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Genetic Variation and Geographical Diversity for Seed Storage Proteins of Seventeen Durum Wheat Populations Collected in Algeria

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Abstract

Variation in the HMW and LMW glutenin subunits of a collection of 856 accessions of botanic durum wheat collected in Algeria and divided *a priori* according to their agronomical and morphological traits in 17 populations were analyzed using SDS-PAGE. Among the 16 alleles identified at the *Glu-1* loci, two were new. The first named *Glu-B1i$_1$* encoding for two bands located between 17 and 18 which was assigned the nomenclature 17_1 and 18_1. The other named *Glu-B1e$_1$* codes two bands similar to 20x and 20y but with faster mobility, which were named $20x_1$-$20y_1$. At Glu-3 and Glu-2 loci, 19 alleles were identified, where the allele named *Glu-B3ab* (encoding for subunits 2-8-9-13-16) was considered as new. Global genetic diversity index (H) was relatively high (0.34). A final core collection of 21 accessions was selected. All the different geographical areas and the allelic diversity at the *Glu-1*, *Glu-2* and *Glu-3* polymorphic loci were represented in this core which represents a minimum of wheat Algerian genetic resources that could be used for dedicated breeding programs.

Keywords: Algerian botanic durum wheat, core collection, glutenin subunits, SDS-PAGE, variability

Introduction

In the mid-nineteenth century, Mendel-and later other pioneering plant geneticists- provided knowledge of plant genetics that made it possible to dramatically increase the production potential of agriculture. However, the wonders of crop improvement have undeniably resulted in the erosion of genetic diversity of many cultivars. Similarly to what has happened in many other crops, the advent of new and homogenous wheat varieties led to the progressive abandonment of the locally adapted, unimproved and genetically more diverse landraces and botanic old cultivars (Reif *et al.,* 2005). In some instances, these biological resources have been lost due to ignorance or late realization of their importance. To prevent further erosion, large collections and gene banks have been compiled. But given the fact that diversity should be usable by and accessible to breeders, its quantitative and qualitative evaluation and knowledge of its genetic structure are preconditions for the definition of plant improvement strategies and for the management of genetic resources. The study and evaluation of these large collections and their distribution over a wide range of agro-climatic environments will definitely increase our knowledge of the genetic diversity of the crop. It will also help identify geographical regions as potential donors of desirable traits for breeding purposes. Durum wheat (*Triticum turgidum* L. *var durum*) represents only approximately 8% of the wheat production worldwide and 90% of its production has its origin in the Mediterranean region (Ammar *et al.,* 2000). In Algeria, *T. turgidum* ssp. *durum* Desf. em. Husn. is grown much more widely than *Triticum aestivum* L. This crop has been cultivated extensively for many centuries. During this long period, the species has undergone considerable diversification encouraged by the notable diversity of climates which resulted in the very wide genetic diversity of the species. Certain agronomic and morphological traits have already been used to describe the genetic diversity of durum wheat collections (Cecarelli *et al.,* 1987; Van hintum and Elings, 1991). Biochemical markers like storage proteins whose polymorphism has already been described, offer the opportunity to analyze genetic diversity independent of environmental influences (Nakamura, 2001). Biochemical, genetic and molecular aspects of high and low molecular weight glutenin subunits have received a great deal of attention in recent years due to their importance in determining the flour processing propeties of cultivated wheat. The first studies of the molecular aspects of quality in the world wheat collections revealed low variability for some protein components with a clear influence on bread making performance (Shewry *et al.,* 1989). For this reason, the search for species that could contribute genes for quality improvement is of great importance in most wheat breeding programmes (Jauhar, 1993). Genetic resources of good quality wheat germplasms are crucial to sustaining global wheat production now and in the future. Wheat germplasms embody a wide range of genetic diversity that is critical to enhancing and maintaining the quality potential of wheat, as they provide new sources of allele quality. The new alleles detected can be studied to determine their value in improving the quality of wheat. The concept of the core collection was introduced by Frankel and Brown (1984) with the aim of minimizing costs and improving the exploitation of the gene banks. A core collection is a limited sample resulting from a vaster collection that is selected to represent genetic diversity as well as possible uses.

The objective of this study was to evaluate the polymorphism of high and low molecular weight glutenin markers of a large number of samples of *Triticum durum* collected in Algeria with the aim of selecting representative samples to create a core collection and to provide information needed for plant breeding programmes. Understanding the composition of high and low molecular weight glutenin subunits will promote the use of wheat germoplasm in the development of cultivars.

Materials and methods

Plant material

A total of 856 accessions of durum wheat collected in Algeria were analyzed for high and low molecular weight glutenin alleles. These accessions were collected jointly by the Technical Institute of Field Crops (Constantine, Algeria) and the International Center for Agricultural Research in Dry Areas (Aleppo, Syria), during the year 1988-1989. The accessions originated from different regions of Algeria were grouped *a priori* into 17 varieties according to their agronomical and morphological traits (Tab. 1). The germoplasm is currently being conserved by ICARDA. The study of morphological characters of the 17 populations was carried out at the agricultural experimental station of the Technical Institute of Field Crops (ITGC) in Khroub, during the 1995/1996, 1996/1997, 1997/1998 agricultural campaigns (Boudour, 2005).

Protein extraction, separation and nomenclature

Proteins were extracted from the brush of kernels following the sequential procedure described by Singh *et al.* (1991) and were separated using Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) at an acrylamide concentration of 10.8% according to the method of Singh *et al.* (1991). High Molecular Weight Glutenin (HPM-Glu) alleles were identified using the nomenclature of Payne and Lawrence (1983) completed by Branlard *et al.* (1989) and Low Molecular Weight Glutenin (LMW-Glu) alleles were identified using the nomenclature of Nieto-Taladriz *et al.* (1997). The new subunits and alleles identified were designated according to McIntosh *et al.* (2008). Some standard hexaploid and tetraploid varieties were included to compare and classify the subunits detected.

Statistical analyses

The frequency of glutenin alleles at each glutenin locus was calculated for the genotypes belonging to each population and for the whole collection. Genetic diversity was evaluated through the *D*-index (Weir, 1996), according to the following expression: $D_i = 1 - \Sigma p_{ij}^2$, where p_i is the proportion of the j^{th} allele at locus i^{th}. Roger's distances (Roger, 1972) were calculated on the allele frequency matrix. Cluster analysis was performed using BIOSTAT-1.

Results

Tab. 2 summarizes the allelic composition and frequencies of the HMW and LMW glutenin subunits encoded by the *Glu-1* and *Glu-3* loci respectively.

Fig. 1 shows the profiles of subunits in some accessions. In our interpretation of the results, the alleles with frequencies less than 5% were defined as rare in order to compare the distribution of alleles within and between different populations (Cabalerro *et al.,* 2004).

Allelic variation at Glu-1 loci

At *Glu-1* loci encoded for HMW-GS, each locus contains two tightly linked genes (Harberd *et al.,* 1986) encoding subunits designated x- and y- type based on their molecular weights and biochemical characteristics (Payne *et al.,* 1981). However, because of the silencing of some genes, only one to three HMW subunit genes are expressed in different durum wheat accessions: one or two subunits are always expressed by the *Glu-B1* locus, and one or none (the null allele) by the *Glu-A1* locus. When only one subunit is expressed by *Glu-B1* or *Glu-A1* loci, this is always the x type (Shewry *et al.*, 2003). In these loci, up to 16 allelic forms were found. Four different alleles were detected at *Glu-A1* and 12 at *Glu-B1*. Although four alleles were observed at the *Glu-A1* locus, the null allele (*Glu-B1c*) was found in most genotypes of all populations studied (97.78%). The *Glu-A1a* allele (encoding subunit 1) which appeared at a lower frequency (1.05%) was considered to be rare (Cabalerro *et al.,* 2004); it was present in one genotype of each one of the following populations: 'Obscurum', 'Fastuasum', 'Melanopus', 'Murciense', 'Lencomelan' and in two genotypes of the 'Reichenbachi' population. The *Glu-A1b* and *Glu-A1VI* alleles (encoding the 2* and 2*** subunits respectively) were also considered as rare, as each was only present in two genotypes of two populations among the 17 populations analyzed.

At the *Glu-B1* locus, a total of 12 alleles were detected of which two alleles and three bands have not been previously described. The *Glu-B1e* allele (encoding the subunit pair 20x-20y), *Glu-B1d* (subunits 6-8) and *Glu-B1f* (sub-

Tab.1. Local Algerian durum wheat accessions included in the study

Variety	Genotype identifier [a]
1) 'Apulicum' (3)	IG6884, IG6355, IG6356
2) 'Africanum' (3)	IG5805, IG5806, IG5808
3) 'Obscurum' (3)	IG7752, IG7755, IG7756
4) 'Algeriense' (6)	IG6766, IG6775, IG6778, IG6781, IG6782, IG6795
5) 'Fastuasum' (6)	IG10228, IG11049, IG11944, IG11945, IG 12202, IG12203
6) 'Alexandrinum' (8)	IG22413, IG22414, IG22415, IG22416, IG22417, IG22503, IG22583, IG22629
7) 'Melanopus' (11)	IG6933, IG6937, IG6998, IG7015, IG7053, IG7054, IG7055, IG7056, IG7752, IG7755, IG7756,
8) 'Circumflescum' (14)	IG17559, IG17562, IG17563, IG17564, IG17565, IG17566, IG17567, IG17568, IG17569, IG17570, IG17571, IG17572, IG17574, IG17575;
9) 'Affine '(26)	IG14273, IG14274, IG14275, IG14276, IG14277, IG14278, IG14279, IG14280, IG14281, IG14282, IG14283, IG14284, IG14285, IG14286, IG14287, IG14288, IG14289, IG14290, IG14291, IG14292, IG14293, IG14294, IG14295, IG14296, IG14297, IG14299,
10) 'Lencurum' (28)	IG14301, IG14302, IG14303, IG14304, IG14305, IG14306, IG14307, IG14308, IG14309; IG14310, IG14311, IG14313, IG14314, IG14315, IG14316, IG14317, IG14318, IG14320, IG14321, IG14322, IG14323, IG14324, IG14325, IG14326, IG14328, IG14329, IG14330, IG14331;
11) 'Aegyptiacum' (42)	IG14332, IG14333, IG14334, IG14335, IG14336, IG14337, IG16257, IG16268, IG16269, IG16271, IG16320, IG16321, IG16417, IG16418, IG16419, IG16420, IG16433, IG16434, IG16435, IG16436, IG16437, IG16438, IG16439, IG16445, IG16446, IG16447, IG16448, IG16449, IG16457, IG16458, IG16459, IG16468, IG16469, IG16470, IG16481, IG16482, IG16484, IG16491, IG16492, IG16494, IG16495, IG16497;
12) 'Valenciae' (50)	IG16498, IG16499, IG16500, IG16501, IG16502, IG16503, IG16504, IG16505, IG16506, IG16507, IG16508, IG16509, IG16549, IG16550, IG16551, IG16554, IG16555, IG16556, IG16557, IG16558, IG16559, IG16561, IG16562, IG16563, IG16564, IG16565, IG16566, IG16567, IG16568, IG16569, IG16571, IG16572, IG16573, IG16574, IG16575, IG16576, IG16577, IG16578, IG16579, IG16580, IG16581, IG16582, IG16583, IG16584, IG16585, IG16586, IG16587, IG16588, IG16589, IG16590;
13) 'Murciense' (58)	IG16596, IG16599, IG16600, IG16601, IG16602, IG16603, IG16650, IG16654, IG16667, IG16668, IG16669, IG16670, IG16683, IG16684, IG16685, IG16686, IG16687, IG16688, IG16689, IG16691, IG16692, IG16694, IG16695, IG16696, IG16697, IG16698, IG16699, IG16700, IG16701, IG16702, IG16703, IG16704, IG16705, IG16706, IG16707, IG16708, IG16714, IG16716, IG16717, IG16718, IG16719, IG16721, IG16722, IG16723, IG16724, IG16725, IG16726, IG16727, IG16730, IG16731, IG16732, IG16733, IG16734, IG16735, IG16736, IG16737, IG16738, IG16739;
14) 'Hordeiforme' (75)	IG16740, IG16741, IG16742, IG16743, IG16744, IG16748, IG16749, IG16754, IG16755, IG16756, IG16757, IG16758, IG16759, IG16760, IG16761, IG16762, IG16765, IG16767, IG16768, IG16769, IG16771, IG16772, IG16780, IG16781, IG16792, IG16793, IG16794, IG16795, IG16796, IG16797, IG16798, IG16799, IG16800, IG16801, IG16802, IG16803, IG16804, IG16810, IG16811, IG16812, IG16813, IG16814, IG16815, IG16816, IG16817, IG16818, IG16819, IG16820, IG16821, IG16824, IG16825, IG16826, IG16827, IG16828, IG16829, IG16840, IG16841, IG16842, IG16843, IG16844, IG16845, IG16846, IG16847, IG16848, IG16849, IG16850, IG16851, IG16852, IG16854, IG16855, IG16858, IG16857, IG16858, IG16859, IG16860
15)'Italicum' (142)	IG16861, IG16862, IG16863, IG16864, IG16865, IG16866, IG16867, IG16868, IG16869, IG16870, IG16871, IG16872, IG16873, IG16874, IG16875, IG16876, IG16877, IG16879, IG16880, IG16881, IG16882, IG16883, IG16885, IG16886, IG16887, IG16891, IG16893, IG16894, IG16895, IG16896, IG16897, IG16898, IG16899, IG16900, IG16901, IG16902, IG16903, IG16904, IG16905, IG16906, IG16907, IG16908, IG16909, IG16910, IG16911, IG16913, IG16914, IG16915, IG16916, IG16917, IG16918, IG16919, IG16920, IG16921, IG16922, IG16923, IG16924, IG16925, IG16926, IG16927, IG16928, IG16929, IG16930, IG16931, IG16933, IG16934, IG16936, IG16937, IG16938, IG16939, IG16940, IG16941, IG16942, IG16943, IG16944, IG16945, IG16946, IG16947, IG16948, IG16949, IG16950, IG16951, IG16952, IG16953, IG16954, IG16956, IG16957, IG16958, IG16959, IG16960, IG16961, IG16962, IG16963, IG16964, IG16965, IG16966, IG16967, IG16968, IG16969, IG16970, IG16984, IG16985, IG16986, IG16987, IG16988, IG16989, IG16992, IG16993, IG17005, IG17006, IG17007, IG17008, IG17009, IG17010, IG17011, IG17017, IG17024, IG17026, IG17027, IG17030, IG17031, IG17032, IG17033, IG17034, IG17035, IG17045, IG17079, IG17086, IG17087, IG17088, IG17089, IG17090, IG17091, IG17093, IG17094, IG17095, IG17058, IG17059, IG17071, IG17072, IG17073, IG17074;
16) 'Reichenbachi' (176)	IG17075, IG17076, IG17077, IG17078, IG17046, IG17047, IG17048, IG17050, IG17052, IG17053, IG17054, IG17055, IG17056, IG17057, IG17096, IG17097, IG17099, IG17100, IG17101, IG17103, IG17104, IG17105, IG17107, IG17109, IG17110, IG17111, IG17112, IG17113, IG17114, IG17115, IG17116, IG17117, IG17118, IG17119, IG17120, IG17121, IG17124, IG17125, IG17126, IG17127, IG17128, IG17129, IG17130, IG17142, IG17143, IG17145, IG17146, IG17147, IG17148, IG17149, IG17151, IG17152, IG17153, IG17154, IG17155, IG17156, IG17158, IG17162, IG17163 IG17164, IG17165, IG17179, IG17180, IG17181, IG17182, IG17183, IG17184, IG17185, IG17186, IG17188, IG17189, IG17190, IG17191, IG17192, IG17193, IG17194, IG17195, IG17196, IG17197, IG17198, IG17199, IG17200, IG17201, IG17203, IG17204, IG17205, IG17207, IG17208, IG17209, IG17210, IG17211 , IG17212, IG17215, IG17216, IG17217, IG17218, IG17220, IG17221, IG17222, IG17223, IG17224, IG17225, IG17226, IG17227, IG17228, IG17229, IG17230, IG17231, IG17233, IG17234, IG17235, IG17236, IG17237, IG17238, IG17239, IG17240, IG17241, IG17244, IG17245, IG17246, IG17247, IG17248, IG17249, IG17250, IG17251, IG17252, IG17253, IG17254, IG17255, IG17256, IG17257, IG17260, IG17263, IG17265, IG17266, IG17268, IG17269, IG17270, IG17271, IG17272, IG17273, IG17274, IG17275, IG17276, IG17277, IG17278, IG17279, IG17280, IG17281, IG17282, IG17284, IG17285, IG17286, IG17287, IG17288, IG17289, IG17292, IG17293, IG17294, IG17295, IG17296, IG17297, IG17298, IG17299, IG17300, IG17301, IG17302, IG17303, IG17308, IG17309, IG17310, IG17311, IG17312, IG17313, IG17314, IG17315;
17)'Lencomelan' (206)	IG17316, IG17317, IG17318, IG17319, IG17320, IG17321, IG17322, IG17323, IG17324, IG17352, IG17326, IG17327, IG17328, IG17329, IG17330, IG17331, IG17332, IG17333, IG17334, IG17339, IG17341, IG17342, IG17343, IG17344, IG17345, IG17346, IG17347, IG17348, IG17349, IG17350, IG17351, IG17353, IG17354, IG17355, IG17356, IG17357, IG17358, IG17359, IG17360, IG17361, IG17362, IG17363, IG17364, IG17365, IG17370, IG17371, IG17372, IG17373, IG17374, IG17375, IG17376, IG17377, IG17378, IG17394, IG17395, IG17397, IG17398, IG17399, IG17400, IG17401, IG17402, IG17404, IG17407, IG17708, IG17409, IG17411, IG17412, IG17413, IG17414, IG17415, IG17416, IG17417, IG17418, IG17419, IG17420, IG17421, IG17422, IG17423, IG17424, IG17425, IG17426, IG17427, IG17428, IG17429, IG17430, IG17431, IG17432, IG17433, IG17434, IG17435, IG17436, IG17437, IG17438, IG17439, IG17440, IG17441, IG17442, , IG17443, IG17444, IG17445, IG17447, IG17448, IG17449, IG17451, IG17452, IG17453, IG17454, IG17456, IG17457, IG17458, IG17459, IG17460, IG17461, IG17462, IG17463, IG17464, IG17465, IG17466, IG17467, IG17468, IG17469, IG17470, IG17471, IG17472, IG17473, IG17475, IG17476, IG17477, IG17478, IG17480, IG17481, IG17482, IG17483, IG17484, IG17485, IG17486, IG17487, IG17488, IG17489, IG17490, IG17491, IG17492, IG17493, IG17494, IG17495, IG17496, IG17497, IG17498, IG17499, IG17500, IG17501, IG17502, IG17503, IG17504, IG17505, IG17506, IG17507, IG17508, IG17509, IG17510, IG17511, IG17512, IG17513, IG17514, IG17515, IG17516, IG17517, IG17518, IG17519, IG17520, IG17521, IG17522, IG17523, IG17524, IG17525, IG17526, IG17527, IG17528, IG17530, IG17531, IG17532, IG17533, IG17534, IG17535, IG17536, IG17537, IG17538, IG17539, IG17540, IG17541, IG17542, IG17543, IG17544, IG17546, IG17547, IG17547, IG17547, IG17548, IG17549, IG17550, IG17551, IG17552, IG17553, IG17556, IG17557, IG17558

a the references number are codes from ICARDA Bank Germoplasm

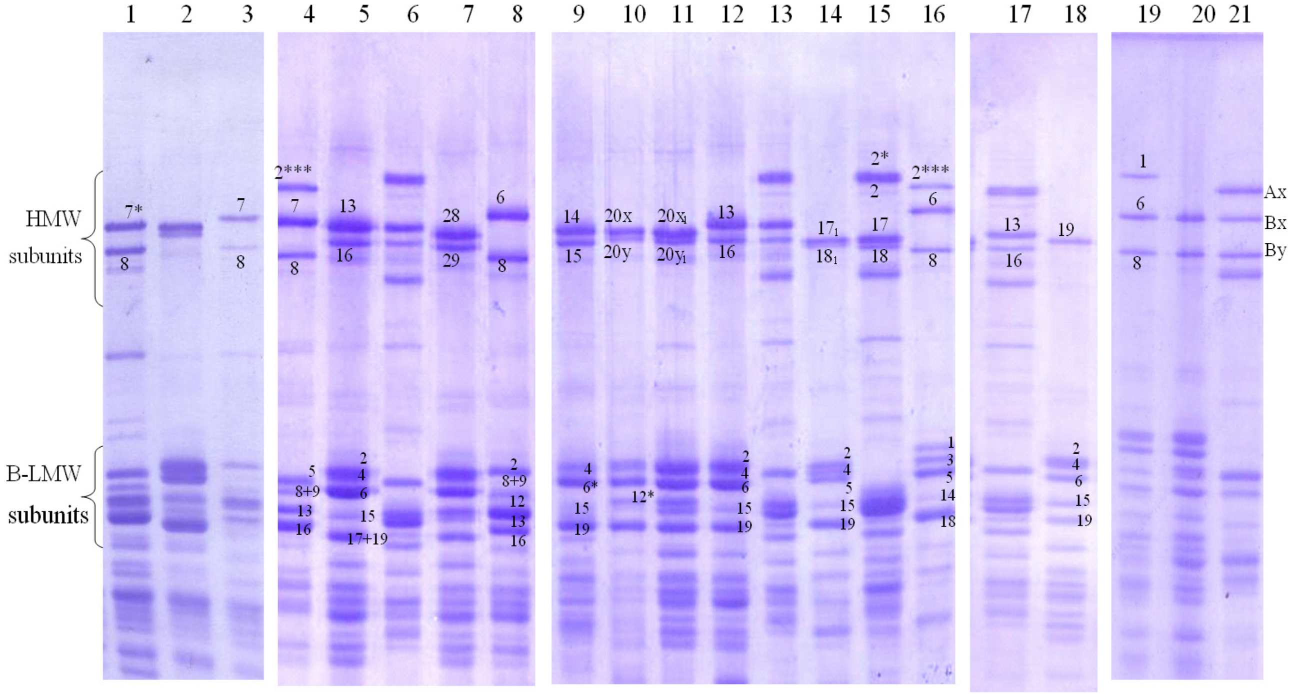

Fig. 1. SDS-PAGE patterns of high molecular weight and low molecular weight glutenin subunits of endosperm proteins of accessions from some populations of local durum wheat collected in Algeria: 1. 'Africanum' IG5806, 2. 'Circumflescum' IG17564, 3. 'Fastuasum' IG11049, 4. 'Hordeiforme' IG16802, 5.'Fastuasum' IG11945, 6. T1: 'Atlas 66', 7. 'Lencomelan' IG17409, 8. 'Melanopus' IG17565, 9. 'Algerience' IG6766, 10. 'Reichenbachi' IG17112, 11. 'Lencurum' IG14311, 12. 'Affine' IG14287' 13. T1: 'Atlas 66, 14' 'Affine' IG14288, 15.T2: 'Prinqual', 16. 'Lencurum IG14320, 17. T1: 'Atlas' 66, 18. 'Aegyptiacim' IG16420, 19. 'Murciense' IG16684, 20.' Italicum' IG16893, 21. T3: 'Clément'

units 13-16) were the most frequent with 31.54%, 25.70% and 21.38% frequency, respectively. The higher frequencies of these alleles were found in the three major populations in this collection (Italicum with 142 accessions, 'Reichenbachi' with 176 accessions and'Lencomelan' with 206 accessions). Another relatively high frequency (12.73%) allele was *Glu-B1h* (encoding subunit pairs 14-15). The other six allelic forms that were identified (*Glu-B1b, Glu-B1u, Glu-B1z, Glu-B1i, Glu-B1r, Glu-B1ag*) encoding subunits 7-8, 7*-8, 7-15, 17-18, 19, 28-29 respectively. The four new bands were rarely expressed in this collection. They appeared at lower frequencies in only a few populations and were completely absent in the others. Accession IG-5806 of 'Africanum' population has subunit 7 of the pair 7+8 (Fig. 1. line 1) which migrates faster than subunit 7 of the pair 7+8 in Chinese Spring. This subunit is similar to subunit 7* of the pair 7*+8 observed by Pogna *et al.* (1989). One of the two new alleles was unique to the 'Lencurum' population and was found in only two accessions (IG-14303 and IG-14326). This allele encodes two subunits of which one had slightly faster mobility than that of band 20x encoded by the *Glu-B1e* allele, the other new band moved slightly faster than subunits 20y and 16, (Fig. 1, line 10 and 11). These subunits were designated $20x_1$ and $20y_1$ respectively and their encoding allele was named *Glu-B1e*$_1$. The second new allele was detected in accessions IG-14311 and IG-14331 of the 'Lencurum' population and in IG-14288 of the 'Affine' population (Fig. 1. line 14), this allele codes for two subunits with mobility between bands 17-18 controlled by *Glu-B1i* allele (Fig. 1. line 15). We named these new bands 17_1 and 18_1 and their encoding allele *Glu-B1i*$_1$.

Allelic variation at Glu- B2 and Glu-3 loci

This set of durum wheat germplasm displayed abundant allelic variation of LMW-GS. A total of 19 alleles were identified (Tab. 2), six at the *Glu-A3* locus (*Glu-A3a, Glu-A3b, Glu-A3c, Glu-A3d, Glu-A3e, and Glu-A3h*), 10 at *Glu-B3* (*Glu-B3a, Glu-B3b, Glu-B3d, Glu-B3e, Glu-B3f, Glu-B3g, Glu-B3h, Glu-B3i, Glu-B3j, Glu-B3ab*) the last (coding for 2-8-9-13-16) being considered as new, and three at *Glu-B2* (*Glu-B2a, Glu-B2b, Glu-B2c*). The low molecular weight glutenin subunits identified were those previously described by Nieto-Taladriz *et al.*, (1997) except subunit 12* (Fig. 1, lane 10) which was identified by Martinez *et al.*, (2004) and 6* (Fig. 1, lane 9) encoded by the allele *Glu-B3j* which was identified by Lerner *et al.* (2004). For the B-LMW glutenins, the most common allelic composition was *Glu-A3a-Glu-B3a-Glu-B2a* encoding the pattern (2-4-6-12-15-19), 39% of the collection analyzed was characterized by this pattern.

The most common allele at *Glu-A3* was *Glu-A3a* encoding subunit 6 with a frequency of 83.06%, this allele was present in genotypes from every population analyzed, followed by *Glu-A3h* (null) with 10.5%; this allele was found in genotypes from nearly all populations and was absent in only two (Afrinacum and 'Algeriense'). The five other alleles classified as rare were all detected at lower frequencies of between 0.58% and 2.22%. At the *Glu-B3* locus, the frequency of allele *Glu-B3a* was 80.05%, it was present in many accessions in all populations except 'Africanum'.

Tab. 2. Alleles of HMW and LMW subunits of glutenin found in the 856 local Algerian *T. durum* wheat

		Varieties																
Locus Allele	total	Apulicum	Africanum	Obscurum	Fastuasum	Algerience	Alexandrinum	Melanopus	Circumflescum	Affine	Lencurum	Aegyptiacum	Valenciae	Murciense	Hordeiforme	Italicum	Reichenbachi	Lencomelan
Subunits	856	3	3	3	6	6	8	11	14	26	28	42	50	57	75	142	176	206
Glu-A1																		
a: 1	1.05	/	/	33.33	16.66	/	/	9.09	/	/	/	/	/	3.50	/	/	1.70	0.48
b: 2*	0.70	/	33.33	/	/	/	/	/	/	/	/	/	10	/	/	/	/	/
VI: 2***	0.46	/	/	/	/	/	/	/	/	/	10.71	/	/	/	1.33	/	/	/
c: null	97.78	100	66.66	66.66	83.33	100	100	90.91	100	100	89.28	100	90	96.5	98.66	100	98.3	99.52
Glu-B1																		
d: 6-8	25.70	33.33	/	66.66	50	66.66	75	72.72	42.85	53.84	28.57	14.28	42	64.91	62.66	8.45	11.93	11.65
b: 7-8	3.35	/	/	/	/	/	12.5	/	/	7.69	7.14	/	4	8.77	9.33	4.22	0.56	0.48
u: 7*-8	0.12	/	33.33	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
z: 7-15	0.47	/	/	/	/	/	/	/	/	/	/	/	/	3.50	/	1.41	/	/
f: 13-16	21.38	/	/	/	16.66	/	/	27.27	/	11.54	3.57	73.8	4	/	10.66	83.10	5.11	3.40
h: 14-15	12.73	/	/	/	/	16.66	12.5	/	21.42		21.43	4.77	/	7.03	4	1.41	15.34	29.12
i: 17-18	0.47	/	/	33.33	/	/	/	/	/	3.84	/	/	2	1.75	/	/	/	/
r: 19	0.23	/	/	/	/	/	/	/	/	/	/	2.38	/	/	1.33	/	/	/
e: 20x-20y	31.54	33.33	66.66	/	33.33	16.66	/	/	35.71	23.09	21.43	4.77	48	/	12	1.41	67.04	44.66
ag: 28-29	2.57	/	/	/	/	/	/	/	/	/	3.57	/	/	/	/	/	/	0.48
e: 20x1-20y1	0.23	/	/	/	/	/	/	/	/	/	7.14	/	/	/	/	/	/	10.20
i: 171-181	0.35	/	/	/	/	/	/	/	/	3.84	7.14	/	/	/	/	/	/	/
Glu-A3																		
a: 6	83.05	33.33	33.33	66.66	66.67	100	75	72.72	64.28	61.54	71.43	95.23	18	91.37	84	95.07	83.52	93.20
b: 5	1.87	33.33	66.66	/	16.66	/	/	/	7.14	11.54	7.14	/	/	1.72	/	/	2.84	/
c: 6-10	0.83	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	1.70	1.94
d: 6-11	0.93	/	/	/	/	/	/	/	14.28	/	/	/	/	3.44	4	/	0.57	/
e: 11	0.58	/	/	/	/	/	/	/	/	19.22	/	/	/	/	/	/	/	/
h: null	12.72	33.33	/	33.33	16.66	/	25	27.27	14.28	7.7	21.42	4.76	82	3.44	12	4.92	11.36	3.85
Glu-B3																		
a:2-4-15-19	80.05	33.33	/	66.66	83.33	66.66	75	72.72	57.15	50	46.43	90.47	56	55.17	57.33	91.54	89.77	95.14
b:8-9-13-16	1.63	33.33	33.33	33.33	/	/	/	/	/	/	7.14	7.14	/	1.72	4	0.71	/	0.48
d:2-4-15-17-19	5.22	/	33.33	/	/	/	/	/	7.14	/	25	/	/	32.75	17.33	0.71	/	1.94
e:2-4-15-16-18	0.23	/	/	/	/	16.66	/	/	/	/	/	/	/	/	/	0.71	/	/
f:2-4-15-17	2.21	/	33.33	/	/	16.66	/	/	/	3.84	/	/	/	6.89	9.33	/	1.14	1.45
g:2-4-15-16	1.40	/	/	/	/	/	/	/	14.28	7.7	3.57	/	2	/	/	1.41	2.27	/
h:1-3-14-18	0.47	/	/	/	/	/	/	9.09	/	3.84	7.14	/	/	/	/	/	/	/
i:7-8-14-18	0.93	/	/	/	/	/	/	/	7.14	26.92	/	/	/	/	/	/	/	/
j:4-6*-15-19	1.05	/	/	/	/	/	12.5	/	/	3.84	3.57	/	/	/	/	0.71	2.84	/
ab:2-8-9-13-16	6.77	33.33	/	/	16.66	/	12.5	18.18	14.28	3.84	7.14	2.38	42	3.44	12	4.22	3.98	0.97
Glu-B2																		
a: 12	70.16	50	66.66	66.66	83.33	83.33	100	90.90	78.56	34.61	67.86	21.42	84	82.75	77.33	12.68	92.04	95.63
b: null	28.66	50	33.33	33.33	16.66	/	/	9.09	21.43	65.39	32.14	78.57	16	17.23	22.66	87.32	5.68	4.37
c: 12*	0.58	/	/	/	/	16.66	/	/	/	/	/	/	/	/	/	/	2.27	/

Tab. 3. Genetic Index diversity

Locus	total	Apuli-cum	Africa-num	Obscu-rum	Fastua-sum	Algerie-nce	Alexan-drinum	Melan-opus	Circum-flescum	Affi-ne	Lencu-rum	Aegyp-tiacum	Valen-ciae	Murci-ense	Hordei-forme	Itali-cum	Reichen-bachi	Lenco-melan
		3	3	3	6	6	8	11	14	26	28	42	50	57	75	142	176	206
DGlu-A1	0.04	0.00	0.44	0.44	0.27	0.00	0.00	0.16	0.00	0.00	0.21	0.00	0.18	0.06	0.02	0.00	0.03	0.01
DGlu-B1	0.57	0.66	0.44	0.44	0.61	0.50	0.40	0.43	0.64	0.63	0.76	0.40	0.58	0.54	0.57	0.29	0.51	0.69
DGlu-A3	0.30	0.66	0.44	0.44	0.49	0.00	0.37	0.39	0.54	0.56	0.37	0.09	0.29	0.16	0.27	0.09	0.24	0.12
DGlu-B3	0.35	0.66	0.66	0.44	0.27	O.50	0.40	0.43	0.61	0.29	0.72	0.17	0.50	0.58	0.61	0.14	0.19	0.11
DGlu-B2	0.42	0.50	0.44	0.44	0.27	0.27	0.00	0.16	0.44	0.45	0.45	0.33	0.26	0.28	0.35	0.22	0.12	0.08
Di	0.34	0.48	0.48	0.44	0.38	0.25	0.23	0.31	0.45	0.39	0.50	0.20	0.36	0.32	0.36	0.14	0.22	0.20

Two other *Glu-B3* alleles (*Glu-B3d* and *Glu-B3*) were considered relatively abundant (>5%) (Cabalerro *et al.*, 2004) with approximately the same frequencies: 5.22% and 6.77% respectively.

Genetic diversity

The indices (D_i) of genetic diversity at each of the five loci of the 17 wheat populations are presented in Tab. 3. Most of the individual populations as well as the whole collection dispalyed relatively high levels of genetic diversity.

The average total gene diversity index (D_i) was 0.34. The *Glu-A1* locus had the lowest genetic variation (0.04) because it was monomorphic (D_i=0, expression of only one allele *Glu-A1c*) in seven populations: 'Apulicum', 'Algeriense', 'Alexandrinum', 'Circumflescum', Affine, 'Aegyptiacum' and' Italicum'. The highest genetic index diversity was at *Glu-B1* with an average total of 0.57. This locus was polymorphic in all populations. The extent of HMW and LMW glutenin subunit polymorphism varied among populations: the highest allelic diversity (0.50) was found in 'Lencurum' (28 accessions), with 10 alleles at *Glu-1* and nine at *Glu-3,* which also contained the two new alleles found at *Glu-1* and the new allele k at *Glu-B2*. 'Italicum', 'Reichenbachi' and 'Lencomelan', which have the highest numbers of accessions (142, 176 and 206 accessions respectively), showed the lowest diversity with D_i of 0.14, 0.22 and 0.20 respectively.

Cluster analysis based on HMW and LMW allelic frequencies

Cluster analysis based on HMW and LMW allelic frequencies was performed to determine the diversity among the durum wheat populations studied. The dendrogram (Fig. 2) obtained from Roger's (1972) genetic distances of durum wheat population revealed several groups.

The 'Africanum' population (composed of only tree accessions) is very different from other populations. At Roger's distance of 0.32, all populations split into two groups, one containing the two populations (Italicum and 'Aegyptiacum') which have a high level of similarity, while the second is further divided into two subgroups: the first comprising only one population ('Valencia'), while the other is divided evenly into two groups, one comprising two populations ('Affine' and 'Apulicum') and the other containing the majority of the collection. The mean value of Roger's distances was 0.30 (Fig. 2), at this distance, the dendrogram as a whole revealed high degree of similitude in the genetic diversity of proteins among the majority of populations. At distance 0.15, the dendrogram shows that the majority of populations could be clustered into two subgroups, the first comprising the population 'Hordeiforme' and the second a major group containing the nine remaining populations: 'Lencomelan', 'Reichenbachi',

Tab. 4. Representative of proposal core collection

Accessions	Geographical localisation	HMW-GS	LMW-GS	Morphological characters[a]
		Diagram's	Diagram's	
1. 'Apulicum' IG 6355	Guelma, Constantine,Bourdj-	Nul, 20x-20y	2-4-6-12-15-19	*Red ear pubescent, elongated, triangular shaped pyramid with a slightly
	Bouarreridj, Relizane, Saida			flattened compact. The free glumelles are extended by desnse black be-
				ards. The grain is white, large and translucent and the straw is hollow.
2. 'Africanum' IG5806	Toughourt , Biskra	Nul, 7*-8	5-7-8-12-14-18	*White ear pubscent, elongated, narrow slightly flattened with a rather lo-
				w compactness. The glumelles are extended by long black beards little
				different or parallel. The grain is red, big and hunch back and the hollow
				straw.
3. 'Obscurum' IG7756	Tebessa, Tiaret, Relizane	1, 17-18	2-6-8-9-12-13-16	*Black hairless ear, semi compact pyramidal form or fusiform. The glume-
				lles are extended by barbs black or bluish black, long and duvergent. Th-
				e grain is red, large, elongated hump and hollow straw generally 1/4th of
				the stem is hollow.
4. 'Fastuasum' IG11049	Medea, Relizane	Nul, 7-8	2-8-9-12-13-16	*Ear white pubscent. The glumelles are extended by barbs straight or pa-
5. 'Fastuasum' IG11945	***	Nul, 13-16	2-4-6-15-17-19	rallel, nearly long, red grain, large, elongated hump and hollow to semi-
				hollow straw.
6. 'Algerience' IG6766	Relizane, Tebessa	Nul, 14-15	4-6*-15-19	*Red hairless ear, semi-compact. The glumelles are extended by black be-
				ard curved at the base sometimes divergent, red grain, large, elongated
				hump and hollow straw.
7. 'Alexandrinum' IG22415	Annaba	Nul, 6-8	2-8-9-12-13-16	*Same morphological characters as those of Algerience polulation.
8. 'Melanopus' IG17565	Guelma, Constantine, Bourdj-	Nul, 6-8	2-8-9-12-13-16	*White pubscent ear, lying more or less flattened with a rather low comp-
	bouarreridj			actness, long black beard divergent or parallel to grain white, large, elon-
				gated hump and hollow straw usually hollow to semi-hollow.
9. 'Circumflescum' IG17564	Motaguanem	Nul, 20x-20y	2-4-6-11-12-15-19	*White pubscent ear, fusiform, the glumelles are extended by long curved
				barbs at the base and grain red, large, elongated hump, hollow to semi-
				hollow.
10. 'Affine' IG14288	Mostaguanem, Annaba and	New (171-181)	2-4-5-15-19	*White glabrous ear, straight, narrow, loose, white parallel beards some-
11. 'Affine' IG14299	Medea	Nul, 13-16	2-4-11-15-17	times divergent, red grain elongated hump and, hollow or semi-hollow
				str.aw
12. 'Lencurum' IG14311	Oran and Annaba	New (20x1-20y1)	2-4-6-12-15-17-19	*White glabrous ear, sometimes nearly rectangular sometimes pyramid

Tab. 4. Representative of proposal core collection (Continous)

Accessions	Geographical localisation	HMW-GS	LMW-GS	Morphological characters^a
13. 'Lencurum' IG 14320	***	2^{***}, 6-8	1-3-5-14-18	with low compactness, with slightly divergent white bear, a medium white or amber grain, and hollow straw.
14. 'Aegyptiacum' IG16420	Tiaret,Bousaada, Toughourt	Nul, 19	2-4-6-15-19	*Red pubscent ear, compact, pyramid, of prolonged glumelles by little divergent red bread, red grain, elongated and large hump and semi-hollow straw.
15. 'Valenciae' IG16554	Biskra, Bousaada, Toughourt	2^{*}, 17-18	2-4-6-12-15-16	*Same morphological characters as those of 'Lencurum'polulation.
16. 'Murciense' IG16684	Setif, Guelma, Constantine, bourdjbouarreridj.	1, 7-8	2-4-6-12-15-19	*Ear red, glabrous somewhat loose with glumelles extended by long red beard semi-divergent, red grain, medium hump and half filled straw.
17. 'Hordeiforme' IG16802	Tiaret and Tebessa	2^{***}, 7-8	5-8-9-13-16	*Same morphological characters as those of Murcience population.
18.' Italicum' IG16893	Batna and Bousaada	Nul, 7-8	2-4-6-12-15-19	*Same morphological characters as thoseof 'Fastuasum' population.
19.' Italicum' IG16919	***	Nul, 7-15	2-4-6-10-15-16-18	
20. 'Reichenbachi' IG 17112	Saida and Tebessa	Nul, 20x-20y	2-4-6-12*-15-19	*White hairless ear, loose, straight, narrow, with low divergent black beard, medium sized red grain, humped with hollow, semi hollow or 3/4th widening straw.
21.'Lencomelan' IG17409	Oran and Annaba	Nul, 28-29	2-4-6-12-15-19	*Same characters as thoseof 'Reichenbachi' population.

a: according to Boudour, L. (2005)

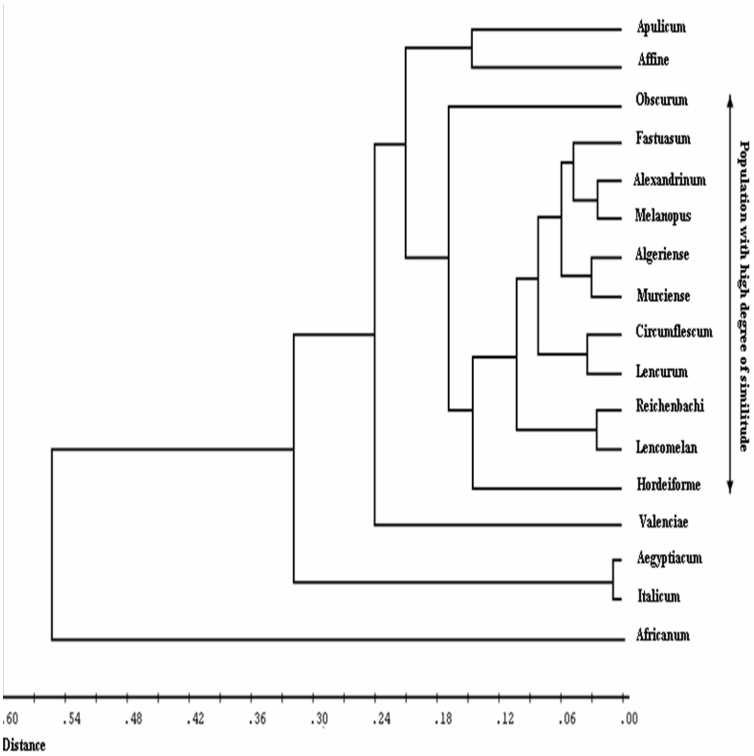

Fig. 2. Cluster analysis based on allele frequency in each local Algerian durum wheat population

'Lencurum', 'Circumflescum', 'Murciense', 'Algeriense', 'Melanopus', 'Alexandrinum', and 'Fastuasum'. The results of cluster analysis (Fig. 2.) showed no relation between allelic variation and the geographical distribution of the populations analyzed. For example, the nine populations in the same cluster at the distance 0.18 do not have the same geographic origins and come from completely different climates: some are distributed throughout the Mediterranean climate and littoral areas ('Lencurum', 'Circumflescum', 'Lencomelan', etc.), others are from the tellian Atlas ('Melanopus' and 'Murcience') or from semi-arid areas ('Fastuasum' and 'Algeriense') and the arid Saharan regions ('Alexandrinum' and 'Rechenbachi'). Another example concerns the two populations 'Africanum' and 'Valenciae', which are very distant based on their HMW-GS and LMW-GS composition, but were collected at the same sites.

Morphological characters

Ten morphological characters were selected to study similarities among populations. These morphological characters were included in this study as a complementary approach to confirm the choice of accessions for the core collection. Cluster analysis (Fig. 3) showed five groups, each composed of two populations that were very close morphologically: 'Lencurum' and 'Valenciae', 'Hordeiforme' and 'Murciense', 'Reichnbachi' and 'Lencomelan', 'Fastuasum' and 'Italicum', and 'Alexandrinum' and 'Algerience'.

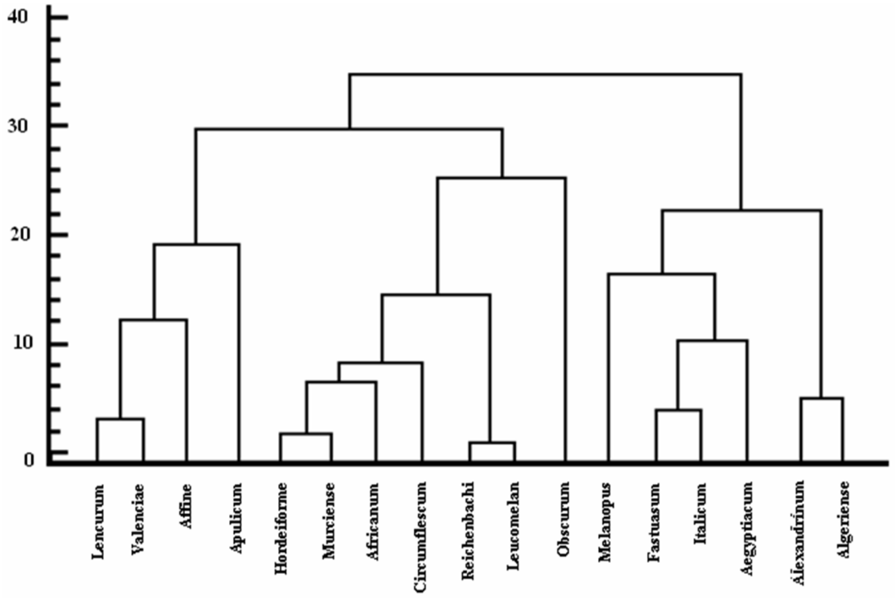

Fig. 3. Cluster analysis based on morphological characters in each botanic durum wheat population

Choice of representative accessions for the core collection

In order to constitute the core collection, the geographic origin of the accessions was used as a priority and at least one representative accession was selected per location. The choice of the accessions in this core collection (Tab. 4) was based on two other factors: some morphological characters and the genetic variability of SG-HPM and FPM. Since no significant correlations were found between these three factors, the choice of the accessions for the core collection was made by selecting accessions covering all the variability of the parameters studied. For this reason, we always chose accessions that were sufficiently distant from one another.

Discussion and conclusions

Nowadays, the genetic variability of wheat cultivars is decreasing as a consequence of the genetic erosion of cultivated wheat. It is thus important to search for new genes for wheat improvement. With this objective, many studies have been performed to evaluate the materials conserved in germplasm banks and core collections (Balfourier et *al.,* 2007) using molecular markers as tools. Previous investigations showed that old varieties and landraces as well as closely related *Triticum* species of cultivated wheat possess considerable allelic variation in HMW and LMW-GS composition (Yan *et al.,* 2003; Pflüger *et al.,* 2001; Vallega and Waines, 1987). These rich genes resources may play an important role in improving wheat quality.

In our study, we detected allelic variation at *Glu-1*, *Glu-3* and *Glu-B2* loci in 856 accessions of durum wheat collected in Algeria and divided *a priori* according to their agronomical and morphological traits into 17 populations to create a core collection. The germoplasm from ICARDA-GenBank had not been previously investigated for the genetic diversity of their gluten compositions. The results obtained showed rather high polymorphism in HMW-GS and LMW-GS variation, especially for *Glu-B1* and *Glu-*

B3 loci. Altogether, 53 glutenin patterns were detected, including 22 for HMW and 21 for B-LMW glutenin subunits. A total of 35 different alleles were identified for the five glutenin loci studied *Glu-A1* (4), *Glu-B1* (12), *Glu-A3* (6), *Glu-B3* (10) and *Glu-B2* (3). Three new alleles were found, two at *Glu-B1* and one at *Glu-B3*. For the *Glu-B1* locus, some alleles are very frequent: more particularly the alleles *Glu-B1e* and *Glu-B1d* encoding subunit pairs (20x-20y and 6-8) respectively. These results are in agreement with those of Branlard *et al.* (1989), who found that these two alleles occurred in 33.5% and 26.3% of the 502 varieties of durum wheat originating from 23 countries; however, most accessions of durum wheat analyzed do not have subunit 7-8 encoded by *Glu-B1b* (present in only 3.03% of accessions) contrasting with the higher frequency (25.9%) found in the durum wheat of 502 varieties. The alleles *Glu-B1f* (13-16) and *Glu-B1h* (14-15), which were common among the accessions in our study (21.37% and 12.73%, respectively), were rarely expressed in the world collection analyzed by Branlard *et al.* (1989) i.e. in only 5.5% and 0.6%, respectively. Moragues *et al.* (2006) in their study of 63 durum wheat landraces from the Mediterranean basin, reported three alleles in *Glu-A1* and 14 alleles at the *Glu-B1* locus, where *Glu-B1e* and *Glu-B1b* were the most frequent (30.16% and 23.81%, respectively). Similar results were found by Carrillo (1995), who studied 200 varieties of Spanish durum wheat landraces. Conversely Hailu *et al.* (2006) in a collection of 120 Ethiopian tetraploid wheat germplasm analyzed found *HMW* glutenin subunits 7-8 to be the most frequent and 6+8 to be rare. For the *B-LMW* glutenins, seven, eleven and three allelic variants were observed at Glu-*A3, Glu-B3 and Glu-B2* respectively, which is nearly the same genetic variability as that reported by Nieto-Taladriz *et al.,* (1997) in a collection of 88 durum wheat cultivars originating from different areas. The most common allelic composition was *Glu-A3a-Glu-B3a-Glu-B2a* (36%). Comparing the frequencies of glutenin subunits found in Algerian durum wheat landraces (Cherdouh *et al.,* 2005) and those found in durum varieties grown in Algeria (no published results), higher diversity was found in the local durum wheat collection analyzed in the present study. This information could be useful for selecting local varieties with improved quality and also as a source of genes to develop new lines when breeding for quality as shown by the observed high frequencies of alleles related to grain quality: *Glu-B1b* (7+8), *Glu-B1d* (6+8) (Du Cros, 1987) and *Glu-A1a* (subunit 1) (Kaan *et al,* 1993; Aghai *et al.,* 1996), *Glu-B3a* (2+4+15+19), *Glu-B3c* (2+4+14+15+19), *Glu-A3h* (nul), *Glu-A3a* (6) (Carillo *et al.,* 2000). The development of the phytogenetic resources requires not only their conservation but also their use. Through this study, which took into account the genetic variability of HMW-GS and LMW-GS and the variability of morphological characters and the geographical location of populations, we were able to reduce the collection of 856 to only 21 accessions. This is the minimum number of accessions and molecular markers that would be needed to undertake a complete evaluation of the representative subsets formed with chosen accessions. Assembling a core collection of accessions is one way of streamlining the process of evaluating genetic diversity. For instance, having a small representative sample of accessions should make it easier to look for allelic variation in genes of interest for quality and thus facilitate their use in breeding programs.

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