

## The Genetic Diversity of Gliadins in *Aegilops geniculata* from Algeria

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### Abstract

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The gliadins of the wild wheat *Aegilops geniculata* represent a valuable gene pool in breeding for bread making quality. The genetic diversity of gliadins in *A. geniculata* was studied among 36 of its accessions, collected in the north of Algeria, using acid polyacrylamide-gel electrophoresis (Acid-PAGE). In total, sixty-one polymorphic bands and 35 gliadin patterns were identified. Twenty-eight different bands and 34 patterns were found in the  $\omega$ -gliadin region, 13 polymorphic bands and 33 patterns for  $\gamma$ -gliadins, 12 bands and 34 different patterns for  $\beta$ -gliadins and eight bands in combination resulted in 25 different patterns in the  $\alpha$ -gliadin zone. Thirty-five patterns were found for each of the *Gli-1* ( $\gamma/\omega$  region) and *Gli-2* ( $\alpha/\beta$  region) loci. The genetic diversity index (*H*) was higher for  $\omega$ -gliadins (0.968), followed by  $\gamma$ - and  $\beta$ -gliadins (0.964 and 0.961, respectively), and the lowest value was detected in  $\alpha$ -gliadin patterns (0.944). Cluster analysis based on Ward's method divided the analysed collection into five separated groups in which genetic diversity did not follow the geographical distribution. The polymorphism observed in the electrophoretic patterns highlights close correlations between bioclimatic features and some  $\omega$ -gliadin proteins.

**Keywords:** Acid-PAGE; gliadin patterns; polymorphism; wild wheat

*Aegilops geniculata* is an annual autogamous, allotetraploid grass (genome formula: MMUU,  $2n = 4x = 28$ ). Studies identified *Aegilops comosa* ( $2n = 2x = 14$ , MM) as the donor of the M genome and *Aegilops umbellulata* ( $2n = 2x = 14$ , UU) as the donor of the U genome of this species (HAMMER 1980). This jointed goat grass has been identified as a source of genes for resistance to biotic and abiotic factors, as well as genes responsible for the grain quality improvement (ZAHARIEVA *et al.* 2001a, b). *Aegilops geniculata* exhibits a high genetic diversity for glutenins (BANDOU *et al.* 2009; MEDOURI *et al.* 2014). The combination of glutenins and gliadins is crucial for the bread-making quality of wheat (SHEWRY *et al.* 2003). Gliadins are the main class of wheat seed storage proteins, and are mainly monomeric, high in proline and glutamine, and contribute to dough physical characteristics (WRIGLEY *et al.*

2006). Gliadins are divided into four different classes according to their mobilities on Acid-PAGE ( $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\omega$ -gliadin families) (WOYCHIK *et al.* 1961), however some genetic studies suggested that  $\alpha$ - and  $\beta$ -gliadins are very similar and only three types of gliadins ( $\alpha/\beta$ ,  $\gamma$  and  $\omega$ ) are classified (BIETZ *et al.* 1977; KASARDA *et al.* 1983). Their main coding loci, *Gli-1* and *Gli-2*, are located on the short arms of the homoeological group-1 and group-6 chromosomes of wheat and relatives, respectively (PAYNE *et al.* 1984). Each *Gli* locus codes for a group of gliadin polypeptides that are inherited as a block (Mendelian unit). The *Gli-1* loci are tightly linked to the corresponding LMW glutenin subunit genes (*Glu-3*) (SINGH & SHEPHERD 1988). Genes coding for most  $\alpha$ - and  $\beta$ -gliadins are located on the short arm of group 6 chromosomes at the *Gli-2* loci (PAYNE *et al.* 1984; RUIZ & CARRILLO 1993), whereas *Gli-1* loci, on the

short arm of group 1 chromosomes, code for most  $\gamma$ - and  $\omega$ -gliadins. Some slow-moving  $\beta$ -gliadins were attributed to be controlled by *Gli-A1* locus (PAYNE 1987; TATHAM & SHEWRY 1995; CIAFFI *et al.* 1997). Gliadins have received little attention in the genus *Aegilops*, except for some species (*Aegilops cylindrica*, *Aegilops biuncialis*) (KOZUB *et al.* 2012; KHABIRI *et al.* 2013), and their genetic variation had not been previously described in *Aegilops geniculata*. The aim of the present investigation is to assess the genetic variability of gliadin proteins in a local collection of the tetraploid species *Aegilops geniculata* collected throughout the north of Algeria and appreciate their correlation with some bioclimatic parameters.

## MATERIAL AND METHODS

**Plant material.** In this work, 36 accessions were used to study the polymorphism and genetic diversity of gliadin proteins in the Algerian wild wheat *Aegilops geniculata*. This germplasm was collected from various ecogeographical areas in the north of Algeria. Each sampling site was characterised by its geographic coordinates (latitude, longitude and altitude Alt (m a.s.l.)) and some bioclimatic parameters (average annual rainfall Pm (mm), the average of the minimum temperatures of the coldest month Tm (°C), the average of the maximum temperatures of the hottest month TM (°C) and classical Emberger's coefficient Q2) (Table 1).

**Gliadin analysis.** Gliadins were extracted from a single seed following the procedure of CABALLERO *et al.* (2004). Electrophoretic separation of gliadins was performed according to the standard Acid-PAGE method described by CABALLERO *et al.* (2004). At least five grains per accession were analysed, only accessions with homogeneous gliadin patterns in the five seeds analysed were retained for data analysis.

**Data analysis.** The data obtained from Acid-PAGE was scored for the presence (1) or absence (0) of the bands and entered as a binary data matrix, which were subjected to tree clustering using Statistica 6 software based on Ward's method. The genetic diversity for each gliadin pattern was calculated as per NEI (1973) as  $H = 1 - \sum P_i^2$ , in which  $H$  is the genetic variation index, and  $P_i$  is the proportion of a particular pattern in each group of  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\omega$ -gliadins separately. The mean value of  $H$  was calculated for all the four groups of gliadins. In order to examine the correlation between gliadin polymorphism and climatic parameters, a Principal Component Analysis

(PCA) was performed on the 36 Algerian accessions by including the gliadin band frequencies per accession and the most important climatic parameters (Pm, TM, Tm, Q2 and Alt).

## RESULTS AND DISCUSSION

**Gliadin electrophoretic patterns.** Among the 36 accessions analysed, up to 61 different bands were detected assuming that the bands with the same relative mobility represent the same protein. These bands were grouped into patterns at each of the four zones of gel ( $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\omega$ -gliadins). The patterns within each gliadin group of  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\omega$  were identified by comparing banding patterns of each accession with all the other accessions. Figure 1 shows the variations detected using Acid-PAGE for some representative samples. Table 2 presents the number of gliadin bands and patterns and the genetic diversity in gliadins for the accessions of *Aegilops geniculata*.

A total of 28 different mobility bands and 34 gliadin patterns were identified in the  $\omega$ -gliadin zone. Bands range from six to 11 in each  $\omega$ -gliadin pattern.  $\omega$ -gliadin bands 13 and 10 had the highest frequencies of 55.55% and 52.77%, respectively, whereas the lowest frequency of  $\omega$ -gliadin bands was found out in band 1 and 2, which were present together in only one accession (G32) with 0.03%. Each accession presented its unique  $\omega$ -gliadin pattern except three accessions which shared the same pattern (G17, G18 and G19), patterns with eight bands being the most frequent (30.55%).

In the  $\gamma$ -gliadin area, 13 bands were detected, the most frequent band was 11 present in 20 accessions, while band 2 was considered as rare and was detected in 4 accessions of the collection. Thirty-three different  $\gamma$ -gliadin patterns were found, with 30 specific patterns to each accession, every  $\gamma$ -gliadin pattern includes from two to seven bands. Patterns with four  $\gamma$ -gliadin bands dominated over all others, being present in the half of the collection.

Twelve bands were encountered in the  $\beta$ -gliadin zone, whose combination formed 34 different  $\beta$ -gliadin patterns, 32 patterns were specific to each accession, and each pattern contains from three to eight bands. Twenty-eight percent of the collection presented patterns with five  $\beta$ -gliadin bands. The eleventh  $\beta$ -gliadin band was the most abundant band and was found in 30 accessions, followed by bands eight and four with 58.33% (21 accessions) and 55.55%

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Table 1. Locations and ecological parameters of different *Aegilops geniculata* accessions used in this study

Accession	Province	Latitude	Longitude	Alt (m a.s.l.)	Pm (mm)	Tm (°C)	TM (°C)	Q2
G1	Annaba	37°02'56.98"N	7°23'19.39"E	132	712	8.2	28.1	122.72
G2	Guelma	36°22'08.93"N	7°13'59.93"E	798	564	1.9	32.1	64.06
G3	Oum Bouaghi	35°56'17.26"N	6°55'17.26"E	1064	462	0.4	31.3	51.28
G4	Batna 1	35°28'29.88"N	6°02'28.77"E	1055	329	0.3	33.4	34.09
G5	Batna 2	35°30'47.40"N	6°05'14.04"E	1080	390	2.0	32.0	44.59
G6	Batna 3	35°33'08.81"N	6°10'18.73"E	1034	335	0.7	32.6	36.02
G7	Constantine 1	36°16'N	6°42'E	623	540	2.8	32.5	62.36
G8	Constantine 2	36°18'08.13"N	6°27'50.96"E	850	552	3.0	32.2	64.84
G9	Constantine 3	36°31'42.48"N	6°34'31.56"E	412	704	3.2	31.4	85.63
G10	Constantine 4	36°20'25.71"N	6°37'25.87"E	580	590	3.3	32.0	70.51
G11	Constantine 5	36°20'N	6°37'E	564	624	3.3	32.0	74.58
G12	Constantine 6	36°20'N	6°37'E	564	624	3.3	32.0	74.58
G13	Constantine 7	36°20'N	6°37'E	564	624	3.3	32.0	74.58
G14	Constantine 8	36°20'06.65"N	6°37'02.61"E	607	767	7.1	28.5	122.94
G15	Skikda	36°35'51.34"N	6°45'05.14"E	406	818	8.2	29.3	132.97
G16	Jijel	36°47'03.98"N	5°43'21.80"E	188	562	2.5	31.3	66.93
G17	Mila 1	36°21'02.43"N	6°17'57.58"E	830	562	2.5	31.3	66.93
G18	Mila 2	36°20'53.03"N	6°17'45.90"E	824	562	2.5	31.3	66.93
G19	Mila 3	36°21'08.37"N	6°17'38.82"E	887	562	2.5	31.3	66.93
G20	Mila 4	36°22'42.43"N	6°20'13.72"E	679	659	6.2	31.3	90.05
G21	Bejaia 1	36°29'05.84"N	4°33'44.94"E	187	731	7.8	30.0	112.94
G22	Béjaia 2	36°37'31.42"N	4°42'50.42"E	81	420	1.2	33.1	45.16
G23	Bordj Bouariridj	36°06'04.26"N	4°33'55.57"E	907	506	0.2	30.9	56.53
G24	Bouira	36°33'33.96"N	3°34'31.86"E	128	896	6.2	32.0	119.12
G25	Tizi Ouzou	36°43'54.04"N	4°17'54.66"E	129	791	7.0	30.9	113.52
G26	Blida	36°32'48.32"N	2°48'23.72"E	70	736	2.5	30.6	89.84
G27	Medéa	36°20'33.96"N	2°46'04.83"E	385	609	1.1	30.1	72.03
G28	Tissemsilt	36°00'36.95"N	2°09'11.39"E	575	593	6.0	33.5	73.96
G29	Ain Defla	36°18'58.41"N	2°25'46.37"E	382	405	6.6	32.6	53.43
G30	Chlef	36°14'45.40"N	1°14'17.86"E	135	348	6.8	31.1	49.12
G31	Relizane	35°55'35.18"N	0°47'33.24"E	49	347	8.3	27.8	61.04
G32	Mascara	35°30'21.85"N	0°07'17.76"E	124	368	7.5	29.0	58.71
G33	Mostaganem	35°54'44.96"N	0°04'46.54"E	94	450	4.8	29.7	61.99
G34	Sidi Bel Abbes	35°14'20.82"N	0°37'03.24"E	465	341	3.5	32.6	40.19
G35	Saida	34°50'N	0°09'E	1134	529	2.1	31.5	61.72
G36	Tiaret	35°20'N	1°19'E	1009	633	3.9	32.1	76.99

Alt – altitude; Pm – average annual rainfall; Tm – average of the minimum temperatures of the coldest month; TM – average of the maximum temperatures of the hottest month; Q2 – Emberger's coefficient

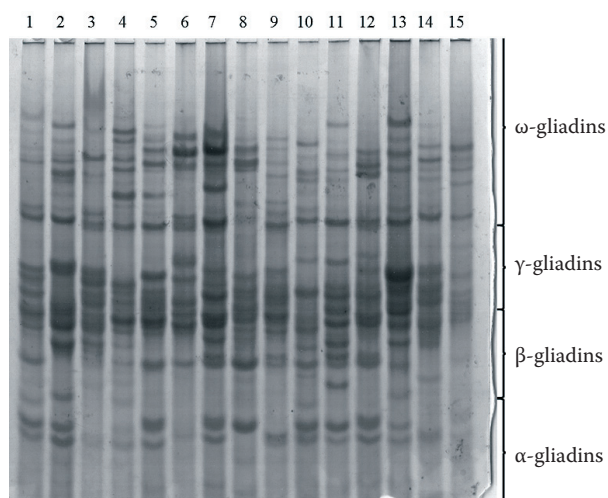


Figure 1. Variations detected using Acid-PAGE for some representative samples of *Aegilops geniculata*; 1: G1, 2: G2, 3: G3, 4: G4, 5: G5, 6: G6, 7: G7, 8: G8, 9: G9, 10: G10, 11: G11, 12: G12, 13: G13, 14: G15, 15: G16

(20 accessions), respectively. The lowest frequency  $\beta$ -gliadin band was detected in five accessions.

The  $\alpha$ -gliadin zone showed limited variation compared to the previous zones, 25  $\alpha$ -gliadin patterns were found resulting from the combination of eight bands. Each  $\alpha$ -gliadin pattern counts for one to six bands. The fastest moving band ever (band 8) was detected in most accessions (88.88%). Band 5 was also considered frequent and was encountered in 24 accessions of the collection under analysis.

Considering the four zones together, 35 gliadin patterns were identified, this diversity was higher than that revealed in the tetraploid wild wheat *Aegilops cylindrical*, in which the population with the highest number of gliadin bands had 16 bands (KHABIRI *et al.* 2013), whereas in our study 29 bands were revealed in G19 (Table 2). Also HARSCH *et al.* (1997) found 30 different bands only when analysing 16 spelt cultivars using densitometry methods.

The results show that the diversity in  $\omega$ ,  $\gamma$  and  $\beta$  zones was higher than that of  $\alpha$ -gliadin zone, which is in agreement with results reported by ALIYEVA *et al.* (2012) in Azerbaijani synthetic branched spike wheat accessions. However, results of ZAEFIZADEH *et al.* (2010) showed higher variation in  $\gamma$  and  $\omega$  gliadins than in  $\alpha$  and  $\beta$  gliadins in durum wheat landraces from the northwest of Iran and Azerbaijan. This may be either due to greater staining intensity of the  $\alpha$ - and  $\beta$ -gliadins, or the separation of these proteins may not be complete in a 1-D electrophoresis system (SEWA *et al.* 2005). Although care was taken to separate all the

Table 2. Number of gliadin bands, patterns, and the genetic diversity in gliadins for the accessions of *Aegilops geniculata*

Accessions	No. of bands				
	$\omega$	$\gamma$	$\beta$	$\alpha$	$\alpha + \beta + \gamma + \omega$
G1	9	4	4	3	20
G2	7	4	4	3	18
G3	7	5	5	3	20
G4	8	3	4	5	20
G5	8	3	6	4	21
G6	6	5	3	1	15
G7	6	4	6	5	21
G8	10	2	6	5	23
G9	9	4	5	4	22
G10	7	5	5	3	20
G11	8	3	5	3	19
G12	7	3	6	3	19
G13	8	4	4	3	19
G14	9	4	5	4	22
G15	7	4	8	2	21
G16	8	4	3	3	18
G17	9	4	6	6	25
G18	9	4	6	6	25
G19	9	7	7	6	29
G20	6	4	6	2	18
G21	7	4	4	4	19
G22	8	4	4	3	19
G23	10	4	5	3	22
G24	8	3	5	3	19
G25	11	4	4	3	22
G26	6	3	4	2	15
G27	8	5	5	3	21
G28	8	2	6	2	18
G29	7	4	5	2	18
G30	8	5	4	4	21
G31	8	5	5	4	22
G32	11	3	6	3	23
G33	10	4	5	4	23
G34	9	5	5	4	23
G35	11	5	5	4	25
G36	9	4	5	4	22
Range of bands	6–11	2–7	3–8	1–6	15–25
No. of patterns	34	33	34	25	35
<i>H</i> index	0.968	0.964	0.961	0.944	0.959

*H* – genetic variation index

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bands, more than one protein may be present in a band. TANAKA *et al.* (2003) and CABALLERO *et al.* (2004) also reported larger variation in  $\gamma$ - and  $\omega$ - gliadins than in  $\alpha$ - and  $\beta$ -gliadins in Japanese cultivars and in Spanish spelt wheat, respectively. The gene coding for most  $\alpha$  and  $\beta$ -gliadins occurs at the *Gli-2* loci (PAYNE 1987); in our study, 35 different combinations were found assuming these two zones together, 34 accessions had a specific combination pattern. Most of  $\omega$ - and  $\gamma$ -gliadin genes are located at the *Gli-1* loci (PAYNE 1987), the combination of their different patterns would give different allelic variants. Combined together,  $\omega$ - and  $\gamma$ -gliadins give 35 different combinations in our study.

The variation found among the analysed accessions is higher than that found in other materials, thus HARSCH *et al.* (1997) found 30 bands in sixteen spelt wheat cultivars. SEWA *et al.* (2005) reported 45 different gliadin bands in 157 Indian wheats, CABALLERO *et al.* (2004) detected up to 72 different bands in 403 lines of spelt wheats. ZAEFIZADEH *et al.* (2010) also found 66 bands among 46 durum wheat landraces, ALIYEVA *et al.* (2012) detected 62 bands when analysing 68 accessions of synthetic wheat, whereas in our study we found 61 different mobility bands in only 36 accessions of *Aegilops geniculata*.

**Diversity index.** The genetic diversity ( $H$ ) based on gliadin patterns, observed in accessions of this species from Algeria ( $H = 0.959$ ), showed higher polymorphism than in 17 *Aegilops cylindrica* populations from Iran ( $H = 0.085$ ) (KHABIRI *et al.* 2013). Furthermore, this variability was higher than that found in durum wheat landraces in other countries; England, Italy and the former Yugoslavia with  $H = 0.676$ ,  $0.754$  and  $0.728$ , respectively (METAKOVSKY *et al.* 1994). In addition,

the variability found in Indian wheats (*Triticum aestivum* L.),  $H = 0.875$  (SEWA *et al.* 2005), and in durum wheat landraces in the northwest of Iran and Azerbaijan,  $H = 0.900$  and  $H = 0.854$ , respectively (ZAEFIZADEH *et al.* 2010) was lower than that revealed in the Algerian collection. This variation was also much higher than that of Spanish spelt wheat,  $H = 0.703$  (CABALLERO *et al.* 2004), Spanish common wheat,  $H = 0.844$  (METAKOVSKY *et al.* 2000),  $H = 0.804$  (RUIZ *et al.* 2002) and French common wheat,  $H = 0.714$  (METAKOVSKY & BRANLARD 1998).

The high level of genetic diversity of the tetraploid species *Aegilops geniculata* collected in Algeria could be caused initially by the variation of ecological conditions. Probably, as it was suggested for cultivated wheat, wild wheat might undergo intense geographical and microgeographic differentiation in adjusting their genotypes to the environment (METAKOVSKY *et al.* 2000).

By taking into account the genetic diversity indexes among 36 genotypes for  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\omega$  regions separately, the highest genetic diversity index was found for  $\omega$ -gliadin ( $H = 0.968$ ), whereas the lowest value was determined for  $\alpha$ -gliadins ( $0.944$ ) (Table 2); this agrees with that found in Azerbaijan synthetic branched spike wheat (ALIYEVA *et al.* 2012). This important genetic diversity might be caused by the wide geographical distribution of the genotypes.

**Cluster analysis.** The genotype grouping of the collection can be seen in Figure 2. Cluster analysis of gliadin bands using Ward's method classified the collection into 5 main clusters at a level distance of  $d = 38$ . The resulting clusters showed a clear separation of *Aegilops geniculata* accessions. Cluster 1 included three ac-

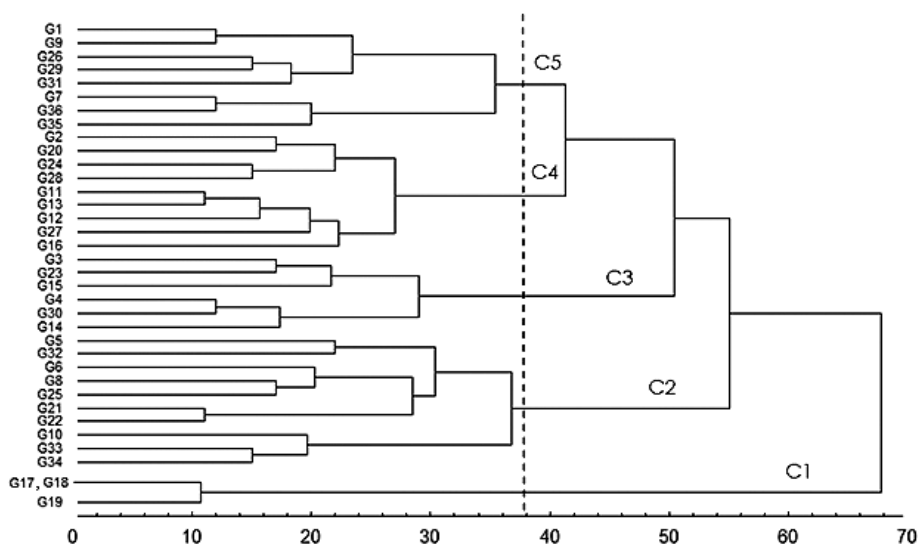


Figure 2. Cluster analysis of *Aegilops geniculata* accessions from Algeria based on gliadin polymorphism using Ward's method

cessions coming from close sites (G17, G18 and G19) having the same pattern in the  $\omega$  zone, in this case the genetic diversity follows geographical distribution. Whereas all the remaining 33 accessions are aligned in 6 clusters at a level distance of  $d = 32$ . Each of the six clusters gathers accessions from different geographical areas. Some exceptions are pointed out; cluster 2 gathered Béjaia 1 and Béjaia 2 accessions (G21 and G22), besides Batna 2 and Batna 3 accessions (G5 and G6). In addition, cluster 4 included three accessions from Constantine (G11, G12 and G13). KHABIRI *et al.* (2013) evaluated the relationships among 17 populations of *Aegilops cylindrica* by gliadin polymorphism and found that genetic diversity did not follow the geographical distribution. ZAEFIZADEH *et al.* (2010) found no correlation between genetic diversity and the geographical distribution of durum wheat landraces studied. As published by ZILLMAN and BUSHUK (1979), the electrophoregram of gliadins is not affected by the area of growth. The results of cluster grouping justify the high level of genetic variation among *Aegilops geniculata* accessions from Algeria.

**Gliadins and bioclimatic correlations.** In order to investigate the relationships between gliadin diversity and bioclimate, a PCA was performed on the 36 North Algerian accessions, taking into account both the gliadin frequencies and the four quantitative parameters of the Mediterranean bioclimate (Pm, Tm, TM and Q2 with the altitude). The preliminary PCA (by taking into ac-

count the four zones) shows that only bands from the  $\omega$ -zone presented significant correlations with studied parameters (data not shown), therefore bioclimatic correlations were performed only considering the  $\omega$ -gliadin zone. The first three axes accounted for 33.67% of the total variation (13.74%, 10.97% and 8.95%, respectively). The distribution of the relative contribution of each variable to the total variance of the two first axes is well represented by projection of vectors (Figure 3), which indicates the direction of maximum variation in plane 1–2. Only four  $\omega$ -gliadin markers (B8, B3, B14 and B9) showed a close relationship with the climatic variables. B8 shows a strong positive correlation with Pm. Band B3 was also linked to Pm but with a strong negative correlation. B14 as well as B9 were highly correlated with Tm with positive and negative values, respectively. B14 was also linked highly negatively with altitude. The  $\omega$ -gliadin band or marker (B14) is thus very interesting because it is correlated with two bioclimatic parameters (Tm and Alt), and could be an indicator of populations coming from uplands or slopes with significant rainfall.

This study on gliadin variability pointed out that the wide distribution of the tetraploid wild wheat *Aegilops geniculata* in North Algeria is made possible by an adaptive variation. In this study, the genetic polymorphism of gliadin coding loci of *Aegilops geniculata* collected in Algeria was characterized. As a result of our work, the gliadin analysis has shown that Algerian *Aegilops geniculata* germplasm is highly polymorphic and rather unique. The  $\omega$ -region was the most polymorphic followed by  $\gamma$ ,  $\beta$  and  $\alpha$ , respectively. Thus electrophoresis of gliadins may be used to detect variability among wild wheat genotypes to identify new sources of variation that could be used in crop improvement programs. The observed variation is highlighted by the significant correlation between gliadin polymorphism and ecological parameters. Further experiments with different molecular markers will allow a better understanding of the role of ecological factors in the genetic differentiation and evolution in this annual polyploid species.

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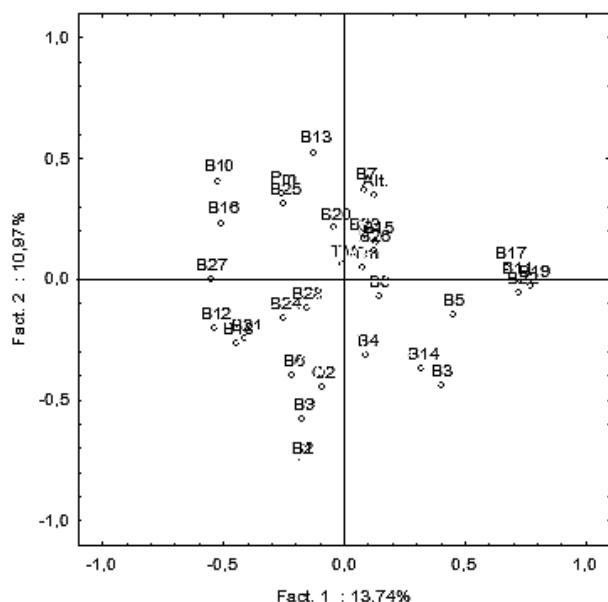


Figure 3. The relative loading of each studied variable ( $\omega$ -gliadins bands (B), Pm (mm), Tm ( $^{\circ}$ C), TM ( $^{\circ}$ C), Q2, Alt. (m)) on the principal components of 36 Algerian accessions of *Aegilops geniculata*

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