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Agronomic, nutritional and nutraceutical aspects of durum wheat (*Triticum durum* Desf.) cultivars under low input agricultural management

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Abstract

Among cereals, durum wheat has a central role in the Italian diet and economy, where there is a historical tradition of pasta making. In the present study, we evaluated the nutrient and nutraceutical properties of 2 old and 6 modern durum wheat varieties grown under low input agricultural management. Considering the lack of available data on the adaptability of existing durum wheat varieties to the low input and organic sectors, the research aimed at providing a complete description of the investigated genotypes, considering the agronomic performance as well as the nutrient and phytochemical composition. The experimental trials were carried out at the same location (Bologna, Northern Italy) for two consecutive growing seasons (2006/2007, 2007/2008). No clear distinction between old and modern varieties was observed in terms of grain yield (mean values ranging from 2.5 to 4.0 t/ha), highlighting that the divergence in productivity, normally found between dwarf and non-dwarf genotypes, is strongly reduced when they are cropped under low input management. All durum wheat varieties presented high protein levels and, in addition, provided remarkable amounts of phytochemicals such as dietary fibre, polyphenols, flavonoids and carotenoids. Some of the investigated genotypes, such as Senatore Cappelli, Solex, Svevo and Orobel, emerged with intriguing nutritional and phytochemical profiles, with the highest levels of dietary fibre and antioxidant compounds. The study provided the basis for further investigations into the adaptability of the durum wheat genotypes to low input management, for the selection of genotypes characterised by higher yield and valuable nutrient and nutraceutical quality.

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Introduction

Italy is the first world producer of pasta and has the highest *pro capita* consumption rates (http://www.internationalpasta.org). Due to the long tradition of pasta making, intense efforts have been made in the past to select durum wheat (*Triticum durum* Desf.) genotypes with the best agronomic performance. An increase in productivity was achieved during the first decades of the 20th century after the introduction of the Senatore Cappelli variety selected by Nazareno Strampelli from North African landraces (Scarascia Mugnozza, 2005). After the *Green Revolution* (in the 1970s), the dwarf genes were introduced in Italy through breeding between the International Maize and Wheat Improvement Center (CIMMYT) short straw lines and local durum wheat varieties. This led to new durum wheat cultivars with high yield and improved technological quality (De Vita *et al.*, 2007a, 2007b). Selection procedures were carried out to obtain genotypes that perform well under intensive crop management, but their adaptability to organic and low-input farming was never considered. At the moment, organic agriculture lacks crop varieties that can respond to the specific demands of this sector (*i.e.* high nitrogen use efficiency, competitiveness against weeds, resistance to biotic and abiotic stresses) as recently reviewed by Lammerts van Bueren *et al.* (2011). It is likely that the crossbreeding between old durum wheat genotypes and dwarf selection lines allowed some traits typical of old varieties to be maintained, such as wide adaptability and rusticity (Scarascia Mugnozza, 2005). These characteristics could play a key role in the choice of cultivars suitable for low-input farming. Moreover, the breeding programmes always focussed on improving yield and technological properties (*i.e.* gluten quality) and did not give much consideration to the nutritional and nutraceutical importance of wheat consumption in the human diet (Peccetti and Annicchiarico, 1998; Motzo *et al.*, 2004). Recently, Fardet (2010) provided a complete description of the bioactive compounds present in whole wheat grains (including dietary fibre, polyphenols and carotenoids) and Gil *et al.* (2011) reported on the numerous studies that have demonstrated a direct relationship between wholegrain consumption and prevention of chronic diseases (*i.e.* diabetes, colon cancer). In addition, there is an increasing interest among both consumers and industries in the identification of specialty products with particular health-promoting properties, especially in the organic and functional food sectors. In this context, considering the scarce availability of literature data concerning the nutritional and

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nutraceutical properties of Italian durum wheat varieties, this study aimed to provide a comparative evaluation of the agronomic performance and the nutrient and phytochemical composition of old and modern durum wheat genotypes grown under low-input agricultural management.

Materials and methods

Grain samples and field experiments

Grain samples included 8 Italian durum wheat varieties (*Triticum turgidum* ssp. *durum*), including two old (Senatore Cappelli, Urria) and 6 modern (Anco Marzio, Iride, Levante, Orobel, Solex, Svevo) genotypes. Year of release and description of the pedigree of the varieties investigated are given in Figure 1. Trials were carried out at the experimental farm of the University of Bologna, Cadriano (44° 33' N, 11° 21' E, 32 m asl), Italy, during the growing seasons 2006/2007 and 2007/2008. The soil at the experimental farm is classified as fine silty, mixed, mesic, Udic Ustochrepts and has a silty loam texture with 380, 375 and 245 g kg^{-1} of sand, silt and clay respectively. The pH (1: 2.5 soil to water) is 7.9 and organic carbon is 8.5 g kg^{-1}. The field experiments were carried out using a randomised block design with 3 replicates (plot dimension 6×5 m). Data on monthly mean, minimum and maximum temperature recorded during the two growing seasons are shown in Table 1. During the 2-year study period, the total rainfall differed mostly during springtime, with lower rainfall in May and more abundant values in June of 2006/2007, as compared to the same month of the second year. As regards the temperature recorded during the first year, in general, higher values were observed as compared to 2007/2008. In particular, the period from April to June (2006/2007), which corresponds to the heading, anthesis and maturity stages of the crop, was hotter than that of the second growing season. Each genotype was grown in plots (6×5 m) according to a low nitrogen input agrotechnique (nitrogen fertilization with 10 kg N ha^{-1} applied before sowing and 20 kg NO_3 ha^{-1} applied at leaf sheath lengthening stage). Weeds were controlled by hand and no herbicide (or other pesticide) treatment was applied throughout the entire crop cycle. Whole-grain samples were ground to semolina in a stone mill, immediately cooled to -20°C, and stored at this temperature until analysis.

Chemicals

Folin-Ciocalteu reagent, gallic acid, catechin, β-carotene were purchased from Sigma-Aldrich (St. Louis, MO, USA). Lutein and zeaxanthin were obtained from Extrasynthèse (Genay, France). HPLC-grade methanol and acetonitrile were purchased from Carlo Erba (Milan, Italy). All other chemicals and solvent were of analytical grade.

Yield and grain quality traits

The recorded data included plant height (cm), grain yield (t/ha), harvest index (HI) (ratio of grain yield to grain-plus-straw yield), 1000-kernel weight (g) and test weight (kg/hL). One thousand-kernel weight was obtained as the mean value of 3 replicates of 100 seeds from each plot. Test weight was calculated using a Shopper chondrometer equipped with a 1 L container and reported as kg/hL without reference to the moisture content.

Macronutrients

Grain protein was measured using the Kjeldahl procedure (N 5.7) (American Association of Cereal Chemists, method 46-12) (AACC, 1995). The gluten content was determined using the method previously described by Kieffer *et al.* (2007). Lipid analysis was carried out according to the AOAC (1990) using chloroform-methanol (2:1 v/v) to extract lipid compounds. Starch was enzymatically hydrolysed and

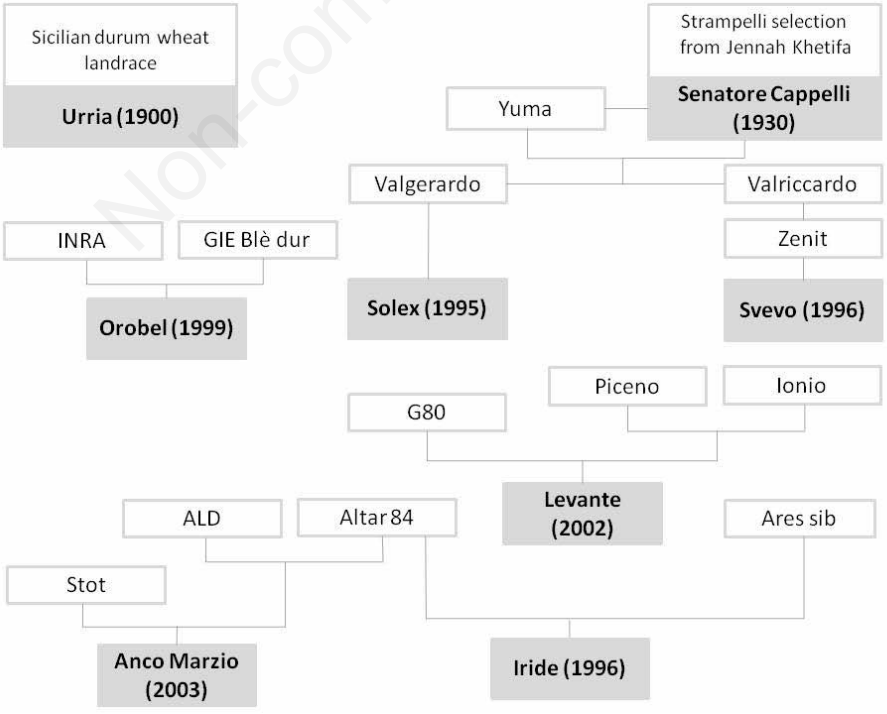

Figure 1. Genealogy and year of release of the durum wheat varieties investigated.

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measured using a Megazyme assay kit (Megazyme International Ireland Ltd, Wicklow, Ireland), as described by Di Silvestro *et al.* (2012).

Dietary fibre and resistant starch analysis

Total (TDF), insoluble (IDF) and soluble (SDF) dietary fibre content was determined using a Megazyme assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland), following the enzymatic/gravimetric method described by Prosky *et al.* (1988). The procedure was based on the sequential enzymatic digestion of 1 g of flour, using heat-stable α-amylase, protease and amyloglucosidase to remove protein and starch. The solution was then filtered to obtain the IDF residue and the filtrate was treated with 95% heated ethanol to precipitate the SDF. Both residues were dried and corrected for protein, ash and blank for final calculation of the IDF and SDF content. Resistant starch (RS) was determined with the Megazyme assay kit (Megazyme International Ireland Ltd.), as described by Marotti *et al.* (2012). After overnight α-amylase and amyloglucosidase digestion, soluble starch was removed with 95% and 50% ethanol consecutive washes. The pellet was dissolved in potassium hydroxide solution, hydrolysed with amyloglucosidase and quantified using a glucose oxidase-peroxidase reagent.

Phenolic compounds

Free and bound phenolic compounds were extracted as previously described by Dinelli *et al.* (2011) with cold 80% ethanol followed by acid and alkaline hydrolyses. Extracts were analysed for the polyphenol quantification using the colorimetric procedure based on the Folin-Ciocalteu reagent, as described by Singleton *et al.* (1999). Appropriated dilutions of extracts were oxidised using Folin-Ciocalteu reagent, and the reaction was neutralised with sodium carbonate. After 120 min, the absorbance of resulting blue colour was measured at 765 nm and converted using a gallic acid calibration curve. Furthermore, free and bound phenolic extracts were analysed for flavonoid content following the colorimetric method previously described by Dinelli *et al.* (2011), using catechin as a standard.

Carotenoids

Carotenoid content was determined according to Hentschel *et al.* (2002) and Topuz and Ozdemir (2003). Pigments were first extracted as described by Di Silvestro *et al.* (2012), then analysed using an RP-HPLC (Beckman Coulter Inc., Miami, FL, USA) consisting of a Gold 126 multisolvent pump, photodiode array detector Beckman 168 and a Spark Holland autosampler. For separation, a Waters RP C18 column (4.6 mm×250 nm, 5 µm) was employed. The binary gradient (acetone-water, initially 75:25) was run at a flow rate of 1 mL/min for 5 min, then increased to 95:5 in 5 min and maintained at this level for 10 min. Carotenoids were detected at 450 nm and identified by comparing retention time and spectra with those of lutein, β-carotene and zeaxanthin standards.

Statistical analyses

Data were subjected to analysis of variance (ANOVA, Tukey's honest significant difference multiple comparison) using Statistica 6.0 software (2001; StatSoft, Tulsa, OK, USA). The effects of genotype and growing season and their interaction were tested for significance by two-way ANOVA. Significance between means was determined by least significant difference values for $P<0.05$. A linear discriminant analysis (LDA) was performed using Statistica 6.0 software (StatSoft). LDA was applied to the standardised data matrix of the nutrient and bioactive compounds obtained for the investigated durum wheat varieties from both cropping years. It allowed scoring of the cases as a function of the first two roots to provide a picture of the similarities among genotypes. LDA is a multivariate technique that increases the informative level on the variables that mainly influence similarities and differences between samples (Rodriguez-Delgado *et al.*, 2002). LDA is also a statistical method used to find a linear combination of features (discriminant functions) that characterises or separates two or more classes of objects or events (McLachlan, 2004). The statistical significance of each discriminant function was evaluated on the basis of Wilks' Lambda factor, which ranges from 1.0 (no discriminatory power) to 0.0 (perfect discriminatory power).

Results and discussion

Yield and commercial quality

The main agronomic traits and commercial quality parameters of the investigated durum wheat varieties are reported in Table 2. Grain yield significantly varied among genotypes and ranged from 2.5 to 4.0 t/ha. The highest yield value was obtained for the old variety Urria 12 (4.0 t/ha) along with other durum wheat genotypes, such as Iride (3.8 t/ha), Orobel (3.9 t/ha), Solex (3.9 t/ha) and Svevo (3.9 t/ha). The lowest pro-

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Table 1. Monthly data recorded for rainfall (mm), mean, minimum and maximum temperature (°C) during the two cropping seasons (2006/2007, 2007/2008) at the experimental location (Cadriano, Bologna, Northern Italy).

	Rainfall (mm)		Mean temperature (°C)		Minimum temperature (°C)		Maximum temperature (°C)	
Month	2006/07	2007/08	2006/07	2007/08	2006/07	2007/08	2006/07	2007/08
October	11.6	143.8	16.9	14.4	12.9	11.0	21.3	21.0
November	41.8	17.0	10.7	8.7	7.4	5.8	14.3	12.2
December	28.4	41.0	6.4	4.8	3.8	2.1	9.4	8.1
January	10.6	48.6	6.7	6.2	4.0	3.8	10.2	9.1
February	34.6	16.2	8.3	6.9	5.2	3.5	11.9	10.9
March	100.4	54.2	10.9	10.6	6.8	6.6	15.1	14.8
April	19.8	30.8	16.8	13.8	11.3	9.4	22.2	18.5
May	41.8	133.2	20.5	18.4	15.5	13.5	25.8	23.1
June	131.8	87.8	23.2	22.6	18.8	17.8	28.2	27.9
July	1.2	10.4	27.0	26.4	21.0	20.3	32.9	31.0

ductivity was obtained for the modern variety Anco Marzio (2.5 t/ha), while Levante and Senatore Cappelli showed intermediate values (3.3 t/ha). The observed grain yield was lower than values previously reported for modern and old durum wheat varieties grown under low and high levels of nitrogen inputs (Quaranta *et al.*, 2010; De Vita *et al.*, 2007b; Giunta *et al.*, 2007). These studies described high variability comparing durum wheat varieties released before and after the Second World War, reporting a significant rise in productivity of the newest varieties due to increasing spike fertility and density (spikes per square metre). However, this result was not confirmed in our study as there was no difference between the two old genotypes investigated and the other durum wheat varieties, suggesting that without high nitrogen inputs old and modern cultivars are comparable in terms of grain yield. Further studies focussing on how old and modern cultivars differ for each yield component may go further to explain the comparison between grain yield formation processes. The environmental conditions significantly affected the observed grain yield. During the second crop cycle, an increase in productivity was recorded compared to the previous year (3.8 and 3.4 t/ha, respectively), probably due to quite lower mean temperature and more homogenous rainfall distribution during the grain filling period (May-July). Indeed, the air temperature during grain filling may affect translocation rates of carbon and nitrogen compounds, determining the yield and the final protein concentration of wheat kernel (Vaccari *et al.*, 2007). The wheat varieties investigated evidently differed in plant height. During the 1970s, breeding programs introduced the dwarf habitus in the Italian varieties through the introgression of the short-straw genes (*Rht*) of Norin 10 (*Triticum aestivum* L.) (De Vita *et al.*, 2007a, 2007b). In the present study, modern varieties showed plant height values ranging from 66.9 cm (Anco Marzio) to 80.0 cm (Svevo). In contrast, the old wheat genotypes presented significantly taller straw: 116.0 cm (Urria 12) and 121.2 cm (Senatore Cappelli) (Table 2). As expected, the highest plant height corresponded to the lowest HI values: 0.21 and 0.22 for Senatore Cappelli and Urria 12, respectively. All the modern varieties presented significantly higher HI values: within 0.24-0.37. The increase in HI as a function of the year of cultivar release was described in detail in previous studies concerning both durum and common wheat genotypes (De Vita *et al.*, 2007b; Giunta *et al.*, 2007). Besides the dwarf habitus, the rise in HI values was associated with the highest number of kernels per square metre, while other parameters such as number of spikelets per spike and spike length, did not show a clear trend in relation to breeding improvements. The HI values observed in the present study

Table 2. Yield, harvest index, plant height, test weight and 1000-kernel weight of the durum wheat genotypes investigated.

Variety	Grain yield (t/ha)	Harvest index	Plant height (cm)	Test weight (kg/hL)	1000-kernel weight (g)
Anco Marzio	2.5^{c}	0.24^{d}	66.9^{c}	64.0^{c}	33.9^{c}
Iride	3.8^{a}	0.30^{b}	76.2^{bc}	73.6^{b}	35.0^{c}
Levante	3.3^{b}	0.30^{b}	72.7^{bc}	75.0^{ab}	38.3^{b}
Orobel	3.9^{a}	0.36^{a}	70.9^{bc}	78.2^{a}	41.1^{a}
Senatore Cappelli	3.3^{b}	0.21^{e}	121.2^{a}	78.3^{a}	41.9^{a}
Solex	3.9^{a}	0.37^{a}	70.0^{bc}	77.2^{ab}	39.1^{b}
Svevo	3.9^{a}	0.27^{c}	80.0^{b}	74.7^{ab}	33.9^{c}
Urria 12	4.0^{a}	0.22^{de}	116.0^{a}	78.4^{a}	41.2^{a}
Year					
2006/2007	3.4^{b}	0.28^{a}	83.8^{a}	75.3^{a}	38.0^{a}
2007/2008	3.7^{a}	0.29^{a}	84.6^{a}	74.5^{a}	38.0^{a}
Interaction V x Y	ns	ns	ns	ns	ns

^{a,b,c,d,e}Means followed by different letters are statistically different at P<0.05. ns, not significant.

Table 3. Nutrient and dietary fibre composition of the durum wheat varieties investigated expressed as g per kg of wholegrain semolina.

Variety	Protein g/kg	Gluten g/kg	Starch g/kg	Lipid g/kg	TDF g/kg	SDF g/kg	SDF/TDF %	RS g/kg
Anco Marzio	147.4^{ab}	84.8^{d}	544.7^{ab}	29.2^{cd}	193.5^{bc}	25.8^{c}	13.4^{cd}	3.8^{cd}
Iride	156.7^{a}	101.6^{b}	514.0^{ab}	32.3^{ab}	204.9^{a}	21.8^{d}	10.8^{e}	4.5^{c}
Levante	147.3^{ab}	103.4^{b}	526.7^{ab}	30.4^{bc}	189.0^{c}	26.9^{c}	14.2^{c}	5.0^{bc}
Orobel	137.8^{b}	88.2^{d}	568.6^{a}	22.4^{e}	181.8^{d}	24.6^{c}	13.6^{cd}	3.0^{d}
Senatore Cappelli	163.8^{a}	120.1^{a}	505.0^{b}	27.4^{d}	199.0^{ab}	24.2^{c}	12.2^{de}	8.2^{a}
Solex	149.2^{ab}	96.9^{c}	528.0^{ab}	30.7^{bc}	202.3^{a}	32.9^{b}	16.3^{b}	3.7^{cd}
Svevo	159.6^{a}	101.2^{b}	536.7^{ab}	33.7^{a}	173.4^{e}	37.4^{a}	21.5^{a}	6.0^{b}
Urria 12	157.9^{a}	103.1^{b}	506.9^{b}	29.1^{cd}	192.6^{bc}	21.4^{d}	11.1^{e}	6.1^{b}
Year								
2006/2007	143.5^{b}	94.5^{b}	540.3^{a}	20.9^{b}	188.7^{b}	26.7^{a}	14.3^{a}	6.8^{a}
2007/2008	161.4^{a}	105.3^{a}	517.4^{b}	38.2^{a}	195.5^{a}	27.0^{a}	14.0^{a}	3.3^{b}
Interaction V x Y	**	***	ns	ns	***	***	***	ns

TDF, total dietary fibre; SDF, soluble dietary fibre; RS, resistant starch; ns, not significant. ^{a,b,c,d,e}Means followed by different letters are statistically different at P<0.05. *P<0.05; **P<0.01; ***P<0.001.

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were lower than those commonly obtained for durum wheat grown under conventional (high N input) farming, but aligned with values reported for low nitrogen input management (De Vita *et al.*, 2007b; Giunta *et al.*, 2007). All the investigated durum wheat varieties investigated presented test weight values lower than market requests (>80 kg/hL) (De Vita *et al.*, 2007a) (Table 2). The modern variety Anco Marzio showed the lowest test weight (63.9 kg/hL). Orobel and the old genotypes Senatore Cappelli and Urria 12 presented test weights close to the commercially accepted threshold limit (78.2-78.4 kg/hL), showing a possible higher adaptability to the low input growing conditions. These genotypes also presented the highest 1000-kernel weight, within 41.1-41.9 g (Table 2) and, therefore, showed the best grain commercial quality. The remaining durum wheat varieties had significantly lower 1000-kernel weight, with Anco Marzio, Iride and Svevo showing the lowest values (33.9, 35.0 and 33.9 g, respectively). The kernel weight obtained aligned with data previously reported for old and modern durum wheat varieties by De Vita *et al.* (2007b). The environmental conditions had no impact on commercial quality parameters (test weight, 1000-kernel weight) as no differences were observed when the two growing seasons were compared.

Macronutrient profile

The macronutrient composition of the old and modern durum wheat varieties is presented in Table 3. The investigated durum wheat genotypes showed protein content ranging from 137.8 to 163.8 g/kg (Table 3). The modern variety Orobel presented the lowest protein yield, while several genotypes, including the old (Senatore Cappelli, Urria 12) and modern (Iride, Svevo) ones, showed significantly higher protein content. All the varieties studied presented protein amounts that were higher than the minimum industry standard set at 125 g/kg (De Vita *et al.*, 2007a). The observed protein levels were in line with those previously reported for old and modern durum wheat varieties cropped under low N input management (Quaranta *et al.*, 2010; De Vita *et al.*, 2007b) and were also comparable with those obtained at higher nitrogen rates by Giuliani *et al.* (2011). The protein accumulation was shown to be highly dependent on genotype, soil fertility (available N) and environmental conditions (temperature, water) (Giuliani *et al.*, 2011; Dupont *et al.*, 2006). In our study, the growing season was observed to have a big effect on protein content, with a significant increase from 143.5 to 161.4 g/kg during the second year compared to the previous one. The significant interaction between variety and year (Figure 2) clearly shows that the rise in protein levels was mainly the result of the highest accumulation rates in the grains of Iride, Solex, Svevo and Senatore Cappelli, while the remaining genotypes presented stable protein amounts between years. This finding suggests that, under low input conditions, the protein yield is mainly determined by the nutrient use efficiency of each wheat genotype. Besides the protein quantity, durum wheat genotypes may vary greatly in protein quality and composition as a result of differences in genetic and agricultural management. The technological properties of the modern durum wheat varieties were improved by selecting genotypes with high gluten content and quality (*i.e.* strength, tenacity, extensibility). However, the technological quality is only guaranteed when these genotypes are cropped under high nitrogen supply (conventional agriculture)

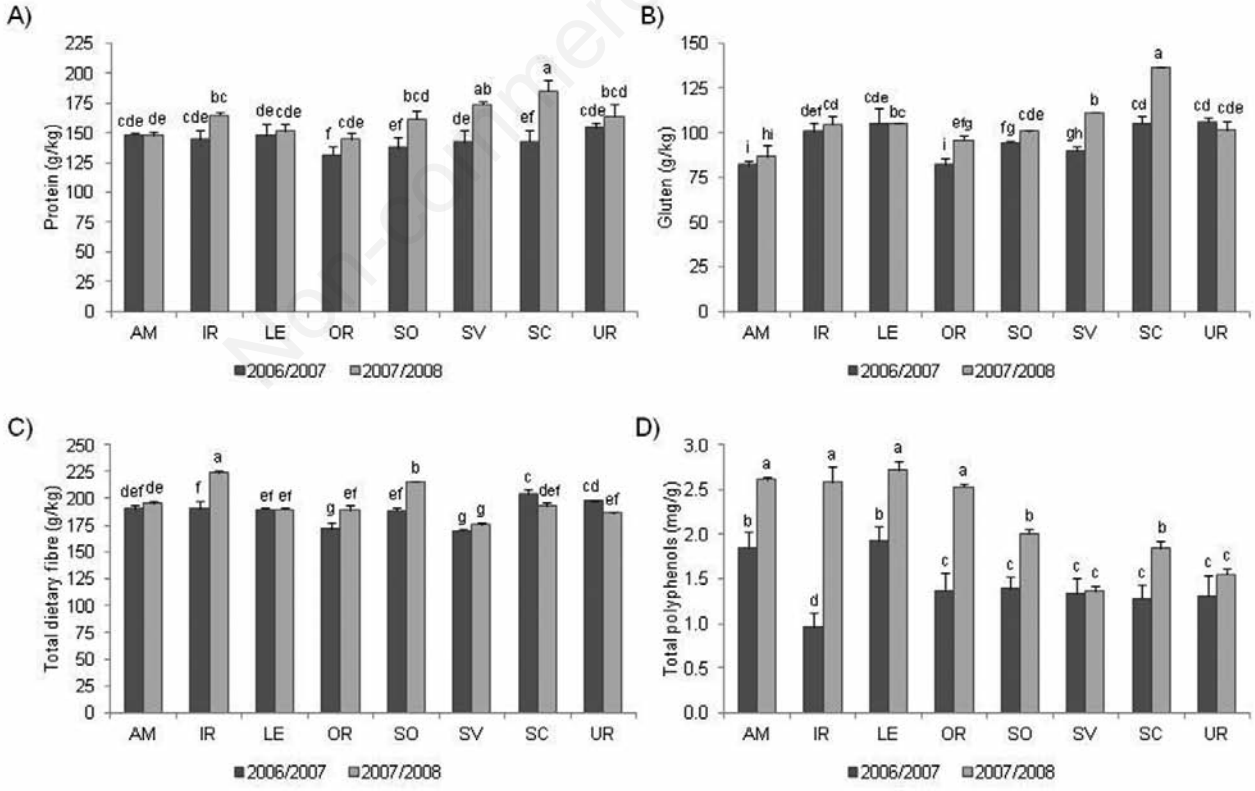

Figure 2. Significant interactions observed between variety and year for (A) protein, (B) gluten, (C) total dietary fibre and (D) total polyphenol content (±standard error). a,b,c,d,e,f,g,h,i, means followed by different letters are statistically different at P<0.05; AM, Anco Marzio; IR, Iride; LE, Levante; OR, Orobel; SC, Senatore Cappelli; SO, Solex; SV, Svevo; UR, Urria.

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(Giuliani *et al.*, 2011). In our study, the highest gluten content was obtained for the old variety Senatore Cappelli (120.1 g/kg), while some modern cultivars, such as Anco Marzio, Orobel and Solex, presented very low gluten levels: within 84.8-96.9 g/kg (Table 3). The effect of the cropping year was also observed for the gluten content that significantly increased during 2007/2008, as Orobel, Svevo and Senatore Cappelli responded to favourable growing conditions with a higher accumulation of gliadin and glutenin proteins (Figure 2). The durum wheat varieties investigated presented a total starch amount ranging from 505.0 to 568.6 g/kg (Table 3). As expected, an inverse relationship between protein and starch levels was observed, with Senatore Cappelli and Urria 12 showing the lowest starch accumulation (505.0 and 506.9 g/kg, respectively), while Orobel had the highest (568.6 g/kg). The effect of environmental growing conditions on macronutrient accumulation was also observed when comparing the mean value of starch content for each year; this showed a decrease from 540.3 to 517.4 during 2007/2008. Starch levels agreed with those previously reported for wholegrain semolina (Labuschagne *et al.*, 2009). High variability was observed among durum wheat varieties for lipid content (Table 3). The highest values were obtained for the modern cultivars Svevo (33.7 g/kg) and Iride (32.4 g/kg), while Orobel had the lowest fat content (22.4 g/kg). High levels of fats in wholegrain semolina can affect the shelf life of the final product causing rancidity and loss of yellow pigments (due to lipoxygenase, peroxidise and polyphenol oxidase activities) (Aalami *et al.*, 2007), but lipids are also interesting from a nutraceutical point of view, as they include compounds belonging to the vitamin E complex (tocopherols, tocotrienols) that exert strong antioxidant activity (Borrelli *et al.*, 2008; Shewry *et al.*, 2010).

Dietary fibre content

Dietary fibre is the predominant bioactive component of wholewheat grain. Due to its multiple health benefits, which include prevention of colon cancer, prebiotic activity and modulation of blood glucose and insulin levels, dietary fibre makes a big contribution to the nutraceutical value of wheat-based products (Charalampopoulos *et al.*, 2010). As regards dietary fibre content, there was a big difference between the wheat varieties investigated in terms of both soluble and insoluble fibre components (Table 3). TDF ranged from 173.4 g/kg (Svevo) to 204.9 g/kg (Iride), while SDF was between 21.4 g/kg (Urria 12) and 37.4 g/kg (Svevo). Considering the SDF/TDF ratio, the modern varieties Solex and Svevo emerged with the highest proportion of SDF (16% and 22% of the total amount, respectively). On the contrary, Iride, Senatore Cappelli and Urria 12 had the lowest values of SDF percentage (10%, 11% and 12%, respectively), showing a preponderance of insoluble fibre components. TDF content was higher than that previously reported by Gebruers *et al.* (2008) for wholemeal durum wheat. However, the difference may be explained by the strong influence that genotype and environmental conditions have on the accumulation of fibre components in wheat grain (Gebruers *et al.*, 2010; Shewry *et al.*, 2010). The significant interaction between variety and year (Figure 2) showed that the durum wheat genotypes with the highest TDF amount (Iride, Solex) were those most influenced by the environmental conditions, while the other varieties had stable fibre content over the growing seasons. These results again underline the important effect of climate and growing factors and the need for in-depth investigation of the genotype *per* environment effects on the accumulation of fibres in the grain. The present research also evaluated RS content, a fraction of the starch that cannot be digested by human gastrointestinal enzymes and is fermented by the colon microflora, exerting high prebiotic activity (Sajilata *et al.*, 2006; Fuentes-Zaragoza *et al.*, 2010). For its physiological properties, RS is commonly referred to as a dietary fibre component. Very different levels of RS were detected in the investigated durum wheat genotypes (Table 3). The old variety Senatore Cappelli was the richest in RS (8.2 g/kg) whereas the lowest value was obtained for Orobel (3.0 g/kg). As previously observed for total dietary fibre, the growing season was seen to have a big effect on resistant starch content. In contrast to protein, lipid and TDF that increased during 2007/2008, all the durum wheat varieties presented a large reduction in RS content in the second year (mean value 6.8 and 3.3 for 2006/2007 and 2007/2008, respectively). This confirmed the considerable impact that environmental factors may have on starch composition (*i.e.* amylose/amylopectin ratio) in the wheat grain (Labuschagne *et al.*, 2009; Flagella, 2006). Available literature data generally agree with RS values obtained in the second year rather than in the previous year (Ragaee *et al.*, 2006; Marotti *et al.*, 2012).

Table 4. Polyphenol, flavonoid and carotenoid content of the durum wheat varieties investigated expressed as mg (polyphenols, flavonoids) and µg (carotenoids) per g of wholegrain semolina.

Variety	Free polyphenols mg/g	Total polyphenols mg/g	Free flavonoids mg/g	Total flavonoids mg/g	Lutein µg/g	Total carotenoid µg/g
Anco Marzio	0.62^{b}	2.28^{a}	0.18^{ab}	0.41^{a}	1.61^{e}	3.35^{d}
Iride	0.46^{c}	1.81^{c}	0.17^{bcd}	0.46^{a}	1.63^{e}	3.34^{d}
Levante	0.60^{b}	2.36^{a}	0.19^{a}	0.64^{a}	2.55^{c}	5.41^{b}
Orobel	0.72^{a}	2.00^{b}	0.17b^{cd}	0.47^{a}	2.82^{b}	5.86^{a}
Senatore Cappelli	0.39^{d}	1.60^{de}	0.17b^{cd}	0.47^{a}	1.71^{e}	3.40^{d}
Solex	0.47^{c}	1.74^{cd}	0.15^{d}	0.46^{a}	1.50^{e}	3.28^{d}
Svevo	0.46^{c}	1.39^{f}	0.16^{cd}	0.45^{a}	3.23^{a}	6.09^{a}
Urria 12	0.33^{e}	1.49^{ef}	0.18^{abc}	0.43^{a}	2.19^{d}	3.98^{c}
Year						
2006/2007	0.71^{a}	1.51^{b}	0.15^{b}	0.42^{b}	2.15^{a}	4.36^{a}
2007/2008	0.30^{b}	2.15^{a}	0.18^{a}	0.52^{a}	2.16^{a}	4.32^{a}
Interaction						
V x Y	***	***	**	ns	ns	ns

^{a,b,c,d,e,f}Means followed by different letters are statistically different at P<0.05. **P<0.01; ***P<0.001. ns, not significant.

Phenolic compound content

Polyphenols are the most representative antioxidant compounds of the wheat grain. They are mainly made up of phenolic acids and flavonoids (Dinelli *et al.*, 2009). In wheat kernel, polyphenols can be found in the free soluble and the bound insoluble forms, both possessing strong antiradicalic power as well as anticancer and anti-inflammatory properties (Thompson, 1994; Jacobs *et al.*, 1998). The phenolic composition of the investigated durum wheat varieties is presented in Table 4. Regarding total polyphenol content, statistically significant differences were observed among genotypes. Anco Marzio and Levante varieties had the highest amounts (2.28 and 2.36 mg/g, respectively), Svevo showed the lowest content (1.39 mg/g), while the remaining durum wheat genotypes presented intermediate values. High variability was also observed in the free soluble fraction of polyphenols: range 0.33 mg/g (Urria 12) to 0.72 mg/g (Orobel). The biosynthesis and accumulation of phenolic compounds during kernel development is known to be highly dependent on the wheat variety and environmental conditions (abiotic and biotic stresses) (Stracke *et al.*, 2009; Bellato *et al.*, 2013). The impact of environmental effects was seen in a comparison of the mean values obtained for each growing season (1.52 and 2.15 mg/g for the first and the second year, respectively). The variety *per* year significant interaction (Figure 2) confirmed that all the durum wheat genotypes investigated had increased phenolic amounts during the second growing season, except for Svevo and Urria 12 that did not vary between years. All durum wheat varieties had comparable total flavonoid content (0.41-0.64 mg/g) (Table 4), but varied in the soluble/insoluble compound ratio, with Levante showing the highest (0.64 mg/g) and Solex the lowest (0.15 mg/g) free flavonoid amount. As observed for polyphenols, the effect of the cropping year led to a significant increase in the flavonoid content in all the durum wheat genotypes investigated (mean values 0.43 and 0.52 for 2006/2007 and 2007/2008, respectively). Most literature data report on the phenolic content exclusively in terms of free soluble fraction without considering the insoluble fraction that accounts for 50-75% of the total amount (Dinelli *et al.*, 2009). The free phenolic contents obtained in the present study were higher than those previously reported for soluble compounds (Bellato *et al.*, 2013; Ciccoritti *et al.*, 2013), but were in line with those reported by Fares *et al.* (2010) that also took into consideration the insoluble phenolic fraction.

Carotenoid content

Carotenoids are pigments responsible for the bright yellow colour of semolina and pasta and, therefore, determine one of the main factors affecting durum wheat quality and consumer choice. From a nutraceutical point of view, these pigments are particularly interesting as they act as powerful antioxidant agents and can lower the incidence of age-related macular degeneration of the human eye (Beatty *et al.*, 2000; Bernstein *et al.*, 2002). In durum wheat, carotenoid content has been reported to be a highly heritable trait (Borrelli *et al.*, 2008). This is in agreement with the lack of variability observed in our study between growing seasons (Table 4). However, statistically significant differences were observed among genotypes for total carotenoid and lutein (which is the most representative pigment in durum wheat) content. The modern varieties Orobel and Svevo had the highest carotenoid levels (5.87 and 6.09 µg/g, respectively). This is not surprising given the breeding improvements that selected durum wheat genotypes for higher yellow index values (Flagella, 2006). The lowest pigment contents (3.28-3.40 µg/g) were obtained for Anco Marzio, Iride, Senatore Cappelli and Solex varieties. Lutein accounted for approximately half of the total carotenoid amount and ranged from 1.50 µg/g (Solex) to 3.23 µg/g (Svevo) (Table 4). The observed pigment contents were in general agreement with those previously reported for old and modern durum wheat varieties (Hung *et al.*, 2011; Digesù *et al.*, 2009; Panfili *et al.*, 2004).

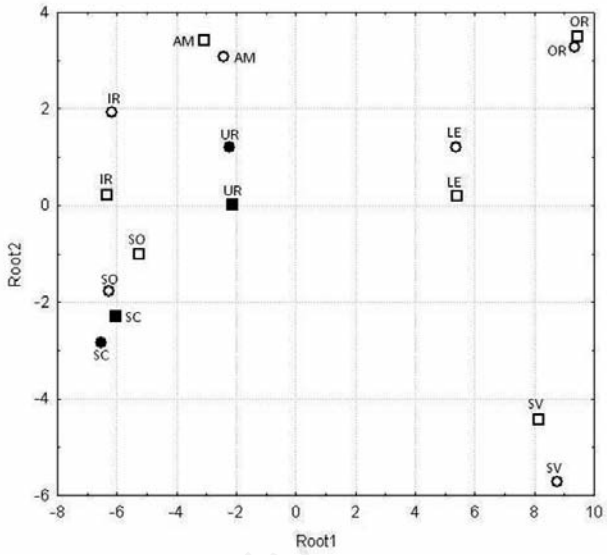

Figure 3. Scatterplot of the 6 modern (empty symbols) and 2 ancient (solid symbols) durum wheat varieties from both growing seasons according to the nutrient and phytochemical content defined by the first two canonical functions (Root 1 and Root 2, representing 71% and 13%, respectively, of cumulated variability). Circle and square symbols refer to the first (2006/2007) and second (2007/2008) growing season, respectively. AM, Anco Marzio; IR, Iride; LE, Levante; OR, Orobel; SC, Senatore Cappelli; SO, Solex; SV, Svevo; UR, Urria.

Linear discriminant analysis

LDA has been used to explain the observed variability in nutrient and phytochemical content of the wheat genotypes investigated harvested in two successive growing seasons (2006/2007, 2007/2008) Figure 3 shows the scatterplot of the wheat samples on the space defined by the first two canonical functions.

The Wilks' lambda value was 0.0000218 (significant at $P<0.000001$), indicating the high discriminatory power of the applied model to classify wheat genotypes based on nutrient and phytochemical composition. The score of the cases in the plot did not show a clear differentiation between the two growing seasons or between old and modern durum wheat genotypes. For each genotype, the close position between samples grown in the first (2006/2007) and second (2007/2008) cropping year indicated that this environmental parameter had almost no influence on the investigated nutritional and phytochemical variables. In addition, the two old varieties (Senatore Cappelli, Urria) are clustered together with the modern varieties Iride, Anco Marzio, Solex. The remaining modern varieties (Orobel, Levante, Svevo) are clustered in a clearly separate group. As shown by the values of canonical functions standardised within variances, the described clusterisation of the cases along the first canonical function (Root 1) resulted strongly influenced by the content of lipids, total dietary fibres, free polyphenols, lutein and total carotenoids (first canonical discriminant function equal to -2.01, -0.75, 1.02, 0.68 and 0.69, respectively). For example, a gradient goes from the negative to the positive part of Root 1 that can be associated with increased content of free polyphenols, lutein and carotenoids. In complete contrast, the total dietary fibre content increases from the

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positive to the negative part of Root 1, as shown by the score of Iride, Solex and Senatore Cappelli varieties which had the highest TDF values. The clusterisation of the cases along second canonical function (Root 2) resulted strongly influenced by the gluten, soluble dietary fibre, resistant starch and free flavonoid content (second canonical discriminant function equal to -0.97, -0.77, -1.15 and 0.93, respectively). The free flavonoid content increases from the negative to the positive arm of Root 2, determining the position of the Anco Marzio, Levante and Urria 12 varieties that showed the highest values, while gluten, resistant starch and soluble dietary fibre content has an opposite trend. According to the results obtained, the cropping year did not have a strong effect on kernel composition of primary and secondary metabolites. The particular phytochemical content of wheat grains mainly reflected their genealogy (Figure 1). Urria and Senatore Cappelli were originally selected from North African landraces, and Senatore Cappelli was in the breeding line of Solex. Anco Marzio and Iride shared a common parental line, while Orobel, Svevo and Levante were selected from crosses with durum semi-dwarf lines, originally obtained from North America.

Conclusions

The study highlighted that there was no great difference in agronomic performance between old and modern durum wheat varieties grown under low input agricultural management. While common wheat modern varieties were obtained directly from selected CYMMIT lines and are definitely distinct from the oldest genotypes, the dwarf durum wheat genotypes were selected by breeding the local Italian durum varieties (such as Senatore Cappelli, Urria 12 and others) with dwarf and semi-dwarf lines. Consequently, the genome of the modern durum wheat cultivars may include some traits of the ancient genotypes allowing the persistence of useful features for low-input management, such as wide adaptability and rusticity. Moreover, some of the genotypes investigated, such as Senatore Cappelli, Solex, Svevo and Orobel, presented intriguing nutritional and phytochemical profiles, with high levels of dietary fibre and antioxidant compounds. The study provided the basis for further investigation into the adaptability of the durum wheat genotypes to low input management, and for the selection of genotypes characterised by high yield and valuable nutrient and nutraceutical quality.

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