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Molecular characterization of storage proteins for selected durum wheat varieties grown in different environments

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ABSTRACT

The variations of the amounts of individual high molecular weight glutenin subunits (HMW-GS), of the ratios HMW-GS*y* to HMW-GS*x* and HMW-GS to low molecular weight glutenin subunits (LMW-GS) and of protein content were evaluated for eight durum wheat cultivars in two regions using four fertilizer combinations during two successive years. All measured parameters showed significant variation with genotypes (G), environments (E) and fertilizers (F). The interaction E × G × F was highly significant for glutenin amount variation. Amongst cultivars possessing HMW-GS 20, landraces seem to better value the N-fertilizer use for the accumulation of HMW-GS*y* than high yielding cultivars. Both HMW-GS*y* to HMW-GS*x* and HMW-GS to LMW-GS ratios were found to be positively correlated ($p < 0.05$) with total protein content.

1. Introduction

The main storage proteins of cereals are gliadins and glutenins. Glutenins are polymeric fractions composed of high molecular weight and low molecular weight glutenin subunits (HMW-GS and LMW-GS) linked with intermolecular disulfide bonds. The HMW subunits are classified into two types on the basis of their molecular values and sequences: *x*-type which migrates more slowly on SDS-PAGE and *y*-type which migrates faster (Shewry and Tatham, 1990). The HMW-GS are the most studied protein fractions because of their relationship to gluten strength and bread-making quality (MacRitchie et al., 1990). The ratios between the different components of proteins have been shown to reflect the qualitative changes in durum wheat quality (Flagella et al., 2010; Triboï et al., 2000). A high HMW-GS to LMW-GS ratio has been associated with a better technological performance (Flagella et al., 2010). Fertilization and water availability are considered the most important environmental factors affecting protein content and composition. Nitrogen-supply has been shown to be accompanied by an increase of grain protein content, gliadin and glutenin content and the gliadin to glutenin ratio (Triboï et al., 2000). A water deficit through the growing season was shown to be accompanied with an increase in protein content and in HMW-GS to LMW-GS ratio (Flagella et al., 2010).

The aim of this paper was to assess the glutenin variation of eight durum wheat cultivars in different environments using the ratios HMW-GS*y* to HMW-GS*x* and HMW-GS to LMW-GS.

2. Material and methods

2.1. Cultivars and experimental conditions

The experiment was laid out as a randomized 3 block design with a factorial set of treatments that included 8 genotypes and 4 fertilizer treatments of nitrogen and potassium combinations (N_0K_0, N_0K_1, N_1K_0 and N_1K_1), each block including 32 experimental units. The eight cultivars were composed of 'Chili', 'Biskri', 'Mahmoudi', 'INRAT69' 'Karim', 'Razzak', 'Khiar' and 'Omrabiaa'. The

Abbreviations: CE, capillary electrophoresis; CIMMYT, International Maïze and Wheat Improvement Center; E, environment; F, fertilizer; G, genotype; HMW-GS, high molecular weight glutenin subunit; ICARDA, International Center for Agricultural Research in the Dry Areas; INRAT, Institut National de Recherches Agronomiques de Tunis; K, potassium; LMW-GS, low molecular weight glutenin subunit; N, nitrogen; P, protein content; SA, semi-arid; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SH, sub-humid.

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experiment was repeated in two regions of Tunisia: semi-arid (Kef) and sub-humid (Bousalem) and during two growing seasons (2007 and 2008).

2.2. Glutenin extraction

A glutenin extraction was carried out on 3 samples from each variety as described by Fu and Kovacs (1999) and Rhazi et al. (2009) with some modifications. Samples of 30 mg of flour were stirred for 15 min at room temperature with 1 ml of 0.08 M Tris–HCl buffer (pH = 7.5) containing 50% propan-1-ol, then they were centrifuged at 15,900 g for 10 min at 15 °C. The supernatant was discarded (eliminating albumins, globulins and gliadins). The pellet was added to 600 μl of the buffer containing Tris–HCl 0.2 M (pH = 9.2), 2% (w/v) of SDS and 1% of DDT, and dispersed by sonication with amplitude of 40% for 40 s using a stepped microtip probe of 3 mm diameter (ultrasonic Processor, Sonics, model 75,038) then the mixture was maintained at a temperature of 60 °C for 20 min and centrifuged at 12,500 g for 10 min at 20 °C.

2.3. Separation of glutenin subunits by CE LabChip 90®

Analysis of the glutenin subunits was carried out on capillary electrophoresis (CE) LabChip 90® plateform as described by Rhazi et al. (2009). The 384 samples (8 genotypes × 4 fertilizer treatments × 4 environments × 3 blocks) were used for quantitation of HMW-GS and estimation of HMW-GS to LMW-GS ratio and HMW-GS*y* to HMW-GS*x* ratio. Results were expressed as percentage of the time corrected area of individual HMW-GS to the total HMW-GS area. The cultivar set was analyzed 3 times using multiple Chips.

2.4. Protein content evaluation

Grain protein content (P) was determined using the NIRS system (near infrared spectroscopy using a Perten-Inframatic-8600).

2.5. Statistical methods

Analysis of variance was carried out using proc anova and option proc mixed of SAS (version 9.1). When first or second order interactions were significant, the option PDMix800 was used to compare means. Proc corr was used to evaluate relationships between protein content and ratios between the different protein components.

3. Results and discussion

3.1. Wheat properties

The durum wheat genotypes used in this study were composed of four landraces 'Chili', 'Biskri', 'Mahmoudi' and 'INRAT69' and four high yielding varieties 'Karim', 'Razzak', 'Khiar' and 'Omrabiaa'. Landraces and high yielding varieties are known for their contrasting qualities (Daaloul Bouacha et al., 2014). Using CE Lab-On-Chip technique, different Glu-B1 HMW-GS patterns were identified: 6 + 8, 7 + 8 and 20 (Fig. 1). The high yielding varieties 'Karim', 'Razzak' and 'Khiar' possessed the subunits 7 + 8. 'Biskri' possessed the 6 + 8 composition and 'Chili', 'Mahmoudi', 'INRAT69' and 'Omrabiaa' possessed the HMW-GS 20.

3.2. Impact of genotypes (G), environments (E), fertilizers (F) and their interactions on total protein content and quantitative parameters of glutenins

In this study, a combination of one region (sub-humid or semi-arid) and one cropping season (2007 or 2008) was considered as a separate environment (E). HMW-GS*y* and HMW-GS*x* are complementary and their distribution followed an inverse order of classification amongst cultivars and they were found to be negatively correlated. The strong relationship between these two fractions is due to the fact that they are very tightly linked genes on each locus. Therefore, only the results from HMW-GS*y* were reported.

3.2.1. Genotypic impact

Genotype was a significant source of variation for individual HMW-GS amounts, for HMW-GS to LMW-GS ratios and for HMW-GS*y* to HMW-GS*x* ratios (Table 1). This is in accordance with Luo et al. (2000) indicating that the content of HMW-GS and LMW-GS was genetically controlled. Furthermore, Fuertes-Mendizábal et al. (2010) found that the relative proportions of HMW-GS subunits were strongly conserved by genetic determination.

HMW-GS to LMW-GS ratios showed significant ($p < 0.05$) inter-genotype variations. Karim showed the highest ratio with a mean of 25.10 followed by Biskri and Khiar with respectively 23.3 and 23.29 (Table 2). Cultivar Chili showed an intermediate ratio with a mean of 19.75. Mahmoudi and INRAT69 were comparable to Chili with similar values ($p > 0.05$). The lowest HMW-GS to LMW-GS ratio was observed with Omrabiaa. These variations were attributed to a genotype factor which was considered the only significant source for the quantitative variation of HMW-GS and LMW-GS (Luo et al., 2000).

The HMW-GS relative amounts varied significantly with genotypes (Table 2). Cultivar Biskri showed the highest amounts of HMW-GS*y* with a mean of 38.04%, followed by Mahmoudi, Karim, Razzak and Khiar with similar values ranging from 21.60% to 22.14%. Omrabiaa showed the lowest amount of HMW-GS*y* with a mean of 17.22%.

Changes in the relative quantity of HMW-GS were also reflected in the variation of HMW-GS*y* to HMW-GS*x* ratios. Comparison amongst the HMW-GS*y* to HMW-GS*x* ratio means of the eight cultivars according to their HMW-GS composition showed that

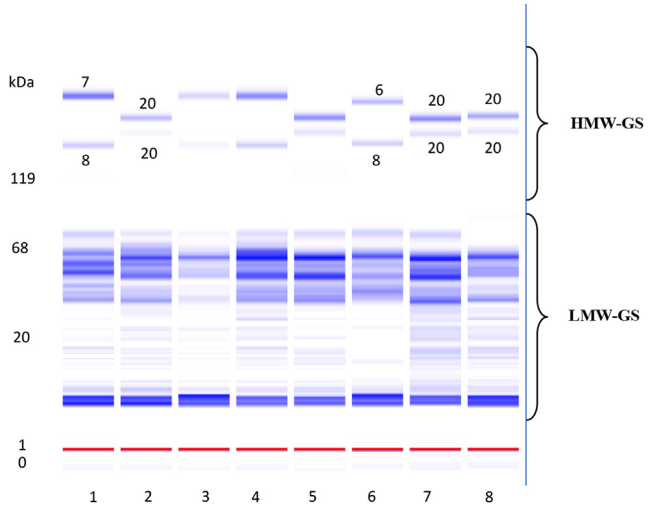

Fig. 1. Gel image overlay of glutenin subunits from cultivars Karim, Omrabiaa, Razzak, Khiar, Chili, Biskri, Mahmoudi and INRAT69, respectively: 1, 2, 3, 4, 5, 6, 7 and 8, separated by EC LabChip 90.