Allelic variation at high-molecular weight and low-molecular weight glutenin subunit genes in Moroccan bread wheat and durum wheat cultivars

Fatima Henkrar1,2,3,4 • Jamal El-Haddoury3 • Driss Iraqi2 • Najib Bendaou4 • Sripada M. Udupa1

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Abstract Glutenin is a major protein fraction contributing to the functional properties of gluten and dough. The glutenin constitutes 30–40% of the protein in wheat flour and about half of that in gluten. It is essential to identify correct glutenin alleles and to improve wheat quality by selecting alleles that exert favorable effects. Moroccan wheat cultivars are unique in West Asia and North Africa region, since many of them possess resistance to Hessian fly, a pest, which is becoming important in other countries in the region. Hence, these cultivars are being used as donor for the resistance in the breeding program. Here, we determine the allelic variation in high-molecular weight glutenin subunits (HMW-GS) and low-molecular weight glutenin subunits (LMW-GS) in Moroccan cultivars of bread and durum wheat using the gene-specific PCR markers. In 20 cultivars of bread wheat, 9 different allele variants were detected at HMW-GS and 13 different allele variants were detected at LMW-GS, in which the alleles Glu-A1b (2*), Glu-B1i (17 + 18), Glu-B1c (7*7 + 9), Glu-D1d (5 + 10), Glu-A3c, Glu-B3 h, and Glu-D3b were the most frequent. In 26 cultivars of durum wheat, less allelic variation was found: seven different allele variants at HMW-GS and six different allele variants at LMW-GS were identified, in which the major alleles were Glu-A1c (null), Glu-B1b (7 + 8), Glu-B1e (20), Glu-A3c, and Glu-B3d. The mean value of the genetic diversity for the glutenin loci was 0.502 in bread wheat and 0.449 in durum wheat. Most of the glutenin alleles carried by Moroccan bread wheat cultivars impart good bread-making quality. Most of the durum wheat glutenin alleles were related to low strength dough or poor quality and need to be improved. To improve quality of Moroccan durum wheat, essentially, Glu-A1c and Glu-B3d alleles of the genes should be replaced with the better alleles through breeding.

Keywords Moroccan wheat • Glutenin • HMW-GS • LMW-GS • PCR markers • End-use quality

Introduction

Glutenin proteins are the most important protein group which determines bread-making quality of bread wheat (Triticum aestivum L.) and pasta making quality of durum wheat (Triticum turgidum L.). It contributes to the ability of dough to rise and maintain its shape as it is baked. Glutenin strength differs with varieties of wheat. It is a highly heterogeneous mixture of polymers consisting of a number of different high- and low-molecular-weight glutenin subunits (HMW-GSs and LMW-GSs) linked by disulfide bonds (Veraverbeke and Delcour 2002), resulting in variability in gluten strength among wheat varieties. The HMW-GSs comprise about 20–30% of the glutenin (Shan et al. 2003) and play a key role in determining wheat gluten and dough elasticity. The HMW-GSs presented a high level of polymorphism. Therefore, the HMW-GSs are of immense importance in wheat breeding and genetics. A complex locus Glu-1 encodes HMW-GS. Glu-1 complex
markers are designed to differentiate the x-type glutenin subunit and y-type glutenin subunit polypeptides (Shewry et al. 1992). In each chromosome, the Glu-1 locus contains two closely linked genes that encode for the glutenin loci. The PCR-based markers are available to discriminate the important alleles that are functionally different, such as Ax2 and Ax2*, Bx7 and Bx7*, Bx8 and By8*, Bx14–By15, and Bx20 for LMW-GS, and several alleles overlapping for LMW-GS. Hence, characterization of HMW-GS and LMW-GS genes at the DNA level and development of functional markers are needed for the discrimination of different-alleles in wheat breeding. In wheat, many functional markers are developed for the glutenin loci. The PCR-based markers are available to discriminate the important Glu-1 alleles Dx5, Dy10, Ax2*, Bx7, Bx7*, Bx17, By8, and By9 (Ahmad 2000; Ma et al. 2003; Butow et al. 2004; Lei et al. 2006). Similarly, several markers are designed to differentiate the Glu-3 alleles at Glu-A3, Glu-B3, and Glu-D3 (Zhang et al. 2004; Zhao et al. 2007a, b; Wang et al. 2009).

In Morocco, several bread wheat and durum wheat cultivars have been released over the years. In recent years, bread wheat and durum wheat cultivars with the Hessian fly (Lhaloui et al. 2000, 2005) resistance have been developed and released for cultivation to tackle this pest problem in arid and semi-arid regions. Arrihane and Aguilal varieties of bread wheat were released in 1998. For durum wheat, the varieties Irden, Nassira, Chaoui, and Amria were released in 2003, Marouane in 2005, and Icamor in 2006. These resistant cultivars are useful donors for other countries in the North Africa and West Asia regions, where the Hessian fly is emerging as an important pest in recent times. However, these cultivars are not yet characterized for HMW-GS and LMW-GS variability, which is useful for marker-assisted selection in the breeding when those cultivars used as parents in the breeding program.

In Morocco, some studies were realized on the allelic variation in prolamin protein, namely, glutenin and gliadin. Using SDS-PAGE, Bakhella and Branlard (1997) observed the predominance of subunit 2*–5–17–18–10 in 44 Moroccan bread wheat cultivars and landraces, and predominance of 6–8 and 20 in 39 Moroccan durum wheat cultivars and landraces with respect to HMW-GS. In that abstract, no details regarding the landraces or cultivars used and their HMW-GS alleles were available. Zarkti et al. (2010) using also SDS-PAGE for characterization HMW-GS and LMW-GS of 23 Moroccan durum wheat landraces reported that the majority of the landraces possess the null subunit at Glu-A1 and 20x + 20y at Glu-B1. However, information on HMW-GS and LMW-GS variability in the Moroccan cultivars of bread wheat and durum wheat is not available. Thus, the objective of the present study was to determine the allelic variation at Glu-1 and Glu-3 glutenin loci in 20 Moroccan bread and 26 Moroccan durum wheat cultivars released until 2006 using gene-specific PCR markers. The allelic information at Glu-1 and Glu-3 glutenin loci based on PCR-based technique, a non-destructive method, will be helpful for transferring useful alleles through genomic-assisted improvement of wheat.

Materials and methods

Plant materials

Total of 20 bread wheat and 21 durum wheat varieties (Table 1; Henkrar et al. 2015a, b) and 5 additional durum wheat varieties, Isly (released in 1988), Massa (released in 1988), Anouar (released in 1993), Sboula (released in 2000), and Chaoui (released in 2003) were used to characterize the glutenin alleles at Glu-A1, Glu-B1, Glu-D1, Glu-A3, Glu-B3, and Glu-D3 loci. Five exotic cultivars with known glutenin subunit composition (Tables 1, 2) were used as controls to confirm the exact fragment amplified.

DNA extraction and gene-specific marker analysis

Genomic DNA was extracted from leaves at seedling stage using a CTA (cetyltrimethylammonium bromide) protocol of Saghai-Maroof et al. (1984) with slight modification (Udupa et al. 1999). Quality and quantity of the isolated DNA were determined on 1.0% (w/v) agarose gels by comparing bands to known concentrations of lambda DNA. The PCR reactions were performed in a total volume of 10 μL, containing 1X PCR buffer (Promega, USA), 1.5 mM MgCl2, 200 μM of each dNTPs, 10 pmol of each primer, 0.5 U of Taq DNA polymerase, and approximately 50 ng of genomic DNA. All the allele-specific and gene-specific PCR primers were synthesized (Sigma-Genosys, Germany) according to published sequence information: Ax2*/Ax1/Axnull (Lafiandra et al. 1997), Ax2* (De Bustos...
et al. 2000), Dx5/Dx2, Dy10/Dy12, and Bx7 (Ahmad 2000), Bx/Bx7*/Bx6 (Butow et al. 2004), By8/By8*/By9/By18*/By20* (Lei et al. 2006), Glu-A3 (Zhang et al. 2004), Glu-B3 (Wang et al. 2009), and Glu-D3 (Zhao et al. 2007a, b). The amplification programs and electrophoresis conditions of the PCR assays were based on the references mentioned above. The PCR products were separated in ethidium bromide-stained 1.2 or 1.5% (w/v) agarose gels run in 1× TBE buffer and exposed to UV light to visualize DNA fragments.

Statistical analysis

The gene diversity, number of alleles, and PIC value were calculated using the PowerMarker software (Ver. 3.0; Liu and Muse 2005). The glutenin relationship between cultivars was visualized as a dendrogram using the PowerMarker and MEGA5 software (Tamura et al. 2011). The Neighbor-joining tree was constructed using the frequency-based distance for the shared allele.

Results

Allelic variation in bread wheat cultivars

HMW-GS and LMW-GS composition of 20 Moroccan bread wheat cultivars based on gene/allele-specific PCR analysis are shown in Table 3. The frequencies of different alleles identified were calculated and schematized in Fig. 1.

A total of nine different allele variants were detected at HMW-GS. Three subunits (1, 2*, and null) were identified at Glu-A1 locus, and the sum of the frequency of the two active types 1 (Glu-A1a) and 2* (Glu-A1b) was 85%. While the rest were null-type gene Glu-A1c. There were four subunit pairs at Glu-B1 locus 7*–8 (Glu-B1u), 7–8* (Glu-B1al), 7/7*–9 (Glu-B1c), and 17–18 (Glu-B1i). Among them, the subunit pairs 7/7*–9 and 17–18 had highest proportion, 35% for each. At Glu-D1 locus, the predominant HMW-GS were the combination 5–10 (Glu-D1d) at frequency of 85%. Then, 15% were for the combination 2–12 (Glu-D1a).

In LMW-GS, 13 different allele variants were identified. At Glu-A3 locus, five alleles were found (b, c, d, e, and i) among which Glu-A3c occurred in 50% of the cultivars. Glu-B3 appears to be highly polymorphic in this set of cultivars. Out of the six alleles (b, fg, g, i, h, and j), alleles Glu-B3h and Glu-B3i were predominant and showed a high frequency of 35% and 29%, respectively. The cultivars Nasma, Rajae, and Salama did show any alleles using the available allele-specific PCR markers for Glu-B3. This indicates that these cultivars had other allele types, not able to be identified using the present PCR markers and involve the SDS-PAGE technique. In addition, no allele was amplified in variety Tilila using the same set of allele-specific primers. The variety Tilila had a 1BL.1RS translocation and was derived from Veery’s (Jlibene et al. 1996), which has been characterized to have the allele j (Gupta et al. 1994). Furthermore, according to Gupta et al. (1994), the allele Glu-B3j is associated with the translocated chromosome1BL.1RS. Thus, Tilila had the allele Glu-B3j. At Glu-D3 locus, two alleles were identified, Glu-D3a and Glu-D3b with a frequency of 5 and 95%, respectively.

| Cultivar    | Glu-A1 | Glu-B1 | Glu-D1 | Alleles | References     |
|-------------|--------|--------|--------|---------|----------------|
| Chinese-Spring | 7 + 8  | 2 + 12 | c, b, a| Bekes et al. (2008a) |
| Annuello    | 1      | 7* + 8 | 2 + 12 | a, u, a | Bekes et al. (2008a) |
| Pavon-76    | 2*/1   | 17 + 18| 5 + 10 | b/a, i, d| Bekes et al. (2008a) |
| Stylet      | 1      | 7 + 9  | 5 + 10 | a, c, d | Bekes et al. (2008a) |
| Yecora-Rojo | 1      | 17 + 18| 5 + 10 | a, i, d | Bekes et al. (2008a) |

Table 2 LMW-GS composition of exotic cultivars used in this study as controls

| Cultivar    | GluA3 | GluB3 | GluD3 | References     |
|-------------|-------|-------|-------|----------------|
| Chinese-Spring | a     | a     | a     | Bekes et al. (2008b) |
| Annuello    | b     | b     | b     | Bekes et al. (2008b) |
| Pavon-76    | b     | h     | e?    | Bekes et al. (2008b) |
| Stylet      | c/e   | h     | c     | Bekes et al. (2008b) |
| Yecora-Rojo | d     | h     | a     | Bekes et al. (2008b) |
Furthermore, the gene diversity of the individual loci varied widely (Table 5). The lowest value was 0.095 showed at Glu-D3 locus that exhibited only two different alleles a and b in bread wheat and 0 at Glu-A1 locus in durum wheat due to the overwhelming presence of the null-type gene Glu-A1c. The highest value was 0.770 at Glu-B3 in bread wheat and 0.701 at Glu-B1 in durum wheat. The neighbor-joining dendrogram (Fig. 2) clustered the two species in separated groups. The bread wheat cultivars were highly divergent than the durum wheat cultivars.

Discussion

HMW-GS variations in some old varieties and landraces of bread wheat and durum wheat from Morocco were previously investigated using SDS-PAGE technique (Bakhella and Branlard, 1997). Zarkti et al. (2010) studied HMW-GS and LMW-GS variation in 23 local landraces of durum wheat using SDS-PAGE technique. The SDS-PAGE base technique is destructive and can be carried out only after the harvest of the grains and may not be handy for marker-assisted selection.

However, the HMW-GS and LMW-GS variations in the recently released bread wheat and durum wheat varieties from Morocco are lacking. Moreover, all the previous works on HMW-GS and LMW-GS variability in Moroccan durum wheat due to the overwhelming presence of the null-type gene Glu-A1c. The highest value was 0.770 at Glu-B3 in bread wheat and 0.701 at Glu-B1 in durum wheat. The neighbor-joining dendrogram (Fig. 2) clustered the two species in separated groups. The bread wheat cultivars were highly divergent than the durum wheat cultivars.

Allelic variation in durum wheat cultivars

The HMW-GS and LMW-GS compositions of 26 Moroccan durum wheat cultivars are summarized in Table 4 and their frequencies are presented in Fig. 1. Less allelic variation was found in the Moroccan durum wheat compared to the bread wheat: six different allele variants at Glu-1 (HMW-GS) and seven allele variants at Glu-3 (LMW-GS). At Glu-A1, the null type was present in all cultivars studied (100%), and no active type was detected. Five alleles identified at Glu-B1 loci, with subunits 6–8 (Glu-B1d), 7–8 (Glu-B1b), 7/7–9 (Glu-B1c), 17–18 (Glu-B1i), and 20 (Glu-B1e), in which the subunit pairs 7–8 and 20 were the predominant with 38 and 35%, respectively. Among the three alleles detected at Glu-A3 loci, Glu-A3c was the most frequent (62%). The Glu-B3 locus exhibited four alleles (d, i, g, and h) and Glu-B3d was the major allele with high frequency of 58%. In this locus, Oum-Rabia, Tensift, and Icamor did show any allele using the available PCR markers.

Genetic diversity

The mean value of the gene diversity for the glutenin loci was 0.502 in bread wheat and 0.449 in durum wheat. Furthermore, the gene diversity of the individual loci varied widely (Table 5). The lowest value was 0.095 showed at Glu-D3 locus that exhibited only two different alleles a and b in bread wheat and 0 at Glu-A1 locus in durum wheat due to the overwhelming presence of the null-type gene Glu-A1c. The highest value was 0.770 at Glu-B3 in bread wheat and 0.701 at Glu-B1 in durum wheat. The neighbor-joining dendrogram (Fig. 2) clustered the two species in separated groups. The bread wheat cultivars were highly divergent than the durum wheat cultivars.

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wheat varieties were based on SDS-PAGE technique, which uses the harvested grains and destructive and is not useful for making selection at early stage of plant growth.

In this study, we analyzed the allelic variation of HMW-GS and LMW-GS glutenin loci in the 20 bread wheat and 26 durum wheat varieties representing the most important and recently developed cultivars in Morocco using gene/allele-specific PCR. Many of the recently developed varieties carry resistance to the Hessian fly, which is an important pest in semi-arid regions of Morocco. Because of climate change, the problem of this pest is spreading to other areas in Morocco, the North Africa and many other wheat-producing countries. The Moroccan varieties could be used as donors in wheat presumptive breeding in many counties in the semi-arid regions. Therefore, knowledge of allelic variation at Glu-1 and Glu-3 loci is very important for selection of suitable parents for crossing and marker-assisted selection of the Hessian resistance and better end-use quality (Henkrar et al. 2016).

Alleles present at each of the Glu-1 and Glu-3 loci can have a large combined effect on dough properties and suitability for specific end-products (Appelbee 2007; Eagles et al. 2006; Gupta et al. 1994). With correct classification of glutenin alleles, it is possible to improve wheat quality by selecting alleles that exert favorable effects and allelic combinations (Eagles et al. 2002). Therefore, in this study, we revealed the allelic variation of HMW-GS and LMW-GS glutenin subunit composition in 46 Moroccan wheat cultivars using PCR markers. 9 different allele variants at HMW-GS and 13 different allele variants at LMW-GS were identified in 20 cultivars of bread wheat. Six different allele variants at HMW-GS and seven allele variants at LMW-GS were noticed in 26 cultivars of durum wheat.

![Fig. 1 Frequency of alleles at different Glu loci in the 20 Moroccan bread wheat cultivars (a) and 26 Moroccan durum wheat cultivars (b)](image-url)
Allelic variation in bread wheat cultivars

The HMW-GS composition 2* (b), 7/7*–9 (c), 17–18 (i), and 5–10 (d) was the most frequent. Odenbach and Mahgoub (1988) found that the HMW glutenin subunits 2*, 7*–9, and 5*–10 were associated with large sedimentation volumes. Ram (2003) reported also that the combination of Glu-A1b, Glu-B1i, and Glu-D1d alleles exhibited the highest dough strength and can be used as combination to improve bread-making quality. For Glu-A1 locus, the two active types of HMW-GS 1 and 2* were detected at high frequency (85%) which appears to be a better baking quality allele and confers better values for the

Table 4  HMW-GS and LMW-GS composition in Moroccan durum wheat cultivars using gene-specific PCR markers

| Cultivar     | HMW-GS | LMW-GS |
|--------------|--------|--------|
|              | Glu-A1 | Glu-B1 | Glu-A3 | Glu-B3 |
| Karim        | null (c) | 7–8 (b) | c   | d   |
| Ourgh        | null (c) | 7–8 (b) | c   | d   |
| Oum-Rabia    | null (c) | 7–8 (b) | c   | –   |
| Sarif        | null (c) | 6–8 (d) | i   |   |
| Amjad        | null (c) | 20 (e)  | c   | d   |
| Marzak       | null (c) | 7–8 (b) | d   | d   |
| Jawhar       | null (c) | 20 (e)  | c   | d   |
| Anouar       | null (c) | 7–8 (b) | c   | g   |
| Massa        | null (c) | 7–8 (b) | c   | h   |
| Isly         | null (c) | 6–8 (d) | d   | d   |
| Sebou        | null (c) | 17–18 (i) | d | d |
| Tensift      | null (c) | 20 (e)  | c   | –   |
| Merjana      | null (c) | 7–8 (b) | c   | d   |
| Tomouch      | null (c) | 20 (e)  | c   | d   |
| Tarek        | null (c) | 6–8 (d) | c   | d   |
| Belbachir    | null (c) | 7–8 (b) | c   | d   |
| Icamor       | null (c) | 20 (e)  | c   | –   |
| Maroune      | null (c) | 7–8 (b) | d   | h   |
| Nassira      | null (c) | 7*–9 (c) | c   | d   |
| Chaoui       | null (c) | 20 (e)  | d   | i   |
| Amria        | null (c) | 20 (e)  | d   | i   |
| Cocorit      | null (c) | 6–8 (d) | g   | h   |
| Irden        | null (c) | 20 (e)  | d   | i   |
| Kyperonda    | null (c) | 17–18 (i) | d | d |
| Sboula       | null (c) | 7–8 (b) | c   | d   |
| Selbera      | null (c) | 20 (e)  | g   | d   |

Table 5  Number of alleles, Gene diversity and PIC value of HMW-GS and LMW-GS in Moroccan bread and durum wheat cultivars

| Marker | Bread wheat | Durum wheat |
|--------|-------------|-------------|
|        | No. of alleles | Gene diversity | PIC | No. of alleles | Gene diversity | PIC |
| Glu-A1 | 3           | 0.555       | 0.491 | 1           | 0.701       | 0.649 |
| Glu-B1 | 4           | 0.690       | 0.628 | 5           | 0.541       | 0.465 |
| Glu-D1 | 2           | 0.255       | 0.222 | –           | –           | –   |
| Glu-A3 | 5           | 0.660       | 0.611 | 3           | 0.555       | 0.515 |
| Glu-B3 | 6           | 0.754       | 0.717 | 4           | 0.555       | 0.465 |
| Glu-D3 | 2           | 0.095       | 0.090 | –           | –           | –   |
| Mean   | 3.667       | 0.502       | 0.460 | 3.250       | 0.449       | 0.407 |

Allelic variation in bread wheat cultivars

The HMW-GS composition 2* (b), 7/7*–9 (c), 17–18 (i), and 5–10 (d) was the most frequent. Odenbach and Mahgoub (1988) found that the HMW glutenin subunits 2*, 7*–9, and 5*–10 were associated with large sedimentation volumes. Ram (2003) reported also that the combination of Glu-A1b, Glu-B1i, and Glu-D1d alleles exhibited the highest dough strength and can be used as combination to improve bread-making quality. For Glu-A1 locus, the two active types of HMW-GS 1 and 2* were detected at high frequency (85%) which appears to be a better baking quality allele and confers better values for the
quality parameters than allele null (Luo et al. 2001). The same subunit had been previously described by Giraldo et al. (2010) in set of Spanish wheat landraces. Likewise, the same subunit had been found in Argentinean bread wheat (Lerner et al. 2009). However, these results are quite different to those observed in China and French bread wheat, where the allele Glu-A1c (null type) was the most frequent (Yan et al. 2007; Branlard et al. 2003).

For Glu-B1 locus, four alleles were detected. The most frequent alleles were 77*-9 (Glu-B1c) and 17–18 (Glu-B1i). Both alleles have high sedimentation volume, but allele 17–18 (Glu-B1i) has greater effect on sedimentation and mixograph (Carrillo et al. 1990b; Ram 2003). The allele Glu-B1a which affects negatively the dough properties was not detected in our cultivars. Previous studies reported the predominance of allele 7–9 (Glu-B1c) in varieties from US, Argentina and Pakistan (Shan et al. 2007; Lerner et al. 2009; Tabasum et al. 2011). Ma et al. (2003) identified that alleles 17–18 (Glu-B1i) and 7–8 (Glu-B1b) were the major alleles in Australian wheat. In the bread wheat varieties of France and China, allele 7–8 (Glu-B1b) was the most predominant (Yan et al. 2007; Branlard et al. 2003).

At Glu-D1, Payne (1987) proved that allelic variation at Glu-D1 locus had greater effects than other loci on bread-making quality. According to Gupta et al. (1989, 1994), subunit combination 5 + 10 is associated with good bread-making quality, whereas subunit combination 2 + 12 associated with poor bread-making quality. 85% of cultivars studied possessed combination 5 + 10 (Glu-D1d). Similar allelic distribution discovered in Argentinean bread wheat (Lerner et al. 2009). Nevertheless, studies on Spanish, French or Asian bread wheat (Giraldo et al. 2010; Yan et al. 2007; Terasawa et al. 2011) have reported the predominance of 2 + 12.

For LMW-GS, the Glu-3 alleles have been already ranked according to their R_{max} (maximum dough resistance). The Glu-A3 alleles ranked as b > d > e > c, the Glu-B3 alleles ranked as i > b = a > e = f = g = h > c and the Glu-D3 alleles ranked as e > b > a > c > d (Gupta and Shepherd 1988; Gupta et al. 1989, 1990, 1994; Gupta and MacRitchie 1994; Makowsky et al. 1990). In the examined cultivars, the allele Glu-A3c represented 50%, and according to R_{max}, this allele is associated with low dough resistance and ranked poor quality. Lerner et al. (2009) and Shan et al. (2007) found also similar results and predominance of allele c at Glu-A3 locus in Argentinean and US bread wheat cultivars. At Glu-B3, the alleles Glu-B3h and Glu-B3i were the most frequent. The allele Glu-B3i is associated with high gluten strength, while allele Glu-B3h is related to intermediate gluten quality. Comparing the Glu-B3 variation with other studies, our results is totally different to the results of US, Argentinean and French wheat in which the allele g was the most frequent (Shan et al. 2007; Lerner et al. 2009; Giraldo et al. 2010). The allelic variation at the Glu-D3 was limited to the presence of two alleles Glu-D3a and Glu-D3b. The allele Glu-D3b was the major allele in Moroccan bread wheat (95%) and generally reported to be associated with good quality (Lerner et al. 2009). This result is similar to the results of Argentinean and US wheat (Lerner et al. 2009; Shan et al. 2007), but different to those observed in French wheat were the allele Glu-D3c was the predominant.

### Allelic variation in durum wheat cultivars

The null-type gene Glu-A1c related to less extensible or medium elastic dough (Branlard et al. 2003) was the only allele present in the 26 cultivars of durum wheat. The Glu-B1b, Glu-B1e, and Glu-B1d were predominant with 38, 35, and 15%, respectively. Glu-B1b is considered the best allele in relation to quality; Glu-B1d slightly poorer than Glu-B1b and Glu-B1e is considered the poorest (Carrillo et al. 1990a). Like in the bread wheat, the predominant allele at Glu-A3 was allele c with 61% in the durum wheat cultivars of Morocco. At the Glu-B3 locus, the allele Glu-B3d was the most frequent (65%) which had a medium to weak dough properties (Cornish et al. 1993; Luo et al. 2001). For the Glu-A3 and Glu-B3, our results were quite different from the Spanish durum landraces (Aguiriano et al. 2008), in which they reported the predominance of allele a for both locus. Compared to bread wheat, durum wheat was less variable in glutenin alleles.

### Conclusion

The results obtained in this report describing the allelic compositions of Moroccan bread and durum wheat cultivars may have high allelic variability. From this analysis, two points were important. Our results obtained using PCR markers are similar to those reported previously by Bakhella and Branlard (1997) and Zarki et al. (2010) for HMW-GS proteins in which they use SDS-PAGE. Hence, this study proves the efficiency of molecular markers to identify the correct glutenin alleles, in a non-destructive way. In general, Moroccan bread wheat cultivars carried alleles associated to good bread-making quality. However, in durum wheat cultivars, most of the alleles related to low strength dough and need to be improved. Even though many of the durum wheat cultivars and some of the bread wheat cultivars having genes for resistance to the Hessian fly could be used as donors in the breeding program, the glutenin alleles such as Glu-A1c and Glu-B3d should be avoided during selection in the breeding program.
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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest in the publication.

Ethical standards The experiment complies with the ethical standards as per the current laws of Morocco in which it was performed.

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References

Agudriano E, Ruiz M, Fité R, Carrillo JM (2008) Genetic variation for glutenin and gliadins associated with quality in durum wheat (Triticum turgidum L., ssp. turgidum) landraces from Spain. Span J Agric Res 6:599–609. doi:10.5424/sjar/200806-353
Ahmad M (2000) Molecular marker-assisted selection of HMW glutenin alleles related to wheat bread quality by PCR-generated DNA markers. Theor Appl Genet 101:892–896. doi:10.1007/s001229051558
Appelbee M-J (2007) Quality potential of gluten proteins in hexaploid and related wheat species. PhD thesis, University of Adelaide, Australia
Bakhella M, Branlard G (1997) High-molecular-weight glutenin subunits composition of Moroccan durum and common wheat-varieties. Sci des Aliment 17:487–496
Bekes F, Cavanagh CR, Martinov S, Bushuk S, Wrigley CWF (2008a) The gluten composition of wheat varieties and genotypes PART II. Composition table for the HMW subunits of glutenin, 3rd edn. http://www.aaccnet.org/initiatives/definitions/Documents/GlutenFree/II_HMW_Subunits.pdf
Bekes F, Wrigley CWF, Cavanagh CR, Martinov S, Bushuk S (2008b) The Gluten Composition of Wheat Varieties and Genotypes PART III. Composition table for the LMW subunits of glutenin, 3rd edn. AACC International. http://www.aaccnet.org/initiatives/definitions/Documents/GlutenFree/III_LMW_Subunits.pdf. Accessed 26 July 2017
Branlard G, Dardevet M, Amiour N, Igrejas G (2003) Allelic diversity of HMW and LMW subunits and omega gliadins in French bread wheat (Triticum aestivum L.) Genet Resour Crop Evol 50:669–679. doi:10.1023/A:1025077005401
Butow BJ, Gale KR, Ikej J, Juhász A, Bedő Z, Tamás L, Gianibelli MC (2004) Dissemination of the highly expressed Bx7 glutenin subunit (Glu-B1a/b) in wheat as revealed by novel PCR markers and RP-HPLC. Theor Appl Genet 109:1525–1535. doi:10.1007/s00122-004-1776-8
Carrillo JM, Vazquez JF, Orellana J (1990a) Relationship between gluten strength and glutenin proteins in durum wheat cultivars. Plant Breed 104:325–333. doi:10.1111/j.1439-0523.1990.tb00443.x
Carrillo JM, Rousset M, Quislet CO, Kasarda DD (1990b) Use of recombinant inbred lines of wheat for study of associations of high-molecular-weight glutenin subunit alleles to quantitative traits I. Grain yield and quality prediction tests. Theor Appl Genet 79:321–330. doi:10.1007/BF01186074
Cornish GB, Burridge PM, Palmer GA, Wrigley CW (1993) Mapping the origins of some HMW and LMW glutenin subunit alleles in Australian wheat germplasm. In: Wrigley CW (ed) Proceedings of 43rd Australian Cereal Chemistry Conference, Royal Australian Chemical Institute, Melbourne, Australia, pp 255–260
Cornish GB, Békes F, Allen HM, Martin JM (2001) Flour proteins linked to quality traits in an Australian doubled haploid wheat population. Aust J Agric Res 52:1339–1348. doi:10.1071/AR01060
De Bustos A, Rubio P, Jouve N (2000) Molecular characterization of the inactive allele of the gene Glu-A1 and the development of a set of AS-PCR markers for HMW glutenins of wheat. Theor Appl Genet 100:1085–1094. doi:10.1007/s001220051390
Eagles HA, Hollambay GJ, Gororo NN, Eastwood RF (2002) Estimation and utilisation of glutenin gene effects from the analysis of unbalanced data from wheat breeding programs. Aust J Agric Res 53:367–377. doi:10.1071/AR01074
Eagles HA, Cane K, Eastwood RF, Hollambay GJ, Kuchel H, Martin PJ, Cornish GB (2006) Contributions of glutenin and puroindoline genes to grain quality traits in southern Australian wheat breeding programs. Aust J Agric Res 57:179–186. doi:10.1071/AR05242
Gianibelli MC, Gupta RB, Lafiandra D, Margiotta B, MacRitchie F (2001) Polymorphism of high Mr gluten subunits in Triticum tauschii: characterization by chromatography and electrophoresis methods. J Cereal Sci 33:39–52
Giraldo P, Rodriguez-Quijano M, Simon C, Vazquez JF, Carrillo JM (2010) Allelic variation in HMW glutenins in Spanish wheat landraces and their relationship with bread quality. Span J Agric Res 8:1012–1023. doi:10.5424/sjar/201008-1394
Gupta RB, MacRitchie F (1994) Allelic variation at glutenin subunit and gliadin loci, Glu-1, Glu-3 and Gl-1 of common wheats. II. Biochemical basis of the allelic effects on dough properties. J Cereal Sci 19:19–29. doi:10.1016/j.cresc.1994.1004
Gupta RB, Shepherd KW (1988) Low-molecular weight glutenin subunits in wheat: their variation inheritance and association with bread-making quality. In: Miller TE, Koebner RMD (ed) Proceedings of the 7th International Wheat Genetics Symposium, Institute of Plant Science Research, Cambridge, pp 943–949
Gupta RB, Shepherd KW (1990) Two-step one-dimensional SDS-PAGE analysis of LMW subunits of glutenin. I. Variation and genetic control of the subunits in hexaploid wheats. Theor Appl Genet 80:65–74. doi:10.1007/BF00224384
Gupta RB, Singh NK, Shepherd KW (1989) The cumulative effect of allelic variation in LMW and HMW glutenin subunits on dough properties in the progeny of two bread wheats. Theor Appl Genet 77:57–64. doi:10.1007/BF00292316
Gupta RB, Bekes F, Wrigley CW, Moss HJ (1990) Prediction of wheat quality in breeding on the basis of LMW and HMW glutenin subunit composition. In: O’Brien L, Ellison FW, Hare RA, Mackay MC (eds) Proceedings of the 6th Wheat Breeding Society of Australia (Inc), Tamworth, Australia, pp 217–225
Gupta RB, Bekes F, Wrigley CW (1991) Prediction of physical dough properties from glutenin subunit composition in bread wheats: correlation studies. Cereal Chem 68:328–333
Gupta RB, Paul JG, Cornish GB, Palmer GA, Bakes F, Rathjen AJ (1994) Allelic variation at glutenin subunit and gliadin loci, Glu-1, Glu-3 and Gl-1 of common wheats. I. Its additive and interaction effects on dough properties. J Cereal Sci 19:9–17. doi:10.1016/j.cresc.1994.1003
He ZH, Liu L, Xia XC, Liu JJ, Pena RJ (2005) Composition of HMW and LMW glutenin subunits and their eVacs on dough
properties, pan bread, and noodle quality of Chinese bread wheats. Cereal Chem 82:345–350. doi:10.1094/CC-82-0345

Henfr K, El-Haddoumy J, Ouabhou H, Nsarellah N, Iraqi D, Bendaul N, Udupa SM (2015a) Genetic diversity and its temporal changes in improved bread wheat cultivars of Morocco. Rom Agric Res 32:19–25. http://www.inca-fundulea.ro/rar/nr32/rar32.3.pdf. Accessed on 27 July 2017

Henfr K, El-Haddoumy J, Ouabhou H, Nsarellah N, Iraqi D, Bendaul N, Udupa SM (2015b) Genetic diversity reduction in improved durum wheat cultivars of Morocco as revealed by microsatellite markers. Sci Agric 73:134–141. doi:10.1590/0103-9016-2015-0054

Odenbach W, Mahgoub El-S (1988) Relationships between HMW glutenin subunit composition and the sedimentation value in reciprocal sets of inbred backcross lines derived from two winter wheat crosses. In: Proceedings of 7th International Wheat Genetics Symposium, Cambridge, England, pp 987–991

Payne PI (1987) Genetics of wheat storage proteins and the effects of allelic variation on breadmaking quality. Ann Rev Plant Physiol 38:141–155. doi:10.1146/annurev.pp.38.060187.001041

Ma W, Zhang W, Gale KR (2003) Multiplex-PCR typing of high molecular weight glutenin alleles in wheat. Euphytica 134:51–60. doi:10.1023/A:1026191987804

Variation for glutenin and waxy alleles in the US hard winter wheat germplasm. J Cereal Sci 37:129–137. doi:10.1016/j.jcs.2006.09.007

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphisms in barley: mendelian inheritance, chromosomal location, and population dynamics. Proc Nat Acad Sci 81(24):8014–8018

Tabasum A, Iqbal N, Hameed A, Arshad R (2011) Evaluation of Pakistani wheat germplasm for bread quality based on allelic variation in hmw glutenin subunits. Pak J Bot 43:1735–1740

Shewry PR, Halford NG, Tatham AS (1992) High molecular weight subunits of wheat glutenin. J Cereal Sci 15:105–120. doi:10.1016/S0733-5210(09)80062-5

Tabasum A, Iqbal N, Hameed A, Arshad R (2011) Evaluation of Pakistani wheat germplasm for bread quality based on allelic variation in hmw glutenin subunits. Pak J Bot 43:1735–1740

Shewry PR, Halford NG, Tatham AS (1992) High molecular weight subunits of wheat glutenin. J Cereal Sci 15:105–120. doi:10.1016/S0733-5210(09)80062-5

Terasawa Y, Clayshulte SR, Haley SD, Byrne PF (2003) Variation for glutenin and waxy alleles in the US hard winter wheat germplasm. J Cereal Sci 45:199–208. doi:10.1016/j.jcs.2006.09.007

Terasawa Y, Takata K, Hirano H, Kato K, Kawahara T, SasaTaka T, SasaTama T (2011) Genetic variation of high-molecular-weight glutenin subunit composition in Asian wheat. Genet Resour Crop Evol 58:283–289. doi:10.1007/s10722-010-9573-5

Uduma SM, Robertson LD, Weigand F, Baum M, Kahl G (1999) Allelic variation at the vfaA microsatellite loci in a World Collection of Chickpea (Cicer arietinum L.) Germplasm. Mol Gen Genet 261:354–363. doi:10.1007/s004380050976

Veraverbeke WS, Delcour JA (2002) Wheat protein composition and glutenin and waxy alleles in the US hard winter wheat germplasm. J Cereal Sci 45:199–208. doi:10.1016/j.jcs.2006.09.007

Luo C, Griffin WB, Bramlard G, McNeil DL (2001) Comparison of low- and high-molecular-weight wheat glutenin allele effects on flour quality. Theor Appl Genet 102:1088–1098. doi:10.1007/s001220000439

Ma W, Zhang W, Gale KR (2003) Multiplex-PCR typing of high molecular weight glutenin alleles in wheat. Euphytica 134:51–60. doi:10.1023/A:1026191987804

Ma W, Zhang W, Gale KR (2003) Multiplex-PCR typing of high molecular weight glutenin alleles in wheat. Euphytica 134:51–60. doi:10.1023/A:1026191987804

Metakovský EV, Wrigley CW, Bekes F, Gupta RB (1990) Gluten polypeptides as useful genetic markers of dough quality in Australian wheats. Aust J Agric Res 41:289–306. doi:10.1071/AR9900289

Zarki K, Ouabhou H, Hilali A, Udupa SM (2010) Detection of genetic diversity in Moroccan durum wheat accessions using agro-morphological traits and microsatellite markers. Afr J Agric Res 5:1837–1844. doi:10.5977/AJR090249

Zhang W, Gianibelli MC, Rampling L, Gale KR (2004) Characterisation and marker development for low molecular weight glutenin genes from Glu-A3 alleles of bread wheat (Triticum

aestivum L.). Theor Appl Genet 108:1409–1419. doi: 10.1007/s00122-003-1558-8

Zhao XL, Ma W, Gale KR, Lei ZS, He ZH, Sun QX, Xia XC (2007a) Identification of SNPs and development functional markers for LMW-GS genes at Glu-D3 and Glu-B3 loci in bread wheat (Triticum aestivum L.). Mol Breed 20:223–231. doi: 10.1007/s11032-007-9085-y

Zhao XL, Xia XC, He ZH, Lei ZS, Appels R, Yang Y, Sun QX, Ma W (2007b) Novel DNA variations to characterize low molecular weight glutenin Glu-D3 genes and develop STS markers in common wheat. Theor Appl Genet 114:451–460. doi: 10.1007/s00122-006-0445-5