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Influence of allelic prolamin variation and localities on durum wheat quality

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ABSTRACT

Thirty-seven varieties of a Mediterranean durum wheat collection grown in Tunisia and Spain were analysed for their allelic composition in prolamins, as well as their protein concentration, sodium dodecyl sulphate sedimentation (SDSS) test and mixograph parameters. Genotype was a greater source of variation in all measurements than locality. Uncommon high and low molecular glutenin subunits (HMW-GS and LMW-GS) were found (V and 2•• subunits at *Glu-A1*, 13 + 16 at *Glu-B1*, 5* subunit and *ax* allele at *Glu-A3*). The rare combinations 2 + 4+14 + 18 and 8 + 9+13 + 16+18 subunits at the *Glu-B3* locus were found. *Glu-A3ax* had a positive influence on SDSS and mixograph parameters. Of all the prolamins, those that have the B-LMW-GS composition *aaa* (for *Glu-A3, Glu-B3* and *Glu-B2* loci, respectively), when associated with the *Glu-A1c* and *Glu-B1d* gave the best semolina quality. By contrast, semolina quality is poor when this same composition is associated with the *Glu-A1c* and *Glu-B1e* and even poorer when associated with the *Glu-A1c* and *Glu-B1f*. In addition, the cultivars with B-LMW-GS allelic composition *aab* (for *Glu-A3, Glu-B3* and *Glu-B2* loci, respectively), when associated with the *Glu-A1c* and *Glu-B1d,* gave high quality, whereas when associated with the *Glu-A1c* and *Glu-B1e* or with *Glu-A1o* and *Glu-B1f*, the quality was very poor.

1. Introduction

Durum wheat (*Triticum turgidum* L.ssp. *durum* (Desf.)) is the preferred raw material for the production of pasta worldwide and couscous in North Africa and the Mediterranean region. Semolina/flour protein content and gluten composition are the main factors that determine the quality of durum wheat cultivars and pasta cooking quality (DuCros, 1987). Environment and potential interaction between environment and genotype are also important factors influencing the durum wheat grain quality parameters (Lerner et al., 2004).

The genetic improvement of protein content has been particularly hampered, not only by strong environmental influences, but also by the fact that a negative correlation has frequently been found between grain yield and seed protein content in segregating populations in all cereals (Marchylo et al., 2001). Two types of gluten protein: gliadin and glutenin (prolamins), have been studied to establish their relationships with pasta quality. In durum wheat, prolamins are coded by complex loci located in chromosomes of homeologous groups 1 and 6. The loci coding for the high molecular weight glutenin subunits (HMW-GS) are located on the long arms of chromosomes 1A and 1B and are called *Glu-A1* and *Glu-B1*, respectively (Payne et al., 1982), and the loci coding for the low molecular weight glutenins (LMW-GS) are located on the short arms of the same chromosomes (Singh and Shepherd, 1988), and are called *Glu-A3* and *Glu-B3*, respectively. Closely linked to these, the loci *Gli-A1* and *Gli-B1* encode the ω- and some γ-and β-gliadins (Singh and Shepherd, 1988). The loci *Gli-A2* and *Gli-B2* encode some

Abbreviations: BDR, Breakdown Resistance; HMW-GS, High Molecular Weight Glutenin Subunits; LMW-GS, Low Molecular Weight Glutenin Subunits; MT, Mixing Time; SDSS, Sodium Dodecyl Sulphate Sedimentation.

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γ-, β-, and α-gliadins and are located on the short arms of 6A and 6B chromosomes, respectively. Other minor loci involved in the expression of LMW-GS: *Glu-B2* and *Glu-B4*, have also been identified (Ruiz and Carrillo, 1993).

Previous studies have used the γ-gliadins 45 and 42 as markers for good and poor gluten quality, respectively (DuCros et al., 1982.) This is due to the genetic linkage with LMW-GS (Payne et al., 1984). In fact, pasta cooking quality and gluten strength were initially related to the negative and positive effects of the low molecular glutenin subunit patterns LMW-1 and LMW-2, respectively (Payne et al., 1984). The HMW-GS appear to have less critical effects than the LMW-GS on the gluten strength of durum wheat (Vázquez et al., 1996), but this has not been clearly established because of limited genetic variability at the *Glu-1* loci present in modern durum wheat cultivars used in published studies (Sissons et al., 2005). In this context, some studies have shown the existence of variability and new alleles for quality traits in durum wheat landraces (Aguiriano et al., 2008) and in related species (Sissons and Batey, 2003).

Combinations of SDS-sedimentation test, mixograph parameters and protein content have been found to be good predictors of cooked pasta quality (Dick and Quick, 1983). The primary objective of the work presented here was to use these tests to evaluate Mediterranean durum wheat cultivars and landraces for pasta quality in field trials sown in Tunisia and Spain to determine the influence of the environment on gluten strength. Secondary objectives were to analyse the variability and the genetic control of HMW-GS and B-LMW-GS in the cultivars in order to determine the allelic variation at each of the loci involved and to study any new alleles found and evaluate the effect of allelic variation on gluten strength.

2. Materials and methods

2.1. Plant materials

Thirty-seven old and modern durum wheat cultivars from Mediterranean countries (two from Italy, twenty two from Spain and thirteen from Tunisia) were collected (Table 1). All genotypes were sown in a randomized complete-block design with two replicates per genotype during the 2010–2011 season at two localities: Experimental Station of the Center of Biotechnology (Borj-Cédria, Tunisia) (36°42′ N, 10° 28′ E) and the Agronomy Engineers School Experimental Station (Madrid, Spain) (40° 25′ N, 3° 42′ W). In this site the soil was a well-drained Typic Xerorthents, with a sandy loam texture. The mineral fertilization was applied once in pre-sowing, 350Kg/Ha of 9N, 18 P_2O_5, 27 K_2 O. Throughout the growing season (November through July), the natural rainfall received 28, 78, 41, 30, 50, 57, 61, 29, 0 mm of water, respectively. The monthly average air temperature ranged from 6.2 °C in December to 25.1 °C in July. The Experimental Station is located in northern Tunisia and belongs to the semi-arid bioclimatic stage. The soil is loamy-clay classified as Xerofluvent. A mineral fertilization was applied, in pre-sowing for phosphorus (60 kg of P_2O_5 per ha) and post-sowing nitrogen (100 kg of ammonitrate at 33.5% (N)) with two fractions (lifting and tillering). The natural rainfall was 45, 50, 59, 34, 38, 27, 16, 8 mm of water during November to June, respectively. The site had air temperature that ranged from 8.4C° in January to 26.8C° in June.

2.2. Electrophoretic analysis

Glutenins were extracted using 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) (Payne et al., 1980). Gliadins were fractionated by A-PAGE (Lafiandra and Kasarda, 1985). HMW-GS alleles at *Glu-1* loci were identified using the nomenclature of Payne and Lawrence (1983). B-LMW-GS alleles at *Glu-3* and *Glu-2* loci were identified as described by Nieto-Taladriz et al. (1997).

2.3. Quality evaluation

A sample of grain was randomly taken from each replicate, cleaned and used for the determination of quality, therefore, four values for each quality test of the analyzed variety were obtained. Some of the varieties did not have material of the two repetitions of both locations. Three values of each quality parameter were recorded for the varieties: Chili, Clarofino de Balazote, Derbessi, Inrat69, Mahmoudi, Richi and Swabaa Algia, whereas, only two values were obtained for Farto and Recio Cañihueco. Protein content, on a 14% moisture basis, was estimated by near-infrared reflectance analysis (NIR) using a Technicon Infralyzer 300. Gluten strength was estimated by the SDS-sedimentation (SDSS) test according to Dick and Quick (1983). Rheological properties were determined by Mixograph of 10 g whole wheat meal (Finney and Shogren, 1972). The mixograph parameters measured were: mixing development time (MT), maximum peak height (MH), height at 3 min after the peak (H3), and resistance to breakdown (BDR) (percentage difference between MH and H3). The high values of SDSS and MT and lower of BDR were accorded to good quality.

2.4. Statistical analyses

The growing environment and allelic variation at each locus were considered as sources of variation. The data were analysed using SAS statistical software (SAS institute, Cary, NC). ANOVA and differences between mean values were analysed using the Duncan method for multiple comparisons at $P = 0.05$. PROC GLM and PROC MIXED were used to accommodate the unbalanced data. Pearson correlation coefficients were calculated to determine the relationships between mean values of the test results.

3. Results and discussion

3.1. Electrophoresis for prolamin composition

The electrophoretic analysis of prolamins carried out on five grains of each cultivar gave identical patterns for each one of the 37 cultivars. Electrophoretic analysis of the prolamins was also carried out on the same gel for the two replicates of each cultivar from the two localities in order to determine flour homogeneity. Table 1 shows the composition of the 37 cultivar prolamins analysed and Fig. 1 shows the composition of some cultivar prolamins.

3.1.1. HMW-GS (Glu-A1/Glu-B1)

Five different HMW subunits encoded by the *Glu-A1* locus were found (Table 1). These were the 1, 2* and Null subunits (*a*, *b* and *c* alleles, respectively, according to Payne and Lawrence (1983)) and two rare subunits (V and 2••). The V subunit (allele *o*) was only found in two landraces and the 2•• subunit (allele *y*) was only found in one Spanish landrace (Table 1). The most frequent subunit found was the Null subunit, while the 1 and 2* subunits were only found in Spanish landraces. The high frequency of the Null glutenin subunit of the *Glu-A1* locus has also been described in other studies of durum wheat from Mediterranean countries (Carrillo et al., 1990; Cherdouh et al., 2005; Nazco et al., 2014). The rare subunit V has been found previously in landraces of durum wheat and *Triticum dicoccum* (Vallega and Mello-Sampayo, 1987) and *Triticum turanicum* (Rodriguez-Quijano et al., 2010). The 2•• subunit was observed in some bread wheat genotypes (Giraldo et al., 2010).