agronomy

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Antioxidant Activity, Phenolic Content, and Antioxidant Gene Expression in Genetic Resources of Sorghum Collected from Australia, Former Soviet Union, USA, Sudan and Guadeloupe

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Abstract: Functionality based on the biological activity of sorghum such as antioxidant activity is known worldwide for its excellence. In this study, we investigated the reactive oxygen species (ROS) scavenging activity, total phenolic and flavonoid contents, phenol compounds, and changes in antioxidant gene expression in sorghum seed cells collected from five countries (Australia, former Soviet Union, USA, Sudan, and Guadeloupe). Sorghum seeds were obtained from 12 genetic resources (K159041, K159042, K159078, K159081, K159088, K159089, K159093, K159097, K159100, K159096, K159048, and K159077). ROS scavenging activity was analyzed using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,20-azinobis 3-ethylbenzothiazoline-6-sulfonate (ABTS). K159097 showed high antioxidant activity values of 33.52 ± 0.70 μg/mL (DPPH) and 271.06 ± 13.41 μg/mL (ABTS), respectively. The reducing power of the resources improved in a concentration-dependent manner, and 10 sorghum resources, except K159078 and K159048, showed high reducing power. K159042 had the highest total phenol content (231 ± 2.17 mg·GAE/g), and K159081 had the highest total flavonoid content (67.71 ± 5.38 mg·QE/g). Among the six phenolic compounds (protocatechuic acid, caffeic acid, p-coumaric acid, ferulic acid, taxifolin, and naringenin) analyzed, the compound with the highest content was taxifolin (203.67 ± 4.99 mg/L in K159093). K159041, K159042, and K159048 had the highest expression levels of superoxide dismutase (SOD), ascorbate peroxidase 1 (APX1), and catalase (CAT), which are indicators of antioxidant activity. An evaluation of the diversity of sorghum provided useful information on antioxidant activity, physicochemical content, and antioxidant gene expression in seed cells, suggesting that sorghum can be used as a biomaterial from natural resources.

Keywords: antioxidant activity; ascorbate peroxidase; catalase; phenolic compounds; sorghum seeds; superoxide dismutase

Citation: Seo, J.W.; Ham, D.Y.; Lee, J.G.; Kim, N.Y.; Kim, M.J.; Yu, C.Y.; Seong, E.S. Antioxidant Activity, Phenolic Content, and Antioxidant Gene Expression in Genetic Resources of Sorghum Collected from Australia, Former Soviet Union, USA, Sudan and Guadeloupe. *Agronomy* **2023**, *13*, 1698. https://doi.org/10.3390/agronomy13071698

Academic Editor: Salvatore Camposeo

Received: 8 June 2023
Revised: 20 June 2023
Accepted: 23 June 2023
Published: 25 June 2023

1. Introduction

Sorghum is a C4 crop native to Africa and was cultivated in Asia, Africa, and Central America. It is a food crop that grows well even when the annual rainfall is <400 mm, because it requires only half the amount of corn and less fertilizer [1]. Currently, global sorghum production is known to be over 61 million tonnes, and Israel, Jordan, France, and Italy showed the highest yields in small-scale cultivation. Sorghum is the most cultivated crop among grain sorghum, sorghum, and broomcorn, depending on its use, and is an important grain resource, followed by rice, barley, wheat, and corn worldwide [2]. Broomcorn has

no nodes on the ear, and the skin of the fruit is difficult to thresh; therefore, it is used only as a seed. Since ancient times, it was used by the private sector to promote digestion, maintain body temperature, and protect the stomach [2]. Recently, as studies on the biological functionality of sorghum grew, there were reports on its active ingredients, such as flavonoids and various phenolic components, including tannins [3]. Polyphenol extracts from sorghum exhibit antioxidant activity and inhibit the activity of enzymes related to cholesterol biosynthesis [4]. Biologically active substances related to polyphenols in sorghum suppress blood LDL (low density lipoprotein)-cholesterol and increase HDL (high density lipoprotein)-cholesterol and have excellent hypocholesterolemic effects [5]. Compared to cereals such as wheat, oats, and millet, the cholesterol-lowering effect of sorghum was known for a long time [5].

Recently, phenolic compounds were recognized as substances that provide health benefits by reducing oxidative stress. Phenolic acids, flavonoids, and tannins belonging to these phenolic compounds are also present in sorghum grains [6]. In particular, large amounts of flavonoids are present in the seed coat of sorghum, and the enhancement in their content appears to be related to the color or thickness of the seed coat [7]. Sorghum is a crop that adapts well to environments with insufficient moisture; therefore, it is easy to grow even in regions with irregular precipitation distributions and high temperatures [8]. Studies on the relationship between the biological activity of sorghum and the precipitation and climate of the growing region were conducted; however, it is difficult to determine their correlation because sorghum grows well even in a barren climate [9]. A study on the antioxidant activity and nutritional value of alfalfa leaves found that they were greatly influenced by climate according to seasonal changes [10]. The ascorbic acid content in potatoes varies considerably among cultivars exposed to different environments in Europe [11]. In addition, the mineral content of potatoes is influenced by various factors, including the cultivar and growing site [12]. It was reported that the sorghum genotype has a reduced photosystem II efficiency under high salinity stress, leading to a decrease in CO_2 and stomatal closure, which inhibits leaf expansion, and so, the salinity level should also be considered when selecting a cultivation area [1,13]. In sorghum, active components, such as phenol content, correlate with the environment and the local climate in which they are grown.

ROS cause oxidative damage upon exposure to various biotic and abiotic stress conditions, and when they accumulate in excessive amounts, they impair biomolecules, induce cell damage, and activate cell death mechanisms [14,15]. When plants are stressed, redox homeostasis is maintained by activating related enzymes to combat oxidative stress [16,17]. These enzymes protect against stress and detoxify ROS. The enzymes involved in this enzymatic antioxidant system include APX, dehydroascorbate reductase (DHAR), glutathione reductase (GR), monodehydroascorbic acid reductase (MDHAR), SOD, and CAT [18]. Compounds involved in the opposite non-enzymatic antioxidant system include ascorbate, glutathione (GSH), proline, and α-tocopherol, which regulate gene expression during biotic and abiotic stress responses [19,20]. Such an antioxidant system makes plants resistant to stress and regulates the expression of signal transduction genes in plant growth promotion pathways [21]. Several reports revealed the relationship between different antioxidant enzyme activities; however, few studies revealed the relationship among the transcription levels of antioxidant enzyme genes.

In this study, we aimed to investigate the changes in antioxidant activity and total phenol, total flavonoid, and phenolic contents in sorghum according to the climate of each cultivation area by using sorghum genetic resources collected from Australia, the former Soviet Union, USA, Sudan, and Guadeloupe, and to verify the relevance between the expression level of *APX*, *SOD*, and *CAT* genes and antioxidant activity.

2. Materials and Methods

2.1. Plant Material and Extract Manufacture

The genetic resource numbers were K159041, K159042, K159078, K159081, K159088, K159089, K159093, K159097, K159100, K159096, K159048, and K159077. Sorghum seeds were obtained from 12 genetic resources collected from five regions of the National Agrobiodiversity Center, National Institute of Agricultural Sciences, Rural Development Administration, South Korea. The collection regions for each sorghum genetic resource were Australia, the former Soviet Union, USA, Sudan, and Guadeloupe. Sorghum seeds (2 g) were finely crushed using a grinder (HG-7113; Haeger, Barcelona, Spain) and extracted by soaking in 100% methanol at room temperature for 48 h at 10 times the weight of the seeds. The extract was filtered through a filter paper (Watman No. 42) and concentrated under reduced pressure using a rotary vacuum concentrator (EYELA N-1110, Tokyo Rikakikai Co. Ltd., Tokyo, Japan) via heating in a water bath at 45 °C.

2.2. Measurement of ROS Scavenging Activity

To measure ROS scavenging ability, the DPPH and ABTS methods were used. For DPPH free radical scavenging activity, 1 mL of 0.15 M DPPH solution was mixed with 4 mL sorghum extract diluted with methanol. After quantifying the final concentrations of samples to 10, 50, 100, and 500 μg/mL, DPPH was added and allowed to react at room temperature for 30 min. Thereafter, a UV spectrophotometer (Multiskan FC Microplate Photometer, Thermo Fisher Scientific Inc., Waltham, MA, USA) was used to measure the absorbance of samples at 517 nm [22]. For ABTS free radical scavenging activity, the ABTS solution was obtained by mixing 2.6 mM potassium persulfate and 7.4 mM ABTS in a 1:1 ratio and, subsequently, leaving them to react in the dark for 15 h. After mixing 100 μL of the diluted sample with 90 μL of ABTS solution at concentrations of 100, 500, and 1000 μg/mL, the solutions were allowed to react at room temperature for 10 min. Thereafter, the absorbance was measured at 734 nm using a UV spectrophotometer [23]. The values calculated using the DPPH and ABTS methods were expressed as RC_{50} (the concentration of the compound that reduced the value of the control group, to which no compound was added, by 50%).

2.3. Measurement of Reducing Power

The reducing power was determined by mixing 100, 500, and 1000 μL of 100% methanol extract (10 μg/mL) with 500 μL of 0.2 M sodium phosphate buffer (pH 6.6) and 500 μL of 1% potassium ferricyanide at 50 °C for 20 min. Then, 2.5 mL of 10% trichloroacetic acid was added. After centrifuging this reaction solution at 1000 rpm for 10 min, 500 μL of the supernatant was separated, and the reaction solution was mixed with 500 μL distilled water and 100 μL 1% ferric chloride. The absorbance was measured at 700 nm using a UV spectrophotometer (UV-1800, SHIMADZU Corp., Kyoto, Japan) [24].

2.4. Analysis of Total Phenolic and Flavonoid Contents

The total polyphenol content in sorghum genetic resources was determined according to the method by Appel et al. (2001) with minor modifications [25]. After adding 400 μL of distilled water to 20 μL of 2 mg/mL sorghum sample, 40 μL of 2N Folin–Ciocalteu phenol reagent (Sigma-Aldrich, St. Louis, MO, USA) was added and stirred. Thereafter, 400 μL of 30% Na_2CO_3 was added to this solution and reacted for 1 h. The absorbance was measured at 765 nm using a UV spectrophotometer (Multiskan FC Microplate Photometer, Thermo Fisher Scientific Inc., Waltham, MA, USA). The total flavonoid content was checked by adding 100 μL of 10% aluminum nitrate and 1 M potassium acetate to 500 μL of the sample at a concentration of 1000 ppm, and the absorbance was measured at 415 nm using a UV spectrophotometer (Multiskan FC Microplate Photometer, Thermo Fisher Scientific Inc., Waltham, MA, USA) [26]. Gallic acid (Sigma-Aldrich) and Qucetin (Sigma-Aldrich) was used to quantify the total polyphenol and total flavonoid, and there were expressed as mg gallic acid equivalents (GAE) and mg qucetin equivalents per gram dry weight.

2.5. Phenol Component Analysis Using High-Performance Liquid Chromatography (HPLC)

Samples were prepared at a concentration of 10,000 μg/mL using 100% methanol, filtered through a 0.45 μm syringe filter (Hyundai Micro Co. Ltd., Seoul, Republic of Korea), and used for subsequent experiments. Protocatechuic acid, caffeic acid, ferulic acid, p-coumaric acid, taxifolin, and naringenin were used as standards. Taxifolin was diluted to 100, 250, and 500 μg/mL, whereas the others were diluted to 12.5, 25, and 50 μg/mL. Phenolic content was analyzed using a HPLC Agilent 1260 series (Agilent Technologies Inc., Santa Clara, CA, USA) instrument and an HC-C18 column (Agilent Technologies Inc.). The wavelength was set to 288 and 360 nm, column temperature to 25 °C, and the sample injection volume to 10 μL. HPLC water (solvent A) and acetonitrile (solvent B) were used as mobile phases, and 0.1% formic acid was added. The flow rate was 0.5 mL/min, and the linear gradient elution was 5–15% B (5 min), 15–50% B (40 min), 50–70% B (2 min), 70–100% B (1 min), 100% B (7 min), 100–5% B (1 min), and 5% B (9 min).

2.6. Expression Confirmation of Antioxidant Genes Using qPCR

Total RNA extraction for cDNA synthesis from 12 sorghum seeds was performed using TRIzol reagent (Thermo Fisher Scientific Inc.). The total RNA was quantified using a Microvolume Spectrophotometer (Keen Innovative Solutions, Daejeon, Republic of Korea). cDNA was synthesized using PrimeScript™ RT Master Mix (Perfect Real Time; Takara Korea Biomedical Inc., Seoul, Republic of Korea), and the total RNA content was adjusted. The CronoSTAR 96 Real-Time PCR System (Takara Korea Biomedical Inc.) was used for real-time PCR analysis. The conditions were as follows: initial denaturation at 95 °C for 30 s with a total volume of 25 μL, 40 cycles of 2-step amplification (denaturation 95 °C for 5 s, annealing at 60 °C for 30 s), and an experimental melting step at 95 °C for 1 min, 60 °C for 15 s, and 98 °C for 5 s. The expression levels of *SOD*, *APX1*, *CAT*, and housekeeping gene (pp2a), known as antioxidant genes in sorghum, were evaluated using the method by Bruno et al. (2020) [27].

2.7. Statistical Analysis

All data are expressed as the mean ± standard deviation of >3 replicates of the experiment. One way analysis of variance was used, and Duncan's multiple range test was used at $p < 0.05$. IBM SPSS Statistics 26 was used for statistical analysis, and the presence or absence of significance was indicated using different letters.

3. Results and Discussion

3.1. Analysis of the Climate, Morphological Characteristics, and Antioxidant Activity of 12 Sorghum Genetic Resource Seeds

The sorghum genetic resources provided by the National Agrobiodiversity Center, National Institute of Agricultural Sciences, and Rural Development Administration in South Korea are shown in Figure 1. These data were collected from each of the five regions as indicated in Figure 1. The five regions were Australia, the former Soviet Union, the USA (Nebraska, Texas, and Virginia), Sudan, and Guadeloupe. Among the five regions, the average annual temperature of the sorghum seed collection area was lowest in Virginia, USA (−18.3 °C), and highest in Sudan (35.79 °C). The highest annual average precipitation was recorded in Virginia (USA) at 1086 mm, while Texas (USA) reported the lowest at 27.25 mm (Table 1).

The morphological characteristics of the sorghum resources used for antioxidant activity analysis were observed (Figure 2). The shapes of seven sorghum seeds, K159041, K159042, K159081, K159088, K159089, K159100, and K159077, were ovoid, and the remaining resources were classified as globose (yellow brown): K159081 (reddish-brown), K159089 (black), K159093 (yellow brown), K159048 (ivory), and K159077 (light brown). The remaining seeds (K159041, K159042, K159088, K159097, K159100, and K159096) had mixed colors (Table 2).

Figure 1. Locations of *Sorghum bicolor* genetic resources collected from seven different regions. (**A**) Australia, New South Wales, (**B**) former Soviet Union, (**C**) United States, Nebraska, (**D**) United States, Texas, (**E**) United States, Virginia, (**F**) Sudan, Kordofan, (**G**) Guadeloupe, Basse-Terre.

Table 1. Average annual temperature and annual precipitation of *Sorghum bicolor* collection regions.

Location	Annual Average Temperature (°C)		Annual Average Precipitation (mm)
	Low	High	
Australia, New South Wales	16.00	26.00	863.60
Former Soviet Union	8.00	18.50	488.58
United States, Nebraska	2.30	16.60	882.00
United States, Texas	3.80	35.50	27.25
United States, Virginia	−18.30	25.00	1086.00
Sudan, Kordofan	23.05	35.79	104.44
Guadeloupe, Basse-Terre	20.55	31.11	75.73

Figure 2. Seed shape and colors of *Sorghum bicolor* genetic resources collected from seven different regions.

Table 2. Morphological characteristics of collected *Sorghum bicolor* seeds.

Accession No.	Color	Shape
K159041	Brown and black	ovoid
K159042	Light brown and black	ovoid
K159078	Yellow brown	globose
K159081	Reddish brown	ovoid
K159088	Yellow brown and black	ovoid
K159089	Black	ovoid
K159093	Yellow brown	globose
K159097	Yellow brown and light brown	globose
K159100	Reddish brown and yellow brown	ovoid
K159096	Dark brown and yellow brown	globose
K159048	Ivory	globose
K159077	Light brown	ovoid

To investigate the antioxidant activity of sorghum by measuring ROS scavenging activity, the DPPH and ABTS methods were used. K159097 showed the highest DPPH free radical scavenging activity (33.52 ± 0.70 μg/mL), and K159078 showed the lowest (582.83 ± 219.07 μg/mL). There were numerical differences among the nine resources, K159041, K159042, K159081, K159088, K159089, K159093, K159097, K159100, and K159096, for DPPH analysis; however, no significant differences were observed. These nine resources showed higher ROS-scavenging activity in DPPH analysis than the remaining three resources. In ABTS analysis, K159097, K159081, K159096, K159089, K159042, K159093, K159088, K159100, K159041, K159077, K159048, and K159078 showed ROS scavenging activity in the order of high. Compared with the DPPH analysis, the ABTS analysis showed statistically significant ($p < 0.05$) results among the 12 resources; the values of the resources with the highest and lowest ROS scavenging activities measured in most DPPH analyses correlated with each other, indicating that there was a relationship among the 12 sorghum resources (Table 3). This confirmed the reliability of the overall data.

Table 3. Antioxidant activities of *Sorghum bicolor* genetic resources collected using DPPH and ABTS analyses.

Accession No.	DPPH Activity (RC_{50})	ABTS Activity (RC_{50})
K159041	61.01 ± 2.26 ^{a}	536.45 ± 11.63 ^{cd}
K159042	35.64 ± 1.08 ^{a}	362.74 ± 1.91 ^{ab}
K159078	582.83 ± 219.07 ^{c}	1252.60 ± 33.84 ^{e}
K159081	71.63 ± 0.56 ^{a}	336.77 ± 24.39 ^{ab}
K159088	55.56 ± 1.92 ^{a}	430.01 ± 7.81 ^{bc}
K159089	38.28 ± 1.50 ^{a}	356.29 ± 7.05 ^{ab}
K159093	43.84 ± 1.53 ^{a}	380.56 ± 8.44 ^{ab}
K159097	33.52 ± 0.70 ^{a}	271.06 ± 13.41 ^{a}
K159100	59.30 ± 1.62 ^{a}	465.96 ± 42.51 ^{bcd}
K159096	44.36 ± 0.67 ^{a}	343.50 ± 12.07 ^{ab}
K159048	−289.44 ± 22.99 ^{b}	2874.26 ± 252.45 ^{f}
K159077	186.80 ± 14.33 ^{b}	569.89 ± 25.47 ^{d}

Values represent mean ± S.D. of data obtained from three independent experiments. Duncan's Multiple Range Test at 5% level (DMRT, $p < 0.05$). Significant statistical differences are indicated by different letters.

To measure the reducing power and ROS scavenging activity of sorghum, the absorbance of each sorghum resource was measured using the reducing power method. The reducing power measured according to the concentration of each sorghum seed extract confirmed that there was a correlation between the reducing power results according to the concentrations of 100, 500, and 1000 μg/mL. At the highest concentration of 1000 μg/mL, K159078 and K159048 showed absorbance values ≤0.8, indicating lower reducing power

than other sorghum resources (Figure 3). This demonstrated the same trend as the antioxidant activity values obtained using DPPH and ABTS between sorghum resources; it was confirmed that the reducing power also correlated with ROS scavenging activity.

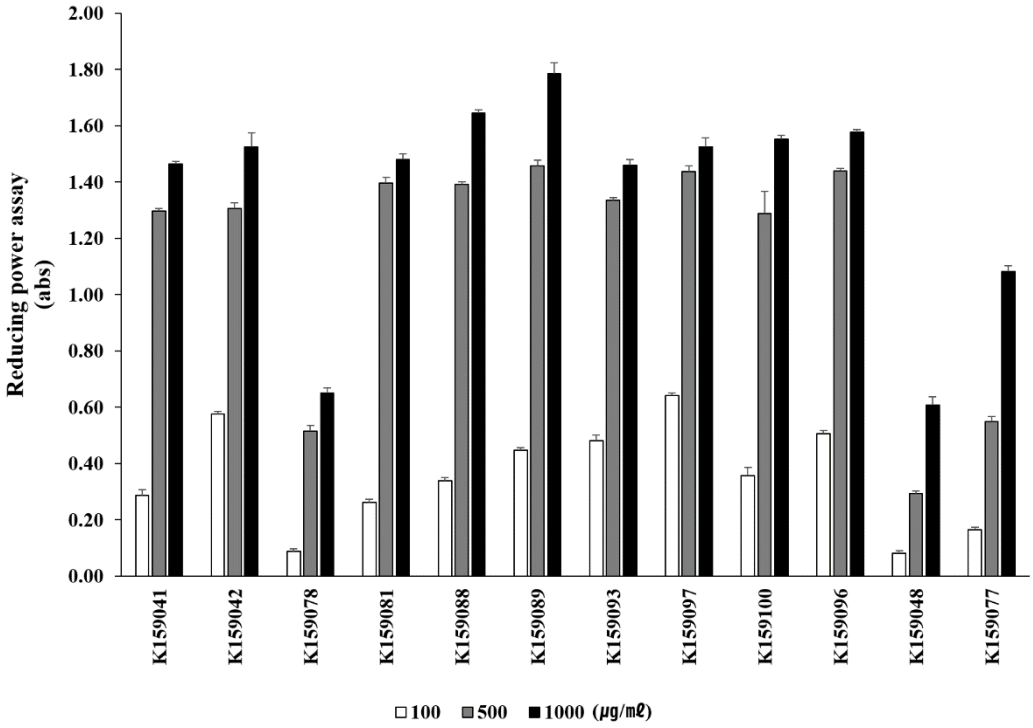

Figure 3. Reducing power analysis depending on concentrations from seed extract of *Sorghum bicolor* genetic resources collected from seven different regions. Values represent mean ± S.D. of data obtained from three independent experiments.

Measurement of the antioxidant activity of the 12 sorghum resources used in this study confirmed that there were no significant differences in seed shape, color, and antioxidant activity. Analysis of the antioxidant activity according to the collection area revealed that the USA sorghum resources had the highest antioxidant activity, while the Sudan (K159048) and former Soviet Union (K159078) resources had the lowest antioxidant activity. The differences in antioxidant activity according to the genetic characteristics of sorghum were analyzed. In addition, the range of antioxidant values (RC_{50}) between sorghum resources measured in this experiment was 33.52 ± 0.70 to 582.83 ± 219.07 μg/mL for DPPH analysis and 271.06 ± 13.41 to 2874.26 ± 252.45 μg/mL for ABTS analysis, showing a wide range of values. This was similar to the results of a study that investigated a broad spectrum of antioxidant activities in sorghum collected from 15 different regions [28]. An analysis of five different sorghum genotypes collected in India revealed that red sorghum had high antioxidant activity [29]. Shen et al. (2018) and Wu et al. (2016) also reported similar results. However, in the present study, the seed shape and color did not affect antioxidant activity. Grain color is one of the reasons that lead to differences in total phenol and flavonoid contents between these cultivars, but it can also be caused by other reasons such as genetic variation and growing environment [30,31].

3.2. Total Phenolic and Flavonoid Contents

The total polyphenol content of the 12 sorghum resources ranged from 16.33 ± 0.32 mg·GAE/g to 231.21 ± 2.17 mg·GAE/g. The difference between the highest and lowest total polyphenol content was >14-fold. K159048, which had the lowest ROS-scavenging ability, showed the lowest polyphenol content, and a correlation with antioxidant activity was established. The highest total phenol content was observed in

K159042 (231.21 ± 2.17 mg·GAE/g) and K159041 (125.71 ± 0.91 mg·GAE/g) collected from the same area, and a significant difference ($p < 0.05$) in total phenol content was noted. A difference in the total phenol content among sorghum resources collected from the same area (the former Soviet Union) was also observed for K159078 and K159081. The difference in their total phenol content was more than 4-fold (Table 4). It is possible that even genetic resources collected from the same region can cause such differences in the genetic characteristics and inherent properties of the resources; nonetheless, other studies showed similar results [32]. A study of sorghum populations by Ghimire (2021) showed similar results, with a significant difference in total phenol content between resources collected in the same country. This study confirmed the correlation between the antioxidant activity variation pattern and total phenol content. Among the 12 genetic resources, K159081 demonstrated the highest total flavonoid content of 67.71 ± 5.28 mg·QE/g. The lowest total flavonoid content was observed for K159078 (16.46 ± 5.38 mg·QE/g), which showed a >4-fold difference between the same resources collected from the former Soviet Union region. This was also observed for the total phenol content. The genetic characteristics of the plant, climate, and exposure environment lead to variations in total flavonoid content [33,34].

Table 4. Content comparison for total phenol and total flavonoid in *Sorghum bicolor* genetic resources collected.

Accession No.	Total Phenol Contents (mg·GAE/g)	Total Flavonoid Contents (mg·QE/g)
K159041	125.71 ± 0.91 ^{h}	17.94 ± 0.36 ^{c}
K159042	231.21 ± 2.17 ^{a}	17.17 ± 0.14 ^{c}
K159078	32.14 ± 0.30 ^{k}	16.46 ± 2.78 ^{c}
K159081	147.54 ± 1.07 ^{f}	67.71 ± 5.38 ^{a}
K159088	139.08 ± 1.55 ^{g}	21.48 ± 1.38 ^{c}
K159089	199.00 ± 0.99 ^{c}	47.04 ± 22.10 ^{b}
K159093	162.39 ± 1.65 ^{e}	17.02 ± 1.03 ^{c}
K159097	216.02 ± 5.52 ^{b}	23.72 ± 1.51 ^{c}
K159100	120.60 ± 1.43 ^{i}	22.32 ± 0.09 ^{c}
K159096	182.32 ± 0.89 ^{d}	17.00 ±0.27 ^{c}
K159048	16.33 ± 0.32 ^{l}	24.93 ± 1.08 ^{c}
K159077	80.95 ± 1.73 ^{j}	41.18 ± 5.11 ^{b}

Values represent mean ± S.D. of data obtained from three independent experiments. Duncan's Multiple Range Test at 5% level (DMRT, $p < 0.05$). Significant statistical differences are indicated by different letters.

3.3. Phenol Composition Analysis

The phenolic content was measured using the sorghum seed extract. The six phenolic compounds examined in this study were protocatechuic acid, caffeic acid, p-coumaric acid, ferulic acid, taxifolin, and naringenin. Protocatechuic acid content ranged from 7.63 ± 0.03 mg/L to 21.14 ± 0.09 mg/L. The content of caffeic acid ranged from 0.46 ± 0.02 mg/L to 10.73 ± 0.11 mg/L and that of p-coumaric acid ranged from 2.40 ± 0.07 mg/L to 22.44 ± 0.94 mg/L. The content of ferulic acid ranged from 0.86 ± 0.03 mg/L to 4.21 ± 0.30 mg/L, while that of taxifolin ranged from 2.30 ± 0.32 mg/L to 203.67 ± 4.99 mg/L. Naringenin was not detected in four (K159041, K159081, K159100, and K159077) of the 12 resources; its content ranged from 5.54 ± 0.33 mg/L to 43.93 ± 0.49 mg/L in eight resources. K159089 had the highest protocatechuic acid, caffeic acid, ferulic acid, and naringenin contents. K159097 had the highest p-coumaric acid content, while K159093 had the highest taxifolin content. Ferulic acid was the phenolic compound with the lowest content in all the 12 sorghum seeds. Statistical analysis revealed a significant component of the content of six phenolic compounds in sorghum resources ($p < 0.05$) (Table 5).

Table 5. Analysis of phenolic compounds from seed extracts of *Sorghum bicolor* genetic resources collected using HPLC analysis.

Accessions	Protocatechuic Acid	Caffeic Acid	*p*-Coumaric Acid	Ferulic Acid	Taxifolin	Naringenin
K159041	12.64 ± 0.11 ^{g}	1.86 ± 0.02 ^{f}	8.49 ± 0.43 ^{c}	2.40 ± 0.21 ^{b}	32.11 ± 0.07 ^{h}	ND
K159042	19.54 ± 0.27 ^{b}	3.95 ± 0.19 ^{c}	7.83 ± 1.08 ^{c}	1.55 ± 0.04 ^{cd}	189.27 ± 2.61 ^{b}	20.69 ± 0.76 ^{b}
K159078	7.63 ± 0.03 ^{j}	4.09 ± 0.09 ^{c}	7.83 ± 1.98 ^{c}	2.23 ± 0.08 ^{b}	2.30 ± 0.32 ^{j}	5.54 ± 0.33 ^{g}
K159081	11.21 ± 0.16 ^{i}	1.48 ± 0.03 ^{g}	3.18 ± 0.05 ^{e}	2.15 ± 0.15 ^{b}	134.10 ± 0.71 ^{c}	ND
K159088	14.63 ± 0.25 ^{de}	4.40 ± 0.16 ^{b}	5.94 ± 1.47 ^{d}	2.15 ± 0.26 ^{b}	66.27 ± 3.21 ^{de}	1.92 ± 0.74 ^{h}
K159089	21.14 ± 0.09 ^{a}	10.37 ± 0.11 ^{a}	8.69 ± 0.33 ^{c}	4.21 ± 0.30 ^{a}	19.11 ± 0.42 ^{i}	43.93 ± 0.49 ^{a}
K159093	14.37 ± 0.11 ^{e}	2.51 ± 0.22 ^{d}	11.27 ± 0.21 ^{b}	2.47 ± 0.12 ^{b}	203.67 ± 4.99 ^{a}	14.09 ± 0.40 ^{d}
K159097	12.04 ± 0.22 ^{h}	1.24 ± 0.02 ^{g}	22.44 ± 0.94 ^{a}	1.43 ± 0.26 ^{d}	61.83 ± 2.02 ^{ef}	7.25 ± 0.24 ^{f}
K159100	13.31 ± 0.83 ^{f}	1.92 ± 0.34 ^{f}	4.90 ± 0.60 ^{d}	2.32 ± 0.30 ^{b}	71.52 ± 10.52 ^{d}	ND
K159096	12.14 ± 0.18 ^{gh}	2.24 ± 0.03 ^{e}	2.63 ± 0.14 ^{e}	1.51 ± 0.10 ^{cd}	59.32 ± 1.88 ^{f}	11.95 ± 0.24 ^{e}
K159048	14.90 ± 0.10 ^{d}	4.47 ± 0.05 ^{b}	3.03 ± 0.06 ^{e}	1.77 ± 0.05 ^{c}	2.36 ± 0.66 ^{j}	17.78 ± 0.30 ^{c}
K159077	16.90 ± 0.28 ^{c}	0.46 ± 0.02 ^{h}	2.40 ± 0.07 ^{e}	0.86 ± 0.03 ^{e}	51.88 ± 1.74 ^{g}	ND

Values represent mean ± S.D. of data obtained from three independent experiments. Duncan's Multiple Range Test at 5% level (DMRT, $p < 0.05$). Significant statistical differences are indicated by different letters.

Phenolic compounds are widely distributed throughout the plant kingdom and have various structures and molecular weights. When a phenolic hydroxyl group is combined with a macromolecule, it exhibits physiological functions such as antioxidant, anticancer, and antibacterial properties [35]. Polyphenolic compounds present in cereals were reported to exhibit excellent antioxidant properties [36]. Phenolic acids are classified as simple substances that are abundant in the seed coat of almost all sorghum seeds [37]. The contents of gallic acid, chlorogenic acid, caffeic acid, ellagic acid, and p-coumaric acid were examined in a study on the change in antioxidant activity according to the seed roasting temperature and maintenance conditions of phenolic compounds in sorghum seed extracts [38]. In the study by Punia et al. (2021), the contents of nine phenolic compounds in five sorghum varieties were analyzed; the highest taxifolin content reported was 34.96 ± 0.23 mg/L, which was significantly lower than that in the present study (203.67 ± 4.99 mg/L). In their study, the contents of naringenin and other phenolic compounds were also significantly lower than those in the present study. This is because the sorghum genetic resources in this study had a significantly higher phenolic content, and the use of antioxidants and functional foods was considerably high.

3.4. Differences in Expression Levels of Antioxidant-Related Genes in Sorghum Genetic Resources

The correlation between antioxidant activity and the expression of antioxidant enzyme genes was examined by designed primers in the cells of 12 sorghum cultivars (Table 6). The transcription levels of *APX1*, *SOD*, and *CAT* were compared in seed cells. The highest *SOD* activity was observed in K159041, followed by K159048, K159042, and K159081; the remaining resources showed little expression of *SOD* in seed cells. Unlike *SOD*, the expression patterns of *APX1* and *CAT* correlated with the resources. The expression levels of *APX1* and *CAT* were significantly higher in K159041, K159042, and K159048 (Figure 4). This indicated that in resources with high antioxidant activity in DPPH and ABTS assays, ROS scavenging activity did not correlate with the expression levels of antioxidant enzyme genes at the cellular level. Several studies reported results for the enzymatic activity of APX1, SOD, and CAT; however, only a few studies reported the correlation between gene expression levels and ROS scavenging activity [39–41]. Antioxidant enzymes typically include SOD, CAT, and APX, and their function is to protect plants from active oxygen under environmental stress, thereby inducing the resistance mechanism against stress. Antioxidant enzymes show physiological activity related to biological health via various adult disease prevention and anti-aging functions, as well as plant defense mechanisms, revealing their value as a health supplement [42]. This finding is in agreement with that

of a previous study examining the antioxidant activity of plant-derived natural pigments, wherein plants with the highest antioxidant activity (DPPH assay performed using various plant extracts) and those with high APX, SOD, and CAT activities differed from each other in various patterns [43]. This is because in experiments such as DPPH and ABTS assays, organic solvents are used for extraction and antioxidant activity is determined via absorbance. However, the gene expression of antioxidant enzymes is determined via total RNA extraction, and the expression level detected is examined according to a specific gene sequence. Therefore, the results obtained using different experimental methods could vary. This indicates that the expression levels of *APX*, *SOD*, and *CAT*, which play a role in inactivating H_2O_2 in the cells of sorghum resources, may differ.

Table 6. Primer sequences used in qPCR analysis to detect the transcriptional expression of *APX*, *SOD* and *CAT* genes.

Gene Reference ID	Primer Sequence (5′ → 3′)
SOD	Forward: TCGAGTCAAGGCTCACGAAA Reverse: CTGGCGACTTCTTGGTCTCC
CAT	Forward: GGCAAGTCCCACTACGTCAA Reverse: AGCTGCTCGTTCTCGTTGAA
APX1	Forward: AGAGCGGTCTGGTTTTGAGG Reverse: GAGCTTGAGGTGGGCTTCTT
pp2a	Forward: AACCCGCAAAACCCCAGACTA Reverse: TACAGGTCGGGCTCATGGAAC

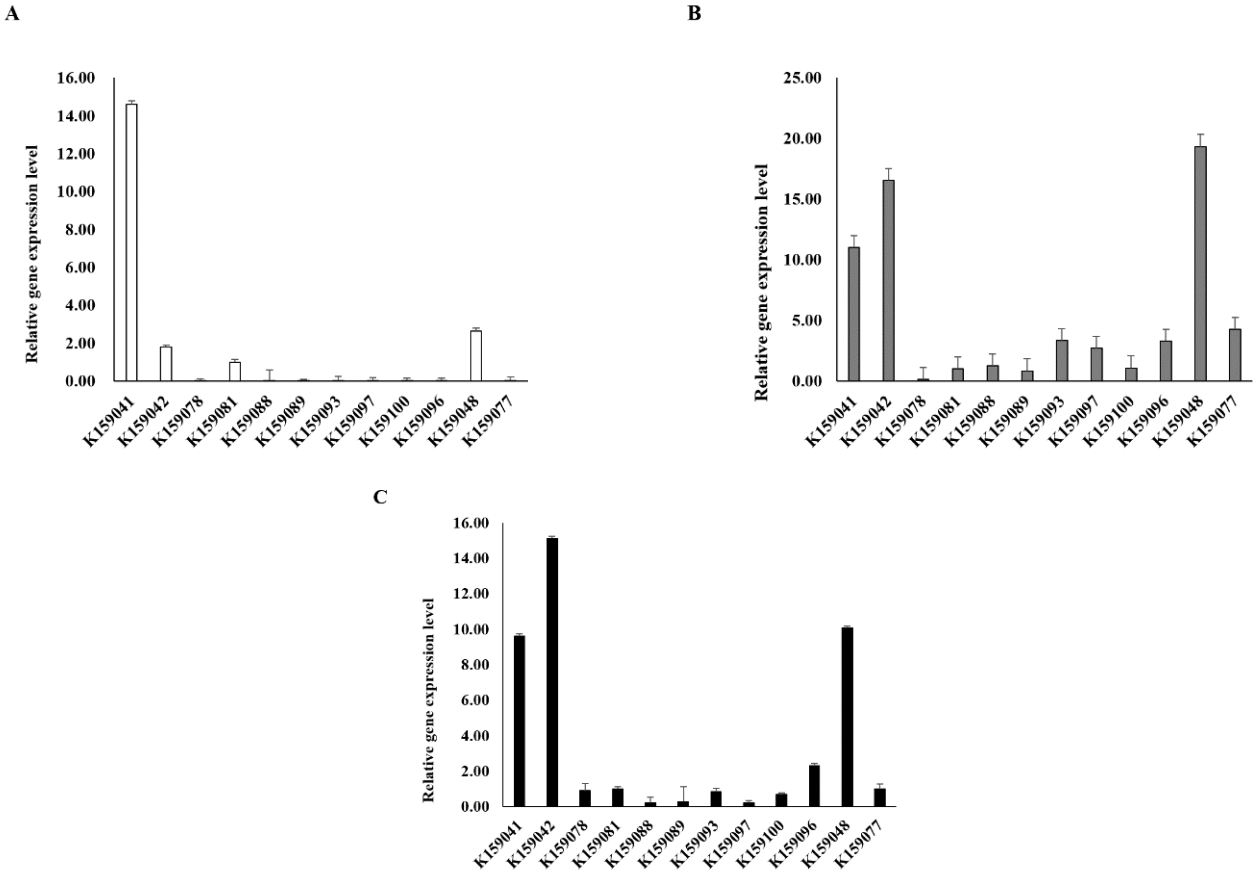

Figure 4. Comparative analysis of transcriptional level for antioxidant genes related to ROS scavenging activity using total RNA isolated from seeds of *Sorghum bicolor* genetic resources collected from seven different regions. (**A**) SOD, (**B**) APX1, (**C**) CAT. Values represent mean ± S.D. of data obtained from three independent experiments.

4. Conclusions

Based on the data from this study, the antioxidant activity of sorghum resources adapted and grown in cultivation regions with different climate conditions did not correlate with the precipitation, temperature, or type of climate of the cultivation region. The differences in antioxidant activity varied according to the genetic characteristics of each sorghum resource. Among the six verified phenolic compounds, taxifolin showed the highest content. In addition, K159041, 159042, and 159048, which showed high levels of *SOD*, *APX*, and *CAT* antioxidant enzymes.

Author Contributions: Conceptualization, E.S.S. and M.J.K.; methodology, J.W.S., D.Y.H., J.G.L. and N.Y.K.; supervision, C.Y.Y. and E.S.S.; investigation, E.S.S.; writing, E.S.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are contained within the article.

Acknowledgments: This study was supported by the Bioherb Research Institute, Kangwon National University, Republic of Korea.

Conflicts of Interest: The authors declare no conflict of interest.

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