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RESEARCH ARTICLE

Genetic variability of high molecular weight glutenin subunits in bread wheat from continental Portugal, Madeira and Canary Islands

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Abstract The genetic variability of high molecular weight glutenin subunits (HMWGS) composition at the *Glu-1* loci in bread wheat (*Triticum aestivum* L.) was studied electrophoretically using the SDS–PAGE in 3,470 individuals representing 159 populations originated from the Canary Islands (Spain), the Archipelago of Madeira (Portugal) and the continental Portugal. A total of 25 alleles were detected, resulting in 69 different allele combinations. The geographical distribution of the high molecular weight glutenin alleles confirms historical data regarding circulation of wheat germplasm between the Iberian Peninsula and Madeira and between Madeira and the Canary Islands and vice versa.

Keywords Canary Islands · Genetic variability · High molecular weight glutenin subunits · Madeira Island · Portugal

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Introduction

Wheat was introduced to the Atlantic Archipelagos of Madeira and Canary five hundred years ago. Madeira was colonised first in the beginning of the fifteenth century. The Portuguese settlers brought wheat that was cultivated on the island to provide food for steadily growing local population. During the first six decades Madeira farmers produced high volumes of the crop and the surplus of wheat grain was exported to the Azores, Canary and Maghreb and thus was an important resource for the Portuguese explorations along the West Coast of Africa (Vieira 1988). From the seventies of the fifteenth century, a great portion of the arable land of Madeira was converted to production of new lucrative crops including sugar cane and grapes, while wheat plots were relegated to the less fertile land. As a consequence, wheat yields drastically declined and crop production was insufficient to feed the Madeira population. This situation imposed an urgent need to increase area of land devoted to wheat cultivation. Therefore, new production areas were developed on the neighbouring Archipelagos of Canary and Azores (Vieira 1984; Freitas de Meneses 1995). The commercial flow of wheat seeds among the Azores, Canary and Madeira Archipelagos over the course of several centuries resulted in the accumulation of high wheat diversity on these islands (dos Santos et al. 2009).

Seed storage proteins are a result of genome expression and contain extensive genetic variation in wheat landraces (Chaparzadeh et al. 2008; Terasawa et al. 2009). Therefore, they are considered to be good criteria for genetic diversity studies (Porceddu et al. 1998). The high molecular weight glutenin subunits (HMWGS) are storage proteins synthesized in the seeds of wheat and related species (Lawrence and Shepherd 1981; Shewry et al. 1995). The HMWGS are encoded by the *Glu-1* loci located on the long arms of the homoelogous group one chromosomes, with each locus comprising two linked genes encoding x- and y-types subunits (Lawrence and Shepherd 1981, Payne 1987, Wan et al. 2005). Consequently, three loci (*Glu-A1*, *GluB1* and *Glu-D1*) encoding six HMWGS are present in hexaploid wheat (*Triticum aestivum*, 2n=6x=42, AABBDD), which originated 10,000 years ago from natural hybridization between tetraploid wheat (2n = 4x = 28, AABB) and diploid *Ae. tauschii* (2n = 2x = 14, DD) (Feldman et al. 1995; Feldman 2000). The subunits differ in the molecular weight (higher for x-type), number of cysteine residues, and repetitive motifs. Silencing of specific genes results in variation in the number of expressed subunits from three to five, while allelic variation in subunits encoded by the expressed genes leads to polymorphism of x-type and y-type subunits, which can be separated by the SDS–PAGE (Payne and Lawrence 1983; Payne et al. 1987; Vallega 1988; Feng et al. 2004). The HMWGS have been successfully used to assess the genetic diversity of wheat germplasm from different countries (Lawrence 1986; Nevo and Payne 1987; Lagudah et al. 1987; Morgunov et al. 1990; Caballero et al. 2001; Branlard et al. 2003; Hua et al. 2005; Tohver 2007; Shan et al. 2007; Li et al. 2009).

The main goal of the present study was to analyze the HMWGS composition of a collection of wheat accessions collected from the continental Portugal, Madeira and the Canary Islands.

Materials and methods

Plant material

A total of 159 *Triticum aestivum* L. accessions were used in this study (Table 1), among them 22 accessions originated from the continental Portugal (the Vasconcellos (1933) collection), 63 from Madeira and 74 from the Canary Islands. The seeds were provided by the ISOPlexis Gene Bank at the University of Madeira, Centro de Biodiversidad de Tenerife, Banco de Germoplasma de España, Cabildo de La Palma and Maria Teresa Carvalho e Vasconcelos, Instituto Superior de Agronomia (ISA). The standard wheat cultivars (Gabo, Marquis, Payne, Atlas 66, Federation, Florida 301, Cheyenne, Florence, David, Forlani, Champlein, Atlas 50, Chinese Spring) were kindly provided by Dr. Harold Bockelaman from the National Small Grains Collection, USDA-ARS.

SDS–PAGE analysis

Proteins were extracted from individually crushed endosperm seeds wholemeal. Samples (20 mg) were extracted once with 1 ml dimethyl sulfoxide (DMSO) and twice with 1 ml 50% propan-1-ol to remove gliadin, albumin and globulin proteins (mixing each time on a vortex mixer for 10 s and centrifuging 10 min at 16,000 *g*). The full range of glutenin subunits was then extracted at 65°C for 30 min with 200 ml of 1% SDS solution containing 1% dithiothreitol (DTT) followed by centrifugation for 10 min (Uthayakumaran et al. 2006).

Proteins were fractionated by electrophoresis in a vertical SDS–PAGE gel in a discontinuous Tris–HCl–SDS buffer system (pH 6.8/8.8) following the ISTA Standard Method for the SDS–PAGE (Cooke 1992). The gels were stained with Coomassie blue and visually analysed for allele identification and compared with the patterns of known genotypes (standards). The nomenclature of the HMWGS was followed as proposed by Payne and Lawrence (1983).

Data analysis

The following genetic variability parameters were calculated for all the populations using the POPGENE 1.32 program (Yeh and Boyle 1997): observed number of alleles per locus (na), effective number of alleles

Table 1 Plant material and allelic composition of GLU-1 alleles

Accession number	Common name	Origin	Alleles
2943	Egípcio	Mainland (Portugal)	b; d; g
3100	Tremês branco	Mainland (Portugal)	b; d; a
3085	Rieti	Mainland (Portugal)	a; b; d
3526	Mocho de esp. Branca	Mainland (Portugal)	b; f; g
17712	Serrano	Mainland (Portugal)	a; d; a
2936	Mestiço	Mainland (Portugal)	a/b; e/g; a
3643	Precoce	Mainland (Portugal)	b; d/f/g; a/d
2984	Funchal	Mainland (Portugal)	b; a; a/h
7940	Ribeiro	Mainland (Portugal)	b; d; g
7938	Almadense	Mainland (Portugal)	a; an; a
7501	Saloio	Mainland (Portugal)	a/b; an/g; a
3136	Transmontano	Mainland (Portugal)	b; b; a
3172	Ideal	Mainland (Portugal)	b; a/ag; g
3077	Mirandês	Mainland (Portugal)	b; a; h*
3153	Alentejano	Mainland (Portugal)	b; d; a
2977	Ardito	Mainland (Portugal)	b; aj; a
3844	Galego rapado	Mainland (Portugal)	b; g; a
3094	Santareno	Mainland (Portugal)	b; f; g
3081	Temporão de Coruche	Mainland (Portugal)	b; a; a
3188	Tremês ruivo	Mainland (Portugal)	b; e; a
3067	Mocho ou rapado	Mainland (Portugal)	b; f; d
3105	Grécia	Mainland (Portugal)	b; g; c
ISOP00072	T. Branco	Madeira (Portugal)	b; a/b/f; a
ISOP00073	T. Galhoto	Madeira (Portugal)	b/c; f/h; a
ISOP00074	T. Raposo	Madeira (Portugal)	b/c; a/c/f; a
ISOP00076	T. Serra	Madeira (Portugal)	b; d/f/h/ae; a/g
ISOP00077	T. Vermelho	Madeira (Portugal)	b/c; b/f/ae; a
ISOP00079	T. Cabeiro	Madeira (Portugal)	b/c; a/b/f; a
ISOP00080	T. Preto	Madeira (Portugal)	c; a/b/f/g/e; a/b/d
ISOP00083	T. Barbela	Madeira (Portugal)	b/c; f; a
ISOP00084	T. Pardo	Madeira (Portugal)	b/c; b/f/u; a
ISOP00085	T. Leacock	Madeira (Portugal)	b/c; f; a
ISOP00087	T. Leacock	Madeira (Portugal)	b/c; c/f; a/g
ISOP00089	T. Raposo	Madeira (Portugal)	b/c; a/e/f/g; a
ISOP00092	T. Branco	Madeira (Portugal)	b/c; b/f; a
ISOP00093	T. Canalha	Madeira (Portugal)	b/c; a/b/f; a
ISOP00094	T. Douradinho	Madeira (Portugal)	b; a/f; a/g
ISOP00097	T. Sem Pragana	Madeira (Portugal)	b/c; b/d/f/g/u; a/b/d/g
ISOP00098	T. Peladinho	Madeira (Portugal)	b/c; c; a/b/d/g
ISOP00100	T. Mouro	Madeira (Portugal)	b; u; a
ISOP00101	T. do Mato	Madeira (Portugal)	b; f; a
ISOP00102	T. Branco	Madeira (Portugal)	b; b/c/f; a
ISOP00103	T. Cana Roxa	Madeira (Portugal)	b; a/f; a
ISOP00105	T. Branco	Madeira (Portugal)	b/c; f; a/g

Table 1 continued

Accession number	Common name	Origin	Alleles
ISOP00107	T. Temporão	Madeira (Portugal)	b/c; b; a
ISOP00108	T. Arroz	Madeira (Portugal)	b; f; a
ISOP00110	T. Rapadinho	Madeira (Portugal)	b/c; a/f; a
ISOP00111	T. Galhoto	Madeira (Portugal)	b/c; f; a/g
ISOP00112	T. Doiradinho	Madeira (Portugal)	b; f/g; a
ISOP00113	T. do Porto Santo	Madeira (Portugal)	b; b/f; a
ISOP00114	T. Rapado (Branco)	Madeira (Portugal)	a/b/c; c/e/f; a/b/d/g
ISOP00115	T. Rapadinho	Madeira (Portugal)	a/b/c; b/c/f/ag; a/b/g
ISOP00116	T. Rapado	Madeira (Portugal)	b/c; b/g/c; a/g
ISOP00117	T. Rapado	Madeira (Portugal)	a/b/c; b/c; a/d
ISOP00118	T. Rapadinho	Madeira (Portugal)	b; a/f; a
ISOP00119	T. Raposinho	Madeira (Portugal)	b; f/g; a
ISOP00120	T. Raposo	Madeira (Portugal)	b; f/g; a
ISOP00123	T. Maçaroquinho	Madeira (Portugal)	b/c; b/u; a
ISOP00238	T. do Cedo	Madeira (Portugal)	b; f; a
ISOP00239	T. Rapado Branco	Madeira (Portugal)	b/c; f/g; a
ISOP00241	T. Novo	Madeira (Portugal)	b/c; d/f; a/g
ISOP00242	T. de 3 meses	Madeira (Portugal)	b/c; b; a
ISOP00243	T. Cana Roxa	Madeira (Portugal)	b/c; a/f/g; a
ISOP00246	T. mais largo em cima	Madeira (Portugal)	b/c; a/c/d/f/g/u; a/b/d
ISOP00247	T. Mentana	Madeira (Portugal)	b/c; a/b; a
ISOP00248	T. Galhoto	Madeira (Portugal)	b/c; a/b/f/g; a
ISOP00278	T. Leacock	Madeira (Portugal)	b/c; b; a
ISOP00124	T. Galhoto	Madeira (Portugal)	b; a; a
ISOP00191	T. Branco	Madeira (Portugal)	b/c; d/f; a
ISOP00258	T. Canoco	Madeira (Portugal)	b; a/f; a
ISOP00264	T. Leacock sem P.	Madeira (Portugal)	b/c; b/d/g; a/d
ISOP00288	T. Potuguês	Madeira (Portugal)	a/b; c; a/d
ISOP00007	Trigo	Madeira (Portugal)	b/c; f/g; a/c/d
ISOP00078	T. Leacock	Madeira (Portugal)	b; b; a
ISOP00025	Trigo	Madeira (Portugal)	c; f; b
ISOP00289	T. Leacock com barbas	Madeira (Portugal)	c; f; b
ISOP00319	Trigo	Madeira (Portugal)	b/c; b/g; a/b/d
ISOP01484	T. rapadinho	Madeira (Portugal)	b; b/f; a
ISOP01357	Trigo	Madeira (Portugal)	b/c; f/g; a/d
ISOP01485	T. rapadinho de baixo Glúten	Madeira (Portugal)	b; b/f; a
ISOP01322	T. Branco	Madeira (Portugal)	b/c; e/f; a/d
ISOP01269	Trigo	Madeira (Portugal)	b; a; a
ISOP01298	Raposo	Madeira (Portugal)	c; d;c
1*	Trigo	Canary Islands (Spain)	a/b; a/d/g; a
2*	Trigo Marroquín	Canary Islands (Spain)	a/b; b/e/u; d
3*	Trigo rápido	Canary Islands (Spain)	a/b; b; a/d
4*	Trigo Blanco	Canary Islands (Spain)	a; b/u/v; a/d
5*	Trigo Peloño	Canary Islands (Spain)	a/b; b/f/g; a/c/d

Table 1 continued

Accession number	Common name	Origin	Alleles
6*	Trigo morisco	Canary Islands (Spain)	b; b/d/e/u; g
001	Barbilla blanco	Canary Islands (Spain)	b; d; a
002	Barbilla colorado	Canary Islands (Spain)	b; d; a
003	Barbilla blanco	Canary Islands (Spain)	b; d; a
004	Barbilla colorado	Canary Islands (Spain)	b; d/f; a
005	Barbilha Blanco	Canary Islands (Spain)	b; d; a
006	Barbilla colorado	Canary Islands (Spain)	b; b/f; a
007	Barbilla	Canary Islands (Spain)	b; d/f/h; a
008	Marrueco	Canary Islands (Spain)	b; d; a
009		Canary Islands (Spain)	c; d; a
010	Marrueco	Canary Islands (Spain)	c; a/e; a
011	Colorado	Canary Islands (Spain)	b; f; a
012		Canary Islands (Spain)	b; f; a
BGE18651	Arisnegro de Tenerife	Canary Islands (Spain)	b; a/d; a
BGE24864	Trigo	Canary Islands (Spain)	b; d; a
BGE29104	Trigo	Canary Islands (Spain)	b; g; a
BGE29105	Trigo	Canary Islands (Spain)	b; f; g
BGE13760	Isla de Fuerteventura	Canary Islands (Spain)	b; f; a
BGE29103	Trigo	Canary Islands (Spain)	a/b; a/f/h; a/d
BGE18226	T. colorado	Canary Islands (Spain)	a/b; a/b; a/d
BGE13754	Morisco blanco	Canary Islands (Spain)	b; f; a
BGE24863	Trigo	Canary Islands (Spain)	b; d; a
BGE13761	Isla de Fuerteventura	Canary Islands (Spain)	b; f; g
BGE13160	Morisco rojo	Canary Islands (Spain)	b; d/f/g; a
BGE31122	Trigo de la tierra	Canary Islands (Spain)	b/c; b; a
BGE31123	Trigo pelon	Canary Islands (Spain)	b; v; a
BGE20366	T. alto	Canary Islands (Spain)	b/c; d/e; a/g
CBT00724	T. di alto	Canary Islands (Spain)	b; aj/f/g; a
CBT00685	T. de alto	Canary Islands (Spain)	b/c; d/f; a
CBT00684	T. Jallado	Canary Islands (Spain)	b/c; b/d/f; a
CBT00686		Canary Islands (Spain)	b/c; d/f; a
CBT00527	T. barbilla marrueco	Canary Islands (Spain)	b/c; d/f/g; a
CBT00524	Barbilla blanco	Canary Islands (Spain)	b; d/f; a
CBT00522	Barbilla blanco	Canary Islands (Spain)	b; b/d/f; a/d
CBT00598		Canary Islands (Spain)	b/c; a/d/f; a
CBT00622	Barbilla	Canary Islands (Spain)	b; d/f; a
CBT00608	Barbilla	Canary Islands (Spain)	b; d; a
CBT00398	Morisco	Canary Islands (Spain)	b; b/d/f; a
CBT00391		Canary Islands (Spain)	b; f; a/d
CBT00306	T. colorado	Canary Islands (Spain)	b; f; a/d
CBT00528	Barbilla marrueco	Canary Islands (Spain)	b/c; a/d/e/g; a
CBT00379	T. morisco	Canary Islands (Spain)	b; b/e; a
CBT00384		Canary Islands (Spain)	b; f; a
CBT00307	Plaganudo	Canary Islands (Spain)	a/b/c; a/b/d; a

Table 1 continued

Accession number	Common name	Origin	Alleles
CBT00383	T. del país	Canary Islands (Spain)	b; d/h/an; a
CBT00521	Barbilla colorado	Canary Islands (Spain)	b; b/f/aj; a
CBT00520	Barbilla blanco	Canary Islands (Spain)	a/b; d/f; a
CBT00243	Barbilla	Canary Islands (Spain)	b; d/f; a
CBT00242	Arisnegro de Tenerife	Canary Islands (Spain)	b/c; a/b/e/f/i; a
CBT00241	Arisnegro	Canary Islands (Spain)	b/c; d/f/ag; a
CBT00240	Marroquí	Canary Islands (Spain)	b/c; b/d/f/i; a/d
CBT00377	Marroquí	Canary Islands (Spain)	b/c; d/f; a
CBT00293	Morisco	Canary Islands (Spain)	b; d/g; a
CBT00308	Marrueco	Canary Islands (Spain)	b; a/b/d/g; a
CBT00239	Colorado de Tacoronte	Canary Islands (Spain)	b; a/d/f; a
CBT00244	Arisnegro	Canary Islands (Spain)	b/c; a/d/g; a
CBT00523	Barbilla colorado	Canary Islands (Spain)	b; d/f; a
CBT00526	T. barbilla marrueco	Canary Islands (Spain)	b/c; f/aj; a
CBT00623	T. barbilla	Canary Islands (Spain)	b; d/f; a
CBT00309	Marsello	Canary Islands (Spain)	b; b/d/f/g/an; a
146	Trigo peloño	Canary Islands (Spain)	a/b/c;a/b/f; a/d/g
148	Trigo rápido	Canary Islands (Spain)	a/b; a/b; d
149	Trigo blanco	Canary Islands (Spain)	a/b/c; a/b; a/d
150	Trigo peloño	Canary Islands (Spain)	a/c; b/e/f; a/d
151	Trigo marroquin	Canary Islands (Spain)	b; b/I; d
152	Trigo Blanco	Canary Islands (Spain)	a/b; b; c
215	–	Canary Islands (Spain)	b; e; a
217	Trigo pelón	Canary Islands (Spain)	a; b; d
222	Trigo elón	Canary Islands (Spain)	c; f; a

(ne), Shannon's information index (S), average heterozygosity, genetic diversity (He), genetic differentiation (Fst) and. Gene flow (Nm).

The genetic identify (I) values were calculated among the populations. The Nei's genetic distances (D) were used to generate a clustered dendrogram based on unweighted pair-group method using the *MEGA* version 4 program (Tamura et al. 2007).

Results

The frequencies of 25 alleles identified at the 3 loci encoding HMWGs are shown in Table 2. Three alleles were identified at *Glu-A1*, sixteen at the *Glu-B1* and six at the *Glu-D1* locus. At the *Glu-A1* locus, three alleles were detected in the wheat accessions originated from the Canary and Madeira Islands and two from the continental Portugal (Table 2). *Glu-A1b* was the most common allele with a frequency of 84.4% among the accessions from continental Portugal, 79.35% among the Canary accessions and 65.82% among the Madeira accessions. The frequency of *Glu-A1a* was 15.6% in the Portuguese accessions, 11.4% in the Canary accessions and only 1.38% in the accessions from Madeira. On the other hand, the allele *Glu-A1c* had the highest frequency of 32.8% in the Madeira wheats followed by 9.24% in the Canary wheats, while it was not detected in wheat accessions from the continental Portugal.

In wheat accessions originated from the Canary Island, the allele *f* of *Glu*-B1locus was the most frequent allele (31.43%) followed by *Glu-B1d* (29.43%). In the Madeira wheat the *Glu-B1f* and *Glu-B1b* loci were the more frequent alleles, representing 47.09 and 16.51% of landraces, respectively. In the continental accessions the *Glu-B1d* locus shown the highest frequency (24.77%) followed by *Glu-B1a*

Table 2 The frequencies (%) of high molecular weight glutenin subunits (HMWGS) in bread wheat (*Triticum aestivum* L.)

Locus	Allele	Subunits type	Canary	Madeira	Portugal (mainland)
	a	1	11.40	1.38	15.60
	b	2*	79.35	65.82	84.40
Glu-A1	c	Null	9.24	32.80	
	a	7	5.24	11.01	17.43
	b	7 + 8	15.72	16.51	8.17
	c	7 + 9		8.47	
	d	6 + 8	29.43	2.65	24.77
	e	20	3.85	2.75	7.34
	f	13 + 16	31.43	47.09	15.60
Glu-B1	g	13 + 19	6.16	5.93	14.68
	h	14 + 15	1.08	0.32	
	i	17 + 18	1.08		
	an	6	0.31		5.5
	aj	8	1.23		4.59
	ae	18*		0.63	
		6 + 9		0.53	
	ag	7*	0.31	0.63	0.92
	u	7* + 8	2.62	2.75	
	af	7* + 9	1.54		
	a	2 + 12	80.28	80.53	54.12
	b	3 + 12		6.03	
	c	4 + 12	1.23	0.95	4.59
Glu-D1	d	5 + 10	12.33	6.24	9.17
	g	2 + 10	6.16	6.24	23.85
	h	2 + 12*			8.26

(17.43%). The subunit type *18**, *7 + 9* and *6 + 9* were detected only in the Madeira wheat and the alleles *Glu-B1i*, *Glu-B1an*, *Glu-B1aj* and *Glu-B1af* (Fig. 1) were absent in the Madeira accessions. The *Glu-B1i* and *Glu-B1af* (Fig. 1) alleles were present only in the Canary wheat, while the continental accessions shown only nine of the seventeen alleles found at the *Glu-B1* locus.

At the *Glu-D1* locus, six alleles were detected with *Glu-D1a* being the preponderant allele in the Canary (80.28%), Madeira (80.53%) and in the continental (54.12%) accessions. The *Glu-D1 h* allele was detected only in the Old Portuguese wheat (8.26%).

The allele *Glu-D1d* associated with good bread making quality (Payne et al. 1987) was detected among the Canary, Madeira and mainland Portuguese accessions, showing the highest frequency in the wheat originated from the Canary Islands (12.33%). Subunit 3 + 12 (*Glu-D1b*) was present only in Madeira accessions (6.03%) (Fig. 1) whereas 4 + 12 (*Glu-D1c*) (Fig. 1) appeared in all the three regions, with a low frequency of 1.23, 0.95 and 4.59% the Canary, Madeira and continental Portugal accessions, respectively. The low frequency of these two subunits can be an advantage, because there are associated with poor bread making quality (Payne et al. 1987). A total of 69 allele combinations were observed in our data, with the most common being *Glu-A1b/Glu-B1f/Glu-D1a*.

The genetic data for the *Glu-1* loci are summarized in the Table 3. The effective number of alleles (ne), the expected heterozygosity (He) and the Shannon information index shown the highest values at the *Glu-A1* loci in the wheat originated from Madeira. The highest value of genic diversity (Fst) detected among in the wheat accessions from the continental Portugal, while similar values of gene flow in all loci were found in the Madeira and in Portuguese wheat's described by Vasconcellos (1933).

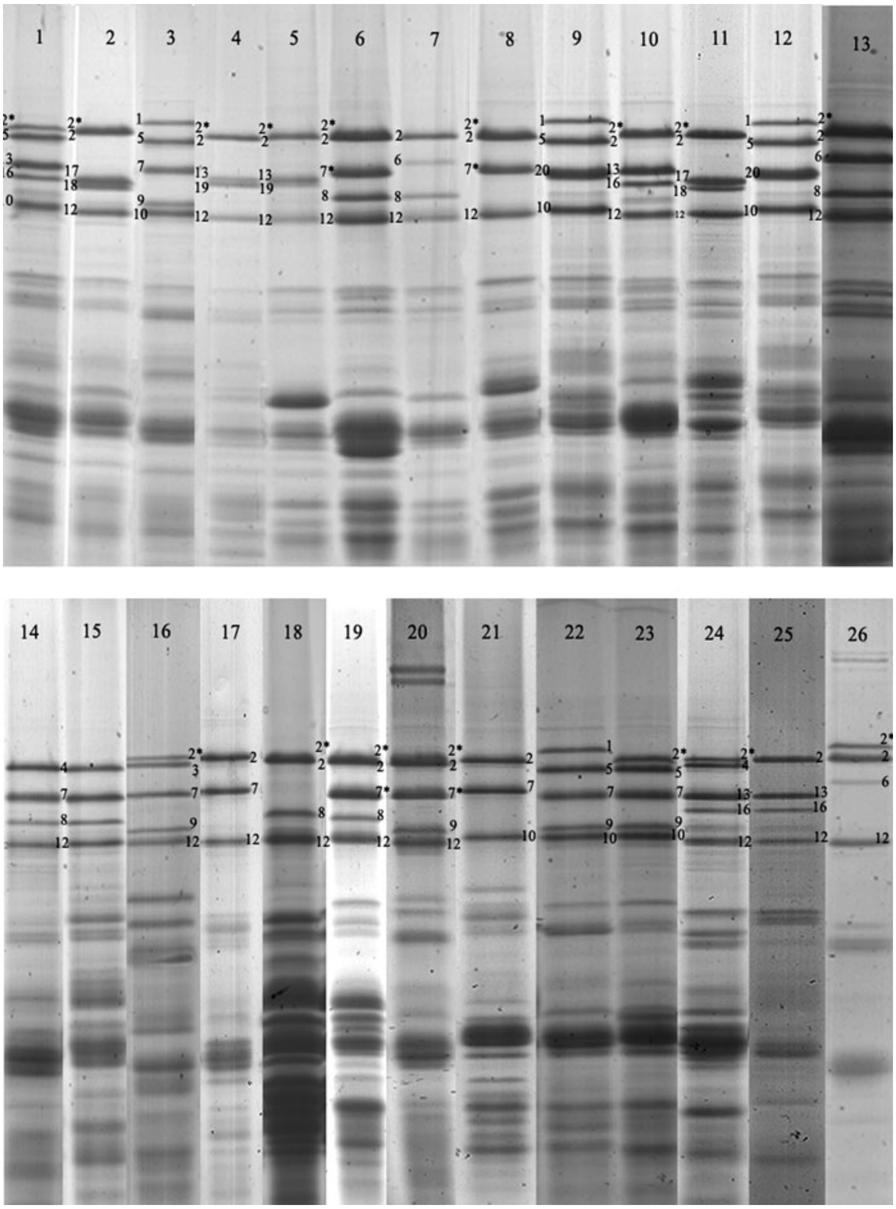

Fig. 1 SDS–PAGE patterns of HMW—GS from some accessions, representative of the most uncommon allelic variants detected at the *Glu-1* loci. *Lanes* as follows: *1* (Florida 301); *2* (Gabo); *3* (Marquis); *4* (Florence); *5* (CBT00308); *6* (ISOP00097); *7* (3172); *8* (ISOP00089); *9* (Federation); *10* (006); *11*(CBT00240); *12* (2*); *13* (1*); *14* (Champlein); *15* (152); *16* (ISOP00098); *17* (ISOP00093); *18* (3077); *19* (ISOP00248); *20* (CBT00526); *21* (ISOP00105); *22* (Marquis); *23* (ISOP00288); *24* (ISOP00072); *25* (ISOP00087); *26* (CBT00309)

At the *Glu-B1* locus the Portuguese wheat's (the Vasconcellos collection) shown the highest values for effective number of alleles (ne), the Shannon information index (S), expected heterozygosity (He) and genic diversity (Fst). In general the wheat's from the Madeira and Canary Islands exhibited a similar variation of genetic parameters.

At the *Glu-D1* locus the Canary and Madeira wheat accessions had similar values, while the Portuguese ones shown higher levels of genetic variations except average heterozygosity.

The mean genic diversity (Fst) for al loci were 0.54 (Madeira), 0.66 (Canary) and 0.92 (Old Portuguese) wheat's. These results indicate a high diversity among the populations from the three different geographical regions.

A UPGMA dendrogram was constructed based on Nei's (1973) genetic distance for all populations (Fig. 2). All populations were grouped into two main clusters (I and II). While the majority of accessions were included in the cluster II, the accession 152 and 00010 originating from Canary (La Palma) Islands

Table 3 Genetic diversity parameter based on the *Glu-1* loci in bread wheat originated from Canary, Madeira and Mainland Portugal

Locus	Origin	Size	na	ne	S	He	Ave.Het	Fst	Nm
	Canary	1298	3	1.54	0.49	0.349	0.121	0.67	0.13
Glu-A1	Madeira	1,890	3	1.84	0.7	0.459	0.252	0,44	0.32
	Portuguese	218	2	1.35	0.43	0.263	0.029	0.89	0.31
	Canary	1,298	13	4.56	1.77	0.781	0.311	0.6	0.16
Glu-B1	Madeira	1,890	13	3.65	1.77	0.726	0.297	0.6	0.17
	Portuguese	218	9	6.38	1.98	0.843	0.073	0.91	0.02
	Canary	1,298	4	1.51	0.54	0.34	0.073	0.79	0.07
Glu-D1	Madeira	1,890	5	1.52	0.73	0.34	0.168	0.55	0.21
	Portuguese	218	5	2.72	1.24	0.63	0.029	0.95	0.01
	Total	3,470	8.6667 ± 7.3711	2.6977 ± 1.8119	1.1544 ± 0.6884	0.5260 ± 0.2314	0.2048 ± 0.0569	0.615	0.16

Observed number of alleles (na); Effective number of alleles (ne); Shannon's Information index (S); expected heterozygosity (He); average heterozygosity; genetic differentiation (Fst); Nm = Gene flow estimated from Fst = 0.25(1 − Fst)/Fst

were contained within the cluster I. The cluster II was divided into 33 subclusters, of which eight encompassed accessions from Madeira (subcluster # 5, 8, 11, 14, 21, 23, 24 and 27), five contained accessions from Canary (subcluster # 13, 16, 19, 26 and 32), nine had accessions from Madeira and Canary (subcluster # 2, 3, 6, 10, 12, 22, 25, 28 and 31), six contained accessions from Madeira, Canary and Portugal (subcluster # 9, 15, 17, 18, 29 and 30) and five included wheat accessions from Canary and Portugal (subcluster # 1, 4, 7, 20 and 33).

Discussion

The analysis of variation in storage protein can provide important information for the assessment of genetic variability of plant germplasms (Igrejas et al. 1999).

The accessions analyzed in this study included landraces from Madeira (Portugal), the Canary Islands (Spain) and the continental Portugal. Twenty six alleles were detected at all loci. The 69 haplotypes were found at the HMW glutenin loci owing to the combination of the allelic variants at Glu-A1, Glu-B1 and Glu-D1. The *Glu-A1b/Glu-B1f/Glu-D1a* haplotype was the most frequent in all three geographical regions. The average genic diversity (Fst) in each locus displayed high values, which points to the existence of genic variability among the Madeira, Canary and Portuguese (the Vasconcellos collection) wheat populations and to a low level of immigration. The latter suggests that the populations are well structured and are of a different geographical origin.

The cluster analysis confirmed the historic information about the introduction and the spread of wheat across the studied regions. Initially, wheat was introduced by settlers from Portugal to the Island of Madeira and the majority of the land was dedicated to this crop. Excess of crop yield left after satisfying the needs of local population was sold to the mainland of Portugal, Maghreb, Azores and the Canary Islands. The Madeiran wheat grain was also used to support the Portuguese exploration of the cost of northern of Africa and in the Gulf of Guinea (de Albuquerque and Vieira 1987; Vieira 1987, 1988). The cluster 9, 15, 17, 18, 29 and 30 seem to match with this historic data. From the seventies of the fifteenth century, a great part of the land was converted to sugar cane and grape

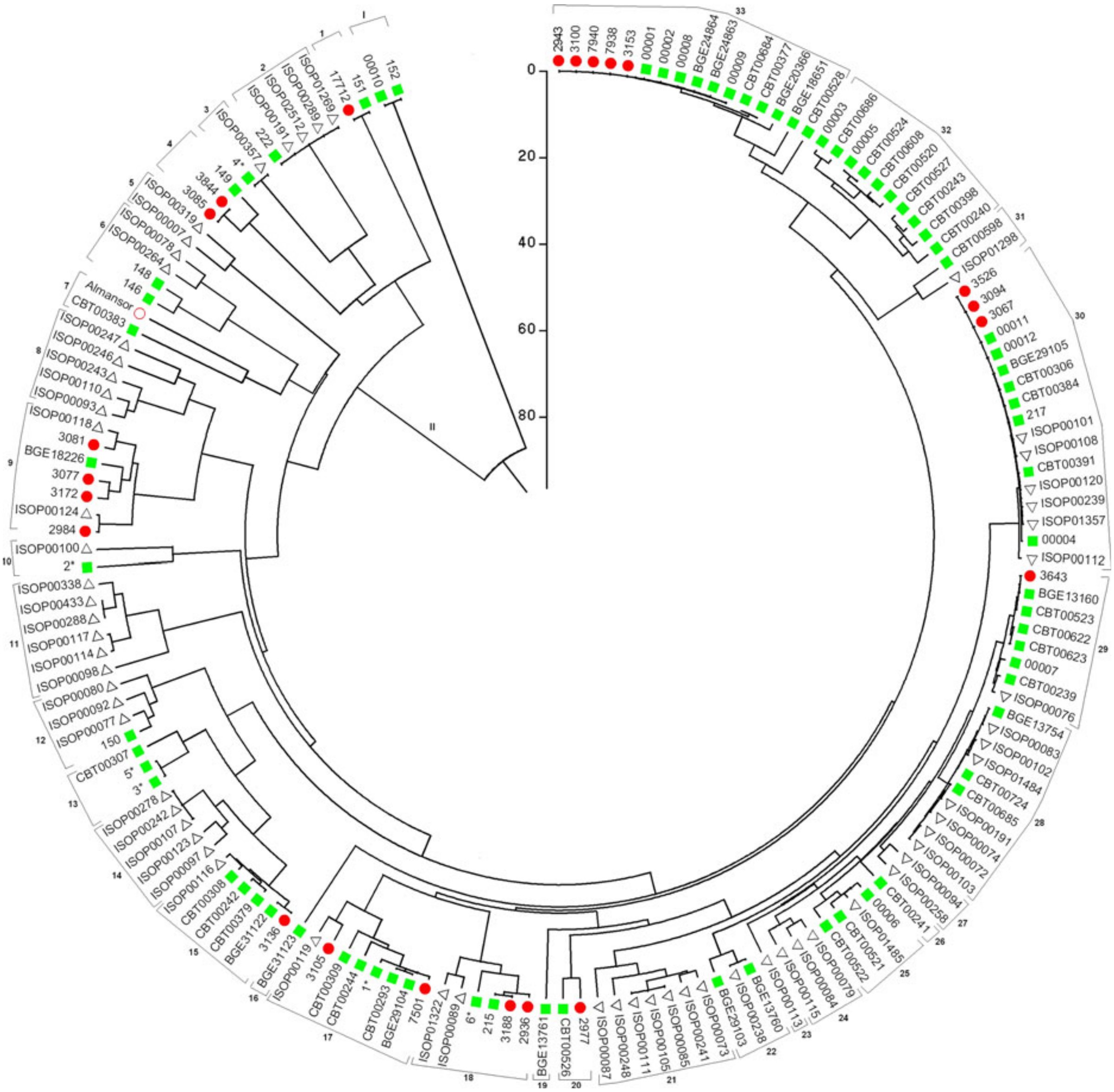

Fig. 2 Dendrogram based on unweighted pair-group method. The accessions marked with *filled circle* originated from mainland Portugal, with *open triangle* from Madeira, and with *filled square* from Canary Islands

production and wheat cultivation was transferred to less adequate land, which resulted in substantial reduction of wheat yields. To compensate for decline of wheat production on Madeira new fertile areas in the region were identified and put into cultivation including the Azores Islands and the Canary Islands (Vieira 1984). Subsequently, in the XV, XVI and XVII centuries an intensive trade was development among the archipelagos of Madeira, Canary and Azores. Madeira imported wheat from Azores and the Canary Island and exported for both archipelagos sugar and wine (Vieira 1983, 1984; Freitas de Meneses 1995). The subcluster 2, 3, 6, 10 and 12 included landraces from Madeira and La Palma (Canary Islands), which reflects the wheat trade activities between the two islands. La Palma had played an important role in the commerce of wheat to Madeira and it was a popular immigration destination of residents of Madeira who introduced the sugar production technology to this island (Vieira 1984).

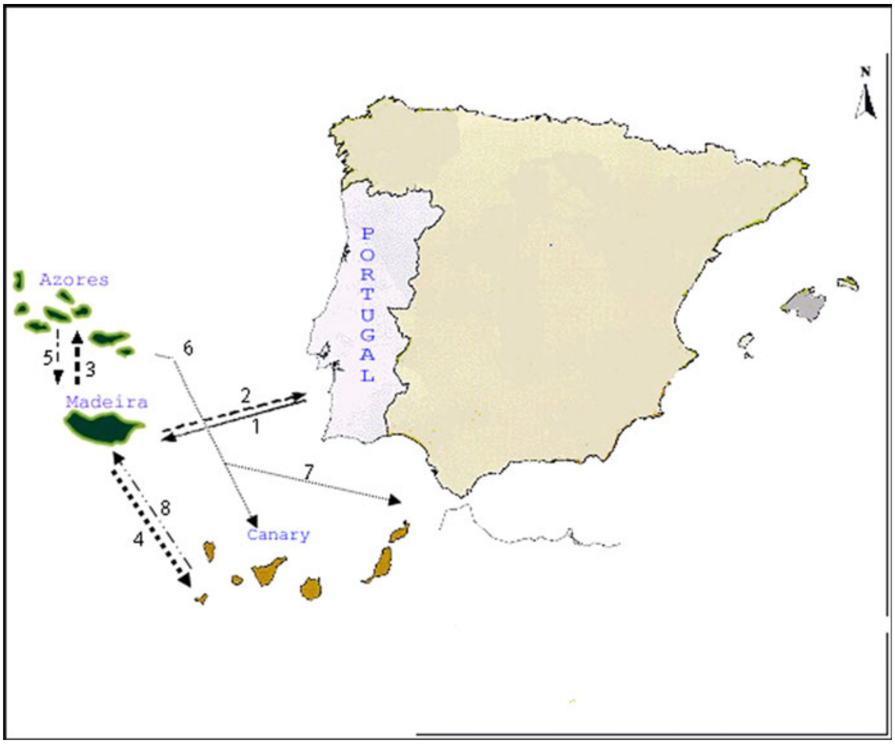

Fig. 3 The routes of the wheat exchange among Iberian Peninsula and the Atlantic Islands (Azores, Madeira and Canary) and north of Africa. In the fifteenth century the wheat was introduced in Madeira from Mainland (*1*), in the seventies was export from this Island to mainland Portugal (*2*), Azores (*3*) and Canary Islands (*4*). In the end of fifteenth and sixteenth and seventeenth centuries Madeira imported wheat from Azores (*5*) and Canary Islands (*8*); Canary Islands (*6*) and North Africa (*7*) imported wheat from Azores Islands in the sixteenth and seventeenth centuries

Tenerife, the other island of the Archipelago of Canary, has also played an important role in the commercial exchanges with Madeira. This historical fact could be supported by the analysis of clusters 15, 22, 25 and 28. The Branch I includes only sample from La Palma, which may suggest another origin of these samples. The three archipelagos (Madeira, Azores and Canaries) for several centuries have played a key role in the navigations and connections among Europe, America and Asia. All exchanges of wheat grain that happened during this period led to the accumulation of a great agricultural diversity on these islands, which is now stored in germplasm banks in Madeira and Canary islands. To prevent the genetic erosion produced by genetic drift in accessions stored in germplasm banks as result of their management (Hammer 2003), it is important to promote the utilisation of these local landraces by the farmers.

We propose that geographical distribution of the high molecular weight glutenin alleles confirms the existence of wheat exchanges from the Iberian Peninsula to Madeira and from Madeira to the Canary Islands and vice versa (Fig. 3). Also, the high genetic diversity among the population of each island supports the hypothesis of different wheat introductions to both archipelagos. Data from the Azores Islands were not included because wheat as a crop is presently extinct in this region.

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