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Effects of temperature increase, through spring sowing, on antioxidant power and health-beneficial substances of old and new wheat varieties

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ABSTRACT

The effects of heat stress on the potentially health-beneficial compounds of two old durum wheat genotypes, Timilia and Cappelli and a more recent cultivar, Claudio, were analysed following sowing in winter and in spring. Grain profiling was performed for content of: resistant and not-resistant starch, carotenoids, tripeptide glutathione, GSH and glutathione disulfide, GSSG. Hydrophilic and lipophilic grain extracts were analysed for polyphenol content and antioxidant capacity using two *in-vitro* assays: 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical quenching and Fremy's salt radical quenching. The antioxidant activities of hydrophilic and lipophilic extracts determined by the two tests were not related. ANOVA and multivariate discriminant analyses showed that genotypic effects had the most significant role in the determination of grain quality. Spring sowing increased the lipophilic/hydrophilic polyphenol ratio, across all of the genotypes. It also enhanced the content of resistant starch (+68%) for Claudio and GSH (+14%) for Timilia. In contrast, if sown in winter rather than spring, Cappelli accumulated more carotenoids (+8.6%) and Timilia accumulated more resistant starch (+81%). Spring sowing had a detrimental effect on yield, but a positive effect on 1000 kernel weight. Consequently, it may lead to a net accumulation of healthy substances and not a relative increase due to grain shrivelling.

1. Introduction

On the basis of predictive climate models, the consequence of global warming across Europe is expected to be thermal stress, rather than drought (Semenov and Shewry, 2011). Abiotic stress during kernel ripening could have a detrimental impact on wheat yield, as it can result in lightweight and shrivelled seeds (Farooq et al., 2011). At the same time, the metabolic adaptation of plants, which is necessary to counteract potential damage to biological tissues from excessive heat, can affect their content of health-beneficial components (Singh et al., 2008).

Although how such molecules play a role in mammals in terms of their health-promoting and/or lifespan-increasing properties is not completely understood (Poljsak and Milisav, 2013), benefits can result from their increased intake. In fact, health beneficial compounds can become deficient not only due to incorrect eating habits, but also to alterations in macro and micro-nutrients caused by environmental stress or by storage, transport and the processing of foods (Giusti et al., 2008).

Antioxidants (Dinelli et al., 2009; Fratianni et al., 2013; Heimler et al., 2010) and high quality starch components (Lafiandra et al., 2010) are some of the phytochemicals of particular interest in durum wheat, in terms of their health-beneficial effects. Wheat starch is mainly composed of amylopectin and amylose. Amylose is an essential component in the so-called resistant starch, which is the carbohydrate fraction that is not broken down by human

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enzymes in the small intestine (Chawla and Patil, 2010). This resistant starch has similar effects to those of dietary fibre, as it contributes to faecal bulk, reduces plasma cholesterol and the glycaemic index (Chawla and Patil, 2010). At least 31 antioxidants, or groups of compounds, with antioxidant properties can be found in the whole grain of cereals (Fardet, 2010). The low-molecular-weight thiol, glutathione, is a powerful antioxidant that plays a central role in plant tolerance to abiotic stress (Colville and Kranner, 2010), as well as in human health. Dietary glutathione can be absorbed in the upper section of the small intestine, and after oral introduction, its plasma concentration increases (Jones et al., 1992). Carotenoids (including carotenes and xanthophylls) are lipid-soluble antioxidants that protect against excess solar radiation reaching the chloroplasts (Hirschberg, 2001), and are involved in the biosynthesis of vitamin A in humans (Zile, 1998). Polyphenols are a large group of micronutrients that defend plants against oxidative stress and pathogens. These antioxidants are present in wheat (Heimler et al., 2010) and appear to help prevent cancer, cardiovascular and neurodegenerative diseases, with efficacies and bioavailabilities that largely depend on their content and nature (Manach et al., 2004).

In this study, to investigate the qualitative changes in the sources of the antioxidant power, polyphenol content and antioxidant potential were analysed on hydrophilic and lipophilic extracts of the grain. The radical scavenging activities of these hydrophilic and lipophilic extracts were determined following two *in-vitro* assay systems: 1,1-diphenyl-2-picrylhydrazyl radical (DPPH$^{\bullet}$) quenching and Fremy's salt radical quenching. The antioxidant activities were determined using these two quenching systems, as the antioxidant activities determined can change depending on the analytical methods used. To the best of our knowledge, there have been no other reports in the literature on the screening of the antioxidant activities of different solvent extracts and fractions of durum wheat grain.

Researchers strive to find worldwide sources of biodiversity in terms of the content and composition of health-beneficial compounds in plant tissues. It is widely recognised that old plant genotypes are sources of biodiversity (Heimler et al., 2010; Dinelli et al., 2009). In Italy, the old landraces and varieties, which underwent intensive breeding programmes at the beginning of the twentieth century, were replaced after the Second World War with the modern semi-dwarf and high-yielding cultivars.

Our research provides an evaluation of the agronomic performance and grain quality of three durum wheat genotypes, in relation to different sowing dates. Durum wheat is a facultative crop, and delaying sowing provides a more natural way for the plants to be exposed to higher temperatures in the field, while the majority of previous studies have been conducted in controlled environments. Spring sowing also allows plants to be exposed to higher temperatures throughout the whole of their growth cycle. To avoid the superimposition of drought stress on this high temperature stress, the spring sown wheat was supplied with supplemental irrigation, during the ripening phase.

2. Experimental

This study was carried out in the fields of the CER (Cereal Research Centre), outside Foggia (Italy). Two old genotypes of wheat cultivars (*Triticum turgidum* L. var. *durum*) were used: Cappelli was released during the 1920s; Timilia is a landrace (Venora et al., 2002)), often sown in the spring in Sicily. Claudio is a more recent high-yielding cultivar, which was released in 1998 (Heimler et al., 2010).

The experiment was laid out in a split-plot design with 3 repetitions. Two sowing dates were implemented as the main plot: winter sowing (on 19 December 2011) and spring sowing (on 3 March 2012). Each main plot was divided into 3 sub-plots (1.35×7.50 m^2) to accommodate different wheat genotypes. Fertilisation was administered at stem elongation, using 300 kg/ha ammonium nitrate. No disease infections were noted during the plant growth. The seedling stands were measured 25 days after sowing. In duplicate within each plot, 1-m-long rows were randomly selected and the numbers of seedlings/row were counted. In June, three supplemental irrigations were carried out to ensure that spring sowing had adequate water availability, compared to winter sowing. The total irrigation amount was 58.2 mm and was calculated according to the FAO Penman–Monteith method (Allen et al., 1998), considering a monthly reference evapotranspiration of 132.9 mm and an average crop coefficient of 0.44. At harvesting, grain yield was determined by harvesting the whole plot. Hectolitre weight and 1000 kernel weight and all the analytical determinations were performed on one grain sample from each plot, and hence in triplicate for each genotype.

2.1. Resistant and not-resistant starch

The contents of resistant and not-resistant starch were determined using Megazyme resistant-starch kits (K-RSTAR, Megazyme International Ireland Ltd, Bray, Ireland). Briefly, the kernels (0.1 g) were ground in a porcelain mortar and incubated in a shaking water bath with 4 ml pancreatic α-amylase and amyloglucosidase, for 16 h at 37 °C. The reaction was terminated by adding 4 ml 99% ethanol:methanol solution (95:5; v/v), followed by centrifugation at $1500 \times g$ for 10 min. The supernatants were recovered and separated into different tubes, while the pellets were washed twice by resuspension in 4 ml 50% ethanol:methanol (95:5; v/v) aqueous solution, with the previous centrifugation repeated. The supernatants were combined and diluted 5-fold in ethanol to measure the content of non-resistant starch. The resistant starch that remained in the pellets was dried by decanting and then dissolved in 2 ml 2 M KOH, with stirring for 20 min in an ice-water bath using a magnetic stirrer. This solution was neutralised by the addition of 8 ml 1.2 M sodium acetate buffer, pH 3.8 and the starch was hydrolysed after incubation with 330 units of amyloglucosidase, in a water bath at 50 °C for 30 min. After this incubation, the extracts were centrifuged as described above and the supernatants were separated to measure the amounts of resistant starch. Finally, the D-glucose content was measured using a colour reaction, with aliquots of 0.1 ml of both supernatants incubated with 3 ml glucose oxidase/peroxidase reagent, in a water bath at 50 °C for 20 min, and then read at 510 nm. The total starch content is the sum of the resistant starch and the non-resistant starch calculated against a D-glucose standard, which is converted to g glucose and then to starch (assuming the equivalence of 1 g glucose to 0.9 g starch) per 100 g kernel dw.

2.2. Proteins and carotenoid

For each plot sample, two semolina sub-samples were analysed using a near-infrared (NIR) scanning spectrophotometer (FOSS XDS rapid Content TM analyser) for proteins and carotenoid. The instrument was calibrated for protein content by the Kjeldahl method, using the factor $5.7 \times$ N% for conversion, in accordance with the 46-13 procedure (AACC, 2000). The instrument was calibrated for the contents of carotenoid pigments according to the previous American Association of Cereal Chemists (AACC) standard method 14-50 (AACC, 2000), based on the extraction of total pigments with water-saturated 1-butanol and subsequent spectrophotometrical measurement with β-carotene as the reference.