ISSN 1479-2621
Plant Genetic Resources: Characterization and Utilization (2011) **9**(2); 240–242
doi:10.1017/S1479262111000451

Allelic variation at the *EF-G* locus among northern Moroccan six-rowed barleys

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Abstract

A germplasm panel of 52 six-rowed barley landraces from northern Morocco was analysed by a Cleaved Amplified Polymorphic Sequences (CAPS) assay of a fragment of the elongation factor G (*EF-G*) gene. Forty-nine of these accessions carried allele A, and the other three carried allele D. The latter all originated from a narrow region close to the border with Algeria, whereas the former were represented across the whole collection area. Since six-rowed D allele carriers are present in North Africa, along with both two-rowed cultivated and wild barleys, it is likely that the European six-rowed barley varieties carrying the D allele have Moroccan parentage.

Keywords: elongation factor G; *Hordeum vulgare*; Morocco; row type

Introduction

The row type of the barley (*Hordeum vulgare*) spike is controlled by the *vrs1* locus located on chromosome 2H. The *vrs1* locus encodes a HD-ZIP I type transcription factor specific to barley (Komatsuda *et al*., 2007), which may interact with the other HD-ZIP I transcription factor encoded by *HvHox2*, which is a gene highly conserved in cereal species (Sakuma *et al*., 2010). The six-rowed spike is restricted to cultivated barley (subsp. *vulgare*), and is believed to have evolved from the two-rowed type (Komatsuda *et al*., 2007) around the time of the crop's domestication (*c*. 6500 BCE) (Harlan, 1995). The cDNA sequence cMWG699 (Graner *et al*., 1991), which is a fragment of an elongation factor gene *EF-G*, maps within 0.1 cM of the *vrs1* locus (Komatsuda *et al*., 1999a), and has been applied for phylogenetic studies in the *Hordeum* and related species (Komatsuda *et al*., 1999b, 2009; Blattner, 2009). The two co-dominant *EF-G* alleles (A and K, defined by a *Taq*I Cleaved Amplified Polymorphic Sequences (CAPS) assay) are generally correlated with, respectively, the six- and two-rowed type, although some recombinant genotypes existed (Komatsuda *et al*., 1999a). When the assay was applied to a collection of 65 cultivated barley accessions, Tanno *et al*. (1999) uncovered a third allele D of *EF-G*, which was restricted to six-rowed types. A wider survey of 464 accessions showed that the A allele is distributed world-wide, whereas the D allele only occurs in southern Europe (Tanno *et al*., 2002). The D allele has, however, also been identified in one two-rowed cultivated barley (cv. Palmella Blue) from an unidentified north African country and all the seven Moroccan wild barley applied (subsp. *spontaneum*) from Morocco. Molina-Cano *et al*. (1987) have suggested that Moroccan wild barley was a possible progenitor of southern European cultivated barleys, and given the probability that the six-rowed spike evolved from the two-rowed one, it is likely that the D allele originated in Moroccan subsp. *spontaneun*. If this supposition is correct, then the expectation is that the allele should still be represented among Moroccan six-rowed barleys. Thus, here we set out to screen a collection of six-rowed Moroccan barleys for their allelic constitution at the *EF-G* CAPS marker.

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Materials and methods

A germplasm panel of 52 six-rowed landraces collected from northern Morocco (Kuwabara *et al.*, 1988) was assembled (Supplementary Table S1, available online only at http://journals.cambridge.org). Their collection sites ranged from sea level to an altitude of >2000 m (Fig. 1). Four seeds from each accession were grown to isolate a single pure line to provide a source of DNA, which was extracted from leaves of 9-d-old seedlings following the protocol described by Komatsuda *et al.* (1998). This DNA provided the template for PCR amplification of the *EF-G* fragment, based on the primer pair T7-3 and T3-3 (Tanno *et al.*, 1999). The resulting amplicons were *Taq*I restricted as described by Komatsuda *et al.* (1998). A further Derived Cleaved Amplified Polymorphic Sequences (dCAPS) assay was based on the primer pair T7-3 and T3-4 (5'-AACTCTGAGAATAAAATGGCTAGCG), using *Hba*I restriction to recognize the additional nucleotide polymorphism between the A and the D allele (Tanno *et al.*, 1999).

Results

The *EF-G* amplicon resolved as a single fragment of size ~490 bp in all 52 accessions. Its digestion with *Taq*I produced two restriction patterns, corresponding to the A and D alleles (Tanno *et al.*, 1999). Forty-nine of these accessions carried allele A, and the other three carried allele D (Supplementary Table S1, available online only at http://journals.cambridge.org). The A allele carriers were distributed across the whole collection region, whereas the D allele ones originated from a narrow area around the city of Ojuda (Fig. 1), which lies close to the border with Algeria, at an altitude of 1250–1560 m (Supplementary Table S1, available online only at http://journals.cambridge.org). The second dCAPS analysis did not reveal any polymorphism among the A allele accessions, so no A–D intermediate (as found in a Chinese cultivar by Komatsuda *et al.*, 1999b) was present.

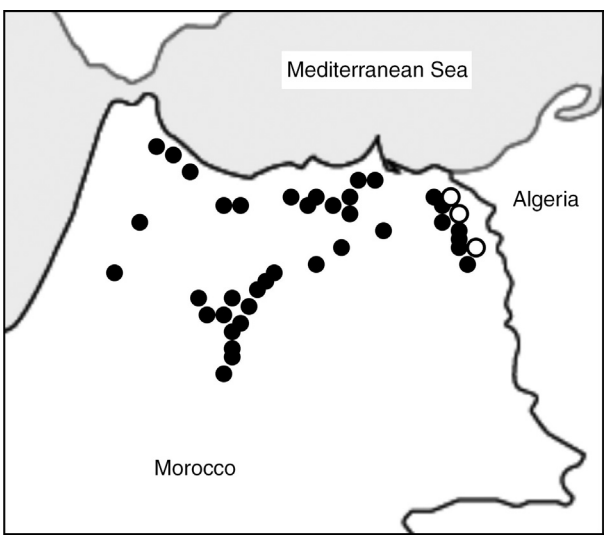

Fig. 1. Geographical distribution of the *EF-G*/*Taq*I polymorphism across northern Morocco. Closed circle, A allele; open circle, D allele.

Discussion

The *EF-G* fragment has offered a means of exploring the evolution of the six-rowed ear. The distribution of alleles within this sequence has suggested that the A and D alleles arose prior to the appearance of the six-rowed ear, thus implying that a two-rowed barley carrying the A allele was the ancestor of the six-rowed barleys carrying the A allele, and likewise a D allele two-rowed type was the ancestor the current D allele six-rowed types (Tanno *et al.*, 1999, 2002). The hypothesis that the six-rowed D allele carriers evolved from a Moroccan two-rowed D allele individual, before spreading toward southern Europe, requires that six-rowed D allele carriers can be located in Morocco.

In that event, we found three such accessions among a collection of 52 six-rowed northern Moroccan landraces. The result confirms the plausibility that the progenitor of six-rowed D allele carriers is a wild barley from Morocco. D allele wild barleys are distributed throughout Algeria, Tunisia, Egypt and Spain (T. Komatsuda, unpublished data). The result also supports the suggestion made by Molina-Cano *et al.* (1987) that, on the basis of some isozyme and morphological data, a number of Spanish six-rowed barleys were descended from Moroccan subsp. *spontaneum* types.

Tanno *et al.* (1999) have shown that the *EF-G* fragment A and D alleles reflect four nucleotide substitutions, and that the age of the divergence between these alleles is in the range 138,000–830,000 years. The evolution of the six-rowed spike appears to have occurred independently in an A allele and a D allele carrier (Komatsuda *et al.*, 2007; Saisho *et al.*, 2009). Here, we have reported that the D allele is present in the Moroccan population of six-rowed (cultivated) barley, while Tanno *et al.* (2002) showed that it is also present in Moroccan subsp. *spontaneum*. Thus, the likelihood is that the lineage of six-rowed barley D allele carriers involved a Moroccan subsp. *spontaneum* followed by a Moroccan two-rowed *vulgare*.

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https://doi.org/10.1017/S1479262111000451 Published online by Cambridge University Press

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