

ASSESSMENT OF WINTER WHEAT ADVANCED LINES BY USE OF MULTIVARIATE STATISTICAL ANALYSES

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This study was conducted to evaluate 49 advanced lines of winter wheat (*Triticum aestivum* L.) for their morphoagronomic traits and to determine best criteria for selection of lines to be included in future breeding program. The material was assessed in two years experiment at two locations, using RCBD design with three replications. Ten quantitative traits: plant height, number of fertile tillers, spike length, number of spikelets per spike, number of grains per spike, weight of grain per spike and per plant, fertility, biological yield and harvest index were evaluated by PCA and two-way cluster analysis. Three main principal components were determined explaining 71.391% of the total variation among the genotypes. One third of the variation is explained by PC1 which reflects the genotype yield potential. PC2 and PC3 explained 25.22% and 15.49% of the total variance, mostly in relation to the plant height and spike components, respectively. Biplot graph revealed strongest positive association between spike length, number of spikelets and biological yield and between number of tillers, weight of grains per spike and per plant. Two-way cluster analysis resulted with a dendrogram with one solely separated genotype, superior for all traits and two main clusters of genotypes defined with wide genetic diversity especially between the groups within the second cluster. Genotypes with high values for specific traits will be included in the future breeding programmes. Classification of genotypes and the extend of variation among them illustrated on the heatmap has proved to be practical tool for selecting genotypes with desired traits in the early stages of the breeding process.

Keywords: advanced lines, cluster analysis, PCA, productive traits, wheat

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INTRODUCTION

The improvement of wheat yield has been achieved mainly through wide use of germplasm and different breeding strategies, but the rate of genetic gain in wheat yield potential have steadily decreased in recent decades. Most of the research efforts in the past years were focused on improving wheat resistance to biotic and abiotic stress (BRAUN *et al.*, 2010), through modernization in plant breeding approaches (HUSSAIN, 2015) and use of agriculture biotechnology (HAGGAG *et al.*, 2015). The primary objective was to develop widely adapted and stable wheat germplasm with increased productivity and efficiency in water (HUB *et al.*, 2015) and nutrient use (HAWKESFORD, 2014; MAJEED *et al.*, 2015). These breeding directions were enforced with the evidence of climatic changes. Wheat production will be reduced due to heat stress and drought, especially in agro-climatically marginal areas. According to ASSENG *et al.* (2015) it is estimated that global production will fall by 6% for each °C of further temperature increase. In this regard, wheat is one of the most sensitive crops of the major staples. The efforts of wheat breeders have already resulted with new cultivars with enhanced grain yield in many drier producing areas (MANES *et al.*, 2012).

Basic research in photosynthesis and radiation use efficiency suggests that further improvements in yield are possible (ZHU *et al.*, 2010). This complex trait is quantitative genetic parameter resulting from the expression of many productive components influencing the yield *per se* (MAURYA *et al.*, 2014). Present breeding programs employ modern and traditional breeding techniques to obtain genetic progress in yield. Most of them are directed to accumulate yield potential traits and to create new germplasm. Trait-based hybridization strategies aim to achieve their simultaneous expression in elite germplasm. For that purpose, instead of conventional hybridization of parents with high and low adaptation, crosses between high-yielding and well adapted parents are used for examination of genetic and phenotypic diversity among the progeny (BUSTOS *et al.*, 2013). Desirable lines will have a combination of traits with a maximum individual gain that will finely result in enhanced productivity and quality.

The magnitude of genetic diversity in elite germplasm is significantly narrowed leading to lower adaptability to abiotic and biotic stresses (BARANWAL *et al.*, 2012; AREMU, 2012). Therefore, many researchers exploit the existing variability in wheat germplasm pools in order to distinguish genetically diverse material for hybridization (KAHRIZI *et al.*, 2010; SAJJAD *et al.*, 2011; RAUF *et al.*, 2012). Indirect selection of potential superior lines with desirable traits in early generations based on yield components is one of the most important breeding strategies. These lines could be further on used as parental material for creation of new varieties (ALI *et al.*, 2008).

To classify breeding lines according to traits of interest and their effect on the yield, principal component and cluster analyses are proved as useful tools. Tracing the total variation into its components aids to harnessing the friable genetic variation within the breeding material. These techniques are used by many breeders and researchers for improvement of wheat traits via indirect selection based on particular characters (KHODADADI *et al.*, 2011; BEHESHTIZADEH *et al.*, 2013; DEGEWIONE and ALAMEREW, 2013; KRZYSKO *et al.*, 2013; KHAN *et al.*, 2015; MISHRA *et al.*, 2015; DUTAMO *et al.*, 2015).

For this purpose, present study was conducted to i) perceive the association and the scope of genetic variation among wheat advanced breeding lines based on productive traits, ii) to determine the relationship among yield and yield components and iii) to ascertain which characteristics could be used for indirect selection further on in the breeding program.

MATERIALS AND METHODS

Plant material and experimental design

This study was conducted with 49 advanced lines of winter wheat (*Triticum aestivum* L.) obtained by positive selection of hybrid progenies. The experiment was carried out during two years (2013/14 and 2014/15) at two locations, Skopje and Gradsko, in Macedonia. RCBD design was applied with three replications. Each plot consisted of 10 rows, 1 m long, with 12cm distance between rows and 10 cm in the row. During the growth season, standard crop management practices were applied. Ten plants from each plot were randomly collected for analyses, selected from the middle rows. Following quantitative traits were recorded: plant height (PH), number of fertile tillers (NT), spike length (SL), number of spikelets per spike (NS), number of grains per spike (NG), weight of grain per spike (WGS), weight of grain per plant (WGP), fertility (F), biological yield (BY) and harvest index (HI).

Statistical analyses

The mean values of the genotypes for the analyzed quantitative traits were analyzed with different packages in the R 3.0.3 statistical software. Principal component analysis (PCA) was performed by using “psych” package (REVELLE, 2014) where data matrix is standardized by default, meaning that component scores are standard scores (mean=0, sd = 1). Components were extracted until the eigen value > 1. Two-way cluster analysis was carried out with the quantitative traits that were highly correlated with the first three principal components. Euclidian distance between the genotypes was calculated by use of standardized values of the selected traits. The obtained values were normalized according to ROLDAN-RUIZ *et al.* (2001) and cluster dendrogram was created by UPGMA method. The optimal number of clusters was estimated by multiscale bootstrap resampling for evaluation of uncertainty in hierarchical cluster analysis (SUZUKI and SHIMODAIRA, 2013). For each cluster, the *p*-values were calculated (a value between 0 and 1) and the clusters with approximately unbiased (AU) *p*-values larger than 95% after 1000 bootstrapping replications were taken to be strongly supported by data. This analysis resulted with a dendrogram which was used as a row dendrogram in the heatmap, while the column dendrogram was obtained in relation to the row dendrogram. Two-way cluster was generated by use of “heatmap.plus” package (DAY, 2012).

RESULTS AND DISCUSSION

Principal component analysis

PCA represents multivariate technique which can be effectively used for identification of components that are positively or negatively correlated with a certain plant character. Characters with high correlation gave higher input to the variation pattern of the accessions, thus they could be used as effective traits for indirect selection of plants in early generations.

By the application of principal component analysis three PCs were determined with eigen values > 1. They accounted for 71.391% of the variation among the wheat genotypes used in this study (Table 1).

Table 1. Principal components scores for the analyzed yield components

Characters	Factor loadings		
	PC1	PC2	PC3
PH	0.104	0.479	-0.140
NT	0.290	0.007	-0.462
SL	0.179	0.193	0.563
NS	0.286	0.233	0.497
NG	0.298	-0.450	0.217
WGS	0.430	0.030	0.061
WGP	0.517	0.050	-0.185
F	0.109	-0.563	-0.113
BY	0.313	0.306	-0.318
HI	0.374	-0.248	0.062
SS loadings	3.067	2.522	1.550
Proportion of Variance	30.673	25.222	15.495
Cumulative Variance	30.673	55.895	71.391

One third of the total variation is explained by the first PC that is positively correlated with all traits. The significant breeding parameters, weight of grains per spike and weight of grains per plant that directly influence the yield performance were highly correlated with PC1. They are followed by harvest index and biological yield, traits with indirect impact on the yield. This correlation suggests that PC1 reflects the yield potential of each genotype. The second PC explained 25.22% of the total variance, mostly in relation to the plant height. This component was highly negatively correlated with fertility and consequently with the number of grains and harvest index. The last component explains the smallest proportion of the variation (15.49%). It was strongly positively associated with spike length, number of spikelets and number of grains per spike and can be regarded as spike component. This component was negatively associated mainly with plant growth (number of tillers, biological yield and plant height) differentiating lines according to their vegetative characteristics.

PC analysis revealed that principal discriminatory characters representing the clusters were SL, WGP, NS and PH, with their positive contributions as well as and F and NT with their negative contributions to the genetic diversity of the analyzed lines. Similar results for plant height and grain yield obtained by PCA are reported from KHAN *et al.* (2015) and MISHRA *et al.* (2015). Beside these two characters, DEGEWIONE and ALAMEREW (2013), BARANWAL *et al.* (2013), MAQBOOL *et al.* (2010), AJMAL *et al.* (2013) and JANMOHAMMADI *et al.*, 2014 determined that NS, SL and NT are also highly contributing to diversification of genotypes. On the contrary, MEENA *et al.* (2014) found out that along with the plant height and grain yield, biological yield and harvest index are contributing to diversity.

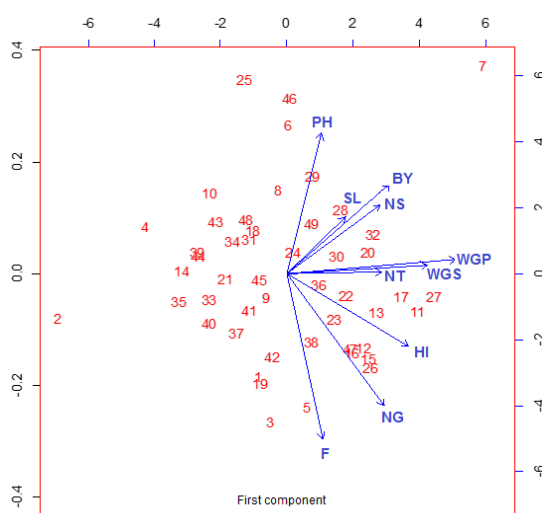


Figure 1. Biplot of PC1 and PC2 representing correlation between the genotypes and quantitative traits

PC1 and PC2 factor loadings were plotted on Figure 1, where location of 49 genotypes displays the relation to the values of their characters. YAN and RAJCAN (2002) noted that cosine of the angle between trait vectors approximates the correlation coefficient between the traits, where, according to YAN and KANG (2003), correlation is positive if angles are acute ($<90^\circ$) and negative if angles are obtuse ($>90^\circ$).

The strongest positive association in this study indicated by acute angles between the vectors was determined between SL, NS and BY and between NT, WGS and WGP. All traits from these two groups are also positively correlated. Two of them, BY and WGP, have highest magnitude of effect on the yield, determined by their vectors length (YAN and TINKER, 2005). The first group of traits (SL, NS and BY) is positively correlated with PH, negatively with NG and F, while almost not correlated with HI. The second group (NT, WGS and WGP) is in lower positive correlation with HI and NG and almost not correlated with F. Plant height (PH) showed high magnitude as well and negative association with HI, NG and F, which is good position as shorter genotypes are preferred in the wheat breeding programs. The last three traits are in positive correlation and have high effect on the yield. Certain variations between the original data and plot display are expected having in mind that PC1 and PC2 explain only 86.4% of the total variation. Location of the genotype, i.e. its distance from the biplot origin measures how it differs from a hypothetical “average” genotype located at the biplot origin that has an average level for all traits (YAN and FREGEAU-REID, 2008). Long vectors of the genotypes 7, 25 and 2 indicate that they have extreme values for one or more traits, out of which genotype 7 is particularly superior line for its high positive values of almost all traits.

Two-way cluster analysis

First two PCs refer to the largest amount of genotypes variability and highly correlated traits with these components were used for two-way cluster analysis. The analysis resulted with a

dendrogram representing the genetic distance (degree of similarity) among 49 wheat genotypes. According to the analyzed data based on 1000 iterations, one genotype (7) did not belong to any cluster. Genotype 7 is solely separated and most distanced from the others due to its high values for all traits except for fertility and harvest index. All other genotypes were classified in two main clusters, out of which, only genotypes 2 and 14 belonged to the first one, and the rest of the genotypes form the second cluster. In the second cluster, two main subclusters can be identified (marked with red rectangles), consisted of genotypes that are very similar considering analyzed traits (Figure 2). This analysis reveals the presence of wide genetic diversity among the experimental material, according to grouping within the main clusters.

The traits were classified in two groups as well, each of them divided on 2 subgroups (Figure 3). NS and SL along with BY and PH belonged to the first group. The second group of traits has one subgroup consisted of NG and F and other subgroup where NT, WGP, HI and WGS where classified.

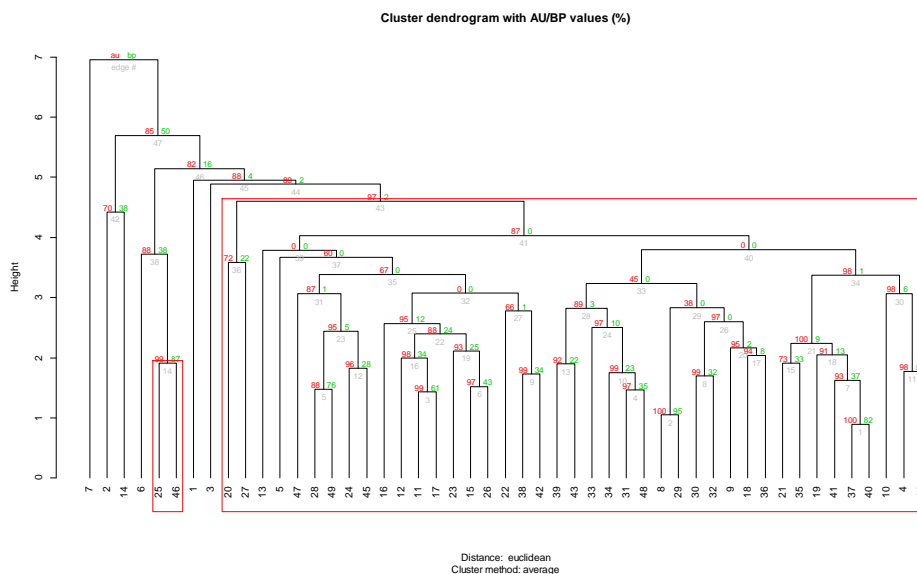


Figure 2. Dendrogram representing clustering of the analyzed wheat lines

First subcluster of genotypes (2 and 14) is characterized with high values for biological yield and plant height, low values for number of grains and fertility and moderate values for the other analyzed traits. The second subcluster could be divided in 2 main groups which is noticeable on the heat map (Figure 3). Most of the genotypes from the first group (20-42) have high values for all traits with few exceptions mainly for number of spikelets, spike length and number of tillers. The genotype 26 can be also considered as superior due to its high values for all traits except for very low plant height that is desirable characteristic for wheat. The second group of genotypes (39-44) of cluster II could be further on divided on a subgroup of genotypes (39-36) with high values for number of spikelets and spike length and high to moderate values

for the other traits and a subgroup with genotypes 21-44 with moderate to low values for most of the analyzed traits.

Superior genotypes in some clusters might be usefully involved in hybridization programmes for obtaining transgressive segregating lines with high genetic yield potential. Beside genotype 7 which could be a candidate for releasing variety, all other genotypes not belonging to any cluster (2, 14, 6, 1 and 3) could be used in the breeding program for improvement of certain characteristics for which they show high values. For example, the genotypes 3 and 1 represent desirable parents for increasing fertility and harvest index, respectively, as they have the highest values for these traits.

Clustering of wheat breeding material for tracking superior genotypes was applied by a number of researchers. According to the existing diversity within the analyzed genotypes and different methods for the applied analysis, number of clusters in their studies varies from nineteen (MEENA *et al.*, 2014), seven (KHODADADI *et al.*, 2011), six (MAQBOOL *et al.*, 2010 and DEGEWIONE and ALAMEREW, 2013), five (AJMAL *et al.*, 2013), four (BARANWAL *et al.*, 2013 and KHAN *et al.*, 2015) to three (AHMAD *et al.*, 2014). Similar to our results, MISHRA *et al.* (2015) performed genotype x trait cluster analysis and found two major clusters with several subclusters.

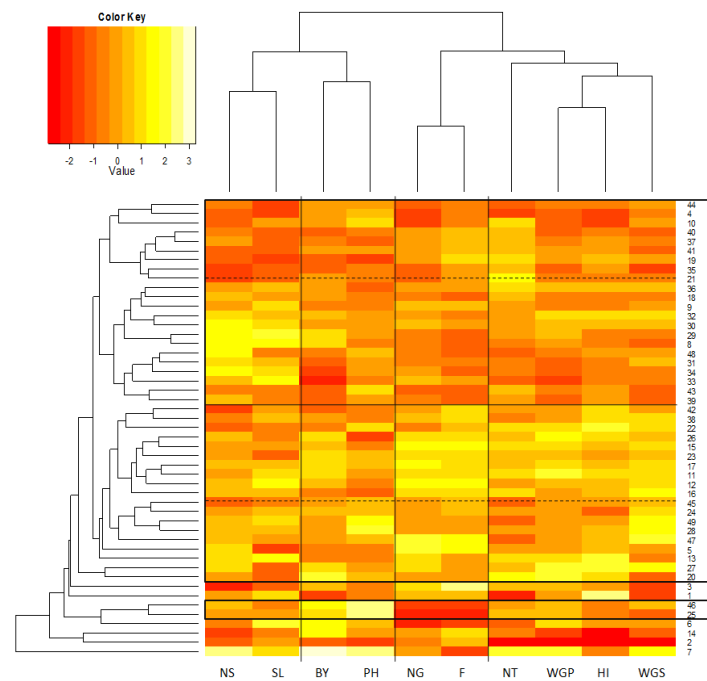


Figure 3. Two-way cluster with classification of genotypes according to the effect of the analyzed traits

CONCLUSIONS

The existing genetic variability between the wheat genotypes analyzed in this study was effectively evaluated by PCA and two-way cluster analysis. Three main PCs explained over 71% of the variation among the genotypes. Spike length, weight of grains per plant, number of spikelets, plant height, fertility and number of tillers were principal discriminatory traits. Information obtained by biplot graph will be used for selecting parents with high values of traits that are positively associated and have high effect on the yield. Genotypes 7, 25 and 2 with extreme values for more traits will be included in the future breeding plans, even more genotype 7 will be exploited as a candidate for direct release as variety.

Two-way clustering enabled effective overview and easy determination of the breeding material that share common set of characteristics. Two main clusters of genotypes were defined with wide genetic diversity especially between the groups within the second cluster. Genotypes from the first group of the second cluster with lower values for spike characters and tillering potential should be crossed with the genotypes from the second group that have high values for these characteristics. The genotype 26 also represents a superior line with high values for all traits and very low value for the height of the plants. The extend of variation among the advanced lines and their classification was clearly illustrated on the heatmap, presenting this technique as an practical tool for selecting genotypes for desired traits in the early stages of the breeding process.

In general, based on germplasm classification, diverse parents from various clusters could be integrated in planning and broadening of breeding programme by increased use of heterosis and genetic diversity. Superior lines based on their performance for the targeted character should be used in hybridization plans for improvement of different plant characters and genetic yield potential. This will reduce the overall time and labor required to screen large germplasm for potential breeding material.

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PROCENA NOVIH SORATA ZIMSKE PŠENICE PRIMENOM STATISTIČKE MULTIVARIANTNE ANALIZE

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Izvod

Izvršena je evaluacija 49 novih sorata zimske pšenice (*Triticum aestivum* L.) u cilju sagledavanja morfoagronomskih osobina u cilju određivanja najboljeg kriterijuma za selekciju sorata koje će biti uključene u budući program oplemenjivanja. Materijal je ocenjivan u toku dve godine na dve lokacije, primenom RCBD dizajna, u tri ponavljanja. Ocenjivano je deset (10) kvantativnih osobina: visina biljke, broj fertilnih grančica, dužina klasa, broj klasića u klasu, broj zrna u klasu, težina zrna po klasu i po biljci, plodnost, biološki prinos i žetveni indeks su ocenjivani primenom PCA i klaster analizom na dva načina. Tri glavne principijal komponente su determinisane objašnjenjem 71.391% ukupnog variranja među genotipovima. Trećina variranja je objašnjena sa PC1 koji predstavlja potencijal prinosa genotipa. PC2 i PC3 objašnjava 25.22% i 15.49% ukupne varijanse, uglavnom u odnosu prema visini biljke i komponenata klasa. Biplot grafikon potvrđuje najjaču pozitivnu asocijaciju između dužine klasa, broja klasića i biološkog prinosa i između broja grančica, težine zrna i težina zrna po klasu i po biljci. Genotipovi sa visokim vrednostima za specifične osobine će biti uključeni u buduće programe oplemenjivanja.

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