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Grain filling duration and glutenin polymerization under variable nitrogen supply and environmental conditions for durum wheat

Roberto Ferrise [a], Marco Bindi [a], Pierre Martre [b,c,*]

[a] Department of Agri-food Production and Environmental Sciences, University of Florence, Piazzale delle Cascine 18, 50144 Florence, Italy
[b] INRA, UMR1095 Genetics, Diversity and Ecophysiology of Cereals, 5 Chemin de Beaulieu, F-63 039 Clermont-Ferrand, Cedex 02, France
[c] Blaise Pascal University, UMR1095 Genetics, Diversity and Ecophysiology of Cereals, F-63 177 Aubière, France

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ABSTRACT

The end-use value of durum wheat (*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.) is mainly governed by its grain protein concentration and composition. Adjustment of variables to compensate for inter-annual and location variations in semolina quality leads to high cost for processors in the wheat industry. A better understanding of the mechanisms governing environmental variations of grain protein composition is thus required. Here, a field experiment was setup in a Mediterranean environment with the aim to analyze the effect of sowing date and nitrogen (N) fertilization on the dynamics of grain dry mass, water, protein composition and glutenin polymer size distribution for durum wheat cv. Creso. The results indicated that (1) grain dry mass accumulation was related to grain water concentration and stopped at 44.9% independently of the growing conditions and N supply; (2) during the grain filling period as well as at ripeness maturity, the quantity of the different protein fractions scaled with the quantity of N per grain; (3) SDS-extractable glutenin polymers were produced continuously until the same grain water concentration as the dry mass deposition was reached; (4) SDS-unextractable polymeric proteins were found as early as 7 days after anthesis and their rate of accumulation increased sharply when grain dry mass was 60% of its final value and proceeded at a constant rate until ripeness maturity, thus suggesting that the insolubilization of glutenin polymers is not directly related to the rapid loss of water after physiological maturity, but rather to the continuous dehydration of the grain.

1. Introduction

In contrast with other cereals, most of the wheat production is used after processing, mainly by the pasta industry in the case of durum wheat (*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.), which require specific functional properties. These properties largely depend on structures and interactions of the grain storage proteins gliadin and glutenin (Gras et al., 2001; Shewry and Halford, 2002). Customers are becoming more discriminating in their quality requirements and inter-annual variability in product quality is becoming less acceptable, particularly for premium products (Marchylo et al., 2001). Therefore, adjustment of variables to compensate for inter-annual and location variations in semolina quality lead to high cost for processors in the wheat industry. A better understanding of the mechanisms governing environmental variations of grain protein composition is thus required.

The gliadin fraction accounts for 20% to 30% of total grain protein content and consists mainly of single chain polypeptides with molecular mass (M_r) ranging from 15 to 60 × 10^3, but most are in the narrow range 25 to 40 × 10^3 (Bunce et al., 1985; Wieser, 2007). The glutenin fraction accounts for 30% to 45% of total grain protein content and consists of very high M_r polymers (5 × 10^5 to more than 1 × 10^7) stabilized by inter-chain disulfide bonds and are partially insoluble in denaturing sodium dodecyl sulfate (SDS) solutions (Wieser et al., 2006). Glutenin polymers are made of low (LMW-GS; M_r 32 to 35 × 10^3; D'Ovidio and Masci, 2004) and high (HMW-GS; M_r 67 to 88 × 103; Gao et al., 2010) molecular mass subunits and account for 5% to 10% and 20% to 30% of total grain protein content, respectively. The rheological properties of wheat gluten and dough depend on the balance between monomeric gliadins and polymeric glutenins and most importantly on the M_r distribution of the latter (Weegels et al., 1996; MacRitchie, 1999; Don et al., 2003). The ratio of SDS-unextractable polymeric proteins (UPP) to total polymeric

Abbreviations: DAA, days after anthesis; DM, dry mass; EPP, total SDS-extractable polymeric proteins; GWM, mass of water per grain; SD, sowing date; SDS, sodium dodecyl sulfate; SPP, small-size polymeric proteins; LPP, large-size polymeric proteins; N, nitrogen; TPP, total polymeric proteins; UPP, SDS-unextractable polymeric proteins; %GWC, percent grain water concentration.

* Corresponding author at: INRA, UMR1095 Genetics, Diversity and Ecophysiology of Cereals, 5 Chemin de Beaulieu, Cedex 02, F-63 039 Clermont-Ferrand, France. Tel.: +33 473 624 351; fax: +33 473 624 457.
E-mail addresses: roberto.ferrise@unifi.it (R. Ferrise), marco.bindi@unifi.it (M. Bindi), pierre.martre@clermont.inra.fr (P. Martre).

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proteins (TPP) has often been used as a measure of the distribution of the M_r of glutenin polymers (e.g. Gupta et al., 1993; Larroque and Bekes, 2000) and has consistently been linked to dough and gluten rheological properties (Southan and MacRitchie, 1999; Morel et al., 2000; Ohm et al., 2009).

Extended periods of high temperature are common in most durum wheat-growing areas of the world and above optimal temperature is one of the major environmental factors affecting durum wheat yield and quality. Weaker dough from grains that experience one or several days of very high temperature (>30 °C) has been related to a marked decrease in the proportion of UPP, independently of changes in total grain protein concentration or proportions of gliadin classes or glutenin subunits (Ciaffi et al., 1996; Corbellini et al., 1998; Wardlaw et al., 2002; Don et al., 2005). Nitrogen (N) supply has strong effect on both grain protein concentration and composition. Higher rate of N application tend to increase the gliadin-to-glutenin ratio and to decrease the proportion of UPP, resulting in higher dough extensibility (e.g. Johansson et al., 2004, 2005; Godfrey et al., 2010). Significant interactions between growing temperature and N supply in grain protein composition and the size distribution of glutenin polymers have been reported (Johansson et al., 2005; Malik et al., 2011), which complicate the interpretation of their own effects under variable environmental conditions.

Some studies have suggested that the formation of UPP in both durum and bread wheat (*T. aestivum* L.) may also be controlled by the water status of critical grain structures and is triggered by the sharp increase in grain water loss at physiological maturity (Stone and Nicolas, 1996; Carceller and Aussenac, 1999, 2001). In contrast, other studies have suggested that the formation of UPP start during the grain filling period (Zhu and Khan, 1999; Panozzo et al., 2001; Johansson et al., 2005; Jia et al., 2012), maybe when a threshold concentration of monomer subunits or small-size polymers is reached (Gupta et al., 1996). Moreover, how growing conditions and N supply affect the dynamics of glutenin polymer formation in relation to the cessation of grain dry mass accumulation and changes in %GWC is largely unknown. These aspects of glutenin polymer formation (i.e. rate and duration of the accumulation) are particularly important to understand how the environment modifies the grain protein composition and to develop a phenomenological model of grain protein accumulation and polymer formation in response to environmental variations.

The aim of this work was thus to study the effect of sowing date and N fertilization, two important management practices, on the dynamics of grain dry mass, water mass and concentration, and protein composition and glutenin polymer size distribution for the durum wheat cv. Creso grown in the field in a Mediterranean environment. In particular, we aimed at testing the following hypothesis: (1) the quantity of the different grain protein fractions scales with the quantity of N per grain and the scaling relationships are independent of the growth conditions; (2) the formation of glutenin polymers is related to changes in grain water concentration; (3) the insolubilization of glutenin polymers is not directly related to the rapid loss of water after physiological maturity, but rather to the continuous dehydration of the grain and thus starts early during the grain-filling period.

2. Materials and methods

2.1. Experiment set up, plant sampling and grain dry mass, water mass and nitrogen determination

Rain-fed field experiments were carried out at the University of Florence, Italy (11°13′E, 43°46′N; 42 m elevation) during the 2002–2003 and 2004–2005 growing seasons (hereafter referred as 2003 and 2005, respectively). Seeds of the durum wheat (*T. turgidum* L. subsp. *durum* (Desf.) Husn.) cultivar Creso were sown on 11 December 2002 and 05 November 2004 (normal sowing, treatments termed 03SD1 and 05SD1, respectively), and 27 January 2003 and 18 January 2005 (late sowing, hereafter 03SD2 and 05SD2, respectively). Creso is a semi-dwarf Italian durum wheat cultivar, characterised by very limited cold requirements and a high sensitivity to photoperiod (Motzo and Giunta, 2007). Due to its moderate but constant yield and its great adaptability to Italian environmental conditions, it has been widely cultivated during past decades and is still in use (Arduini et al., 2006).

Three N treatments were applied with a total of 0, 6, and 18 g N m^{-2} (hereafter N0, N6, and N18, respectively). The treatments were arranged in a split-plot design with three blocks, where the main plots corresponded to the sowing dates. For a detailed description of the experiment set up and data collection the reader is referred to Ferrise et al. (2010). Daily weather data were recorded on a weather station adjacent to the field plots. Thermal time was calculated by summing daily degree-days, which were calculated as the daily mean air temperatures above a base temperature of 0 °C (Cao and Moss, 1989).

Within each subplot, 20 plants were randomly tagged and their phenological development determined as described in Tottman (1987) by daily inspections in the field. For each treatment, 16 mainstems were randomly collected at 5–7 days intervals starting at growth stage (GS) 71 (grain water ripe) in 2003 and at GS 65 (anthesis) in 2005. Spikes were hand threshed and grains were counted. Grain dry mass (DM) was determined on subsamples (ca. one-third of the whole sample weight) after oven drying at 80 °C for 48 h. Average single grain water mass (GWM) was calculated as the difference between fresh mass and DM divided by the number of grains of the subsample.

The remaining grains were freeze-dried and milled to whole flour using a rotor mill (Cyclomill, PBI, UK). Grain total N concentration was determined with the Dumas method (AOAC method no. 7.024) using a FlashEA 1112 NC Analyzer (Thermo Electron Corp., Waltham, MA, USA) and was expressed on a DM basis. Grain protein concentration was calculated by multiplying grain N concentration by 5.62 (Tkachuk, 1966; Mossé et al., 1985).

2.2. Protein extraction

Whole flour samples (75 mg) were stirred for 2 h at 60 °C in the presence of 7.5 mL of a 0.1 M sodium phosphate buffer (pH 6.9) containing 2% (v/v) sodium dodecyl sulfate (SDS), and were then centrifuged for 30 min at $37.5 \times 10^3 \times g$ at 20 °C (Dachkevitch and Autran, 1989) to obtain a supernatant (SDS-extractable protein fractions). The Pellets were then stirred for 10 min at room temperature in the presence of 7.5 mL of the same extractant, and the resulting dispersion was sonicated for 30 s at 10 W and 22.5 kHz (Daniel and Triboi, 2002), using a 3-mm diameter probe mounted on a XL—Microson™ ultrasonic cell disruptor (Misonic Inc., NY, USA). After centrifugation (30 min, $37.5 \times 10^3 \times g$, 20 °C), the supernatants (SDS-unextractable protein fractions) were collected. Both extracts were filtered through 0.20 μm regenerated cellulose filters (Titan2 HPLC filtration systems, Sun SRi, TN, USA) and stored at −20 °C in sealed HPLC vials until analysis.

2.3. Protein fractionation and quantification by size-exclusion high performance liquid chromatography

Proteins in the SDS-extractable and -unextractable fractions were fractionated by size-exclusion high performance liquid chromatography (SE-HPLC) using a Bio-Tek Kontron HPLC (Bio-Tek Instruments, Inc., VT, USA). A Kroma System 2000 V1.83 was used to control the pump and for acquisition and