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Genetic Diversity of High and Low Molecular Weight Glutenin Subunits in Algerian *Aegilops geniculata*

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Abstract

Aegilops geniculata Roth is an annual grass relative to cultivated wheat and is widely distributed in North Algeria. Endosperm storage proteins of wheat and its relatives, namely glutenins and gliadins, play an important role in dough properties and bread making quality. In the present study, the different alleles encoded at the four glutenin loci (*Glu-M1, Glu-U1, Glu-M3* and *Glu-U3*) were identified from thirty five accessions of the tetraploid wild wheat *A. geniculata* collected in Algeria using Sodium dodecyl Sulfate - Polyacrylamide Gel Electrophoresis (SDS-PAGE). At *Glu-M1* and *Glu-U1* loci, encoding high molecular weight glutenin subunits (HMW-GS) or A-subunits, 15 and 12 alleles were observed respectively, including one new subunit. B-Low molecular weight glutenin subunits zone (B-LMW-GS) displayed a far greater variation, as 28 and 25 alleles were identified at loci *Glu-M3* and *Glu-U3* respectively. Thirty two subunits patterns were revealed at the C subunits- zone and a total of thirty four patterns resulted from the genetic combination of the two zones (B- and C-zone). The wide range of glutenin subunits variation (high molecular weight glutenin subunits and low molecular weight glutenin subunits) in this species has the potential to enhance the genetic variability for improving the quality of wheat.

Keywords: alleles, electrophoresis, goatgrass, glutenins, polymorphism

Introduction

A. geniculata is an annual, autogamous, allotetraploid jointed goatgrass deriving from natural hybridization between *Aegilops comosa* (2*n*=2*x*=14, MM) and *Aegilops umbellulata* (2*n*=2*x*=14, UU) (Kilian *et al.,* 2011; Van Slageren, 1994). This tetraploid species is particularly interesting in wheat quality improvement especially as a source of resistance to various diseases, pests, drought and salinity (Zaharieva *et al.,* 2001 a, b).

The endosperm proteins of cultivated wheat, namely glutenins are important components of gluten which provides the unique viscoelastic properties indispensible for bread-making (Payne, 1987). Glutenins are aggregates of high molecular weight glutenin subunits (HMW-GS) or A-subunits and low molecular weight glutenin subunits (LMW-GS) or B-, C- and D-subunits, linked together by inter-molecular disulphide bonds. On SDS-PAGE, and according to known amino acid sequences, the group of A-subunits shows molecular mass ranging from 65.000 to 90.000 (Lagrain *et al.,* 2013). The group of B subunits is slower moving with apparent molecular weight of about 40.000 to 50.000 comparing to C-subunits which ranges from 30.000 to 40.000. Thus the A-, B- and C- designations are based on their relative mobilities on SDS-PAGE (slower to faster, respectively) (Gianibelli *et al.,* 2001; Masci *et al.,* 1993). The D-subunits group, migrating slightly slower than B-group was presumably called D because it was discovered after the assignment of A, B and C designations (Payne and Corfield, 1979). The HMW-GS are encoded by the complex *Glu-1* loci present on the long arm of group 1 homoeologous chromosomes and are further subdivided into high molecular x-type and low molecular y-type subunits tightly linked pairs of genes encoding one x-type and one y-type subunits. The LMW-GS account for about one-third of the seed protein and roughly 60% of glutenin proteins, are mostly encoded by genes located on the short arms of the group 1 chromosomes, which form a multigene family (*Glu-3* loci), closely linked to the *Gli-1* loci containing genes encoding omega- and gamma-gliadins. This fraction of seed proteins play an important role in determining dough properties and the quality of wheat food products (Branlard *et al*., 2001; Gupta *et al*., 1991, 1994; Howitt *et al*., 2006). The copy number of LMW-GS genes in wheat was estimated to range from 10-20 to 30-40 (D'Ovidio and Masci, 2004; Liu *et al.,* 2010; Zhang *et al.,* 2013). Many studies were carried out to analyse the genetic diversity of *Aegilops geniculata* which is considered as source of new genes (Bandou *et al.,* 2009; Monte *et al.,* 1999; Zhang *et al.,* 1996). However, no research has been conducted on the allelic composition of HMW and

LMW-GS in this species. The objective of this study was to determine the allelic composition at four glutenins loci (*Glu-M1*, *Glu-U1*, *Glu-M3* and *Glu-U3*) encoding for HMW (A-subunits) and LMW-GS (B- and C-subunits) within *A. geniculata* accessions.

Materials and methods

Plant material

Thirty five accessions of *Aegilops geniculata* originating from Algeria were analyzed for high and low molecular weight glutenin subunits. This collection was sampled throughout North of Algeria after seed maturation. Diploid progenitors; *Aegilops comosa* and *Aegilops umbellulata* kindly provided by the International Center for Agricultural Research in the Dry Areas (ICARDA) were used to assign components of the tetraploid species to M or U genomes.

Protein analysis

Glutenins were extracted from the brush of kernels following the sequential procedure described by Singh *et al.* (1991) and were separated using SDS-PAGE according to the method of Singh *et al.* (1991).

Nomenclature

To allocate components of the HMW-GS and B- LMW-GS patterns of the tetraploid species *A. geniculata* to U or M genomes, comparison of glutenins electrophoretic patterns between *A. geniculata* accessions and diploid progenitors' accessions (*A. comosa* and *A. umbellulata*) was made.

The nomenclature of subunits and alleles of HMW-GS (Glu-1 locus) corresponds to the terminology of Rodriguez-Quijano *et al.* (2001), prefixed with the letter M for bands from A. comosa and the letter U for bands from *A. umbellulata*. For LMW-GS (B-and C-subunits), the different bands were numbered according to their mobility order in SDS-PAGE and a nomenclature was proposed for B-subunits.

Results

During the initial analysis of HMW-GS and LMW-GS patterns from different accessions of the collection, it was realized that almost all of them were unique in their overall-banding patterns; and only one accession was found to consist of two biotypes of HMW-GS with respect to LMW-GS. All the genes for B-glutenin subunits are located on the short arms of group 1 chromosomes of wheat and relatives (Gupta, 1991). However, Gupta *et al.* (1995) found a few C type LMW-GS that are controlled by genes on other chromosome arms. Furthermore, another study has confirmed that two C-subunits are encoded by genes located at the short arm of the chromosome 6 (Felix *et al.,* 1996). For these reasons, the allelic variation of LMW-GS patterns of *A. geniculata* accessions at the *Glu-3* loci has been done considering only the B-subunits. For C-subunits, a typology has been carried out. Thus, to simplify their analysis, LMW-GS patterns were studied separately for B- and C-subunits.

Characterization of A-, B- and C- glutenin subunits patterns

Important variability was observed for A-glutenin subunits. A total of twenty nine patterns were revealed from the analysis of different *A. geniculata* accessions. Twenty three patterns were specific to one accession each. All of the accessions analyzed possessed four subunits (Fig. 1) in the A-zone except two accessions (G4 and G32 corresponding to pattern 4 and pattern 27 respectively) which showed three subunits each (Fig. 2). Likewise, extensive variation in the number and relative mobility of B-subunits was observed in the material under analysis. Considering the two loci together (*Glu-M3* and *Glu-U3*), up to thirty three different patterns were identified and are shown in Figure 3.A. The number of glutenin subunits present in the B-zone varied from two to seven (patterns 9 and 18: Fig. 3.A), and some B-subunits appear to be common in several patterns. A clear resolution was obtained and it was possible to make a diagram of all the proteins present in the C-zone. Thirty two different patterns in C-subunits were observed, representing a slightly lower variation than that observed in B-subunits. The number of C-subunits varied between one (pattern 6 Fig. 3.B) and six subunits, and only one accession (G12) presented six C-subunits (pattern 32: Fig. 3.B), while 82.86% of the collection had three to five C-subunits. Accessions with the same C-subunits and different B-subunits were observed (G11and G34). Considering the two zones together (B- and C-subunits), each accession exhibited from four to eleven LMW-GS, which result in thirty four different patterns.

Allelic variation at Glu-1 loci

Twenty seven alleles were detected at the two loci together. Fifteen alleles were found at the *Glu-M1* locus (Tab. 1). Alleles *Glu-M1l* and *Glu-M1i* were the most frequent with 20.00% and 14.29% respectively, followed by allele *Glu-M1h* at a frequency of 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 and alleles *Glu-M1c* and *Glu-M1d* were found in two accessions each. The seven remaining alleles (*Glu-M1a,*

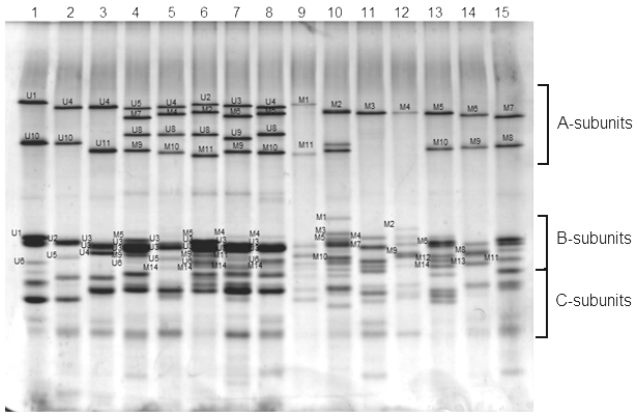

Fig. 1. SDS-PAGE patterns of HMW and LMW in some accessions of *Aegilops geniculata* collected in Algeria: 4: G1; 5: G2; 6: G3; 7: G15 and 8: G23; 1, 2 and 3: *A. umbellulata*; 9, 10, 11, 12, 13, 14 and 15: *A. comosa*

Fig. 2. Diagrammatic representation of HMW-GS patterns found in *Aegilops geniculata* from Algeria

Glu-M1b, Glu-M1f, Glu-M1g, Glu-M1 j, Glu-M1k and *Glu-M1o*) were rare in the investigated material with 2.86%. At the *Glu-U1* locus, up to twelve alleles were detected including a subunit which has not been previously described, characterized by a slightly slower mobility comparing to subunit 8 (Fig. 4.A), this novel subunit identified at the *Glu-U1* locus (designated *new*) was detected in two accessions (G26 and G27). The *Glu-U1f* allele (encoding the subunit pair 5+8) and *Glu-U1d* (subunits 4+9) were predominant with frequencies of 25.71% and 20.00%, respectively. Alleles *Glu-U1a*, *Glu-U1c* and *Glu-U1j* were found in three accessions each. Alleles *Glu-U1b*, *Glu-U1g* and *Glu-U1i* appeared in 5.71 % of the collection each, whereas alleles *Glu-U1e*, *Glu-U1h* besides to alleles *Glu-U1k* and *Glu-U1l* coding for (4+ *new*) and (6+ *new*) respectively; were considered to be rare and have been detected in only one accession each (Tab. 2).

Allelic variation at Glu-3 loci in zone B

This set of *A. geniculata* germplasm displayed abundant allelic variation for B-LMW-GS. A total of 53 alleles were identified at the B-zone (Tab. 1), 28 at the *Glu-M3* locus (Fig. 4 B) and 25 at *Glu-U3* locus (Fig. 4 C). At the *Glu-M3* locus, approximately all the alleles were detected with the same frequency (2.86%) except alleles *Glu-M3a*, *Glu-M3k*, *Glu-M3m*, *Glu-M3q* and *Glu-M3y* coding respectively for (null form, 5+9, 7+13, 4+7+13 and 7+9+12+13) which were present with 5.71% each. Only *Glu-M3d* (encoding subunit 9) had a frequency of 8.57%. At the *Glu-U3* locus, eighteen alleles were detected in one accession each. Five alleles (*Glu-U3b*, *Glu-U3d Glu-U3g*, *Glu-U3i* and *Glu-U3m*) were detected in two accession each (5.71%). The most frequent allele (*Glu-U3u* coding for subunits 3+3'+6) was detected in four accessions (11.43% of the collection), *Glu-M3t* (encoding subunit 3' alone) was less frequent with 8.57%.

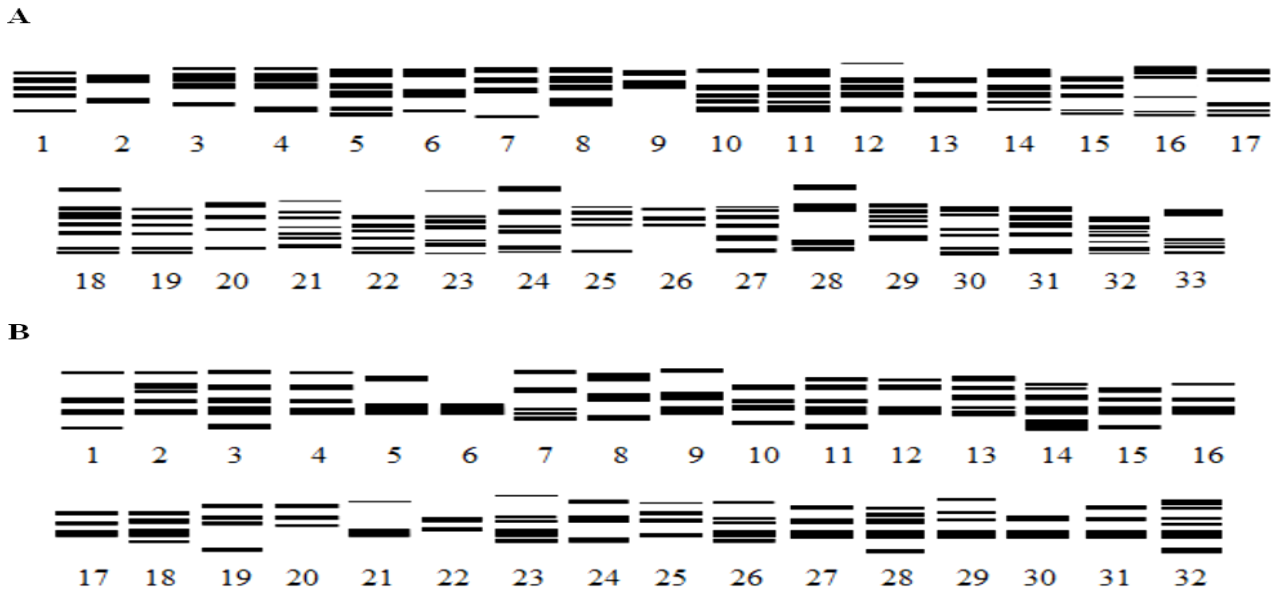

Fig. 3. Diagrammatic representation of (A): B-glutenin subunits patterns and (B): C-glutenin subunits patterns present in *Aegilops geniculata* accessions

Tab. 1. Allelic composition of HMW and B-LMW glutenin subunits of *Aegilops geniculata* accessions

Accessions	Province	*Glu-M1*	Allele	*Glu-U1*	Allele	*Glu-M3*	Allele	*Glu-U3*	Allele
G1	Annaba	7+9	*n*	5+8	*f*	5+9	*k*	3+3'+6	*u*
G2	Guelma	4+10	*d*	4+8	*a*	Nul	*a*	3+5	*m*
G3	Oum Bouaghi	2+11	*c*	2+8	*b*	5+9	*k*	3+3'+6	*u*
G4	Batna 1	7	*m*	5+8	*f*	7+13	*m*	2+4+6	*t*
G5	Batna 2	2+8	*a*	4+8	*a*	Nul	*a*	2+4+6	*t*
G6	Batna 3	2+11	*c*	5+8	*f*	6+8+13	*r*	1	*b*
G7	Constantine 1	7+9	*n*	4+9	*d*	2+12	*f*	2+6	*k*
G8	Constantine 2	5+10	*i*	5+9	*g*	4+6+7+11	*u*	1+3	*g*
G9	Constantine 3	4+11	*f*	7+8	*j*	8+9+12+13	*z*	1+2	*f*
G10	Constantine 4	5+10	*i*	4+9	*d*	6+7+9+13	*w*	1	*b*
G11	Constantine 5	6+9	*l*	4+9	*d*	7+13	*m*	3+3'+4+5+6	*y*
G12	Constantine 6	5+10	*i*	4+9	*d*	10+12+13	*s*	2+5	*j*
G13	Constantine 7	4+9	*e*	4+9	*d*	2+7+13	*o*	2+3'+6	*s*
G14	Constantine 8	5+9	*k*	2+8	*b*	4+13	*i*	1+3+3'+5	*w*
G15	Skikda	6+9	*l*	3+9	*c*	4+11	*h*	3+3'	*l*
G16	Jijel	7+10	*o*	5+8	*f*	2+6+7+11+13	*aa*	3+5	*m*
G17	Mila 1	5+8	*h*	3+9	*c*	4+7+13	*q*	2+3'	*i*
G18	Mila 2	5+8	*h*	3+9	*c*	4+7+13	*q*	2+3'	*i*
G19	Mila 3	6+10	*g*	6+9	*h*	7	*c*	1+3	*g*
G20	Béjaia 1	5+10	*i*	7+8	*j*	11	*e*	1+3+3'	*p*
G21	Béjaia 2	6+9	*l*	5+8	*f*	5+7	*j*	Null	*a*
G22	Bordj Bouariridj	6+9	*l*	5+8	*f*	9	*d*	1+3'+5+6	*v*
G23	Bouira	5+10	*i*	4+8	*a*	4	*b*	3+3'+6	*u*
G24	TiziOuzou	7+9	*n*	5+8	*f*	9	*d*	2+3'+5+6	*x*
G25	Blida	6+9	*l*	4+9	*d*	3+9	*g*	3+3'+6	*u*
G26	Médéa	4+10	*d*	4+ new	*k*	9	*d*	3'+6	*o*
G27	Tissemsilt	6+9	*l*	6+ new	*l*	9+12	*n*	2+3'+5	*r*
G28	Ain Defla	2+9	*b*	5+8	*f*	7+9+12+13	*y*	3'	*d*
G29	Chlef	6+8	*j*	7+8	*j*	5+13	*l*	2+4+6	*t*
G30	Relizane	5+8	*h*	4+10	*e*	5+11+12+13	*v*	3	*c*
G31	Mascara	6+9	*l*	6+10	*i*	7+9+12+13	*y*	1+3'	*h*
G32	Mostaganem	7	*m*	5+9	*g*	2+7+9+13	*t*	1+3+6	*q*
G33	Sidi Belabbes	5+8	*h*	6+10	*i*	4+6+9	*p*	6	*e*
G34	Saida	4+9	*e*	4+9	*d*	3+5+6+9+10+12	*ab*	3'	*d*
G35	Tiaret	4+9	*e*	5+8	*f*	6+7+10+13	*x*	3+6	*n*

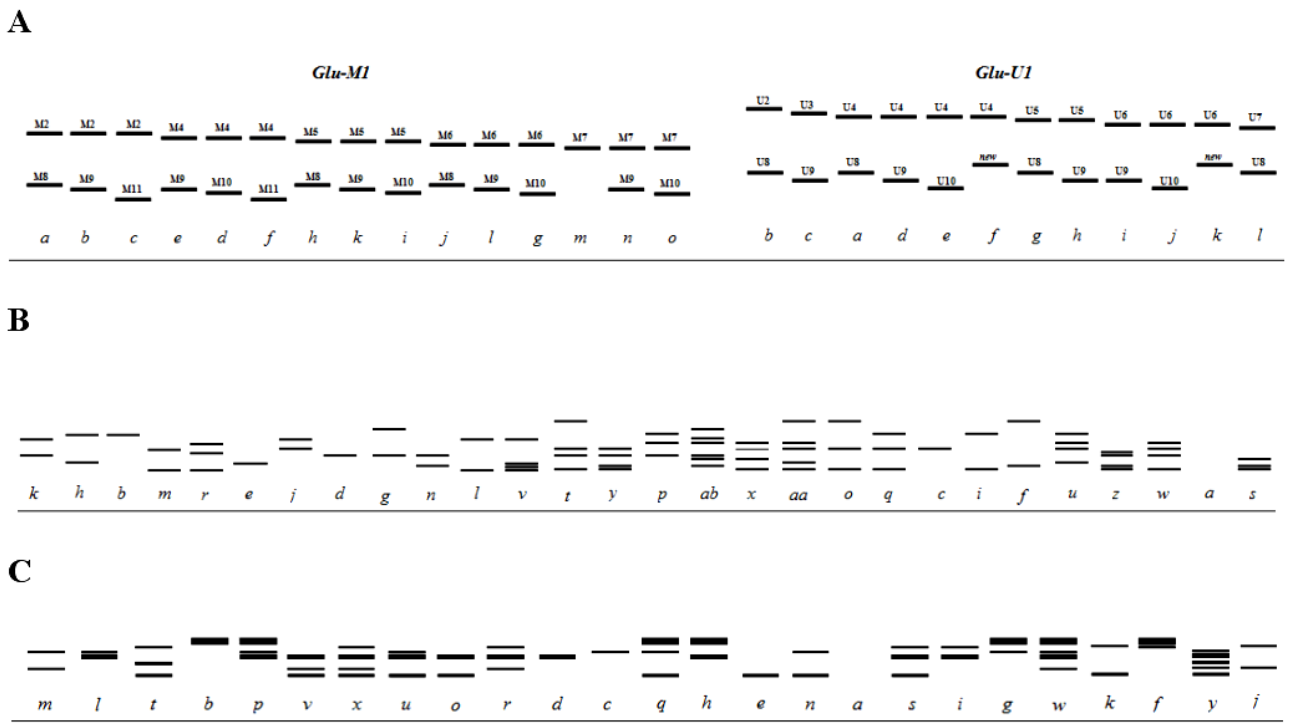

Fig. 4. Schematic representation of the mobility on SDS-PAGE of the different alleles encoded at (A): *Glu-M1* and *Glu-U1* loci, (B): *Glu-M3* and (C): *Glu-U3* loci found in *Aegilops geniculata* accessions studied

Tab. 2. Allele frequencies at HMW glutenin subunits and genetic index diversity at the *Glu-M1* and *Glu-U1* loci in *Aegilops geniculata* accessions

Locus	Allele	Subunits	Frequency	(%)
Glu-M1	*a*	2+8	1	2.86
	b	2+9	1	2.86
	c	2+11	2	5.71
	e	4+9	3	8.57
	d	4+10	2	5.71
	f	4+11	1	2.86
	h	5+8	4	11.42
	k	5+9	1	2.86
	i	5+10	5	14.29
	j	6+8	1	2.86
	l	6+9	7	20.00
	g	6+10	1	2.86
	m	7	2	5.71
	n	7+9	3	8.57
	o	7+10	1	2.86
	H index			**0.90**
Glu-U1	*b*	2+8	2	5.71
	c	3+9	3	8.57
	a	4+8	3	8.57
	d	4+9	7	20.00
	e	4+10	1	2.86
	f	5+8	9	25.71
	g	5+9	2	5.71
	h	6+9	1	2.86
	i	6+10	2	5.71
	j	7+8	3	8.57
	k	4+ *new*	1	2.86
	l	6+ *new*	1	2.86
	H index			**0.86**

Discussion

In this study, we analyzed the variation of HMW and LMW-GS in a set of thirty five *A. geniculata* accessions. Concerning the *Glu-1* loci, twenty seven alleles were detected; representing fifteen alleles at the *Glu-M1* locus and twelve alleles at the *Glu-U1* locus. This diversity is higher than that revealed in its diploid parents; *A. comosa* and *A. umbellulata,* where eleven and eight alleles were found at the *Glu-M1* and *Glu-U1* loci respectively (Rodriguez-Quijano *et al.,* 2001). A total of nineteen HMW subunits were revealed, resulting in twenty nine subunits patterns, higher than that found by Bandou *et al.* (2009) who analyzed the variability within fourteen *A. geniculata* populations collected in North Algeria and detected sixteen HMW-GS bands which in combination generated twenty eight phenotypes. In our study, the *Glu-M1* locus displayed the highest level of variation (45.71%) comparing to the *Glu-U1* locus (34.29%), this finding was also observed by Rodriguez-Quijano *et al.* (2001), who revealed 33.3% and 27.6% for the *Glu-M1* and *Glu-U1* loci respectively. Kozub *et al.* (2011) when analyzing a collection of *A. biuncialis* (UU MbMb) from Ukraine have noticed that the *Glu-M1* was more polymorphic (10 alleles at the *Glu-M1* locus and 8 alleles at the *Glu-U1* locus). Moreover the genetic diversity index at the *Glu-M1* locus (H=0.90) was slightly higher than the *Glu-U1* locus (H=0.86). HMW-GS alleles controlling the x-type subunit only were detected in two accessions (G04 and G32) at *Glu-M1* locus (allele *Glu-M1m*). Alleles coding for the x-type subunit only were encountered in diploid and tetraploid *Aegilops* species (Kozub *et al.,* 2011; Rodriguez-Quijano *et al.,* 2001), common at the *Glu-A1* locus and rare at the *Glu-D1* in cultivated wheat (Fernández-Calvín and Orellana, 1990; Payne and Lawrence, 1983; Saponaro *et al.,* 1995).

Extensive variation in the number and relative mobilities of the B-glutenin subunits was observed in *A. geniculata;* fifty three alleles were counted at the two loci for B-LMW-GS which in combination result in thirty three subunits patterns. This variability was slightly higher than that observed in *Aegilops tauschii* for the same zone by Gianibelli *et al.* (2002), who found thirty patterns, however it was lower than that revealed by Caballero *et al.* (2004) who detected 46 patterns in spelt wheat. Moreover, variation found in our study was higher than that revealed in Algerian durum and bread wheat (Bellil *et al.,* 2012, 2014 a, b; Cherdouh *et al.,* 2005), and much higher than that reported in other collections of durum wheat (Nieto-Taladriz *et al.,* 1997; Ruiz *et al.,* 1998) and in some wheat cultivars (Branlard *et al.,* 2003; Gupta and Shepherd, 1990). This indicates the importance of the collection under analysis as a source of new genes for LMW-GS. In some cases, difference in B-LMW patterns was limited in only one subunit (band); for example, accessions G1 and G25 (pattern 1 and 12: Fig. 3.A), this could be explained by the silencing of the corresponding gene. Quantitative variations were frequently detected, thus strongly stained bands could indicate the presence of this gene in a high copy number or the presence of one subunit or more with the same apparent molecular weight. In some studies, the high gene copy number was attributed to be the cause of the high level of HMW-GS 7 coded by *Glu-B1* locus in a landrace and a cultivar of hexaploid wheat (D'Ovidio *et al.,* 1997; Lukow *et al.,* 1992).

For the C zone, thirty two patterns were found, much higher than that detected by Caballero *et al.* (2004) in spelt wheat; however Gianibelli *et al.* (2002) revealed forty three patterns in a collection of *A. tauschii*, much higher than that we found. In contrast to our study, Gianibelli *et al.* (2002) observed more polymorphism for the C-LMW-GS zone than the B-LMW-GS zone. Like in B-subunits zone, differences between some patterns in the C zone lay only in one subunit as revealed in pattern 3 and pattern 11 (Fig. 3.B). Differently to B-subunits patterns, some patterns could be distinguished only by the presence or the absence of only one specific subunit, for example patterns 30 and 31 differed by the presence of an extra subunit in the latter pattern (Fig. 3.B). These results provide more evidence that B and C subunits could be encoded by two separate gene subfamilies although they are closely linked (Gupta and Shepherd, 1990). The presence of accessions with identical B-subunits patterns and different C-subunits patterns and vice-versa reinforce earlier studies suggesting that some C-subunits are encoded by genes at different loci (Felix *et al.,* 1996; Gupta and Shepherd, 1993). The important number of band patterns found in this study (thirty four, thirty two and thirty four patterns for; B-subunits, C-subunits and B- and C-subunits together respectively) is higher than that reported in durum and bread wheat studies giving

possibilities to enhance dough properties by introducing new genes of quality.

In conclusion, this work shows that Algerian *A. geniculata* exhibited a wide range of variation in HMW-GS and LMW-GS, including new subunits and alleles, this indicates that this species is an important source for increasing genetic variability in cultivated wheat and those alleles are likely to be useful via wheat breeding programs to provide improvement to flour properties.

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