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Polymorphism at High Molecular Weight Glutenin Subunits and Morphological Diversity of *Aegilops geniculata* Roth Collected in Algeria

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A collection of 35 accessions of the tetraploid wild wheat *Aegilops geniculata* Roth (MM, UU) sampled in northern Algeria was evaluated for morphological and biochemical variability. Morphological and ecological analyses based on morphological traits and bioclimatic parameters, respectively, were assessed using principal component analysis (PCA). Accessions were differentiated by width characters, namely spike's width, and a weak relationship between morphological traits and ecological parameters was found. Polymorphism of high molecular weight (HMW) glutenin subunits was carried on by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Among accessions analyzed, 27 alleles were identified at the two loci *Glu-M1* and *Glu-U1:* resulting in twenty-nine patterns and a nomenclature was proposed. Two alleles at the *Glu-U1* locus expressed a new subunit with a slightly slower mobility than subunit 8. These results provide new information regarding the genetic variability of HMW glutenin subunits, as well as their usefulness in cultivated wheat quality improvement.

Keywords: *Aegilops geniculata,* Algeria, morphology, HMW glutenin subunits

Abbreviations: ICARDA: International Center for Agricultural Research in the Dry Areas, PCA: Principal Component Analysis, SDS-PAGE: sodium dodecyl sulphate-polyacrylamide gel electrophoresis

Introduction

The genus *Aegilops* L., consists of 11 diploid, 10 tetraploid and 2 hexaploid species (Van Slageren 1994), with extremely diverse genomic formula, representing the D, S, U, C, N and M genomes. These species can be used as donors of new alleles to increase bread making quality (Kilian et al. 2011). *Aegilops geniculata* Roth is an annual self-fertile, allotetraploid species ($2n = 4x = 28$) with the genomic formula MMUU; belonging to tribe Triticeae Dumort., subtribe Triticinae Griseb (Hammer 1980; Masci et al. 1992; Van

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Slageren 1994; Kilian et al. 2011). Its M genome derives from the species *Aegilops comosa*, and the U genome is related to the species *Aegilops umbellulata* (Bandou et al. 2009). This species has a wide distribution around the Mediterranean Sea, especially in southern Europe. In Algeria, *Aegilops geniculata* is found in a wide range of climatic conditions, showing adaptations to various environmental constraints. Moreover, this species is a valuable source of resistance to various diseases and pests (Farooq et al. 1996; Zaharieva et al. 2001a). Studies based on morphological and phenological characters were already assessed and a high variation between *Aegilops geniculata* populations was found, and this was explained by its adaptation to different ecogeographical environments (Perrino et al. 1993; Zaharieva et al. 1999).

Storage proteins, in particular high molecular weight glutenin subunits, are directly associated with the level of bread making quality (Gianibelli et al. 2002). The HMW glutenin subunits are controlled by the complex *Glu-1* loci present on the long arm of the group 1 homologous chromosomes of wheat and relatives which contains two tightly linked genes coding for a subunit of lower mobility and another of faster mobility, termed x- and y-type, respectively (Kozub et al. 2011). Some genetic diversity studies based on proteins and 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 (Fernández-Calvín and Orellana 1990; Rodriguez-Quijano et al. 2001; De Bustos and Jouve 2006; Sun et al. 2006). There are few genetic diversity studies in *Aegilops geniculata,* except some works using RFLP and RAPD polymorphism (Zhang et al. 1996; Monte et al. 1999) or proteins (Bandou et al. 2009). However, no genetic diversity studies based on allelic identification have been carried out in *Aegilops geniculata.*

The aim of the present study was to analyze the morphological variation, to allocate and to identify alleles at the high molecular weight glutenin subunits loci *Glu-M1* and *Glu-U1* in a set of 36 accessions of *Aegilops geniculata* collected in various ecogeographical areas throughout northern Algeria.

Materials and Methods

Sampling and plant material

The collection included 35 accessions of *Aegilops geniculata* sampled in various eco-geographical locations of northern Algeria, extended from East to West including the coastal plains and the steppic highlands, according to an ascending aridity from North to South (Fig. 1). In each sampling site, plants were randomly collected after the seed maturation. Each sample location was characterized by its altitude (Alt.), the Mediterranean bioclimatic coefficient of Emberger Q2, the average annual rainfall (Pm), the average minimum temperature of the coldest month during the *Aegilops*-growth cycle (January) (Tm), and the average maximum temperature of the hottest month (July) (TM) (Table 1).

Aegilops umbellulata and *Aegilops comosa* were also used to allocate components of the HMW glutenin subunits patterns of the tetraploid species *Aegilops geniculata* to M or U genomes. The collection of diploid species is provided by ICARDA.

Table 1. Sampling sites and main bioclimatic parameters

Accession	Provenance	Alt.	Pm	Tm	TM	Q2	Bio-climate
G01	Chetaibi	132	712	8.2	28.1	122.72	Hu
G02	Oued Zenati	798	564	1.9	32.1	64.06	SH
G03	Ain Fekroune	1064	462	0.4	31.3	51.28	SA
G04	Ain Touta	1055	329	0.3	33.4	34.09	SA
G05	Lambridi	1080	390	2	32	44.59	SA
G06	Fesdis	1034	335	0.7	32.6	36.02	SA
G07	Khroub	623	540	2.8	32.5	62.36	SA
G08	Chettaba	850	552	3	32.2	64.84	SA
G09	Beni Hmidane	412	704	3.2	31.4	85.63	SH
G10	Chaab ‘Rssas 1	580	590	3.3	32	70.51	SA
G11	Chaab ‘Rssas 2	564	624	3.3	32	74.58	SA
G12	Chaab ‘Rssas 3	564	624	3.3	32	74.58	SA
G13	Chaab ‘Rssas 4	564	624	3.3	32	74.58	SA
G14	Ain El Bey	607	633	3.9	32.1	76.99	SA
G15	Boumeziane	406	767	7.1	28.5	122.94	SH
G16	Boghdir	188	818	8.2	29.3	132.97	Hu
G17	Mila 1	830	562	2.5	31.3	66.93	SA
G18	Mila 2	824	562	2.5	31.3	66.93	SA
G19	Mila 3	887	562	2.5	31.3	66.93	SA
G20	Akbou	187	659	6.2	31.3	90.05	SH
G21	Sidi Aich	81	731	7.8	30	112.94	Hu
G22	Yachir	907	420	1.2	33.1	45.16	SA
G23	Kadiria	484	506	0.2	30.9	56.53	SH
G24	Tizi Ouzou	129	896	6.2	32	119.12	Hu
G25	Blida	267	791	7	30.9	113.52	SH
G26	Chiffa	385	736	2.5	30.6	89.84	SH
G27	Thneiet El Had	575	609	1.1	30.1	72.03	SA
G28	Khemis Meliana	382	593	6	33.5	73.96	SH
G29	Ouled Fares	135	405	6.6	32.6	53.43	SH
G30	Matmer	49	348	6.8	31.1	49.12	SA
G31	Sig	124	368	7.5	29	58.71	SH
G32	Mostaganem	94	347	8.3	27.8	61.04	SH
G33	Sidi Brahim	465	450	4.8	29.7	61.99	SA
G34	Saida	1134	341	3.5	32.6	40.19	Ar
G35	Tiaret	1009	529	2.1	31.5	61.72	Ar

Alt. altitude (m), Pm annual rainfall (mm), Tm the average minimum temperature of the coldest month (°C), TM the average maximum temperature of the hottest month (°C), Q2 Emberger coefficient, H humid, SH sub-humid, Ar Arid, SA semi-arid

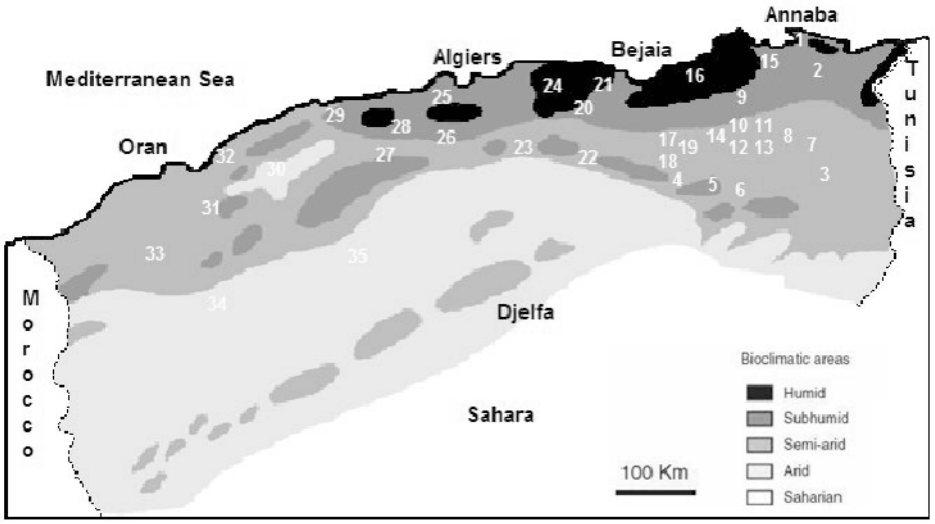

Figure 1. Distribution of the studied *Aegilops geniculata* Roth accessions in the different eco-geographical regions of northern Algeria. Bioclimatic limits according to Stewart (1974)

Morphological analysis

Fifteen morphological traits related to inflorescence, spike, spikelet and caryopsis and independent traits were studied in the morphological analysis. Measurements were individually assessed on ten randomly chosen individuals per accession. The measured characters were: diameter of spike (K), width of: glume and lemma (M, Q), caryopsis (U) and rachis (S); also length of: spike (C), glume and lemma (L, P), caryopsis (T) and rachis (R), besides to some independent characters: number of the awns of superior glume of the first spikelet (N), number of awns of the lemma of the same spikelet (w), number of grains per spike (G), number of total spikelets per spike (E), number of fertile spikelets per spike (F).

Protein analysis and nomenclature

Glutenins were extracted following a sequential procedure (Singh et al. 1991) from single seed of *Aegilops geniculata* species. Electrophoresis of glutenin subunits was performed using SDS-PAGE according to Singh et al. (1991). The nomenclature of HMW-GS (*Glu-1* loci) corresponds to that of Rodriguez-Quijano et al. (2001), prefixed with the letter M for bands from *Aegilops comosa* and the letter U for bands from *Aegilops umbellulata.*

Statistical analysis

Data of the morphological analysis was assessed using PCA which was applied to the 350 individuals of the collection. Correlation between morphological traits and ecological parameters (Alt., Pm, Tm, TM and Q2) was assessed using another PCA on the average of analyzed accessions. Allelic frequencies were calculated at each glutenin locus. The ge-

netic diversity at each locus was calculated as follows: $H = 1 - \Sigma Pi^2$, with H and Pi denoting the genetic variation index and the frequency of the number of alleles at the locus, respectively (Nei 1973). The level of variation was estimated dividing the number of patterns found by the number of accessions. Cluster analysis was performed using SPSS Version 20.0.

Results

Morphological analysis

The PCA performed on 350 individuals and based on fifteen traits showed a particular distribution of studied characters (Fig. 2a). Characters related to inflorescence; diameter of the spike (K), width and length of the glume (M, L), width and length of the lemma (Q, P), width and length of the grain (U, T) and width of the rachis were negatively correlated with the two axis. Moreover, independent traits; number of spikelets per spike (E), number of fertile spikelets per spike (F) and number of grains per spike (G) were more correlated between them, negatively correlated with axis 1 and positively correlated to the axis 2. The length of the spike (C) not correlated with the inflorescence characters, was correlated with independent ones. The remaining traits; number of awns of the superior glume and of the lemma (N, W) and length of the rachis (R) form a group positively correlated with axis, with (R) and (N) contributed weakly to PC 1 but had a significant loading in relation to PC 2 . Morphological analysis results showed the contribution of each group of traits to the global phenotypic variation, which differ depending on the nature of traits; inflorescence traits or independent traits.

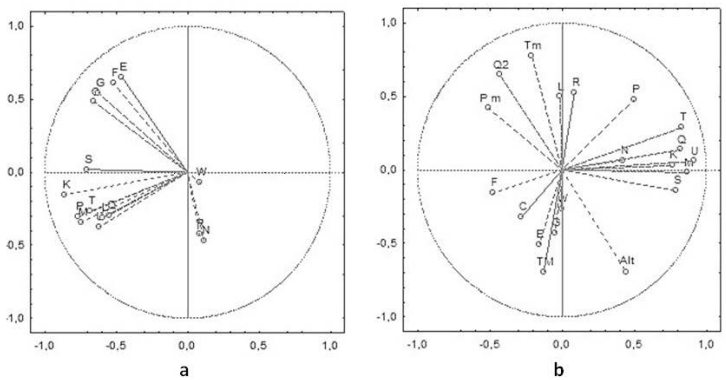

Figure 2. Principal component analysis of *Aegilops geniculata* accessions based on a: fifteen morphological traits K, M, Q, U and S character group of diameter and widths; C, L, P, T and R character group of lengths; N, W, G, E and F; independent characters, b: correlation between morphological characters and ecological parameters (Q2, Pm, TM, Tm and Alt)

Correlation between morphological characters and ecological parameters

The PCA was based on morphological characters by taking in account the five ecological parameters; altitude (Alt.), (Pm), (TM), (Tm) and Q2 (Fig. 2b). The length of the superior glume of the first spikelet (L) was weakly correlated with Tm (the average of the minimum temperature of the coldest month). The traits E (number of spikelets per spike), G (number of grains per spike) and W (number of awns of the lemma) were correlated with TM (the average of the maximum temperature of the hottest month), with E which was strongly correlated with TM. The TM parameter (the average of the maximum temperature of the hottest month is an indicator of arid and semi-arid areas with hot summer, but this opposed with G02 and G25 growing under sub-humid conditions and having an average of 3.40 and 3.50 of spikelets per spike, respectively. The same observation with the trait (G) which exhibited a moderate correlation with TM, however, accessions collected under humid bioclimates G21 and G24 presented an average of grains per spike of 4.20 and 3.50, respectively.

Allelic variation at Glu-1 *loci in* Aegilops geniculata

In order to verify the homogeneity of analyzed accessions, electrophoresis had been carried out on at least five grains per accession; obtained patterns showed that all the accessions are homogeneous for HMW glutenin subunits except only one accession which was excluded from incoming protein analysis.

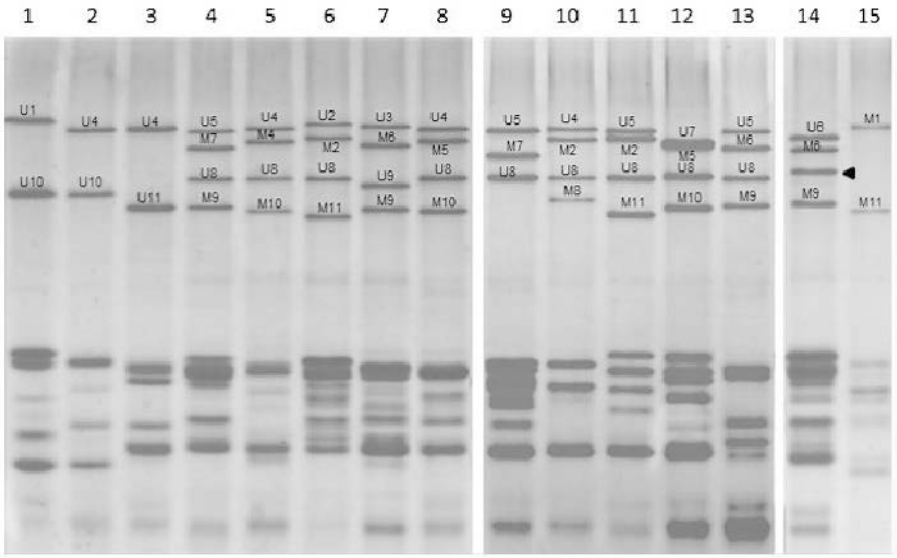

Figure 3. SDS-PAGE patterns of high molecular weight glutenin subunits in Algerian *Aegilops geniculata.* 1, 2 and 3: *Aegilops umbellulata,* 15: *Aegilops comosa,* 4–14: *Aegilops geniculata* accessions; 4: G1, 5: G2, 6: G3, 7: G15, 8: G23, 9: G4, 10: G5, 11: G6, 12: G20, 13: G21, 14: G27. Arrow's head indicates the new subunit

Aegilops geniculata accessions showed extensive variation for HMW glutenin subunits for both *Glu-M1* and *Glu-U1* loci (Fig. 3), resulting in twenty nine patterns among accessions analyzed, twenty-three patterns were specific of one accession each (Table S1*).

At *Glu-1* loci encoded for HMW glutenin subunits, each locus contains two tightly linked genes encoding subunits designated x- and y-type based on their molecular weights and biochemical characteristics. A total of twenty-seven alleles were identified at the two loci: *Glu-M1* and *Glu-U1* (Table S1). Fifteen alleles were found at the *Glu-M1* locus (*Glu-M1a, Glu-M1b, Glu-M1c, Glu-M1d, Glu-M1e, Glu-M1f, Glu-M1g, Glu-M1h, Glu-M1i, Glu-M1j, Glu-M1k, Glu-M1l, Glu-M1m, Glu-M1n* and *Glu-M1o*) (Fig. S1). The predominant allele was *Glu-M1l* which occurred at 20.00%, followed by the allele *Glu-M1i* encoding the pair 5+10 with 14.29%, the allele *Glu-M1h* was relatively frequent (11.46%). Alleles *Glu-M1e* and *Glu-M1n* have been detected each in 8.57% of the collection. The allele *Glu-M1m* coding for subunit 7 only (Fig. 3), besides to alleles *Glu-M1c* and *Glu-M1d* coding for subunit pairs (2+11), (4+10), respectively, were found in two accessions each. Seven different alleles (*Glu-M1a, Glu-M1b, Glu-M1f, Glu-M1g, Glu-M1 j, Glu-M1k* and *Glu-M1o*) were considered rare with 2.86%.

At the *Glu-U1* locus, a total of 12 alleles were detected of which one subunit has not been previously described. This new subunit has a slightly slower mobility than subunit 8 (Fig. S1). The *Glu-U1f* allele (encoding the subunit pair 5+8) and *Glu-U1d* (subunits 4+9) were the most frequent with 25.71%, 20.00%, respectively. Alleles *Glu-U1a, Glu-U1c* and *Glu-U1j* encoding, respectively, subunits pairs: (3+9, 4+8, 7+8) were less frequent with 8.57% each. Alleles *Glu-U1b, Glu-U1g* and *Glu-U1i* appeared in two accessions each. Finally, alleles *Glu-U1e, Glu-U1h* and alleles *Glu-U1k* and *Glu-U1l* coding for (4+ *new*) and (6+ *new*), respectively; were rare and have been detected in only one accession each.

Cluster analysis

The cluster analysis (Fig. S2) based on HMW allelic frequencies was performed to determine the diversity among the Algerian *Aegilops geniculata* accessions analyzed. This cluster analysis separated the collection into two major clusters at a level distance 25.

The first cluster is composed of fourteen accessions collecting from distant geographical regions and growing under two close bioclimates (semi-arid and sub-humid). For the same cluster, a strong relation between accessions from semi-arid bioclimate (G08, G05, G17, G18, G33 and G30) was observed comparing to those sampled from sub-humid regions (G09 and G29, G02 and G26, G23, G20).

The second cluster including the most part of the studied collection (21 accessions) is divided into two groups. The first group (I) is split into two subgroups, the first one comprising four accessions collected under arid and semi-arid bioclimates (G13, G34, G35 and G07), the second subgroup contains accessions originated from all the bioclimatic conditions, with a high level of similarity at distance 10. The second group (II) linked to the first one at a level distance 23, is composed of nine accessions (G03, G14, G01, G24,

* Further details about the Electronic Supplementary Material (ESM) can be found at the end of the article.

G28, G16, G06, G04 and G19). G03 and G14 accessions originated from semi-arid areas form the first subgroup of group (II) with a moderate similarity at a level distance 13. The second one contains four accessions belonging to warm humid and sub-humid bioclimates (G01, G24, G26 and G16) with a considerable level of similarity, and three accessions (G04, G06 and G19) sampled from close semi-arid stations. G04 and G06 displayed a high level of similarity, however G19 had a low level of similarity ($d = 17.5$) comparing to the two previous accessions. The results of cluster analysis showed no relation between allelic variation and the ecogeographical distribution of the accessions analyzed. For example, the nine accessions in the same cluster at the distance 23 do not have the same geographic origins and come from completely different climates: some are distributed throughout the humid climate (G01, G24 and G16), others are from the semi-arid bioclimate (G03, G14, G04, G06 and G19) or from sub-humid areas (G28). Other examples concern the two accessions 'G11' and 'G12', which are very distant, based on their HMW glutenin subunits composition, but, were collected at the same sites; and the two accessions G13 and G34 which have the same HMW-GS composition, but were collected at very distant sites.

Discussion

Morphological diversity and polymorphism of HMW glutenin subunits have been studied in *Aegilops geniculata* Roth collected in northern Algeria from different eco geographical environments.

Traits expressing thickness of spike (K, M, Q and U) and length of grain and glume (T and L) (Fig. 2) were those differentiating better between accessions, especially spike's width (K). Frequently, taxonomists and florists used the length of glumes, lemmas and awns to differentiate species and subspecies (Quezel and Santa 1962; Hammer 1980). In this study, characters of length of the lemma and awns discriminated less the studied accessions than thickness characters.

The distribution of morphological and ecological traits in PCA showed a weak correlation between them for the majority of the studied parameters. Traits of the number of spikelets per spike (E), the number of grains per spike (G) and the number of awns of the lemma (W) were correlated with the ecological factor TM. However, by taking in account all the morphological traits, there was no strong relationship between morphological traits and ecological parameters. Perrino et al. (1993) when analyzing the morphological diversity of Italian populations of *Aegilops geniculata* revealed that morphological characters were highly related with ecological conditions. Moreover, Zaharieva et al. (2003a, b) observed a moderate correlation between morphological characters and ecogeographical conditions when analyzing *Aegilops geniculata* and other *Aegilops* species populations sampled in Bulgaria. Bandou et al. (2009) analyzed populations of *Aegilops geniculata* collected in Algeria and distinguished between climatic clusters by the traits of length of rachis and caryopsis and number of awns, which were strongly correlated with ecological factors, particularly winter and summer temperatures and altitude. Some authors says that collecting sites having the same ecological conditions could be grouped together on the

basis of the morphological variation (Nevo et al. 1982; Spagnoletti-Zeuli and Qualset 1987) while others claim in favor of no strong relationship between morphological traits and eco-geographical conditions (Vojdani and Meybodi 1993). In this study, the comparison of morphological variation between ecogeographical regions and the analysis of the correlations between morphological traits and ecological factors suggests that the morphological variability could be explained by the adaptation to different ecological environment. An important adaptation to a large range of rainfall from semi-arid to humid areas was observed in this collection (from 329 mm to 896 mm). This adaptation was also observed by Bandou et al. (2009) in populations of *Aegilops geniculata* collected from Algeria (from 400 mm in semi-arid areas, to 1350 mm in humid regions). Zaharieva et al. (2001b, 2003a, b) observed a wide variation in the adaptation to drought conditions in *Aegilops geniculata* and other *Aegilops* species from different geographic origins, and found that *Aegilops geniculata* was widely distributed in low rainfall areas in Bulgaria (Zaharieva et al. 2004). Baalbaki et al. (2006); noted an important resistance to drought stress in a collection from the semi-arid region of Lebanon.

A high polymorphism of HMW glutenin subunits was found among *Aegilops geniculata* accessions studied. A total of nineteen HMW subunits was revealed. All the subunits identified by Rodriguez-Quijano et al. (2001) at the *Glu-M1* locus were detected in this study except subunits 1 and 3. At the *Glu-U1* locus, two subunits were absent; subunits 1 and 11. The novel subunit identified at the *Glu-U1* locus (designated *new*) was detected in two accessions (G26 and G27). Twenty-seven alleles were detected; representing fifteen alleles at the *Glu-M1* locus and twelve alleles at the *Glu-U1* locus among accessions analyzed. This diversity is higher than that revealed in its diploid progenitors *Aegilops comosa*, and *Aegilops umbellulata,* eleven and eight alleles at the *Glu-M1* and *Glu-U1* loci, respectively (Rodriguez-Quijano et al. 2001). In *Aegilops geniculata* populations analyzed by Bandou et al. (2009), the frequency analysis of glutenin subunits revealed a large variability between populations and sixteen bands of HMW glutenin subunits generated twenty-eight phenotypes. The *Glu-M1* locus displayed the highest level of variation (45.71%) in *Aegilops geniculata* accessions analyzed comparing to the *Glu-U1* locus (34.29%), this finding was also observed by Rodriguez-Quijano et al. (2001), who found 33.3% and 27.6% for the *Glu-M1* and *Glu-U1* loci, respectively. Kozub et al. (2011) when analyzing a collection of *Ae. biuncialis* (UUMbMb) from Ukraine have noticed that the *Glu-M1* was more polymorphic. Moreover the genetic diversity index at the *Glu-M1* locus (H = 0.90) was higher than the *Glu-U1* locus (H = 0.86). HMW glutenin subunits alleles controlling the x-type subunit only were detected in two accessions (G04 and G32) at the locus *Glu-M1* (allele *Glu-M1m*). The allele encoding the x-component only at the *Glu-M1* locus was also identified in one accession of *Aegilops comosa* by Rodriguez-Quijano et al. (2001), and in one accession of *Aegilops biuncialis* analyzed by Kozub et al. (2011). Alleles coding for the x-type subunit only are common at *Glu-A1* locus, encountered among alleles at the *Glu-B1* locus and uncommon at the *Glu-D1* locus in cultivated wheat (Payne and Lawrence 1983; Fernández-Calvín and Orellana 1990; Saponaro et al. 1995). In this study, nineteen bands (HMW glutenin subunits) were detected result-

ing in twenty-nine different patterns from the analyzed collection. In fact, cytogenetic studies based on C-banding and FISH (Fluorescence In Situ Hybridization), revealed an important intraspecific diversity in *Aegilops geniculata,* and claim that it is still undergoing the speciation process due to hybridization associated with chromosomal rearrangements (Badaeva et al. 2004).

In conclusion, this study showed that Algerian *Aegilops geniculata* Roth has an extensive allelic variation in HMW glutenin subunits. Its wide distribution in various geographical regions and the considerable adaptation in extremes bioclimatic conditions make this species of a potential interest in cultivated wheat quality improvement, especially resistance to abiotic stress, for this further molecular studies are intended.

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Electronic Supplementary Material (ESM)

Electronic Supplementary Material (ESM) associated with this article can be found at the website of CRC at http://www.akademiai.com/content/120427/

Electronic Supplementary *Table S1.* Allelic composition at *Glu-M1* and *Glu-U1* loci found in *Aegilops geniculata* accessions collected in Algeria

Electronic Supplementary *Table S2.* Allele frequencies at HMW glutenin subunits and genetic index diversity at the *Glu-M1* and *Glu-U1* loci in *Aegilops geniculata* accessions collected in Algeria

Electronic Supplementary *Figure S1.* Schematic representation of the mobility on SDS-PAGE of the different HMW glutenin subunits alleles encoded at *Glu-M1* and *Glu-U1* loci found in *Aegilops geniculata* accessions studied

Electronic Supplementary *Figure S2.* Dendrogramm based on HMW glutenin subunits polymorphism, showing the relationships among accessions of *Aegilops geniculata* Roth sampled in Algeria