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The genetic diversity and variation in crude protein content of wheat (*Triticum aestivum* L.) promising cultivars for breeding in Albania

Zróżnicowanie genetyczne i zmienność zawartości białka surowego obiecujących genotypów pszenicy (*Triticum aestivum* L.) do hodowli w Albanii

Summary. The genetic diversity and variation in crude protein content among eleven wheat genotypes, comprising three elite local genotypes and eight wheat genotypes of foreign origin were investigated in the present study. Variability was evidenced in grain protein content estimated by biuret test, it ranged from 9.5 to 13.9% with mean of 11.58%. Comparative analysis between local and introduced wheat genotypes revealed that the local genotypes had lower protein content than those of foreign origin. Fourteen polymorphic RAPD markers were used to assess genetic diversity among selected wheat varieties. The mean similarity among wheat genotypes was 67%. Genetic similarity among local wheat varieties was higher (83%) than among those of foreign origin (66%). The wheat genotypes were grouped into two main clusters on UPGMA dendrogram constructed based on Dice similarity coefficients. A clear clustering of genotypes according to the origin was observed. This clustering was also supported by principal coordinate analysis (PCoA) results. There was no observed clustering based on the protein content. The data revealed that local wheat genetic had narrow genetic diversity, however the wheat genotypes of foreign origin constitute a promising material to be employed in breeding programs aiming the increase of wheat protein content and genetic diversity.

Key words: *Triticum aestivum*, RAPD, genetic diversity, total protein content

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important cultivated cereals in the world. Genetic improvement of cereals, including wheat had primarily focused in the im-

proving yield traits and selecting superior genotypes. These practices led to narrowing of genetic diversity in this crop and as consequence increasing its vulnerability to biotic and abiotic stresses [Mir et al. 2012]. Moreover, the improper conservation of genetically improved germplasm worsens results in genetic erosion in these crops. Therefore, more emphasis should be placed to the monitoring of genetic diversity [Fu 2006]. Genetic erosion of crop landraces including wheat was observed in many countries [Sthapit et. al. 2020] as well as in Albanian wheat landraces [Hammer et al. 1996]. Incorporating new germplasm by breeding programs have been able to maintain a quantitative level of genetic diversity in wheat over time [Sthapit et. al. 2020]. Hence, it is crucial to assess the existing genetic diversity of local wheat germplasm as well as of the wheat genotypes of foreign origin that showed best adaptability of the eco-climatic conditions. Various molecular marker techniques have been used to assess the genetic diversity in order to enhance the efficiency of plant breeding in wheat. RAPD marker assay provides a simple low-cost technique that has been efficiently applied in genetic diversity studies in wheat [Mandoulakani et al. 2010, Abdellatif and Abouzeid 2011, Cifci and Yagdi 2012, Khaled et al. 2015, Al-Kaab et al. 2016].

Several studies assessing the variability in yield and agronomic traits of wheat germplasm in Albania have been carried out [Xhulaj et al. 2019, Xhulaj and Gixhari 2020, Xhulaj and Koto 2022]. To our knowledge the molecular characterization and comparative assessment of genetic diversity of local and introduced wheat genotypes was not previously investigated. Therefore the present study aims to determine the genetic diversity of local and some selected well adapted Italian wheat varieties by molecular markers. To investigate the variation in crude protein content, to provide knowledge on the flour quality of wheat cultivars. The knowledge will contribute to the sustainable use of local varieties explore the potential of introduced varieties for breeding purposes and implementing best breeding practices to increase the genetic diversity and quality of wheat germplasm.

MATERIAL AND METHODS

Plant material and total protein content analysis: In this study, seeds wheat (*Triticum aestivum* L.) were kindly provided by Agriculture Technology Transfer Center of Lushnje. A total of 11 wheat genotypes from which three genotypes Progresi, Dajti and LVS were the most cultivated local varieties and eight genotypes Arabia, Tiepolo, Solehio, Andana, Anforeta, Masacio, Stendal and Sobald were of Italian origin, introduced and tested about their adaptability and some important qualitative and yield traits.

The mean weight of 1000 seeds was calculated for each cultivar based on the three weight repetition of 1000 manually counted seeds. To evaluate the total protein content of sample set, extraction of protein1 from dried grains of wheat was needed. Extraction was performed from 100 g of each feedstock. Dry wheat grains were grinded to form a fine flour. Proteins were extracted from the ethanol treated with distilled water, 5% NaCl, 70% ethyl alcohol and 0.1 N NaOH to obtain different fractions of proteins [Tan et al. 2012]. The whole procedure was performed at room temperature for 30 min. Quantitative determination of protein content in wheat extracts was evaluated by Biuret test. Bovine serum albumin was used to generate standard curves. A calibration curve of absorbance versus protein concentration was prepare using a series of protein solution of known concentration. Clean, dry cuvettes were used for each sample and 1–2 ml of the test solution

and deionized water was added in the respective test tubes. Biuret reagent was added to all test tubes, shaken well and left to incubate up to 10 min. Color change was observed. A deep purple colored product of Biuret complex is formed when Biuret reagent reacts with the protein in the mixture. Spectrophotometric methods were used to measure the absorbance at 540, using UV-1600PC Spectrophotometer. All extractions and analyses were carried out in ten replicates and then the results were expressed as mean ± standard deviation (mean ±SD).

DNA isolation and RAPD analysis. The seeds of each accession (30 seeds/each cultivar) were sown into pots containing peat moss for germination, in 22–25°C, occasionally irrigated until full germination. The DNA isolation was carried out from fresh leaves of two weeks seedlings by using CTAB method as described by Kump and Javornik [1996]. The genetic diversity among accessions was assessed by means of fourteen decametric markers (RAPD). The PCR assay was performed in a 15 µl reaction mixture containing 1x reaction PCR buffer, 2mM $MgCl_2$, 0.2 mM of each dNTP, 0.2 µM primer and 0.3 U of *Taq* DNA polymerase (Thermo Scientific) and 20 ng of DNA template. The amplification protocol consisted of 94°C for 1.5 min, 36–40 cycles of 94°C for 30 s, 36°C–40°C for 45 s, 72°C for 1 min followed by final elongation at 72°C for 5 min. The PCR products were visualized by conventional 1.5% agarose gel electrophoresis, stained with ethidium bromide and photographed under UV illumination. The DNA banding patterns were analyzed PyElph version 1.4 software [Pavel and Vasile 2012], the presence (1) and the absence (0) of each amplified fragment for each accession and RAPDs was recorded in a binary matrix. Number of scored bands (NSB), number of polymorphic bands (NPB) and polymorphism percentage (PP) were calculated for each primer used. The polymorphism information content (PIC) was computed for each locus according to Botstein et al. 1980, while the discrimination power (D) was calculated according to Tessier et al. [1999]. In addition, the assay efficiency index (AEI) as a ratio of total polymorphic fragments obtained in the analysis and the total number of primers used was provided. Marker Index (MI) was calculated based on Powell et al. [1996] and the probability of an individual in a population being heterozygous in the locus, expected heterozygosity (H) was estimated according to Liu [1998].

The cluster analysis was performed based on Dice similarity matrix and unweighted pair group method with arithmetic mean (UPGMA) and a dendrogram of relationships was constructed, in addition principal coordinate analysis (PCoA) to investigate the distribution of variation based on the wheat genotypes origin and total protein content was performed using the PAST v.3.06 package [Hammer et al. 2001].

RESULTS

Protein content variation. The weight of 1000 grain and the total protein content as the indicators of yield and nutrition quality were determined for eleven wheat genotypes. The grain weight (g/1000 seeds) ranged from 36 g in Solehio to 44.6 g in Sobald and Anforeta. While protein content ranged from 9.5 to 13.9% with an average 11.58%. The local variety Dajti showed the lowest concentration of protein content (9.5%) while the highest concentration resulted in Italian variety Solehio (13.9%) – Table 1. Three local Progresi, Dajti LVS wheat genotypes had higher seed weight (43.3–45 g) but lower protein content compared to the introduced Italian wheat genotypes (Tab. 1).

Table 1. Total protein concentration and 1000 grain weight (g) in wheat genotypes

Genotype	TKW (g)	Protein concentration (%)
Progresi	43.3	12.9 ±0.7
Dajti	43.6	9.5 ±0.13
LVS	45.0	9.7 ±0.21
Arabia	38.4	10.7 ±0.2
Tiepolo	45.0	11.25 ±0.3
Soleiho	36.0	13.9 ±0.9
Andana	43.6	13.4 ±0.1
Anforeta	44.6	11.74 ±0.3
Masacio	41.6	11.5 ±0.17
Stendal	36.4	10.9 ±0.1
Sobald	44.6	11.9 ±0.2

TKW – Thousand Kernel Weight.

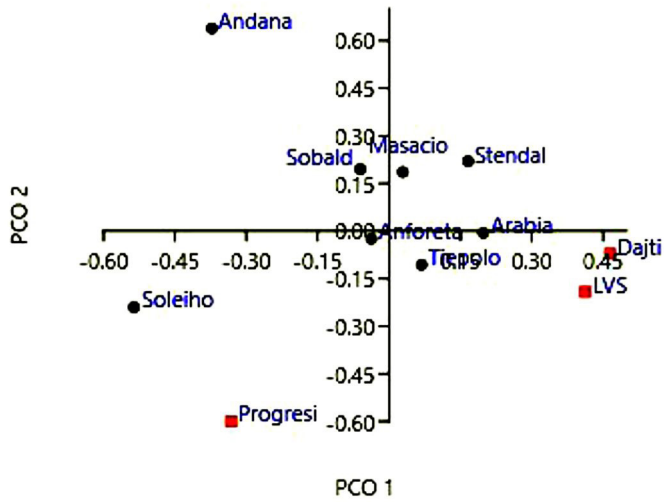

Fig. 1. The PCoA based on the crude protein content of wheat genotypes

The PCoA analysis was carried out to investigate the distribution of variability of the total protein concentration in our sample set. The two principal coordinates (PCO1 and PCO2) explained 84% of the protein concentration variability. An admixture pattern was observed in the scatterplot, the wheat genotypes did no show clustering (Fig. 1).

RAPD assay and genetic diversity indices. To assess genetic diversity among 11 wheat accessions the RAPD assay was employed. The analysis of our sample set with 14 RAPD primers generated 66 different fragments with a mean of 4.71 bands per locus. The lowest number of amplified fragments (3) was detected by using primers OPJ12, OPJ20 and OPA18, whereas the highest number of fragments (7) was amplified by using

primer OPJ04. 87.9% of the total fragments were found to be polymorphic, while the percentage of polymorphism (PP) ranged from 50–100%. The size of amplified products varied between 100 bp (OPJ20) to 2000 bp (OPJ04). The AEI was 4.14 indicating the efficiency of the RAPD assay in our wheat accession discrimination. The mean polymorphic information content was 0.403, it ranged from 0.26–0.59 in the OPA16 and OPA 08, respectively. 92.8% (13) of primers used in this study showed PIC value higher than 0.35. In this study, the Marker index (MI) values ranged from 0.01 (OPJ04 and OPJ12) to 0.6 (OPA17 and OPA08), with a mean of MI = 0.18. The discriminating power (D) showed a mean value of 0.582, it varied from 0.248 to 0.895 in OPAE10 to OPJ04, respectively. Expected heterozygosity (H) ranged from 0.316 (OPA16) to 0.664 (OPA08) with a mean value of H = 0.5. The informativeness of RAPD assay and the genetic diversity indices obtained in the analysis of sample set were presented in the Table 2.

Table 2. Diversity indices of RAPD loci across wheat genotypes

Primer	Size range (bp)	NSB	NPB	PP %	H	PIC	MI	D
OPA17	1200–400	4	4	100	0.498	0.374	0.021	0.781
OPA01	1200–200	5	5	100	0.488	0.369	0.025	0.676
OPJ04	2000–300	7	6	85.7	0.444	0.345	0.013	0.895
OPJ12	800–400	3	2	66.6	0.492	0.371	0.019	0.814
OPA07	1200–200	4	5	100	0.617	0.564	0.616	0.378
OPP10	600–300	4	3	75	0.495	0.372	0.025	0.707
OPJ20	500–100	3	2	66.6	0.485	0.367	0.025	0.661
OPA18	400–200	3	3	100	0.495	0.372	0.020	0.801
OPAE10	600–200	5	5	100	0.500	0.449	0.500	0.248
OPAG04	1000–400	6	6	100	0.454	0.351	0.026	0.579
OPA08	1000–400	4	4	100	0.664	0.591	0.664	0.345
OPA13	1500–500	6	5	83.3	0.477	0.363	0.026	0.636
OPA15	1000–300	5	5	100	0.564	0.501	0.564	0.278
OPA16	1500–400	6	3	50	0.316	0.266	0.023	0.357
Mean		4.64	4.14	87.6	0.501	0.403	1.183	0.582
AEI		4.1	–	–	–	–	–	–

NSB – number of scored bands, NPB – number of polymorphic bands, PP – polymorphism percentage, H – expected heterozygosity, PIC – polymorphic information contents, MI – marker index, D – discriminating power.

Cluster analysis. The genetic relatedness among eleven wheat genotypes was carried out based on pairwise similarity. The dendrogram of relatedness obtained grouped genotypes into two main clusters with a mean similarity of 67%. The similarity ranged from 47% between LVS and Sobalt to 92% between Tiepolo and Solehio. The genotypes Andana, Masacio, Stendal and Sobald grouped together in the first cluster sharing 63% of similarity among them. The second group comprised genotypes Arabia, Tiepolo, Solehio and Dajti, LVS, Progresi which share 79% of similarity among them. However, a clear

clustering of wheat genotypes of Albanian origin was observed in the second subcluster of the cluster II, sharing a similarity of 83% among them. The genetic diversity among all wheat genotypes of Italian origin was 66% (Fig. 2).

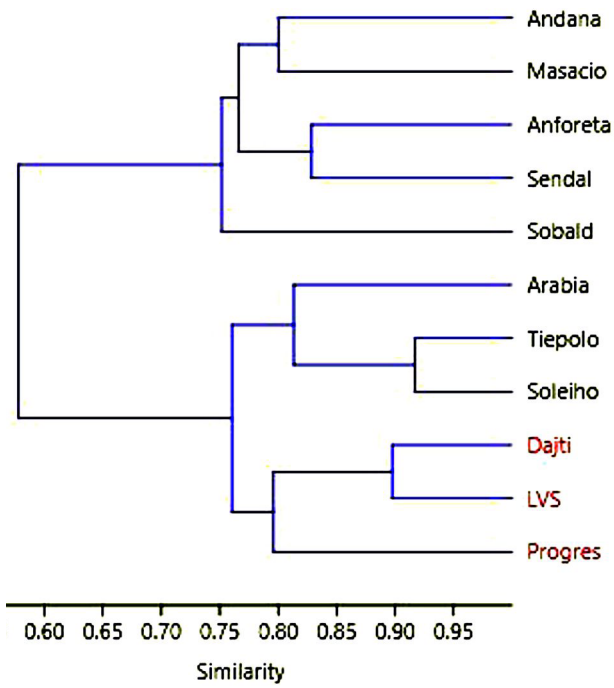

Fig. 2. UPGMA dendrogram of genetic similarities based on RAPD data based on Dice coefficient among wheat genotypes

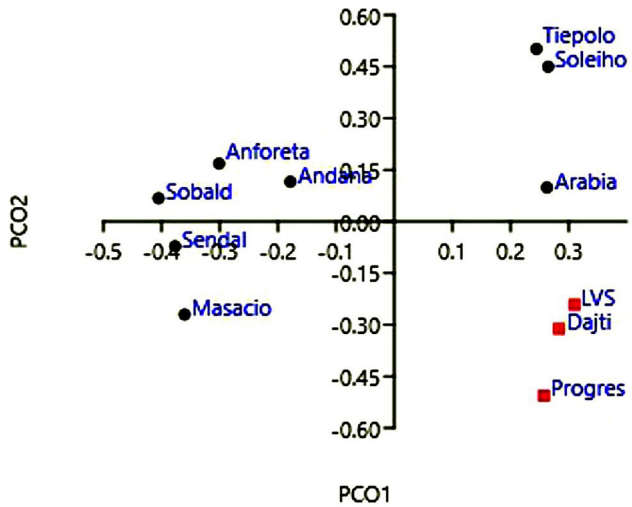

Fig. 3. The PCoA based on the geographical origin of wheat genotypes

The principal coordinate analysis (PCoA) based on the geographical origin showed that the first two principal coordinates explain 41.5% and 14.2% of the total variation, respectively. Wheat varieties clearly clustered according to their origin (Fig. 3) in accordance with the clustering in UPGMA dendrogram. The PCO1 clustered wheat genotypes in the same groups observed in the dendrogram, the local varieties LVS, Dajti and Progresi clustered distinctly. In addition, the Tiepolo, Arabia and Solehio clustered separately from the other foreign wheat varieties, the same differentiation was also observed in the dendrogram given in the Figure 3.

DISCUSSION

The grain total protein percentage is an important component, in determination the quality and the nutritional value of wheat, as it impacts the quality of the bread and other flower deriving food products. The crude protein in wheat varieties was reported to be in the range of 8–15% [Khan et al. 2013] depending on genetic wheat varieties composition. The results obtained in this study showed that the analyzed genotypes had medium-high protein content with an average of 11.58%. Furthermore, comparative analysis between local and introduced wheat genotypes revealed that the local genotypes had lower protein content than those of Italian origin. This may be due to the process of selecting genotypes with higher yields and better adaptability to local ecological conditions carried out in the past. This was also supported by the higher estimated grain weight (43.3–45.0 g), as one of the yield components, of three local genotypes Progresi, Dajti LVS compared to the introduced wheat genotypes (Tab. 1). The principal coordinate analysis performed on eleven wheat genotypes based on their crude protein concentration showed no clear clustering. Similarly, no grouping of wheat varieties was reported in other studies [Punia et al. 2019]. However, a significant correlation between variation in protein content and functional, pasting, color and antioxidant properties of wheat flours was observed [Punia et al. 2019], indicating the protein content as an important trait related to quality. Moreover, the variation of external factors associated with crop might explain the admixture observed in our PCoA analysis, as the protein content varies depending on genetic composition and environmental factors.

In the present study eleven wheat were genotyped by using 14 RAPD primers. To evaluate the RAPD assay usefulness in germplasm evaluation several indices were calculated. Our results showed that RAPD markers employed were highly informative, the mean PIC value was 0.403. 13 out 14 markers had a remarkable level of the informativeness showing PIC > 0.35, indicating the usefulness of these markers in the evaluation of wheat germplasm, thus confirming the reliability of our results. Three out of fourteen markers showed a PIC value higher than 0.5 (OPA07, OPA08 and OPA15). Theoretically, PIC for dominant markers as RAPD markers have values from 0–0.5, slightly higher PIC values were attributed to their equal distribution [Chesnokov and Artemyeva 2015], as well as the low number of samples in our analysis might be another factor of PIC values greater than 0.5 observed in our study. However, PIC values greater than 0.5 in RAPD markers were also reported by Cifci and Yagdi [2012]. The marker index (MI) as another indicator of the usefulness of the RAPD markers in revealing diversity among our sample set showed a mean value of 0.185 which was lower than MI mean values reported in previously published studies which employed RAPD markers in genetic diversity evaluation

in wheat [Khaled et al. 2015]. The RAPD assay efficiency was also supported by the obtained high assay efficiency index (AEI = 4.1) and discriminating power index (D = 0.58).

The knowledge on the genetic diversity of wheat genotypes facilitates the efficient exploitation of the germplasm. Therefore, we investigated the level of genetic diversity among eleven wheat varieties based on RAPD markers. The results showed low levels genetic diversity among analyzed wheat genotypes, they shared the mean similarity of 67%. The diversity was lower within local wheat genotypes (83%), whereas genotypes of foreign origin shared a similarity of 66% among them. The resulted mean value of polymorphic fragments obtained in our study was lower (4.6) than the mean polymorphic fragments reported by other studies on wheat where the RAPD assay was used [Cifci and Yagdi 2012, Khan et al. 2015], moreover the mean expected heterozygosity obtained in our results had average value (H_{mean} = 0.5). In overall, the results indicate narrow genetic diversity of wheat genotypes under study. The cluster analysis based on UPGMA and principal coordinates analysis (PCoA) based on the geographic origin of wheat genotypes showed a clear grouping, the local varieties were clustered distinctly from Italian ones (Fig. 2 and Fig. 3). Differentiation of wheat genotypes according to their origin was reported also by Abdellatif and Abouzeid [2011].

A low level of genetic diversity reduces the ability of the species to cope with adverse abiotic conditions or evolving pathogens, thus high diversity is crucial for species survival especially in climate change scenario [Najaphy et al. 2011]. Therefore, effective utilization is recommended to preserve local elite genetic material which is well adapted and commercially relevant. In addition, wheat genotypes of foreign origin constitute a promising material to be employed in breeding programs aiming the increase of protein content and genetic diversity.

CONCLUSION

The data obtained in this study confirmed the efficiency of RAPD assay to assess genetic diversity of wheat germplasm. The present study revealed low protein content and genetic diversity of local wheat genotypes compared to the introduced foreign wheat genotypes. The selection for yield and local conditions adaption might be the reason of this high similarity observed in local wheat varieties. This information is relevant to avoid genetic erosion of local germplasm and could be helpful for wheat breeders to efficiently maximize the use of genetic resources.

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