Evaluation of diversity in some genotypes of Algerian durum wheat using agronomical and biochemical markers

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Abstract. *Atoui A, Boudour L, Chaib G, Boudersa N. 2021. Evaluation of diversity in some genotypes of Algerian durum wheat using agronomical and biochemical markers. Biodiversitas 22: 2005-2011.* In Algeria, wheat occupies a preponderant place regarding its food richness, and its agronomic characteristics. Quantification and characterization of the local genetic material of different species constitute a strategic axis for the improvement of these species. To quantify the diversity that may exist between nine genotypes belonging to the durum variety "*valenciae*" mainly cultivated in Algeria, biochemical and agronomical parameters were studied. The obtained results revealed highly significant differences for all measured agronomical parameters and a significant positive relationship between grain yield and its compounds. Protein profiles obtained using SDS-PAGE allowed us to split the total seed proteins. The obtained gel has shown different bands with molecular weights ranging from 17 to 122.09 KDa of which ten monomorphic bands, eight polymorphic bands and Three Unique bands in G6 and G9. The total protein polymorphism revealed an intra-varietal variability. This study will help breeders better select genotypes to be used in the Algerian wheat breeding program.

Keywords: Agronomical, biochemical markers, diversity, durum wheat, SDS-PAGE

INTRODUCTION

Since the discovery and domestication of cereals by the first cultivators of ancient civilizations, wheat has always been at the center of political, economic and social issues of the highest rank. Being a major staple food crop in the world, wheat provides an indispensable source of dietary, energy and nutrients to a growing population with high living standards rise in both developed and developing countries (Wang et al. 2020). The cultivation of many old varieties and replacing them with improved varieties has contributed to the loss of many varieties that resulted from processes of adaptation and interaction with the surrounding environment throughout the ages, hence, it is important to preserve genetic resources in a manner that guarantees stability and increase in production and preserving genetic diversity from loss.

Durum wheat (*Triticum turgidum* var. durum) is the most cultivated species in the Mediterranean basin with an arid and semi-arid climate (Bonjean et al. 2016). However, in Algeria the genetic variability of cultivated wheat has decreased dramatically as a result of the importation of foreign varieties despite the traditional local varieties. Currently, researchers are interested in studying introduced varieties that have become widely cultivated in Algeria, and local varieties have been abandoned in both field research and agriculture. Estimating and characterizing genetic variability is a fundamental problem in plant breeding at all levels in a breeding program (Amallah et al.

2016). Therefore, genetic resources of native landraces of Algerian wheat represent a heritage that must be preserved, in addition, the investigation of its different agricultural potentials will maximize its exploitation and ensure sustainable production. The study of the diversity and characterization of these genetic resources are essential to create new varieties with good quality, high efficiency, adapted to climatic variations and disease resistant (Aguiriano et al. 2006).

Several adaptive traits, such agro-morphological, and physiological characters, participate in the improvement of tolerance to different biotic and abiotic stresses, adaptative mechanisms are therefore important to give regularity to wheat production under different environments (Bahlouli et al. 2005). Many researchers have investigated agronomic traits to estimate the genetic diversity and to characterize the genetic resources in wheat (Boudour et al. 2011; Bellatreche et al. 2017, Kirouani et al. 2019)**,** lentil **(**Gaad et al. 2018), and barley (Karkour et al. 2019) in biochemical traits like SDS-PAGE (Chehili et al. 2017, Shuaib et al. 2007) and molecular traits (Zarkti et al.2010, Belattare et al. 2016, Adoui et al. 2017). This study was conducted to preserve plant genetic resources from erosion and to valorize our varietal heritage. The aim of our study is to enhance the value of phylogenetic resources of native durum wheat cultivated in Algeria by the analysis of morph-physiological variation, yield and the investigation of polymorphism within the *valenciae* variety using biochemical markers.

MATERIALS AND METHODS

Plant materials and field conditions

The study material consisted of nine Algerian durum wheat genotypes belonging to the *valenciae* variety, taken from Dr. Boudour's collection (2004-2006).

The experiment was conducted during 2017-2018 season under rainfed conditions at El Khroub experimental farm station of ITGC (Institut Technique des Grandes Cultures) located 14 km southeast of Constantine district, Algeria. With an altitude of 640m, a latitude of 6.67 East, and longitude 36.67 North. The seeds were planted in 1 m rows in each plot with 20 cm distance between rows, in approximately 2 to 3 cm depth.

Phenotypic measurements

The accessions were evaluated for the seven agronomical traits: plant height (PH, cm) measured at maturity from the ground level to the top of the ears, not included beards; spike length (SL, cm) measured from the base of the ear to the tip, not included beards; total chlorophyll content (TCHC, SPAD), at the middle of the flag leaves was determined at heading stage using a portable chlorophyll meter (SPAD); thousand kernel weight (TKW, g) determined by weighing directly using a precision balance; number of spike per square meter (N spike/m²) determined by counting at the maturity stage; the average number of grains per spike (NG/S) and grain yield (GY, t ha⁻¹) (obtained by calculation based on the performance components using the following formula: The number of spike per square meter (N spike/m²) ×number of grains per spike (NG/S)× thousand kernel weight (TKW)/1000.

Biochemical analysis

Protein extraction

Biochemical analysis was conducted at National Research Center for Biotechnology, Constantine, Algeria (C.R.Bt). Protein was extracted from dry seeds, after seeds in fine grinding. An appropriate quantity (0.13 g) of the powder was taken into a 1.5 ml Eppendorf tube. Protein extraction phosphate buffer (300 µl) was added to the extracts and centrifuged at 150.000 rpm for 15 min at 4C°.

Electrophoresis (SDS-PAGE)

Just before starting electrophoresis process, supernatant was mixed (2:1) with buffer containing 0.5 M Tris-HCl, 4% SDS, 20% glycerol, 0.04% 2-Mercaptoethanol and 1% bromophenol blue was added to the tube. The sample was vortexed for 5 min, and heated in water bath at 90C° for 5

min to denature the protein prior to load on gel. After that, the protein samples were subjected to one dimensional (SDS-PAGE) in a gel slab of 1 mm thickness (5% stacking gel = 2.5 cm height and 15% separating gel= 5.5 cm height) as described by Laemmli (1970). Electrophoresis was performed with a discontinuous buffer system in a vertical electrophoresis unit. The gel was run until the bromophenol blue marker had reached the bottom of the gel.

The gel was stained in a staining solution (10%Ethanol; 6.6% acetic acid 0.05% Coomassie Brilliant Blue G-250) for overnight. The gel was destained in the de-staining solution (40% Ethanol; 7% acetic acid) overnight until the color of background disappeared.

Finally, gels were photographed and scanned using Bio-Rad software. Detailed analysis of protein band patterns in terms of band number, mobility of protein bands, staining intensity, band percentage, and the determination of molecular weight of each band.

Statistical analysis

Phenotypic data

Data were analyzed using analysis of variance (ANOVA) performed for each variable at the P<0.05 significance level. Treatment means were compared statistically using Student-Newman-Keuls test at p=0.05. Principal component analysis (PCA). The relationships between variables were examined using simple correlation analysis based on Pearson's Correlation Coefficient.

Biochemical analysis data

The presence of the bands was identified as 1 and absence was identified as 0, were entered in a data matrix. The similarity matrix thus generated was used to construct dendrogram. All statistical analyzes were performed with XLSTAT 2014 software.

Table 2. Meteorological data during 2017/2018 season

Month	Rainfall (mm) Average	Temperature (C°) Max.	Min.	Average
Sep.	33	28.5	20.2	24.35
Oct.	18.5	2 1	16.58	18.79
Nov.	120.7	13.20	11.60	12.37
Dec.	62.5	9.87	8.55	9.22
Jan	28.4	7.88	7.10	7.88
Feb	45	8.07	6.74	7.39
Mars	115.6	10.47	8.95	9.74
Apr.	54.3	11.91	10.38	11.10
Mai.	59.1	14.34	12.72	13.61
Jun	12.9	18.70	16.54	17.81

Table 1. Physical and chemical properties of the soil at the experimental site

Soil textures	Particle size distribution (%) Course Sand	Fine sand	Course silt	Fine silt	Clay	Real density (g/cm³)	Electrical conductivity (mS/cm)	pH	Organic matter (%)	Soil depth (cm)
Clay soil	3	11	3	29	55	2.2	0.5	7.6	1.49	0-30
	3	11	3	3	44	2.2	1.7	7.6	1.45	30-60
	13	18	4	6	60	2.2	0.6	7.9	1.37	60-90

RESULTS AND DISCUSSION

Mean performance of yield and its components

Morphological markers are already known as effective tools for estimating the genetic diversity of wheat (Al Khanjari et al. 2008). For all the studied characters as observed in Table 3. Analysis of variance revealed highly significant effect (P < 0.001) for seven quantitative characters.

The results showed that the maximum PH was obtained in G4 (158.67cm), followed by G7 (155.83cm), while the lowest value was obtained in G8 (86.00 cm) and G9 (76.83cm), the rest genotypes had intermediate values for PH. Plant height is a basic criterion for selecting wheat varieties, especially in dry areas (Benmahammed 2005; Labdelli 2011). According to Allam et al. (2015), the taller varieties are the most drought-tolerant.

For the SL, the values ranged from 10.33 cm to 7.67 cm, where G4 scored the best value and G9 scored the lowest value. According to Guo et al. (2018) spike morphology traits can be used for the improvement of grain yield in wheat.

The genotypes with the highest plant height were distinguished by the highest spike length while the genotypes with the short spike were distinguished by the short spike; these results are in agreement with those obtained by Boudour (2006).

The NG/S varies between 64.67 and 51 grains. The highest values were noted at G8, G7and G2 followed by G4, G5, G9 and G6 with values of the order of 59.33; 58; 56.67 and 54.67, respectively. The lowest average was recorded at G1 and G3 with 51 grains. Knezevic et al. (2012) have shown that the number of seeds per spike is very important in the variability of yield and depends on the fertility of the spikelet. According to Polat et al. (2015), improving yield necessarily requires reasoning about the number of seeds per spike which explains 75% of the variations in yield.

The N spike/m² registered the highest at G3 (173 spike/m^2), whereas the G1 recorded lowest value (99 spike/m^2). Garcia Del Moral et al. (1991) reported that number of spike/m^2 and grain spike^{-1} seriously decreased due to water deficit in durum wheat. Water limitation can cause severe competition between the different plant organs for photosynthesis assimilates during the stem elongation.

The TKW is one of the important common traits in improving yield, the studied genotypes showed close values, where G3 showed the best value, while G6 showed the lowest value. According to Benbelkacem and Kellou (2000), the TKW is generally low because it is strongly linked to the effects of the environment at the time of grain formation and filling. A lack of water after flowering combined with high temperatures leads to a decrease in TKW by altering the speed and/or duration of filling, resulting in scalding of the grains.

The grain yield of wheat is the product of the interaction of a large number of its components Mohtasham et al. (2014). Grain yield per plant can be improved by selecting genotypes with more spikelets per spike (Ashfaq

et al. 2003). According to the results obtained the genotype G2 and G7 recorded the highest grain yield with 4.85 t ha-^{1}; 4.72 t ha-^{1} respectively, the lowest grain yield was the G1with 2.14 t ha-^{1} and G6 with 2.30 t ha-^{1}.

The THCC ranged from 55.40 to 70 SPAD in the studied genotypes, the values were close, G2 showed the highest values, G1 showed the lowest. Chlorophyll content can serve as a guide for nitrogen management in agricultural systems. Hence, investigating leaf chlorophyll in crops could be of benefit to boost production (Kizilgeci et al. 2019). The results of the study showed a positive relationship between the total chlorophyll content and the grain yield. This corresponds to the results of Bavec and Bavec (2001) who observed that significant and positive correlations were found between the chlorophyll content and the grain yield at the heading stage of winter wheat. Liu et al. (2017) reported that it is possible to derivate the lines having higher Chlorophyll content than their parents at heading and later growing stages Chlorophyll content measurements.

Correlation analysis

Correlation coefficients between all agronomical are illustrated in Table 4. PH shows positive association with LS (r=0.89). The THCC was highly significantly and positively correlated with GY (r= 0.97); N spike/m² (r= 0. 94); TKW (r=0.66) and NG/S (r= 0. 54). Barutcular et al. (2016) noted that there is a significant positive relationship between total chlorophyll content values at the heading stage and grain yield; they can also be used as selection criteria to identify high grain yield and quality durum wheat genotypes at both rain-fed and irrigated environments. Yildirim et al. (2013) and Jahan et al. (2019) have pointed out that grain yield could be predicted by total chlorophyll content.

The TKW had a significant positive correlation with the N spike/m² (r= 0. 68) and GY (r=0. 77). These results contrast with those of Sinha et al. (2006) and Gelalcha and Hanchinal (2013) where non-significant relationship was reported between 1000 kernel weight and yield per plant in irrigated conditions.

On the other side, the GY was highly significant positively correlated with N spike/m² (r= 0.95). Fellahi et al. (2017) note that the yield is more related to the number of spike, and remains independent of the weight of 1000 grains which does not show significant correlation with the number of spike. According to Ferdous et al. (2010) and Safarova et al. (2019) the number of grains/spike, the weight of 1000 and the number of spikes per plant contribute positively to yield and therefore need to be included in a selection index to improve yield.

Principal component analysis

A principal component analysis was performed from seven variables. We noticed the first axis alone explains 47.71% of the information, the first two axes developed 69.46% (Figure 1), therefore, these two axes had better summarize information provided by all the initial variables. On the positive side, the first components (axis) integrate information relating to the variation of the variables THCC,

NG/S, GY, N spike/m², TKW. This axis could be defined as an axis of productivity. The LH and LS is positively correlated with axis 2 on the positive side, this axis could be defined as an axis of morphology (Table5). Considering the significance of axes; we analyzed the distribution of Genotypes. Along axis 1 oppose the genotypes G4, G2, G5, G7, G1, and G6. On the positive side of the axis the G4, G2, G5, G7 were characterized by high THCC, on the negative side, G1, G6 is distinguished by low GY, N spike/m² and THCC. On the negative side of axis 2, the G3, G8, and G9 were also characterized by low PH and low LS (Figure 2).

The principal component analysis reveals 3 distinct groups which are differentiated by agronomical and grain yield. (i) Group 1 = G2, G4, G5, G7, (ii) Group2 = G1, G2, (iii) Group3 = G3, G8, G9.
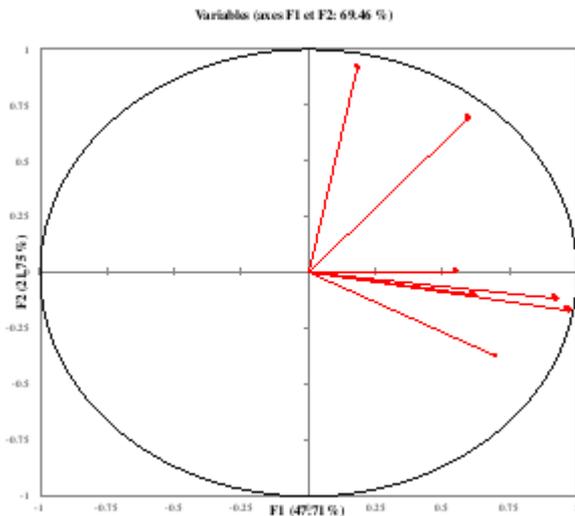

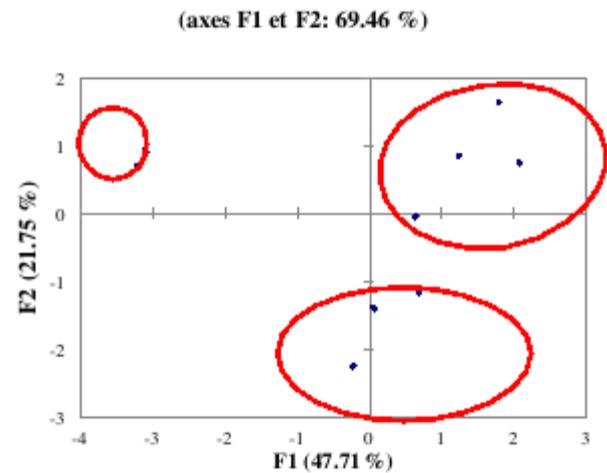**Figure 1.** Correlation graph of seven analyzed parameters.

Figure 2. Distribution of genotypes in function of two principal components.

Table 3 . Mean performance of yield and agronomical attributes for genotypes under rain fed conditions

Genotypes	PH, cm)	spike length (SL, cm)	Total chlorophyll content (THCC)	number of grains per spike (NG/S)	thousand kernel weight (TKW in g)	number of spike per square meter (N spike/m²)	grain yield (GY, t ha-1)
G1	129,67 [d]	8.67[a]	55.40 [a]	51[a]	42.27 [ab]	99 [a]	2 .1 4 [a]
G2	148[f]	9.89 [b c]	79 [a]	60[abc]	49.67 [d]	172.63[f]	4.85 [c]
G3	123[c]	8.20 [a]	71.93 [b]	51[a]	50.25 [d]	173[f]	4.43 [bc]
G4	158.67[g]	10. 33 [c]	77.63[c]	59.33[abc]	45.15 [abc]	165.7[de]	4.43 [b c]
G5	141.67 [e]	8.67[a]	78.90 [c]	58 [abc]	48.07 [cd]	169.8 [e f]	4.72 [c]
G6	146 [e f]	9.50 [b]	57. 33 [c]	54.67[ab]	38.28 [a]	110 [b]	2.30 [a]
G7	155.83 [g]	10 [b c]	77.27[c]	61[bc]	42.38 [ab]	171.11 [e f]	4.42 [b c]
G8	86 [b]	8 [a]	69.09 [b]	64.67[c]	46.42 [c]	130.89 [c]	3.91 [b]
G9	76.83 [a]	7.67 [a]	71.23 [b]	56.67 [abc]	45.15 [b c]	159.29 [d]	4.0 7 [b]
Pr > F	< 0.0001	< 0.0001	< 0.0001	0.0026	< 0.0001	< 0.0001	< 0.0001

Note: Values followed by the same letter(s) are not significantly different according to Newman-Keuls test at P = 0.05 level

Table 4 Correlation matrix of yield and agronomical attributes for genotypes under rainfed conditions

Variables	PH	SL	THCC	NG/S	TKW	N spike/m²	GY
PH	1						
SL	0.8951***	1					
THCC	0.2071	0.2716	1				
NG/S	-0.0702	0.2235	0.5428**	1			
TKW	-0.1704	-0.2523	0.6600**	0.1229	1		
N Spike/m²	0.1736	0.1820	0.9460***	0.3009	0.6845**	1	
GY	0.0734**	0.1205	0.9796***	0.4928	0.7718**	0.9575***	1

Note: ** (P<0.01); *** (P<0.0001)

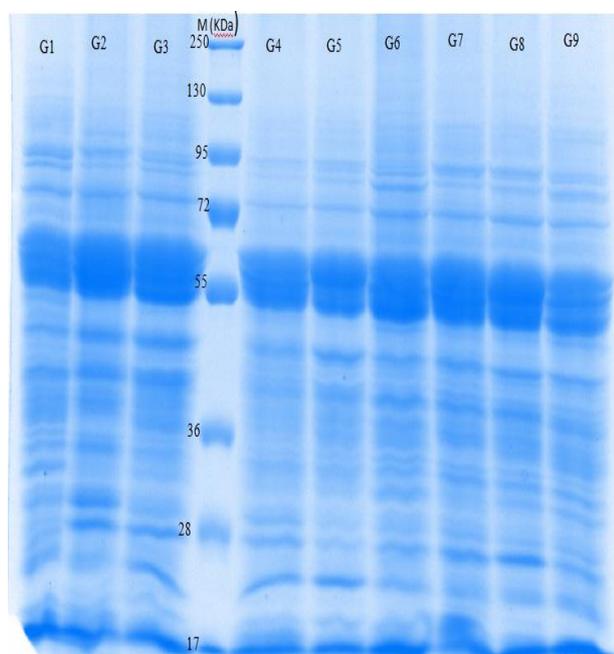**Table 5.** Projection of the different traits studied on the tow axes of the principal components analysis

Traits	LP	SL	THCC	NG/S	TWS	N spike/m²	GY
Axis 1	0.1833	0.5943	**0.6134**	**0.5512**	**0.7010**	**0.9205**	**0.9665**
Axis 2	**0.9217**	**0.6956**	-0.0966	0.0026	-0.3743	-0.1126	-0.1640
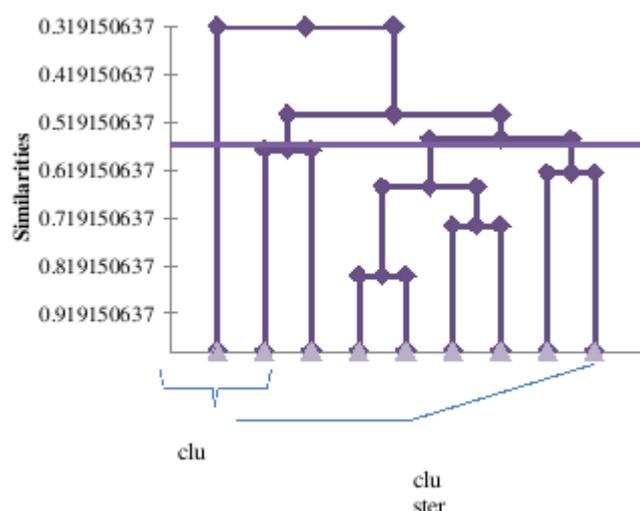
Figure 3. Dendrogram represents the genetic relationship among durum wheat genotypes using SDS-PAGE of total seed protein

Figure 4. Total seed protein profile of wheat in nine genotypes using SDS-PAG.

Biochemical analysis

Seed protein content is an important factor influencing flour quality (Laino et al. 2010), protein markers are widely used to identify genetic polymorphism within different varieties of wheat (Amallah et al. 2016). The results obtained through electrophoresis of the total proteins (Figure 3; Table 6; and Table 7) showed a different number of bands and their molecular weights. There were 21 bands with molecular weights ranged from 17 to 122.09 KDa and polymorphism (38.09%). Al-Tamimi and Al-Rufaye (2018) observed a 15% low polymorphism using SDS-PAGE for total wheat grain proteins. Where, reduced polymorphism between genotypes of wheat was reported by Shuaib et al. (2007). The resulted profile comprises ten monomorphic bands and eight polymorphic bands. Three Unique bands in G6 (122.09 KDa) and G9 (34KDa) (21.15 KDa), respectively. The presence of unique bands increases the chance to have a unique fingerprint and differentiate genotypes (Sood et al. 2007). Variation was observed in density bands, but this variation was not taken into consideration.

Table 6. Results of SDS-PAGE for durum wheat genotypes: The presence of band was (+) while absence was (-)

Fragment size in KDa	G1	G2	G3	G4	G5	G6	G7	G8	G9
122 .09	-	-	-	-	-	-	+	-	-
115.19	+	-	+	+	-	+	+	+	+
106	+	+	+	-	+	+	+	+	+
93.1	+	-	+	-	+	+	+	+	+
90	+	+	+	+	+	+	+	+	+
84	-	+	+	-	-	+	+	+	+
70	+	+	+	+	+	+	+	+	+
56	+	+	+	+	+	+	+	+	+
46	+	+	+	+	+	+	+	+	+
40	+	-	+	-	+	-	+	-	+
38	+	+	+	+	+	+	+	+	+
34	-	-	-	-	-	-	-	-	+
33	+	+	+	+	+	+	+	+	+
32	+	+	+	-	-	+	+	+	+
31	+	-	-	-	-	+	+	+	+
30	+	+	-	-	-	+	+	-	+
28	+	+	+	+	+	+	+	+	+
25	+	+	+	+	+	+	+	+	+
2115	-	-	-	-	-	-	-	-	+
1908	+	+	+	+	+	+	+	+	+
17	+	+	+	+	+	+	+	+	+

Table 7. Number and type of the bands produced by SDS-PAGE of seed protein as well as the percentage of the polymorphism detected in the nine wheat genotypes

Polymorphism %	Main bands	Unique bands	Monomorphic bands	Polymorphic bands	Molecular size in KDa
38. 09	21	3	10	8	10-250

The dendrogram (Figure 3) shows the clustering of the studied nine wheat genotypes based on the data obtained from (SDS-PAGE) profiles. It was revealed that at similarity coefficient level of 0. 319; two major clusters (I and II) are separated. The first cluster (I) comprised the genotype G9. The second cluster (II) splits into two groups at similarity coefficient level of 0. 559. The first group comprised the genotypes G2 and G6 that are grouped together at similarity coefficient level of 0. 569. The second group splits into two subgroups at similarity coefficient level of 0.559. Under the first group includes the genotypes G1; G7; G3 and G8 at similarity coefficient level of 0.657, and under the second group consist the genotypes G4 and G5 are groups that are close to each other at similarity coefficient level 0.678.

In conclusion, our study results showed that there was variability between the nine genotypes under rain-fed conditions. Genotypes with better chlorophyll content recorded the best grain yield. Biochemical results were highly significant; indeed 21 bands were recorded with a molecular weight ranging from 17 to 122.09 KDa showing polymorphism with 38.09% between the genotypes under study. In conclusion, the results obtained through the agro-morphological and biochemical traits showed the existence of an interesting intra-varietal variability. This variability must be preserved and enhanced using the plant material with characters of interest and a well-known genetic potential still available in Algeria.

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