



Phenolics in the bran of waxy wheat and triticale lines[☆]

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ABSTRACT

The present study was designed to determine total phenolic acid contents (TPC) and compositions of bran from newly developed near-isogenic waxy wheat and triticale translocation lines. Two waxy wheat sets, Svevo (durum) and N11 (bread wheat), consisting of partial and waxy null lines and four sets of triticales (GDS7, Trim, Rhino and Rigel) having translocations at 1A.1D and 1R.1D with high molecular weight glutenin subunits (HMW-GS) 5 + 10 and 2 + 12 were investigated. Similar to non-waxy wheat, ferulic acid was the predominant phenolic acid found in waxy wheats analyzed. Two other major phenolics include *p*-coumaric and vanillic acids followed by lesser quantities of syringic acid. Waxy lines had higher TPC than the parent line in the N11 set, whereas the Svevo set showed the opposite trend. TPC of waxy bread wheats were correlated with amylose fractions in which the order was complete waxy < double waxy nulls < single waxy nulls. Lines with HMW-GS 2 + 12 have lower TPC than other lines in each group of triticales, except the Trim set. TPC was negatively correlated ($r = -0.41$; $p > 0.1$) with bran yields in triticale lines studied, indicating that variation in phenolics was not only due to bran yields but also to genotypic differences.

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1. Introduction

The increasing demand for natural food antioxidants has hastened research to extract the biologically active substances from a variety of raw materials (Diaz-Reinoso et al., 2006). Increased consumption of phenolic compounds has been correlated with a reduced risk of cardiovascular diseases and certain types of cancers (Arts and Hollman, 2005; Jacobs et al., 1998; Kris-Etherton et al., 2002). Significant levels of antioxidant activities have been detected in wheat and wheat-based food products (Adom and Liu, 2002; Baublis et al., 2000; Yu et al., 2002).

Wheat (*Triticum aestivum* L.) is among the most extensively cultivated crops in the world. Waxy wheats (amylose-free, consists of essentially all amylopectin in its starch) have been developed via classical breeding and genetics (Nakamura et al., 1995) and it was

reported to have unique properties in bread making with retardation of staling and formation of a new texture in breads and noodles. It is also considered as a good material for making whole-wheat bread (Hung et al., 2006, 2007). Therefore, interest in waxy wheat is high and is expected to grow over the next few years. Triticale (*Triticosecale* Wittmack) is a hybrid crop developed by crossing wheat (*Triticum* spp.) and rye (*Secale cereale*) that combines the properties of both parental cereals (Salmon et al., 2001). Triticale is intended to have high yield potential and grain quality of wheat and resistance to pathogens of rye. The nutritional value of triticale is close to that of wheat and rye (Chapman et al., 2005; Salmon et al., 2008). The area under triticale cultivation has been increasing very slowly (Arseniuk and Oleksiak, 2002) but steadily (Varughese et al., 1996) all over the world, with current cultivation at 3.9 million ha with a production of 14 million tons (FAO, 2008).

Previous studies reported that phenolic acids in wheat grains are mostly in the bound form and exist in bran associated with cell wall materials (Adom and Liu, 2002; Li et al., 2008; Liyana-Pathirana and Shahidi, 2006). Triticale and waxy wheat by-products such as bran may serve as sources of valuable phenolics for food and nutraceutical applications. Although antioxidant activity and phenolic acid profiles of cereals have been reported extensively (Andersson et al., 2008; Verma et al., 2009; Zielinski and Kozłowska, 2000), only a few recent studies are available on bioactive components of triticale and waxy wheat (Hosseinian and Mazza, 2009; Hung et al., 2009; Menga

Abbreviations: GAE, gallic acid equivalents; HMW-GS, high molecular weight glutenin subunits; HPLC, high performance liquid chromatography; NIL, near-isogenic lines; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; TPC, total phenolic acids content.

[☆] Names are necessary to report factually on available data; however, the U.S. Department of Agriculture neither guarantees nor warrants the standard of the product, and use of the name by the U.S. Department of Agriculture implies no approval of the product to the exclusion of others that may also be suitable.

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et al., 2010). On the other hand, literature on the genotype effect and correlation with phenolics in those grains is limited.

Different waxy null alleles affect the amylose content and other functional properties in various ways. For instance, the reduction of amylose content due to these null alleles was as follows: $Wx-A1B1D1 > Wx-B1D1 > Wx-A1B1 > Wx-A1D1 > Wx-B1 > Wx-D1 > Wx-A1$ (Miura et al., 1994, 1999). Wild types (presence of all three *Wx* proteins) contain 25–28% amylose (Graybosch, 1998; Yamamori et al., 2000), whereas waxy lines lack all three *Wx* loci (waxy) and contain in the order of 0–3% amylose (Nakamura et al., 1995; Yasui et al., 1997). Variation in amylose/amylopectin ratios has been shown to contribute to differences in physicochemical properties, ultimately affecting the quality of end-use products (Hung et al., 2006). However, it is also important to determine whether the genetic changes that results in variation in the starch compositions also have any impact on other bioactive components presented in the bran material.

Near-isogenic waxy wheat and triticale translocation lines used in this study differ at one or more genetic loci coding for storage proteins. These lines have been used for functionality studies, and the information can be successfully used to manipulate functional properties predictably in a breeding situation. Though the breeding program for the genetic lines used in this study was targeted mainly for changes in composition of proteins, we cannot ignore the unintentional changes that might occur in bioactive components of wheat bran during the development of new genetic materials. The aim of the present study was to determine and compare the phenolics contents and compositions of newly developed near-isogenic waxy wheat and triticale translocation lines.

2. Materials and methods

2.1. Samples

Two waxy wheat lines (Svevo—a durum wheat and N11—a bread wheat) were used in this study. These waxy wheats were developed at the Department of Agrobiological & Agrochemistry, University of Tuscia, Viterbo, Italy. They were grown in Viterbo during 2005–2006. Detailed description of near-isogenic waxy wheat lines used in this study is provided in Table 1. All these lines were developed for at least 6 generations, backcrossed to the same parent and thus are near-isogenic lines (NIL). Samples were run on SDS-PAGE to identify the HMW-GS composition.

Four sets of triticale translocation lines, GDS7, Trim, Rhino, and Rigel were used in the study. Triticale lines were grown in Viterbo during 2005–2006. These lines were developed to evaluate the effect of the specific translocation types on bread making performance. Brief description of the lines used along with HMW-GS

Table 1
Description of waxy wheat lines used in the study along with their bran yields.

Sample Name	Type of wheat	Variation	Bran yield (%)
Svevo set (Null, 7 + 8)			
Svevo wheat	Durum wheat	Parent line	50.6
Svevo waxy	Complete waxy	Null at A1 ⁻ /B1 ⁻	53.0
Svevo waxy A1 ⁻	Partial waxy	Null at A1 ⁻	52.0
Svevo waxy B1 ⁻	Partial waxy	Null at B1 ⁻	61.3
N11 set (1, 7 + 8, 2 + 12)			
N11 wheat	Bread wheat	Parent line	46.9
N11 waxy	Complete waxy	Null at A1 ⁻ /B1 ⁻ /D1 ⁻	53.0
N11 waxy A1 ⁻	Partial waxy	Null at A1 ⁻	50.0
N11 waxy B1 ⁻	Partial waxy	Null at B1 ⁻	49.9
N11 waxy D1 ⁻	Partial waxy	Null at D1 ⁻	53.1
N11 waxy A1 ⁻ /B1 ⁻	Partial waxy	Null at A1 ⁻ /B1 ⁻	53.4
N11 waxy A1 ⁻ /D1 ⁻	Partial waxy	Null at A1 ⁻ /D1 ⁻	56.7
N11 waxy B1 ⁻ /D1 ⁻	Partial waxy	Null at B1 ⁻ /D1 ⁻	55.2

composition is presented in Table 2. All the triticale sets have 13 + 16 subunits in common at the *Glu-B1* loci.

The rationale behind the selection of these two different groups of waxy wheat samples was to include a complete set of partial and waxy null lines with the same parental background of bread wheat (N11) and durum wheat (Svevo). In this way, we can observe the effect of each of the waxy null lines on contents of phenolics, both with-in and between the two different kinds of wheat i.e. durum and bread wheat. Similarly, four different sets of triticales were selected for analysis in which all the sets contain similar translocation lines.

Waxy wheat and triticale samples were tempered to 15.5% moisture, 15–16 h prior to milling. Tempered samples were milled in a Brabender Quadrumat Junior Mill (C.W. Brabender, Duisburg, Germany) following method AACC 26-50 (AACC, 2000). Bran yields of waxy wheat and triticale lines are presented in Table 1 and Table 2, respectively. Bran samples were ground in a coffee grinder (Black and Decker CBG5, Miami Lakes, FL) for 1 min and sieved by a 30 µm sieve (The W.S. Tyler, Mentor, Ohio) prior to extraction.

2.2. Extraction of phenolic compounds

The method used to extract phenolics was based on the method described by Kim et al. (2006). Triticale and waxy wheat bran samples were defatted twice by stirring in hexane at a 1:4 ratio (w/v) for 1 h at ambient temperature. The mixture was filtered through Whatman #1 filter paper. Defatted bran samples were dried at room temperature and hydrolyzed by mixing 2 M NaOH in 80% methanol at a 1:5 ratio (w/v) for 3 h to release both bound and free phenolic compounds. The mixture was acidified to pH = 2.0 ± 0.1 with 8 M HCl and filtered through glass wool. The clear mixture was extracted three times with 150 ml of diethyl ether. Combined ether fractions were dried over anhydrous sodium sulfate. The ether extract was evaporated to dryness and the final residue was reconstituted with 80% methanol to a final volume of 5 ml for 1 g bran.

2.3. Determination of total phenolic content (TPC)

The total polyphenolic contents of the extracts were assayed by the Folin–Ciocalteu assay (Folin and Ciocalteu, 1927; Singleton

Table 2
Description of triticale lines used in the study along with their bran yields.

Sample ID	Translocation	HMW-GS on chromosome			% Bran yield
		1A	1B	1R	
GDS7 set					
GDS7	Parent line	Null	13 + 16		59.8
GDS7 1R.1D 5 + 10	1R.1D	Null	13 + 16	5 + 10	62.2
GDS7 1R.1D 2 + 12	1R.1D	Null	13 + 16	2 + 12	67.3
GDS7 1A.1D 5 + 10	1A.1D	5 + 10	13 + 16		58.9
Trim set					
Trim	Parent line	1	13 + 16		62.2
Trim 1R.1D 5 + 10	1R.1D	1	13 + 16	5 + 10	61.2
Trim 1A.1D 2 + 12	1A.1D	2 + 12	13 + 16		64.1
Trim 1A.1D 5 + 10	1A.1D	5 + 10	13 + 16		67.4
Rhino set					
Rhino	Parent line	Null	13 + 16	<i>Sec-3</i>	56.4
Rhino 1R.1D 5 + 10	1R.1D	Null	13 + 16	5 + 10	59.2
Rhino 1A.1D 2 + 12	1A.1D	2 + 12	13 + 16	<i>Sec-3</i>	57.7
Rhino 1A.1D 5 + 10	1A.1D	5 + 10	13 + 16	<i>Sec-3</i>	53.8
Rigel set					
Rigel	Parent line	Null	13 + 16		53.8
Rigel 1R.1D 5 + 10	1R.1D	Null	13 + 16	5 + 10	65.9
Rigel 1A.1D 2 + 12	1A.1D	2 + 12	13 + 16		54.5
Rigel 1A.1D 5 + 10	1A.1D	5 + 10	13 + 16		63.1

and Rossi, 1965) with slight modifications. The 0.3 ml methanol extract was mixed with 2 ml diluted Folin–Ciocalteu reagent and 1.6 ml of 7.5% Na₂CO₃. The mixture was stirred and kept at ambient temperature for 2 h. Absorbance at 765 nm was recorded using a spectrophotometer (Philips PU 8625, UK). A calibration curve ($r^2 = 0.999$) was prepared using gallic acid at 0.03–0.20 mg/4 ml assay solution. Total phenolic acids in the methanol extracts were expressed as gallic acid equivalents (GAE).

2.4. Determination of individual phenolic acids – HPLC analysis

Individual phenolic acids in the bran extracts were analyzed by a Hewlett Packard 1100 Series high performance liquid chromatograph equipped with UV detector (Hewlett-Packard, Palo Alto, CA) and Phenomenex Jupiter C18 (250 × 4.60 mm, 10 μ, 300 Å; Phenomenex, Torrance, CA) column. The mobile phase of water with 0.05% trifluoroacetic acid (solvent A) and 30% acetonitrile, 10% methanol, 59.95% water and 0.05% trifluoroacetic acid (solvent B) were used at a flow rate of 1.0 ml/min. Total run time was 50 min and the gradient program was as follows: 10% B–12% B for 16 min, 12%–38% for 9 min, 38% B–70% B for 7 min, 70% B–85% B for 8 min and 85% B–10% B for 10 min. There was 5 min of post-run for reconditioning. The injection volume was 20 μl. Detection was done at 280 nm. Identification and quantification of phenolic acids in samples were performed comparing to chromatographic retention times and areas of external standards. Phenolic acid standards used for peak identification were ferulic acid, vanillic acid, syringic acid, gallic acid, *p*-hydroxybenzoic acid, chlorogenic acid, caffeic acid, *p*-coumaric acid and trans-cinnamic acid that were purchased from Sigma and Aldrich (Sigma–Aldrich Corporation, St. Louis, MO) and used without further purification (97% and higher purity). The eight different concentrations of phenolic acid standards were used in calibration curves ranging from 3.9 μg/mL to 500 μg/mL. For all the standard calibration curves, r^2 values varied from 0.978 to 0.999. All samples were prepared and analyzed in triplicate.

2.5. Statistical analysis

All extraction runs and analyses were carried out in triplicate and in randomized order with mean values being reported. Analysis of variance (ANOVA) of the results was performed using the General Linear Model procedure of SAS (Software Version 9.1 SAS Institute Inc., Cary, NC). Statistical significance was declared at $P < 0.05$.

3. Results and discussion

3.1. Phenolic acid composition of waxy wheats

Ferulic acid was the major phenolic acid (~92%) found in waxy wheats similar to non-waxy wheat lines (Beta et al., 2005; Moore et al., 2006) followed by *p*-coumaric and vanillic acid and syringic acids (Table 3). Caffeic, *t*-cinnamic and *p*-hydroxybenzoic acids were present in minor quantities. Significant differences were observed among samples for different phenolic acids; however, dissimilar trends were noted for two waxy sets in accordance with previous studies that showed phenolic acid concentrations varied according to wheat genotype (Gelinas and McKinnon, 2006; Irmak et al., 2007; Moore et al., 2006). All the developed lines have higher total phenolic acid content in the case of the N11 set, whereas the parent line has highest phenolic acids in the case of the Svevo set. Within the developed lines of the N11 set, single nulls/partial waxy have the highest total phenolics (1386–1304 μg/g bran) followed by the triple null (complete waxy) and double nulls (partial waxy). On the other hand, complete waxy has the highest total phenolics

Table 3

Phenolic acid composition (μg/g bran) of waxy wheat bran analyzed by HPLC*.

Sample Name	FA	<i>p</i> -CM	VA	SA	Others	Total
Svevo set						
Svevo wheat	1116 ^a	44.3 ^a	16.4 ^a	4.8 ^a	18.1 ^a	1200 ^a
Svevo waxy	948 ^b	39.5 ^b	14.2 ^{ab}	4.3 ^a	14.0 ^c	1020 ^b
Svevo waxy A1 ⁻	899 ^c	28.1 ^c	13.3 ^b	4.1 ^a	15.2 ^{bc}	960 ^b
Svevo waxy B1 ⁻	891 ^c	26.9 ^c	13.2 ^b	4.2 ^a	17.1 ^{ab}	952 ^b
N11 set						
N11 wheat	1006 ^h	28.7 ^e	28.1 ^{ab}	5.4 ^e	20.9 ^{ab}	1089 ^f
N11 waxy	1150 ^d	31.2 ^e	29.4 ^a	5.7 ^e	19.3 ^b	1236 ^d
N11 waxy A1 ⁻	1253 ^a	75.1 ^a	26.0 ^b	9.5 ^{ab}	22.1 ^{ab}	1386 ^a
N11 waxy B1 ⁻	1199 ^c	45.1 ^b	26.3 ^b	9.9 ^a	23.4 ^a	1304 ^c
N11 waxy D1 ⁻	1239 ^b	46.8 ^b	20.2 ^{cd}	9.5 ^{ab}	23.6 ^a	1339 ^b
N11 waxy A1 ⁻ /B1 ⁻	1142 ^e	44.4 ^b	19.3 ^d	6.6 ^{de}	22.2 ^{ab}	1235 ^d
N11 waxy A1 ⁻ /D1 ⁻	1126 ^f	38.0 ^d	22.1 ^c	8.0 ^{bc}	19.6 ^b	1214 ^e
N11 waxy B1 ⁻ /D1 ⁻	1113 ^g	41.5 ^c	18.4 ^d	7.4 ^{cd}	21.7 ^{ab}	1202 ^e

abcddefgh Means with the same letter in same column of same group are not significantly different at $P < 0.05$.

*FA = Ferulic acid; *p*-CM = *p*-coumaric acid; VA = Vanillic acid; SA = Syringic acid; Others = Total of *t*-cinnamic acid, caffeic acid and *p*-hydroxybenzoic acid.

(1020 μg/g bran) among all the developed lines of the Svevo set. Hung et al. (2009) reported that the total phenolic contents of the free and bound phenolic extracts of waxy wheat flour fractions gradually increased in order from the inner to the outer. For instance, free + bound ferulic acid concentration of the outermost fraction was found to be 533 μg/g flour which was highest among all flour fractions. When comparing ferulic acid concentrations of the bran fraction of the waxy wheat samples, it is expected to be found higher since it is well known that phenolic acids in wheat grain are concentrated in the bran fraction of the kernels (Onyeneho and Hettiarachchy, 1992).

3.2. Total phenolic content in waxy wheats

The TPC analyzed from the Folin assay using a UV spectrophotometer was compared with the total individual phenolics from HPLC (Fig. 1). In general, TPC values from the Folin assay were higher than HPLC due to the use of standards restricted only to major phenolics in HPLC. The extraction procedure we employed could release both free and bound phenolics and thus the data reported comprises both forms of phenolic acids. The concentrations of phenolics obtained in this study are in agreement with previous findings for non-waxy wheats (Kim et al., 2006; Verma et al., 2009). Although there is no information available on phenolic acid contents of waxy wheat bran to date, polyphenol contents of grain and flour of waxy wheats have been reported (Hung et al., 2009; Takata et al., 2007). Both the grain and flour polyphenol contents of complete waxy (null at ABD) were highest for two sets of waxy NILs studied (Takata et al., 2007). A similar trend with the single waxy wheat NIL was reported in another study (Takata et al., 2005). The highest TPC value among our samples was observed for N11 waxy wheat (single null A1) which correlates with HPLC results. The parent line has slightly higher total phenolics than all the developed lines in the case of the Svevo set.

Since the phenolic acids are concentrated in the bran, correlations were generated between bran yields and TPC as measured by HPLC and UV spectrophotometer. A weak correlation ($r = 0.08$) of TPC (measured by HPLC and UV) with bran yields was observed for N11 bread waxy wheat, whereas, in the case of the Svevo set, correlations ($r = -0.07$, UV results; $r = -0.58$, HPLC results) were negative. However, these correlations are not statistically significant.

One of the major differences between waxy and non-waxy wheats is the amylose fractions, in which waxy wheats are amylose-free. Ascending order of amylose content in a set of waxy lines are complete waxy < waxy double null < waxy single nulls

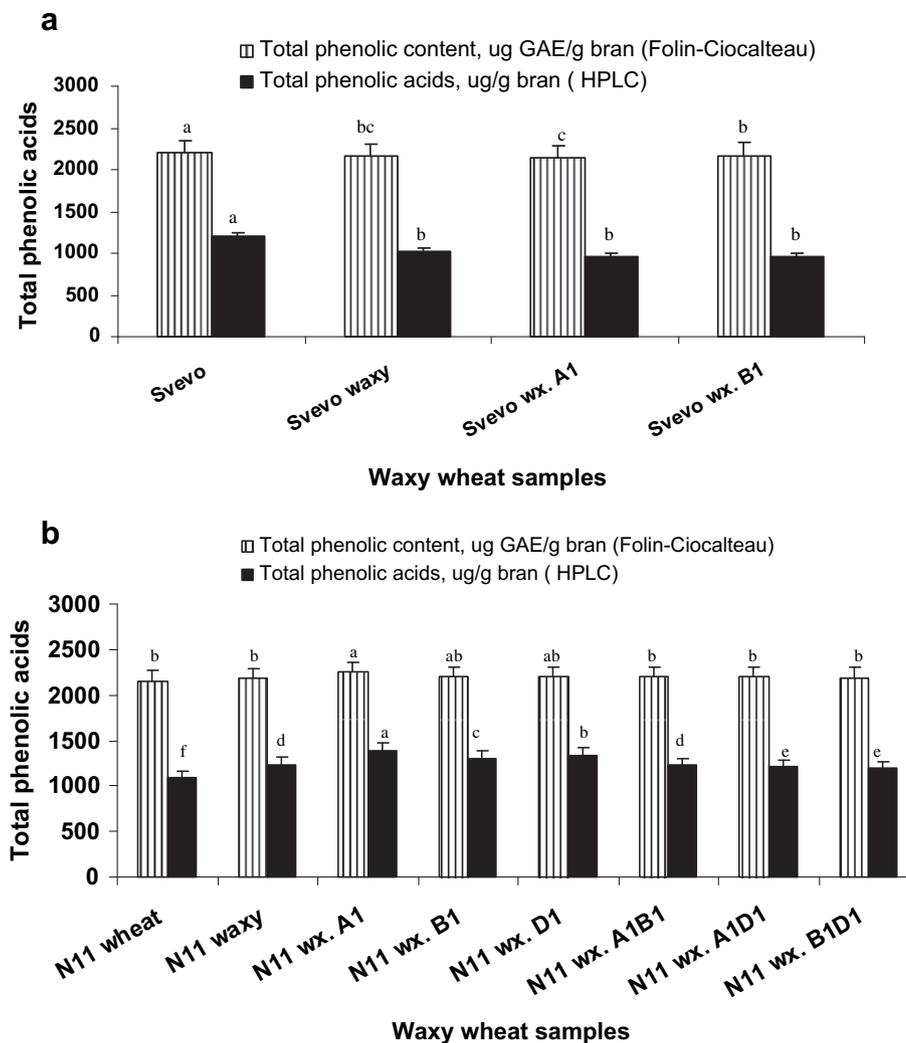


Fig. 1. Comparison of total bran phenolic contents of waxy wheat genotypes analyzed by the Folin–Ciocalteu assay (UV) and HPLC. (A) Svevo set and (B) N11 set. The vertical bars represent the SD ($n = 2$), and values denoted by the same letter are not significantly different ($P < 0.05$).

(Miura et al., 1994, 1999; Nakamura et al., 1995; Yasui et al., 1997). In the present study, TPC values measured by UV and HPLC of N11 waxy wheat has the order of: complete waxy < waxy double null < waxy single nulls. This is an interesting observation that concentrations of bioactive components such as phenolics correlate with amylose fractions of the starch. On the other hand, durum waxy wheat (Svevo set) showed an exactly opposite trend in which complete waxy has higher TPC than single nulls, which does not correlate with amylose fractions. However, to confirm these trends of reduction of TPC with amylose fractions in waxy bread wheats, further studies are required with samples grown under different environmental conditions for at least 2–3 growing seasons.

3.3. Phenolic acid composition of triticales

More than 90% of the phenolic acids were in the form of ferulic acid (845–1501 $\mu\text{g/g}$ bran) in all the triticales lines (Table 4). The highest ferulic acid content was found in the Rigel 1R.1D 5 + 10 sample. The other major phenolic found was *p*-coumaric acid. Phenolics such as *t*-cinnamic, caffeic, syringic and vanillic acids were found in minor quantities. Similar individual bran phenolic acid compositions were reported for cultivar Ultima, atriticales variety, by Hosseinian and Mazza (2009).

Among individual phenolic acids, ferulic acid, which is a major one, was lowest with 2 + 12 subunits in all the sets analyzed except in Trim, in which it was highest (1286 $\mu\text{g/g}$ bran). Within 5 + 10 HMW-GS lines, the 1R.1D of Rigel and Rhino sets showed higher phenolic acid contents than the 1A.1D line, whereas the opposite trend was observed in Trim and GDS7 lines.

In general, at least one of the developed lines showed lower values for corresponding phenolic acids than all other developed lines and the parent line. Ferulic acid (1000 mg/g dry matter) is the most abundant phenolic acid followed by sinapic (130 mg/g) and *p*-coumaric (60 mg/g) acids in the whole rye crop (Andreasen et al., 1999), which is one of the parents for triticales. On the other hand, Hosseinian and Mazza (2009) reported vanillin to be the second major phenolic followed by *p*-coumaric acid and then vanillic acid in triticales bran. The other parent line, wheat bran, has ferulic, *p*-coumaric and vanillic as major phenolics. Wheat bran also contains caffeic, chlorogenic, genistic, syringic and *p*-hydroxybenzoic acids in minor quantities (Onyeneho and Hettiarachchy, 1992).

3.4. Total phenolic contents of triticales

Total phenolic contents (TPC) of GDS7, Trim, Rhino and Rigel triticales sets are shown in Fig. 2a, b, c and d, respectively. Rigel and

Table 4
Phenolic acid composition ($\mu\text{g/g}$ bran) of triticales analyzed by HPLC*

Sample Name	Ferulic	<i>p</i> -coumaric	Others	Total
GDS7 set				
GDS7	1053 ^b	28.8 ^b	26.9 ^c	1109 ^b
GDS7 1R.1D. 5 + 10	956 ^c	21.7 ^c	28.6 ^{bc}	1006 ^c
GDS7 1R.1D. 2 + 12	911 ^d	23.7 ^c	31.4 ^b	966 ^d
GDS7 1A.1D. 5 + 10	1210 ^a	37.3 ^a	35.7 ^a	1283 ^a
Trim set				
Trim	1087 ^{ab}	29.7 ^c	17.3 ^{ab}	1134 ^b
Trim 1R.1D. 5 + 10	920 ^b	21.1 ^d	13.5 ^c	955 ^c
Trim 1A.1D. 2 + 12	1286 ^a	42.5 ^a	19.4 ^a	1348 ^a
Trim 1A.1D. 5 + 10	1039 ^b	33.1 ^b	14.6 ^{bc}	1087 ^{bc}
Rhino set				
Rhino	1226 ^a	28.2 ^a	14.9 ^a	1269 ^a
Rhino 1R.1D. 5 + 10	1186 ^{ab}	31.8 ^a	14.2 ^a	1232 ^a
Rhino 1A.1D. 2 + 12	845 ^c	17.0 ^b	13.3 ^a	875 ^c
Rhino 1A.1D. 5 + 10	1063 ^b	17.0 ^b	14.4 ^a	1094 ^b
Rigel set				
Rigel	1180 ^b	30.2 ^b	14.9 ^a	1225 ^b
Rigel 1R.1D. 5 + 10	1501 ^a	41.7 ^a	13.0 ^a	1556 ^a
Rigel 1A.1D. 2 + 12	984 ^b	33.7 ^{ab}	13.5 ^a	1031 ^b
Rigel 1A.1D. 5 + 10	1120 ^b	18.4 ^c	14.9 ^a	1153 ^b

^{abcd}Means with same letter in same column of same group are not significantly different at $P < 0.05$.

*Others = Total of *t*-cinnamic, caffeic, syringic and vanillic acids.

Trim lines with 2 + 12 subunits had higher TPC values than other samples. This is a very interesting result since the HMW-GS pair 2 + 12 is well known for its contribution to weak dough properties and inferior bread baking potential. However, it has higher TPC values in triticales. The TPC of triticale was found to be 940 $\mu\text{g/g}$ of grain as reported by Zdunczyk et al. (2006). Hosseinian and Mazza (2009) reported 2849 $\mu\text{g/g}$ bran for TPC of bound phenolics of triticale. The average TPC of triticale bran samples in this study was 2182 $\mu\text{g/g}$ bran. The difference in bran phenolics from previous

studies might be attributed to the presence of more phenolic acid contents in the bran part and also the extraction method we employed to release these compounds.

The total of individual phenolics measured by HPLC did not match the TPC values obtained from UV measurement. This is because we employed standards only for the reported individual phenolic acids and there might be more of those compounds present that had increased the TPC values in UV analysis. In addition to ferulic and *p*-coumaric acids, significant amounts of syringic acid, vanillic acid and vanillin were also reported as bound phenolics in triticale bran (Hosseinian and Mazza, 2009). The variation in phenolics distribution can be attributed to the different extraction method used in the present study. It is interesting to note that triticale bran had higher TPC and individual ferulic acid content (840–1500 μg ferulic/g of bran) than either of the parent wheat lines (90–230 μg ferulic/g of bran; Zhou et al., 2004) or rye (900–1100 μg ferulic/g of grain; Andreassen et al., 1999).

All triticale samples were combined to run the correlations between TPC and bran yields. Unlike in N11 and Svevo waxy wheats, TPC as measured by UV spectrophotometry was correlated negatively ($r = -0.41$) with bran yields which was statistically significant ($p > 0.10$). Nevertheless, these weak correlations of TPC with bran yields indicates that, the variations in TPC values are not simply due to their differences in bran yields but mainly to genotypic differences. Similar findings were reported for non-waxy wheats by Li et al. (2008).

Findings from this study are preliminary, since the samples used for analysis were collected from one growing season and one location only. Also, it is important to analyze the waxy wheat samples for starch fractions and properties. Further studies are required to confirm the possible impact of presence of waxy null alleles on concentrations of bioactive components. Similarly, studies are planned on triticale translocation lines to see the possible influence of changes in protein composition on the amounts of phenolic acids.

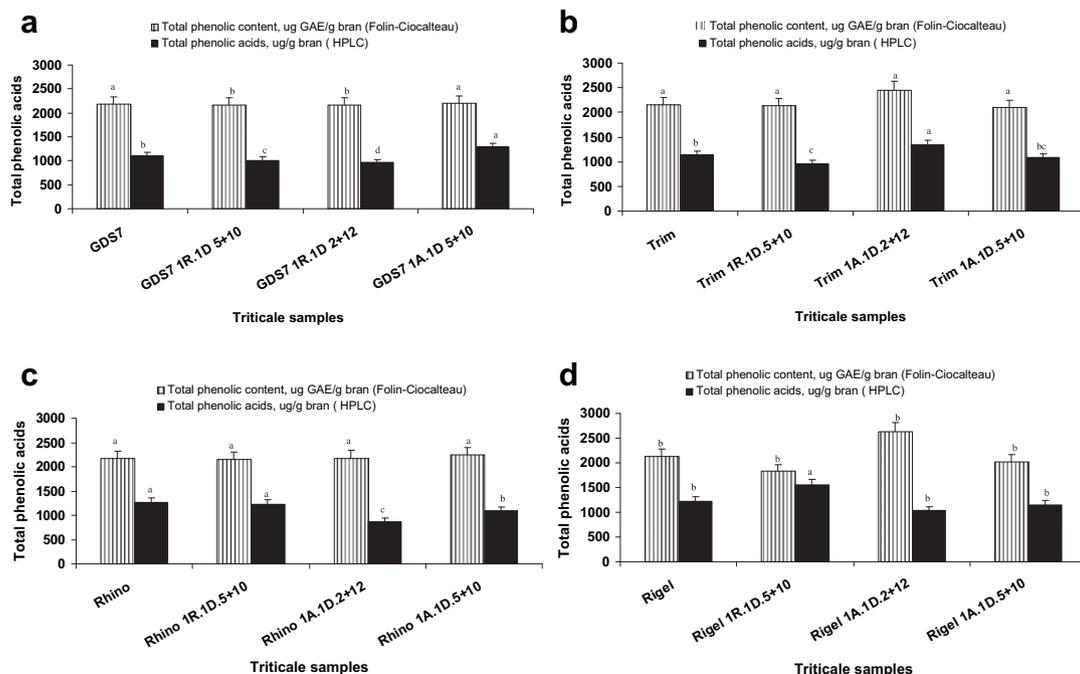


Fig. 2. Comparison of total bran phenolic contents of triticale genotypes analyzed by the Folin–Ciocalteu assay (UV) and HPLC. (A) GDS7 set (B) Trim set (C) Rhino set and (D) Rigel set. The vertical bars represent the SD ($n = 2$), and values denoted by the same letter are not significantly different ($P < 0.05$).

4. Conclusions

Near-isogenic waxy wheat and triticale translocation lines used in this study differ at one or more loci coding for storage proteins. The present study was designed to determine if any unintentional changes occur in phenolic acid components of wheat and triticale brans during the development of their genetic lines, originally intended to alter the protein composition. Preliminary studies indicate that, similar to non-waxy wheats, ferulic acid was the major phenolic acid in both waxy sample sets examined. All the developed lines have higher total phenolic acid content in the case of the N11 set, whereas the parent line has highest phenolic acids in the case of the Svevo set. Within the developed lines of the N11 set, single nulls/partial waxy have the highest total phenolics. In waxy bread wheat, TPC values were in correlation with amylose fractions in which the order was complete waxy < waxy double nulls < waxy single nulls. However, further studies are required to confirm these preliminary trends.

Among triticale translocation lines, with the lines having 5 + 10 HMW-GS, the 1R.1D of Rigel and Rhino sets showed higher phenolic acid contents than the 1A.1D line, whereas the opposite trend was observed in Trim and GDS7 lines. TPC was negatively correlated with bran yields for triticales lines studied which indicates the variation in TPC might be due to genotypic differences. Lines with HMW-GS 2 + 12 have lower TPC than other lines in each group of triticale samples, except for Trim set.

Although there are variations among waxy wheat and triticale samples for phenolics, further research is required to confirm the trends observed in this study with the samples grown under different conditions and seasons over a period of time. Also, in the case of waxy wheats, it is important to study the starch properties on the same set of samples to seek a possible correlation to the bioactive components.

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References

American Association of Cereal Chemists, 2000. Approved Methods 26–50, tenth ed. AACC, St. Paul, MN.

Adom, K.K., Liu, R.H., 2002. Antioxidant activity of grains. *Journal of Agricultural and Food Chemistry* 50, 6185–6187.

Andersson, A.A.M., Kamal-Eldin, A., Fras, A., Boros, D., Aman, P., 2008. Alkylresorcinols in wheat varieties in the HEALTHGRAIN diversity screen. *Journal of Agricultural and Food Chemistry* 56, 9722–9725.

Andreasen, M.F., Christensen, L.P., Meyer, A.S., Hansen, A., 1999. Release of hydroxyinnamic and hydroxybenzoic acids in rye by commercial plant cell wall degrading enzyme preparations. *Journal of the Science of Food and Agriculture* 79, 411–413.

Arseniuk, E., Oleksiak, T., 2002. Production and breeding of cereals in Poland. In: E. Arseniuk (Ed.), *Proceedings of 5th International Triticale Symposium*, IHAR, Radzikow, Poland, 11–20.

Arts, I., Hollman, P., 2005. Polyphenols and disease risk in epidemiologic studies. *American Journal of Clinical Nutrition* 81, 317S–325S.

Baublis, A., Decker, E.A., Clydesdale, F.M., 2000. Antioxidant effects of aqueous extracts from wheat based ready-to-eat breakfast cereals. *Food Chemistry* 68, 1–6.

Beta, T., Nam, S., Dexter, J.E., Sapiirstein, H.D., 2005. Phenolic content and antioxidant activity of pearled wheat and roller-milled fractions. *Cereal Chemistry* 82, 390–393.

Chapman, B., Salmon, D.F., Dyson, C., Blackley, K., 2005. Spring and winter triticale for grain, forage and value-added. *Alberta Agriculture, Food and Rural Development Bulletin*, 1–65.

Diaz-Reinoso, B., Moure, A., Dominguez, H., Parajo, J.C., 2006. Supercritical CO₂ extraction and purification of compounds with antioxidant activity. *Journal of Agricultural and Food Chemistry* 54, 2441–2469.

Folin, O., Ciocalteu, V., 1927. On tyrosine and tryptophane determination in proteins. *Journal of Biological Chemistry* 73, 627–650.

Food and Agriculture Organization of the United Nations (FAO), 2008. The FAO Statistical Database, 2008 Available at: <http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567>.

Gelinas, P., McKinnon, C.M., 2006. Effect of wheat variety, farming site, and bread-baking on total phenolics. *International Journal of Food Science and Technology* 41, 329–332.

Graybosch, R.A., 1998. Waxy wheats: Origin, properties, and prospects. *Trends in Food Science and Technology* 9, 135–142.

Hosseini, F.S., Mazza, G., 2009. Triticale bran and straw: potential new sources of phenolic acids, proanthocyanidins, and lignans. *Journal of Functional Foods* 1, 57–64.

Hung, P.V., Maeda, T., Morita, N., 2006. Waxy and high-amylose wheats-characteristics, functionality and uses. *Trends in Food Science and Technology* 17, 448–456.

Hung, P.V., Maeda, T., Morita, N., 2007. Dough properties and breadmaking quality of flours with whole waxy wheat flour substitution. *Food Research International* 40, 273–279.

Hung, P.V., Maeda, T., Miyatake, K., Morita, N., 2009. Total phenolic compounds and antioxidant capacity of wheat graded flours by polishing method. *Food Research International* 42, 185–190.

Irmak, S., Jonnala, R.S., MacRitchie, F., 2007. Effect of genetic variation on phenolic acid and policosanol contents of pegaso wheat lines. *Journal of Cereal Science* 48, 20–26.

Jacobs, D.R., Meyer, K.A., Kushi, L.H., Folsom, A.R., 1998. Whole grain intake may reduce risk of coronary heart disease death in postmenopausal women: the Iowa Women's Health Study. *American Journal of Clinical Nutrition* 68, 248–257.

Kim, K.-H., Tsao, R., Yang, R., Cui, S.W., 2006. Phenolic acid profiles and antioxidant activities of wheat bran extracts and the effect of hydrolysis conditions. *Food Chemistry* 95, 466–473.

Kris-Etherton, P.M., Hecker, K.D., Bonanome, A., Coval, S.M., Binkoski, A.E., Hilpert, K.F., Griel, A.E., Etherton, T.D., 2002. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. *American Journal of Medicine* 113, 71S–88S.

Li, L., Shewry, P.R., Ward, J.L., 2008. Phenolic acids in wheat varieties in the HEALTHGRAIN diversity screen. *Journal of Agricultural and Food Chemistry* 56, 9732–9739.

Liyana-Pathirana, C.M., Shahidi, F., 2006. Importance of insoluble-bound phenolics to antioxidant properties of wheat. *Journal of Agricultural and Food Chemistry* 54, 1256–1264.

Menga, V., Fares, C., Troccoli, A., Cattivelli, L., Baiano, A., 2010. Effects of genotype, location and baking on the phenolic content and some antioxidant properties of cereal species. *International Journal of Food Science and Technology* 45, 7–16.

Miura, H., Tanii, S., Nakamura, T., Watanabe, N., 1994. Genetic control of amylose content in wheat endosperm starch and differential effects of three Wx genes. *Theoretical and Applied Genetics* 89, 276–280.

Miura, H., Araki, E., Tarui, S., 1999. Amylose synthesis capacity of the three Wx genes of wheat cv Chinese spring. *Euphytica* 108, 91–95.

Moore, J., Liu, J.-G., Zhou, K., Yu, L., 2006. Effects of genotype and environment on the antioxidant properties of hard winter wheat bran. *Journal of Agricultural and Food Chemistry* 54, 5313–5322.

Nakamura, T., Yamamori, M., Hirano, H., Hidaka, S., Nagamine, T., 1995. Production of waxy (amylose-free) wheats. *Molecular and General Genetics* 248, 253–259.

Onyeneho, S.N., Hettiarachchy, N.S., 1992. Antioxidant activity of durum wheat bran. *Journal of Agricultural and Food Chemistry* 45, 1644–1648.

Salmon, D.F., McLelland, M., Schoff, T., Juskiw, P.E., 2001. Triticale. *Alberta Agriculture, Food and Rural Development Bulletin*. [http://www1.agric.gov.ab.ca/\\$department/deptdocs.nsf/all/agdex127](http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/agdex127).

Salmon, D.F., McLelland, M., Schoff, T., Juskiw, P.E., 2008. The growth potential of triticale in Western Canada. *Alberta Agriculture and Rural Development Bulletin*. [http://www1.agric.gov.ab.ca/\\$department/deptdocs.nsf/all/fcd4230](http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/fcd4230).

Singleton, V.L., Rossi, J.A., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture* 16, 144–158.

Takata, K., Nishio, Z., Iriki, N., Tabiki, T., Funatsuki, W., Yamauchi, H., 2005. Comparison of quality characteristics of waxy wheat using near-isogenic line. *Breeding Science* 55, 87–92.

Takata, K., Yanaka, M., Fujita, Y., Ishikawa, N., 2007. Evaluation of the grain and flour quality in near-isogenic wheat lines with waxy and double-null Wx proteins. *Breeding Science* 57, 79–83.

Varughese, G., Pfeiffer, W.H., Pena, R.J., 1996. Triticale: a successful alternative crop (Part 1&2). *Cereal Foods World* 41, 635–645, 474–482.

Verma, B., Hucl, P., Chibbar, R.N., 2009. Phenolic acid composition and antioxidant capacity of acid and alkali hydrolysed wheat bran fractions. *Food Chemistry* 116, 947–954.

Yamamori, M., Fujita, S., Hayakawa, K., Matsuki, J., Yasui, T., 2000. Genetic elimination of starch granule protein, SGP-1, of wheat generates and altered starch with apparent high amylose. *Theoretical and Applied Genetics* 101, 21–29.

Yasui, T., Matsuki, J., Sasaki, T., Yamamori, M., 1997. Waxy endosperm mutants of bread wheat (*Triticum aestivum* L.) and their starch properties. *Breeding Science* 47, 161–163.

- Yu, L., Haley, S., Perret, J., Harris, M., Wilson, J., Qian, M., 2002. Free radical scavenging properties of wheat extracts. *Journal of Agricultural and Food Chemistry* 50, 1619–1624.
- Zielinski, H., Kozłowska, H., 2000. Antioxidant activity and total phenolics in selected cereal grains and their different morphological fractions. *Journal of Agricultural and Food Chemistry* 48, 2008–2016.
- Zdunczyk, Z., Flis, M., Zielinski, H., Wroblewska, M., Antoszkiewicz, Z., Juskiewicz, J., 2006. In vitro antioxidant activities of barley, husked oat, naked oat, triticale, and buckwheat wastes and their influence on the growth and biomarkers of antioxidant status in rats. *Journal of Agricultural and Food Chemistry* 54, 4168–4175.
- Zhou, K., Su, L., Yu, L.L., 2004. Phytochemicals and antioxidant properties in wheat bran. *Journal of Agricultural and Food Chemistry* 52, 6108–6114.