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Molecular characterization of the *Glu-Ay* gene from *Triticum urartu* for its potential use in quality wheat breeding

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Abstract

Triticum urartu Thum. *ex* Gandil. is a wild species identified as A-genome donor for polyploid wheats, which could be used as gene source for wheat breeding. The high-molecular weight glutenin subunits are endosperm storage proteins that are associated with bread-making quality. In *T. urartu*, these proteins are encoded by the *Ax* and *Ay* genes at the *Glu-A^{u}1* locus. The *Ay* gene of 17 *Glu-A^{u}1* allelic variants previously detected in this species has been analysed using PCR amplification and digestion of the PCR products with two endonucleases (*Dde*I and *Pst*I). The combination of two restriction patterns has revealed variations between the active and inactive alleles, and within each type. This variation, especially that detected among the active alleles, could enlarge the high-quality genetic pool of modern wheat and be used for bread-making quality improvement in durum and common wheat.

Keywords: electrophoresis; genetic resources; glutenin; quality breeding; wild wheat

Introduction

Nowadays, global climate change is one of the major problems facing humanity. For crops, this will require the release of new cultivars able to adapt to a changing environment, without reducing quality standards or affecting industrial food production according to the demands of a population highly sensitized to food quality (Godfray *et al*., 2010). Several different studies have suggested that relatives and wild progenitors of wheat species could be interesting candidates for enlarging the gene pool of cultivated wheats (Hajjar and Hodgkin, 2007). At the diploid level, the main species of wild diploid wheat are *Triticum monococcum* L. ssp. *aegilopoides* Link em. Thell. (syn. *T. boeoticum*; $2n = 2x = 14$; A^mA^m) and *T. urartu* Thum. *ex* Gandil ($2n = 2x = 14$; A^uA^u), the latter species having been identified as the A-genome donor of polyploid wheat (Dvorak *et al*., 1993).

The presence and variability of the endosperm storage proteins are associated with the bread-making quality of wheat. These proteins are divided into two main groups (gliadins and glutenins) according to their molecular characteristics (Payne, 1987). Glutenins are also divided into high molecular weight (HMWGs) and low molecular weight (B-LMWGs and C-LMWGs) subunits. The HMWGs, encoded by genes at the *Glu-1* loci located on the long arm of group-1 homoeologous chromosomes being the best studied (Payne, 1987), have been associated with bread-making quality in common wheat (Cornish *et al*., 2006). Each *Glu-1* locus contains two tightly linked genes that encode for two types of HMWGs, called *x*- and *y*-type (Harberd *et al*., 1986), although some of these genes are not expressed in cultivated wheats. In particular, the A*y* subunit is absent in all durum and common wheats, while it is expressed in wild diploid and tetraploid wheats (Waines and Payne, 1987; Ciaffi *et al*., 1993), its presence

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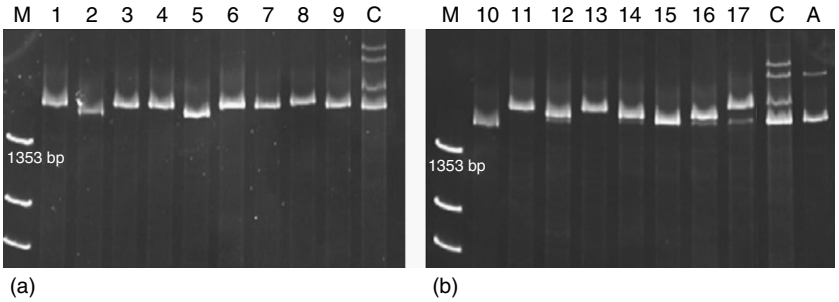

Fig. 1. PAGE separation of PCR products from the *Ay* genes. (a) active *Ay* alleles; (b) inactive *Ay* alleles. M, X174 DNA-Hae III digest; C, cv. Cheyenne; A, cv. Alaga.

being associated with an increase in bread-making quality in wheat (Ciaffi *et al.*, 1995).

Alvarez *et al.* (2009) showed that the introgression of *T. urartu* genome in durum wheat affects the gluten strength. However, these materials were developed from a single line, whereas Caballero *et al.* (2008) found as many as 17 allelic variants for the *Glu-A^{u}1* locus in a large collection of *T. urartu*. The *Ax* gene was found to be active in all these alleles, while the *Ay* subunit was detected in nine of them.

The aim of the present study was the molecular characterization of the allelic variants for the *Ay* gene detected by Caballero *et al.* (2008) to obtain additional data prior to their potential introgression in wheat.

Materials and methods

Seventeen accessions of *T. urartu* that have the allelic variants found by Caballero *et al.* (2008) were analysed.

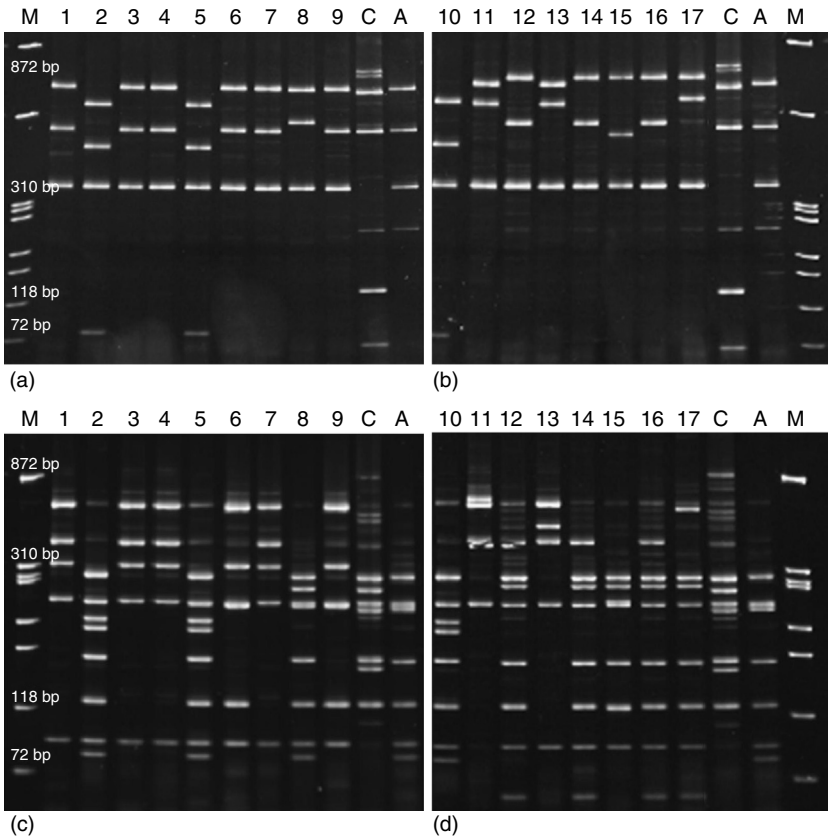

Fig. 2. PAGE separation of PCR products from the *Ay* genes digested with *Dde I* and *PstI* (up and down, respectively). (a and c) digestion patterns of active *Ay* alleles; (b and d) digestion patterns of inactive *Ay* alleles. M, X174 DNA-Hae III digest; C, cv. Cheyenne; A, cv. Alaga.

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DNA isolation was carried out from young leaf tissue using the cetyl trimethyl ammonium bromide method (Stacey and Isaac, 1994).

The primers reported by D'Ovidio *et al.* (1995) were used to amplify the complete coding sequence of the Ay gene. PCR reactions mixtures were carried out in a final volume of 20 µl composed of 1 × Taq PCR buffer (Promega), 125 ng of template DNA, 0.6 µM of each forward and reverse primer, 1.5 mM $MgCl_2$, 0.2 mM of each deoxyribonucleotide and 1 U of *Taq* DNA polymerase (Promega). DNA was subjected to an initial denaturation step at 95°C for 5 min, and the amplification conditions were 35 cycles at 95°C for 1 min, 60°C for 1 min and 72°C for 2 min, followed by a final incubation step at 72°C for 8 min.

The amplicons (PCR products) were separated in polyacrylamide gel electrophoresis (PAGE) gel with a discontinuous Tris–HCl buffer system (pH: 6.8/8.8) at a polyacrylamide concentration of 8% (w/v, crosslinker (C): 1.68%). These amplicons were digested using *Dde*I and *Pst*I endonucleases and separated in PAGE gel with a discontinuous Tris–HCl buffer system (pH: 6.8/8.8) at a polyacrylamide concentration of 10% (w/v, C: 1.68%).

Results and discussion

The amplification of the complete coding sequence in the accessions with and without active *Ay* subunits revealed a single band of around 1500 bp, although with some small differences in size among them, in the accessions with *Ay* active subunits as well as in others with inactive ones (Fig. 1). This is in agreement with the findings of Caballero *et al.* (2008), who detected four *Ay* active subunits with differences in their mobility.

Some studies have suggested that digestion with endonucleases could be a useful tool to evaluate the internal differences between these alleles (Lafiandra *et al.*, 1997; Alvarez *et al.*, 1998). The amplicon digestion with *Dde*I (Fig. 2(a) and (b)) showed three restriction patterns between the active *Ay* alleles, while in the inactive ones, five restriction patterns were identified. In the case of the digestion with *Pst*I (Fig. 2(c) and 2(d)) four patterns were revealed for the active *Ay* alleles and six for the inactive ones. Although, in general, the restriction patterns of the active alleles were different from those of the inactive alleles, one of the patterns of the active alleles (lanes 2 and 5), together with the pattern of lane 10 (of an inactive allele), showed the same restriction pattern with both digestions (Fig. 2, lanes 2, 5 and 10).

On the other hand, all the restriction patterns found in these *T. urartu* lines differed from those of the inactive *Ay* allele present in cv. Cheyenne, while some of them showed similarities with the *Ay* alleles detected in cv. Alaga (Fig. 2).

In some cases, only the combined use of the restriction patterns from both endonucleases evidenced differences between alleles. The actives alleles shown in lanes 1, 3, 4, 6, 7 and 9 were similar in total size (Fig. 1) and *Dde*I digestion (Fig. 2); however, the *Pst*I digestion separated these alleles into two groups. The first group (lanes 1, 3, 4 and 7) presented five bands or fragments, whereas the second group (lanes 6 and 9) showed six. The difference was that fragment 2 of group 1 was digested into two fragments for group 2: one fragment comigrated with the band 3 and the other produced a new band of approximately 120 bp that also appeared in the rest of the restriction patterns. The same occurred for *Dde*I digestion in the inactive alleles, where the alleles showed in lanes 11 and 13 presented similar restriction pattern, but the *PstI* digestion indicated that the two alleles are different.

Although further research needs to be carried out in the future, such as the sequencing of these *Ay* alleles, the results of this study demonstrate that the *Ay* alleles presented in *T. urartu*, both active and inactive ones, are different from the alleles found in cultivated wheat. For breeding purposes, the variation detected for the active alleles would permit to expand the high-quality gene pool of the cultivated wheats. Consequently, the evaluation and characterization of the genetic resources of this wild species are very important for its conservation and potential use in wheat breeding programmes.

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