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Genotype and Environmental Variation in Phenolic Content, Phenolic Acid Composition, and Antioxidant Activity of Hard Spring Wheat

ARCHIE MPOFU, HARRY D. SAPIRSTEIN, AND TRUST BETA*

Department of Food Science, University of Manitoba, Winnipeg, Manitoba, R3T 2N2 Canada

The health-promoting effects of whole-grain wheat likely derive from phenolic compounds and other antioxidants that also make wheat a potential source of functional food ingredients. The objective of this study was to determine the effects of genotype and growing environment on the phenolic contents and antioxidant activities of alcohol-soluble extracts from commercial wheat cultivars. Total phenolic contents (TPCs), antioxidant activities (AOAs), and concentrations of six phenolic acids were measured in six red- and white-grained hard spring wheat genotypes grown at four diverse locations in Western Canada during the 2003 crop year. There were significant differences among genotypes and environments for TPC, AOA, and concentrations of all the phenolic acids measured. The predominant indicators of antioxidant potential, i.e., TPC, AOA, and ferulic acid (FA) concentration were highly intercorrelated ($r > 0.72$). For these indices, the Canada Western (CW) Red Spring wheat cultivars Neepawa and AC Elsa had the highest levels, whereas an analogous CW hard white spring wheat cultivar, AC Snowbird, had the lowest levels. Grain color did not appear to be a factor in the expression of antioxidant-related parameters. For both TPC and AOA, as well as for vanillic acid, syringic acid, and ferulic acid, environmental effects were considerably larger than genotype effects. Neither growing temperature nor rainfall from anthesis to maturity appeared to be related to the environmental variation that was observed. Genotype × environment interaction was small for all parameters compared with genotype and location effects and was significant only for TPC. Genotype variation for antioxidant properties indicates that it would be possible to select for these quantitative traits in a breeding program. However, the significant environmental variation observed would delay and/or complicate this process.

KEYWORDS: Wheat; genotype by growing location; antioxidant activity; total phenolic content; DPPH

INTRODUCTION

Regular consumption of whole-grain foods has been found to be associated with reduced total mortality (*1*, *2*), as well as reduced risk of coronary heart disease (CHD) (*3*), ischemic stroke (*4*), and type 2 diabetes (*5*, *6*). Health-beneficial properties of whole-wheat grains can be largely ascribed to the presence of phytochemicals in the diet that reduce oxidative stress, thereby reducing the risk of chronic diseases. Wheat is a critically important commodity worldwide. It is grown on more land area than any other commercial crop and is the most important food grain source for humans. Quantification of health-beneficial phytochemicals present in whole grain and its products is important for the breeding and marketing of wheat based on its potential to promote health in line with increasing consumer demands for healthier foods.

Wheat has significant levels of antioxidants (*7*−*14*). Among the different antioxidants present in wheat, phenolic compounds seem to have the greatest potential of being beneficial to health (*15*). Phenolic compounds inhibit lipid peroxidation by scavenging free radicals such as hydroxyl radicals (HO•) and peroxyl radicals (ROO•) resulting in the formation of low energy phenolic radicals whose energy is not sufficient to promote lipid oxidation at biologically significant rates (*16*). Wheat phenolic compounds exist in free, bound and soluble conjugated forms (*7*). Ferulic, *p*-coumaric, and vanillic acids are the most dominant free phenolics and are found together with other phenolics including caffeic, chlorogenic, gentisic, syringic, and *p*-hydroxybenzoic acids (*7*).

The wheat genotype (G), the environment (E) in which wheat is grown, and possibly genotype−environment (G × E) interactions can likely strongly influence the levels of grain antioxidants. The literature is, however, relatively deficient on this topic. Three hard winter wheat varieties (Akron, Trego, and Platte) grown in a single field location differed significantly in their capacities to quench free radicals using scavengers such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-di-(3-ethylbenzthiazoline-6-sulfonate) (ABTS) radicals (*9*). Growing location had a strong influence on the antioxidant activity of

* To whom correspondence should be addressed. Address: Department of Food Science, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2. Phone: (204) 474-8214. Fax: (204) 474-7630. E-mail: Trust_Beta@UManitoba.CA.

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Table 1. Environmental Conditions at the Growing Locations and Outcomes for Test Weight and Protein Content Across Genotypes

location	soil zone	soil pH	temp[a] (°C)	rainfall[a] (mm)	test weight[b] (kg/hL)	protein[b] (%)
Melfort	transition	6.4	19.8	15.2	83.8 ± 0.5	13.8 ± 0.7
Swift Current	black and gray brown	7.3	22.2	3.3	74.1 ± 0.8	15.7 ± 0.6
Regina	transition	8.1	22.6	34.5	82.9 ± 0.8	13.7 ± 0.7
Winnipeg	black and brown black	7.2	21.6	48.7	82.5 ± 1.3	10.1 ± 0.3

[a] Average per location from anthesis to maturity. [b] Mean ± standard deviation.

pearled wheat fractions of a leading Canadian bread wheat cultivar, Superb (*13*). Yu et al. (*11*) reported significant effects of growing conditions, including the number of hours exceeding 32 °C, on the antioxidant properties of Akron, a hard red winter wheat variety. Genotype and growing location had significant effects on the antioxidant activity and phenolic content of flours of Akron, Trego, and Platte wheat grown at five locations in Colorado; the relative contributions of G and E to total variation were, however, not reported (*12*). To our knowledge, there have been no reports on the relative contributions of the genotype and growing environment of wheat to its phenolic composition and antioxidant activity. This study was carried out to evaluate the effects of genotype, growing location, and genotype−environment interactions on the total phenolic content, phenolic acid composition, and antioxidant activity of six wheat genotypes grown in the Canadian Prairie region.

MATERIALS AND METHODS

Sample Description. The wheat samples used in this study were all grown in the 2003 crop year, and the harvested wheat was of sound milling condition. The samples comprised six western Canadian wheat genotypes representing three commercial classes with different technological qualities. Neepawa [Canada Western Red Spring (CWRS)], AC Elsa (CWRS), AC Barrie (CWRS), Superb (CWRS), AC Vista (Canada Prairie Spring White), and AC Snowbird [Canada Western Hard White Spring (CWHWS)]. The six genotypes were grown in triplicate according to a split-plot design, at each of four locations: Regina, Swift Current, and Melfort (Saskatchewan) and Winnipeg (Manitoba). Accordingly, the total number of samples was 72. Wheat in each growing environment was planted in a randomized complete block design with replicates as blocks.

Table 1 summarizes the environmental conditions at the locations, as well as some physical and chemical properties of the wheat (test weight and protein) that reflect typical differences in those growing locations. Test weight averaged across locations was lowest for wheat genotypes grown in Swift Current because of the low amounts of rainfall in this traditionally semi-arid growing location, which attenuated normal grain filling, resulting in relatively high protein contents (**Table 1**). At the other extreme, protein content was lowest for genotypes grown in Winnipeg, mainly because of relatively high amounts of precipitation during the grain filling period. Samples were each ground to pass through a 0.8-mm screen in a model 3100 falling number laboratory mill (Perten Instruments, Springfield, IL) prior to chemical analysis.

Determination of Total Phenolic Content. Total phenolic content was determined using the Folin−Ciocalteau method (*17*) modified by Gao et al. (*18*) as described previously (*13*). Briefly, ground wheat samples (200 mg) were extracted with acidified methanol (HCl/methanol/water, 1:80:10, v/v) (4 mL) at room temperature for 2 h. The extracts obtained were oxidized with Folin−Ciocalteu reagent, and the reaction mixture was neutralized with sodium carbonate. The mixture was incubated at room temperature for 90 min, and its absorbance was measured at 725 nm. Acidified methanol was used as the blank. Ferulic acid was used as the standard, and results are expressed as ferulic acid equivalents per gram of wheat. All analyses were performed in duplicate

Determination of Antioxidant Activity as DPPH Scavenging Capacity. Antioxidant activity was measured using a modified version of the Brand-Williams et al. (*19*) DPPH radical scavenging method described by Beta et al. (*13*). Ground wheat samples were extracted with 100% methanol over a period of 2 h at ambient temperature. The extracts were mixed with DPPH solution (6×10^{-5} mol/L of methanol). The absorbance (A) of the mixture at 515 nm was determined at 0 and 30 min. Methanol was used as a blank, and antioxidant activity (AOA) was calculated as percent discoloration

$$\text{percent discoloration} = \left(1 - \frac{A \text{ of sample } t = 30 \text{ min}}{A \text{ of control } t = 0 \text{ min}}\right) \times 100$$

The tests were all carried out in duplicate.

Determination of Phenolic Acid Composition. *Sample Preparation.* Wheat samples were hydrolyzed according to the method of Krygier et al. (*20*) with some modifications. Ground wheat samples (2 g) were hydrolyzed using 4 M NaOH (60 mL) for 4 h under nitrogen. The pH of the resulting mixture was adjusted to between 1.5 and 2 using 6 M ice-cold HCl, and the pH-adjusted mixture was centrifuged at 13 000 rpm (Sorvall RC5C, Sorvall Instruments, DuPont, Wilmington, DE) for 15 min. The supernatant was extracted three times with ethyl acetate (70 mL), and the organic phase was retained, dried by adding anhydrous Na_2SO_4 (1 g), and filtered. The filtrate was evaporated to dryness at 35 °C in a rotary vacuum evaporator (RE III Rotavapor, Büchi, Switzerland). The residue was redissolved in 50% methanol (4 mL) and then filtered through a 0.45-μm nylon filter. The filtrate was stored in the dark at −20 °C and subsequently analyzed by HPLC.

HPLC Analysis. HPLC analysis was performed on a Waters model 2695 chromatograph instrument (Waters, Mississauga, ON, Canada) equipped with a Waters 2996 photodiode array detector. Phenolic acids were separated on a reverse-phase Waters μBondapak C18 column (3.9 × 300 mm) with a gradient of solvent A [water containing 1% (v/v) HAc] and solvent B (100% methanol) for 33 min at a flow rate of 1.5 mL/min. The solvent gradient was programmed as follows: at 0 min, 15% B; at 10 min, 20% B; at 16 min, 23% B; at 24−28 min, 27% B; and at 30−33 min, 15% B. Phenolic acids in the eluents were monitored at 280 nm. The following phenolic acids were quantified: ferulic acid (FA), *o*-coumaric acid (OCA), *p*-coumaric acid (PCA), syringic acid (SA), caffeic acid (CA), and vanillic acid (VA). Identification of the phenolic acids was accomplished by comparing the retention times of peaks in wheat samples to those of phenolic acid standards. The HPLC analyses were carried out in duplicate.

Statistical Analysis. Data for all locations were combined and analyzed by analysis of variance (ANOVA). The ANOVA was performed with the general linear model (GLM) of the SAS software package (release 8.2) (SAS Institute, Cary, NC) using the split-plot design and analyzing genotype and environment as fixed effects. Genotype (G), environment (E), and G × E effects were determined using replicates as blocks with measurements from duplicate analyses for each plot. Data from each location were additionally analyzed individually by ANOVA as randomized complete block designs. Comparison of means was done at the 5% significance level using Duncan's multiple-range test. Correlation analyses were performed with the PROC Corr procedure of the SAS software package using the Pearson correlation test.

RESULTS

Genotype and Environmental Effects. There were highly significant differences ($p < 0.0001$) among the six genotypes for total phenolic content (TPC), AOA, and concentrations of all of the phenolic acids assayed (**Table 2**). Differences among genotypes at each location were also highly significant for all of these parameters. Averaged across growing environments, TPC was highest for AC Elsa and Neepawa (1990 and 1985 μg/g, respectively), followed by AC Barrie, Superb, and AC Vista. AC Snowbird had the lowest TPC of 1709 μg/g. Differences in TPC were significant ($p < 0.05$) for data combined across all locations and when analyzed on a by-

Table 2. Mean Total Phenolic Content, Antioxidant Activity, and Phenolic Acid Concentrations of Six Wheat Genotypes[a,b]

genotype	total phenolic content (μg/g)	AOA (% DPPH discoloration)	vanillic acid (μg/g)	caffeic acid (μg/g)	syringic acid (μg/g)	*p*-coumaric acid (μg/g)	ferulic acid (μg/g)	*o*-coumaric acid (μg/g)
AC Barrie	1894b ± 86.6	14.22b ± 0.81	9.69a ± 1.18	12.87a ± 2.78	13.31b ± 4.34	28.45d ± 3.57	417.69b ± 38.65	146.08c ± 36.53
AC Elsa	1990a ± 172.4	15.06a ± 1.34	9.69a ± 1.18	10.38b ± 1.89	16.05a ± 2.92	29.53d ± 3.49	441.02a ± 45.51	187.92b ± 50.26
Neepawa	1985a ± 82.3	14.61ab ± 0.86	9.45ab ± 1.38	12.26a ± 2.41	13.18b ± 3.03	32.64c ± 3.82	436.63a ± 31.97	188.36b ± 31.37
AC Snowbird	1709d ± 169.9	13.21d ± 1.03	9.01b ± 1.07	7.60c ± 1.02	12.80bc ± 2.86	34.24b ± 3.07	371.04d ± 32.02	145.51c ± 32.04
Superb	1776c ± 87.0	13.47cd ± 0.74	8.17c ± 1.24	9.81b ± 2.32	11.61c ± 1.61	23.93e ± 1.98	378.50d ± 22.44	229.17a ± 35.45
AC Vista	1803c ± 108.8	13.99bc ± 0.95	7.77c ± 1.27	7.70c ± 0.74	12.78bc ± 2.48	37.22a ± 3.36	403.92c ± 25.80	165.74c ± 18.03

[a] Values expressed as mean ± standard deviation. [b] Means in the same column followed by different letters are significantly different ($p < 0.05$).

Table 3. Mean Total Phenolic Content, Antioxidant Activity, and Phenolic Acid Concentrations of Wheat Grown at Four Locations[a,b]

growing location	total phenolic content (μg/g)	AOA (% DPPH discoloration)	vanillic acid (μg/g)	caffeic acid (μg/g)	syringic acid (μg/g)	*p*-coumaric acid (μg/g)	ferulic acid (μg/g)	*o*-coumaric acid (μg/g)
Melfort	1794c ± 103.3	13.59c ± 0.85	7.75d ± 0.95	9.71b ± 2.16	10.25d ± 1.49	31.42b ± 5.39	383.60c ± 25.82	210.10a ± 39.85
Regina	1746d ± 157.1	13.62c ± 1.14	9.49b ± 1.06	8.99b ± 2.43	12.79c ± 1.74	29.87c ± 4.77	390.84c ± 32.14	156.43c ± 37.99
Swift	1889b ± 119.1	14.17b ± 1.00	10.28a ± 0.83	11.90a ± 3.37	14.01b ± 1.72	28.23d ± 3.81	408.59b ± 32.44	161.04c ± 39.02
Current Winnipeg	2009a ± 119.9	15.00a ± 1.04	8.32c ± 1.13	9.80b ± 2.08	16.11a ± 3.86	34.49a ± 4.93	449.51a ± 43.29	180.96b ± 43.98

[a] Values expressed as mean ± standard deviation. [b] Means in the same column followed by different letters are significantly different ($p < 0.05$).

location basis. AC Snowbird had the lowest TPC at every location except Winnipeg, where, although it had the second lowest TPC, the value was similar to that of Superb, which had the lowest TPC. In Swift Current, AC Snowbird had the lowest TPC; its TPC was, however, not significantly different from those of AC Barrie, AC Vista, and Superb. AC Elsa had the highest TPC in all locations but Regina, where it was joint second highest with AC Barrie. Neepawa had a TPC similar to that of AC Elsa in Melfort and Swift Current. Neepawa had the highest TPC in Regina and was second highest after AC Barrie in Winnipeg.

Mean AOA among genotypes ranged between 13.21% and 14.22% (**Table 2**). Similarly to the TPC results, AC Elsa had the highest AOA; its AOA was significantly different from those of all other genotypes except Neepawa. The AOA of Neepawa was not significantly different from those of AC Barrie and AC Vista but was significantly different from those of Superb and AC Snowbird, which had the lowest AOA. AOA differences were significant for data combined across all locations and for data analyzed location by location.

Significant differences were found in the concentration of each phenolic acid per genotype averaged across growing environments (**Table 2**), as well as at each location when results were evaluated by location. Moreover, the patterns of variation for phenolic acid concentrations were dissimilar among genotypes (**Table 2**). Ferulic acid was the predominant phenolic acid, representing approximately 63% of the total content of individual phenolic acids averaged over genotypes and environments. The overall mean concentration of ferulic acid ranged across genotypes from 371 to 441 μg/g. Similarly to the results for TPC and AOA, the genotypes with the highest ferulic acid concentrations were AC Elsa and Neepawa, with AC Snowbird and Superb having the lowest concentrations. In contrast, Superb had the highest concentration of *o*-coumaric acid (OCA) (229 μg/g), which was the second most prominent phenolic acid that was measured; OCA comprised approximately 25% of the total content of individual phenolic acids averaged over genotypes and environments. The remaining phenolic acids, VA, CA, SA, and PCA, comprised about 13% of the total phenolic acids that were quantified.

There were significant differences among growing environments for TPC, AOA, and concentrations of all of the phenolic acids assayed (**Table 3**). Winnipeg had the highest TPC; AOA; and FA, PCA, and SA concentrations. Swift Current had the highest VA and CA concentrations and the second highest TPC, AOA, and FA and SA concentrations. With one exception, there was no significant relationship between phenolic acid content, composition, or AOA and either total rainfall or average temperature during the kernel development period (refer to **Table 1**). OCA concentration per location was negatively correlated ($r = -0.970$, $p < 0.05$) to average temperature during grain filling. However, given the limited number of site year results available to this study, this relationship must be interpreted with caution.

Intercorrelations among Total Phenolics, Antioxidant Activity, and Phenolic Acids. There were highly significant ($p < 0.01$) and strong correlations between TPC and AOA ($r = 0.73$), between TPC and concentration of FA ($r = 0.84$), and between AOA and FA ($r = 0.72$) (**Table 4**). Other positive and significant correlations, but of lesser magnitude, were found between FA and SA ($r = 0.61$), between AOA and SA ($r = 0.47$), between TPC and CA ($r = 0.45$), between VA and SA ($r = 0.41$), between SA and VA ($r = 0.41$), between FA and CA ($r = 0.40$), and between FA and PCA ($r = 0.30$). There were significant, negative correlations ($p < 0.05$) between VA and OCA ($r = -0.40$), between CA and PCA ($r = -0.38$), between VA and PCA ($r = -0.32$), and between OCA and PCA ($r = -0.27$).

Relative Influence of Genotype and Environment and G × E Interactions. The magnitude of the variance components of genotype and environment and G × E interactions indicates their relative importance for specific antioxidant activities and related parameters. These results are summarized in **Table 5**, which compares the proportions of mean squares to the total mean square of each variance component. Similarly to mean difference results for the measured parameters (**Tables 2** and **3**), variance due to genotype and environment was significant for TPC, AOA, and all of the phenolic acids. Depending on the measured parameter, the combination of genotype and environment variance explained from 87% (AOA) to 96% (TPC) of the total variance. For both TPC and AOA, as well as for FA, VA, and SA, environmental effects were considerably larger than genotype effects, by as much as 52% and 53% for TPC and FA, respectively, and by 37% for AOA. Interestingly, for

Table 4. Correlation Coefficients between Total Phenolic Content, AOA, and Phenolic Acid Concentrations[a]

	total phenolic content	AOA[b]	vanillic acid (VA)	caffeic acid (CA)	syringic acid (SA)	*p*-coumaric acid (PCA)	ferulic acid (FA)
AOA[b]	0.729**						
vanillic acid (VA)	0.213	0.143					
caffeic acid (CA)	0.449**	0.205	0.510**				
syringic acid (SA)	0.581*	0.474**	0.406**	0.151			
p-coumaric acid (PCA)	0.101	0.166	–0.323*	–0.38*	0.152		
ferulic acid (FA)	0.842**	0.718**	0.222	0.396*	0.609**	0.299*	
o-coumaric acid (OCA)	0.107	0.064	–0.397*	0.013	–0.172	–0.265*	0.07

[a] * = significant ($p < 0.05$); ** = highly significant ($p < 0.01$). [b] AOA (% DPPH discoloration).

Table 5. Variance Components (Percent of Total Mean Squares) for Genotype (G), Growing Environment (E), and G × E Interaction Effects for Total Phenolic Content, Antioxidant Activity, and Phenolic Acid Composition of Six Wheat Genotypes Grown at Four Locations[a]

variance component:	G[b]	E[c]	G × E[b]	G × block/E	block/E
degrees of freedom:	5	3	15	40	8
parameter					
total phenolic content (μg of ferulic acid equivalents/g)	38.12***	57.86***	3.34***	0.38	0.29
antioxidant activity (% DPPH discoloration)	36.99***	50.61***	6.71	4.00	1.68
vanillic acid (μg/g)	24.42***	71.09***	0.79	1.43	2.28
caffeic acid (μg/g)	60.89***	29.29***	1.93	2.03	5.87
syringic acid (μg/g)	17.34***	70.91***	3.63	1.66	6.45
p-coumaric acid (μg/g)	63.54***	30.61***	1.17	0.70	3.98
ferulic acid (μg/g)	36.86***	56.57***	2.34	0.95	3.29
o-coumaric acid (μg/g)	46.74***	41.44***	2.76	2.45	6.61

[a] * = significant ($p < 0.05$); ** = significant ($p < 0.01$); *** = highly significantly ($p < 0.0001$). [b] Significance is based on genotype × block/E mean square. [c] Significance is based on block/E mean square.

Table 6. Total Phenolic Content Means and Coefficients of Variation (CV) of Six Wheat Genotypes Grown at Each of Four Locations[a]

	location				
genotype	Melfort	Regina	Swift Current	Winnipeg	CV (%)
AC Barrie	1862a	1837b	1844b	2030b	4.84
AC Elsa	1902a	1793b	2039a	2227a	9.42
Neepawa	1884a	1987a	2032a	2037b	3.57
AC Snowbird	1630c	1505d	1765b	1936cd	10.81
Superb	1736b	1677c	1821b	1873d	4.91
AC Vista	1747b	1680c	1830b	1956c	6.59

[a] Means in the same column followed by different letters are significantly different ($p < 0.05$).

two other phenolic acids, viz., CA and PCA, relative genotype variance (61% and 63%, respectively) was substantially greater than environmental variance (29% and 31%, respectively). Genetic and environmental variances related to the phenolic acid OCA were comparable (47% and 41%, respectively).

Despite the large influences of both genotype and environment on essentially all response factors, the variance attributable to G × E interactions was small (<4% of total variance) and was significant ($p < 0.0001$) only for TPC (**Table 5**). Genotype–environment interactions become significant depending on the extent of crossover interactions, i.e., significant change of ranking of cultivars over environments and/or magnitude of genotype variation across growing environments when rankings are unaffected. The small level of G × E interaction is partly reflected in the similar patterns of genotype-by-location means and location-by-genotype means for TPC (**Table 6**). The G × E interaction appears to stem partly from a significant change in ranking, relative to other cultivar TPC levels, of cultivar Neepawa (increased in Regina) and cultivar Elsa (increased in Winnipeg). Comparing all genotypes together, the greatest effect of G × E interactions for TPC was found jointly for AC Snowbird and Elsa, which had coefficients of variation (average CV = 10.1%) across growing locations more than double that of the average of the other four cultivars (CV = 4.4%). The TPC of cultivar Neepawa was least affected by environment across growing locations (CV = 3.6%), indicating the relative stability of this genotype for TPC.

DISCUSSION

The preeminence of wheat as a food crop is mainly due to the presence of a unique viscoelastic gluten protein complex that makes it the only cereal grain suitable for the manufacture of leavened bread. Significant antioxidant levels have been found in wheat, indicating its importance in a healthy diet to reduce the risk of many chronic diseases, as well as its potential as a source of functional food ingredients. Several authors have concluded that the antioxidant properties of wheat or wheat products are significantly influenced by the genotype and/or the environment in which wheat is grown, without quantifying the relative contributions of these factors (*7*, *11*, *13*, *21*, *22*). This study is the first to document the relative contribution of genotype and environmental conditions to the antioxidant properties of wheat genotypes grown in different locations. It is also the first to document predominant indicators of antioxidant activity of western Canadian wheat cultivars, which are important in international wheat commerce.

There were highly significant genotype differences in TPC, AOA, and all phenolic acids assayed. The CWRS wheat cultivars AC Elsa and Neepawa had the highest TPC, AOA, and FA concentrations compared to other genotypes. CWHWS wheat AC Snowbird had the lowest TPC, AOA, and FA concentration. Its AOA and FA concentration were, however similar to those of Superb, another CWRS wheat. The Canada Prairie Spring White wheat AC Vista had midrange AOA, FA concentration, and TPC values. Accordingly, no link was

identified between wheat color (red vs white) and antioxidant properties. A similar observation was made by Beta et al. (*13*).

Compared to genotype effects, the effects of the growing environment on TPC; AOA; and the simple phenolic acids FA, VA, and SA was even larger. This result might be partly due to relatively close genetic relationships among some of the genotypes used in this study, which would limit genetic variability accordingly. However, the nature of the location effects was not apparent in this study. There was no clear link between levels of any of the predominant indicators of antioxidants (TPC, AOA, and FA concentration) and environmental effects such as rainfall or temperature that otherwise had very significant effects on grain properties such as test weight and protein content. For example, the Swift Current and Winnipeg locations were the most diverse locations in this study according to weather patterns related to rainfall. The impact of a very dry grain development period in Swift Current and timeliness of ample precipitation in Winnipeg resulted in crops with extreme differences in protein content for western Canadian hard spring wheat and very large differences in bulk density or test weight; wheat samples of all genotypes harvested from the Winnipeg location were of high test weight (82.5 kg/hL) and low protein content (10.1%) compared to genotypes grown in Swift Current (test weight 74.1 kg/hL, protein content 15.7%). Moreover, the high-protein wheat grown in Swift Current had technological properties for breadmaking that were very different from those obtained from the low-protein wheat grown in Winnipeg (results not shown). Despite these very large differences in grain properties, the Swift Current and Winnipeg locations generated wheat with mean total phenolic contents, antioxidant activities, and phenolic acid concentrations that were more similar to each other than to wheat grown in either Melfort or Regina, Saskatchewan (**Table 3**). It is known that plant phenolic compounds can be influenced by defense reactions related to pathogen attack (*23*). For example, FA, which is the major phenolic acid in wheat, has been found to be a significant variable correlated with resistance to wheat midge (*24*) and *Fusarium* head blight (FHB) (*25*). However, no significant effects of midge, FHB, or damage by any other pest or pathogen were evident in the samples that were studied.

Yu et al. (*11*) reported significant effects of growing conditions, including the number of hours exceeding 32 °C, on the antioxidant properties of Akron, a hard red winter wheat variety. The average temperature at growing locations was shown to have effects on the antioxidant properties of strawberries (*26*). Emmons and Peterson (*27*) found significant cultivar effects for AOA and significant cultivar, location, and cultivar × location interaction effects for the concentration of total free phenolic contents in oats. The cause of location effects was not identified in that study.

Phenolic compounds have potent antioxidant activities (*16*). Significant correlations between the TPC and AOA of whole wheat or milling fractions of wheat have been reported in the literature (*13*, *21*, *28*). Zhou et al. (*21*) also reported significant correlations between FA concentration and the concentrations of *p*-hydroxybenzoic, vanillic, coumaric, and syringic acids. As correlation coefficients for AOA and TPC and for AOA and FA concentration were higher than other significant correlations, FA and TPC levels are likely to be good indicators of AOA. Total phenolic content ranged between 1709 and 1990 μg of FA equivalents/g. The values were twice as high as levels reported by Li et al. (*28*) for four Chinese wheat samples, an indication of genotypic and/or environmental effects. Beta et al. (*13*) found TPC values of between 1300 and 5300 μg of FA equivalents/g in different mill fractions of wheat with the highest concentrations (>4000 μg of FA equivalents/g) in fractions from first and second pearlings. The larger TPC range reported by Beta and others (*13*) reflects the increase in phenolic content of wheat from the inner to the outer parts of the grain. Whole-wheat samples were used in the current study. Hence, lower and less variable TPC values were observed.

Several phenolic compounds are present in wheat. These include phenolic acids; alkylresorcinols; flavanoids, and phenolic acid diacyl glycerols, phenolic aldehydes, and ferulates (*29*). Phenolic acids present in wheat include ferulic, *p*-coumaric, vanillic, caffeic, chlorogenic, gentisic, syringic, and *p*-hydroxybenzoic acids (*7*). Ferulic acid was the predominant phenolic acid, accounting for between 49.7% and 64.8% of the total amount of measured phenolic acids in the wheat samples. This observation was in agreement with the findings of Onyeneho and Hettiarachchy (*7*), Adom et al. (*10*), Zhou et al. (*21*), and Zhou and Yu (*22*). Significant levels of syringic, vanillic, caffeic, and *p*- and *o*-coumaric acids were also detected, in line with observations by Li et al. (*22*). Levels of these phenolic acids were lower in our study, likely because of varietal differences.

Antioxidant activity (AOA) was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (*19*). This assay is based on the measurement of the reducing ability of antioxidants toward DPPH. AOA was calculated as percentage discoloration and ranged between 13.21% and 14.22%, with higher percentages indicating higher AOAs. Using a similar extraction protocol and AOA method, AOAs of between 2.5% and 26% were previously obtained (*13*). The differences in the magnitude of the ranges are likely linked to the increase in concentration of phenolics in wheat from the inner to the outer parts of the grain. Wheat antioxidants are concentrated in the bran (*13*, *21*). Significant antioxidant activity has, however, also been detected in the endosperm (*30*) and in wheat germ (*31*).

Conclusion. Both the genotype and growing location manifest significant differences in the total phenolic content, antioxidant activity, and phenolic acid composition of wheat. Genotype effects were highly significant ($p < 0.0001$). CWRS wheats AC Elsa and Neepawa had significantly higher antioxidant properties than the other genotypes examined. Location effects were also highly significant ($p < 0.0001$), and wheat grown in Winnipeg had the highest predominant indicators of antioxidant potential: TPC, AOA, and FA concentration. Location effects were greater for TPC, AOA, and FA concentration than genotype effects. Interaction effects were much smaller than the genotype or location effects, and were significant only for TPC. Genotype differences for TPC, AOA, VA, SA, FA, CA, PCA, and OCA indicate that it would be possible to select for these quantitative traits in a genotype development program. The significant effects of environment must, however, be considered. More research is needed to investigate the cause of location effects, extend the study to several years, and determine the heritability of the antioxidant properties. As the DDPH assay is only one of several measures of antioxidant activity, it would be of interest to determine G × E effects on other antioxidant activity parameters such as the oxygen radical absorbance capacity (ORAC), LDL oxidation, or lipid peroxidation in future studies.

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