# Effects of Genotype and Environment on the Antioxidant Properties of Hard Winter Wheat Bran

JEFFREY MOORE,[†] JIAN-GUO LIU,[§] KEQUAN ZHOU,[#] AND LIANGLI (LUCY) YU*,[†]

Department of Nutrition and Food Science and Department of Mathematics, University of Maryland, College Park, Maryland 20742, and Department of Food Science and Technology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061

Recent consumer interest in controlling and preventing chronic diseases through improved diet has promoted research on the bioactive components of agricultural products. Wheat is an important agricultural and dietary commodity worldwide with known antioxidant properties concentrated mostly in the bran fraction. The objective of this study was to determine the relative contributions of genotype (G) and growing environment (E) to hard winter wheat bran antioxidant properties, as well as correlations of these properties to growing conditions. Bran samples of 20 hard winter wheat varieties grown in two locations were examined for their free radical scavenging capacities against DPPH, ABTS cation, peroxyl (ORAC), and superoxide anion radicals and chelating properties, as well as their total phenolics and phenolic acid compositions. Results showed significant differences for all antioxidant properties tested and multiple significant correlations between these properties. A factorial designed analysis of variance for these data and pooled previously published data showed similar results for four of the six antioxidant properties, indicating that G effects were considerably larger than E effects for chelating capacity and DPPH radical scavenging properties, whereas E was much stronger than G for ABTS cation radical scavenging capacity and total phenolics, although small interaction effects (G × E) were significant for all antioxidant properties analyzed. Results also showed significant correlations between temperature stress or solar radiation and some antioxidant properties. These results indicate that each antioxidant property of hard winter wheat bran is influenced differently by genotype and growing conditions.

**KEYWORDS: Hard wheat; antioxidant; phenolic; chelating; genotype; environment; genotype × environment interaction**

## INTRODUCTION

Recent consumer interest in controlling and preventing chronic diseases through improved diet has promoted research on the bioactive components of agricultural products and their physiological mechanisms. Antioxidants, one type of bioactives, have been linked in epidemiological studies to reductions in the risk of chronic diseases such as cancer, cardiovascular disease, and diabetes (*1*, *2*). Antioxidants are thought to prevent damage to important biomolecules such as DNA, proteins, and lipids through several possible mechanisms such as chelation of transition metals, quenching of free radicals, and stimulation of antioxidative enzyme activities (*1*−*4*).

Wheat is an important dietary staple and economic commodity with an estimated 2005/2006 production of 616.4 million metric tons worldwide (*5*). Numerous studies have found significant antioxidant properties in wheat grain including chelation of $Fe^{2+}$ and $Cu^{2+}$ and scavenging capacities against numerous free radicals including DPPH, ABTS cation, peroxyl, superoxide anion, and hydroxyl radicals (*6*−*23*). Five phenolic acids were detected in hard wheat bran, and four of them were also present in soft wheat grain (*16*, *19*). These phenolic acids are believed to contribute to the proposed health benefits of wheat grain and grain fractions (*24*). Ferulic acid is the predominant phenolic acid present in wheat, concentrated mostly in the bran fraction of both hard and soft wheat varieties (*13*, *15*, *16*, *19*, *22*, *25*, *26*). These previous studies of wheat antioxidants showed that wheat grain and fractions from individual wheat samples may differ in their contents of natural antioxidants, suggesting the potential to enhance wheat antioxidant capacity though improved agricultural practices such as growing a selected wheat variety under optimal conditions.

It is widely accepted that genotype (G) and growing environment (E) as well as their interaction (G × E) are the primary factors examined in the attempt to optimize phenotypic traits in agricultural crops. If significant environmental effects exist, individual environmental parameters may also be examined for their correlations to phenotypic traits. A number of studies have

* Corresponding author [telephone (301) 405-0761; fax (301) 314-3313; e-mail Lyu5@umd.edu].
[†] Department of Nutrition and Food Science, University of Maryland.
[§] Department of Mathematics, University of Maryland.
[#] Virginia Polytechnic Institute and State University.

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separated and quantified how the variance in baking qualities among wheat samples can be attributed to genotype and environmental conditions (*27*−*32*) and examined the correlation of environmental parameters such as heat stress to these baking qualities (*28*). Studies have also found antioxidant properties to be influenced by various environmental factors for several plants or crops. Wang and others (*32*) found antioxidant properties in strawberries to be increased by temperature stress, whereas an opposite trend was found for antioxidant properties in spearmint (*33*). Spinach antioxidants were found to be influenced by growing season (*34*) and antioxidant properties in *Hypericum brasiliense* by temperature, water stress, and light intensity (*35*). Previous studies of wheat antioxidant properties also have suggested that genotype and environment may influence these properties (*13*, *14*, *17*, *19*, *21*), and three studies have found correlations between selected antioxidant properties in hard wheat bran and solar radiation or temperature stress (*12*, *16*, *18*). No comprehensive studies, however, have separated the effects of genotype and environment and quantified their contributions to individual antioxidant property variances.

This study was conducted to further elucidate the effects of G, E, and G × E on the antioxidant properties of wheat bran by quantifying their separate contributions to antioxidant property variance. This study also investigated the effects of environmental parameters including solar radiation and temperature stress on the antioxidant properties of hard winter wheat bran. These analyses were performed using antioxidant property data from 20 hard wheat varieties, each grown in two locations, as well as pooled data from our three previous studies (*12*, *16*, *18*). The results of this study not only contribute useful antioxidant property data for Colorado-grown hard winter wheat bran but also separate and quantify the effects of genotype and environment on these properties, which will be useful for crop breeders and growers attempting to produce wheat with high levels of natural antioxidants.

## MATERIALS AND METHODS

**Hard Winter Wheat Bran Sample.** Bran samples of 20 hard winter wheat varieties each grown during the 2001 growing season in two locations were provided by Dr. Scott Haley in the Department of Soil and Crop Sciences at Colorado State University, Fort Collins, CO, and used in this study. All varieties were adapted for production in Colorado and the West Central Plains and included the red varieties Prowers 99, Co 99534, Verango, Stanton, Alliance, CO980607, CO980630, CO99508, CO980376, Akron, Enhancer, CO980719, Kalvesta, Wichita, and Halt, as well as the white varieties Trego, Avalanche, Lakin, Gmt10002, and Intrada. Each variety was grown at Walsh and Burlington testing locations, both nonirrigated sites in eastern Colorado. Agronomic practices at both locations were considered to be typical for wheat production in eastern Colorado. Grain samples were harvested in each location and cleaned using seed cleaners to remove all nongrain debris present.

**Chemicals and Reagents.** Disodium ethylenediaminetetraacetate (EDTA), 2,2′-bipyridyl, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), fluorescein (FL), lauryl sulfate sodium salt, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), hypoxanthine (HPX), xanthine oxidase (XOD), and nitro blue tetrazolium solution (NBT) were purchased from Sigma-Aldrich (St. Louis, MO). β-Cyclodextrin (RMCD) was purchased from Cyclolab R&D Ltd. (Budapest, Hungary). 2,2′-Azobis(2-aminopropane) dihydrochloride (AAPH) was obtained from Wako Chemicals USA (Richmond, VA). All other chemicals and solvents were of the highest commercial grade and used without further purification.

**Extraction and Testing Sample Preparation.** Bran samples were ground to 40-mesh powder using a micromill manufactured by Bel Art Products (Pequannock, NJ). Five grams of each ground bran sample was extracted with 50 mL of 50% acetone for 15 h under nitrogen at ambient temperature and subsequently filtered through Whatman no. 1 filter paper (Whatman Int., Maidstone, U.K.). No volume adjustment was made because all samples were extracted under the same conditions and volume change was minimal. All filtrates in sealed containers were kept in the dark under nitrogen at room temperature until further antioxidant analysis and subjected to further treatment for phenolic acid analysis.

**$ABTS^{\bullet+}$ Scavenging Capacity.** Radical scavenging capacity of the 50% acetone wheat bran extracts were measured against $ABTS^{\bullet+}$ generated according to previously reported protocols (*15*, *36*). Twenty microliters of wheat extracts was diluted with 800 μL of 50% acetone to create working sample solutions. Trolox solution (0.5 M) in 7% RMCD was diluted in 50% acetone to create working Trolox standards. $ABTS^{\bullet+}$ radicals were generated by oxidizing a 5 mM aqueous solution of ABTS with manganese dioxide under ambient temperature for 30 min. The final reaction mixture contained 1.0 mL of $ABTS^{\bullet+}$ solution with an absorbance of 0.7 at 734 nm and 80 μL of 50% acetone for the control or 80 μL of the working sample or standard solution. The absorbance after 1 min of reaction time was measured at 734 nm. Results were calculated using a standard curve prepared with Trolox and expressed as micromoles of Trolox equivalents (TE) per gram of bran.

**Chelating Capacity.** $Fe^{2+}$ chelating activity was determined using a previously reported 2,2′-bipyridyl competition assay (*37*). The final reaction mixture contained 20 μL of the 50% acetone wheat bran extract or EDTA standard, 100 μL of 1 mM $FeSO_4$ solution, 300 μL of 10% hydroxylamine−HCl, 800 μL of 1 M Tris-HCl buffer (pH 7.4), and 400 μL of 2,2′-bipyridyl solution (0.1% in 0.2 M HCl). Absorbance was measured at 522 nm to determine chelating activity using EDTA as a standard. Results are expressed as milligrams of EDTA equivalents per gram of wheat bran.

**$DPPH^{\bullet}$ Scavenging Activity.** The 50% acetone extracts of wheat bran samples were examined to estimate their $DPPH^{\bullet}$ scavenging properties according to a previously reported procedure using the commercial stable $DPPH^{\bullet}$ (*10*). Briefly, fresh $DPPH^{\bullet}$ solution was added to the bran extracts and 50% acetone for blank, and absorbance at 517 nm was measured at 0, 0.5, 1, 2, 5, and 10 min. The initial concentration was 100 μM for $DPPH^{\bullet}$ in all reaction mixtures. $DPPH^{\bullet}$ radical scavenging capacity was estimated as the difference in absorbance between samples and a blank at 10 min of reaction and expressed as percent $DPPH^{\bullet}$ remaining.

**ORAC Assay.** The ORAC assay was conducted with FL as the fluorescent probe and the 50% acetone extracts according to a protocol described previously (*15*, *38*). With the exception of samples and Trolox standards, which were prepared with 50% acetone, all other reagents were prepared in 75 mM phosphate buffer (pH 7.4). The final reaction mixture contained 0.067 μM FL, 60 mM AAPH, and 300 μL of 50% acetone wheat bran extract or 50% acetone for blank. The fluorescence of each assay mixture was recorded every minute for 1 h at ambient temperature. Excitation and emission wavelengths were 490 and 515 nm, respectively. Trolox equivalents (TE) were calculated using the relative area under the curve for samples compared to a Trolox standard curve prepared under the same experimental conditions. Results are expressed as micromoles of TE per gram of wheat bran.

**Superoxide Anion Radical ($O_2^{\bullet-}$) Scavenging Capacity.** The $O_2^{\bullet-}$ scavenging capacity of the wheat bran extracts was measured using the HPX/XOD system following a previously described procedure (*15*, *39*). XOD, HPX, and NBT solutions were prepared in 50 mM phosphate buffer (pH 7.4). Reaction mixtures contained 200 μL of XOD with an activity of 0.56 unit/mL, 700 μL of 2 mM HPX, 200 μL of 0.34 mM NBT, and 100 μL of the wheat bran extract. The decrease in absorbance at 560 nm was measured after 7 min of reaction. Results are expressed as percent $O_2^{\bullet-}$ remaining.

**Total Phenolic Contents.** The 50% acetone extracts were analyzed for total phenolic contents following a laboratory procedure using the Folin−Ciocalteu reagent (*10*). The Folin−Ciocalteu reagent was prepared by refluxing a mixture of sodium molybdate, sodium tungstate, concentrated hydrochloric acid, and 85% phosphoric acid for 10 h and then reacting it with lithium sulfate, and oxiding it with a few drops of bromine. The resulting solution was filtered and used for testing. The

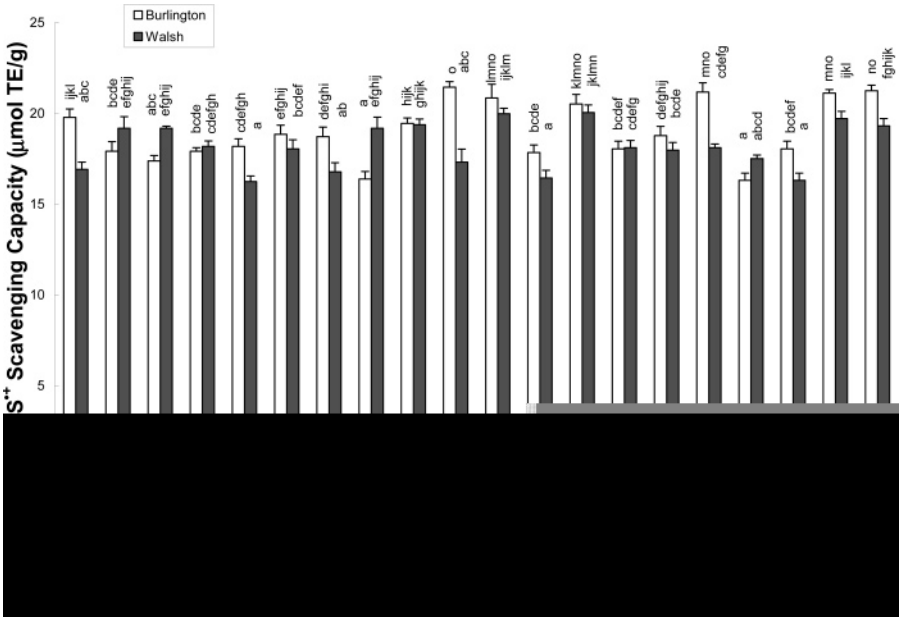


**Figure 1.** ABTS•+ radical scavenging capacity of hard winter wheat bran. Results are expressed as micromoles of Trolox equivalents per gram of hard winter wheat bran ($\mu$mol of TE/g). All tests were conducted in triplicate, with mean values reported. The vertical bars represent the standard deviation of each data point. Values marked by the same letter are not significantly different ($P < 0.05$).

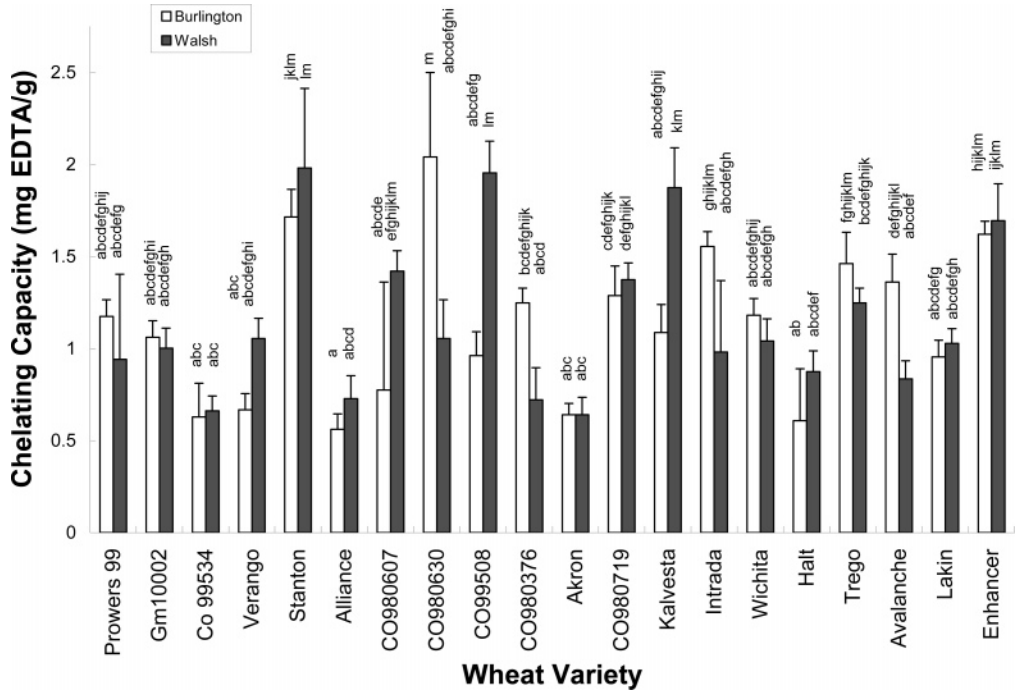


**Figure 2.** Chelating capacity of hard winter wheat bran. Results are expressed as milligrams of EDTA equivalents per gram of wheat bran. All tests were conducted in triplicate, with mean values reported. The vertical bars represent the standard deviation of each data point. Values marked by the same letter are not significantly different ($P < 0.05$).

final reaction mixture contained 500 $\mu$L of the Folin−Ciocalteu reagent, 100 $\mu$L of the wheat bran extracts, 1.5 mL of 20% sodium carbonate, and 6 mL of distilled deionized water. After 2 h of reaction, the absorbance at 765 nm was measured to calculate the total phenolic contents (TPC) in samples using gallic acid as a standard. Results are expressed as milligrams of gallic acid equivalents per gram of wheat bran (mg of GAE/g).

**Phenolic Acid Composition.** The phenolic acid composition of 50% acetone wheat extracts was determined using a previously described purification procedure (*40*) and separation conditions (*15*, *41*). Briefly, after removal of acetone, the wheat bran extracts were hydrolyzed with 4 N NaOH at 55 °C for 4 h under nitrogen and then acidified with 6 N HCl and extracted with ethyl ether/ethyl acetate (1:1, v/v). After removal of the organic solvents at 25 °C using a nitrogen evaporator, the solid residue was redissolved in methanol and filtered through a 0.45 $\mu$m membrane and kept under nitrogen in the dark until HPLC analysis. The phenolic acid composition of the methanol solution was analyzed using reverse-phase HPLC with a Phenomenex C18 column (250 mm × 4.6 mm). The phenolic acids were separated using a linear gradient elution program with mobile phase solvents A (acetic acid/$H_2O$, 2:98, v/v) and B (acetic acid/acetonitrile/$H_2O$, 2:30:68, v/v/v). Solvent gradient with a flow rate of 1.5 mL/min was programmed from 10 to 100% B in 42 min. Phenolic acid identification was performed

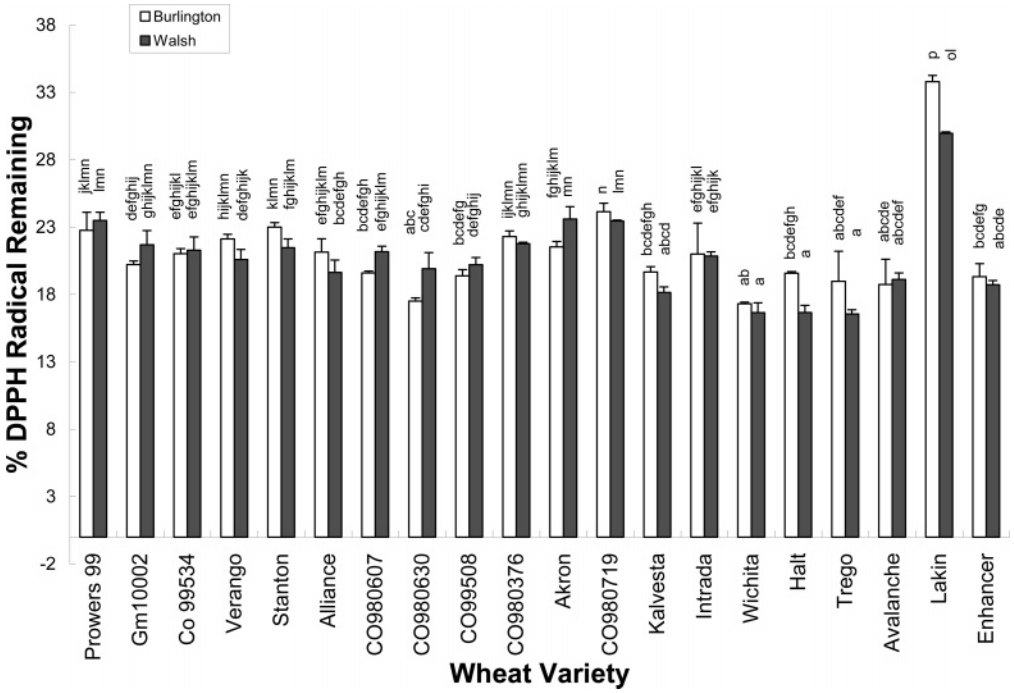


**Figure 3.** DPPH radical scavenging capacity of hard winter wheat bran. The percent DPPH radical remaining was determined at 10 min for each reaction. All tests were conducted in triplicate, with mean values reported. The vertical bars represent the standard deviation of each data point. Values marked by the same letter are not significantly different ($P < 0.05$).

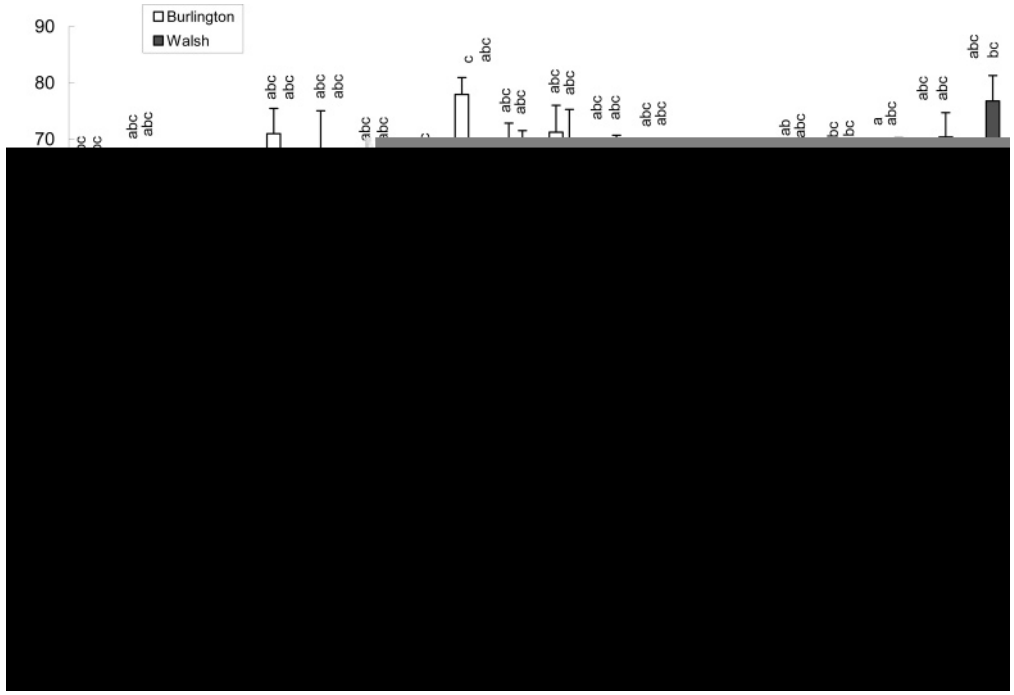


**Figure 4.** Oxygen radical scavenging capacity of hard winter wheat bran. Results are expressed as micromoles of Trolox equivalents per gram of hard wheat bran ($\mu$mol of TE/g). All tests were conducted in duplicate, with mean values reported. The vertical bars represent the standard deviation of each data point. Values marked by the same letter are not significantly different ($P < 0.05$).

by comparing the retention time of the peaks in wheat bran extract with that of the standard compounds.

**Statistical Analysis.** All statistical analyses were performed using SPSS (ver. 10.0.5, 1999, SPSS Inc., Chicago, IL). Data were analyzed by analysis of variance (ANOVA). Genotype and environment contributions to variance were determined using a factorial design with three replicates and the general linear model (GLM) using genotype and environment as fixed effects. Replicates were random samples taken within test plots at each growing location. Comparison of means was performed using Tukey's HSD post-hoc testing. Correlation analyses were performed using a two-tailed Pearson's correlation test. Statistical significance was declared at $P < 0.05$.

## RESULTS AND DISCUSSION

Growing evidence has shown that whole foods such as whole grains and not their purified components have the best correlation to reduced risk for chronic diseases (*1*, *42*, *43*). Whole

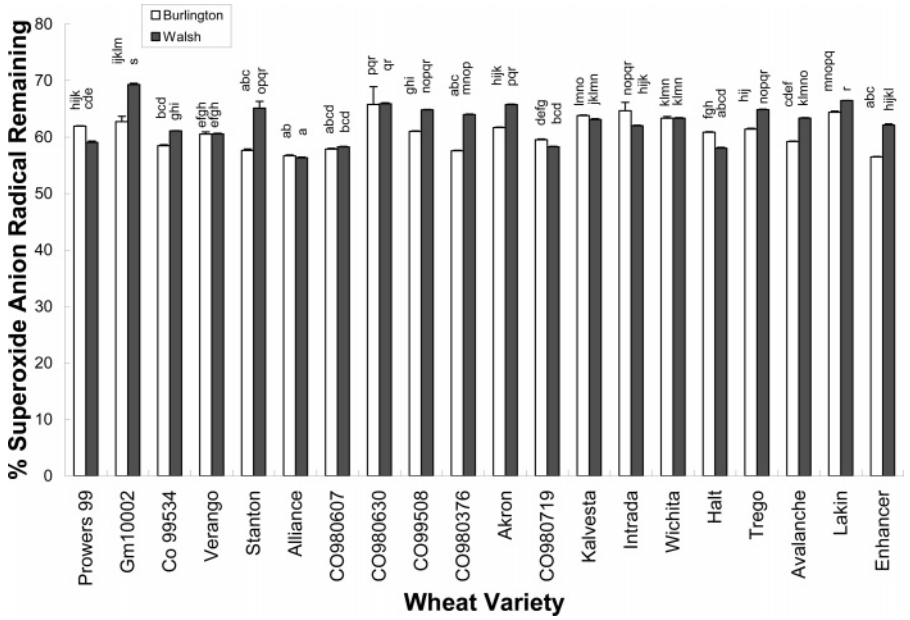


**Figure 5.** Superoxide anion radical ($O_2^{\bullet -}$) scavenging capacity of hard winter wheat bran. The percent $O_2^{\bullet -}$ remaining was determined at 7 min for each reaction. All tests were conducted in triplicate, with mean values reported. The vertical bars represent the standard deviation of each data point. Values marked by the same letter are not significantly different ($P < 0.05$).

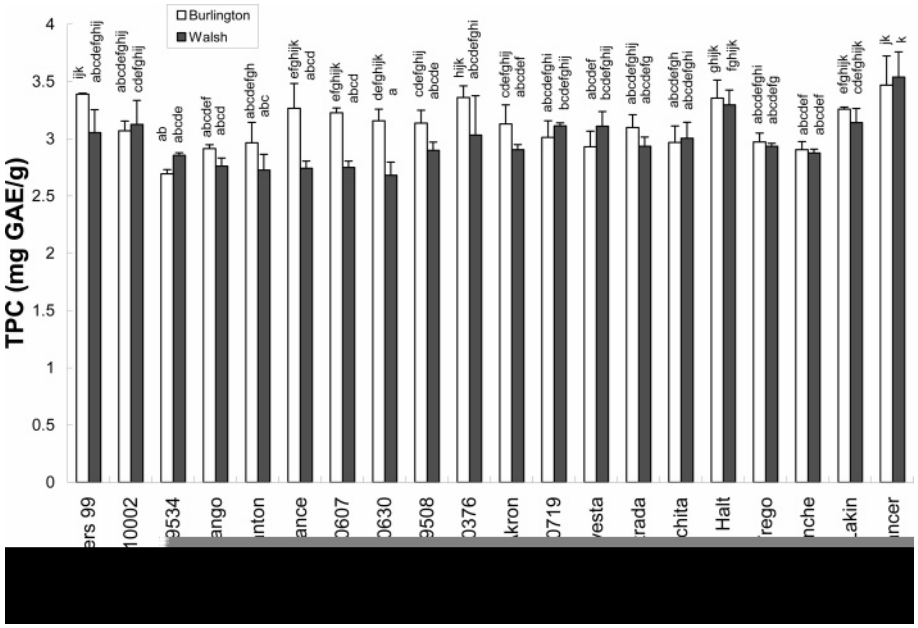


**Figure 6.** Total phenolic contents of hard winter wheat bran. Results are expressed as milligrams of gallic acid equivalents (GAE) per gram of hard wheat bran. All tests were conducted in triplicate, and mean values are used. The vertical bars represent the standard deviation of each data point. Values marked by the same letter are not significantly different ($P < 0.05$).

grains in particular have been shown in 43 of 45 epidemiological studies to reduce the risk of cancer (*24*). Given the volume of wheat utilized in the diet worldwide and its potential to help manage chronic diseases, it is important to advance our understanding of the potential approaches to improve the health beneficial capacities of wheat and its fractions. The present study examined antioxidant properties of bran samples from an additional 20 hard winter wheat varieties and elucidated the relative contributions of genotype and environment as well as the interaction between genotype and environment to these properties. This information could be used to optimize and enhance the antioxidant properties of wheat through improved crop breeding programs, management of environmental conditions, or a combination of both.

**Antioxidant Properties of the Bran Samples of the 20 Hard Winter Wheat Varieties Grown at Two Locations.** Reported in **Figures 1**−**6** and **Table 1** are the individual antioxidant properties for all 40 wheat bran samples examined in this study. $ABTS^{\bullet +}$ scavenging capacity had a range of 16.2 to 21.5 $\mu$mol of Trolox equiv/g (**Figure 1**), $Fe^{2+}$ chelating capacities ranged from 0.56 to 2.04 mg of EDTA equiv/g (**Figure 2**), and TPC values were from 2.7 to 3.5 mg of GAE/g (**Figure 6**). All of these ranges were comparable to previous results for hard wheat bran samples (*14*, *15*) and higher than

**Table 1.** Phenolic Acid Composition of 20 Hard Wheat Varieties Grown in Two Locations[a]

| growing location and variety[b] | *p*-hydroxybenzoic acid ($\mu$g/g of bran) | vanillic acid ($\mu$g/g of bran) | syringic acid ($\mu$g/g of bran) | coumaric acid ($\mu$g/g of bran) | ferulic acid ($\mu$g/g of bran) |
|---|---|---|---|---|---|
| W.Prowers 99 | 8.23 ± 0.16 | 11.85 ± 0.15 | 29.02 ± 0.92 | 6.58 ± 0.38 | 115.56 ± 0.43 |
| B.Prowers 99 | 24.26 ± 5.22 | 14.81 ± 0.16 | 37.61 ± 0.12 | 8.98 ± 0.04 | 142.54 ± 0.04 |
| W.Gm10002 | 11.20 ± 1.25 | 14.94 ± 0.07 | 45.97 ± 0.05 | 5.09 ± 1.52 | 122.40 ± 3.26 |
| B.Gm10002 | 16.02 ± 4.91 | 19.04 ± 1.79 | 45.38 ± 1.47 | 4.84 ± 1.36 | 114.47 ± 1.07 |
| W.Co 99534 | 9.57 ± 0.11 | 15.38 ± 0.06 | 37.29 ± 0.23 | 5.96 ± 0.07 | 127.33 ± 0.56 |
| B.Co 99534 | 9.10 ± 0.13 | 17.79 ± 0.11 | 37.52 ± 0.12 | 6.20 ± 0.02 | 108.80 ± 0.36 |
| W.Verango | 7.21 ± 0.77 | 9.42 ± 0.11 | 19.51 ± 2.65 | 5.67 ± 0.16 | 119.08 ± 9.31 |
| B.Verango | 11.54 ± 0.64 | 15.47 ± 0.02 | 32.08 ± 0.10 | 7.12 ± 0.08 | 137.35 ± 0.66l |
| W.Stanton | 9.32 ± 0.07 | 12.17 ± 0.29 | 31.92 ± 0.24 | 7.33 ± 0.04 | 108.36 ± 1.04 |
| B.Stanton | 25.12 ± 8.93 | 34.21 ± 22.26 | 40.50 ± 1.03 | 8.19 ± 0.83 | 117.13 ± 16.92 |
| W.Alliance | 17.88 ± 5.69 | 14.45 ± 0.73 | 39.30 ± 0.50 | 5.84 ± 0.00 | 142.37 ± 0.52 |
| B.Alliance | 8.89 ± 0.20 | 16.48 ± 1.13 | 39.18 ± 0.27 | 8.60 ± 1.71 | 146.38 ± 1.86 |
| W.CO980607 | 21.31 ± 3.99 | 13.71 ± 0.14 | 32.31 ± 0.07 | 5.05 ± 0.34 | 114.23 ± 0.72 |
| B.CO980607 | 18.61 ± 2.32 | 17.98 ± 1.28 | 41.52 ± 1.11 | 5.50 ± 0.97 | 118.85 ± 0.84 |
| W.CO980630 | 16.17 ± 3.89 | 15.06 ± 1.16 | 31.30 ± 1.01 | 5.80 ± 0.02 | 106.73 ± 0.33 |
| B.CO980630 | 29.34 ± 2.41 | 18.64 ± 0.07 | 42.79 ± 0.69l | 6.47 ± 0.01 | 124.50 ± 0.40 |
| W.CO99508 | 12.38 ± 0.10 | 14.41 ± 0.04 | 35.17 ± 1.00 | 5.32 ± 0.18 | 123.08 ± 0.44 |
| B.CO99508 | 34.08 ± 6.27 | 16.78 ± 3.11 | 43.27 ± 0.04 | 8.06 ± 0.51 | 133.70 ± 0.17 |
| W.CO980376 | 8.30 ± 0.03 | 12.89 ± 0.04 | 36.31 ± 0.28 | 5.01 ± 0.01 | 106.60 ± 0.27 |
| B.CO980376 | 25.39 ± 6.02 | 18.69 ± 0.41 | 43.30 ± 0.08 | 8.42 ± 0.01 | 163.93 ± 0.67 |
| W.Akron | 29.16 ± 8.51 | 16.94 ± 0.88 | 41.54 ± 0.51 | 5.66 ± 0.07 | 129.96 ± 0.40 |
| B.Akron | 22.93 ± 8.16 | 17.81 ± 1.63 | 57.06 ± 1.78 | 11.59 ± 0.05 | 142.62 ± 0.10 |
| W.CO980719 | 14.56 ± 0.22 | 14.04 ± 0.01 | 30.68 ± 0.24 | 5.08 ± 0.79 | 115.72 ± 1.01 |
| B.CO980719 | 15.25 ± 0.96 | 19.80 ± 0.15 | 36.91 ± 0.02 | 5.58 ± 0.09 | 102.73 ± 0.16 |
| W.Kalvesta | 10.31 ± 0.54 | 12.78 ± 0.08 | 35.11 ± 0.03 | 2.54 ± 0.11 | 151.68 ± 2.19 |
| B.Kalvesta | 18.31 ± 0.01 | 16.71 ± 0.07 | 45.02 ± 0.36 | 8.70 ± 0.22 | 162.27 ± 0.19 |
| W.Intrada | 13.99 ± 0.87 | 15.23 ± 0.07 | 36.79 ± 0.08 | 4.94 ± 0.15 | 109.64 ± 0.20 |
| B.Intrada | 28.32 ± 0.02 | 16.37 ± 0.07 | 31.70 ± 0.25 | 4.60 ± 0.11 | 89.42 ± 0.12 |
| W.Wichita | 18.98 ± 1.78 | 33.12 ± 0.31 | 55.70 ± 0.57 | 5.81 ± 0.09 | 130.54 ± 2.55 |
| B.Wichita | 11.49 ± 0.59 | 17.59 ± 0.04 | 36.45 ± 0.52 | 7.02 ± 0.08 | 130.06 ± 0.65 |
| W.Halt | 10.81 ± 4.46 | 12.92 ± 1.01 | 26.73 ± 0.05 | 4.47 ± 0.13 | 115.90 ± 0.82 |
| B.Halt | 20.73 ± 0.80 | 19.32 ± 0.46 | 45.37 ± 0.50 | 7.96 ± 0.19 | 174.47 ± 4.38 |
| W.Trego | 21.85 ± 1.35 | 13.08 ± 0.23 | 32.49 ± 0.56 | 6.35 ± 0.20 | 111.35 ± 2.00 |
| B.Trego | 11.10 ± 0.07 | 15.16 ± 0.40 | 33.27 ± 0.01 | 3.69 ± 0.02 | 90.93 ± 3.09 |
| W.Avalanche | 10.00 ± 1.51 | 14.83 ± 2.48 | 28.36 ± 1.82 | 3.92 ± 0.49 | 90.34 ± 8.46 |
| B.Avalanche | 10.53 ± 0.10 | 17.11 ± 0.00 | 30.79 ± 0.11 | 3.70 ± 0.16 | 98.54 ± 0.39 |
| W.Lakin | 8.27 ± 0.46 | 7.07 ± 0.08 | 10.07 ± 0.20 | 7.10 ± 0.07 | 93.11 ± 0.58 |
| B.Lakin | 17.88 ± 0.22 | 20.24 ± 0.08 | 36.42 ± 0.05 | 16.99 ± 0.04 | 142.23 ± 0.37 |
| W.Enhancer | 12.65 ± 0.15 | 17.83 ± 0.20 | 47.06 ± 0.83 | 7.24 ± 0.02 | 160.06 ± 0.62 |
| B.Enhancer | 12.72 ± 0.01 | 22.22 ± 0.02 | 56.51 ± 0.10 | 8.28 ± 0.01 | 193.92 ± 0.14 |

[a] Data expressed as mean ± standard deviation ($n = 3$). [b] W, Walsh; B, Burlington (growing locations in Colorado).

those for eight varieties of soft wheat whole grain (*19*) and Swiss red wheat grain (*15*), all using similar extraction procedures and testing conditions for each assay.

DPPH$^{\bullet}$ scavenging capacity results are reported as percent DPPH$^{\bullet}$ remaining after 10 min of reaction and ranged from 16.6 to 33.8%, lower (higher in scavenging capacity) than previously published results from Trego wheat bran samples grown in four locations extracted with ethanol but tested under similar assay conditions (**Figure 3**) (*16*). ORAC values of the 20 wheat bran samples from the two locations ranged from 45.0 to 78.0 $\mu$mol of Trolox equiv/g (**Figure 4**), a range similar to previous results for seven hard wheat bran samples (*14*) and higher than those for eight varieties of soft wheat whole grain (*19*), both using similar extraction procedures and analytical conditions. $O_2^{\bullet-}$ scavenging capacities were from 56.3 to 69.3% expressed as percent $O_2^{\bullet-}$ remaining (**Figure 5**). These results were comparable to results for the seven hard wheat bran samples extracted under similar conditions (*14*).

Phenolic acid composition results showed ranges of 7.2−34.1, 7.1−34.2, 10.1−57.1, 2.5−17.0, and 89.4−193.9 $\mu$g/g for *p*-hydroxybenzoic, vanillic, syringic, coumaric, and ferulic acids, respectively (**Table 1**). These results are similar to those found by Zhou and others (*14*) for bran samples of the seven wheat varieties from different countries and by Zhou and others (*16*) for Trego hard wheat bran grown in five locations, all analyzed using similar experimental conditions.

In summary, these data support the assumption that both genotype and environment may have significant effects on the antioxidant properties of hard winter wheat. These results also support the conclusions of numerous previous studies that wheat bran is rich in antioxidants and that the predominant phenolic acid present in wheat is ferulic acid (*9*, *14*, *15*, *19*, *21*, *22*).

**Effects of G and E on Wheat Antioxidant Properties.** To separate and quantify the contributions of G, E, and G × E interactions on wheat antioxidant property variance, a 2 × 20 factorial designed ANOVA was performed on all data from the 20 hard wheat varieties grown in two locations. The magnitude of variance proportion (percent total mean squares) attributed to G, E, and G × E indicates their relative significance in determining each antioxidant property. Results showed that G and E, as well as the interaction between G and E, significantly influenced all antioxidant properties of wheat bran except the chelating capacity and ORAC (**Table 2**). For ABTS$^{\bullet+}$ and $O_2^{\bullet-}$ scavenging properties and TPC, E contributed the highest proportion of total variance, ranging from 60 to 68%, G contributed 22−27%, and G × E contributed 8−14% (**Table**

**Table 2.** Proportions of Variance (Total Mean Squares) Attributed to Genotype (G), Environment (E), and G × E Interaction for 20 Hard Wheat Varieties Grown in Two Locations[a]

| antioxidant property | variance component | | |
|---|---|---|---|
| | G | E | G × E |
| ABTS•+ scavenging capacity (μmol of TE/g) | 25.39*** | 60.07*** | 13.95*** |
| chelating capacity (mg of EDTA/g) | 64.14*** | 1.79 | 30.22*** |
| DPPH• scavenging capacity (% remaining) | 85.78*** | 7.35*** | 5.88*** |
| ORAC (μmol of TE/g) | 56.13*** | 0.53 | 29.16*** |
| $O_2^{\bullet-}$ scavenging capacity (% remaining) | 26.60*** | 62.90*** | 10.26*** |
| TPC (mg of GAE/g) | 21.61*** | 68.32*** | 8.02*** |
| *p*-hydroxybenzoic acid (μg/g) | 12.38*** | 72.46*** | 13.50*** |
| vanillic acid (μg/g) | 9.28*** | 76.95*** | 10.63*** |
| syringic acid (μg/g) | 17.94*** | 74.14*** | 7.87*** |
| coumaric acid (μg/g) | 12.59*** | 79.20*** | 7.95*** |
| ferulic acid (μg/g) | 31.66*** | 56.99*** | 11.09*** |

[a] Results expressed as percent of total variation (mean squares) from factorial design ANOVA using genotype and environment as fixed effects. Results without asterisks were not significant at $P < 0.05$; ***, highly significant ($P < 0.001$). TE and GAE stand for Trolox equivalents and gallic acid equivalents, respectively.

**Table 3.** Proportions of Variance (Total Mean Squares) Attributed to Genotype (G), Environment (E), and G × E Interaction for Three Hard Wheat Varieties Grown in Five Locations[a]

| antioxidant property | variance component | | |
|---|---|---|---|
| | G | E | G × E |
| ABTS•+ scavenging capacity (μmol of TE/g) | 33.28*** | 51.33*** | 15.06*** |
| chelating capacity (mg of EDTA/g) | 59.56*** | 25.64*** | 13.99*** |
| DPPH• scavenging capacity (% remaining) | 88.58*** | 7.84*** | 3.57*** |
| ORAC (μmol of TE /g) | 25.63*** | 51.75*** | 22.23*** |
| $O_2^{\bullet-}$ scavenging capacity (% remaining) | 2.82 | 27.83 | 54.22** |
| TPC (mg of GAE/g) | 5.35*** | 79.54*** | 15.00*** |

[a] Results expressed as percent of total variation (mean squares) from factorial design ANOVA using genotype and environment as fixed effects. Data used in analysis were previously reported (*12*, *16*, *18*). Results without asterisks were not significant at $P < 0.05$; ***, highly significant ($P < 0.01$); **, significant ($P < 0.05$). TE and GAE stand for Trolox equivalents and gallic acid equivalents, respectively.

**2**). For chelating capacity, DPPH• scavenging ability, and ORAC, G contributed the highest proportion of total variance, ranging from 56 to 86%, whereas E was only significant for DPPH at 7% and G × E contributed 6−30%. For all phenolic acid composition data, E contributed the highest proportion of total variance, ranging from 57 to 79%. Although revealing significant information for determining the separate effects of G and E on antioxidant properties, the scope of these results is limited by the small number of growing conditions involved.

To further investigate the contributions of G, E, and G × E on antioxidant properties and increase the scope of our results, we additionally conducted a similar statistical analysis (3 × 5 factorial design) on previously collected data for bran samples of three hard winter wheat varieties (Akron, Trego, Platte) grown at five locations in eastern Colorado (Akron, Burlington, Julesburg, Walsh, Fort Collins) (*12*, *16*, *18*). Results shown in **Table 3** indicate significant G, E, and G × E effects for all antioxidant properties except G and E for $O_2^{\bullet-}$ scavenging. E was the main contributor to total variance for ABTS•+ scavenging property, ORAC, and TPC, whereas G was the main contributor for chelating and DPPH• scavenging ability and G × E interaction for $O_2^{\bullet-}$ scavenging property.

**Table 4.** Correlation Analysis of Growing Conditions and Antioxidant Properties for Three Hard Wheat Varieties Grown in Five Locations[a]

| environment parameter | antioxidant property[b] | | | | | |
|---|---|---|---|---|---|---|
| | ABTS | CHEL | DPPH | ORAC | SUPER | TPC |
| total solar radiation | –0.392** | –0.443** | –0.236 | 0.102 | –0.246 | 0.147 |
| hours exceeding 32 °C | –0.127 | 0.036 | 0.158 | –0.112 | –0.052 | –0.417** |

[a] Data used in analysis were previously reported (*12*, *16*, *18*). Total solar radiation and the hours during which the temperature exceeded 32 °C were measured during the 6-week grain-filling period. Total solar radiation measured with units of MJ $m^{-2}$. Results shown as Pearson correlation coefficients with indicated level of significance. Data without asterisks were not significant at $P < 0.05$; ***, very significant ($P < 0.001$); **, highly significant ($P < 0.01$); *, significant ($P < 0.05$). [b] ABTS, ABTS•+ scavenging capacity; CHEL, chelating capacity; DPPH, DPPH• scavenging capacity; SUPER, $O_2^{\bullet-}$ scavenging capacity; ORAC, oxygen radical absorbing capacity; TPC, total phenolic contents.

Evaluation of both sets of data for hard winter wheat bran revealed that all antioxidant properties were significantly altered by the interaction between environment and genotype (G × E), but G or E showed stronger influence than that of G × E (except $O_2^{\bullet-}$ scavenging property for the 15 wheat bran data), indicating that either G or E may be the predominant contributor to these antioxidant properties. These data suggest that E may play the most important role in determining ABTS•+ scavenging property and TPC, whereas G may have the strongest effect for DPPH• scavenging ability and chelating properties. Results also indicated that phenolic acid composition may be more affected by E than G. Results that differed between both sets of data, for example, ORAC, which had significantly different G and E effects seen in **Tables 2** and **3**, can be explained by the fact that each analysis used different data including different numbers of factors and growing environments. For example, **Table 3** data from five growing environments, which had more environmental variability compared to data from two locations in **Table 2**, would be expected to yield different results. This also points to the need for further studies using more data and replications. Taken together, the results suggest that individual antioxidant properties are significantly affected by either G or E, and how G or E may alter wheat antioxidant property may also depend on the assay used to estimate the antioxidant property.

**Effects of Individual Environmental Factors on Wheat Bran Antioxidant Properties.** Given the present results indicating that growing environment (E) may be a significant factor affecting some antioxidant properties for hard wheat bran, it would be useful to further determine which individual environmental factors are the main contributors to this environmental variance. Previously published data on bran samples of three hard winter wheat varieties grown in five locations from our laboratory showed significant correlations between antioxidant properties and environmental factors, but were analyzed individually by variety (*12*, *16*, *18*). Because pooling these data together would provide a more powerful assessment of these correlations, an analysis was performed in this study using total solar radiation and temperature stress (hours above 32 °C) data from the grain-filling time frame. Correlation analysis detected three significant negative correlations between solar radiation and ABTS•+ scavenging activity, solar radiation and chelating capacity, and TPC and temperature stress (**Table 4**).

**Table 5.** Significant Correlations between Antioxidant Properties for 20 Hard Wheat Varieties Grown in Two Locations[a,b]

| | ABTS | CHEL | DPPH | SUPER | ORAC | TPC | FERUL | COUM | PH | VAN | SYR |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ABTS | | | | | | | | | | | |
| CHEL | –0.18 | | | | | | | | | | |
| DPPH | 0.248 | –0.17 | | | | | | | | | |
| SUPER | 0.033 | 0.171 | 0.11 | | | | | | | | |
| ORAC | 0.292 | 0.161 | 0.156 | –0.076 | | | | | | | |
| TPC | 0.503** | 0.068 | 0.09 | –0.142 | 0.349* | | | | | | |
| FERUL | 0.723** | –0.036 | –0.078 | –0.261 | 0.235 | 0.550** | | | | | |
| COUM | 0.544** | –0.174 | 0.568** | –0.015 | 0.243 | 0.351* | 0.478** | | | | |
| PH | 0.239 | 0.099 | –0.033 | 0.06 | 0.272 | 0.193 | 0.188 | 0.267 | | | |
| VAN | 0.154 | 0.099 | –0.104 | –0.205 | 0.053 | 0.175 | 0.264 | 0.252 | 0.388* | | |
| SYR | 0.397* | –0.027 | –0.287 | –0.099 | 0.146 | 0.363* | 0.624** | 0.293 | 0.404** | 0.655** | |

[a] Results shown as Pearson correlation coefficients with indicated levels of significance. Results without asterisks were not significant at ($P < 0.05$); *, $P < 0.05$; **, $P < 0.01$. [b] ABTS, $ABTS^{\bullet+}$ scavenging capacity; CHEL, chelating capacity; DPPH, $DPPH^{\bullet}$ scavenging capacity; SUPER, $O_2^{\bullet-}$ scavenging capacity; ORAC, oxygen radical absorbing capacity; TPC, total phenolic contents; FERUL, COUM, PH, VAN, and SYR, compositions of ferulic, coumaric, *p*-hydroxybenzoic, vanillic, and syringic acids, respectively.

**Table 6.** Significant Correlations between Antioxidant Properties for Three Hard Wheat Varieties Grown in Five Locations[a,b]

| | CHEL | ABTS | ORAC | DPPH | TPC | SUPER |
|---|---|---|---|---|---|---|
| CHEL | | | | | | |
| ABTS | 0.212 | | | | | |
| ORAC | –0.103 | 0.361* | | | | |
| DPPH | 0.492** | –0.142 | –0.441** | | | |
| TPC | –0.174 | 0.410** | 0.623** | –0.309* | | |
| SUPER | 0.341 | –0.143 | –0.119 | –0.024 | –0.263 | |

[a] Results shown as Pearson correlation coefficients with indicated levels of significance. Data used in analysis were previously reported (*12*, *16*, *18*). Data without asterisks were not significant at $P < 0.05$; *, significant ($P < 0.05$); **, highly significant ($P < 0.01$). [b] ABTS, $ABTS^{\bullet+}$ scavenging capacity; CHEL, chelating capacity; DPPH, $DPPH^{\bullet}$ scavenging capacity; SUPER, $O_2^{\bullet-}$ scavenging capacity; ORAC, oxygen radical absorbing capacity; TPC, total phenolic contents.

The effects of solar radiation, temperature stress, and drought stress on antioxidant properties have been reported in several studies (*33*−*35*, *44*−*49*). Most of these studies have suggested that environmental stress from sources such as temperature, solar radiation, drought, and excess water result in protective or repair responses in plants including production of antioxidants (*45*, *50*, *51*). Our results do not support this notion for TPC (**Table 4**), showing that it may be significantly decreased by increased temperature stress (hours above 32 °C). Our results also indicate that increased solar radiation may decrease $ABTS^{\bullet+}$ scavenging activity and chelating capacity of wheat grain. For solar radiation, however, no definition has been made to distinguish "normal" from "stressful" conditions, making it difficult to attribute antioxidant changes to "stress".

**Correlations between Antioxidant Properties.** Significant correlations between antioxidant properties for wheat grain and fractions have been previously reported in numerous studies. Beta and others (*21*) and Li and co-workers (*22*) found significant correlations between $DPPH^{\bullet}$ scavenging capacity and TPC for Canadian and Chinese black-grained wheat. Significant correlations between antioxidant properties were also reported for hard wheat grain and its fraction (*14*, *15*) and for Maryland-grown soft wheat grain (*19*). The present study analyzed two sets of data, the bran samples of 20 hard winter wheat varieties grown in two locations and those of 3 hard winter wheat varieties from five locations (*12*, *16*, *18*). Results shown in **Tables 5** and **6** indicate significant correlations among antioxidant activities. Common between the two sets of data were two correlations between TPC and ORAC and between $ABTS^{\bullet+}$ scavenging capacity and TPC. This indicates that for hard winter wheat bran, TPC may be a good indicator of both $ABTS^{\bullet+}$ and peroxyl radical (ORAC) scavenging capacities. Other dissimilar correlations may be a result of the different extraction conditions used for collecting the two sets of data.

In conclusion, results from this preliminary study indicate that genotype, growing environment, and individual environmental parameters could significantly affect antioxidant properties of hard winter wheat grain. For the data analyzed, either genotype or growing environment may have a significant impact on individual antioxidant properties, and the interaction between genotype and environment may have less influence on individual antioxidant properties of wheat grain compared to genotype or environment alone. Furthermore, the assay method for estimating the antioxidant activities may alter the assessment of effects of genotype, environment, and the interaction between the two on antioxidant properties of wheat grain. Environmental parameters including heat stress and solar radiation may also have some significant effects on antioxidant properties of wheat grain. Whereas the scope of the results from this study present preliminary insights into how genotype and growing environment may influence individual wheat antioxidant properties, more in-depth studies are required to better understand these complex relationships. It is therefore recommended that further studies are undertaken using more advanced experimental designs such as randomized complete block split-plot design with more replication and involving more wheat varieties, growing locations and environmental parameters, agricultural practices, and years. In addition, the differences in results dependent on antioxidant property assay also emphasize the importance of using multiple assays and the future challenges of determining which antioxidant property assays are most physiologically important and should therefore be applied to breeding programs aimed at improving wheat antioxidant properties.

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