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Agromorphological Variation in Spontaneous *Aegilops geniculata* Roth Populations Suitable for Mediterranean Conditions

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Abstract: A collection of 13 *Aegilops geniculata* Roth (geniculate goat grass) populations belonging to different regions of Tunisia (in North and Central) was evaluated on the basis of morphological descriptors and agronomical characters. Morphological diversity was analysed using a set of nineteen quantitative traits. Variance analysis, correlation coefficients and principal components analysis (PCA) were performed based on MVSP 3.13 program. Materials were transplanted in the field of the National Institute of Agronomic Research of Tunisia (INRAT) in Ariana after being germinated in Jiffy pots under greenhouse conditions in early November 2002. All measurements were performed on the same randomly selected plants. High significant differences between populations were noted for most morphological traits. Close association was found between most studied traits, with the respect of population origin. The principal components analysis (PCA) of morphological traits indicated geographic information. Indeed, PCA analysis grouped *Ae. geniculata* populations according to genotypic criteria such as: vegetative growth, earliness and high kernel yield. Dj Oust, Sbeitla, Souk Jemaa population originated from dry area, exhibited early heading. This criterion is of importance when plants are cultivated under drought and heat stress in the end of the life plant cycle. Moreover, populations originating from sub humid area have higher vigour compared to those originating from dry environment.

Key words: *Aegilops geniculata* Roth % Morphological variation % Principal component analysis

INTRODUCTION

Aegilops geniculata Roth (= *Ae. ovata* L.) is an annual self-fertile plant [1], allo-tetraploid species (2n= 4x=28) with MU genomes [2], belonging to tribe *Triticeae* Dumort, subtribe *Triticinae* Griseb. It has a wide distribution in Asia and around the Mediterranean Sea region, characterized by a dry summer season with high temperature and high irradiance. Great interest has been focused to the genus *Aegilops* L., which is closely related to *Triticum* constitutes a precious source of economically important traits for wheat improvement [3,4]. In fact, many studies have been elaborated for the assessment of the genetic resources of wheat and wild relative species in particular those of the genus *Aegilops* [5] for particular source of novel variation for resistance to various diseases and pests, drought, salinity and reserve protein quality [6,7]. Therefore, the original habitat of cultivated wheat and/or wild cereals was destroyed or modified by many detrimental activities related to human population growth. In addition, wild cereal sites are mostly degraded by animal overgrazing. These sources of erosion affected the disappearance of numerous local varieties and species. Consequently, loss of diversity is observed especially for gramineous species such as wild wheat and its cultivated derives.

Efficient strategy to solve loss of plant diversity consists of exploiting wild germplasm genomes of wheat species, which preserve a good part of their adaptive factor and diseases tolerance. The introgression of a particular gene constituted the main objective of the improvement of wheat [8,9].

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In Tunisia, the vegetal flora is extremely rich in diverse species of unknown fodder potential. The large variation in environmental conditions had lead to development for each species of numerous ecotypes with genetic pools which constitute a basic material for selection and improvement of varieties adapted to those conditions [10]. Three annual species of *Aegilops* were reported in Tunisia [11]: *Ae. geniculata* Roth, *Ae. triuncialis* L and *Ae. ventricosa* Taush. *Ae. geniculata* Roth is widely distributed while the last two species are quite rare. Its geographical distribution would indicate a large distribution of climatic regions: cold and humid mountains, hot and dry valley. Habitats of *Aegilops geniculata* Roth in the Fertile Crescent differ widely in the humid areas receiving around 800 mm and the lowest arid area with less than 150 mm [12]. *Aegilops*, is equally adapted to areas with altitude ranging from 10 to 900 m.

In order to assess, conserve and update the genetic diversity of the Tunisian *Ae. geniculata* Roth, we initiated a research program. It aims to collect the seeds of local *Ae. geniculata* species from different sites, evaluate their agro mophological and molecular traits of breeding interest, mainly related to vegetative and reproductive growth.

MATERIALS AND METHODS

Collecting missions were conducted in North and Central Tunisia (Cap-Bon, Mogodses, Kroumiry and the Dorsal areas) (Fig. 1), in spring during two years, 2000 and 2001. Thirteen populations of *Aegilops geniculata* have been collected. These wild materials were distributed in a wide range of climatic conditions (Table 1) with various site altitudes. Seeds of each population were germinated in Jiffy pots placed in a greenhouse in early November 2002. Two weeks after emergence they were transplanted in the field of the National Institute of Agronomic Research of Tunisia (INRAT) in Ariana (E, N, 10 m altitude above sea level) on clay alkaline soil (pH = 8.7). The climate is typically Mediterranean, with mild winter and relatively hot summer. The experimental station is located in the upper semi- arid area with an average annual rainfall of 450 mm. The mean annual temperature was 25°C with monthly means ranging from 15°C in January to 35°C in August. Daily minimum and maximum temperatures as well as the rainfall during the experimental year were recorded by the Laboratory of bioclimatology of INRAT. During experimentation, annual rain was 300 mm. Plants were grown in natural conditions (under rain-fed conditions; no pesticides and no fertilizer were applied; weeds were manually eliminated). Complementary irrigation was given when necessary. The 13 populations were planted in completely randomized design with four replicates. Each replication consisted of a 10 plants row. Distance between plants was 70 cm. Rows were 80 cm apart. Heading was noticed 140 days after sowing and the plants were harvested at complete maturity (180 days after sowing). Quantitative characters were evaluated from leaves, ears and seeds from each

Table 1: Characteristics of the origin sites of *Ae. geniculata* populations

Population codes	Sites/province	Altitude (m)	Rainfall (mm)	Bioclimatic area
J	Souk jemaa/Dorsal	870-900	350	Upper semi arid
G	Goussa/North-west	100	351	Sub Humid
Abd	Djebel Abderahmen/Cap bon	400	380	Sub Humid
B	Bizerte/North- Est	300-500	400	Sub Humid
Zg	Zaghouan/Dorsal	175	496	Upper semi arid
N	Nefza/Mogodses	10	720	Sub Humid
M	Mekna/Kroumerie	500	780	Sub Humid
O	Djebel Ouest/Dorsal	400	453	Upper semi arid
Z	Ain Zana/Kroumerie	641	780	Sub Humid
S	Djebel Serj/Dorsal	500	400	Upper arid
Sb	Sbeitla/Center	670	328	Upper arid
T	Tabarka/Kroumerie	170	800	Humid
R	Djebel Ressas/North-Est	47	453	Upper semi arid

Table 2: Agro-morphological measured traits on *Ae. geniculata*

Abbreviations	Traits
DG	Days to germination
DEM	Days to emergence
DL1T1	Days to full expansion of the first leaf
DL2T1	Days to the second leaf emergence
DT1	Days to the first tiller
T1Le	First tiller length (cm)
NLT1	Number of leaves on the first tiller
NT	Total number of tillers
Dfl	Days to flowering
D50%H	Days to 50% of heading
DH	Days to full heading
SpN/E	Spikelet number per ear
E/P	Ears per plant
S/E	Seeds per ear
PHt	Plant height (cm)
ELe	Ear length (cm)
SLe	Seed length (cm)
SN/P	Seeds Number per plant
SW/P	Seeds weight per plant (g)

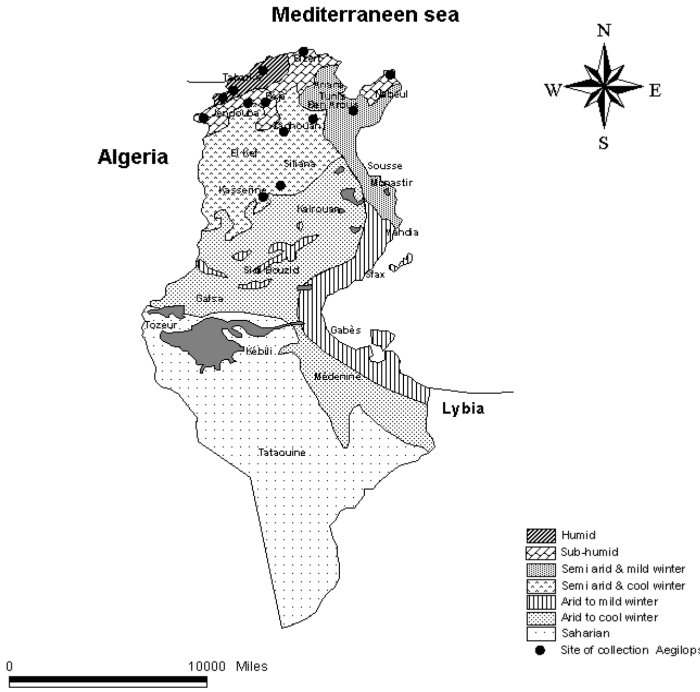

Fig. 1: Distribution sites of Aegilops geniculata in Tunisia

plant. Morphological measurements of 19 traits were assigned in Table 2. Analysis of variance and correlation coefficients were calculated to determine associations between the traits measured. The standardized traits mean values (mean of each trait was subtracted from the data values and the result divided by the standard deviation) were used to perform principal component analysis. This analysis was dealt on the centred and standardized variates using measured data with MVSP 3.13 software [13].

RESULTS

A highly and significant variation (p<0.01) was found among *Ae. geniculata* populations for all morphological traits (Table 3). Replication were homogeneous for the following traits: Days to emergence (DEM), days to full expansion of the first leaf (DL_1T_1), days to the second leaf emergence (DL_2T_1), days to the first tiller (DT_1), spikelet number per ear (SpN/E), seeds per ear (S/E), ear length (ELe), seed length (SLe). However, a significant replication variation was observed for almost half of the studied factors (Table 3).

Matrix of correlation (Table 4) showed different level of significance between traits, indicating most relationships. Moderately high positive associations were detected among traits. To avoid spurious significant results due to examining a large number of correlations, only correlation with <0.01 are given. For example, a positives correlations were detected between days to

Table 3: Variance analysis of *Ae. geniculata* populations

	Ae. geniculata population		Replication	
	MS	F_{ob}	MS	F_{ob}
DG	9.818	25.799**	1.551	4.075**
DEM	89.599	20.578**	4.368	1.003 ns
DL1T1	47.683	4.989**	19.335	2.023 ns
DL2T1	167.55	8.489**	36.963	1.859 ns
DT1	295.159	9.759**	44.567	1.473 ns
T1Le	626.888	15.627**	426.73	10.638**
NLT1	4.1381	12.949**	2.378	7.443**
NT	17787.39	3.524**	68292	13.528**
Dfl	4114.704	29.217**	662.15	4.701**
D50%H	4623.359	32.39**	866.44	6.07**
DH	4705.398	32.732**	722.91	5.029**
SpN/E	0.456	4.083**	0.192	1.719 ns
E/P	16857.72	3.541**	66123	13.888**
S/E	9.151	4.504**	5.192	2.555 ns
PHt	580.999	14.17**	364.46	8.889**
ELe	0.607	6.611**	0.18	1.962 ns
SLe	1.547	8.551**	0.071	0.395 ns
SN/P	439063.7	3.549**	2.00E+06	16.695**
SW/P	132.271	6.085**	303.32	13.953**

Table 4 : Matrix of traits correlations

	DG	DEM	DL_1T_1	DL_2T_1	DT_1	T_1Le	NLT_1	NT	Dfl	$D_{50\%}H$	DH	SpN/E	E/P	S/E	PHt(cm)	ELe(cm)	LeS(cm)	SN/P	SW/P
DG	1																		
DEM	0.59	1																	
DL_1T_1	0.46	0.77*	1																
DL_2T_1	0.72*	0.57	0.74*	1															
DT_1	0.70*	0.51	0.61	0.96*	1														
T_1Le	-0.69*	-0.48	-0.39	-0.65	-0.69*	1													
NLT_1	-0.38	-0.59	-0.34	-0.37	-0.26	0.50	1												
NT	-0.38	-0.16	-0.22	-0.68*	-0.82*	0.51	0.09	1											
Dfl	-0.01	0.27	0.10	-0.18	-0.37	-0.05	-0.63	0.44	1										
$D_{50\%}H$	0.01	0.26	0.11	-0.18	-0.36	-0.04	-0.59	0.41	0.99*	1									
DH	0.02	0.29	0.13	-0.15	-0.33	-0.08	-0.62	0.40	0.99*	0.99*	1								
SpN/E	0.63	0.37	0.29	0.69*	0.75*	-0.53	-0.19	-0.67*	-0.32	-0.32	-0.30	1							
E/P	-0.38	-0.18	-0.20	-0.68*	-0.81*	0.57	0.19	0.99*	0.38	0.36	0.35	-0.66	1						
S/E	-0.46	-0.53	-0.42	-0.25	-0.09	0.50	0.68*	-0.22	-0.68*	-0.65	-0.67*	0.05	-0.16	1					
PHt(cm)	-0.70*	-0.60	-0.47	-0.69*	-0.72*	0.98*	0.61	0.52	-0.10	-0.09	-0.13	-0.52	0.59	0.53	1				
ELe (cm)	-0.62	-0.61	-0.49	-0.55	-0.57	0.80*	0.54	0.31	-0.09	-0.08	-0.12	-0.28	0.36	0.59	0.84*	1			
SLe (cm)	-0.34	-0.22	-0.07	-0.27	-0.26	0.79*	0.64	0.17	-0.32	-0.27	-0.30	-0.13	0.29	0.63	0.78*	0.67*	1		
SN/P	-0.50	-0.45	-0.37	-0.64	-0.72*	0.84*	0.51	0.76*	-0.02	-0.02	-0.06	-0.52	0.81*	0.37	0.86*	0.73*	0.64	1	
SW/P	-0.50	-0.33	-0.27	-0.68*	-0.74*	0.85*	0.54	0.74*	0.06	0.09	0.06	-0.53	0.81*	0.35	0.87*	0.65	0.76*	0.93	1

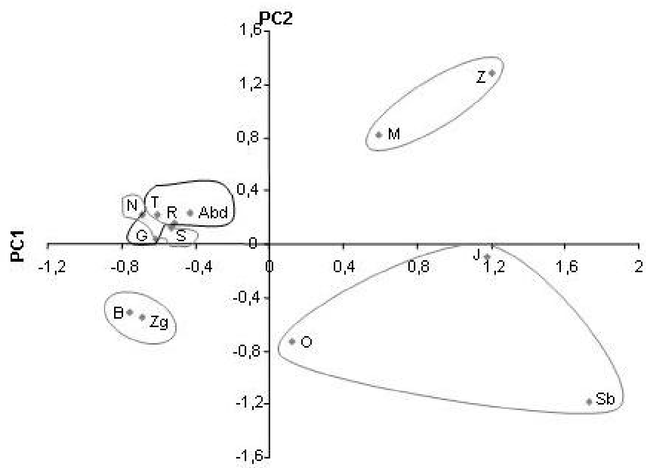

Fig. 2: Principal Component Analysis of Aegilops geniculata populations

the second leaf emergence (DL_2T_1) and days to the first tiller (DT_1) (0.96), between first tiller length (T_1Le) and plant height (PHt) (0.98), first tiller length (T_1Le) and seeds number per plant (SN/P) (0.84), first tiller length (T_1Le) and seeds weight per plant (SW/P) (0.85), between days to flowering (Dfl) and days to 50% of heading ($D_{50\%}H$) (0.99), days to flowering (Dfl) and days to full heading (DH) (0.99), days to 50% of heading ($D_{50\%}H$) and days to full heading (DH) (0.99), between ear per plant (E/P) and seeds number per plant (SN/P) (0.81), ear per plant (E/P) and seeds weight per plant (SW/P) (0.81), between plant height (PHt) and spike length (ELe) (0,84), plant height (PHt) and seeds number per plant (SN/P) (0.86), plant height (PHt) and seeds weight per plant (SW/P) (0.87), between seeds number per plant (SN/P) and seeds weight per plant (SW/P) (0.93). A negatives correlations were observed with: days to germination (DG) and plant height (PHt) (-0,70), days to the first tiller (DT_1) and total number of tillers (NT) (-0.82), days to the first tiller (DT_1) and ear per plant (E/P) (-0.81), days to the first tiller (DT_1) and seeds number per plant (SN/P) (-0.72), days to the first tiller (DT_1) and seeds weight per plant (SW/P) (-0.74).

To gain a better understanding variance sources among *Ae. geniculata* populations, principal component

Table 5 : PCA variable loadings

Morphological traits	PC 1	PC 2	PC 3
DG	0.249	0.008	0.234
DEM	0.212	0.141	0.421
DL1T1	0.19	0.058	0.515
DL2T1	0.28	-0.102	0.26
DT1	0.286	-0.184	0.168
T1Le	-0.3	-0.036	0.156
NLT1	-0.198	-0.278	0.053
NT	-0.224	0.26	0.123
Dfl	0.004	0.426	-0.022
D50%H	0.002	0.417	-0.006
DH	0.014	0.42	-0.007
SpN/E	0.216	-0.186	0.125
E/P	-0.239	0.231	0.182
S/E	-0.156	-0.347	-0.046
PHt	-0.313	-0.058	0.102
ELe	-0.265	-0.094	0.014
SLe	-0.212	-0.175	0.419
SN/P	-0.298	0.014	0.21
SW/P	-0.296	0.042	0.287

analysis (PCA) was carried out. Based on eigenvalues of the order of 1.7 as was suggested by Tomassone *et al* [14], the PCA grouped variables into three components, which explained 83.98% of the total variation. The two first axes were considered as they elucidate the maximum simple variation (respectively 48.32 and 26.38 % of the total variation) with the cumulative variation of 74.70 %. Loading variables and the PCA scores were also calculated (Table 5, Fig. 2). Each principal component was interpreted by its correlation with the original variables. The PC1 accounted for 48.32 % of the variation and showed the largest loading values with phenological, morphological and yield-related traits: Days to germination (DG), days to the second leaf emergence (DL_2T_1), days to the first tiller (DT_1), first tiller length (T_1Le), spikelet number per Spike (SpN/E), ear per plant (E/P), Plant height (PHt), ear length (ELe), seeds number per plant (SN/P), seeds weight per plant (SW/P), whereas, the PC2 accounted for 26.38 % of the variation shared the largest loading values with phenological, morphological and yield-related traits: Number of leaves on the first tiller (NLT_1), total number of tillers (NT), days to flowering (Dfl), days to 50% of heading ($D_{50\%}H$), days to full heading (DH), Seeds per ear (S/E). Considering the plot defined by the PC1 and PC2 and taking in account their projection on the third plan (PC3), most variables were correlated negatively with the first principal component (PC1) ($p < 0.01$). A clear separation of *Ae. geniculata* populations was observed and five main groups can be distinguished (Fig. 2).

The first group positively correlated to the two axes and gatheres Ain Zana and Mekna populations. They are originated from the sub humid area (mild and cool winter) at an altitude of 600 m. Moreover, Dj Abderhamen', 'Goussa', 'Tabarka' and 'Dj Ressas' populations, they are originated from humid and sub humid microclimate, formed the second group. This set is positively correlated to the PC 2 and negatively correlated to the PC 1. The third group is composed by Nefza populations (from the sub humid area) and Dj Serj populations (from the upper arid area). They are positively correlated to the PC 2 and negatively correlated to the PC 1. Bizerte and Zaghouan populations are correlated negatively to the two axes and formed the fourth group. They are also belonging to the sub humid area. The fifth group is composed of Sbeitla, Souk jemaa and Dj Oust populations which are negatively correlated to PC2 and positively correlated to PC1. They are collected from high altitudes (> 400m) of the upper semi arid with mild winter (Fig. 2).

DISCUSSION

In this study, we used agro-morphological traits to assess the variation among 13 *Ae. geniculata* Roth populations collected in North and Central Tunisia. Substantial variation and an important heterogeneity between populations were observed for phenological, morphological and yield-related traits. This result corroborates with that found by Perrino *et al*. [15] between *Ae. geniculata* populations originated from southern Italy and Sicily. This variation was mainly explained by geographical origin, suggesting that plant behaviour resulting from natural selection pressure exerted by the climatic constraints. Furthermore, Roach and Wulff [16] reported that the heterogeneity between populations is due to considerable genetic difference between populations and environmental factors. The morphological variation between populations may reflect the level of adaptation of these populations to the specific conditions of their origin regions.

Moreover, ecological characteristics of the collecting sites were related with the expression of several traits. In Dj Oust, Sbeitla, Souk Jemaa the biomass produced was positively correlated with kernel yield. However, in Mekna and Ain Zana populations (originated of sub humid area with rainfall of 800 mm and high altitudes > 500 m) biomass production was negatively correlated with kernel yield. Populations of Dj Abderhamen, Goussa, Dj Ressas, Nefza, Dj Serj and Tabarka with important vegetative development (high NT, PHt, T_1Le values) and higher grain production were found in sub humid regions with rainfall >400mm.

Agro-morphological diversity among the *Aegilops* populations is further substantiated by principal component analysis, which indicated that the total variation was fairly distributed across all traits. Information obtained throughout principal component analysis may assist plant breeders to identify the number of highly differentiated population for use in crossing and selection programs [17]. The PCA of agro-morphological traits classify populations according to climate regions. It can also, diversifies variations due to micro-climate changes. Several authors have shown that the geographic origin of the collected materiel was sufficient to obtain a reasonable grouping structure [18].

The first group is constructed by Ain Zana and Mekna populations and originated from the sub humid area with mild and cool winter and located at an altitude of 600 m. They are defined by a late heading and flowering (an average of 180 days), a late germination, a weak kernel yield and high biomass production. Jaradat and Humeid [19] also observed that genotypes from cooler sites and located at high elevations (900 m altitude) were characterized by late heading and longer filling periods. Moreover, Dj Abderhamen', 'Goussa', 'Tabarka' and 'Dj Ressas' populations, formed the second group, originated from humid and sub humid microclimate. 'Tabarka' population collected from humid microclimate high land providing a sufficient argument for its belonging to this group, The presence in this second environmental pool of the population Dj Ressas collected from upper semi arid climatic suggested that this population is situated in the limit of the sub humid area with an annual rainfall of 453 mm. The last group is characterized by early germination, high grain yield and biomass production. Seed yield as well as biomass should be considered when evaluating wild populations. So, plants of Dj Abderhamen', 'Goussa', 'Tabarka' and 'Dj Ressas', exhibited high kernel yield (an average of 660 seeds per plant). In this context, Zaharieva *et al* [20] reported an average seed number of 1400 in *Ae. geniculata* plants grown under Mediterranean conditions. She concludes that *Aegilops* plants produce a considerably greater number of spikes and seeds than wheat. The third group is composed by two populations (Nefza from the sub humid area and Dj Serj belonging to the upper arid area). In fact, these populations were found in the same group because they belong to regions with rainfall >400mm. They exhibited early germination, high kernel yield, late heading and flowering, high biomass production.

Bizerte and Zaghouan populations formed the fourth group. They are also belonging to the sub humid area, characterized by weak biomass production and high kernel yield. They exhibited early germination, heading and flowering (flowering after 160 days). Van Slageren [2] reported that the flowering time of *Aegilops* in Europe is from April–May until June–July, depending on the species and their ecogeographical location. This trait enables wild species to escape environmental stress during flowering and ensure seed production. Bizerte, Zaghouan and Dj Abderhamen','Goussa', 'Tabarka' and 'Dj Ressas' genotypes exhibited high plant height (PHt), high number of leaves on the first tiller (NLT_1) and high Seed length (SLe). The last character could be useful in selecting populations with high seedling viability as seed size is directly correlated with the vigour of the seedling [21].

The fifth group is composed of Sbeitla, Souk jemaa and Dj Oust populations, collected from upper semis arid with mild winter and high altitudes (> 400 m). They are defined by late germination, early heading and flowering and weak kernel yield and biomass production. These populations could be selected for their earliness. Dib Ali *et al.* [22] reported that in Mediterranean conditions, early heading could represent an important trait favouring plant survival and reproduction under drought and heat stress in the end of the life cycle on seeds per ear (S/E). This result was also mentioned by Chibani [23] on barley (*Hordeum vulgare* L.). Zaharieva *et al.* [24] observed that earliness was the trait with highest contribution of the inter-population variation to the total variation. In fact, elongation and development events are the most sensitive to climatic factors variation [25] and strong effect of climatic factors on development rate was noted in several crops [26]. Concerning the variability of wild Jordanian emmer, Jaradat and Humeid [19] found that accessions from a dry location are characterized by their high number of spikelets and high productive tilling capacity value. These populations might harbour genes for drought tolerance and possibility to higher photosynthetic ability.

In addition, the restricted geographical area of populations, with a slight climatic difference between the sub humid and the semi arid area, could explain the proximity of most populations. Furthermore, populations originating from sub humid area have higher vigour compared to those originating from dry environment. According to these results, there is clear evidence that variation exists among studied populations. This heterogeneity is of great value in providing material to improve the agriculture systems. Moreover, each population exhibited different behaviour and rich phenotypic diversity may provide valuable resources of important agronomic traits with respect to that variation, which is likely related to the distribution areas. In fact, genetic variation available in wild wheat gene pool is much greater than that found in the cultivated gene pool. In this context, Valkoun *et al.* [3], Holubec *et al.* [4] and Dhaliwal *et al*. [27] reported that wild wheat population (*Ae*. g*eniculata*) provides useful genes for powdery mildew and leaf rust resistance respectively reserve protein quality.

Finally, using molecular markers to understand the genetics and genomic organization of local varieties is of great value for breeding purposes. In fact, molecular markers have been extensively utilized for the study of genetic diversity and interspecific or intergeneric relationships among a number of species of the tribe *Triticeae* [28]. Consequently, a later study using molecular markers on local *Aegilops* species will makes a part of our research.

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