environmental conditions, while Marija and Kavkaz had low grain yield performances. Plant height, number of grains per spike, grain weight per plant and spike weight were the most important traits that differentiated the genotypes. Grain weight per plant was the trait most sensitive to rainfall deficiency conditions. Fundulea 4 showed high productivity only under favorable conditions, while under conditions with reduced rainfall it exhibited drastically reduced productivity. Cluster analysis supported these findings, with seasonal changes in the clustering of genotypes highlighting the importance of selecting varieties based on the environmental context. The observed variations in clustering patterns suggest significant genetic diversity, indicating that Balkan germplasm has strong potential for breeding wheat varieties adapted to specific conditions, such as limited rainfall.

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Conflict of interests. The authors declare that they do not have any conflict of interests.

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