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RESEARCH ARTICLE

Genetic variation for phenolic acids concentration and composition in a tetraploid wheat (*Triticum turgidum* L.) collection

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Abstract Phenolic acid intake through the consumption of whole-wheat foods provides important health benefits associated with reduced risks of cardiovascular diseases and colon cancer. The genetic variation for phenolic acids was extensively studied in common wheat, but a comprehensive survey in tetraploid wheat is lacking. In this study we evaluated the genetic variability for individual and total phenolic acids concentration existing in a large collection of tetraploid wheat (*Triticum turgidum* L.). A 2-year evaluation was undertaken on the whole-meal flour of 111 genotypes belonging to seven *T. turgidum* subspecies including cultivars, landraces and wild accessions. Durum cultivars [*T. turgidum* subsp. *durum* (Desf.) MacKey], had the highest average concentration of total phenolic acids (828.7 μg g^{-1} dm in 2012; 834.5 μg g^{-1} dm in 2013) with amounts varying from 550.9 μg g^{-1} dm to 1701.2 μg g^{-1} dm, indicating a variation of greater than threefold fold. The lowest concentration of phenolic acids was found in *T. turgidum* subsp. *dicoccum* (Schrank ex Schübler) Thell. Rivet wheat (*T. turgidum* L. subsp. *turgidum*) had phenolic acid concentrations similar to those in durum, but less variation was noted among the accessions. On the other hand, the accessions of the four remaining subspecies showed lower phenolic acid concentrations and variation among the accessions as compared to durum. A total of six phenolic acids were identified across the wheat genotypes. The effects of genotype, year and year × genotype were estimated by ANOVA and resulted significant for all phenolic acids. The ratio of genotypic variance to total variance suggested the possibility of improving phenolic acid content in elite wheat germplasm through appropriate breeding programs. Moreover, significant correlations between phenolic acids and other quality characteristics of the grain were detected.

Keywords Genetic variation · Heritability · Phenolic acids · Tetraploid wheats · *Triticum turgidum* · Whole-meal flour

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Introduction

Tetraploid wheats (*Triticum turgidum* L.) comprise a large variability that carries the marks of a long evolutionary history and represents a source of potentially useful, unexploited alleles. Among the

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tetraploid wheats, *T. turgidum* subsp. *dicoccoides* (Körn. ex Asch. et Graebner) Thell.] is the progenitor of durum wheat [*T. turgidum* subsp. *durum* (Desf.) MacKey], which is the preferred raw material for producing pasta, different types of bread and couscous.

Recent findings on the health benefits of whole grains have emphasized the significance of wheat and other cereals as functional foods, which can reduce the risks for several chronic diseases (Liu 2007; Fardet 2010; Durante et al. 2012a, b; Rawat et al. 2013). These health benefits rely on a range of bioactive components contained in the bran and germ portions of whole caryopsides, such as carbohydrate constituents (β-glucans, arabinoxylans and inulin) and a number of phytochemicals (Liu 2007; Durante et al. 2012a, b). In particular, phenolic acids are among the most abundant and ubiquitous metabolites of whole-wheat grains which exert important antioxidant, anti-inflammatory and anti-tumor activity (Drankham et al. 2003; Moore et al. 2005; Laddomada et al. 2015a).

The majority of wheat phenolic acids are insoluble bound due to covalent cross-linkages with cell wall polymers, such as polysaccharides and lignin. Phenolic acids may also occur as soluble conjugated forms that are esterified to sugars and other low molecular mass compounds. Both bound and conjugated phenolic acids can be released from whole-meal wheat flour and wheat bran layers by alkaline hydrolysis for analytical characterization, and for this reason they can be referred as saponified phenolic acids (Li et al. 2008; Parker et al. 2005). Only 0.5–2 % of wheat phenolic acids exists as soluble free forms (Li et al. 2008; Fernandez-Orozco et al. 2010).

From a biological point of view, the health-related effects of bound phenolic acids have been considered less important as compared to the free forms (Manach et al. 2004). In fact, bound phenolics are scarcely digested in the stomach and intestine and, for this reason, are poorly bioavailable. However, it was shown that, by reaching the intestine mostly intact, the bound forms act as potent antioxidants and anti-inflammatory mediators locally, and contribute to a reduced risk for colorectal cancer (Andreasen et al. 2001; Drankham et al. 2003; Vitaglione et al. 2008).

Due to the influence of various biotic and abiotic stimuli on the biosynthetic pathway (Rawat et al. 2013), phenolic acid content in cereal species is largely influenced by environmental factors (Fernandez-Orozco et al. 2010; Menga et al. 2010; Shewry et al. 2010).

Genetic variability for phenolic acids was extensively documented in winter and spring bread wheat genotypes (*T. aestivum* L. subps. *aestivum*). These studies showed a wide variation in the content of phenolic acids, but indicated a low heritability for the trait (Li et al. 2008; Fernandez-Orozco et al. 2010). Indeed, such a detailed diversity screening in tetraploid wheats is lacking. In fact, most studies have considered the various subspecies of *T. turgidum* separately (Serpen et al. 2008; Dinelli et al. 2009), and only a limited number of subspecies and accessions have been analyzed (Brandolini et al. 2013; Giambanelli et al. 2013.

We present the first extensive survey of phenolic acids in a collection of 111 *T. turgidum* genotypes belonging to seven subspecies, which was carried out on one site over two growing seasons. In particular, we show the variability for individual and total saponified phenolic acids and the effects of genotype versus environment and their interaction.

Materials and methods

Plant material

The wheat collection analyzed in this study was composed of a large set of tetraploid wheat genotypes including durum wheat cultivars, landraces and wild accessions of different *T. turgidum* L. subspecies. As it was previously shown, the population structure of this tetraploid wheat collection, partially reflects the evolutionary history of *T. turgidum* subspecies (Laidò et al. 2013). In particular, the collection consisted of 111 accessions grouped into seven subspecies: subsp. *durum* (Desf.) Husn. (64 accessions), subsp. *turanicum* (Jakubz.) A. et D. Löve (8 accessions), subsp. *turgidum* (12 accessions), subsp. *polonicum* (L.) Thell. (8 accessions), subsp. *carthlicum* (Nevski) Á. Löve et D. Löve (3 accessions), subsp. *dicoccum* (Schrank ex Schübler) Thell. (9 accessions) and subsp. *dicoccoides* (Körn. ex Asch. et Graebner) Thell. (7 accessions).

The plant material was grown under conventional farming in the experimental field of the University of Bari, at Valenzano (Bari, Italy) in the 2011–2012 and

2012– 2013 growing seasons. A randomized complete block design (RCB) was used to account for any environmental effect on phenolic acid accumulation in the grain. The RCB was comprised of three replications and plots consisted of 1-m rows, 30-cm apart, with 50 germinating seed per plot.

Phenolic acids analysis

The whole-grain samples were milled using a laboratory mill (Retsch GmbH, Haan, Germany) to yield whole-meal flour of 0.5 mm particle size, which was stored at −20 °C until analysis. Phenolic acids were extracted from 250 mg whole-meal flour. Two subsequent, delipidation steps were carried out using 5 mL of hexane. After centrifugation for 10 min at 6000 × *g* the supernatant was removed and discarded. Ten microliters of internal standard (3,5-dichloro-4-hydroxybenzoic acid at 1.5 mg mL^{-1} in 80:20 v/v ethanol/water) were added to the wet pellet and samples were hydrolyzed with 2 M NaOH for 2 h, with continuous shaking, at 4 °C, in the dark. Samples were then acidified to pH 2 with 12 M HCl and extracted three times with ethyl acetate. The ethyl acetate extracts were combined and dried under nitrogen flux. Dried phenolics were dissolved with 100 μL 80 % methanol and transferred to a clean vial for HPLC analysis. At least two replicates were made for all samples.

The extracted compounds were analysed by HPLC using an Agilent 1100 high-performance liquid chromatography equipped with a photodiode array detector (DAD). Wavelengths of 280, 295 and 320 nm were used to quantify phenolic acids. Phenolic acid separation was achieved by using a Phenomenex-luna 5 μm C18 (2) 100 Å column (250 × 4.6 mm) and a column temperature of 30 °C. The flow rate of the mobile phase was 1.0 mL min^{-1}, and the injection volume was 20 μL. A gradient-elution program was utilized with a mobile phase consisting of acetonitrile (solution A) and 1 % (v/v) H_3PO_4 in water (solution B) as follows: isocratic elution, 100 % B, 0–30 min; linear gradient from 100 B to 85 % B, 30–55 min; linear gradient from 85 B to 50 % B, 55–80 min; linear gradient from 50 B to 30 % B, 80–82 min; post time, 10 min before the next injection. The flow rate of the mobile phase was 1.0 mL min^{-1}, and the injection volume was 20 μL. All phenolic acids were quantified via a ratio to the internal standard (3,5-dichloro-4-hydroxybenzoic acid) added to every sample and using calibration curves of phenolic acid standards having undergone the same extraction procedure. All samples were analyzed in duplicate and concentrations of individual phenolic acids were expressed in micrograms per gram of dry matter. Authentic standards of phenolic acids were obtained from Sigma-Aldrich (Gillingham, UK) and included *p*-hydroxybenzoic acid, vanillic acid, syringic acid, *p*-coumaric acid, sinapic acid and ferulic acid. All standards were prepared as stock solutions at 2 mg mL^{-1} in 80:20 v/v ethanol/water which were stored in the dark at −20 °C. 3,5-Dichloro-4-hydroxybenzoic acid in 80:20 ethanol/water was used as an internal standard at a concentration of 1.5 mg mL^{-1}.

Statistical analyses

Standard procedure for analysis of variance (ANOVA) was carried out to verify the effects of field replications on phenolic acids content of four genotypes included in the tetraploid wheat collection, namely Ciccio, Duilio, Iride and Svevo cultivars. Two factor (genotype and year) ANOVA was performed for each phenolic acid in the combined analysis over years. Broad-sense heritability $\left(h_B^2\right)$ was determined using the variance component estimates with combined analysis. Pearson phenotypic correlation coefficients (r) were calculated among phenolic acids, and between phenolic acids and some yield components (heading time, plant height, grain yield per spike, kernel weight) and quality traits (arabinoxylans, β-glucan, grain protein content, polyphenol oxidase activity) that were evaluated in the growing seasons 2011–2013. MSTATC package Software, Version 2.6. 1998, was used to perform all analyses.

Results and discussion

Variation of total phenolic acid content

Preliminary experiments were carried out on four genotypes (Ciccio, Duilio, Iride, and Svevo) to estimate the significance of differences among replications of the RCB experimental design. Due to the lack of significant

differences (Table 1), for subsequent analysis on the complete wheat collection, 3 g of whole grains from each experimental replication, and corresponding to each genotype, were pooled into one sample, milled and used for phenolic acid analysis.

Values of the means of total saponified phenolic acid concentrations observed in the wheat collection and in the different *T. turgidum* subspecies, are shown in Fig. 1 and Table 2. Considering the complete wheat collection, the mean value of total phenolic acid concentration was 730.4 μg g^{-1} dm in 2012 and 802.5 μg g^{-1} dm in 2013, and a large variation was observed among the genotypes both for 2012 and 2013 (Table 2).

Genotypes of subsp. *durum* showed contents of total phenolic acids that were more stable over the years compared to those of other subspecies (Fig. 1). Also, durum cultivars had the highest average content of total phenolic acids (828.7 μg g^{-1} dm in 2012 and 834.5 μg g^{-1} dm in 2013), with values varying from 550.9 to 1701.2 μg g^{-1} dm, thus indicating a variation of greater than threefold (data not shown). In particular, we found that six durum cultivars, out of 64, showed a total phenolic acid content that exceeded 1000 μg g^{-1} dm in both growing seasons (data not shown). Ambral had the highest level of total phenolic acids (1468.2 μg g^{-1} dm in 2012, and 1701.2 μg g^{-1} dm in 2013), whereas Cosmodur, a cultivar released during the same period as Ambral, had the lowest (671.1 μg g^{-1} dm in 2012 and 577.9 μg g^{-1} dm in 2013). Overall, cultivars Ambral, Pedroso, Primadur and Tito showed the highest and most stable phenolic acid content over the years (data not shown). Actually, previous evidences also showed that the extent of variation due to environmental factors may vary from genotype to genotype, with some genotypes that can be more stable than others (Fernandez-Orozco et al. 2010; Menga et al. 2010).

Based on average content, *turanicum*, *polonicum*, *carthlicum* and *dicoccoides* subspecies had slight lower total phenolic acid content as compared to durum cultivars, and showed less variation among the accessions (Table 2). Likewise, rivet lines (subsp. *turgidum*) had amounts of phenolic acids similar to those of durum, but a lower range of variation was observed (1.8 fold). Finally, on the basis of overall outcome data, the lowest content of total phenolic acids was found in domesticated hulled subsp. *dicoccum* (576.1 μg g^{-1} dm in 2012, and 694.2 μg g^{-1} dm in 2013).

Our results were supported by Giambanelli et al. (2013) who found interesting variation among different old landraces and modern varieties of durum and bread wheat genotypes, without observing an overall superiority of primitive forms on modern wheat varieties. Similarly, a recent study found that durum and bread wheat varieties had a higher content of total phenolic acids compared to that of primitive subspecies, such as *turanicum* and *dicoccum* (Brandolini et al. 2013).

On the whole, this study showed a substantial variation among the tetraploid wheat genotypes for the content of total phenolic acids. In particular, results showed that plant breeding has not caused a decrease in phenolic acid content in modern durum cultivars with respect to old cultivars and primitive subspecies. Therefore, our data indicate that the use of old landraces or wild accessions in breeding programs would not be necessary to improve phenolic acid content in elite wheat germplasm.

Variation of individual phenolic acids

A total of six phenolic acids were identified across the 111 wheat genotypes, namely: ferulic, sinapic, *p*-coumaric, vanillic, syringic and *p*-hydroxybenzoic acids (Table 2). These results are on line with those presented in the genetic diversity screen on common wheats, with the exception of a few minor compounds,

Table 1 Analysis of variance of phenolic acids evaluated in four durum wheat cultivars (Ciccio, Duilio, Iride and Svevo) grown at Valenzano (Bari, Italy) in 2012 using a randomized blocks with three replications

Source of variation	*df*	*p*-Hydroxy benzoic acid	Vanillic acid	Syringic acid	*p*-Coumaric acid	Ferulic acid	Sinapic acid
Replication	2	0.001	0.002	0.009	0.005	1.480	0.131
Genotypes	3	0.585***	0.203***	0.458***	3.678***	629.729***	32.472***
Error	6	0.001	0.003	0.002	0.003	1.119	0.233

df degree of freedom

*** Significant differences at $p = 0.001$

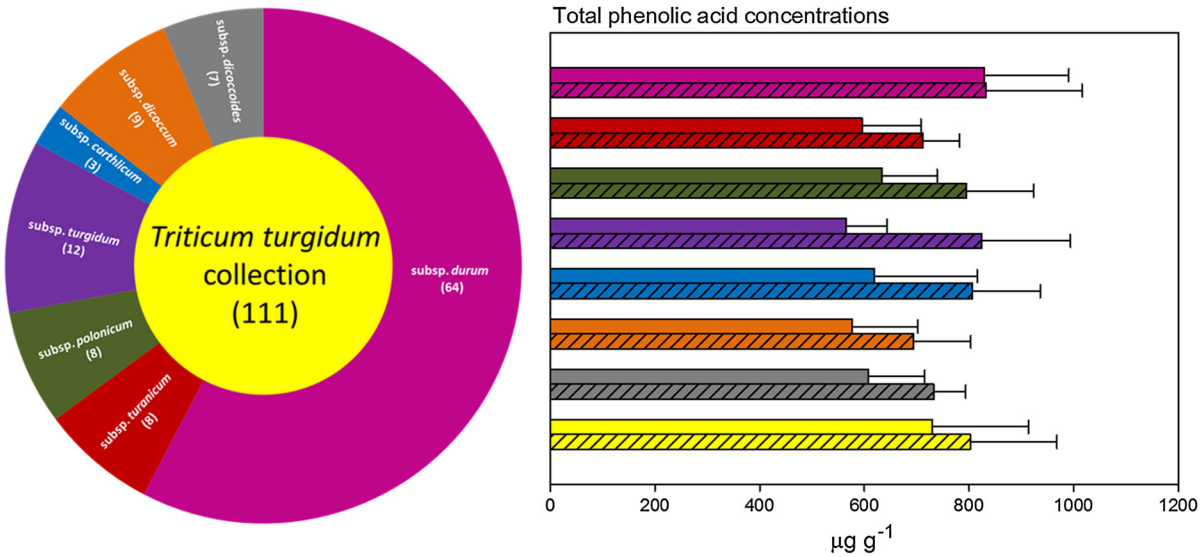

Fig. 1 *Left* Pie chart showing the *T. turgidum* subspecies considered in the study and the number of genotypes (*in brackets*) comprised in each subspecies and in the full wheat collection. The areas corresponding to each *T. turgidum* subspecies are shown in different colors and are proportional to the number of genotypes analyzed. *Right* 2-year means observed for total saponified phenolic acid concentrations. *Plain histograms* 2012 mean values; *dotted-dashed histograms* 2013 mean values. *T. turgidum* subspecies and full collection in histograms are shown with same colors used for the pie chart

namely syringaldehyde, dihydroxybenzoic acid and 2-hydroxycinnamic acid, that were not detected in this study (Li et al. 2008; Fernandez-Orozco et al. 2010).

Considering the whole tetraploid wheat collection, ferulic, sinapic and *p*-coumaric acids were the most abundant phenolic acids. Notably, these acids are derivatives of hydroxycinnamic acid and were previously found to be mainly present as linked to cell wall structural components (Sosulski et al. 1982), and to represent the most abundant phenolic acids in the whole-wheat grain (Li et al. 2008). In particular, ferulic acid contributed 79 % of total phenolic acids, with mean values of 573.7 µg g^{-1} dm in 2012, and of 638.1 µg g^{-1} dm in 2013 (Table 2). Second and third for abundance, sinapic and *p*-coumaric acids accounted for approximately 120 and 25 µg g^{-1} dm, respectively. Minor components were vanillic, syringic and *p*-hydroxybenzoic acids, which contributed for 7, 3 and 2 µg g^{-1} dm, respectively (Table 2). Coefficient of variation (CV), was higher for least represented phenolic acids than for ferulic, sinapic, and total phenolic acids (Table 2), being in agreement with previous results on common wheat (Fernandez-Orozco et al. 2010).

Saponified phenolic acids are among the most abundant forms in the whole-meal wheat flour and may have important implications on human health. Indeed, a growing body of literature showed a more interesting role of saponified phenolic acids as antioxidants with anti-tumor effects, as compared to free forms (Laddomada et al. 2015a, Vitaglione et al. 2008). The occurrence of ester- or ether-linkages to cell wall polymers, is not an hindrance for phenolic acids to exert their biological activity. In fact, intestinal microbes can cleave the bonds, thereby making phenolic acids nutritionally available (Andreasen et al. 2001). Moreover, recent studies evidenced that the structural complexity of bound phenolics could even enforce their health value because, by reaching the colon mostly undigested, they can exert their unique antioxidant and anti-inflammatory activity locally, and contribute to a reduced risk for colorectal cancer (Adreasen et al. 2001; Vitaglione et al. 2008).

Effect of genotype and cropping year on total and individual phenolic acids

The effect of genotype and cropping year was estimated by ANOVA on the overall data (Table 3). Significant effects of genotype, year and genotype × year interaction were found for individual and total

Table 2 Mean, standard deviation (SD) and coefficient of variation (CV %) of individual phenolic acids (μg g^{-1} dry matter) in a tetraploid wheat collection grown at Valenzano (Bari, Italy) in 2012 and 2013

T. turgidum subsp.	Year of release	*p*-Hydroxy benzoic acid		Vanillic acid		Syringic acid		*p*-Coumaric acid		Ferulic acid		Sinapic acid		Total phenolic acids	
		2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
Wheat collection (111)															
General Mean		2.0	2.2	6.7	7.4	3.2	3.1	24.8	26.8	573.7	638.1	120.1	124.9	730.4	802.5
SD		1.3	1.6	2.9	2.6	1.9	1.4	8.4	13.7	154.2	139.4	39.6	47.5	183.1	165.3
CV (%)		66.4	73.3	43.6	35.1	58.5	46.6	34.0	51.3	26.9	21.9	33.0	38.0	25.1	20.6
durum (64)[a]	1991–2008														
Mean (30)		2.3	2.4	5.6	7.8	2.0	3.2	25.8	23.7	623.0	656.3	135.3	128.0	794.0	821.3
SD		1.1	1.3	2.8	2.8	1.1	1.5	7.0	8.4	111.8	145.5	34.4	51.0	128.6	179.1
CV (%)		50.2	55.0	49.7	35.7	52.0	48.7	27.1	35.6	18.0	22.2	25.4	39.8	16.2	21.8
	1971–1990														
Mean (26)		2.1	2.2	5.2	7.3	2.1	3.1	26.3	24.6	700.7	696.7	146.4	125.0	882.7	858.8
SD		1.1	1.1	2.7	2.0	1.1	1.5	7.4	7.7	181.4	194.0	31.5	42.0	200.7	209.9
CV (%)		54.2	49.1	52.3	27.7	50.7	48.4	28.1	31.6	25.9	27.8	21.5	33.6	22.7	24.4
	Before 1971														
Mean (8)		2.0	2.5	7.5	8.5	3.0	3.1	26.5	21.6	620.2	661.4	124.0	107.6	783.4	804.6
SD		1.2	1.3	4.0	3.5	1.8	1.4	5.4	5.4	71.8	74.0	36.0	52.5	90.4	99.0
CV (%)		60.4	50.4	53.4	41.7	59.0	45.2	20.3	25.1	11.6	11.4	29.0	48.8	11.5	12.3
Mean cultivars		2.2	2.3	5.7	7.7	2.2	3.1	26.1	23.8	654.2	673.3	138.4	124.2	828.7	834.5
SD		1.1	1.2	3.0	2.6	1.2	1.5	6.9	7.8	144.8	160.1	33.1	47.8	163.1	184.0
CV (%)		52.3	51.7	52.9	34.2	54.7	47.7	26.5	32.8	22.1	23.8	23.9	38.5	19.7	22.0
turanicum (8)															
Mean		1.0	1.5	7.0	6.9	3.1	2.5	21.4	19.4	476.5	590.5	86.7	91.6	595.8	712.5
SD		0.8	0.8	1.8	1.6	1.1	0.7	7.9	3.3	84.2	70.4	30.0	22.1	112.4	69.6
CV (%)		74.8	51.5	25.3	23.4	35.6	27.8	36.8	17.2	17.7	11.9	34.6	24.1	18.9	9.8
polonicum (8)															
Mean		1.1	1.6	7.1	5.3	4.3	2.6	23.7	30.3	488.8	614.2	108.2	140.5	633.2	794.5
SD		0.7	1.0	1.6	0.7	1.1	0.9	9.2	14.2	82.1	89.8	27.8	51.1	106.0	129.3
CV (%)		62.9	64.8	23.2	12.9	24.5	35.8	38.7	46.9	16.8	14.6	25.6	36.3	16.7	16.3
turgidum (12)															
Mean		1.1	1.5	9.1	7.6	4.9	3.3	22.2	43.0	441.6	613.9	86.8	154.5	565.7	824.0
SD		0.9	0.7	2.1	1.8	1.6	0.9	11.6	28.9	48.5	94.2	23.4	55.5	77.8	170.0

Table 2 continued

T. turgidum subsp.	Year of release	*p*-Hydroxy benzoic acid		Vanillic acid		Syringic acid		*p*-Coumaric acid		Ferulic acid		Sinapic acid		Total phenolic acids	
		2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
CV (%)		78.1	44.9	23.3	24.0	32.0	26.5	52.5	67.3	11.0	15.3	27.0	35.9	13.8	20.6
carthlicum (3)															
Mean		1.2	1.2	8.1	5.6	6.7	3.5	19.4	25.4	528.6	687.5	54.7	83.7	618.7	806.9
SD		1.0	1.3	2.6	2.5	1.9	1.5	7.2	3.5	175.9	136.9	22.4	17.6	196.9	129.1
CV (%)		79.9	108.0	31.9	45.1	29.1	42.9	37.0	13.6	33.3	19.9	41.0	21.0	31.8	16.0
dicoccum (9)															
Mean		3.0	2.2	7.8	5.5	4.1	2.0	25.7	29.2	446.5	535.5	89.6	119.7	576.1	694.2
SD		2.3	1.1	1.3	1.0	1.5	0.3	13.0	11.2	95.0	74.1	28.1	35.6	126.2	109.3
CV (%)		77.4	49.4	17.2	17.3	37.2	16.8	50.7	38.4	21.3	13.8	31.3	29.7	21.9	15.7
dicoccoides (7)															
Mean		3.1	4.1	9.0	10.2	5.7	4.6	23.9	28.2	447.0	559.8	117.9	125.8	607.6	732.6
SD		1.2	4.5	3.2	3.9	2.2	2.3	9.4	13.3	78.9	49.3	38.8	47.7	107.5	60.8
CV (%)		39.3	108.3	35.7	38.3	37.9	49.4	39.2	47.2	17.6	8.8	32.9	37.9	17.7	8.3

[a] In brackets were indicated the number of genotypes for each subspecies

phenolic acids ($p < 0.001$). Broad-sense heritability $\left(h_B^2\right)$ was calculated using the variance component estimates with combined analysis (Table 3). Results showed that heritability values varied among individual phenolic acids, being lower (0.48) for syringic acid and higher (0.70) for ferulic acid. The heritability estimated for total phenolic acid content was 0.63, which was higher than that observed in the diversity screen of bread wheat lines (Fernandez-Orozco et al. 2010; Shewry et al. 2010). In fact, the heritability of phenolic acids registered in bread wheat was particularly low for free and conjugated forms (0.06 and 0.09, respectively) and moderately low for bound and total phenolic acids (0.26 and 0.28, respectively) (Fernandez-Orozco et al. 2010; Shewry et al. 2010). Yet, despite the large variation due to environmental factors, the authors found that some bread wheat genotypes resulted more stable than others (Fernandez-Orozco et al. 2010). The differences in phenolic acids heritability between tetraploid and bread wheat could depend particularly on environmental factors. The tetraploid lines examined in this study were grown in Southern Italy, in a highly different climate as compared to that of northern Europe considered in the HEALTHGRAING diversity screen (Fernandez-Orozco et al. 2010).

To our knowledge, this research has been the first to quantify the effect of genotype and location on individual and total saponified phenolic acids in a large collection of tetraploid wheats. Results showed that by selecting suitable location and eligible genotypes, it would possible to achieve high and more stable phenolic acid contents in durum wheat. The use of durum whole-meal flour with a high content of phenolic acids might contribute to enhancing the health-promoting value of durum-based food products (Laddomada et al. 2015b; Pasqualone et al. 2015).

Correlations of phenolic acids with yield components and quality characteristics

Correlations among different individual phenolic acids and total saponified phenolic acid concentrations are shown in Table 4. Significant correlations ($p < 0.001$) were found between total phenolic acids and ferulic acid ($r = 0.98$ in 2012; $r = 0.54$ in 2013), sinapic acid ($r = 0.74$ in 2012; $r = 0.56$ in 2013) and *p*-coumaric acid ($r = 0.41$ in 2012; $r = 0.47$ in 2013). These results were rather predictable, due to the relative high abundance of ferulic, sinapic and *p*-coumaric acids with respect to the total. A significant correlation was found between vanillic and syringic acids ($r = 0.44$, $p < 0.001$ in 2012 ($r = 0.36$, $p < 0.01$ in 2013) and *p*-hydroxybenzoic and *p*-coumaric acids ($r = 0.44$, $p < 0.001$ in 2012). Due to significant year × genotype interactions (Table 3), some correlations were contradictory over the years (Table 4). In particular, correlations between least abundant phenolic acids, namely *p*-hydroxybenzoic acid and vanillic acid, and *p*-hydroxybenzoic acid and *p*-coumaric acid, that were significant in 2012, were not confirmed in 2013. Also, *r* values between syringic and sinapic acids, and syringic and total saponified phenolic acids, had opposite sign in 2012 and 2013. Actually, correlations between individual phenolic acids have been poorly investigated in literature, and a few and discordant outcomes are available (Zhou et al. 2004; Hernandez et al. 2011). In an extensive and

Table 3 Combined analysis of variance and heritability $\left(h_B^2\right)$ of saponified phenolic acids in a tetraploid wheat collection evaluated at Valenzano (Bari, Italy) in 2 years (2012 and 2013)

Source of variation	*df*	*p*-Hydroxy benzoic acid	Vanillic acid	Syringic acid	*p*-Coumaric acid	Ferulic acid	Sinapic acid	Total phenolic acid
Year (Y)	1	3.702***	19.445***	1.098***	401.927***	476,395.935***	3391.107***	604,268.532***
Genotype (G)	110	6.541***	50.426***	7.382***	391.356***	71,766.494***	5112.566***	94,561.467***
Y × G	110	1.776***	11.102***	3.964***	133.104***	15,333.427***	2513.087***	27,884.704***
Error	222	0.016	0.133	0.039	1.234	155.780	13.374	235.223
h_B^2		0.65	0.69	0.48	0.60	0.70	0.50	0.63

The analysis was carried out on 111 genotypes

df degree of freedom

*** Significant differences at $p = 0.001$

Table 4 Correlations among individual phenolic acids in a tetraploid wheat collection grown at Valenzano (Bari, Italy) in 2012 and 2013

	Year	Vanillic acid	Syringic acid	*p*-Coumaric acid	Ferulic acid	Sinapic acid	Total phenolic acids
p-Hydroxy	2012	0.02	0.19*	0.44***	0.11	0.29**	0.18
benzoic acid	2013	0.52***	0.21*	0.07	−0.06	0.35**	0.10
Vanillic acid	2012		0.44***	−0.03	−0.26**	−0.21*	−0.24**
	2013		0.36**	0.05	0.07	0.19*	0.18
Syringic acid	2012			0.12	−0.38***	−0.33***	−0.37***
	2013			0.27**	0.16	0.38***	0.36***
p-Coumaric acid	2012				0.39***	0.39***	0.41***
	2013				0.21**	0.57***	0.47***
Ferulic acid	2012					0.61***	0.98***
	2013					0.24**	0.54***
Sinapic acid	2012						0.74***
	2013						0.56***

*, **, *** Significant differences at $p = 0.05$, $p = 0.01$ and $p = 0.001$ respectively

complete study on phenolic acid variation in common wheats (Fernandez-Orozco et al. 2010), a high correlation was found between bound and total phenolic acids, which is consistent with our results showing that ferulic acid, making up a large proportion of total phenolic acids, correlated significantly with several individual phenolic acids.

Pearson correlation coefficients (r) were calculated between phenolic acids and other traits and results are shown in Table 5. In particular, we considered the correlations between phenolic acids and yield components (e.g. heading time, plant height, grain yield per spike, kernel weight) (Mangini et al. unpublished data); arabinoxylans (Marcotuli et al. 2015); β-glucan (Marcotuli et al. unpublished data); grain protein content (Mangini et al. unpublished data); polyphenol oxidase activity (Taranto et al. 2015).

Low and positive correlations ($p < 0.01$), were observed between ferulic, sinapic, total phenolic acids and β-glucan, which is one of the major components of dietary fiber in wheat grain. On the other hand, only a few correlations were found between phenolic acids (*p*-hydroxybenzoic and vanillic acids) and arabinoxylans ($p < 0.05$ and $p < 0.01$, respectively). Indeed, stronger correlations were previously observed between phenolic acids and other fiber components, such as lignin, which shares a common biosynthetic precursor with phenolic acids (Fernandez-Orozco et al. 2010).

Correlations between phenolic acids and polyphenol oxidase (PPO) activity were not significant, with the exception of syringic acid ($r = 0.51$, $p < 0.001$). As far as it is known, polyphenol oxidase activity is associated with whole-meal wheat flour and wheat-based products browning (McCallum and Walker 1990; Feillet et al. 2000). In fact, previous studies showed a moderate and positive correlation between free phenolic compounds and polyphenol oxidase activity in the whole-meal flour of tetraploid wheat (Pasqualone et al. 2014). Results here presented refer to saponified phenolic acids and were in agreement with other evidences showing that PPO activation takes place mostly after the addition of water to the wheat flour for pasta or bread processing (Feillet et al. 2000).

Finally, we found that yield components and grain protein content correlated with a number of phenolic acids, although most correlations were not stable over the years due to year × genotype interactions (Table 5). Indeed, while phenolic acids are chiefly concentrated in the bran, no significant correlations were previously found between phenolic acid content and grain properties such as thousand kernel weight, or protein content (Fernandez-Orozco et al. 2010).

Table 5 Correlations among phenolic acids, arabinoxylan (AX), β-glucan (BG), polyphenol oxidase activity (PPO), heading time (HT), plant height (PH), grain yield per spike (GYS), kernel weight (KW) and grain protein content (GPC) in a tetraploid wheat collection grown at Valenzano (Bari, Italy) in 2012 and 2013

	Years[a]	AX	BG	PPO	HT	ALT	GYS	KW	GPC
p-Hydroxy benzoic acid	2012	−0.21*	0.07	0.24*	−0.03	−0.23*	−0.01	−0.08	−0.03
	2013	n.a.	0.03	n.a.	−0.19	−0.25*	0.02	−0.16	−0.23*
Vanillic acid	2012	−0.27**	−0.08	0.31**	0.49***	0.47***	−0.32***	−0.22*	0.34***
	2013	n.a.	0.11	n.a.	−0.09	−0.06	−0.04	−0.07	−0.29**
Syringic acid	2012	−0.08	−0.07	0.51***	0.48***	0.50***	−0.37***	−0.26**	0.51***
	2013	n.a.	0.23*	n.a.	−0.09	−0.03	−0.22*	−0.30**	−0.16
p-Coumaric acid	2012	0.04	0.11	0.09	0.01	−0.22*	−0.02	−0.09	−0.06
	2013	n.a.	−0.01	n.a.	0.33***	0.36***	−0.16	−0.38***	0.20
Ferulic acid	2012	0.16	0.28**	−0.24*	0.30**	−0.48***	0.30**	0.01	−0.37***
	2013	n.a.	0.09	n.a.	−0.13	−0.03	0.05	−0.19	−0.20
Sinapic acid	2012	0.02	0.27**	−0.25*	−0.33***	−0.55***	0.14	−0.04	−0.31**
	2013	n.a.	0.02	n.a.	0.11	0.07	−0.11	−0.30**	0.02
Total phenolic acids	2012	0.13	0.30**	−0.24*	−0.31**	−0.53***	0.27**	−0.02	−0.37***
	2013	n.a.	0.06	n.a.	0.01	−0.05	−0.03	−0.31**	−0.20

n.a data not available

*, **, *** Significant differences at $p = 0.05$, $p = 0.01$, and $p = 0.001$ respectively

[a] The correlation analysis included 98 and 92 accessions in 2012 and 2013 respectively

Concluding remarks

We showed the first comprehensive study of the variability of phenolic acids in a large sample of tetraploid wheat genotypes. Significant effect of genotype was found for total and individual phenolic acids, and the extent of variation due to cropping year, and to year × genotype interaction varied among genotypes. Indeed, the ratio of genotypic variance to total variance was moderately high suggesting that a breeding approach could be considered to increase phenolic acids concentration in durum wheat. We also observed significant correlations between phenolic acids and some quality characteristics of the grain. Moreover, by comparing the different tetraploid subspecies, durum cultivars showed higher concentrations of individual and total phenolic acids. Six cultivars resulted particularly rich in phenolic acids, suggesting that some elite durum varieties could be preferred for improving the health-promoting value of durum end-products in terms of phenolic acid concentration. In fact, recent studies showed that, even though pasta and baking processes result in a lost of phenolic acids in end-products as compared to wholemeal flour, yet an increase of phenolic acids can be achieved in pasta and baking products by using raw materials particularly rich in phenolic acids (Laddomada et al. 2015).

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Compliance with ethical standards

The Authors declare to do not have any relationships or interests that could have direct or potential influence or impart bias on the work. The present research did not involve any human participants and/or animals. The above manuscript publication is approved by all authors and by the responsible authorities where the work was carried out.

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