International Journal of Agronomy and Agricultural Research (IJAAR)
ISSN: 2223-7054 (Print) 2225-3610 (Online)
http://www.innspub.net
Vol. 6, No. 2, p. 8-21, 2015

RESEARCH PAPER OPEN ACCESS

Morphological and genetic variation in *Aegilops geniculata* Roth. from Tunisia

Khaled Mguis[1,2†], Asma Mahjoub[3†], Mejda Abassi[2], Ali Albouchi[2], Zeineb Ouerghi[1], Ben Brahim Nadia[3], Zoubeir Béjaoui[2]

[1]*Unité de physiologie et biochimie de la tolérance aux sels des plantes, Département de biologie FST, Université de Tunis El Manar 2092 Tunis, Tunisia*

[2]*Laboratoire d'écologie Forestière, INERGREF, Rue Hedi Karray, 2080 Ariana, Tunisia*

[3]*Laboratoire des Sciences agronomiques, INRAT, Rue Hedi Karray, 2080 Ariana, Tunisia*

[†]*First and second authors contributed equally in this work.*

Key words: *Aegilops geniculata*. Morphology. Diversity. RAPD.

Article published on February 03, 2015

Abstract

Aegilops geniculata Roth is an annual grass relative to cultivated wheats and is widely distributed in North Africa. In order to understand the diversity of this species, 13 populations collected in different bioclimatic areas in north and central Tunisian were analyzed using morphological and molecular characters. Principal component analyses (PCA) based on the agro-morphological characters allowed the separation of populations in five mainly bioclimatic groups characterized by different morphological patterns. Populations originated from humid coastal areas were characterized by good vegetative development, vigorous spikes and caryopses. Samples collected from mean altitude with sub-humid climate had late germination and a large growing cycle a high biomass production and weak caryopses. Populations collected from intermediate and high mountains with sub-humid and semi-arid conditions presented good fertility and high yield-related. Individuals with early germination, weak vegetative development and high caryopses yield characteristics of the coastal areas and plains in sub-humid and the upper semi-arid climate. Populations originated from steppic highlands in upper arid conditions and mean and high altitudes mountains with upper semi-arid were characterized by low morphological development, weak fertility reduction of yield-related and shortening of growing cycle. Individuals were distinguished successively by Phenological, morphological and Yield-related traits. RAPD analysis based on the phenotypic variability and genetic distances revealed a significant variation within and between populations associated with bioclimatic conditions, in particular winter temperature. Genetic diversity was higher in populations growing under warm bioclimates than in those from cold bioclimates.

***Corresponding Author:** Khaled Mguis ✉ k_guigis@yahoo.fr

Introduction

Aegilops geniculata Roth (= *Ae. ovata* auct.) is an annual, selfing allo-tetraploid species (2n = 4x = 28) with MU genome, belonging to tribe Triticeae Dumort., subtribe Triticinae Griseb. (van Slageren, 1994). This species grows in Mediterranean regions characterized by a dry summer season with high temperature and high irradiance. As with other wild species, it can acclimate to these constraints by escape, avoidance, and tolerance (Colmer *et al.*, 2006). Among the 22 species of the genus *Aegilops*, some species, including *Ae. geniculata*, are particularly interesting as sources of resistance to various diseases and pests, drought and salinity (Gill *et al.*, 1985, Farooq *et al.*, 1996, Rekika *et al.*, 1998; Zaharieva *et al.*, 2001, Mguis *et al.*, 2008). *Aegilops geniculata* Roth (UUMM) has been pursued by Farooq (2002, 2004) as a potential source for improvement of salt tolerance in wheat.

Perrino *et al.* (1993) and Zaharieva *et al.* (1999), using morphological and phenological characters found a high variation between *Ae. geniculata* populations in Italy and Bulgaria, respectively. This variation was mainly explained by the adaptation of this species to different pedoclimatic conditions. Intraspecific variation within *Ae. geniculata* could also be due to the occurrence of natural hybridization with other tetraploid *Aegilops* species sharing the U-genome (Pazy and Zohary, 1965, Zohary, 1965, Kimber and Feldman, 1987). The tendency of these tetraploid species to form mixed populations facilitates further hybridization. Feldman (1965) described many intermediate and introgressed types occurring in mixed populations of *Ae. peregrina* (Hack.) Maire and Weiller, *Ae. biuncialis* Vis. and *Ae. geniculata*. This considerable variability led to some taxonomists to propose different classifications. Maire (1955) included *Ae. geniculata*, together with *Ae. neglecta* Req. ex Bertol, in the same taxon and considered *Ae. neglecta* as a subspecies of *Ae. geniculata*. In Tunisia, *Ae. geniculata* Roth is widely distributed in a large climatic regions: cold and humid mountains, hot and dry valley. Moreover, it is one of the widespread species of the genus showing adaptations to a large range of environmental constraints (Ben Brahim *et al.*, 2002).

Some genetic diversity studies based on protein or DNA polymorphism have been carried out on *Aegilops species*, but more frequently on the diploid species that are the genome donors to polyploid *Aegilops* and *Triticum* species (Fernandez-Calvin and Orellana, 1990, Rodriguez-Quijano *et al.*, 2000, De Bustos and Jouve, 2006, Sun *et al.*, 2006). Fernandez-Calvin and Orellana (1990) analyzed the variation of HMW-GS in diploid *Aegilops* species of section Sitopsis and concluded that *Ae. speltoides* Tausch was the probable donor of the B-genome of wheat. Rodriguez-Quijano *et al.* (2000) analyzed the polymorphism of HMW glutenin subunits in *Ae. umbellulata* Zhuk., *Aegilops comosa* Sibth. and Sm. and *Ae. markgrafii* Greuter L. (donors of the U-, M- and C- genomes, respectively) identified new alleles that could be used in wheat quality improvement. Sun *et al.* (2006) identified two HMW-GS subunits (x and y) from several populations of *Ae. searsii* Feld. and Kis. That were similar to those found on the Glu-1 locus of wheat species. De Bustos and Jouve (2006), analyzing the HMW-GS in three diploids species, *Ae. comosa*, *Ae. uniaristata* Vis. And Ae. speltoides, highlighted new glutenins coded by Glu-Mx, Glu-Unx and Glu-Sy, respectively at these species. They showed that x and y subunits were heterogeneous between species and allowed to differentiate rye from wheat and *Aegilops*. There are few genetic diversity studies in *Ae. geniculata*, except some works using either RFLP (Zaharieva *et al.*, 2001) or RAPD polymorphism (Zhang *et al.*, 1996, Monte *et al.*, 1999). Zaharieva *et al.* (2001) found a large variability between populations originating from different Mediterranean regions and distinguished two groups, from the North and the South of the Mediterranean Sea. Monte *et al.* (1999), by studying the genetic variation for RAPD markers in Spanish populations of *Ae. geniculata*, found a significant correlation between some RAPD bands and ecogeographical factors. They suggested that part of the observed variation could be adaptive.

The objective of this paper is to assess and understand the genetic diversity of *Ae. geniculata* inTunisia in order to improve the management of genetic resources.

Materials and methods

Plant material

Thirteen populations of *Ae. geniculata* were collected in various bioclimatic and ecological conditions of northern and central Tunisia (Fig. 1, Table 1). The collecting sites extended from Kroumiry mountains and the coastal region to the Dorsal areas, including the Cap-Bon, Mogodses, Zaghouan and the steppic highlands, along a gradient of increasing aridity from North to South. Each sampling site was characterized by the main ecological factors of Mediterranean climate using the bioclimatic coefficient (Q_2). The average annual rainfall (P) and the average temperatures of the hottest month in summer (M) and in particular those of the coldest month in winter (m) correlated with altitude were indicating continentality and bioclimatic variants.

Table 1. Characteristics climatic of the origin sites of *Ae. geniculata* Roth populations in Tunisia.

Code	Pop Site	Province	Alt. (m)	*P* (mm)	*m*	*M*	Q_2	Bioclimate
T	Tabarka	Littoral N/W	20	1032	7.2	31.5	148.1	Worm H
N	Nefza	Littoral N/W	10	960	7.5	32	131.2	Worm H
Z	Aine Zana	Kroumery	641	830	1.9	31.8	95.7	Fresh SH
M	Mekna	Kroumery	630	780	2.6	32.5	91.8	Fresh SH
B	Bizerte	N/E	300	653	7.2	30.9	94.3	Worm SH
G	Goussa	N/W	100	663	6.2	35.6	74.3	Mild SH
Abd	Djebel Abderrahmen	Dorsal	637	520	4.9	30.3	69.1	Mild SH
R	Djebel Ressas	N/E	795	453	5.4	33.1	55.3	Temperate Upper SA
Zg	Zaghouan	N/E	175	518	6.6	32.9	67.3	Worm Upper SA
O	Djebel Oust	Dorsal	400	435	5.9	35.6	49.7	Worm Upper SA
J	Souk jemaa	Dorsal	900	503	4.8	32.6	63.1	Temperate Upper SA
S	Djebel Serj	Dorsal	1357	400	3.3	33.7	44.8	Temperate Lower SA
Sb	Sbeitla	Center	670	328	4.5	34.4	37.7	Temperate Upper A

Pop population, N/W north-west, N/E north east, Alt altitude, *P* annual rainfall, *M* and *m* are the of the average maximum temperature of the hottest month and the average of the minimum of the coldest month, respectively, Q_2 Emberger coefficient, H: humid, SH: subhumid, SA: semiarid, A: arid.

Growing conditions

Experiments were carried out during two successive years (20012-2013 and 2013-2014) at National Institute of Agronomic Research of Tunisia (INRAT) in Ariana (E-N, and elevation 10 m above sea level. The mean annual temperature was 25°C with monthly ranging from 15°C in January to 35°C in August).

Seeds of each population were germinated in Jiffy pots placed in a greenhouse in early November. Two weeks after emergence they were transplanted in the field. Plants were grown in natural conditions. The plants of thirteen populations were planted in completely randomized design with four replicates. Each replication consisted of a 5 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).

Morphological analyses

Sixteen characters related to morphology, phenology and yield were recorded for each plant. (Table 2). These characters were measured in ten individuals per population. The morphological characters were selected according to criteria used in taxonomic studies

(Maire, 1955, Kimber and Feldman, 1987) and diversity studies (Zaharieva *et al.*, 2001, 2003a, b).

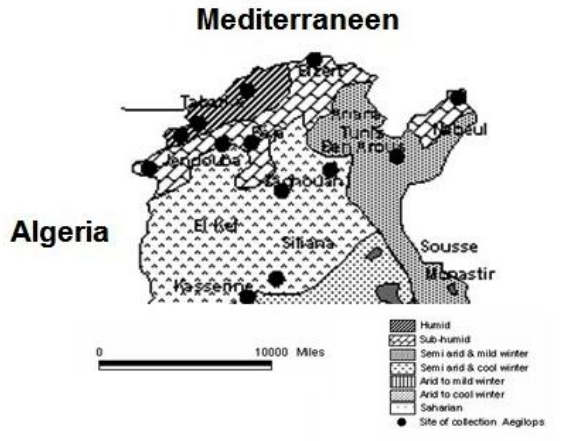

Fig.1. Distribution sites of *Aegilops geniculata* in Tunisia.

RAPD analysis

The genomic DNAs were extracted from young leaves of *Ae. geniculata* populations by the cetylmethylammonium bromide (CTAB) method with minor modification (Murray and Thompson, 1980). After purification, the DNA concentration was spectrophotometrically estimated. DNA integrity was assessed by 0.8% agarose gel electrophoresis (Sambrook *et al.*, 1989). 20 ng of the extracted genomic DNA was diluted and used for PCR amplifications. Some primers which generate variable amplification gave poor amplification products or non-repeatable banding patterns were discarded. Only nineteen primers purchased from Operon Technologies inc. (Alameda, USA) that showed numerous bands, were used for the amplification of random DNA sequences.

Table 2. Agro-morphological characters of *Ae. geniculata* Roth populations used in numerical analysis.

Symbol	Characters
Phenological traits	
G	Time of 50% of germination (days)
L2	Time of appearance of the leaf 2 (days)
T1	Time of appearance of the first tiller (days)
HH	50% of heading (days)
HF	Full heading (days)
FL	Beginning of flowering time (days)
Morphological traits	
TN	Total number of tillers
LN	Number of leaves on the first tiller
PH	Plant height (cm)
SLN	Spikelet number per spike
SL	Length of the spike measured without awns (cm)
SEL	Length of the seed relieved from the first spikelet (mm)
Yield-related traits	
SN	Spikes number per plant
SES	Seeds per spike
SEP	Seeds number per plant
SEY	Seed yield per plant (g)

PCR reactions were performed, in a 25µl volume reaction mixture containing between 20- 40 ng of total cellular DNA, 5µl of 5x Taq DNA polymerase buffer, 0.5µl dNTP (200µM), 0.2µTaq DNA

polymerase (5U/µl) et 1µL de $MgCl_2$ (2.5mM), 25 µM of primer. The reaction mix was overlaid with a drop of mineral water. PCRs were performed using a Biometra UNO II thermal-cycler and involved an initial denaturation step (94°C, 5min), 40 amplification cycles (each 94°C, 30s; 38°C, 1min and 72°C, 1min) and a final extension step (72°C, 10min). Amplification products were analysed by electrophoresis in 2% agarose gels in 1x TAE (Tris Acetate EDTA) buffer and visualized under UV light after Ethidium bromide staining (Sambrook *et al.* 1989). Amplification was performed and only reproducible products were taken into account for further data analysis.

Table 3. Variance analysis, between years, within population (on individuals), populations, between groups.

Characters	Years	Individuals	Populations	Groups
Phenological traits				
G	2.060 ns	4.075 ns	25.799**	2.347 **
L2	1.952 ns	1.859 ns	8.489**	2.078 **
T1	0.698 ns	1.473 ns	9.759**	3.123 **
HH	0.112 ns	6.074 ns	32.390**	4.213 **
HF	0.723 ns	5.029 ns	32.732**	7.809 **
FL	1.653 ns	4.701 ns	29.217**	1.232 ns
Morphological traits				
TN	2.734 ns	10.528 ns	13.524**	2.067 **
LN	0.257 ns	12.949 ns	7.443**	1.543 ns
PH	0.563 ns	8.889 ns	14.171**	3.681 **
SLN	0.024 ns	1.719 ns	4.083**	1.045 ns
SL	1.756 ns	1.962 ns	6.611**	2.436 ns
SEL	0.254 ns	0.395 ns	8.551**	3.917 ns
Yield-related traits				
SN	0.081 ns	13.888 ns	3.541**	0.976 **
SES	0.053 ns	2.555 ns	4.504**	1.544 ns
SEP	1.074 ns	16.695 ns	3.549**	1.439*
SEY	0.065 ns	13.953 ns	6.085**	2.657**

F-probabilities are indicated by symbol: ns = non-significant difference;
* significant differences at $P < 0.05$, ** significant differences at $P < 0.01$.

Statistical analysis

To evaluate the morphological variability of a population, PCA and an analysis of variance (ANOVA) were applied to the 260 individuals. ANOVA was based on the F test which represented a ratio of two variances; one is for example the variance between groups and the other the general variance. Principal component analysis (PCA) was dealt and the genetic distances between the different populations were calculated on the centred and standardized variates using measured data with MVSP 3.13 software. The relationship between the morphological distance matrix and the distances obtained with RAPD markers was analysed. The comparison between the two dendrograms was performed according to the approach developed by Mentel's correlation tests using Mxcomp procedure from NTSYS . The principle of this approach is to compare the observed Z-value or r-value with its permutational distribution according a null hypothesis, which is not difference

between the distance matrix, Z=0. In this comparison, 5000 random permutations were made. The null hypothesis of no correlation is rejected when Mentel statistic falls outside the 0.05 confidence level.

Results

Morphological variability

Variance analysis carried out on all studied traits and earliness assessed during two years of experimentation revealed no significant differences between data obtained during the two years of experimentation (Table 3). So, we used those of the first year of experimentation (20011-2012). Variance analysis revealed no significant difference ($P < 0.05$) within populations for all characters, indicating morphological similarities between individuals within the same populations. Whereas, it exhibited a significant difference ($P < 0.05$) between populations for all characters. Significant differences were expressed mainly for all of traits except beginning of flowering time, number of leaves on the first tiller, length of the spike measured without awns, length of the seed relieved from the first spikelet, length of the seed relieved from the first spikelet and Seeds per spike expressed at group levels.

The PCA analysis performed on the 13 populations and based on sixteen agro-morphological characters explains 83.98 of the total variation with 48.32%, 26.38% and 9.28% for axis 1, 2 and 3. Loading variables and the PCA scores were also calculated (Table 4). Each principal component was interpreted by its correlation with the original variables. The PC1 showed the largest loading values with phenological (L2, T1) morphological (PH, SLN, SL) and yield-related traits (SN, SEP, SEY). Whereas, the PC2 shared the largest loading values with phonological (FL, HH, HF) morphological (LN, TN) and yield-related traits (SES). 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) ($P < 0.05$). 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 it was composed by populations collected from the mountains located at an altitude above 630 m (Aine Zana and Mekna) under fresh sub-humid climates. The plants of this group were characterized by late germination, high biomass production, good fertility, weak seeds and large growing cycle such as the spike emergence, the flowering. The second group positively correlated to the PC 2 and negatively correlated to the PC 1. This group was composed of populations of the coastal area (Tabarka, Nefza). These populations grew under warm humid bioclimate. The individuals of this group were distinguished by large growing cycle, good vegetative development, good fertility, high yield-related, long spikes, spikelets and seeds. The third group included populations from mountains situated at a mean altitude comprised between 637 and 795 m (Abderrahmen, Ressas) and high altitude 1357 m (Djebel Serj). Population of this group were located in the positive part of PC 2 and distributed along negative part of PC 1 according to an increasing gradient of aridity, from mild sub-humid to temperate lower semi-arid. The plants of this group were characterized by reduction of vegetative development and yield-related. The fourth group negatively correlated to the two axes and it was made up of populations collected from the coastal area (Bizerte) and the plains (Goussa and Zaghouan) under Worm, mild conditions, intermediate between the sub-humid and the upper semi-arid climate. The plants of this group were differentiated by They exhibited early germination, an acceleration of growing cycle (heading and flowering, maturity), weak vegetative development and high seed yield. The fifth group negatively correlated to PC2 and positively correlated to PC1 and comprised populations from dorsal mountain (Djebel Oust, Souk Jemaa) and steppic highlands (sbeitla). The plants of this group collected under temperate condition, intermediate between upper semi-arid and upper arid. The plants are characterized by feeble vegetative development, reduction of fertility and yield-related and shortening of growing cycle (spike emergence, flowering).

Table 4. Principal component analysis (PCA) of 16 phenological , morphological and yield-related traits.

Characters	PC1	PC2	PC3
Phenological traits			
G	0.212	0.141	**0.421**
L2	**0.28**	-0.102	**0.515**
T1	**0.286**	-0.184	0.26
HH	0.002	**0.417**	-0.022
HF	0.014	**0.42**	-0.006
FL	0.004	**0.426**	0.123
Morphological traits			
TN	-0.224	**0.26**	0.053
LN	-0.198	**-0.278**	0.156
PH	**-0.313**	-0.058	-0.046
SLN	**0.216**	-0.186	-0.007
SL	**-0.265**	-0.095	0.102
SEL	**-0.212**	-0.175	0.014
Yield-related traits			
SN	**-0.239**	0.231	0.125
SES	-0.156	**-0.347**	0.186
SEP	-0.298	0.014	**0.419**
SEY	**-0.296**	0.042	0.287
Percentage variation	**43.32**	**26.32**	9.28

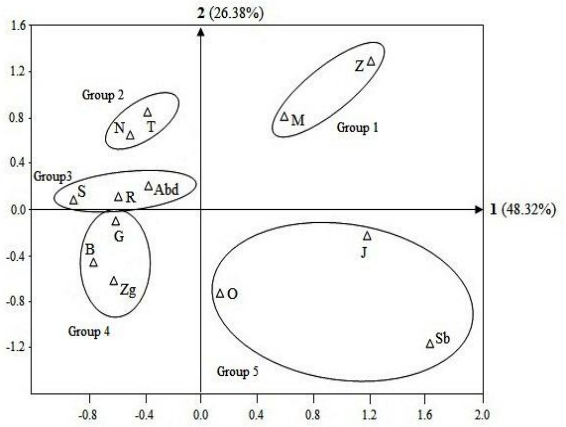

Fig. 2. Principal Component analysis of 13 Tunisian populations belonging to *Ae. geniculata* Roth.

We used the Euclidian distance matrix with linkage average method to draw morphological distances. The dendrogram was constructed as indicated in fig. 3. It shows three main Clusters (I, II and III) according to climatic regions. In fact, the first cluster contains the greatest number of populations originated from humid and sub humid microclimate. This cluster is composed by two sub-clusters. The first one grouped the populations collected from 'Tabarka', 'Nefza' 'Dj Ressas', and 'Dj Abderhamen'. The second sub-cluster formed by populations of 'Bizerte', 'Zaghouan' and 'Goussa'. The average distance between populations of the first sub-cluster varies between 0.10 and 0.16; but that of the second sub-cluster was ranged between 0.14 and 0.23. Cluster II is constituted by five populations. 'Mekna' and 'Ain Zana' originated from the fresh sub humid area at an altitude above 600 m, formed a sub-cluster. They are grouped at the distance of 0.15. 'Dj Serj', 'Dj Oust' and 'Souk jemaa' populations joined the sub-cluster respectively at the distance of 0.29; 0.36; 0.66. They are collected from dorsal located at high altitude from 400 to 1325 m and having a Temperate condition, intermediate between, upper semi-arid and lower sem-arid. Finally, cluster III made up by 'Sbeitla' population collected under temperate upper arid bioclimate from steppic highlands was distinguished by a distance of 0.93.

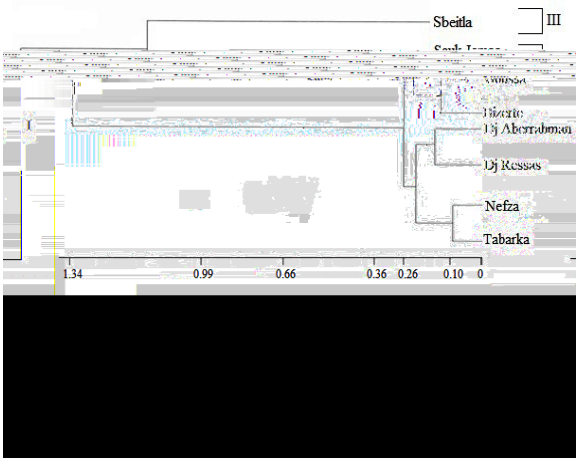

Fig.3. Dendrogram of *Aegilops geniculata* accessions clustered with average linkage method.

Despite that the PCA of populations showed five groups and the dendrogram clustering gave three groups, the most populations gathered in the same group by PCA are also assembled in the same cluster. In fact, 'Ain Zana' and 'Mekna' populations from belong to the same group according to the two

methods. The same remark is given for and for 'Bizerte', 'Zaghouan' and 'Goussa' populations. Also, we remark that 'Souk Jemaa' and 'Dj Oust' populations are included in the same group. The two methods also showed that 'Tabarka' and 'Nefza' and 'Dj Abderhamen', and 'Dj Ressas' populations are associated in the similar sub-groups.

RAPD markers

Analysis of the amplification patterns in *Ae. geniculata* showed a difference by position and number of generated fragments (Fig. 4). A total of 212 DNA fragments were generated by 19 primers with an average of 11.15 fragments per primers and were scored as RAPD markers (Table 5). Of these amplified fragments, 153 were polymorphic (about 71.27%), with an average of 11.76 fragments per population and 8.05 fragments per primers. For each primer, a considerable variation was present for the number of fragments produced (from 9 to 14) and the number of polymorphic fragments (from 3 to 14) that ranged in size from 0.5 to 3 Kb. The number and sizes of the DNA fragments were strictly dependent upon primer sequence.

Table 5. Nucleotide sequence of primers with the number of amplified products and percentages of polymorphic fragments.

Primers	Sequences (5'-3')	Total amplified fragments	Polymorphic fragments	Polymorphism (%)
OPM14	AGGGTCGTTC	9	3	33.33
OPB13	TTCCCCCGCT	12	4	33.33
OPJ18	TGGTCGCAGA	11	7	63.63
OPG02	GGCATGAGC	13	12	92.30
OPF10	GGAAGCTTGG	12	10	83.33
OPD10	GGTCTACACC	9	4	44.44
OPG10	AGGGCCGTCT	11	10	90.90
OPG12	CAGCTCACGA	14	14	100
OPM12	GGGACGTTGG	9	9	100
OPA06	GGTCCCTGAC	14	14	100
OPJ16	CTGCTTAGGG	11	9	81.81
OPJ06	TCGTTCCGCA	9	8	88.88
OPM16	GTAACCAGCC	11	3	27.27
OPD20	ACCCGGTCAC	11	6	54.54
OPD18	GAGAGCCAAC	9	7	77.77
OPE14	TGCGGCTGAG	14	9	64.28
OPA12	TCGGCGATAG	9	7	77.77
OPB05	TGCGCCCTTC	13	10	76.92
OPJ04	CCGAACACGG	11	7	63.63
Total		212	153	71,27

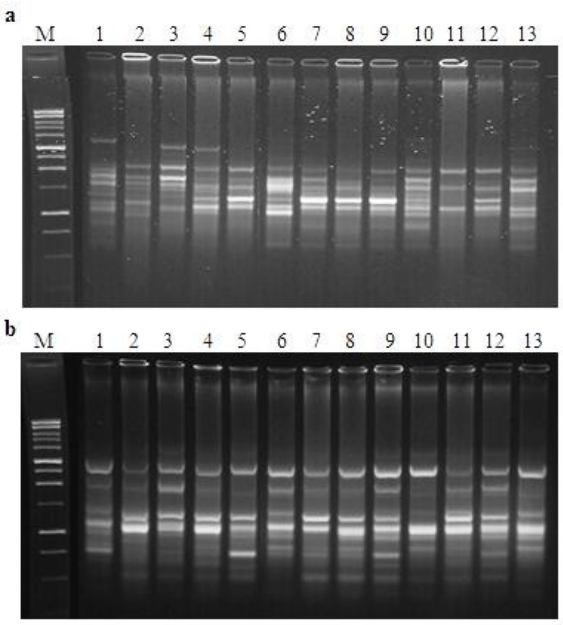

Fig.4. Amplification profile of 13 *Aegilops* accessions OPB05 primer (a) and OPJ18 primer (b) 1= Djebel Ressas, 2= Djebel Serj, 3= Sbeitla, 4= Souk jemaa, 5= Djebel Abderahmen, 6= Ain Zana, 7= Mekna, 8= Goussa, 9=Bizerte, 10= Djebel Oust, 11=Nefza, 12=Tabarka, 13=Zaghouan, M= Molecular weight markers: 1 Kb DNA Ladde.

The number of DNA polymorphic fragments per populations varied from 5.57 in 'Goussa' to 7.26 in 'Djebel Oust' (Table 6). Of the 13 studied populations, 'Dj Oust', 'Tabarka' and 'Ain Zana' population produced the maximum number of DNA polymorphic fragments. This level suggests an efficiency of tested primers. This result showed that the primers OPG02, OPG12, OPM12, OPA06, OPJ06, OPD18, OPG10, OPJ16 and OPA12 were more efficient to assess the genetic relationships, estimate genetic diversity and to explore the DNA polymorphism of studied genotypes. Phylogenetic diagram was drawn using the Unweighted Pair Group Method with the Arithmetic Averaging (UPGMA) algorithm and referring to the similarity rate of 70 dendrogram obtained revealed three main clusters (Fig. 5). The first cluster A is formed by two sub-cluster. The first sub-cluster consisted of 'Dj Ressas', 'Ain Zana' populations, the second one is constituted by 'Bizerte', 'Dj Oust' and 'Zaghouan' populations, their similarities percentage varied between 68.07% and 73.23%. The second cluster B is formed by two sub-clusters. The first sub-

cluster is composed by 'Dj Serj' and 'Sbeitla' populations, the second are constituted by 'Souk jemaa', 'Mekna', 'Goussa' and 'Dj Abderhamen'. The estimated similarity coefficients ranged from75.58% and 79.81%. A high similarity at the level of the DNA was showed between two combinations Mekna and Souk jemaa populations with 82.16%. The third cluster C is formed by 'Nefza', 'Tabarka' populations showing a similarity of 73.70%. *Ae. geniculata* populations exhibited a low level of intra-specific similarity that varied from 59.15% to 82.16%.

Table 6. Number of DNA polymorphic fragments per populations.

Primers	R	S	Sb	J	Abd	Z	M	G	B	O	N	T	Zg
OPM14	7	4	5	4	6	6	4	5	6	6	4	6	6
OPB13	5	4	4	6	6	5	6	5	6	6	5	6	7
OPJ18	5	5	7	6	7	5	7	7	8	6	9	7	6
OPG02	8	7	5	8	7	8	5	6	5	5	7	9	7
OPF10	6	5	6	6	5	6	5	6	8	8	7	6	7
OPD10	6	3	4	5	6	3	4	4	5	6	7	6	7
OPG10	5	5	5	5	6	5	5	5	5	6	7	8	6
OPG12	6	4	8	6	7	8	4	7	6	7	4	6	7
OPM12	5	5	6	7	4	7	6	5	6	6	3	8	5
OPA06	9	10	9	8	7	6	12	9	11	8	5	5	6
OPJ16	5	3	5	1	5	5	1	2	3	6	2	6	5
OPJ06	4	4	4	1	3	2	0	2	6	2	6	5	6
OPM16	9	8	10	8	10	9	9	8	9	9	8	9	9
OPD20	5	6	7	8	7	8	8	8	8	7	5	9	8
OPD18	5	5	7	5	5	5	4	4	5	6	4	5	5
OPE14	9	8	9	8	5	9	8	6	8	12	6	11	11
OPA12	5	6	5	6	8	6	7	4	7	6	7	6	5
OPB05	8	5	7	8	5	9	7	6	8	12	4	6	10
OPJ04	10	7	7	7	6	10	7	7	9	9	9	9	10
Total	122	104	120	113	115	122	109	106	129	138	109	133	133
Average/primers	6.42	5.47	6.31	5.94	6.05	6.42	5.73	5.57	6.78	7.26	5.73	7	7

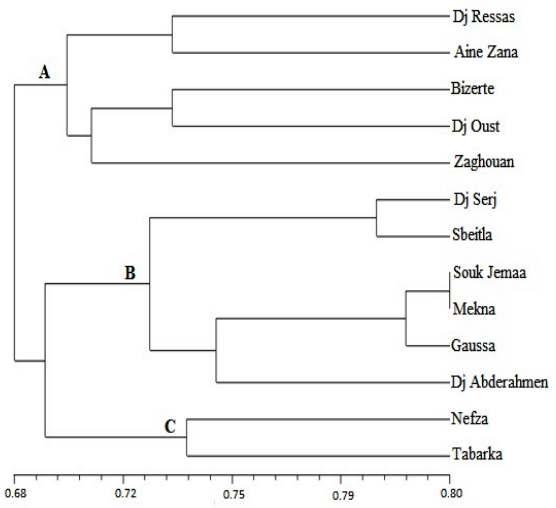

Fig.5. Dendrogram constructed by UPGMA based on RAPD similarity values showing relationships among the 13 *Ae. geniculata* populations.

To provide an objective comparison matrices, generated from RAPD and morphological data, were compared using Mentel test. Not significant and quite low correlation between the dendrograms was obtained (r = -0.268, p = 0.0198) with MxComp procedure from NTSYS programs. In fact, calculated distances between populations by both methods are different

Discussion

Morphological diversity and molecular analysis (RAPD) discriminated the populations of *Ae. geniculata* collected in northern and central Tunisia, in various ecological environments. Characters expressing morphology, phenology and yield (Table 2) highly varied between populations but poorly

differed between groups that reflected environmental conditions of origin (Table 3). They were strongly correlated with ecological factors, particularly winter and summer temperatures and altitude. Taxonomists (Zhukovsky, 1928, Eig, 1929, Hammer 1980, Witcombe, 1983, Kimber and Feldman, 1987) and in particular the regional florists (Battandier and Trabut, 1902, Maire 1955, Quezel and Santa, 1962) often used the length inflorescence characters (spikes, Spikelet and awns) to differentiate species and subspecies. In our study, these characters discriminated less the studied populations than counting characters. The analysis of variance revealed a strong homogeneity within populations and a significant variation between populations for all characters. This variation pattern is specific in most annual colonizing species and particularly in autogamous species (Hamrick and Godt, 1997). The reduction of genetic variation was considered as main factor in colonizing success in most annuals and facilitated their rapid colonization specifically of disturbed sites (Hegde *et al.*, 2002).

The PCA of agro-morphological traits (Fig. 2) showed that the large variability observed between populations was associated to ecological parameters, namely, the mean of annual rainfall, the summer and winter temperatures and the altitude. Indeed, four phenotypes were distinguished. The first one The first one, characterized by late germination, large growing cycle such as the spike emergence, the flowering, a high biomass production, good fertility and weak caryopses, was represented at mean altitude, in fresh sub-humid climate. The second phenotype with large growing cycle, good vegetative development, good fertility, high yield-related, long spikes, spikelets and caryopses. This phenotype distinguished the groups located at coastal areas and plains of warm humid bioclimate and mean and high altitudes of mild and temperate conditions, intermediate between sub-humid to lower semi-arid. The third phenotype was characterized by early germination, an acceleration of growing cycle (heading and flowering, maturity), weak vegetative development and high caryopses yield was mainly present in the coastal areas and plains under worm, mild conditions, intermediate between the sub-humid and upper semi-arid climate. The fourth was characterized by low morphological development Weak fertility reduction of yield-related and shortening of growing cycle was represented at mean and high altitudes of temperate upper semi-arid and steppic highlands of temperate arid.

Perrino *et al.* (1993) analyzing the diversity of Italian populations of *Ae. geniculata* on the basis of morphological characters, showed that the morphological variability was associated with ecological conditions. Zaharieva *et al.* (2003a, b), analyzing numerous populations of *Ae. geniculata* and other *Aegilops* species sampled in Bulgaria noted significant differences between populations for most of the morphological characters and observed a significant correlation between the morphology and the ecogeographical region of the studied populations. The populations of *Ae. geniculata* collected from Tunisia were adapted to a large range of rainfalls, from weak rainfalls (328 mm) in Upper arid areas, to abundant rainfalls (1032 mm) in humid regions. A large variation in the adaptation to drought conditions was observed in a collection of *Ae.geniculata* from different geographic origins by Zaharieva *et al.* (2003a, b) and in a collection from the semi-arid region of Lebanon by Baalbaki *et al.* (2006).

The use of RAPD for identification of cultivars through DNA profiling is the current method of choice in measuring genetic variation within germplasm collections (Hernendez *et al.*, 1999). Consequently, the performance of RAPD markers was evaluated using various parameters such as percentage of polymorphism and clusters formed in the dendrogram. In this study, the high level of polymorphism (71.27%) observed in *Ae. geniculata* populations was obtained by Tao *et al.* (1993) in *Sorghum bicolour* (L) *Moench*. Moreover, this result was also reported by Zaharieva *et al.* (2001). The high level of polymorphism was probably due to the large number of primer used in this experiment. Guadagnuolo *et al.* (2001) affirmed that RAPD

amplifications provided a largest set of polymorphic markers. The variability between populations also reflected the variation due to environmental conditions. The numbers of DNA polymorphic fragments were associated to winter temperatures. The populations growing under warm climates (sub-humid, humid and hyper semi-arid climates) presented the highest values followed by those under mild (sub-humid and humid), moderated (sub-humid, lower semi-arid and hyper arid) and finally those in the cold bioclimates (sub-humid and humid). Nevo *et al.* (1982), using isozyme markers in wild tetraploid wheat, highlighted a similar correlation between specific isozyme loci and some environmental factors.

The populations growing under warm climates showed more genetic variability, compared with those of cold climates. The differences of genetic variability between these populations could be explained by fluctuations of gene flow. The warm temperature in humid and sub-humid environments would constitute a favourable factor which increases the diversity within the populations.

The UPGMA dendrogram for RAPD data showed that the structuration group is not depending on the environmental conditions origin. This result is not in agreement with that found using PCA population loading and the dendrogram of morphological traits. Indeed, the representative dendrograms for the populations based on RAPD markers and morphological traits showed that the overall correspondence between the similarity matrices is low and the correlation between these two dendrograms is not significant (r = -0.268). This result is in agreement with those of Roldan-Ruiz *et al.* (2001) working with 16 ryegrass varieties and reporting very low correlation (r = -0.06) between AFLP and morphological characters. Similar result was found by Campos *et al.* (2005) in Sixty-three Mandarin (*Citrus* spp.) cultivars, noticing a non significant correlation (r = 0.31) between morphological and molecular data. Moreover, our result corroborated with several studies such as European barley varieties (Schut *et al.*, 1997), synthetic hexaploid wheats and their parents (Lage *et al.*, 2003), and Squash germplasm (Ferriol *et al.*, 2004). Hegde *et al.* (2000) observed in diploid and tetraploid wild wheats large-scale morphological similarities and low-level genetic variation, and suggested that these taxa possess similar types of genetic potential in similar ecological interactions which lead them to specialized adaptation. The spread of genetic diversity between populations would be attributed to a fine-scale adaptation to microhabitat such as soil type and climatic regime. They suggested that genetic variation could also be influenced by other micro- and/or macro-evolutionary forces such as meiotic process. Indeed, Badaeva *et al.* (2004), using C-banding and FISH (Fluorescence In Situ Hybridization), observed in *Ae. geniculata* significant intraspecific variability which was due to introgressive hybridization associated with chromosomal rearrangements. They concluded that this species is still undergoing an intensive speciation process. The concentration of diploid species to the Eastern of Mediterranean basin (Zhukovsky, 1928, Eig, 1929, van Slageren, 1994) and their absence from Tunisia confirmed the allopatric origin of *Ae. geniculata*. This species could be considered as young species (Stebbins, 1971) which as not yet finished its speciation. This is in agreement with the conclusions of Badaeva *et al.* (2004).

In conclusion, our genetic and morphological study of *Ae. geniculata* populations from Tunisia showed that all individuals were morphologically homogeneous and that there was little variability within populations. The studied populations clustered into morphological groups adapted to various ecological environments. These groups were differentiated into five main morphological patterns associated to climate, altitude and winter temperature. The wide distribution of *Ae. geniculata* from Kroumiry mountains and the coastal region to the Dorsal areas, including the Cap-Bon, Mogodses, Zaghouan and the steppic highlands, was due to adaptive variation revealed by significant correlations between numbers of DNA polymorphic fragments and bioclimatic

conditions. The populations from cold climates were genetically less diverse than those of warm climates.

References

Baalbaki R, Hajj-Hassan N, Zurayk R. 2006. *Aegilops* species from semiarid areas of Lebanon: variation in quantitative attributes under water stress. Crop Science **46,** 799-806.

Badaeva ED, Amosova AV, Samatadze TE, Zoshchuk SA, Chikida N, Zelenin AV, Raupp WJ, Friebe B, Gill BS. 2004. Genome differentiation in *Aegilops*. 4. Evolution of U-genome cluster. Plant Systematics and Evolution **246,** 45-76.

Battandier JA, Trabut L. 1902. Flore analytique et synoptique de l'Algérie et de la Tunisie. edn. Vve Girault, Alger. p 410.

Ben Brahim N, Sebei H, Gharbi MS. 2002. Collecte des populations *d'Aegilops ovata* auct. et évaluation de la diversité génétique pour l'amélioration du blé. 13th Eds, journées Biologiques, Hammamet, pp : 75.

Campos ET, Gutiérrez Espinosa MA, Warburton ML, Varela AS, Villegas Monter A. 2005. Characterization of Mandarin (Citrus spp.) using morphological and ALFP markers. Interciencia **30 (11),** Caracas. ISSN 0378-1844 version impresa.

Colmer TD, Flowers TJ, Munns R. 2006. Use of wild relatives to improve salt tolerance in wheat. Journal of Experimental Botany **57,** 1059-1078.

De Bustos A, Jouve N. 2006. Characterization and phylogenetic analysis of the genes coding for high molecular wei glutenin subunits in three diploid species of Aegilops. International Journal of Plant Sciences **167,** 359-366.

Eig A. 1929. Monographisch-kritische U¨ bersicht der Gattung Aegilops in German. Feddes Repert **55,** 1–228.

Farooq S. 2004. Salt tolerance in *Aegilops* species: a success story from research and production to large-scale utilization of salt tolerant wheats. In: Taha FS, Ismaial S, Jaradat A (eds) Prospects of saline agriculture in the Arabian peninsula. Amheerst Scientific Publishers, Massachusetts, p. 121-134.

Farooq S. 2002. *Aegilops ovata*: a potential gene source for improvement of salt tolerance of wheat. In: Ahmad R, Malik KA (eds) Prospects for saline agriculture, vol 37. Kluwer Academic Publishers, Dordrecht, p 123-130.

Farooq S, Shah, TM, Asghar M. 1996. Intergeneric hybridization for wheat improvement: V. Production of and metaphase 1 chromosome analysis in F1 hybrids of wheat (*Triticum aestivum*) with *Aegilops ovata* L. Cereal Research Communications **24,** 155-161.

Feldman M. 1965. Further evidence for natural hybridization between tetraploid species of *Aegilops*. sect. *Pleionathera*. Evolution **19,** 162-174.

Fernandez-Calvin B, Orellana J. 1990. High molecular weight glutenin subunit variation in the Sitopsis section of Aegilops. Implications for the origin of the B genome of wheat. Heredity **65,** 455-463.

Ferriol M, Picó B, de Fernandez CP, Nuez F. 2004. Molecular diversity of germplasm collection of squash (*Cucurbita moschata*) determined by SRAP and AFLP markers. Crop Science **44,** 653-664.

Gill BS, Sharma HC, Raupp WJ, Browder IE, Hatchett JH, Harvey TL, Moseman JG, Waines JG. 1985. Evaluation of *Aegilops* species for resistance to wheat powdery mildew, wheat leaf rust, Hussian fly, and greenbug. Plant Disease **69,** 314-316.

Guadagnuolo R, Bianch D, Felder F. 2001. Specific genetic markers for wheat, spelt, and four wild relatives: Comparison of isozymes, RAPDs and wheat microsatellites. Genome **44,** 610-621.

Hammer K. 1980. Vorarbeiten zur Monographischen Darstellung von Wildpflanzen-Sortimenten: Aegilops L. Kulturpflanze **28,** 33-180.

Hamrick JL, Godt MJW. 1997. Effects of life history on genetic diversity in plants species. In: Silvertown J, Franco M, Harper JL (eds) Plant life histories-ecology phylogeny and evolution. Cambridge University Press, London, p 313.

Hegde SG, Valkoun J, Waines JG. 2002. Genetic diversity in wild and weedy *Aegilops*, *Amblyopyrum*, and Secale species-A preliminary survey. Crop Science 42: 608-614.

Hegde SG, Valkoun J, Waines JG. 2000. Genetic diversity in wild wheats and goatgrass. Theoretical and Applied Genetics **101,** 309-316.

Hernendez P, Martin A, Dorado G. 1999. Development of SCARs by direct sequencing of RAPD products: a practical tool for the introgression and marker assisted selection of wheat. Molecular Breeding **5,** 245-253.

Kimber G, Feldman M. 1987. Wild wheat. An introduction. Special Report 353, College of Agriculture, University of Missouri, Columbia, p. 142.

Lage J, Warburton ML, Crossa J, Skovmand B, Andersen SB. 2003. Assessment of genetic diversity in synthetic hexaploid wheat and their *Triticum dicoccum* and *Aegilops* tauschii parents using AFLPs and agronomic traits. Euphytica **134,** 305-317.

Maire R. 1955. Flore de l'Afrique du Nord. Eds Le Chevalier, vol III, Paris, p 65-69.

Mguis K, Ben Brahim N, Albouchi A, Yakoubi-Tej M, Mahjoub A, Ouerghi Z. 2008. Phenotypic responses of the wild wheat relative *Aegilops geniculata* Roth and Wheat (*Triticum durum* Desf.) to experimentally imposed salt stress. Genetic Resource and Crop Evolution **55,** 665-674

Monte JV, Casanova C, Soler C. 1999. Genetic variation in Spanish populations of the genus Aegilops revealed by RAPDs. Agronomie **19,** 419-427.

Murray MG, Thompson WF. 1980. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Research **8,** 4321-4325.

Nevo E, Goldberg E, Beiles A, Brown AHD, Zohary D. 1982. Genetic diversity and environmental associations of wild wheat. *Triticum dicoccoides* in Israel. Theoretical and Applied Genetics **78,** 260-264.

Pazy B, Zohary D. 1965. The process of introgression between Aegilops polyploids. Natural hybridization between *A. variabilis*, *A. ovata* and *A. biuncialis*. Evolution **19,** 385-394.

Perrino P, Laghetti G, Cifarelli S, Volpe N, Spagnoletti-Chichester PL, Zeuli UK. 1993. Wild wheats in southern Italy. In: Damania AB (ed) Biodiversity and wheat improvement. Wiley, Chichester, p. 361-368

Quezel P, Santa S. 1962. Nouvelle flore de l'Algérie et des régions désertiques méridionales. Tome I. Edition du CNRS, Paris, p. 558.

Rekika D, Zaharieva M, Stankova P, Xu X, Souyris I, Monneveux P. 1998. Abiotic stress tolerance in Aegilops species. In: Nachit MM *et al.* Durum Research Network, Proceeding of the SEWANA, South Europe, West Asia and North Africa, ICARDA, Aleppo, Syria, p. 113-118.

Rodriguez-Quijano M, Nieto-Taladriz MT, Carrillo JM. 2000. Polymorphism of high molecular weight glutenin subunits in three species of *Aegilops*. Genetic Resource and Crop Evolution **48,** 599-607.

Roldan-Ruiz L, van Eeuwijk FA, Gilliland TJ, Dubreuil P, Dillmann C. Lallemand J, de Loose M, Baril CP. 2001. A comparative study of molecular and morphological methods of describing

relationships between perennial ryegrass (*Lolium perenne* L.) varieties. Theoretical and Applied Genetics **103,** 1138-1150

Sambrook J, Fritish EF, Maniatis T. 1989. Molecular cloning a laboratory manual. 2 eds. Clod spring Harbor laboratory, New York, p. 165.

Schut JW, Qi X, Stam P. 1997. Association between relationships measures based on AFLP markers, pedigree data and morphological traits in barley. Theoretical and Applied Genetics **95,** 1161-1168

Stebbins GL. 1971. Chromosomal evolution in higher plants. Arnold, London, p. 21.

Sun X, Hu S, Qian W, Hao S, Zhang A, Wang D. 2006. Characterization of HMW glutenin subunits from *Aegilops searsii* and identification of a novel variant HBM glutenin subunit. Theoretical and Applied Genetics **113(4),** 631-641

Tao Y, Manners MM, Ludlow M, Henzell M. 1993. DNA polymorphism in grain *sorghum* (*Sorghum bicolour* L. Moench). Theoretical and Applied Genetics **86,** 679-688.

Van Slageren MW. 1994. Wild Wheats: a monograph of *Aegilops* L. and Amblyopyrum (Jaub.& Spach) Eig (Poaceae). Wageningen Agricultural University Papers, Wageningen, The Netherlands, p. 94-97.

Witcombe JR. 1983. A guide to the species of *Aegilops* L. International Board for plant Genetic Resources. Wheat Program IBPGR, Rome, p. 74.

Zaharieva M, David J, This D, Monneveux P. 1999. Analyse de la diversité génétique d'*Aegilops geniculata* Roth en Bulgarie. Cahiers Agriculture **8,** 181-188.

Zaharieva M, Dimov A, Stankova P, David J, Monneveux, P. 2003. Morphological diversity and potential interest for wheat improvement of three *Aegilops* L. species from Bulgaria. Genetic Resource and Crop Evolution **50,** 507-517

Zaharieva M, Gaulin E, Havaux M, Acevedo E, Monneveux P. 2001. Drought and heat responses in the wild wheat relative *Aegilops geniculata* Roth: potential interest for wheat improvement. Crop Sciences **41,** 1321-1329.

Zaharieva M, Suenaga K, William HM, Mujeeb-Kazi A. 2003. Microsatellite markers for detection *Aegilops geniculata* Roth and U genome chromosomes in wheat background. Annual Wheat Newslett **49,** 75-78

Zhang XY, Wang R, Dong YS. 1996. RAPD polymorphisms in *Aegilops geniculata* Roth. (*Ae. ovata* auct. non L.). Genet Resource Crop Evolution **43,** 429-433.

Zhukovsky PM. 1928. Kritiko-systematischeskii obzor vydov roda *Aegilops* L. (Specierum generis *Aegilops* L. revisio critica). Trudy Prikl Bot Genet Selekc **18,** 417-609

Zohary D. 1965. Colonizer species in the wheat group. In: Baker HG, Stebbins GL (eds) The genetics of colonizing species. Academic Press, New York, p. 403-423.