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An update of low molecular weight glutenin subunits in durum wheat relevant to breeding for quality

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ABSTRACT

The identification of low molecular weight (LMW) glutenin alleles is a valuable tool to select in early generations those lines which combine the best allelic combinations when breeding for quality. The present study was conducted to analyse the allelic variation in glutenins of a core collection of durum wheat with the objectives of increasing the genetic variability available for breeding, and studying the genetic control of some subunits. New and no catalogued allelic variants of glutenins have been described, and the genetic control of some subunits have been determined by genetic analyses. The linkage relationships found among several LMW glutenin subunits and between LMW glutenins and gliadins can be very useful in the accurate identification of some alleles. The findings presented in this study have allowed the updating of the LMW glutenin subunits (catalogued and not catalogued) found so far in durum wheat. This information can be very valuable to assist with the identification of the alleles at the *Glu-3* and the *Glu-B2* loci. Additionally, some new alleles at *Glu-B3* exhibited a positive effect on gluten strength and should be target for future studies.

1. Introduction

In durum wheat (*Triticum turgidum* L.), pasta quality differences between cultivars are strongly dependent upon their allelic variation in endosperm storage proteins, gliadins and glutenins. Both groups of proteins can be defined as prolamins in that they are soluble in alcohol–water mixtures either as protein monomers (gliadins) or as reduced subunits (glutenins) (see Shewry et al., 2003, for a review). The gliadins are traditionally divided based on their mobility in electrophoresis at low pH into four groups: α-, β-, γ- and ω -gliadins. According to their mobility in sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), glutenins can be classified into two groups, high molecular weight (HMW-GS, 70–90 kDa) and low molecular weight (LMW-GS, 20–45 kDa) glutenin subunits.

Genetic analyses indicate that prolamins are encoded by multiple genes at complex loci. The genetic control has been reviewed in detail by Shewry et al. (2003). The HMW-GS are encoded by genes at the *Glu-1* complex loci (*Glu-A1* and *Glu-B1*), present on the long arm of the homeologous group 1 chromosomes. Each locus contains two tightly linked genes encoding subunits designated as x and y type based on their molecular weight and biochemical characteristics. Because of the silencing of some genes, two or one HMW subunit genes are expressed by the *Glu-B1* locus, and one or none (the Null allele) by the *Glu-A1* locus. When only one subunit is expressed by the *Glu-B1* or *Glu-A1* loci, this is always an x type. The B-LMW glutenin subunits are controlled by the *Glu-3* loci (*Glu-A3* and *Glu-B3*), and the *Glu-B2* locus, mapping to the short arms of homeologous group 1 chromosomes. Closely linked to *Glu-3* loci, the loci *Gli-A1* and *Gli-B1* encode the majority of ω-and γ-gliadins, and some β-gliadins. Several molecular and proteomic studies have shown that the major gliadin and LMW-GS loci are clearly multigenic (e.g. Qi et al., 2009). In bread wheat, 14 genes have been identified in *Glu-3* loci (Dong et al., 2010), but taking into account the pseudogenes (very frequent in all prolamin families), this number can go up to 30–40 genes (Beom et al., 2018). Initial studies on durum wheat quality reported that the gliadins γ-42 (*Gli-B1a* allele) and γ-45 (e.g. *Gli-B1b* or *c* allele) were associated with poor and good gluten strength, respectively. Later studies concluded that the B-LMW glutenins were the ultimately responsible for the gluten strength of durum wheat (e.g. Pogna et al., 1990). The HMW-GS have exhibited less significant effects than LMW-GS on durum wheat quality (e.g. Aguiriano et al., 2009; Ruiz and Carrillo, 1995; Vázquez et al., 1996).

The identification of LMW-GS alleles is a valuable tool to select in

Abbreviations: HMW-GS, High Molecular Weight Glutenin Subunits; LMW-GS, Low Molecular Weight Glutenin Subunits; SDS-PAGE, Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis; SDSS, Sodium Dodecyl Sulphate Sedimentation

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early generations lines which combine the best allelic combinations when breeding for quality. According to the Catalogue of Gene Symbols for Wheat (Mcintosh et al., 2013), in durum wheat there are 11, 11 and 3 different allelic variants present at the *Glu-A3*, *Glu-B3* and *Glu-B2* loci, respectively. Protein electrophoresis is still the most widely used method for LMW-GS allelic identification, although some technical limitations involving co-migration of some subunits can led to inaccurate identification of alleles. However, currently, the use of molecular markers for the LMW-GS multigene family allele identification is limited by (i) the high degree of homology among all glutenin genes, (ii) the high presence of pseudogenes and repeat sequences in these loci, and (iii) the lack of a complete genomic reference sequence. Several efforts have been made to develop molecular approaches that could help in the LMW-GS characterisation in bread wheat (Zhang et al., 2012), but their use in durum wheat has to be tested taking into account that the correspondence between durum and bread glutenin alleles has to be first well established. The study of linkage between the specific LMW-GS genes and their protein products is difficult and rarely reported although some recent results are promising (Beom et al., 2018). More studies are needed before molecular markers can be widely applied, so, currently, the best analysis method is still the protein electrophoresis, considering that few laboratories in the world have the expertise to conduct it for large numbers of samples.

The knowledge of glutenin variability in germplasm collections can be very useful for broadening the currently narrow genetic basis of modern wheat cultivars. In this sense, several studies have analysed the variability for B-LMW subunits in durum wheat varieties grown in countries where durum wheat is a significant crop such as Canada (Sissons et al., 2005), and Mediterranean countries (e.g. Babay et al., 2015; Bellil et al., 2014; Ribeiro et al., 2011) and, in some cases, new allelic variability has been described. Nowadays landraces are considered a natural reservoir of the genetic variation within the species and one of the most important sources for potentially favourable alleles to be used in breeding programs. In fact, new B-LMW subunits, some of them with significant influence on gluten strength, have been found only in old cultivars or in landraces (Aguiriano et al., 2008, 2009; Bellil et al., 2014; Brites and Carrillo, 2000, 2001; Martinez et al., 2004). Core collections, where genetic diversity is maximized with minimum repetition, are a favoured approach to efficiently exploring novel variation and enhancing the use of germplasm collections.

In previous research (Ruiz et al., 2013), we created a core collection of durum wheat formed by Spanish landraces of three tetraploid subspecies, which included a high genetic diversity for gliadin alleles (Ht = 0.84). The main objectives of the present study were as follows: (i) to analyse the allelic variation in HMW-GS and LMW-GS of this collection to increase the genetic variability available for breeding, (ii) to study the genetic control of some B-LMW subunits previously assigned by their electrophoretic mobility, and (iii) the update of the LMW- GS found so far in durum wheat to assist other researchers with the accurate identification of the glutenin alleles.

2. Materials and methods

2.1. Plant material

The Spanish core collection of durum wheat (*Triticum turgidum* L.) used in this research comprised 94 genotypes of three subspecies: 10 of *dicoccon* (Schrank) Thell., 32 of *turgidum*, and 52 of *durum* (Desf.) Husn, which were representatives of the entire collection of 555 Spanish landraces and old cultivars of durum wheat preserved at the National Plant Genetic Resources Centre (Ruiz et al., 2013). All the accessions are pure lines derived from original landraces.

2.2. Prolamin analysis

Prolamins were extracted from crushed endosperm halves following a sequential procedure (Singh et al., 1991). Electrophoresis of reduced and alkylated proteins (HMW-GS and LMW-GS) was performed on SDS-PAGE (12% polyacrylamide and 0.9% bisacrylamide gels). All the accessions were analysed in gels of 18 × 24 × 0.1 cm dimensions for a better separation of the protein bands. For an accurate identification of the subunits, each accession was analysed more than twice and at least half of the samples in each gel were standard varieties. For a precise discrimination between Bx7 and $Bx7^{OE}$ (over-expressed allele, 7^{OE}), DNA was extracted with a standard CTAB protocol and the PCR method described in Ragupathy et al. (2008) was applied.

HMW-GS were scored following the nomenclature of Payne and Lawrence (1983). B-LMW subunits were named according to their relative electrophoretic mobility using the numbers described in previous studies (Aguiriano et al., 2008; Brites and Carrillo, 2000; Lerner et al., 2004; Martinez et al., 2004; Nieto-Taladriz et al., 1997; Rodríguez-Quijano et al., 2010). The subunit described as 8* in Aguiriano et al. (2008) was renamed 8a* to differentiate it from the subunit 8* from 'Mourisco Fino' described by Brites and Carrillo (2000). Glutenin alleles at *Glu-3* and *Glu-B2* loci were designated according to the Catalogue of Gene Symbols for Wheat 2013 (Mcintosh et al., 2013). The equivalence with the earlier designations for *Glu-3* alleles is given in Supplementary Table S1. The alleles not catalogued, identified in previous studies or found in the present research, were designated as *new*. The presence/absence of the gliadin γ-51 controlled at the *Gli-A1* locus and the ω- and γ-gliadins encoded by the *Gli-B1* locus were scored for each accession.

Three crosses using durum wheat varieties were carried out to clarify the genetic control of some B-LMW subunits not previously studied: BGE013089 x 'Senatore Capelli' (cross 1), BGE013590 x 'Cocorit' (cross 2) and BGE020948 x 'Cocorit' (cross 3). At least 100 F2 seeds from each cross were analysed for prolamin composition. The LMW-GS that co-segregated with gliadins controlled by genes at the *Gli-B1* locus or with glutenins controlled at the *Glu-B3*, were assigned to the *Glu-B3* locus and those, that were independent and inherited together with subunits controlled by the *Glu-A3* or *Gli-A1*, were assigned to the *Glu-A3* locus. Those LMW-GS inherited as an allelic variant of the subunit 12 from Senatore Capelli or Cocorit encoded by *Glu-B2a* would have been assigned to the *Glu-B2* locus.

2.3. Quality analyses

Gluten strength was determined on 1 g of whole grain flour samples by the SDSS (sodium dodecyl sulphate sedimentation) test, using stoppered 25 ml graduated cylinders. Field experiments were carried out over the season November 2006 to June 2007 in two diverse Spanish locations: Lleida in the North and Alcala de Henares in the Centre of the country. Four check cultivars were common to all experiments (cvs. Don Pedro, Senatore Capelli, Simeto, and Vitron). Restricted maximum likelihood (REML) was used to estimate the variance components and to produce the best linear unbiased predictors (BLUPs) for data from each cultivar/location combination. Since no significant location effect was evident from ANOVA ($F = 1.26$, $P = 0.26$) the average of the two locations was studied. Differences between means were tested using Student's *t*-test at $P = 0.05$. The allelic effect on gluten strength was computed as the difference between the mean SDSS values of genotypes carrying and not carrying a given allele. Taking into account the SDSS values of the check cultivars, mean value of SDSS test was classified as very high (SDSS $>$ 8 as cvs. Simeto and Senatore Capelli), high ($8 \geq$ SDSS ≥ 7 as cv. Vitrón), medium ($7 >$ SDSS ≥ 6, lower than cv. D. Pedro) and low (SDSS < 6).