

Distribution of MWG699 polymorphism in Spanish European barleys

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Abstract: The STS marker MWG699/*TaqI* is closely linked to the *vrs1* locus and has been proposed as a marker of domestication in barley. This study included 257 cultivated barleys of both two- and six-rowed varieties, mainly from the western Mediterranean region. These included many landraces from the Spanish barley core collection, Moroccan landraces, and a set of accessions from other European countries. Restriction analysis of amplified DNA revealed three alleles, as previously described. Most of the two-rowed entries had the same allele, type K. Six-rowed entries showed both types A and D. Indeed, type D was widespread among Spanish landraces and commercial varieties from central Europe. It was also found in some two-rowed landraces originating from Spain and Morocco. Barleys with the D haplotype were predominantly winter types, whereas the A haplotype was evenly distributed among spring and winter types. These results support the existence of two different genetic sources among six-rowed Spanish landraces.

Key words: barley, origin, SBCC, Spanish barley core collection, haplotype.

Résumé : Le marqueur STS MWG699/*TaqI* est fortement lié au locus *vrs1* et il a été proposé qu'il constituerait un marqueur pour la domestication de l'orge. La présente étude portait sur 257 orges cultivées, à deux ou six rangs, provenant surtout de l'ouest de la Méditerranée. La plupart étaient des variétés de pays issues de la « Spanish barley core collection » alors que d'autres étaient des variétés de pays d'origine marocaine ou encore des accessions en provenance de d'autres pays européens. Une analyse de restriction de l'ADN amplifié a révélé trois allèles, tel que décrit antérieurement. La majorité des génotypes à deux rangs portaient le même allèle, K. Les génotypes à six rangs portaient soit l'allèle A ou D. En fait, l'allèle D était très répandu au sein des variétés de pays espagnoles et des variétés commerciales d'Europe centrale. Ce même allèle a également été observé chez certaines accessions à deux rangs provenant d'Espagne ou du Maroc. Les orges de l'haplotype D étaient principalement des orges d'hiver, tandis que l'haplotype A était présent de façon égale au sein des orges de printemps et d'hiver. Ces résultats suggèrent qu'il existerait deux sources génétiques différentes à l'origine des orges à six rangs espagnoles.

Mots clés : orge, origine, SBCC, collection nucléaire d'orges espagnoles, haplotype.

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Introduction

The cMWG699 locus is closely linked (0.1 cM) to the *vrs1* locus, which predominantly controls the formation, development, and fertility of lateral spikelets of *Hordeum vulgare* (Komatsuda et al. 1999a). The fertility of the lateral spikelets is one of the diagnostic genetic traits for the domestication process of barley. It has been generally accepted that *Hordeum spontaneum* gave rise to cultivated two-rowed forms by mutation at the one of the brittle rachis (*Btr1* and

Btr2) loci and that six-rowed forms arose subsequently through mutations at the *vrs1* locus (Komatsuda and Mano 2002).

cMWG699 is a barley cDNA encoding a nuclear DNA homologous to the soybean elongation factor (*EF-G*) gene (Michalek et al. 1999) that has been used for evolutionary studies in the Triticeae (Komatsuda et al. 1999b). Tanno et al. (1999) developed a diagnostic marker for this locus by PCR amplification, followed by *TaqI* digestion. This marker identified three haplotypes in barley, which were named A, D, and K. The analysis of their DNA sequences indicated that each pair of varieties with the same restriction type showed an identical sequence (haplotype). Later, Tanno et al. (2002) studied the distribution of those three haplotypes, and their nucleotide sequences, among wild and cultivated barleys: type K was restricted to two-rowed barleys; six-rowed cultivars were divided into two distinct groups that corresponded to haplotypes A and D. Type I (haplotype A) was distributed widely, whereas type II (haplotype D) was found only in the Mediterranean region. The type I sequence was found in a wild barley accession from Turkmenistan, whereas the type II sequence was also found in a two-rowed

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cultivated barley from North Africa and a wild barley from Morocco. These results led the authors to postulate separate origins for A and D types of six-rowed cultivated barleys.

Previous studies with this marker have shown a very restricted distribution of type D barleys mainly limited to the Mediterranean region. In this paper, the objective was to verify the distribution of the haplotypes of this putative domestication marker in European barleys, especially in the western Mediterranean region, where other reports suggested a possible domestication centre for barley (Molina-Cano et al. 1987).

Materials and methods

This study included 257 cultivated barley accessions, of which 40 were two rowed and 217 were six rowed (Tables 1 and 2). They can be classified into three main groups according to their geographic origin. The largest group comprised Spanish barley landraces that constitute a core collection of national germplasm (Igartua et al. 1998; Lasa et al. 2001). They are inbred lines (148 six rowed, 11 two rowed) derived from the original populations maintained at the National Germplasm Bank. A second group comprised 36 Moroccan *Hordeum vulgare* subsp. *vulgare* accessions (32 six rowed, 4 two rowed), which constitute the subset of Moroccan entries belonging to the United States Department of Agriculture (USDA) world core set. Seed for these entries was kindly provided by the USDA. The third group was a set of accessions from other European countries. They are a sample of old and new cultivars, including some landraces, all widely used in European mainstream barley breeding. Thus, they constitute a representative sample of European barley germplasm (Table 1). Some accessions from European countries were kindly provided by the Nordic genebank (Arnarp, Sweden) and by the CGN genebank from Wageningen (the Netherlands). Another 8 non-European cultivars were also included (Table 1), as were the 8 Moroccan *Hordeum vulgare* subsp. *spontaneum* accessions from Molina-Cano et al. (1997).

Geographical information of the collection sites (longitude, latitude, and altitude) was available for the Spanish landraces. To study geographic distribution, means for these passport variables across haplotypes were calculated for the subgroup of six-rowed Spanish barleys.

Total DNA was extracted from leaves (bulked from 10 plants at the 3 leaves stage) as described by Casas et al. (1998). PCR amplification of cMWG699 and restriction analysis with *TaqI* (Invitrogen, Carlsbad, Calif.) were performed following the methods and primers described by Komatsuda et al. (1998).

Some amplified PCR products were sequenced, to ascertain the equivalence of the haplotypes with the ones described by Tanno et al. (1999). Nucleotide sequences were determined directly from the amplified PCR product, with fluorescence cycle sequencing kits (Applied Biosystems, Foster City, Calif.) and an ABI Prism 377 automated DNA sequencer (Sistemas Genómicos S.A., Valencia, Spain). Two independent amplifications were analysed for each accession to eliminate PCR amplification artefacts. Sequences were compared against the type A *Hordeum vulgare* subsp. *vulgare* 'Azumamugi' cMWG699 DNA sequence (GenBank

accession AY028801) with the aid of the PILEUP program in the GCG software package (version 9.0, Genetics Computer Group, Madison, Wis.).

Results and discussion

A single PCR product of approximately 930 bp was obtained for all the accessions studied. *TaqI* digestion of the amplified DNA revealed the same three haplotypes as those described by Tanno et al. (1999). The overall distribution of the haplotypes was 120 A type, 105 D type, and 32 K type. Two haplotypes, A (115 out of 217) and D (102 out of 217), were detected in 6-rowed barleys. All three haplotypes were found in 2-rowed barleys (Tables 1 and 2). The frequency of type D in this study was much larger than that found by Tanno et al. (2002), and was distributed among landraces and cultivars from both central and southern Europe. Although Tanno et al. (2002) did not find intragroup sequence variation of the D-haplotype accessions, the wide distribution and high frequency of haplotype D in this study led us to explore further the possibility of variations of the nucleotide sequence within those entries. The samples analyzed were five six-rowed landraces (4 from the Spanish core collection and 1 from Morocco); 4 cultivated 6-rowed barleys from France ('Hatif de Grignon'), Croatia ('Ragusa'), Netherlands ('Vindicat'), and Germany ('Vogelsanger Gold'); and the three 2-rowed samples presenting type D (2 from Spain and 1 from Morocco). All of the sequenced samples had the same nucleotide sequence, identical to that of 'Dissa' (Tanno et al. 1999). Previously, Tanno et al. (2002) only found intragroup sequence variation in type A, not in types K or D. Thus, it is quite likely that D is a fairly well-conserved haplotype.

In the present study, most of the 2-rowed entries analysed were type K (32 out of 40), although types A (5 out of 40) and D (3 out of 40) were also found. Types A and K were geographically distributed over all the geographic origins, but type D was only found in 2-rowed landraces from Spain (2) and Morocco (1). This finding agrees with the distribution of haplotypes of 2-rowed cultivated germplasm reported by Tanno et al. (2002) where it was found that the D haplotype was present only in southern Europe and northern Africa, and was absent from Ethiopia, the Middle East and northern central Europe. Interestingly, as previously described by Tanno et al. (2002), all 8 of the existing *H. spontaneum* accessions from Morocco were type D.

Thus, there is a restricted geographic occurrence of D haplotypes for both cultivated and wild two-row barleys in the western Mediterranean region. Also, the nucleotide sequences of D types from 2- and 6-row barleys analysed in this study were identical. Thus, they must share a common origin. The elucidation of the causes that led to this situation is beyond the scope of this study, but several plausible hypotheses can be put forward. One is that the two-rowed D types may have arisen through recombination between the *vrs1* locus of A- or K-type 2-rowed barleys and the *vrs1* alleles of 6-rowed D types. Though this possibility was not favoured by Tanno et al. (2002), it cannot be discarded. 6-rowed D types are abundant in the region (Table 2), and they have very likely coexisted with 2-rowed barleys in (at least) the Iberian peninsula for a long time. Another possibility is

Table 1. Cultivars from European countries other than Spain and non-European genotypes analysed for MWG699/*ItaqI* polymorphism.

(a) European reference set.							
Two row			Six row				
Cultivar	Origin	Cultivation type	MWG699 Haplotype	Cultivar	Origin	Cultivation type	MWG699 Haplotype
Triumph	Germany	Spring	A	Juli	Denmark	Spring	A
Graphic	UK	Spring	A	Kors	Denmark	Spring	A
Beka	France	Spring	K	Vega Svalöf	Denmark-Sweden	Spring	A
Nevada	France	Spring	K	Olli	Finland	Spring	A
Tremois	France	Spring	K	Frisia	Germany	Spring	A
Volga	France	Spring	K	Herse	Norway	Spring	A
Alexis	Germany	Spring	K	Maskin	Norway	Spring	A
Camelot	Germany	Spring	K	Asplund	Sweden	Spring	A
Union	Germany	Spring	K	Edda II	Sweden	Spring	A
Wisa	Germany	Spring	K	Stella	Sweden	Spring	A
Hassan	Netherlands	Spring	K	Bordia	Belgium	Winter	A
Pallas	Sweden	Spring	K	Gerbel	France	Winter	A
Cameo	UK	Spring	K	Plaisant	France	Winter	A
Kym	UK	Spring	K	Dura	Germany	Winter	A
Alpha	France	Winter	K	Athenais	Greece	Winter	A
Clarine	France	Winter	K	Mirco	Italy	Winter	A
Gaelic	France	Winter	K	Ragusa B	Croatia	Winter	D
Hispanic	France	Winter	K	Ager	France	Winter	D
Labea	France	Winter	K	Barberousse	France	Winter	D
Mogador	France	Winter	K	Haïf de Grignon	France	Winter	D
Angora	Germany	Winter	K	Monlon	France	Winter	D
Igri	Germany	Winter	K	Athene	Germany	Winter	D
Tipper	UK	Winter	K	Dea	Germany	Winter	D
				Franka	Germany	Winter	D
				Hauter	Germany-France	Winter	D
				Herfordia	Germany	Winter	D
				Senta	Germany	Winter	D
				Vogelsanger Gold	Germany	Winter	D
				Banteng	Netherlands	Winter	D
				Tapir	Netherlands	Winter	D
				Vindicat	Netherlands	Winter	D

(b) Non-European genotypes.							
Two row			Six row				
Cultivar	Origin	Cultivation type	MWG699 Haplotype	Cultivar	Origin	Cultivation type	MWG699 Haplotype
S-7	ICARDA	Spring	K	Orria	CIMMYT	Spring	A
Logan	USA	Spring	K	S-36	ICARDA	Spring	A
				Steptoe	USA	Spring	A
				Morex	USA	Spring	A
				S-45	ICARDA	Winter	A
				Mammuth	Canada	Winter	D

Table 2. Distribution of MWG699/*TaqI* haplotypes across germplasm groups and cultivation type.

		Spanish landraces ^a			Moroccan landraces ^b			European reference set ^c			Total
		A	D	K	A	D	K	A	D	K	
Two row	Spring	0	2	9	3	1	0	2	0	12	29
	Winter	0	0	0	0	0	0	0	0	9	9
	Total	0	2	9	3	1	0	2	0	21	
Six row	Spring	32	15	0	30	1	0	10	0	0	88
	Winter	31	70	0	1	0	0	6	15	0	123
	Total	63	85	0	31	1	0	16	15	0	

^aSpring or winter type deduced from observations under controlled conditions (F. Ciudad, personal communication).

^bGrowth habit as reported in USDA National Plant Germplasm System databases (<http://www.ars-grin.gov/npgs/>).

^cCultivation type after Baumer and Cais (2000).

the evolution of cultivated D-types from autochthonous 2-rowed *H. spontaneum*, as suggested by Moralejo et al. (1994). This hypothesis cannot be ruled out with the data presented in this report. Other authors question this last hypothesis based on the evidence for a monophyletic origin for all cultivated barleys (Badr et al. 2000) or the suspiciously close molecular resemblance between Moroccan wild and cultivated barleys (Blattner and Badani Méndez 2001).

We did not find any type D sample among the 6-rowed cultivars from Scandinavian countries and only one such sample was found from Morocco. The large proportion (57.4%) of type D samples among Spanish landraces is also remarkable. Type A accessions came from the eastern and southern part of Spain, whereas type D was more prevalent at higher altitudes and latitudes (Table 3). Thus, there are geographic trends of distribution of MWG699/*TaqI* haplotypes in Spanish 6-rowed barleys, which agree with the distribution of frequencies for this marker in 6-rowed barleys beyond the Iberian peninsula to the north, where the D type is more prevalent, and to the South, where the A type is more prevalent. Even so, A and D types could be found all over Spain and most of Europe, suggesting that there were no restrictions to the dispersion of barley populations. Therefore, the preference of one type over the other must have some adaptive significance. Most D-type barleys were winter types and only a few were spring; for the A genotypes, frequencies for spring and winter types were similar. This unbalance was also evident for the European reference set. Fischbeck (1992) pointed out that only a small number of landraces of winter barley had been available for the development of improved cultivars in central and western Europe, as compared with spring barley. Major parents of important winter barley cultivars during the first half of the 20th century were a landrace population from the Netherlands (which originated 'Vindicat'), the Canadian winter barley population Mammuth, the mildew-resistant 'Ragusa', from Dalmatia (Croatia), and the French landrace Hatif de Grignon. Interestingly, 'Hatif de Grignon' and 'Mammuth' (both derived from landraces of the same name), 'Ragusa', and 'Vindicat' all exhibited the D haplotype. However, derivatives of these founder cultivars, produced by recombinations between gene pools of different geographic origin, showed A and D haplotypes.

None of the major loci governing vernalisation and photoperiod responses in barley are close to the *vrs1* locus. Thus, the reason for the predominance of the D haplotype among winter barleys must have a cause other than simple linkage.

Table 3. Relationship of MWG699/*TaqI* haplotype distribution with geographic factors for the 6-row subset of the Spanish barley core collection (148 genotypes).

Geographic factor	MWG699 haplotype		Prob(<i>t</i>) ^a
	A	D	
Longitude	3° 56' E	3° 03' E	0.1412
Latitude	38° 56' N	40° 33' N	0.0006
Altitude	494 m	682 m	0.0005

^aTwo-tailed probability of difference between A and D haplotypes being equal to 0.

There could be an unknown adaptive locus in the vicinity of *vrs1* that might be epistatically related to vernalisation or photoperiod response loci. Hence, the *vrs1* region could be selected along with them. Or, this situation could have also arisen as the result of a bottleneck at the appearance of 6-rowed D-type barleys. There has been a large disagreement about the monophyletic or polyphyletic origin of barley domestication (Abbo et al. 2001; Badr et al. 2000; Molina-Cano et al. 1987; Tanno et al. 2002). Based on the study of wild and cultivated barleys, Tanno et al. (2002) suggested a diphyletic origin of the recessive alleles (*vrs1*) of 6-rowed barley generated from 2-rowed barley (*Vrs1*) by independent mutations. The divergence time of types A and D (4-nucleotide substitution) was calculated as more than 100 000 years (Tanno et al. 1999), much longer than the history of barley cultivation. Archeological evidence shows that domestication of barley took place around 8000 BC in the fertile crescent (Lev-Yadun et al. 2000). The matter of where the D type originated is an open and appealing question, as are other phylogenetic questions that arise from this and other works; however, the interesting point for plant breeders and germplasm users is that these results support the existence of 2 different genetic sources among 6-rowed landraces, characterized by the two MWG699/*TaqI* patterns, A and D. Although both types of barley may have been partially mixed through the years, especially on the Iberian peninsula, geographical distribution of both types still identifies two groups. Their existence should be taken into account when planning barley breeding, especially if they convey different adaptive responses, as suggested in this study.

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