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Variation of total antioxidant activity and of phenolic acid, total phenolics and yellow coloured pigments in durum wheat (*Triticum turgidum* L. var. *durum*) as a function of genotype, crop year and growing area

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This paper reports the results of a study carried out to investigate the effects of genetic and environmental factors on the total antioxidant activity and on the occurrence of certain antioxidant compounds in durum wheat. The content of phenolic acids (PAs) and of total phenolics (TPC), both occurring as soluble free, soluble conjugated and insoluble bound forms, as well as the yellow coloured pigments (YCP) and the level of total antioxidant capacity (TAC) were determined in ten durum wheat genotypes grown in the same experimental field over three consecutive crop years. Three of the investigated genotypes were also grown in other two Italian cultivation areas over the same period. Crop year, genotype, growing area and their interactions significantly affected the variables under study. The content of PAs and TPC was mostly affected by environment, whereas YCP content and TAC level were principally influenced by genetic factors.

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1. Introduction

Regular consumption of cereals, especially as whole grains, has been associated with a reduced risk of several chronic illnesses such as diabetes, cardiovascular diseases and cancer. Many potential mechanisms for this protection effects have been proposed and most of them have been related to the content of nutrients and phytochemicals in whole grains (Slavin, 2004). Whole grains are well known to contain high amounts of dietary fibre, various minerals and vitamins and a variety of bioactive compounds, most of which are antioxidants, which are believed to play an important role in disease prevention and health-promoting effects. These bioactive compounds include, among others, tocopherols, phytosterols, carotenoids and phenolic acids (PAs) (Slavin, 2004).

PAs represent the most common phenolic compounds in cereal grains where they occur in three different forms including free PAs, PAs conjugated to low molecular weight molecules (e.g. sugars, sterols) and PAs bound to cell wall polysaccharides such as arabinoxylans (Adom et al., 2003; Fernandez-Orozco et al., 2010; Li et al., 2008). The bound form has been widely recognized as the most abundant one in wheat, accounting for over 80% of total PAs, followed by the conjugated and lastly by the free form, generally representing 1–2% of total PAs (Fernandez-Orozco et al., 2010; Li et al., 2008). Among all PAs detected in cereals, ferulic acid represents the greatest portion, but also *p*-coumaric, vanillic and other acids are commonly found (Brandolini et al., 2013; Li et al., 2008).

PAs are secondary metabolites which, as many other antioxidants, are synthesized as a part of multifunctional defence system against biotic and abiotic stresses in plants. In particular, they

Abbreviations Used: ABTS, 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid); DHB, 3,5-dichloro-4-hydroxybenzoic acid; dm, dry matter; ESI, electrospray ionization; FAE, ferulic acid equivalent; IS, internal standard; MS, mass spectrometry; PAs, phenolic acids; PCA, principal component analysis; PDA, photodiode array; RP-HPLC, reversed-phase high-performance liquid chromatography; RSD, relative standard deviation; SD, standard deviation; TAC, total antioxidant capacity; TEAC, Trolox equivalent antioxidant capacity; TPC, total phenolic content; YCP, yellow coloured pigments.

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apparently act as defence against herbivores, microbes, viruses or competing plants as well as protecting the plant from oxidation (Lattanzio et al., 2006).

Several studies have shown that different conditions, including genotype and environment, may have a significant impact on the occurrence of antioxidants and, therefore, on the antioxidant activity of cereals (Lv et al., 2013; Nurmi et al., 2010). Genetic effects were considered by Mpofu et al. (2008) who, evaluating data from 11 studies previously carried out, showed that genotype significantly affects total phenolic content (TPC) and ferulic acid concentration in common wheat.

Other studies have investigated environmental effects, such as the role of specific climate parameters (rainfall and temperature) on the content of phenolics, reporting, however, outcomes often in contrast to each other. For instance, some studies have pointed out a negative correlation between high temperature and TPC (Moore et al., 2006; Yu and Zhou, 2004), others have registered an increase in soluble phenols and anthocyanins in wheat subjected to drought and UV-B stresses (Alexieva et al., 2001).

The effect of genotype and environment on the occurrence of antioxidant compounds in cereals has been mostly investigated in common wheat and less in durum wheat. Mpofu et al. (2006) observed that the content of some PAs in hard spring wheat was more affected by environment than genotype, but without showing clear correlation between PA content and atmospheric conditions (i.e. growing temperature and rainfall from anthesis to maturity). More recently, a study within the HEALTHGRAIN Project reported that environment affects PA content (especially the free and conjugated forms) in common wheat, observing that rainfall level before heading period may be a more relevant factor than total precipitation (Fernandez-Orozco et al., 2010). Regarding specifically durum wheat, Menga et al. (2010) observed that TPC was mainly influenced by the growing area and secondly by genotype and growing area × genotype interaction. Additionally, in a preliminary study we observed that genotype, environment and crop year seem to affect the three forms of PAs to different extents (Martini et al., 2014).

The aim of the present study was to examine the effects of genotype, crop year and growing area on the total antioxidant activity (TAC), on the content of certain antioxidant compounds in durum wheat, such as phenolic acids and total phenolic compounds, both occurring in the three different forms, and yellow coloured pigment (YCP). The study has been conducted using 10 different durum wheat genotypes grown in the same experimental field over three consecutive crop years. Three of the considered genotypes were also grown over the same period in other two different Italian agro-climatic regions to investigate the influence of the growing area on the occurrence of the compounds under study and on the total antioxidant activity.

2. Materials and methods

2.1. Chemicals

Analytical-reagent grade formic acid, ethanol, hydrochloric acid, acetic acid, sodium hydroxide, sodium carbonate, Folin–Ciocalteu reagent, ethyl acetate, 1-butanol, and HPLC grade acetonitrile and methanol were obtained from Carlo Erba Reagents (Milan, Italy). Vanillic acid, *p*-hydroxybenzoic acid, syringic acid, *p*-coumaric acid, sinapic acid, ferulic acid, 3,5-dichloro-4-hydroxybenzoic acid (DHB) were purchased from Sigma–Aldrich (Milan, Italy), as well as cellulose, 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), potassium persulfate and 6-hydroxy-2,5,7,8-tetram ethylchroman-2-carboxylic acid (Trolox) for TAC analysis. Deionised water was produced by a Milli-Q unit (Millipore, Bedford, MA, USA).

2.2. Plant material and growing condition

Ten genotypes of durum wheat (*Triticum turgidum* L. var. *durum*) were selected among those largely cultivated in Italy, in order to evaluate the impact of genotype on PAs, TPC, YCP and on TAC. Plants were grown in an experimental field located in Montelibretti (Rome, Italy, 42.14° North latitude, 12.74° East longitude) during 2009/10, 2010/11 and 2011/12 crop years (henceforward called 2010, 2011 and 2012). Among the 10 genotypes, 3 of these (Saragolla, Simeto and Svevo) were grown in other two Italian sites (Jesi, 43.31° North latitude, 13.14° East longitude, and Foggia, 41.46° North latitude, 15.54° East longitude) during the same 3 crop years, in order to investigate the effects of growing area, crop year and their interaction. Both genotypes and agro-climatic areas were selected among those included in a national network of experimental trials on durum wheat grown using conventional practices, annually coordinated by C.R.A.-Unità per la Valorizzazione Qualitativa dei Cereali (http://qce.entecra.it/frduro/dblist3.asp). Furthermore, Foggia and Jesi are representative of the two regions (Puglia and Marche) with the highest durum wheat cultivated surfaces in the South and in the Centre–North of Italy, respectively.

Each genotype was grown in 10 m^2 plots in a randomized block with three replicates, according to agronomic practices traditionally employed in the relative selected areas (Quaranta et al., 2010).

Considering the genotypes, the main grain quality characteristics were similar among environments. During the 3 crop years, the 3 genotypes grown in Montelibretti, Jesi and Foggia had mean test weight of 80.4 ± 3.1, 80.8 ± 3.6 and 80.9 ± 1.6 kg/hL and mean 1000 kernels weight of 45.8 ± 4.5, 47.1 ± 5.3 and 49.0 ± 5.3 g, respectively. Lastly, mean protein content was 13.6 ± 0.7, 14.2 ± 0.9 and 13.1 ± 0.8 % dry matter (dm) in the 3 growing areas (http://qce.entecra.it/frduro/dblist3.asp).

Regarding the environment, climatic conditions (rainfall and temperature) were registered in the 3 sites along the 3 crop years and in particular during the grain filling period (April–June). During this period, mean minimum and maximum temperatures among the 3 areas were 10.6 ± 0.8 and 23.5 ± 0.8 °C (2010), 11.1 ± 0.6 and 25.0 ± 1.3 °C (2011), 10.0 ± 2.2 and 26.9 ± 2.8 °C (2012), respectively (www.sian.it). The highest amounts of rainfall were registered on 2010, although with a high variability among environments: in fact, during the April–June period, mean rainfalls registered in the 3 cultivation fields were 301 ± 170, 128 ± 25 and 160 ± 59 mm on 2010, 2011 and 2012, respectively.

After harvesting, grains were milled by a laboratory mill (Cyclotec, PBI, Milan, Italy) to 1 mm particle size and the resulting samples were immediately stored at −20 °C until their use for the planned analyses. For all samples, moisture was measured just before the chemical analyses on 3 g of milled sample by a Sartorius MA35 thermobalance (Muggiò, Monza-Brianza, Italy) at 120 °C.

2.3. Analysis of phenolic acids (PAs)

The 3 different forms of PAs occurring in durum wheat were individually extracted by a two step extraction method previously described (Nicoletti et al., 2013), using either 250 or 100 mg wholemeal for extracting the less abundant soluble free and conjugated forms and the more abundant insoluble bound PAs, respectively. Briefly, a common extraction step was performed using 80/20 (v/v) ethanol-water solution, then soluble free PAs were extracted twice with ethyl acetate, whereas soluble conjugated PAs were extracted with ethyl acetate after alkaline hydrolysis of the combined supernatants from the extractions with the ethanol-water solution. Insoluble bound PAs, remaining in the pellet from the initial ethanol-water extraction, were released via alkaline hydrolysis with 2.0 M sodium hydroxide and then extracted with

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