## RESEARCH ARTICLE



# Characterization of bread wheat (T. aestivum L.) germplasm stored in Albanian gene bank based on agronomical and biochemical markers

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#### **Abstract**

Study for characterization of genetic diversity presented in the 100-bread wheat (*T. aestivum*) genotypes of local origin, stored in Albanian genebank, using 12 quantitative traits (6 morphological traits and 6 biochemical markers) was carried out. The study was realized in the Experimental Field of Agriculture Institute of Peja (Kosovo) during the 2015-2016 seasons. Variance analysis, cluster on correlation and principal component analysis reveal presence of significant variability between and the association among quantitative traits. Principal component analysis and cluster analysis on correlations divide the whole wheat genotypes into five cluster groups in respect of genetic diversity and similarity among bread wheat accessions. Higher number of the wheat accessions was included into the first cluster group (45 genotypes) and into the fourth cluster group (26 genotypes). Highly significant positive correlations were found between days to flower, plant height, spikelet time, and protein content and gluten traits (coefficient of correlation range from 0.308 to 0.958). Relationships analysis between the quantitative traits and wheat genotypes using principal component analysis show that there are six traits with larger values in PC1 that account for 40.86% of total variance. The study identifies traits with agronomic interest that account for genetic diversity and which will facilitate the maintenance and agronomic evaluation of the wheat collections.

Keywords: genetic diversity; wheat genotypes; quantitative traits.

#### 1. Introduction

Development of wheat cultivars with high production capacity and excellent quality, which meets market requirements, is the goal of each breeding program. The genetic improvement of wheat intends to obtain the best characters of the new cultivar. Realizing of this objective has determined the choice of the parental forms. The genetic variance, genetic gain and heritability estimations are of great importance in plant breeding programmes. Plant breeders estimate genetic variances in their populations so that they can predict the response to selection, determine the best selection and breeding procedure for the populations. The magnitude of heritable variability and more particularly its genetic components is clearly the most important aspects of the genetic constitution of the breeding material, which has a close bearing on its response to selection [7]. Different study results suggest possible parental lines among the bread wheat accessions analyzed can be selected and utilised for sustainable field bread wheat breeding programs [2,3, 10, 11,16,18,19,21]. Some of the bread wheat accessions found in each cluster group could be explained by common parent origin in their pedigree [11, 15]. The study of the Albanian wheat base collection showed presence of high genetic diversity between wheat cultivars created and cultivated in the whole surface of Albania for over 20 years [6, 7]. Plant height for two sets of parents had average approximate value, but the genetic variability and range of the variability within the group was higher than among groups [8]. In the domestic wheat accessions, the content of wet gluten is also high, and ranges from 21.60 % to 38.80 %, with an average value 28.40 % [17]. The protein content, as an indicator of the quality of grain, ranges from 12.47 % to 17.00% with an average value 14.18%, which are considered high values [1].

Study of genetic diversity maintained in the base wheat collection of *T. aestivum* genotypes of local origin, stored in Albanian genebank, using 12 quantitative traits (6 morphological traits and 6 biochemical markers) was the objective of the study for characterization of 100-bread wheat genotypes.

## 2. Material and Methods

**Plant materials:** In this study, we used winter wheat (*Triticum aestivum* L.), 100 accessions by base collection of Albanian Gene Bank, conserved during 1999-2001.

**Experimental site:** The study was conducted in Agriculture Institute of Kosovo, in Peja of the Republic of Kosovo. It lies at an altitude of 513 above sea level and at Latitude 42°41'21.10"N and Longitude 20°19'31.14"E. Climate in this region is continental, the altitude, mean annual temperature 16-17 °C and a yearly precipitation of 700 mm. The soil type is yellow colour on sandy sediment.

**Method:** Experiment carried one replication during the autumn season of 2015/2016. Each accession was planted in 2.5 m long plot with a between-row spacing of 25 cm and within-row spacing 10 cm.

Fertilizer was broadcast on the plots at the rate of 400 kg ha-1, N.P.K. 8:16:20.

At physiological maturity, seeds were harvested and after they were cleaned for analysis.

The traits: sowing-germination (SG), growth class (seasonality), spikelet time (ST), days to flower (DF), falling of plants tillering capacity (Fall) (TC), were investigated.

Grain yield components, plant height (PH) (cm), 1000-seed weight (AW) (g), hectolitre weight (HW) (kg) were analyzed.

Biochemical traits, seed humidity (HC) (%), protein content (PrCo) (%), gluten (Glut) (%) and sediment (Sed) (%) were analyzed.

## Data analysis

Descriptive statistics and Analysis of variance of quantitative agronomical traits was carried out. The mean values, standard deviations, cluster analysis, error of different accessions, subjects of the observed variables, were found significant at the level of  $P_{0.05}$  and  $P_{0.01}$  of probability. Comparing of the eigenvalue for each factor was realized using the minimum eigenvalue criterion [14]. The maximum information for analysis of the relationships among bread wheat accession and morphological traits was received using ordination methods (principal coordinate's analysis) in combination with cluster analyses [13]. The principal variables selected for evaluation of ex situ collections in gene bank [4]. The traits with more significant weighting on respective PC variance can be utilized successfully as quantitative markers for evaluation, characterization and classification of the wheat germplasm [18, 16, 11] stored in gene bank, and in plant breeding programs [21, 20, 9].

# 3. Results and Discussion

ANOVA and cluster analysis showed the presence of variability among the bread wheat (T. aestivum L.) accessions and between quantitative agronomical traits analysed. Highly significant variation was found in all quantitative traits, except for SG and HC not significant at the probability  $P_{0.05}$ . Highly significant variances among agronomical traits as Sed, DF, ST and AW were found. The high amount of genetic variability found in the present study suggests the Albanian bread wheat (T. aestivum L.) germplasm has considerable level of variance available to the breeders and it is sufficient for the creation of new favourable gene combinations. Study results (Table 1) in concordance with [3,18,2,21,10,11,16,19] suggest possible parental lines among the bread wheat accessions analyzed can be selected and utilised for sustainable field bread wheat breeding programs.

**Table 1.** Descriptive statistics and Analysis of variance of 10 quantitative agronomical traits

Traits	Descriptive Statistics		Cluster		Error			_
	Mean	Std.	Mean		Mean		_	
		Deviation	Square	df	Square	df	F	Sig.
SG	17.6500	2.68319	14.583	1	7.124	98	2.047	.156
ST	163.3200**	7.67507	4412.350	1	14.484	98	304.641	.000
DF	167.7400**	7.93104	4839.688	1	14.159	98	341.817	.000

PH	1.0913**	.24326	2.950	1	.030	98	99.419	.000
AW	47.7446**	5.41937	234.655	1	27.275	98	8.603	.004
HW	76.7874**	2.53849	87.933	1	5.612	98	15.668	.000
PrCo	16.1749**	1.37740	46.215	1	1.445	98	31.982	.000
Glut	36.4790**	3.63727	281.820	1	10.489	98	26.868	.000
Sed	62.8059**	8.87530	1484.668	1	64.425	98	23.045	.000
HC	10.7490	.40887	.013	1	.169	98	.079	.779

F – F-ratio; Sig.- significance level (\*) equal to the 0.05 and (\*\*) equal to the 0.01 of probability. df- degree of freedom

Relationships between bread wheat accessions assessed by morphological data and genetic similarity/distances revealed by cluster analyses (Euclidean distances) ranged bread wheat accessions into the five different cluster groups. Analysing the number of cases in each cluster group, results that the first cluster group include 45 bread wheat accessions, the second and the five cluster groups includes each respectively 13 bread wheat accessions and the fourth cluster group includes in total 26 bread wheat accessions. Cluster analysis identified the most number of bread wheat accessions (45 accessions) with similarity between them were included in the first cluster group. The contribution of all these bread wheat accessions on the total variance was found less in comparison with the contribution of bread wheat accessions of the other groups. Similarity among some of the bread wheat accessions found in each cluster group could be explained by common parent origin in their pedigree [11, 15]. The higher estimated genetic distance found especially among bread wheat accessions of different genotypes that can be utilized for genetic improvement without losing genetic diversity in bread wheat germplasm. Clusters were differentiated especially by ST, DF, PH, Sed, HW, AW and Glut agronomical traits significant at the probability  $F < P_{0.01}$ .

Principal components analysis (PCA) on correlations of quantitative agronomical traits identified the variances of the principal components (PC) and the proportion of the total variance accounted for by each factor. Comparing the eigenvalues for each factor using the minimum eigenvalue criterion [14], only three PC were maintained for further analysis. The fourth PC component that account for 9.76% on the total variance, are not retained (Table 2). The maximum information for analysis of the relationships among bread wheat accession and morphological traits was received using ordination methods (principal coordinate's analysis) in combination with cluster analyses [13]. PCA on correlations identified the total variance of the principal components (PC) and the proportion of the variances explained by each factor. All quantitative variables contribute to 100% of total variation. The first three PCs explain 72.66% > 70.0% of the total variation, acceptable for evaluation of ex situ collections in gene bank [4, 13].

**Table 2.** Eigenvalues matrix of principal components analysed (100 bread wheat accessions and 10 quantitative agronomical characters).

Principal Components/factor analysis							
PC No.	Eigenvalue	Percent variance	Cumulative Percent	$\chi^2$	DF	Prob. $> \chi^2$	
1	4.0857	40.857	40.857	1226.89	44.221	<.0001**	
2	2.0220	20.220	61.077	992.699	41.422	<.0001**	
3	1.1581	11.581	72.659	864.707	35.701	<.0001**	
4	0.9767	9.767	82.426	795.713	28.440	<.0001**	
5	0.8265	8.265	90.691	714.559	21.623	<.0001**	

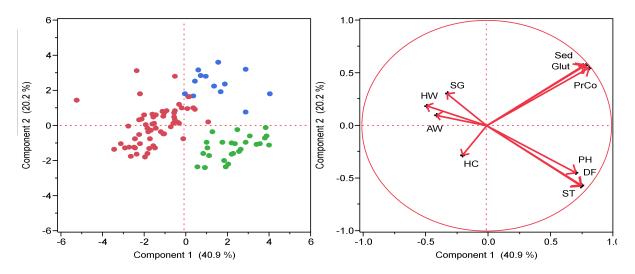
 $<sup>\</sup>chi^2$  – Chi Square, DF – degree of freedom; Prob. – probability; \*\*significance level 0.01 of probability

**Factorial analysis** indicates that the contribution of each bread wheat accession and of each quantitative agronomical trait on the total of variation is not equal. Dimensional scaling of relationships (accession x traits) that accounts for the larger proportion of the total variance in PC1, PC2 and PC3 revealed by PCA identified

highest weighting (PC1 = 40.9%) of total variation was explained by 13 bread wheat accessions included in PC1. There were 26 bread wheat accessions in PC2 that account respectively for 20.2% of total variation (**Figure 1**).

The higher level of variation showed by bread wheat accessions (Cluster II and V), can be explained by the "wild nature" of variation source between these accessions. This proved no any or less breeding activity was carried out with these bread wheat genotypes. Some of these bread wheat genotypes could have interest as possible reserve of desirable traits (genes) for breeding schemes. The lowest weighting of total variation was showed by bread wheat accessions included in the first cluster groups (45 bread wheat accessions). These accessions showing low level of genetic diversity were more uniform. Uniformity of these accession could be ascribed to possible their inclusion in modern breeding programs that usually result in low level of genetic diversity.

Factorial analysis and dimensional scaling for relationships among the quantitative characters, showed the first component (PC1) explained 40.9% of the variation and was positively related to the seven quantitative agronomical traits (ST, DF, PH, PrCo, Glut, Sed and TC) with eigenvalues more than 0.30 and a coefficient of correlation (r) that range from 0.308 to 0.958. Four variables (ST, DF and Sed highly correlated (coefficient of correlation (r) range from 0.710 to 0.933) with nearly the same value of eigenvectors, are the same important to the PC1.



**Figure 1**. Relationships among the 100 bread wheat accessions based on quantitative agronomical traits revealed by principal component analyses.

**Table 3.** Eigenvector values for three principal components of 10 quantitative traits in bread wheat.

Morphological		Eigenvectors				
Quantitative Character	PC1	PC2	PC3			
Sowing –germination	SG	-0.15659	0.21349	-0.26631		
Spikelet Time	ST	0.37406	-0.40235	0.09173		
Days to flower	DF	0.37484	-0.39748	0.09626		
Plant height	PH	0.35283	-0.31357	-0.05637		
Absolute Weight	AW	-0.19845	0.06827	0.60384		
Hectolitre Weight	HW	-0.23793	0.13286	0.28776		
Protein content	PrCo	0.40508	0.38614	0.10231		
Gluten (%)	Glut	0.39299	0.40526	0.12826		
Sediment	Sed	0.38342	0.40281	0.16887		
Humidity Content	HC	-0.09503	-0.19572	0.63668		
Falling of plants	Fall	0.28006	-0.70877	-		
Tillering capacity	TC	0.70711	0.30734	-		

(In bold eigenvectors > 0.30).

The second component (PC2) explained 20.2% of the variation and was positively related to Sed, Glut, PrCo and TC traits. The correlations among these traits range from 0.277 to 0.92. Variables as Glut and Sed (with nearly

the same value of eigenvectors), are the same important to the PC2. In this study traits as PrCo, Glut and Sed of PC2 account for nearly the same amount of variance on PC1 and PC2. The traits ST and DF showed a substantially negative weight on PC2. The third component (PC3) was positively related to AW and HC traits (Table 3, Figure 1). Cluster and principal components analysis on correlations, analysing separately two qualitative traits: Falling of plants (Fall) and Tillering capacity (TC) determined that these two traits were moderately correlated among them (coefficient of correlation r = 0.46). The TC trait showed the most important weight in the PC1 that contribute with 73% of variance on the total variance (Table 3). Good understanding of the most important quantitative agronomical traits in bread wheat can facilitate identification of any individual accession and selection of desirable traits (genes), increasing the information and the representativeness of the wheat germplasm [15] in gene bank. The traits with more significant weighting on respective PC variance can be utilised successfully as quantitative markers for evaluation, characterization and classification of the wheat germplasm [11,16,18] stored in gene bank, and in plant breeding programs [21, 20, 9]. Assessment of the genetic diversity, identification of differences/distances among bread wheat genotypes (where some possible genotypes can be selected as parental lines) and characteristics with potential for future genetic programs within the Albanian genebank bread wheat collection, can be considered as a useful step for sustainable wheat breeding in Albania.

## 4. Conclusions

The field trials accomplished in this study permitted the assessment of the most important agronomical traits and determined the patterns of variation of Albanian bread wheat germplasm with potential for sustainable their future breeding programs.

- PCA results showed the first three PCs account for a substantial proportion of total variation, 72.66%. The percentages of total variation accounted for by each of the first three PCs were 40.9%, 20.2% and 11.58%, respectively. Cluster analysis clearly ranged bread wheat accessions with similarity between them into the five different cluster groups, and allowed the identification of wheat accessions with large variability.
- The study identified the agronomical traits with more significant weighting on PC variance (ST, DF, PH, Sed, HW, AW and Glut) significant at the probability  $F < P_{0.01}$ .
- The significant differences found in the present study show the existence of a high genetic variability among the bread wheat genotypes and quantitative traits analysed, sufficient for selection of desirable traits, and creation of new favourable gene combinations. Possible parental lines among these bread wheat genotypes could be selected and utilised for sustainable field wheat breeding programs.

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