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Abiotic stress induced changes in protein quality and quantity of two bread wheat cultivars

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ABSTRACT

Protein quantity and quality of wheat flour have been found to be influenced by abiotic stress conditions. The aim of this study was to determine the effect of heat, drought and cold stress on protein quantity and quality using reverse phase and size exclusion high performance liquid chromatography in two bread wheat cultivars. Heat stress caused a significant increase in protein content of both cultivars, while drought stress caused a significant protein increase in Duzi. Sodium dodecyl sulphate (SDS) soluble large monomeric proteins were significantly increased by heat stress in both cultivars, and SDS insoluble large polymeric proteins were significantly increased by heat and drought stress in Kariega. SDS insoluble small polymeric proteins were significantly decreased by all three stress conditions in Duzi and heat stress in Kariega. Heat and drought stress had similar effects on protein, but cold stress caused different effects. *GluD1* subunit 2 was least influenced by stress conditions, but *GluD1* subunit 12 was significantly reduced by all stress conditions. *GluA1* subunit 2* and *GluB1* subunit 17 were significantly increased by heat and drought in Kariega. Heat stress had the largest effect on protein quality and quantity, and there was a large genotype effect.

1. Introduction

High temperatures and drought stress are already having a large effect on wheat production, and modelling suggests that this trend will continue, with associated detrimental effects on wheat production (Battisti and Naylor, 2009). Plants have numerous mechanisms, involving changes on molecular, cellular and physiological level, to cope with environmental stresses, with the ultimate goal to produce viable seed. These changes ultimately influence the accumulation of protein and starch in the seed, which directly affect quality (Altenbach, 2012).

Heat and drought stress are often related. In wheat these two types of stress can have severe effects on rheological characteristics of the dough (Li et al., 2013; Zhang et al., 2013). Protein quality as well as the amount (quantity) is important for good bread-making quality (Békés et al., 2006). Both quality and quantity of protein were reported to be influenced by heat and drought stress (Li et al., 2013). Low temperature stress has similarly been implicated in affecting proteins in wheat (Xu et al., 2013). Gluten is a very large component of the protein in wheat and is composed of polymeric glutenins and monomeric gliadins and comprise about 78–85% of total wheat proteins (Horvat et al., 2009). The glutenins consist of low molecular weight (LMW) and high molecular weight (HMW) subunits, associated through inter-chain disulfide bonds (Mamone et al., 2009). HMW glutenin subunits (HMW-GS) play an important role in bread-making quality (Gao et al., 2010; Ji et al., 2012). They are the major determinants of gluten elasticity and comprise about 20–30% of gluten (Shan et al., 2007). Although LMW-GS have received less attention than HMW-GS, they are of importance in determining the quality and end-use properties of grain as their ability to form large aggregates also relates to dough strength (Juhasz and Gianibelli, 2006; Gil-Humanes et al., 2012). Gliadins are another major component of the gluten complex and the most abundant storage protein in wheat seed, amounting to about 40%, by weight of wheat-flour protein (Metakovsky and Graybosch, 2006). Gliadins may have an important contribution to the quality variation of many parameters and providing viscosity and gluten extensibility which is a constituent of dough strength (Gao et al.,

Abbreviations: FPC, flour protein content; HPLC, high performance liquid chromatography; insol, SDS insoluble; LMP, large monomeric proteins; LPP, large polymeric proteins; RP-HPLC, reverse phase HPLC; SDS-PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis; SE-HPLC, size exclusion HPLC; SMP, small monomeric proteins; sol, SDS soluble; SPP, small polymeric proteins.

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2010). The monomeric gliadins and polymeric glutenins are associated with quality differences among wheat cultivars (Liu et al., 2009; Mamone et al., 2009). Studies using size exclusion high performance liquid chromatography (SE-HPLC) revealed that the amount and size distribution of polymeric proteins were important in dough strength and bread-making quality (Gupta et al., 1993). The use of sonication with SE-HPLC made it possible to solubilize proteins and accurately determine all the protein classes (Singh et al., 1990).

2. Materials and methods

2.1. Greenhouse trials

Two hard red spring wheat irrigation cultivars (Duzi and Kariega) were selected for the trials as they both have excellent baking quality characteristics. There are two seed sources of Kariega in South Africa where one source has subunits 2 + 12 and the other has 5 + 10 coded by *GluD1*. In this study the source with subunit 2* at *GluA1*, 17 + 18 at *GluB1* and 2 + 12 at *GluD1* was used, which is exactly the same as for Duzi.

The two cultivars were planted in 3 l pots filled with soil, in the greenhouse. Four treatments were applied to the two cultivars where each treatment was applied to 15 pots per replication, three replications and three plants per pot. A randomized complete block design with two factors, treatments and cultivars, was used. Greenhouse temperatures were set at 15 °C/22 °C (night/day). Fertilization was applied to assure optimal growing conditions. Optimal watering of the pots was done throughout the experiment for the two temperature regimes and the control, and up to the soft dough stage for the drought stress experiment. The trial was done from May to the end of October in 2012 and was repeated in the same time frame in 2013. As soon as the main tillers in each pot reached soft dough stage, treatments commenced. The soft dough stage is when wheat kernels contain approximately 50% moisture and is classified as a value of 85 on the Zadoks scale (Zadoks et al., 1974). The main tiller of each plant was marked with a tag before treatment commenced. For the cold treatment, plants were placed in climate cabinets in the following cycle: 5 °C for 30 min then 1 °C less every 30 min until it reached −5.5 °C, then it was left for 3 h after which it was increased to -2 °C for 30 min, then 0 °C for 30 min; then 2 °C for 30 min; then 5 °C for 30 min; then back to green-house to optimal conditions. This treatment was structured in such a way to closely resemble field conditions in the spring wheat planting areas where cold spells are often experienced after anthesis. For the heat treatment, plants were placed in climate cabinets at 15 °C/32 °C (night/day) temperatures for 72 h and then returned to the green-house. To induce drought stress, watering was withheld until severe wilting was visible and then watering was resumed. The control treatment was left in the green-house under optimal conditions until harvesting. At harvesting the seed of the marked main tillers of the plants of the 15 pots per replication were bulked for each of the treatments and cultivars. The seed was milled to a whole flour with a laboratory mill (IKA A10 Yellowline analysis grinder, Merck Chemicals Pty Ltd). Milled samples were tested with sodium dodecyl sulphate polyacrylamide electrophoresis (SDS-PAGE) and they all tested genetically pure and true to type (data not shown).

2.2. Reverse phase high performance liquid chromatography (RP-HPLC)

Analyses were done according to Vawser and Cornish (2004). Samples were analysed in duplicate. Routine analyses were performed on a Shimadzu 20A HPLC system with Shimadzu PDA detector equipped with a Class-VP™ detector chromatography data system for integration events (Shimadzu Scientific Instruments, Japan) and a 250 × 4.6 mm Jupiter 5uC18 Phenomenex column (Phenomenex Inc., USA). Column temperature was 50 °C; injection volume was 50 µl; quantification was achieved by using a detection wavelength of 210 nm. Absorbance units under the different peaks were calculated according to Vawser and Cornish (2004).

2.3. SDS polyacrylamide gel electrophoresis (SDS-PAGE)

Fraction collection (Gilson FC204 Fraction Collector) was done for every peak eluted during RP-HPLC using the procedure of Vawser and Cornish (2004) to confirm nomenclature of fractions in the two cultivars. The method described by Larroque et al. (1997) was used for concentrating the collected fractions.

A simplified procedure for one dimensional (1-D) separation of glutenin subunits was followed (Singh et al., 1991). Gel preparation was done according to Laemmli (1970). The nomenclature by Payne and Lawrence (1983) was used for identification of HMW-GS.

2.4. Size exclusion high performance liquid chromatography (SE-HPLC)

The procedure of Gupta et al. (1993) was followed and absorbance areas under the different peaks were also calculated using the procedure of these authors. The following fractions were measured at specific time intervals, which were modified slightly from the original: large polymeric proteins (LPP), 4.57–5.54 min; smaller polymeric proteins (SPP), 5.54–6.98 min; large monomeric proteins (LMP) mainly gliadins, 6.98–8.61 min; smaller monomeric proteins (SMP) mainly albumins and globulins, 8.61 up to where the trace cuts the baseline.

2.5. Protein content

Protein content (AACC procedure 46-30) of the flour samples obtained from AACC procedure 44–15A (AACC, 2000), was determined as a percentage.

2.6. Statistical analysis

Analysis of variance was done on the data for both genotypes, four treatments and two seasons using Agrobase as a three factor analysis (2015). An ANOVA was also done for the two cultivars separately for the two seasons combined, in order to determine within each cultivar the effect of stress conditions on the measured characteristics.

3. Results

Cultivar effect was significant or highly significant in the case the SDS soluble large monomeric proteins and small polymeric proteins (Table 1). Since subunits 2 and 18 showed up as one peak on the RP-HPLC (Fig. 1), the point of separation between the two had to be established by fraction collection and then confirmed by running on a gel (Fig. 2). Mean squares of all the subunits identified with RP-HPLC were highly significant or significant, indicating that although the two cultivars had the same banding pattern, there was a significant difference in the amount of the subunits expressed in the two cultivars. Treatment effect was significant or highly significant in the case of flour protein content, the soluble and insoluble large monomeric proteins, the insoluble small monomeric and large polymeric proteins and both the small polymeric protein fractions. The only subunit sensitive to the treatments was subunit 12. Protein content was highly influenced by season, as were