

Comparison of genetic diversity at prolamin loci in Moroccan durum wheat accessions

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Abstract

Morocco is characterized by its tremendous diversity at all levels for various species, including several crop species, such as durum wheat (*Triticum turgidum* L. var. *durum*). The precise identification and characterization of the accessions conserved in the National Genebank of Morocco is of great value for quantifying the extent of genetic diversity within accessions, detecting duplications of genetic materials in germplasm collection, improving and securing durum wheat cultivars in Morocco and in the world. The objective of the present study was to characterize a subset of 23 Moroccan durum wheat accessions held in the genebank for their variability related to quality properties using protein markers of prolamin loci (gliadin and glutenin). The allelic variation at prolamin proteins were studied using polyacrylamide gel electrophoresis namely, A-PAGE and SDS-PAGE. The prolamin differences in the durum accessions were analyzed at the Glu-A1, Glu-A2, Glu-A3/Glu-B3/Glu-B2, and Gli-B1 loci. For the HMW-GS, all the durum wheat accessions studied possess the null subunit at Glu-A1, except for the accession number 4 which possesses the HMW-GS1. At the Glu-B1 alleles, results showed that the majority of accessions possess subunits 20x + 20y or subunit 20; accessions 2, 4, and 19 possess subunits 6 + 8; accession 18 possesses subunit 7 and accessions 6, 7, and 22 possess subunits 7 + 8. The electrophoretic data indicated that the evaluated germplasm encompasses useful variations at prolamin loci. Further investigations are in progress to study the genetic variations in Moroccan durum wheat collection using molecular markers.

Keywords: A-PAGE; Genetic diversity; Gliadin and glutenin genes; SDS-PAGE; *Triticum turgidum* L. var. *durum*

Abbreviations: A-PAGE: acidic-polyacrylamide gel electrophoresis; HMW-GS: high molecular weight-glutenin subunits; LMW-GS: low molecular weight-glutenin subunits; SDS-PAGE: sodium dodecyl sulphate-polyacrylamide gel electrophoresis; SDSS: sodium dodecyl sulphate sedimentation; UPGMA: Unweighted Pair Group Method of Arithmetic Average.

Introduction

Durum wheat (*Triticum turgidum* L. var. *durum*) is an important species of the tribe Triticeae, and is one of the most important food crops in the Mediterranean region, because of its adaptation to semi-arid environments and its unique end products like pasta, couscous, burghul, traditional pan, and others artisanal products. It is an allotetraploid (genome AABB, with a basic chromosome number of $x = 7$ and $2n = 4x = 28$). In Morocco, durum wheat is an economically and nutritionally important cereal crop and ranks third after barley and bread wheat (Belaid et al. 2003). In the mountain and oasis regions of Morocco, durum wheat landraces are still widely grown by farmers. The local durum wheat is highly appreciated by farmers for their adaptation to abiotic stresses and for their good grain and straw quality. Therefore, the landraces represent a promising gene pool for developing new genotypes with good bread making and pasta quality. In wheat, prolamins, class of seed storage polypeptides rich in proline and glutamine amino acids are divided in two groups: gliadins and glutenins, and represent the most important proteins used for assessing genetic diversity and for genotype identification in different species (Nevo and Payne, 1987; Lafiandra et al., 1990; Metakovsky et al., 2000; Ruiz et al.,

2002). According to their electrophoretic mobility, glutenins are subdivided into high molecular weight-glutenin subunits (HMW-GS) and low molecular weight-glutenin subunits (LMW-GS). HMW-GS and LMW-GS form giant protein polymers with a range of different sizes that could reach up to 10 million Daltons (Wrigley 1996). Gliadins are subdivided into α -, β -, γ - and ω -gliadins. In durum wheat, a highly significant correlation has been detected between specific allelic variation of prolamins and gluten strength. It has been reported that cultivars containing γ -45, ω -35 gliadins and LMW-GS pattern-2 possess good durum wheat gluten elasticity and superior pasta cooking quality as compared with those containing its allelic variant γ -42, ω -33, 35 and 38 gliadins and LMW-GS pattern-1 (Damidaux et al., 1978; Payne et al., 1984). Dough strength and baking quality is increased in durum wheat in genotypes expressing some specific HMW-GS. The HMW-GS are encoded by polymorphic genes at the *Glu-1* loci present on the long arms of the group 1 chromosomes (1A and 1B) (Payne and Lawrence, 1983), whereas the most of LMW-GS are encoded by *Glu-3* loci, which are tightly linked to *Gli-1* loci, located on the short arms of group 1 chromosomes (Jackson et al., 1983;

Table 1. Accession number and origin of the durum wheat accessions used in the study

Serial number	Accession number	Origin
1	MGB 6037	Kalaa Sraghna, Morocco
2	MGB 9313	Azilal, Morocco
3	MGB 5968	Morocco
4	MGB 6113	Morocco
5	'Kyperounda'	Morocco
6	MGB 565	Unknown
7	'Oum rabie'	Morocco
8	MGB 5998	Morocco
9	MGB 6066	Azilal, Morocco
10	MGB 33	Morocco
11	MGB 9377	Azilal, Morocco
12	MGB 3281	Morocco
13	MGB 49	Morocco
14	MGB 44429	Kalaa Sraghna, Morocco
15	MGB 3194	Marrakech, Morocco
16	MGB 3047	Morocco
17	MGB 3158	Khenifra, Morocco
18	MGB 44430	Morocco
19	MGB 24	Morocco
20	MGB 9323	Azilal, Morocco
21	MGB 6119	Taza, Morocco
22	MGB 3175	Tetouan, Morocco
23	MGB 6042	Figuig, Morocco

Singh and Shepherd, 1988). Recently a new complex locus for LMW-GS has been reported (Ruiz and Carrillo, 1993; Liu, 1995). Genes encoding gliadin components are located on the short arm of chromosomes of the homoeologous groups 1 (Gli-1 loci) and 6 (Gli-2 loci) of A and B genomes (Joppa et al., 1983). The most durum wheat cultivars could contain 1 to 3 different HMW-GS (3 to 5 subunits in bread wheat) (Waines and Payne, 1987). HMW-GS are used in wheat breeding for selecting the alleles that correlate with quality. Bread-making quality of common wheat flour depends on the number and composition of the HMW-GS, specially, the proportion of subunit Glu-1Ax1 (Payne 1987a and b; MacRitchie et al., 1990). Also dough strength and baking quality is increased in durum wheat genotypes expressing HMW-GS 6+8 and 7+8 compared to those expressing HMW-GS 20 (Ammar et al., 2000). Distinction between durum wheat cultivars possessing LMW-2 or LMW-1 glutenin and type of the polymeric glutenin fraction of HMW is currently scored by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Distinction between genotypes containing allelic variant: γ -45, ω -35 gliadins or allelic variant: γ -42, ω -33, 35 and 38 gliadins is scored by acidic-polyacrylamide-gel electrophoresis (A-PAGE) (Gupta and Shepherd, 1990; Bean and Lookhart, 2000; Kharabian et al., 2008). Knowledge of the genetic variability related to quality properties of local durum wheat landraces and modern cultivars using prolamins markers has a significant impact on the improvement of this crop. This information can assist in fingerprinting and genotype selection of durum wheat accessions with desirable genetic combinations. In this study, we evaluated a set of 23 Moroccan durum wheat accessions, for their diversity related to quality properties using allelic variation in both glutenin and gliadin proteins.

Materials and methods

Plant material

The 23 accessions of durum wheat (*T. turgidum* L. var. *durum*) including two varieties (as controls), 'Kyperounda' (released during 1956), and 'Oum Rabie' (released during 1988) were used in this study. The details of these accessions are presented in Table 1. All the durum wheat accessions analyzed in this work was obtained from the National Genebank of Morocco, Centre Régional de la Recherche Agronomique de Settat, INRA, Settat, Morocco.

Biochemical characterization

The 23 durum wheat accessions were examined for their prolamins composition. Glutenins and gliadins were extracted from at least five randomly single crushed seed for each of the accession. Using the sequential extraction procedure of protein of whole grain flour, following combined protocols of Singh et al. (1991), Pflugger et al. (2001), and Martinez et al. (2004) with minor modifications was applied. Gliadins were extracted from single crushed seed with 1.5 M dimethylformamide (DMF) for 1 hour at room temperature with occasional vortexing, followed by centrifugation for 10 min.

The supernatant containing mainly gliadins was retained and fractionated at pH 3.1 in aluminium lactate buffer by A-PAGE with low catalyst levels (ferrous sulphate and hydrogen peroxide) according to Khan et al. (1985). The residue was then washed twice with 50% 2-propanol to remove any remaining soluble proteins; and glutenins were extracted in an extraction buffer (0.08M Tris-HCl containing 50% 2-propanol, pH 8.5).

After denaturation, glutenin subunits were fractionated by electrophoresis in vertical SDS-PAGE slabs in a discontinuous system. The stacking and separating gels were with 5% and 10% polyacrylamide concentration (w/v) respectively. The Tris-HCl/glycine buffer system of Laemmli (1970) was used. Electrophoresis was performed at a constant current of 30 mA/gel at 18°C for 45 min after the tracking dye migrated off the gel. All the gels were stained overnight with 12% (w/v) trichloroacetic acid solution containing 5% (v/v) ethanol and 0.05% (w/v) Coomassie Brilliant BlueR-250. De-staining was carried out with tap water. The subunits of the controls cultivars 'Kyperounda' and 'Oumrabie' were used as standards. The alleles for high molecular weight and low molecular weight glutenin subunits were named according to Payne and Lawrence (1983) and Nieto-Taladriz et al. (1997), respectively. The gliadin blocks were designated according to Pogna et al. (1990).

Data analysis

Allelic variation at each locus was considered as source of variation. Bands for HMW-GS, LMW-GS glutenin, and γ -45, ω -35 gliadins or γ -42, ω -33, 35 and 38 gliadins were studied. Bands for HMW-GS were scored for the presence (1) or absence (0) for each of 23 samples. The binary data matrix was used to calculate the genetic distance for each pair of accessions using the software PowerMarker version 3.25 (Liu and Muse, 2005). The genetic distance (shared index method) was used for cluster analyses (Unweighted Pair Group Method of Arithmetic Average; UPGMA) and a dendrogram was generated using PowerMarker program.

Table 2. Composition of prolamins in the 23 durum wheat accessions used in the study

Locus	Glutenin			Gliadin
	High molecular weight subunits		Low molecular weight subunits	
	<i>Glu A1</i>	<i>Glu B1</i>	<i>Glu-A3/Glu B3/ Glu B2</i>	
1	Null	20 x + 20 y	LMW-2	ω 35 γ 45
2	Null	6 + 8	LMW-2	ω 35 γ 45
3	Null	20	LMW-2	ω 35 γ 45
4	1	6 + 8	LMW-2	ω 35 γ 45
5	Null	20x + 20y	LMW-2	ω 35 γ 45
6	Null	7 + 8	LMW-2	ω 39 γ 47
7	Null	7 + 8	LMW-1	ω 33-35-38 γ 42
8	Null	20x + 20y	LMW-2	ω 35 γ 45
9	Null	20	LMW-2	ω 35 γ 45
10	Null	20 + 9	LMW-2	ω 35 γ 45
11	Null	20	LMW-2	ω 35 γ 45
12	Null	20	LMW-2	ω 35 γ 45
13	Null	20x + 20y	LMW-2	ω 35 γ 45
14	Null	20	LMW-2	ω 35 γ 45
15	Null	20	LMW-2	ω 35 γ 45
16	Null	20x + 20y	LMW-2	ω 35 γ 45
17	Null	20	LMW-2	ω 35 γ 45
18	Null	7	LMW-2	ω 35 γ 45
19	Null	6 + 8	LMW-2	ω 35 γ 45
20	Null	20	LMW-2	ω 35 γ 45
21	Null	20	LMW-2	ω 35 γ 45
22	Null	7 + 8	LMW-2	ω 35 γ 45
23	Null	20x + 20y	LMW-2	ω 35 γ 45

Results and discussion

The electrophoretic separation of glutenins and gliadins components of the durum wheat cultivars ('Kyperounda' and 'Oumrabie') used as controls is presented in Fig. 1. The cultivar 'Kyperounda' has HMW-GS of 20x + 20y, and LMW-GS of type 2 (LMW2); and the cultivar 'Oumrabie' has HMW-GS of 7 + 8, and LMW-GS of type1 (LMW1). The inheritance of the glutenins and gliadins genes had been determined in previous studies (Joppa et al., 1983; Ruiz and Carrillo, 1993; Nieto-Taladriz et al., 1997; Martinez et al., 2004) and these studies showed that prolamin alleles have effects on the quality properties. The allelic composition of glutenins and gliadins subunits for each accession is given in the Table 2. The prolamins differences in durum accessions analyzed were at Glu-A1, Glu-A2, Glu-A3/Glu-B3/Glu-B2, and Gli-B1 loci. For the HMW-GS, the results of this work showed that all the durum wheat accessions studied possess the null subunit at Glu-A1, except for the accession number 4 which possesses the HMW-GS 1. At Glu-B1, results showed that the majority of accessions (1, 3, 5, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 20, 21, and 23) possess subunits 20x + 20y or subunit 20; accessions 2, 4, and 19 possess subunits 6 + 8; accession 18 possesses subunit 7 and accessions 6, 7, and 22 possess subunits 7 + 8. Based on bands profiles for HMW-GS, a dendrogram was generated and allowed classification of the accessions into 3 groups (Fig. 2). The group A regroups the accessions with subunits 20 (subgroup A1) and the accessions with subunits 20x + 20y (subgroup A2). The group B contains accessions with subunits 6 + 8, whereas group C regroups genotypes with subunits 7 or 7 + 8. Concerning LMW-GS coded at Glu-A3, Glu-B3 and Glu-B2 loci, all accessions possess LMW2, except the cultivars 'Oumrabie' which possesses LMW1. At gliadins loci, all the accessions possess ω 35- γ 45 encoded at Gli-B1, except for the accession 6 which had ω 39- γ 47, and the cultivars 'Oumrabie' which possesses ω 33-35-38 γ 42. In this

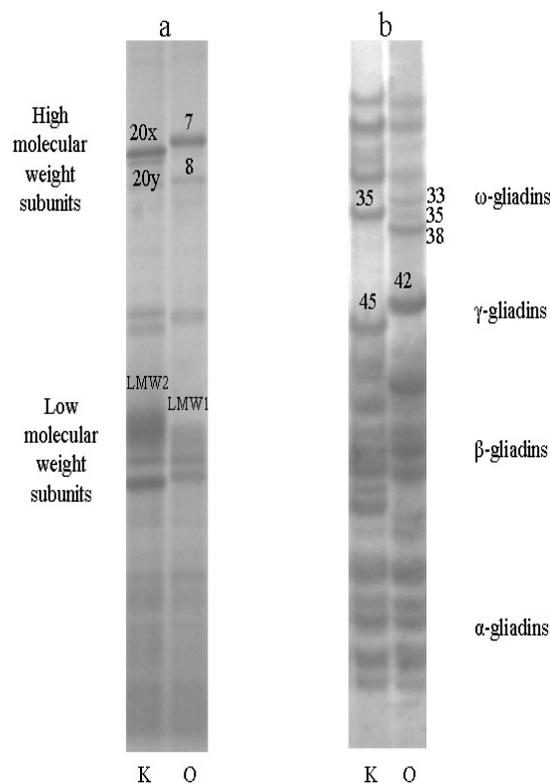


Fig1. SDS-PAGE (a) and A-PAGE (b) separation of the glutenins and gliadins in the cultivars 'Kyperounda' (K) and 'Oumrabie' (O) used as controls. The different prolamin components are indicated along the sides of the gel. The subunits studied are numbered.

sample of durum wheat accessions, Glu-B1 was the most variable among all Glu-1 loci, and the subunit 20 was observed most frequently.

SDS and A-PAGE analyses of prolamins in the durum wheat accessions analyzed indicated that all the genotypes with $\gamma 45$ gliadin component possess low-molecular weight glutenin subunits LMW2, related with good durum wheat gluten elasticity and superior pasta cooking quality (Payne et al., 1984), whereas low molecular weight glutenin subunits LMW1, related with poor durum wheat gluten elasticity and inferior pasta cooking quality (Payne et al., 1984), are present only in genotype with $\gamma 42$ gliadin component. These observations confirm the absence of an intralocus recombination at the Gli-B1 locus in the accessions studied. More recently, studies on the effects of different prolamins alleles on durum wheat quality properties evaluated by the SDS sedimentation, mixograph, micro-alveograph and vitreousness tests and by protein content (Brites and Carrillo, 2001; Martinez et al., 2005) revealed positive effects of the HMW-GS subunit 1 on gluten quality. At the Glu-B1 alleles, these studies indicated that the subunits 6 + 8 were associated with higher SDS sedimentation values than 7 + 8, and 20x + 20y subunits; and the differences between subunits 6 + 8 and 7 + 8 were small, whereas the 20x + 20y subunits had a clear negative influence on gluten quality. Ammar et al. (2000), Shewry et al. (2003), and Sapirstein et al. (2007) reported that the durum wheat genotypes carrying HMW-GS 20 exhibited generally weaker dough properties and inferior baking quality than those expressing HMW-GS 6+8 and 7+8. In our results the composition of HMW-GS showed that the most frequent allele in Glu-A1 is the null allele, and in Glu-B1 are the band 20, bands 6 + 8 and 7 + 8 are presented in the same frequency. The prevalence of LMW2 and $\omega 35$ - $\gamma 45$ gliadins in the accessions studied may reflect past selection pressure exerted on Moroccan durum wheat landraces to meet the demand for good traditional products such as couscous. Also, in Morocco, durum wheat is being used in the production of bread and several types of artisanal products, and suggests that Moroccan durum wheat landraces could be a potential source in the development of durum wheat cultivars suitable for bread, pasta and couscous production.

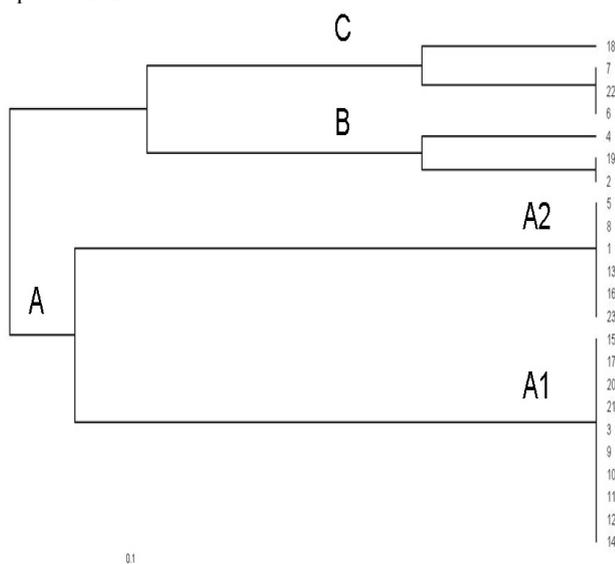


Fig 2. The UPGMA dendrogram of 23 durum wheat accessions based on HMW-GS. A, B and C represent as cluster ranking groups of the accessions; and the numbers at the clusters represent serial numbers of the accession (see Table 1).

Conclusion

Our results showed that the evaluated durum wheat accessions comprised of useful intra-specific polymorphism at prolamins alleles and this polymorphism is maintained by sustainable cultivation of local landraces in different geographical regions of Morocco. The local landraces may form an interesting source of positive quality features in the development of durum wheat genotypes for bread, couscous and pasta-making quality properties.

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