

## Pre-breeding assessment of Macedonian wheat germplasm diversity and yielding potential

Sonja Ivanovska\*, Mirjana Jankulovska, Dane Boshev, Nadica Sandeva Atanasova

*Faculty of Agricultural Sciences and Food, Ss Cyril and Methodius University in Skopje, 16ta Makedonska Brigada 3, Skopje, Republic of North Macedonia*

### Abstract



A wheat breeding program in North Macedonia resulted in a large collection of germplasm. In order to increase the efficiency of using genetic variation within this germplasm, the potential breeding value of 217 genotypes was evaluated through the following traits: duration of heading, flowering, and maturity, plant height, spike length, number of spikelets per spike, number of kernels per spike, and thousand kernel weight. The experiment was carried out in 2016, at two locations (Skopje and Gradsko) with different climatic conditions.

Duration of phenophases was similar in both locations, indicating satisfactory adaptability of the genotypes to different environments. The average values of the productive traits were higher in Skopje, compared to Gradsko, which was expected considering that Gradsko is located in an arid region. Drought conditions predominantly affected the thousand kernel weight, number of kernels per spike, and plant height, as the average values for these traits were 23%, 16%, and 8% (respectively) lower compared to the average values in Skopje. However, some genotypes have shown greater adaptability to both locations and stable productive traits, representing valuable breeding material in order to improve the wheat's potential to resist drought and harsh growth conditions. Except for the differences among the average values, wide ranges of the values on the level of genotypes indicated broad diversity within the germplasm.

According to the Principal component analysis, genotypes were separated into five different groups, while the cluster analysis confirmed wide genetic diversity among the germplasm, resulting in five distinct clusters, each containing many sub-clusters. The genotypes with high values for the number of spikelets per spike, number of kernels per spike, and thousand kernel weight and low values for plant height, separated into group 4 can be used for improving wheat yield potential, as these traits are positively correlated with seed yield.

**Key words:** wheat, principal component analysis, cluster analysis, productive characteristics

### Introduction

Wheat is a major crop contributing to global food security, providing about 20% of global dietary energy and protein intake (Gooding & Shewry 2022). As a most important staple crop cultivated worldwide, it occupies over 220 million of hectares in 2021 with a production of 778.6 million metric tons (FAOSTAT 2021). For the Republic of North Macedonia, as a strategic crop wheat was cultivated on nearly half (70,107 ha) of the

area occupied by cereals (155,542 ha) in 2022 with a production of 224,632 tons (MaxStat 2022). The average yield for the period 2012-2021 was 3285.7 t/ha, varying from 2696 t/ha in 2012 to 3838 t/ha in 2016, largely depending on the climate conditions and on the genetic potential and adaptability of the cultivated varieties. The agricultural production in the Republic of North Macedonia is based on foreign varieties due to the fact that there is no breeding program for any crop, except for wheat, triticale and barley.

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\* Author for correspondence: [s.ivanovska@fznh.ukim.edu.mk](mailto:s.ivanovska@fznh.ukim.edu.mk)

Considering the constantly increasing trend of the world population, subsequently, there is an increased demand for wheat grain. Kettlewell et al. (2023) assume that it is unlikely future wheat demand will be satisfied from the existing cropping area. However, Guarin et al. (2022) suggest that one solution to meet the increased demand is to increase yields on the existing cultivated area through genetic and agronomic improvements.

Breeding programs for wheat genetic improvement are largely focused on using divergent germplasm through different strategies and technologies in order to create high-yielding varieties with increased adaptability, stability, and stress resilience. It is predicted that the climatic changes will reduce global wheat production by 6% for each °C of further temperature increase (Asseng et al. 2015). Khadka et al. (2020) reviewed that many wheat programs comprise selection for all physio-morphological traits with the potential to contribute to drought stress tolerance. Wheat is affected by drought in all growth stages (Sarto et al. 2017; Ding et al. 2018). Senapati et al. (2019) conclude that under climate change in Europe, especially in Southern Europe, high yield potential and stability in wheat may be achieved in varieties tolerant to drought stress during reproductive development. The duration of phenophases and the earliness of wheat varieties can be significant factors for successful wheat production in specific regions. Frantova et al. (2022) suggested that late varieties perform better in the conditions of longer drought, while the early varieties are more suitable for short droughts during the flowering or grain-filling period.

Wheat yield *per se*, is a complex trait depending on many productive components (Maurya et al. 2014), each with a different effect on productivity, depending on the variety and growing conditions. The success of the breeding programs largely depends on the extent of genetic diversity and variability among the genotypes used in the hybridization schemes. Therefore, genotypes and productive traits are separately evaluated in the pre-breeding programs in order to locate superior genotypes for improving the specific traits. Such genotypes are further used for broadening the genetic base, like introgressing genes from landraces and other genetic material into the elite varieties which are intensively used by the breeders. Their impact on genetic gains for improved yield through improved resistance to biotic, and to a much lesser extent to abiotic stresses, is well recognized (Singh et al. 2018). Large research institutions are intensively using precision phenotyping for utilizing the wheat material stored in the genebanks and developing pre-breeding material for stress tolerance breeding programs (Singh et al. 2018; Singh et al. 2019). Therefore, breeders are focusing on creating germplasm with accumulated genes for many productive traits.

Genetic diversity among the breeding material can be successfully estimated on the basis of the productive

traits through quantification of their genetic variation (Li & Gill 2004). Indirect selection of potential superior material with desirable traits can be efficiently performed by cluster and principal component analysis. They are proven useful tools to analyze the genetic diversity and relationships of wheat genotypes (Devesh et al. 2019) and to classify the germplasm according to traits of interest and their effect on the yield or resistance to stresses. Tracing the total variation into its components aids in harnessing the friable genetic variation within the breeding material.

For this purpose, the present study was conducted to determine the association and the scope of genetic variation among different Macedonian wheat genotypes based on several quantitative traits and to ascertain which characteristics could be used for indirect selection further on in the breeding program.

## Material and methods

Field experiments were conducted during the 2015/16 growth season at two locations in North Macedonia: Radishani, near Skopje, and village Gradsko, near Veles, which has more dry climate conditions. A total of 217 Macedonian wheat genotypes were sown in randomized block design with 2 replications for evaluating their agronomic performance and assessing their potential breeding value. Standard agronomic practices for wheat were applied during the vegetative growth season. In both locations, the plot size was 1 m<sup>2</sup>, with a seeding density of 100 grains/m<sup>2</sup>. In order to determine the adaptability of the genotypes, they were phenotyped for three phenological phases (date of heading, flowering, and maturity). Main spikes of 10 plants from each plot per replication, selected from the middle rows of the plots, were used for the measurements of several productive traits: plant height (PH, cm), spike length (SL, cm), number of spikelets per spike (SPS), number of kernels per spike (NSS) and thousand kernel weight (TKW, g).

Based on the average values of the genotypes for the studied traits in both locations, principal component analysis (PCA) and cluster analysis were applied using R 3.5.1. statistical package. The genetic distance was calculated using Ward's distance measure and the UPGMA method was applied to constructing the dendrogram.

## Results and discussion

Comparing the genotype performance in the two locations, notable differences were recorded in the expression of all analyzed traits (Table 1). The date of heading, flowering, and maturity ranged from March 31<sup>st</sup> to April 20<sup>th</sup>, April 25<sup>th</sup> to June 3<sup>rd</sup>, and June 23<sup>rd</sup> to July 3<sup>rd</sup>, accordingly, in Skopje and April 19<sup>th</sup> to May 28<sup>th</sup>,

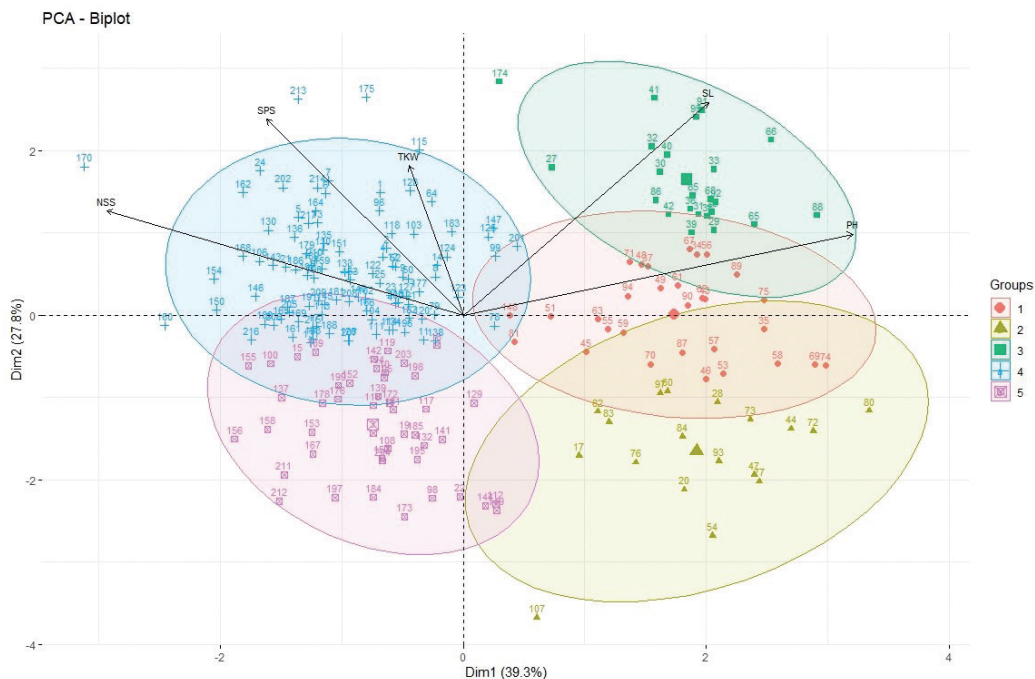
**Table 1.** Average values and variation range of all analyzed genotypes for different productive characteristics

Characteristic		Skopje	Gradsko
Plant height (cm)	Min	55.5	53.0
	Max	152.5	131.0
	Average	96.2	80.6
Spike length (cm)	Min	7.9	7.1
	Max	16.9	18.7
	Average	12.1	12.0
Number of spikelets per spike	Min	18.5	15.0
	Max	29.4	29.9
	Average	23.6	22.7
Number of kernels per spike	Min	14.70	10.80
	Max	98.30	102.00
	Average	57.64	53.04
Thousand kernels weight (g)	Min	18.81	10.48
	Max	53.96	50.00
	Average	40.08	30.76

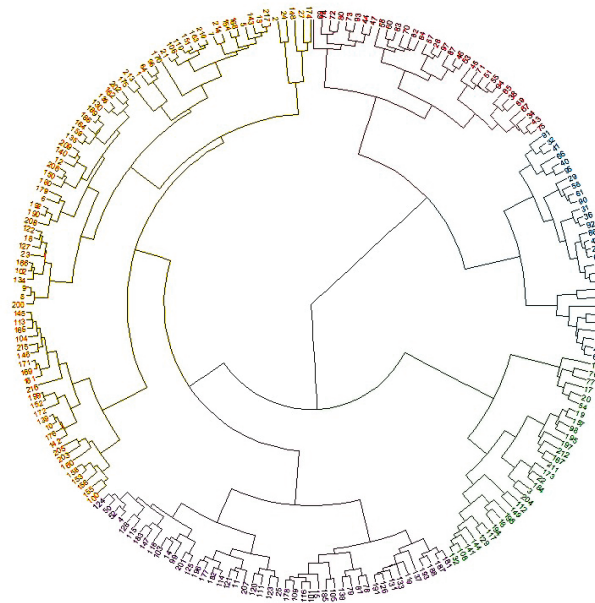
April 24<sup>th</sup> to June 2<sup>nd</sup>, and June 25<sup>th</sup> to July 6<sup>th</sup> in Gradsko. Duration of phenophases was similar in both locations, indicating satisfactory adaptability of the genotypes to different environments. However, the average values of the productive traits were higher in Skopje, compared to Gradsko, which was expected considering that Gradsko is located in an arid region. Drought conditions predominantly affected the thousand kernel weight, number of kernels per spike, and plant height, considering that average values for these traits were

23%, 16%, and 8% (respectively) lower compared to the average values in Skopje.

Khatiwada et al. (2020), in their review paper analyzing drought stress on wheat yield and yield attributing characters also concluded that drought is a serious and common problem for global wheat producers affecting plant height, plant biomass, number of spikes and spikelets, and consequently the expression of the grain yield.



**Graph 1.** Biplot of PC1 and PC2 representing the distribution of genotypes based on analyzed traits (PH, plant height; SL, spike length; SPS, number of spikelets per spike; NSS, number of kernels per spike; TKW, thousand kernel weight)



**Graph 2.** Dendrogram showing the clustering of analyzed wheat genotypes

Cluster and principal component analyses are widely used in studying the genetic diversity among the wheat genotypes available in breeding programs (Devesh et al. 2019; Adilova et al. 2020; Al Lawati et al. 2021). Principal component analysis identified that the PC1 explained 39,3% of total variability of evaluated germplasm, while PC2 explained 27,8%. The genotypes in this experiment can be separated into five different groups according to the average values for quantitative traits. Groups one, two, and three comprised the genotypes with low values for the number of spikelets per spike, the number of kernels per spike, and the thousand kernel weight. The genotypes in group three were characterized with the highest values for plant height and spike length, while the genotypes that belonged to group four had the highest values for the number of spikelets per spike, the number of kernels per spike, and thousand kernel weight (Graph 1). As all of these traits have a direct influence on the yield, the genotypes from group four represent a valuable material for achieving yield gain in the breeding program. The genotypes included in group five have low to medium values for all analyzed traits. These genotypes, along with the genotypes from group four can be included in the breeding program for creating short-stem varieties.

The classification of the genotypes can be observed in Graph 2, where they are clearly differentiated into five distinct clusters, each containing many sub-clusters that reflect the wide genetic diversity among the experimental material. The genotypes with high values for the number of spikelets per spike, number of kernels per spike, and thousand kernel weight and low values for plant height were separated into group one (yellow) and can be included in breeding programs for improving wheat yield potential, as these traits are positive-

ly correlated with seed yield (Mecha et al. 2017; Tabasum et al. 2018; Wolde et al. 2019; Khanal et al. 2020; Sakuma & Schnurbusch 2020; Kumar et al. 2022;). On the other hand, the genotypes classified in group three (blue), characterized by high values for plant height and spike length can be used for breeding wheat for organic production, as these traits are desirable for breeding programs with specific aims (Löschenberger et al. 2008; Sandro et al. 2022).

The results indicate that the magnitude of the genetic diversity among different genotypes on the level of individual traits was significant and should be used for indirect selection to improve grain yield. Such an approach can effectively improve yield, considering the high correlations among yield and yield component traits (Ahmad et al. 2010). Grain yield was found to have significant positive correlations with days to maturity, plant height, kernels per spike, and thousand-grain weight, at both genotypic and phenotypic levels (Mecha et al. 2017, Swailam et al. 2021).

## Conclusion

Different average values among genotypes as well as wide variations of the values on the level of a single genotype indicate broad diversity within the analyzed wheat germplasm. Some genotypes have shown greater adaptability to both locations and stable productive traits. They represent a valuable breeding material and should be used as parents for hybridization in order to improve the wheat's potential to resist drought and harsh growth conditions. Cluster analysis enabled an effective overview of the genotypes that share

a common set of characteristics. According to the principal component analysis, the genotypes from the fourth group can be directly used as parents in the hybridization schemes. The superior genotypes in other clusters, based on their performance for the targeted character, should be used in hybridization plans for the improvement of different characters and genetic yield potential.

## Declaration

This manuscript is original, has not been published before and is not currently being considered for publication elsewhere.

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