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Antioxidant Properties of *Fusarium* Head Blight-Resistant and -Susceptible Soft Red Winter Wheat Grains Grown in Virginia

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Fusarium head blight (FHB) has emerged as a major threat to wheat crops around the world, and it has been hypothesized that wheat antioxidants may play a role against *Fusarium* infections. The current study aimed to determine antioxidant properties of FHB-resistant wheat grains as compared to susceptible wheat. The wheat samples were collected from a single growing location (Warsaw, VA) and the same growing season. The results showed that both FHB-resistant and -susceptible wheat grains exerted strong radical scavenging activities against DPPH• radical [0.91−1.53 μmol of Trolox equivalents (TE)/g], peroxyl radical (15.5−24.5 μmol of TE/g), and hydroxyl radical (15.7−35.8 μmol of TE/g). Their total phenolic contents ranged from 888 to 1117 μg of gallic acid equivalents (GAE)/g. Five phenolic acids including ferulic, syringic, vanillic, caffeic, and *p*-coumaric acids were determined in soluble and insoluble fractions of wheat grains, altogether with a range of 219−389 μg/g. On average, the FHB-resistant wheat group showed significantly higher average values in DPPH• and hydroxyl radicals scavenging activities (30 and 41% higher, respectively) than the FHB-susceptible wheat group.

KEYWORDS: Wheat; *Fusarium* head blight (FHB); antioxidant; phenolic acids

INTRODUCTION

Growing evidence from epidemiological and clinical studies suggests that a diet high in whole grains may have a protective role in reducing the risk of coronary heart disease (*1*−*3*), type 2 diabetes (*4*−*7*), and certain types of cancer (*8*−*12*). A number of phytochemicals in wheat grain, particularly antioxidants and fiber, have been investigated and have demonstrated potential health-promoting properties (*13*−*20*). Wheat grains contain significant levels of diverse natural antioxidants, including flavonoids, phenolic acids, phytic acids, tocopherols, and carotenoids (*15*−*25*). Numerous in vitro experiments showed that wheat antioxidants reduced the availability of transition metals (chelating activity) (*26*), inhibited lipid peroxidation in bulk oil systems and liposomes (*22*, *27*, *28*), protected human low-density lipoprotein (LDL) from oxidation (*29*), and directly reacted with and quenched a wide variety of free radicals such as superoxide, DPPH•, peroxyl, and hydroxyl radicals (*16*, *17*, *19*, *21*, *23*, *24*, *26*, *30*). In vivo research suggests that wheat antioxidants such as phenolic compounds are bioavailable after consumption and exert protective physiological effects such as improving the redox state of immune cells (*31*) and plasma antioxidant status (*32*). Therefore, as one of the most important food grain sources for humans, wheat and wheat-based products may provide substantial dietary levels of natural antioxidants to consumers.

In recent decades, *Fusarium* head blight (FHB) has emerged as a major threat to wheat crops around the world (*33*−*36*). FHB is caused by the fungus *Fusarium graminearum* and may consequently result in serious grain yield and quality losses (*37*−*40*). The fungus also produces mycotoxins that contaminate harvested grain and are a major health concern for both humans and animals (*40*, *41*). For these reasons, research for the cultivation and development of FHB-resistant wheat varieties has been growing rapidly with hopes of improving food safety and profits for wheat producers. FHB resistance of certain wheat varieties has been genetically characterized and mapped to specific chromosome regions (*38*, *40*). Although understanding of the mechanisms for resistance is limited, it has been hypothesized that wheat antioxidants may play a role in preventing *Fusarium* infections (*42*, *43*). A previous study on the interaction between *F. graminearum* and hexaploid wheat reported that antioxidant enzymes in wheat spikes such as superoxide dismutase (SOD), ascorbate peroxidases, and dehydroascorbate reductase (DHAR) were induced after inoculation with *F. graminearum* (*43*). Fungal infection is a common environmental stress and causes the augmentation of oxidative status in plant cells (*44*). The exposure of wheat to oxidative stress or environmental pollutants leads to the activation of

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Table 1. Resistance of Wheat Varieties to *Fusarium* Head Blight (FHB)

wheat variety	FHB incidence[a] (%)	FHB severity[a] (%)	FHB index[a]	FHB resistance[a]
VA04W-389	46	9	4.1	R
VA04W-433	49	11	5.6	R
Ernie	55	11	6.1	R
VA04W-563	61	14	8.7	R
Pioneer-26R15	58	17	9.7	R
Tribute	54	18	9.9	R
Renwood-3260	53	19	9.9	R
VA04W-522	78	19	14.9	S
Pion-2684	68	25	17.1	S
USG-3209	73	24	17.4	S
Sisson	83	25	21.1	S

[a] FHB incidence = percentage of infected spikes; FHB severity = percentage of infected spikelets; FHB index = FHB incidence × severity/100; R and S stand for resistant and susceptible, respectively.

antioxidant enzymes, such as peroxidase expression in wheat seedlings (*45*, *46*). Thus, it would be of interest to determine how the antioxidant properties of FHB-resistant wheat grains differ from those of FHB-susceptible wheat.

Wheat antioxidants are known to be concentrated in bran fractions and exist as ester- or ether-bound conjugates (*16*, *19*, *21*, *47*). Adom and Liu reported that 75% of phenolic compounds in wheat grain were found insoluble-bound (*19*). Also noted was that many factors, particularly genotypes and growing conditions, may have a significant effect on the antioxidant properties of wheat (*15*, *16*, *18*, *23*, *48*−*50*). A recent study indicated that environmental effects were considerably larger than genotype effects on both total phenolic contents and antioxidant activities of wheat grown in western Canada (*15*).

To date, there have been no reports on the antioxidant properties of FHB-resistant wheat grains. The current study, therefore, is aimed to determine the antioxidant properties of FHB-resistant wheat grains as compared to FHB-susceptible wheat. To minimize the environmental interference, all of the FHB-resistant and -susceptible wheat varieties were collected from a single growing location (Warsaw, VA) during the same growing season. The selected wheat grain samples were analyzed for their radical scavenging capacities against DPPH•, peroxyl, and hydroxyl radicals as well as their total phenolic content, both soluble and insoluble phenolic acid profiles. Characterizing antioxidant properties of FHB-resistant/susceptible wheat varieties may provide new opportunities for breeding and promoting the cultivation of value-added varieties rich in health-promoting components that benefit both consumers and local agricultural economies. This investigation may also help to identify prospective molecular markers potentially linked to high antioxidant concentration and/or FHB resistance of wheat varieties.

MATERIALS AND METHODS

Materials. A total of 11 whole wheat grain samples of soft red winter varieties without *Fusarium* infection were used in the current study. These varieties were evaluated by FHB incidence, FHB severity, and FHB index in two field experiments in a previous study, and disease data are presented in **Table 1**. Resistant varieties have FHB incidence below 60% and FHB severity below 20%, or an FHB index below 12. Folin−Ciocalteu reagent, fluorescein (FL), 2,2′-bipyridyl, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and phenolic acid standards were purchased from Sigma-Aldrich (St. Louis, MO), and 2,2′-azobis(2-amino-propane) dihydrochloride (AAPH) was purchased from Wako Chemicals U.S.A. (Richmond, VA). All other chemicals and solvents were of analytical or HPLC grade.

Sample Preparation. Five grams of each wheat grain sample was ground to a fine powder using a micromill and extracted for 15 h with 50 mL of 50% aqueous acetone by shaking at ambient temperature. The extracts were kept in the dark until analyses. Extracts were then examined for oxygen radical absorbance capacity (ORAC) and subjected to ORAC, DPPH• radical, and hydroxyl radical scavenging activities, and total phenolic contents (TPC) assays. For soluble and insoluble phenolic acids determination, the wheat grains samples were extracted by acetone/methanol/water (7:7:6, v/v/v) and prepared according to a previous paper (*16*).

ORAC. The ORAC assay was conducted to measure the peroxyl radical scavenging activity of wheat grain samples with Trolox as antioxidant standard according to the method reported previously (*16*). Fluorescein was used as the fluorescent probe, and peroxyl radicals were generated from AAPH in 75 mM phosphate buffer (pH 7.4). The fluorescence of the reaction mixture was monitored by a Victor^3 multilabel plate reader (Perkin-Elmer, Turku, Finland). Standards and samples were run in triplicate.

DPPH• Scavenging Activity. This high-throughput assay was carried out using a Victor^3 multilabel plate reader (Perkin-Elmer). Briefly, the reaction mixture contained 100 μL of antioxidant extracts and 100 μL of 0.208 mM DPPH• solution. The absorption at 515 nm was determined immediately when the reaction was initiated by gentle shaking. Each plate was read once every minute for 1.5 h. The relative DPPH• scavenging capacities (RDSC) were expressed as millimoles of Trolox equivalents (TE) per gram of sample.

Hydroxyl Radical Scavenging Activity (HOSC). The hydroxyl radicals were generated with a Fenton-like reaction. The reaction mixture contained 170 μL of 9.28×10^{-8} M FL in 75 mM sodium phosphate buffer (pH 7.4), 30 μL of antioxidant samples, 40 μL of 0.1990 M H_2O_2, and 60 μL of 3.43 mM $FeCl_3$; assay reactions were recorded every minute for 3 h with an excitation wavelength of 485 nm and emission wavelength of 535 nm. Trolox was used as antioxidant standard. HOSC values were calculated using the regression equation between Trolox concentration and net area under the FL decay curve. Relative HOSC values were expressed as micromoles of Trolox equivalents (TE) per gram of material and micromoles of TE per micromole of compound for pure compounds.

Total Phenolic Contents. The TPC of wheat grain extracts were determined using Folin−Ciocalteu reagent with gallic acid as standard (*51*). In brief, the appropriate dilutions of extracts were mixed with Folin−Ciocalteu reagent and 20% sodium carbonate at ambient temperature; after 2 h of reaction, the blue color was developed in assay mixture, and the absorbance was recorded at 760 nm. Total phenolic content was expressed as micrograms of gallic acid equivalent per gram of grain.

Phenolic Acid Compositions. The soluble and insoluble phenolic acid compositions of wheat samples were determined according to the method of Moore et al. with some modifications (*16*). The soluble phenolic acids in wheat grains were extracted with acetone/methanol/water (7:7:6, v/v/v); after organic solvents were evaporated under nitrogen, the extractions were further hydrolyzed with 2 N NaOH for 4 h at 45 °C under nitrogen. The resulting mixture was acidified with 6 N HCI and was subsequently extracted with ethyl acetate and ethyl ether (1:1, v/v). The organic phases were collected and dried by a nitrogen evaporator (Labconco, Kansas City, MO). The residue was redissolved in methanol and then filtered through a 0.45 μm filter. The filtrate was stored at −20 °C for HPLC analysis. To prepare insoluble bound phenolic acids in wheat grains, the residues after extraction were hydrolyzed with 2 N NaOH under nitrogen for 12 h. After hydrolysis, the resulting mixture was centrifuged and the supernatant was acidified. The subsequent purifications were the same as for the preparation for soluble phenolic acids.

HPLC analysis was performed on an Agilent 1200 quaternary LC system (Agilent Technologies, Palo Alto, CA) equipped with a photodiode array detector. Phenolic acids were separated on a Phenomenex Luna 5 μm C18 column (250 mm × 4.6 mm) using a linear gradient elution program with a mobile phase containing solvent A

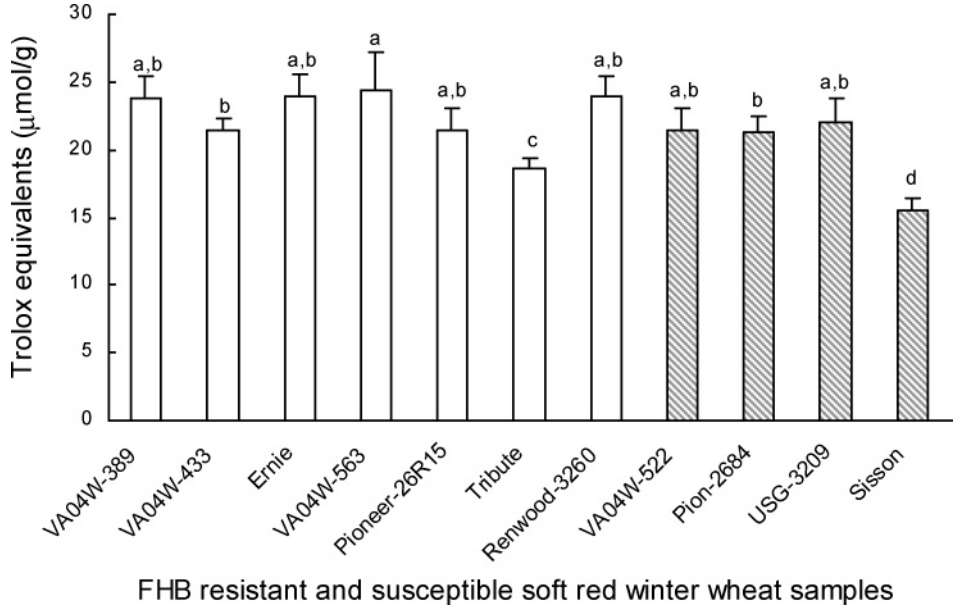

Figure 1. Oxygen radical absorbing capacity of wheat samples. Blank bars represent FHB-resistant wheat, and solid bars stand for FHB-susceptible wheat. Results are expressed as micromoles of Trolox equivalents per gram of wheat grains (mean ± SD, $n = 3$). Bars marked by the same letter are not significantly different ($P < 0.05$).

(acetic acid/H_2O, 2:98, v/v) and solvent B (acetic acid/acetonitrile/H_2O, 2:30:68, v/v/v) (*52*). The solvent gradient was linear programmed from 10 to 100% B in 42 min with a flow rate of 1.0 mL/min. Identification of phenolic acids was accomplished by comparing the retention time and absorption spectra of peaks in wheat samples to that of the standard compounds. Quantification of individual phenolic acids was conducted using total area under each peak with external standards.

Statistical Analysis. Data were reported as mean ± SD for triplicate determinations. To evaluate the differences among the means within FHB-resistant and -susceptible wheat groups, a randomized complete design was utilized using the GLM procedure of SAS (general linear model, SAS, 2003). The least significant difference test (LSD) was performed to separate treatment means ($P < 0.05$). To evaluate the difference among the means between FHB-resistant and -susceptible wheat groups, a nested factorial experiment design was utilized with the wheat varieties as the nested factor followed by LSD test. A two-tailed Pearson's correlation test was conducted to determine the correlations among means.

RESULTS

ORAC. ORAC measures the scavenging capacity of wheat sample extractions against peroxyl radicals using Trolox, a water-soluble vitamin E analogue, as an antioxidant standard, and values are expressed as micromoles of TE per gram of grain. All selected FHB-resistant and -susceptible wheat grains showed strong activity against peroxyl radicals (**Figure 1**). Different wheat lines showed significantly different ORAC values, indicating the potential effect of wheat genotypes on their antioxidant activities. FHB-resistant wheat samples had ORAC values that ranged from 18.6 to 24.5 μmol of TE/g with Tribute showing a significantly lower value than other resistant lines ($P < 0.01$). In the FHB-susceptible wheat group, VA04W-522, Pion-2684, and USG-3209 had similar ORAC values of between 21.3 and 22.0 μmol of TE/g, whereas Sisson had the lowest ORAC value (15.5 μmol of TE/g) among all tested lines ($P < 0.01$). Four wheat lines had ORAC values of >23 μmol of TE/g, including VA04W-563, Renwood-3260, Ernie, and VA04W-389, all of which belonged to FHB-resistant lines. Overall, the FHB-resistant wheat group has an average ORAC value of 22.5 μmol of TE/g, which is greater the 20.0 μmol of TE/g average of the FHB-susceptible wheat group, but the difference was not statistically significant.

DPPH• Radical Scavenging Capacity. The DPPH• radical scavenging activities of FHB-resistant and -susceptible wheat grains were also expressed as micromoles of TE per gram of grain (**Figure 2**). All tested wheat samples showed significant DPPH• radical scavenging activity with a range of 0.91−1.53 μmol of TE/g. FHB-resistant line VA04W-389 showed the greatest DPPH• scavenging activity, whereas Pion-2684, a FHB-susceptible variety, had the lowest activity. FHB-resistant lines had DPPH• radical scavenging values ranging from 1.10 to 1.53 μmol of TE/g, and a significant difference was observed between VA04W-389 (1.53 μmol of TE/g) and Pioneer-26R15 (1.10 μmol of TE/g) ($P < 0.01$). However, all FHB-susceptible wheat lines showed almost the same DPPH• radical scavenging activities with a range of 0.97−1.03 μmol of TE/g, which were significantly lower than those of FHB-resistant lines (except Pioneer-26R15). On average, the FHB-resistant wheat group has a value of 1.27 μmol of TE/g, higher than that of the FHB-susceptible group (0.97 μmol of TE/g). The difference was significant as analyzed by a nested factorial experiment design ($P < 0.01$).

Hydroxyl Radical Scavenging Activity. Strong hydroxyl radical scavenging activities were observed in both FHB-resistant and -susceptible wheats, as shown in **Figure 3**. FHB-resistant line Ernie showed the highest hydroxyl radical scavenging activity of 35.8 μmol of TE/g, which was >2 times higher that that of VA04W-522 (15.7 μmol of TE/g), suggesting that antioxidant activities of wheat samples against hydroxyl radicals were significantly affected by their genotypes. Values of FHB-resistant wheat samples varied from 17.2 to 35.8 μmol of TE/g, whereas FHB-susceptible wheat samples had a range of 15.7−25.5 μmol of TE/g. Statistical analysis revealed that the variations of hydroxyl radical scavenging activities in both FHB-resistant and -susceptible groups were significant ($P < 0.001$). Tribute, USG-3209, and VA04W-522 showed significantly lower hydroxyl radical scavenging activities than other wheat lines. FHB-resistant wheat samples had an average value

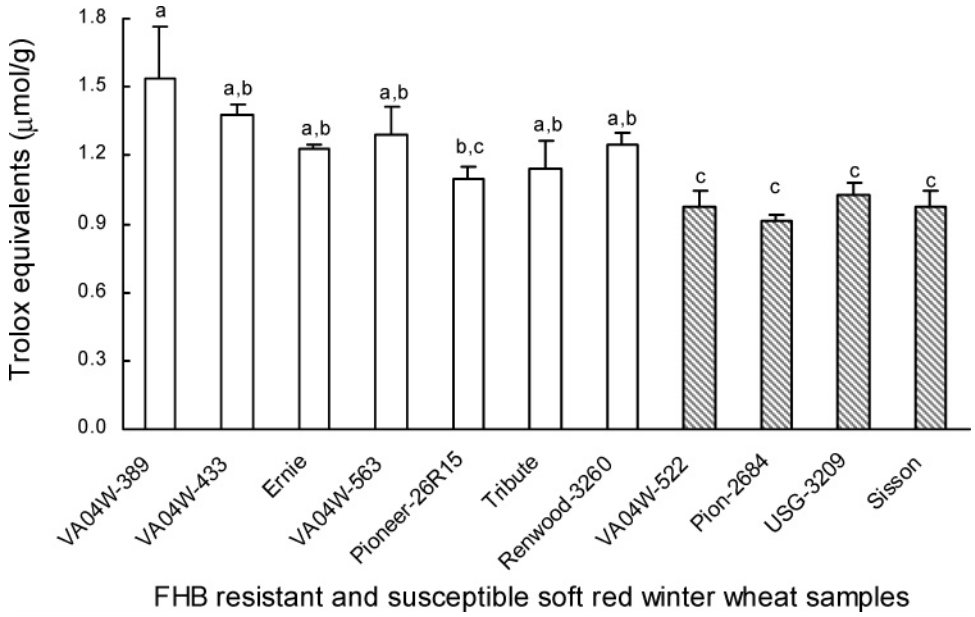

Figure 2. DPPH• radical scavenging activity of wheat samples. Blank bars represent FHB-resistant wheat, and solid bars stand for FHB-susceptible wheat. Results are expressed as micromoles of Trolox equivalents per gram of wheat grains (mean ± SD, $n = 3$). Bars marked by the same letter are not significantly different ($P < 0.05$).

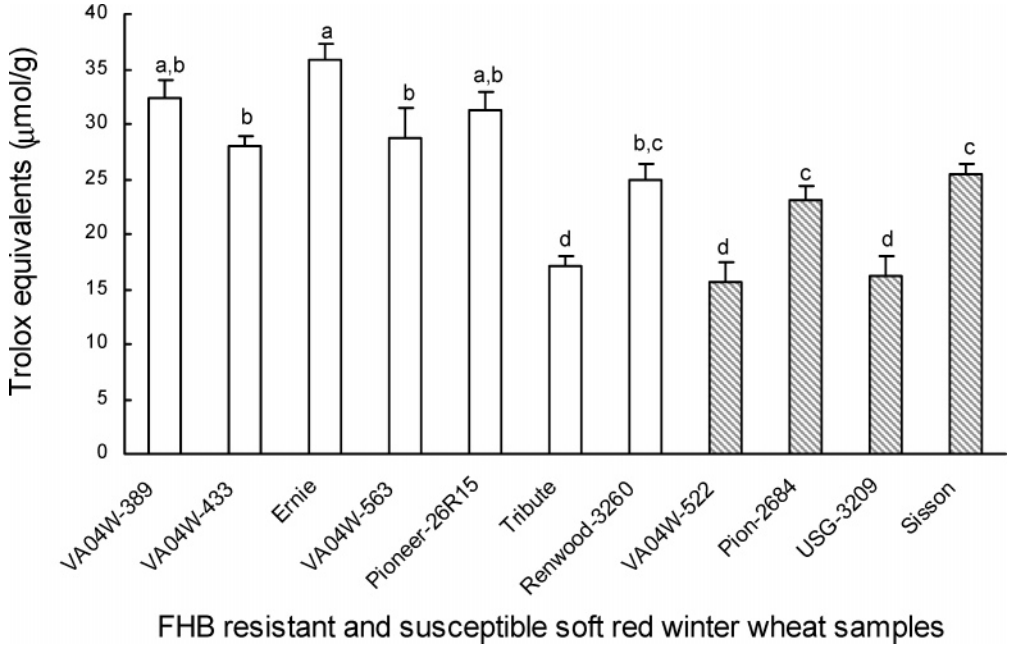

Figure 3. Hydroxyl radical scavenging activity of wheat samples. Blank bars represent FHB-resistant wheat, and solid bars stand for FHB-susceptible wheat. Results are expressed as micromoles of Trolox equivalents per gram of wheat grains (mean ± SD, $n = 3$). Bars marked by the same letter are not significantly different ($P < 0.05$).

of 28.3 μmol of TE/g, significantly higher than that of the FHB-susceptible group (20.1 μmol of TE/g) ($P < 0.01$).

Total Phenolic Contents. The total phenolic contents of FHB-resistant and -susceptible soft red wheat grains were expressed as micrograms of gallic acid equivalents (GAE) per gram of grain (**Figure 4**). TPC values of FHB-resistant wheat samples differed significantly with a range of 907.5−1116.9 μg of GAE/g, suggesting the genetic variances of FHB-resistant wheat grains could potentially affect their phenolic contents. Renwood-3260 and VA04W-433 contained the highest TPC (1116.9 and 1114.9 μg of GAE/g, respectively), followed by VA04W-389 and VA04W-563 (1085.5 and 1030.1 μg of GAE/g, respectively), whereas Ernie, Tribute, and Pioneer-26R15 had the lowest TPC values (907.5−953.8 μg of GAE/g). TPC values of FHB-susceptible wheat samples varied from 888.8 to 1046.0 μg of GAE/g. Sisson contained significantly lower TPC than the other three FHB-susceptible lines. There was no significant difference between the average TPC of FHB-resistant wheat (1024.0 μg of GAE/g) and that of FHB-susceptible wheat (992.2 μg of GAE/g).

Phenolic Acid Compositions. Results for soluble phenolic acid contents in FHB-resistant and -susceptible wheat grains are presented in **Table 2**. Five phenolic acids were detected in the selected wheat samples including 4-hydroxybenzoic, vanillic, syringic, *p*-coumaric, and ferulic acids. Ferulic acid accounted for 64−79 and 58−75% of soluble phenolic acids in FHB-resistant and -susceptible wheat samples, respectively. In the FHB-resistant wheat group, VA04W-433 and VA04W-389

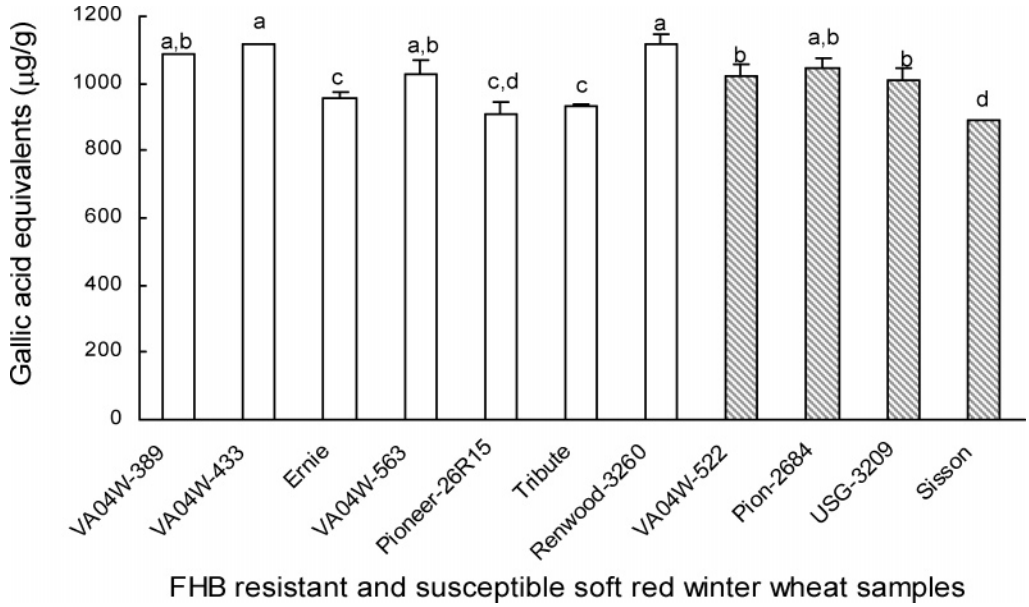

Figure 4. Total phenolic contents of wheat samples. Blank bars represent FHB-resistant wheat, and solid bars stand for FHB-susceptible wheat. Results are expressed as micromoles of trolox equivalents per gram of wheat grains (mean ± SD, $n = 3$). Bars marked by the same letter are not significantly different ($P < 0.05$).

Table 2. Soluble Phenolic Acid Compositions of FHB-Resistant and -Susceptible Wheat Grains

wheat variety	FHB index	4-hydroxybenzoic acid (μg/g)	vanillic acid (μg/g)	syringic acid (μg/g)	*p*-coumaric acid (μg/g)	ferulic acid (μg/g)	total (μg/g)
VA04W-389	4.1	1.54 ± 0.03	4.94 ± 0.06	9.01 ± 0.11	1.26 ± 0.03	40.03 ab ± 0.00	56.79
VA04W-433	5.6	1.57 ± 0.14	3.28 ± 0.30	11.17 ± 1.80	1.95 ± 0.22	40.45 ab ± 0.22	58.43
Ernie	6.1	1.66 ± 0.14	4.99 ± 0.32	9.10 ± 0.73	1.57 ± 0.13	40.62 a ± 1.51	57.94
VA04W-563	8.7	1.42 ± 0.15	3.91 ± 0.20	6.14 ± 0.26	0.99 ± 0.12	37.50 ab ± 0.33	49.97
Pioneer-26R15	9.7	1.24 ± 0.07	4.06 ± 0.25	8.90 ± 0.75	1.10 ± 0.02	36.92 ab ± 2.29	52.23
Tribute	9.9	ND	3.33 ± 0.24	3.91 ± 0.33	0.94 ± 0.08	30.02 de ± 0.42	38.20
Renwood-3260	9.9	ND	4.20 ± 0.28	8.20 ± 0.43	1.06 ± 0.09	37.73 ab ± 0.95	51.19
VA04W-522	14.9	2.72 ± 0.02	4.13 ± 0.00	10.98 ± 0.18	1.10 ± 0.02	26.15 e ± 0.08	45.07
Pion-2684	17.1	ND	2.75 ± 0.07	7.60 ± 0.14	0.49 ± 0.09	19.84 f ± 0.33	30.68
USG-3209	17.4	ND	3.30 ± 0.38	6.22 ± 0.37	1.01 ± 0.15	31.81 cd ± 2.76	42.34
Sisson	21.1	ND	3.73 ± 0.29	7.78 ± 0.53	1.32 ± 0.21	35.66 bc ± 0.40	48.49

Table 3. Insoluble Phenolic Acid Compositions of FHB-Resistant and -Susceptible Wheat Grains

wheat variety	FHB index	vanillic acid (μg/g)	syringic acid (μg/g)	*p*-coumaric acid (μg/g)	ferulic acid (μg/g)	total (μg/g)
VA04W-389	4.1	1.08 ± 0.13	1.08 ± 0.07	2.48 ± 0.23	215.79 e ± 2.59	220.43
VA04W-433	5.6	ND	1.59 ± 0.10	3.61 ± 0.30	246.22 cd ± 2.41	251.41
Ernie	6.1	ND	1.25 ± 0.12	3.10 ± 0.25	272.08 b ± 1.24	276.43
VA04W-563	8.7	ND	0.79 ± 0.05	3.32 ± 0.33	274.05 b ± 2.96	278.16
Pioneer-26R15	9.7	1.21 ± 0.20	1.13 ± 0.00	4.77 ± 0.11	329.73 a ± 3.26	336.84
Tribute	9.9	ND	0.80 ± 0.04	5.68 ± 1.99	241.05 cd ± 0.78	247.52
Renwood-3260	9.9	1.19 ± 0.32	1.21 ± 0.03	2.35 ± 0.05	249.18 c ± 0.40	253.92
VA04W-522	14.9	1.11 ± 0.20	1.32 ± 0.04	2.70 ± 0.01	206.88 e ± 1.34	212.01
Pion-2684	17.1	ND	1.01 ± 0.09	2.18 ± 0.19	185.83 f ± 1.80	189.02
USG-3209	17.4	ND	0.81 ± 0.09	3.82 ± 0.31	273.93 b ± 6.96	278.57
Sisson	21.1	ND	0.99 ± 0.11	2.74 ± 0.27	234.66 d ± 2.52	238.40

showed the highest soluble phenolic acid contents (58.4 and 56.8 μg/g, respectively), whereas Tribute was the lowest (38.2 μg/g). Soluble phenolic acid contents in FHB-susceptible wheat grains had a range of 30.7−48.5 μg/g, with the highest in Sisson and VA04W-522 and the lowest in Pion-2684. Both FHB-resistant and -susceptible wheat groups had a similar range of soluble phenolic contents, and no significant difference was observed between the two groups. **Table 3** shows the results for insoluble phenolic acids in wheat samples. The values in FHB-resistant wheat samples varied from 220.4 to 336.8 μg/g, with ferulic acid accounting for >98% of total insoluble phenolic acids. Pioneer-26R15 had a significantly higher insoluble ferulic acid content than other FHB-resistant wheat lines, whereas VA04W-389 was significantly lower than all others. The observation suggests the potential influences of FHB wheat genotypes on their phenolic acid contents. Insoluble phenolic acids in FHB-susceptible wheat samples ranged from 189.0 to 278.6 μg/g. This range was slightly lower than that of FHB-resistant wheat samples, but no significant difference was observed between two groups.

DISCUSSION

Increased public awareness of the relationship between diet and health has created a growing demand for natural dietary antioxidants. Meanwhile, a number of epidemiological and clinical studies have suggested that regular consumption of whole wheat grain may reduce the incidence of coronary heart disease and certain types of cancer (*1*−*4*, *8*−*12*). This has been partially attributed to the presence of antioxidant compounds in the grain (*30*, *53*). A wide variety of wheat and wheat-based products have been shown to contain significant levels of natural antioxidants, including hard winter wheat (*18*, *54*), hard spring wheat (*15*, *18*), soft winter wheat (*16*−*18*, *47*), durum wheat (*55*, *56*), wheat bran (*21*, *23*, *48*, *51*, *57*), and wheat bread (*58*). This suggests that wheat and wheat-based products may serve as important dietary sources of natural antioxidants. Current research attention has focused on conventional wheat varieties with no information available about antioxidant properties of FHB-resistant wheat despite their growing popularity and cultivation. FHB of wheat causes severe yield and quality losses; the cultivation of resistant varieties would make a substantial contribution to reducing losses from this destructive agricultural disease. Investigation of FHB-resistant wheat varieties for their antioxidant properties may lead to value-added production and consumption of selected varieties with enhanced health and nutritional benefits.

In this study, all FHB-resistant and -susceptible soft red winter wheat grains exerted strong radical scavenging activities against DPPH•, peroxyl, and hydroxyl radicals. The genetic influences were evident as significantly different antioxidant values were observed in both the FHB-resistant and -susceptible groups. The FHB-resistant wheat appeared to have more genetic leverage on the DPPH• radical scavenging activity than the FHB-susceptible wheat did. The individual wheat varieties showed significant variations in their peroxyl radical scavenging activities as measured by the ORAC assay. However, the average ORAC values of FHB-resistant and -susceptible wheat groups showed no significant difference. The ORAC values of the tested wheat samples ranged from 15.5 to 24.5 μmol/g, lower than the 51.5 μmol/g for Swiss red wheat (*52*) and the 32.9−47.7 μmol/g of Maryland-grown soft red winter wheat (*16*). This could partially be explained by genetic variability and experimental conditions used. Correlation tests were performed among the antioxidant activities of the selected samples after being divided into FHB-resistant and -susceptible groups. Only the hydroxyl and DPPH• radical scavenging activities of FHB-resistant wheat samples were significantly correlated ($R^2 = 0.74$, $P < 0.001$). Thus, the measurements of hydroxyl and DPPH• radical scavenging activities may be very useful for breeding programs to screen and select FHB-resistant varieties with higher potential antioxidant properties.

In the FHB-resistant wheat group, no single wheat line showed the highest or lowest antioxidant activities against all three free radicals. VA04W-563 and Ernie showed the highest scavenging activities against peroxyl radicals. Ernie also showed the strongest hydroxyl radical scavenging activity, but VA04W-389 was the best against DPPH• radicals. Tribute displayed the lowest activities against both peroxyl and hydroxyl free radicals, whereas Pioneer-26R15 showed significantly lower DPPH• radical scavenging activity than the other. This observation suggests that wheat genotypes may affect their specific antioxidant activities in different mechanisms, and multi-antioxidant assays should be warranted. In FHB-susceptible wheat samples, no significant difference was observed for their DPPH• radical scavenging activities. Sisson had the highest activities against peroxyl radicals, but it also had the lowest hydroxyl radical scavenging activity. On average, the FHB-resistant wheat group showed significantly higher scavenging activities against both DPPH and hydroxyl radicals (30 and 41% higher, respectively), but not against peroxyl radicals in comparison to the FHB-susceptible group. This indicates that the genetic distinctions between FHB-resistant and -susceptible wheat may alter their antioxidant properties. More research is needed to better understand the differences in antioxidant properties between FHB-resistant and -susceptible wheat as antioxidants may have different mechanisms when reacting with different radicals and cause inconsistent results.

It has been generally accepted that phenolic compounds may significantly contribute to the overall antioxidant properties of wheat grains (*21*, *24*, *47*). Also noted is that wheat genotypes may significantly affect their phenolic contents (*15*, *16*, *18*, *26*, *48*−*51*). In agreement with previous observations, significant differences were observed in both FHB-resistant and -susceptible wheat varieties for their phenolic contents. However, the phenolic contents of selected wheat samples were not correlated with any of the tested free radical scavenging assays. This suggests that some phytochemicals in wheat other than phenolic compounds may also contribute to their antioxidant properties. FHB-resistant wheat had a range of 907.5−1116.9 μg of GAE/g, whereas FHB-susceptible wheat varied from 888.8 to 1046.0 μg of GAE/g. Both ranges were higher than that observed in Maryland-grown soft red winter wheat, which was in the range of 0.4−0.8 μg of GAE/g (*16*), but lower than the 1.8 mg of GAE/g found in Swiss red wheat (*52*) and the 1709−1990 μg/g in hard spring wheat (*15*).

In agreement with the previous studies (*16*, *18*, *21*, *23*), ferulic acid was the predominant phenolic acid in both soluble and insoluble fractions of wheat grain extractions, accounting for >90% of the total phenolic acids in both FHB-resistant and -susceptible wheat samples. Other phenolic acids, such as syringic, vanillic, caffeic, and *p*-coumaric acids, also were detected in the selected wheat samples. FHB-resistant wheat samples had a range of 285−389 μg/g of total phenolic acids; these values were slightly higher for FHB-susceptible wheat, ranging from 220 to 320 μg/g. These values were comparable to the 290−650 μg/g in hard and soft wheat (*18*, *19*), but lower than the 490−650 μg/g in Maryland-grown soft red winter wheat (*16*) and the 577−1255 μg/g in hard spring wheat (*15*). In tested wheat grains, 80−87% of phenolic acids existed in insoluble forms. This range is in line with the findings of Kim et al. (*21*), Adom et al. (*18*), Moore et al. (*16*), and Zhou et al. (*52*), supporting that the majority of phenolic acids in wheat grain are highly cross-linked and bound in cell walls (*47*). Phenolic acids, especially ferulic acid, may be related to some wheat varieties' resistance to *Fusarium*, as some research showed the increase of ferulic acid synthesis from anthesis (*59*). Ferulic acid contents of wheat varieties were reported to be significantly associated with the resistance to midge infestation (*60*). The cross-linkages between phenolic acids and carbohydrates in the cell walls may also contribute to fungal resistance by strengthening the physical barrier (*61*). For these reasons, FHB-resistant wheat grains are expected to contain higher levels of phenolic acids. However, our results did not support this notion, and there was no significant difference between FHB-resistant and -susceptible wheat samples for both soluble and insoluble phenolic acids.

In summary, all selected FHB-resistant and -susceptible soft red winter wheat grains contained significant levels of phenolic compounds and exerted strong scavenging activities against

DPPH•, peroxyl, and hydroxyl radicals. The FHB-resistant wheat group on average showed significantly higher DPPH• and peroxyl radical scavenging activities than the FHB-susceptible wheat group, but no significant differences were observed for their total phenolic contents and phenolic acid profile between the two groups. More research is needed to adequately understand if the differences of antioxidant properties are due to the genetic variations between FHB-resistant and -susceptible wheats. The present data suggested that both FHB-resistant and -susceptible soft red winter wheats grown in Virginia contain significant levels of natural antioxidants, and the former may possess even higher free radical scavenging capacities.

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