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Molecular characterization of *Glu-B3* locus in wheat cultivars and segregating populations

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ABSTRACT

Bread wheat quality constitutes a key trait for the demands of the baking industry as well as the broad consumer preferences. The role of the low molecular weight glutenin subunits (LMW-GS) with regard to bread quality is so far not well understood owing to their genetic complexity and to the use of different nomenclatures and standards for the LMW-GS assignment by different research groups, which has made difficult the undertaking of association studies between genotypes and bread quality. The development of molecular markers to carry out genetic characterization and allele determination is demanding. Nowadays, the most promising LMW gene marker system is based on PCR and high resolution capillary electrophoresis for the simultaneous analysis of the complete multigene family. The molecular analysis of the bread wheat *Glu-B3* locus in F_2 and $F_{4:6}$ populations expressed the expected one-locus Mendelian segregation pattern, thus validating the suitability of this marker system for the characterization of LMW-GS genes in segregating populations, allowing for the successful undertaking of studies related to bread-making quality. Moreover, the *Glu-B3* allele characterization of standard cultivars with the molecular marker system has revealed its potential as a complementary tool for the allelic determination of this complex multigene family.

1. Introduction

Flour derived from bread wheat (*Triticum aestivum* L., AABBDD, $2n = 6x = 42$) encompasses unique dough viscoelastic properties conferred by prolamins, seed storage proteins, which are further subdivided into gliadins and glutenins according to their solubility in aqueous/alcohol solutions.

Gliadins are monomeric proteins, classified into α, β, γ and ω types based on their mobility in A-PAGE, being mainly related to dough extensibility. ω gliadins are rich in glutamine, proline and phenylalanine forming hydrophobic interactions. α/β and γ gliadins are rich in cysteine and methionine and can thus establish disulfide bonds (Shewry et al., 2003). Gliadin genes (*Gli* loci) are located on the chromosomes of homoeologous groups 1 and 6.

Glutenins are polymeric proteins directly related to the viscoelastic properties of wheat flour. On the basis of their mobility in 1D-SDS-PAGE, they can be classified into two groups: the high molecular weight glutenins (HMW-Gs, covering a molecular range of 70–90 KDa) and the low molecular weight glutenins (LMW-GS, comprising a molecular range of 20–45 KDa). They respectively represent 10% and 40% of the prolamins. Both types of glutenins cross-link to generate the glutenin polymer, so far one of the largest macromolecules described in nature (Wrigley, 1996).

Genes encoding the HMW-Gs are located on the group 1 chromosomes, comprising the *Glu-A1*, *Glu-B1* and *Glu-D1* loci with each locus encoding for two polypeptides, which are designated subunits. The type and the number of subunits, between 3 and 5, typify the allelic variation of the HMW-GS subunits and are the most important determinants with regard to the rheological quality of bread wheat dough (Payne et al., 1987), therefore having been deeply studied at a protein level. The allelic characterization is easy to undertake by protein electrophoresis, yet in some cases it might be necessary to resort to other techniques comprising a higher resolution such as RP-HPLC, CE or MALDI-TOF-MS (Gao et al., 2010). In addition, molecular marker use is becoming progressively more frequent. PCR based specific markers for loci *Glu-A1*, *Glu-B1* and *Glu-D1* exist (i.e: Ahmad, 2000; Ma et al., 2003). The extended

Abbreviations: A-PAGE, acid polyacrylamide gel electrophoresis; CE, capillary electrophoresis analysis; HMW-GS, high molecular weight glutenin subunits; LMW-GS, low molecular weight glutenin subunits; 1D-SDS-PAGE, one dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis; 2DE, two dimensional electrophoresis.

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knowledge base with regard to these proteins has substantially contributed to accelerate the genetic improvement of the bread quality trait in breeding programs.

Compared to the HMW-GS, the LMW-GS are a much more complex family, substantially more LMW-GSs are expressed in the kernels of bread wheat and in consequence their contribution to wheat bread quality characteristics is still ill defined.

Based on the first amino-acid of the N-terminal protein, LMW-GS have been classified as LMW-m (methionine), LMW-s (serine) and LMW-i (isoleucine) (D'Ovidio and Masci, 2004). The proposed primary structure of a typical LMW-GS is: a signal peptide of 20 amino acids followed by a short N-terminal domain (13 amino acids), next to a central repetitive domain and concluding with a C-terminal domain, which is further subdivided into three distinctive regions namely, C-terminal I, II and III (D'Ovidio and Masci, 2004). Whereas the signal peptides and the C-terminal I and III regions are extremely well conserved, the repetitive domains are highly polymorphic in length (D'Ovidio and Masci, 2004).

Most of the LMW-GS genes are encoded by the *Glu-A3, Glu-B3* and *Glu-D3* loci, located on the group 1 chromosomes and closely linked to the γ and ω gliadin loci (Gupta and Shepherd, 1990). Part of the bread quality influence attributed to gliadins can be in fact due to the tightly linked LMW-GS loci. Together these genes comprise a multi-gene family whose number has been estimated to vary from 10 to 40 in hexaploid wheat varieties (Huang and Cloutier, 2008). However, the exact copy number of LMW-GS genes is still unknown, mostly due to a lack of efficient methods to distinguish between members of this multigene family. Studies using cDNA and screening of bacterial artificial chromosome (BAC) libraries (Ikeda et al., 2006; Huang and Cloutier, 2008; Dong et al., 2010) have provided a general elucidation of the complexity regarding members of the LMW-GS gene family in wheat varieties. At least four genes coding mostly types LMW-GS-m and i have been identified for the *Glu-A3* locus (from *A3-1* to *A3-4*); three coding mostly type LMW-GS-s for the *Glu-B3* locus (from *B3-1* to *B3-3*) and seven coding only type LMW-GS-m for the *Glu-D3* locus (from *D3-1* to *D3-7*), nonetheless, the number of genes can vary among cultivars and presumably could be higher in view of the fact that a number of genes of this family are still unknown (Dong et al., 2010). In addition, the presence of pseudogenes has also been described for all loci yet their type and number also depend on the cultivar analyzed.

Usage of molecular markers to discriminate different LMW-GS genes has also been investigated by several groups (Dong et al., 2010; Huang and Cloutier, 2008; Ikeda et al., 2006; Lan et al., 2013; Liu et al., 2010; Long et al., 2005; Sharma et al., 2013; Wang et al., 2009,2010; Zhao et al., 2007a,b). Although some of these markers might be useful in breeding programs, the high LMW-GS gene homology and their high content of repetitive domains have made the molecular approach arduous and most molecular markers proposed so far are not very robust, reducing their wide application.

Nonetheless, recently a new molecular marker method was proposed by Zhang et al. (2011a). This method is not locus or allele specific, the ultimate goal is the complete amplification by PCR of all of the LMW-GS coding sequences of a given cultivar followed by their analysis by Capillary Electrophoresis (CE). This is achieved by using a pool of primers designed according to the conserved regions of the LMW gene sequences. So far, this has been revealed as a very powerful marker system to elucidate the complex members of the LMW gene family in different cultivars and isogenic lines (Zhang et al., 2012, 2013).

The detailed characterization of LMW-GS members is important for accurate bread quality studies since the allelic HMW-GS variation is not sufficient to explain all of the variation observed with regard to the bread quality parameter values. The function of the LMW-GS proteins respective to the control of the end use qualities of wheat kernels has been studied in both tetraploid and hexaploid cultivars; the genetic studies having generally found positive contributions of the *Glu-3* loci in connection with parameters related to dough strength, extensibility and bread-making quality (Békés et al., 2006; Gupta and McRitchie, 1994; Ma et al., 2005; Oury et al., 2010; Payne et al., 1987; Zhang et al., 2012). Nowadays, the molecular mechanisms underlying the functional genetic differences of either the orthologous or the allelic *Glu-3* loci have still not been well investigated. The further development and use of molecular markers which support an analysis of the LMW-GS role concerning bread quality is essential while resorting to populations of advanced bread wheat lines. In particular, the detailed characterization of the allelic diversity present at the *Glu-B3* locus exhibiting the highest complexity and variation is important, as it has been established that the most important contribution to bread wheat quality derives from the LMW-GS loci (Zhang et al., 2012).

The objective of this study has been to gain insight into the molecular characterization of the bread wheat *Glu-B3* locus using a molecular marker approach, concomitantly validating the molecular markers in segregating populations and evaluating their possible role in reference to supporting *Glu-B3* specific allele determination in standard cultivars.

2. Material and methods

2.1. Plant material

Two cultivars were selected for having the same composition at the *Glu-A3* and *Glu-D3* loci, but being different for the *Glu-B3* locus: 'Gazul' a hard-grained spring wheat with a higher gluten strength and 'Tigre' a hard-grained winter wheat with a high dough extensibility. Two populations derived from the 'Tigre' × 'Gazul' cross were used for this work, an F_2 population ($N = 95$) and an advanced $F_{4:6}$ population ($N = 65$). Lines were raised at the experimental field of the School of Agricultural Engineering, Technical University of Madrid, Spain (40°26′47.36″ N, 3°44′21.00″ W).

LMW-GS standard cultivars 'Capelle-Desprez', 'Chinese Spring', 'Fengmai 27', 'Gabo', 'Halberd', 'Heilo', 'Nanbu-komugi', 'Norin 61', 'Pepital' and 'Thesee' were selected according to Liu et al. (2010).

2.2. Protein electrophoresis and nomenclature

Prolamin extraction was performed according to Singh et al. (1991). Allelic variation of LMW-GS was determined using Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (1D-SDS-PAGE) as previously described (Payne et al., 1987). LMW-GS were named following Gupta and Shepherd (1990), Jackson et al. (1996), Igrejas et al. (1999) and Liu et al. (2010).

2.3. DNA and RNA isolation and PCR amplification

Genomic DNA was extracted from seeds using the cetyl trimethyl ammonium bromide (CTAB) procedure (Saghai-Maroof et al., 1984). Total RNA samples were prepared from developing seeds (10 days post-anthesis) using the TRIzol reagent according to the manufacturer's protocol (Invitrogen, Carlsbad, CA) and were converted into cDNA using the PrimeScript™ 1st strand cDNA Synthesis Kit (Takara).

The primer sets used for the LMW-GS amplification were modified from Zhang et al. (2011a). To optimize the different amplification, sets of degenerated primers were designed (Table 1). The forward primer of each pair was labeled with