A PCR-based method for discriminating between high molecular weight glutenin subunits Bx7 and Bx7* in *Triticum aestivum* L.

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Abstract

The correct assignment of high molecular weight glutenin subunit variants is a key task in wheat breeding for quality. However, traditional analysis by protein electrophoresis is sometimes difficult and not very precise. This work describes a novel DNA marker for accurate discrimination between the *Glu-B1* locus subunits Bx7 and Bx7*. An analysis of 142 bread wheat varieties from different countries identified a considerable number of misclassified genotypes that could lead to incorrect conclusions in studies of the relationship between glutenin composition and wheat quality.

Wheat gluten proteins are composed of glutenins and gliadins. Glutenins are divided into two groups: high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS) (Payne et al. 1979). Much of the genetic variation in gluten strength can be explained by HMW-GS variability, and it is possible to make an approximate prediction of the breadmaking quality of a given variety knowing its HMW-GS composition (Payne et al. 1987, MacRitchie et al. 1990, Branlard et al. 1992, Cornish et al. 2001).

HMW-GS are encoded by three complex loci, *Glu-A1*, *Glu-B1* and *Glu-D1*, located on the long arms of chromosomes 1A, 1B and 1D, respectively (Payne et al. 1982). Each complex locus consists of two closely linked loci that encode two polypeptides, x-type and y-type subunits, which are differentiated by their mobilities on SDS-PAGE (sodium dodecyl sulphate polyacrylamide).

Characterization of HMW-GS is an indispensable task in breeding programmes focused on bread-making quality. However, in many cases, it is difficult to differentiate the slight differences in the molecular weight of different subunits by standard electrophoretic methods. This difficulty has led to errors in the classification of some wheat varieties.

One of the most important subunits encoded by the *Glu-B1* locus and related to bread quality is the Bx7 subunit. In the first catalogue of HMW-GS (Payne and Lawrence 1983), three alleles of Bx7 subunit were described: the *a* allele for the subunit Bx7, the *b* allele for the pair of subunits Bx7 + By8 and the *c* allele for the pair of subunits Bx7 + By9.

Subsequently, Anderson and Greene (1989) identified a molecular difference between the Bx7 subunits present in varieties 'Cheyenne' and 'Chinese Spring', which are considered the protein standard for the Bx7 subunit. 'Cheyenne' carried a deletion of a hexapeptide (QPGQGQ) in the C-terminal end of the protein. However, both proteins were named Bx7. In the same year, Pogna et al. (1989) described a Bx7 subunit with an electrophoretic mobility slightly higher than the Bx7 standard subunit from 'Chinese Spring'. They named this new subunit as Bx7*, and the Bx7* + By8 pair was named as the *u* allele in the Catalogue of Gene Symbols for Wheat (McIntosh et al. 1990). The wheat standard varieties listed for the *u* allele were 'Fiorello' (Pogna et al. 1989) and 'Norstar' (Ng et al. 1989). Afterwards, this new Bx7* HMW-GS was found linked to the By9 subunit in the varieties 'Neepawa' and 'Bezostaya' (Marchylo et al. 1992). Up to 2010, this Bx7* + Bx9 remained undesignated.

The good quality associated with the Bx7 subunit focused studies on a search for an alternative to SDS-PAGE for its identification. An attempt to use high-performance liquid chromatography (HPLC) proved unsuccessful (Marchylo et al. 1992). For this reason, a number of groups started to work on the development of DNA markers, but many of them were mistaken as they used 'Cheyenne' as the reference Bx7 DNA sequence (D'Ovidio and Anderson 1994, Ahmad 2000, Butow et al. 2003, 2004, Ma et al. 2003). This confusion led to some authors even proposing the existence of new variants that were in fact the standard Bx7 protein (Butow et al. 2003, 2004).

Alberghina et al. (2005) using MALDI-TOF-MS analysis showed that the Bx7 protein from 'Chinese Spring' differed from the predicted protein for the Bx7 reference DNA sequence standard available in the databases for 'Cheyenne'. They detected polymorphism already seen by Anderson and Greene (1989), that is, the indel of a hexapeptide in the C-terminal region of the 'Cheyenne' protein. Despite this, they continued to name the two subunits as Bx7.

Ragupathy et al. (2008) finally linked the Bx7* protein, characterized by electrophoresis, to the molecular variant carrying the deletion of the hexapeptide QPGQGQ in the C-terminal end of the protein. They also provided a DNA marker for another Bx7 variant, the overexpressed subunit named $Bx7^{OE}$, caused by gene duplication (Butow et al. 2004).

This situation has not allowed the correct assignment of Bx7 variants, an important issue owing to their contribution to significant differences in dough properties (Békés et al. 2004). The aim of this work was to provide a PCR screening method for the accurate discrimination between Bx7 and Bx7* variants. With the characterization of more than one hundred varieties, this revealed inconsistencies in the literature.

Materials and Methods

One hundred and forty-five wheat (*Triticum aestivum* ssp. *vulgare* L.) varieties were selected on the basis of carrying any Bx7 variant. 'Chinese Spring' was used as the standard for Bx7, 'Cheyenne' for Bx7* and 'Glenlea' for $Bx7^{OE}$.

HMW-GS were extracted from crushed grain, without removing gliadins, and the extracts were fractionated by SDS-PAGE according to Singh et al. (1991). Nomenclature of HMW glutenin subunits followed Payne and Lawrence (1983) and McIntosh et al. (2010).

DNA extraction followed a standard CTAB protocol (Saghai-Maroof et al. 1984). Overexpression of Bx7 (7^{OE}) was determined by PCR as described in the study by Ragupathy et al. (2008). For Bx7 vs. Bx7* discrimination, the primers Bx7F (5′-CAACTTCTTCACAG-CAGT-3′) and Bx7R (5′-CTAAAGGTGGCAAAGGCGCA-3′) were designed with Primer3 software (http://frodo.wi.mit.edu/) from the sequences of *GluB1*-Bx7 and *GluB1*-Bx7* glutenin subunits available in GenBank (DQ119142 from 'Glenlea' and X13927 from 'Cheyenne', respectively). Sequence alignment was performed with CLC Free Workbench (http://www.clcbio.com). PCR amplification was performed as described in the study by Rodríguez-Quijano et al. (2010) with minor modifications; the programme included twelve cycles of touchdown PCR (from 59°C to 53°C, decreasing 0.5°C per cycle) and then 35 more cycles of standard PCR at 53°C. The results were analysed in 3.5% NuSieve agarose gels (Cambrex, Rockland, ME, USA) stained with GelRed (Biotium, Hayward, CA, USA).

Results

Firstly, wheat varieties were analysed by SDS-PAGE in order to confirm the presence of a Bx7 allelic variant (Fig. 1a). All samples were then analysed by PCR for the identification of the $Bx7^{OE}$ and Bx7* subunits. The Bx7F and Bx7R primers were designed to target an 18-bp deletion present in subunit Bx7* (Fig. 1b). The sizes of the amplification products were 182 bp for Bx7 and 164 bp for Bx7*, and the difference was clearly visible in agarose gels (Fig. 1c). Thirty-one of 142 varieties analysed (21.8% of the total) carried the Bx7 subunit, 98 (69.0%) carried the Bx7*, and 13 (9.2%) carried the $Bx7^{OE}$ (Table 1).

Twenty-seven varieties previously characterized as Bx7 in the AACC catalogue (Wrigley et al. 2006) were identified as Bx7* and four as $Bx7^{OE}$. The varieties 'Impeto' and 'Astral', catalogued as Bx7 + By8 or Bx7* + By8, should actually be assigned as Bx7* + By8 in agreement with Pogna et al. (1989). 'Biggar',

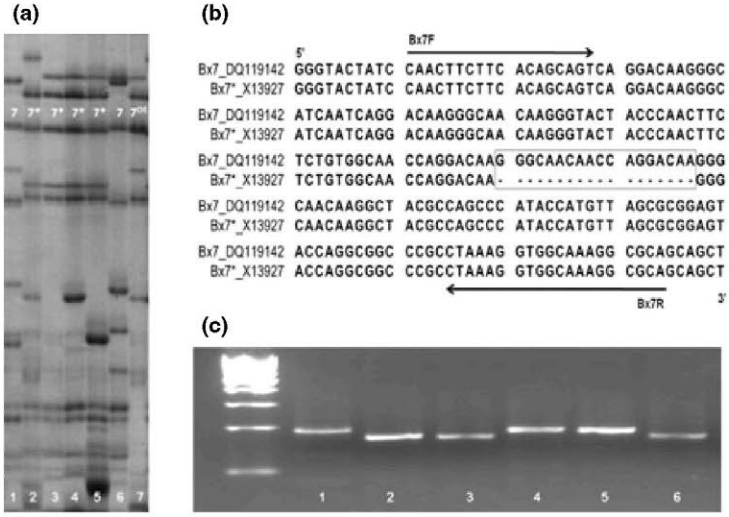

Fig. 1: (a) SDS-PAGE used for screening Bx7 allelic variants from different varieties. Lane 1: 'Chinese Spring', 2: 'Marquis', 3: 'Osona', 4: 'Patizanka', 5: 'Potam 70', 6: 'Pursang', 7: 'Ablaca'. (b) Nucleotide alignment of a region of around 200 nucleotides from HMW-GS genes (locus *Glu-B1*) coding for Bx7* and Bx7 subunits. GenBank accession numbers: DQ119142 corresponds to 'Glenlea' and X13927 corresponds to 'Cheyenne'. The 18-bp indel is highlighted by the box. Arrows indicate the positions of specific primers. (c) PCR products amplified with Bx7F and Bx7R primers. Lanes 1: 'Chinese Spring', 4: 'Pursang' and 5: 'Glenlea' carry the Bx7 or $Bx7^{OE}$ subunits; lanes 2: 'Cheyenne', 3: 'Katepwa' and 6: 'Marquis' carry the Bx7* subunit

Table 1: Distribution of Bx7 allelic variants among 142 bread wheat accessions. Those with changes from previously published data are underlined

Subunit	Standard	Varieties
Bx7*	'Cheyenne'	'Abanto', 'Abel', 'Abrego', 'Adalid', 'Adonay', 'Adonis', 'Alaun', 'Albares', 'Albatros', 'Albero', 'Albinoni', 'Alcotán', 'Alejo', 'Aloda', 'Alto', 'Alud', 'Amarok', 'Andy', 'Anza', 'Apache', 'Ardec', 'Arganda', 'Ariana 8', 'Arminda', 'Arpa 13', 'Arpain', 'Astral', 'Autonomía', 'Bancal', 'Bastión', 'Bezostaya', 'Bolero', 'Borgoya', 'Carat', 'Cargifaro', 'Cartaya', 'Cascogne', 'Cezanne', 'Chapo', 'Dollar', 'Elastic', 'Enesco', 'Escacena', 'Etecho', 'Fiel', 'Fiuza', 'Florence Aurora', 'Fortón', 'Frandoc', 'Gonzalo', 'Greina', 'Hugo', 'Impeto', 'Insengrain', 'Katepwa', 'Krona', 'Lachish', 'Lara', 'Lenta', 'Líbero', 'Lodi', 'Magali', 'Manda', 'Mane Nick', 'Mara', 'Marco', 'Marius', 'Marquis', 'Mecano', 'Mulero', 'Nacar', 'Neepawa 'Nogal', 'Osado', 'Osona', 'Pané 247', 'Patizanka', 'Perla', 'Piron', 'Potam 70', 'Rapor', 'Rescler', 'Resultón', 'Ruy', 'Sansa', 'Sideral', 'Simun', 'Sion', 'Soissons', 'Splendeur', 'Talento', 'Thor', 'Titien', 'Top', 'Trajano', 'Trento', 'Urbión', 'Ysatis', 'Zentos'
Bx7	'Chinese Spring'	'Agrus', 'Amiro', 'Arcole', 'Atlas', 'Babel', 'Boticelli', 'Boulmiche', 'Brigio', 'Brio', 'Caramba', 'Craklin', 'Ecija', 'Festín', 'Gades', 'Galeon', 'Garant', 'Hardi', 'Kilopondio', 'Lozano', 'Paradis', 'Plethore', 'Pursang', 'Soprano', 'Taber', 'Transfer', 'Victor'
$BX7^{OE}$	'Glenlea'	'Ablaca', 'Alcalá', 'Asoros', 'Biggar', 'Búfalo', 'Eduardo', 'Galera', 'Gazul', 'Horzal', 'Jivago', 'Pinzón', 'Sarina', 'Zarco'

catalogued as Bx7 + By8 or $Bx7^{OE}$ + By8*, carries $Bx7^{OE}$ + By8*. In the case of 'Florence Aurora', described as Bx7 + By8 or Bx7 + By9, we observed the Bx7* + By8 combination.

Discussion

The determination of HMW-GS composition in large collections of wheat varieties from different countries has been undertaken in many studies, but there has been insufficient attention given to the discrimination of Bx7, Bx7* and $Bx7^{OE}$ subunits by SDS-PAGE for two reasons: firstly, the electrophoretic mobility of HMW-GS depends very much on polyacrylamide gel concentration and secondly, it was widely considered that the most frequent pair was the Bx7 + By8 combination. Morgounov et al. (1993) determined the HMW-GS composition of more than 1000 varieties from 21 countries and none was described as Bx7* + By8 or Bx7* + By9. Branlard et al. (2003) analysed 200 French varieties and described none with the Bx7* subunit, but listed 41 with the Bx7 subunit and 61 with the Bx7 + By8 pair. More recently, Ribeiro et al. (2011) analysed 262 varieties from Portugal and 47.02% had alleles carrying the Bx7 subunit, but they did not find the Bx7* subunit. Among almost 6000 varieties (of a total of 7830) described in the AACC Catalogue (Wrigley et al. 2006) as having Bx7 allelic variant, 5303 (89.7%) are catalogued as Bx7, 535 (9.1%) as Bx7* and 72 (1.2%) as $Bx7^{OE}$. In the light of the current results, it is likely that many were not correctly identified.

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