한작지(Korean J. Crop Sci.), 68(3): 147~166(2023)
DOI : https://doi.org/10.7740/kjcs.2023.68.3.147

ISSN 0252-9777(Print)
ISSN 2287-8432(Online)

Original Research Article

Assessment of Phenolic Content, Saponin Content, and Antioxidant Activities in Gray, Red, and White Adzuki Bean Germplasm: A Multivariate Analysis

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ABSTRACT Seed color is controlled by several genes and is a key trait in determining the metabolite content and biological activities of legume genotypes. In this study, 296 adzuki bean accessions, including 159 grey, 99 red, and 38 white adzuki beans, were grown in Korea. Variations in total phenolic content (TPC), total saponin content (TSC), DPPH$^{\bullet}$ scavenging activity, ABTS$^{\bullet+}$ scavenging activity, and ferric reducing antioxidant power (FRAP) were assessed and were reported to be in the ranges of 1.52–8.24 mg GAE/g, 14.36–114.22 mg DE/g, 0.23–12.84 mg AAE/g, 1.05–17.66 mg TE/g, and 0.59–13.14 mg AAE/g, respectively, with a wide variation across adzuki beans. Except for DPPH$^{\bullet}$ scavenging activity, the average values declined in the order gray > red > white adzuki beans, each demonstrating a significant variation ($p < 0.05$). White adzuki beans, which showed low metabolite content and antioxidant activity, were clearly separated from the gray and red genotypes using principal component and hierarchical cluster analyses. Moreover, TPC, TSC, and antioxidant activities were strongly correlated, regardless of seed color. Overall, the diversity of the TPC, TSC, and antioxidant activity in a broad population of adzuki bean genotypes was determined. Furthermore, this study found that seed color variation in adzuki beans had a significant effect on the metabolite content and antioxidant activity. Superior accessions with high levels of TPC, TSC, and antioxidant activity were also discovered and could be used for functional plant breeding and human consumption. The findings of this study may be useful for understanding the relationship between seed coat color and metabolite concentration in adzuki beans, paving the way for molecular-level analyses.

Keywords : adzuki bean, antioxidants, polyphenols, saponins, seed color, *Vigna angularis*

Adzuki beans (*Vigna angularis* (Willd.) Ohwi & Ohashi) have been grown and consumed for centuries throughout East Asian countries including Korea, Japan, and China (Chu *et al*., 2021a; Ha *et al*., 2021; Tayade *et al*., 2022). Adzuki beans are high in nutrition and bioactive chemicals that improve human health. As a result, they have long been utilized as a versatile component in a variety of Asian cuisines such as paste, soup, porridge, and pastries (Lee *et al*., 2017a; Yousif *et al*., 2007). Previous research has shown that adzuki beans are good sources of plant-based fats and proteins, as well as minerals including magnesium, iron, potassium, and zinc, all of which are necessary for human wellness (Chu *et al*., 2021b; Sangsukiam & Duangmal, 2022; Sahasakul *et al*., 2022). Furthermore, adzuki beans are high in both soluble and insoluble dietary fibers that may help with weight loss and aid food digestion (Popoola *et al*., 2023). Adzuki beans are also rich sources of secondary metabolites, the most common of which are saponins and polyphenols, which have a synergistic role in regulating reactive radicals in the

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<Received 14 July, 2023; Revised 2 August, 2023; Accepted 2 August, 2023>

human body (Liu *et al*., 2017a, 2017b; Liu & Xu, 2015). Several studies have indicated that adzuki bean phenolic acids, flavonoids, and anthocyanins provide a variety of physiological effects, including antioxidants, anti-inflammatory, anticancer, and antidiabetic properties (Kitano-Okada *et al*., 2012; Kuriya *et al*., 2023; Lee *et al*., 2015a; Sahasakul *et al*., 2022). In general, consuming adzuki beans gives a wide range of health benefits, which has expanded their use in food industries.

Several environmental and genetic factors, including diseases, genotype variation, temperature, post-harvest treatment, and growth conditions, influence the overall metabolite compositions and biological activity of legumes (Hoeck *et al*., 2000; Oh *et al*., 2022). Seed-related traits such as seed size, seed weight, cotyledon color, hilum color, and seed coat color are also additional factors that may influence the chemical makeup of legume seeds (Chu *et al*., 2021a, 2021b; Kim *et al*., 2011; Lee *et al.*, 2008, 2017b). Adzuki beans come in a variety of seed colors, including red, black, gray, yellow, and white. Seed color variation in adzuki beans, like in other legumes, is regulated by numerous genes, and it is one of the key seed-related characteristics that determine metabolite levels and biological activities (Chu *et al.,* 2021b; Horiuchi *et al*., 2015; Redden *et al*., 2009; Zhao *et al*., 2022). Furthermore, it is one of the qualities that influence consumer and farmer preferences, as well as genotype selection in breeding (Kaga *et al*., 2008). As a result, studying the effect of seed color variation on seed metabolite and biological activities in a large population of genetic resources is critical. Furthermore, such studies are required to identify adzuki bean genotypes with high metabolite content that are suited for breeding, conservation, and consumption (Chu *et al*., 2021a; Li *et al*., 2022).

Previously, some studies have shown the metabolite variations in adzuki beans of varying seed coat colors. Black and red adzuki beans, for example, are known to contain more phenolic acids, anthocyanins, and flavonoids than other colors (Chu *et al*., 2021b; Ha *et al*., 2021; Lee *et al*., 2015b). White adzuki beans, on the other hand, are thought to be high in nutrient composition but poor in secondary metabolite contents (Li *et al*., 2022; Nagao *et al*., 2023). Despite these observations, there has been little research that has looked at the impact of seed coat color variation on a large population of adzuki beans. Evaluating the impact of seed coat color variation using a large population of genetic materials could provide a better understanding of its relationship with seed metabolite levels. Moreover, such studies are desirable in order to uncover superior genotypes that could be used for both consumption and breeding as described before (Kaga *et al*., 2008; Li *et al*., 2022). In this study, 296 adzuki beans of various colors, including gray, red, and white were cultivated in Korea, and the diversity of total phenolic content, total saponin content, and antioxidant activities were assessed. Moreover, the effect of seed color variation on each of the parameters was evaluated. This study could serve as a foundation for further research into the relationship between seed color, metabolite concentration, and antioxidant activity in adzuki beans, specifically gray, red, and white genotypes.

MATERIALS AND METHOD

Chemicals and reagents

All of the chemicals and reagents used in this study were of the highest quality and used as obtained. Methanol and ethanol were purchased from Fisher Scientific (Pittsburgh, PA, USA), water from Thomas Scientific (Philadelphia, PA, USA), diosgenin from PhytoLab (Vestenbergsgreuth, Germany), and sulfuric acid from DAEJUNG Chemicals (Siheung, Korea). The other chemicals and reagents, including anhydrous sodium carbonate (Na_2CO_3), vanillin, gallic acid, Folin-Ciocalteu phenol reagent, L-ascorbic acid, sodium carbonate, potassium ferricyanide, trichloroacetic acid, ferric chloride, 1,1-diphenyl-2-picrylhydrazyl radical ($DPPH^•$), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), were obtained from Sigma Aldrich (St. Louis, MO, USA).

Seeds collection, cultivation, and sample preparation

The seeds of 296 adzuki bean genetic materials were obtained from the National Agrobiodiversity Center's gene bank (RDA, Jeonju, Korea). Field cultivation of all adzuki bean varieties was carried out on an experimental field at the Center between June and October 2021. Detailed information regarding cultivation conditions can be found in our recently published paper (Desta *et al*., 2023). Matured seeds were hand-harvested and classified as gray, red, or white genotypes based on seed coat color. Seed samples from each accession were freeze-dried in an LP500 drier (ilSHINBioBase, Dongducheon, Korea). The dried samples were subsequently pulverized using 3 mm stainless TissueLyser II beads (Quagen, Germantown, MD, USA) and stored at -20°C in sealed plastic bags for further analysis.

Seed sample extraction

Seed sample extraction was carried out in accordance with a previously described protocol, with some modifications. Specifically, 0.2 g of powdered sample was combined in triplicate with 2.5 mL of 80% aqueous methanol. The mixture was sonicated for 45 min in the dark at 25 °C. The mixture was then taken off and centrifuged in a high-speed centrifuge (3134 ×g, 15 min) (Labogene, Daejeon, Korea). The upper supernatant was kept, and the extraction procedure was repeated for the residue. The combined supernatant was then used to determine total saponin content, total phenolic content, 1,1-diphenyl-2-picrylhydrazyl radical ($DPPH^•$) scavenging activity, $ABTS^{•+}$ scavenging activity, and ferric-reducing antioxidant power.

Total saponin content (TSC)

The TSC was estimated using a previously known vanillin-sulfuric acid assay, with minor modifications (Xu & Chang, 2009). In brief, 25 μL of sample extract was combined with an equal amount of freshly prepared 8% vanillin (w/v in ethanol) and 250 μL of 72% sulfuric acid (v/v in water). The mixture was incubated in a water bath at 60°C for 10 min before cooling in an ice bath for 15 min. The absorbance was then measured at 544 nm on an Eon Microplate Spectrophotometer (Bio-Tek, Winooski, VT, USA). Diosgenin (0.10~2.00 mg/mL) was used as a standard to plot calibration curves ($R^2 > 0.999$). TSC was then calculated as milligrams of diosgenin equivalent per gram of dried seed sample (mg DE/g).

Total phenolic content (TPC)

The TPC was calculated using the Folin-Ciocalteu colorimetric technique (Xu & Chang, 2009). In brief, 100 μL of sample extract was mixed with 100 μL of Folin-Ciocalteu reagent, and the mixture was incubated at 25 °C in the dark for 3 min. After adding 100 μL of Na_2CO_3 solution (2%), the mixture was further incubated in the dark for 30 min. Finally, the absorbance was measured at 750 nm using an Eon Microplate Spectrophotometer (Bio-Tek, Winooski, VT, USA). Gallic acid (0.025~0.500 mg/mL) was used as a standard to plot calibration curves. TPC was estimated as milligrams of gallic acid equivalents (mg GAE/g) per gram of dried material.

Antioxidant activities

Three in vitro experiments were used to evaluate the antioxidant activity of each adzuki bean, as detailed below, using our previously published methodology (Desta *et al*., 2022). During each experiment, analysis was conducted in triplicate and absorbance was measured with an Eon Microplate Spectrophotometer (Bio-Tek, Winooski, VT, USA).

$DPPH^•$ scavenging activity

During the $DPPH^•$ scavenging activity assay, 100 μL of each sample extract was combined with an equivalent volume of freshly made DPPH-solution (150 μM). The mixture was then incubated for 30 min in the dark at 25 °C, and the absorbance was measured at 517 nm. Calibration curves were plotted using ascorbic acid (2.5~25.0 mg/L, $R^2 > 0.999$), and $DPPH^•$ scavenging activity was calculated as milligrams of ascorbic acid equivalent per gram of dried material (mg AAE/g).

$ABTS^{•+}$ scavenging activity

To determine the $ABTS^{•+}$ scavenging activity, 10 μL of sample extract was combined with 150 μL of $ABTS^{•+}$ working solution before being incubated for 3 min at 25°C in the dark. Then, the absorbance was measured at 734 nm. Trolox was used as a standard to plot calibration curves (10~250 mg/L, $R^2 > 0.999$). $ABTS^{•+}$ scavenging activity was calculated as milligrams of Trolox equivalent per gram of dry material (mg TE/g).

Ferric reducing antioxidant power (FRAP)

For the FRAP test, 60 μL of sample extract was mixed with 150 μL of freshly prepared phosphate buffer (pH: 6.6, 0.2 M) and an equal volume of 1% potassium ferricyanide solution ($K_3Fe(CN)_6$). After 20 min of incubation at 50 °C, 150 μL of 10% trichloroacetic acid was added. Then, the mixture was centrifuged (3134 × g, 10 min), and 100 μL of the supernatant was combined with an equal volume of distilled water and 20 μL of 0.1% ferric chloride solution. After 10 min, the absorbance at 700 nm was measured. Calibration curves were constructed using ascorbic acid (10~150 mg/L, $R^2 > 0.999$), and FRAP was presented in mg AAE/g.

Data analysis

Measurements were taken in triplicate during each experiment, and results are reported as mean standard ± standard deviation

Table 1. Statistical values on the variations of total phenolic content, total saponin content, and antioxidant activities in gray, red, and white adzuki beans grown in Korea.

Variable	Seed coat color	Number of accessions	Minimum	Maximum	Mean	Variation coefficient (%)	Skewness	Kurtosis
TSC (mg DE/g)	Gray	159	15.743	108.239	50.937[a]	41.162	0.694	-0.032
	Red	99	23.258	114.218	42.068[b]	32.146	2.080	7.447
	White	38	14.364	33.850	22.094[c]	21.325	0.409	-0.369
	Total	296	14.364	114.218	44.268	44.542	1.048	0.988
TPC (mg GAE/g)	Gray	159	2.145	8.235	4.161[a]	22.262	1.483	3.902
	Red	99	2.702	6.336	3.787[b]	14.338	1.439	4.535
	White	38	1.522	2.370	1.965[c]	8.060	-0.459	1.181
	Total	296	1.522	8.235	3.754	27.469	0.513	2.155
DPPH (mg AAE/g)	Gray	159	1.127	11.208	3.909[a]	40.082	1.634	3.948
	Red	99	1.196	12.840	4.142[a]	37.358	2.241	10.123
	White	38	0.227	1.164	0.335[b]	43.004	5.228	29.798
	Total	296	0.227	12.840	3.528	54.039	0.720	2.694
ABTS (mg TE/g)	Gray	159	2.361	16.801	8.511[a]	30.450	0.773	0.978
	Red	99	3.840	17.660	7.806[b]	26.760	1.263	4.170
	White	38	1.054	3.231	1.915[c]	26.298	0.347	0.004
	Total	296	1.054	17.660	7.428	41.883	0.099	0.522
FRAP (mg AAE/g)	Gray	159	1.366	10.841	4.257[a]	38.269	1.478	3.587
	Red	99	1.759	13.139	4.141[a]	38.073	2.244	9.660
	White	38	0.588	2.563	1.298[b]	26.473	0.705	4.289
	Total	296	0.588	13.139	3.838	46.787	1.158	3.470

AAE, ascorbic acid equivalent; ABTS, $ABTS^{\bullet+}$ scavenging activity; DE: Diosgenin equivalent; DPPH, $DPPH^{\bullet}$ scavenging activity; FRAP: Ferric reducing antioxidant power; GAE: Gallic acid equivalent; TE, Trolox equivalent; TPC: Total phenolic content; TSC: Total saponin content.
Different superscript letters in a row represent significantly different means ($p < 0.05$).

(SD). Statistical analysis was carried out using the xlstat program (Lumivero, CO, USA). To statistically determine the differences between means, an analysis of variance at $p < 0.05$ was performed. R-software version 4.2 (www.r-project.org) was used for boxplot, principal component (PCA), hierarchical cluster (HCA), and Pearson's correlation analyses.

RESULTS AND DISCUSSION

Variation of TPC and effect of seed color difference

Adzuki beans are well-known for their phenolic content, which plays an important role in health promotion (Wang *et al.*, 2022). The TPC of all 296 adzuki beans was determined using a colorimetric assay, as previously described. Table S1 (Supplemental material) provides the average TPC for each accession, and Table 1 summarizes the statistical data. The TPC was in the range of 1.52~8.24 mg GAE/g with a mean of 3.75 mg GAE/g and a coefficient of variation (CV) of 27.47%. Among the 296 accessions, 52.03% of the adzuki beans had a TPC greater than the total mean value. The highest TPC was found in accession IT-216286, while the lowest was recorded in accession IT-105698. Previously, several studies assessed the level of TPC in various adzuki bean genotypes. For instance, Lee *et al.* (2017a) and Sung *et al.* (2020) independently investigated 209 adzuki bean landraces and 8 adzuki bean cultivars cultivated in Korea and discovered TPC ranges of 1.1~11.7 and 0.52~4.28 mg GAE/g, respectively. In another study, TPC in 150 adzuki beans grown in Korea ranged from 374.5 to 6596.9 μg/g (Kim *et

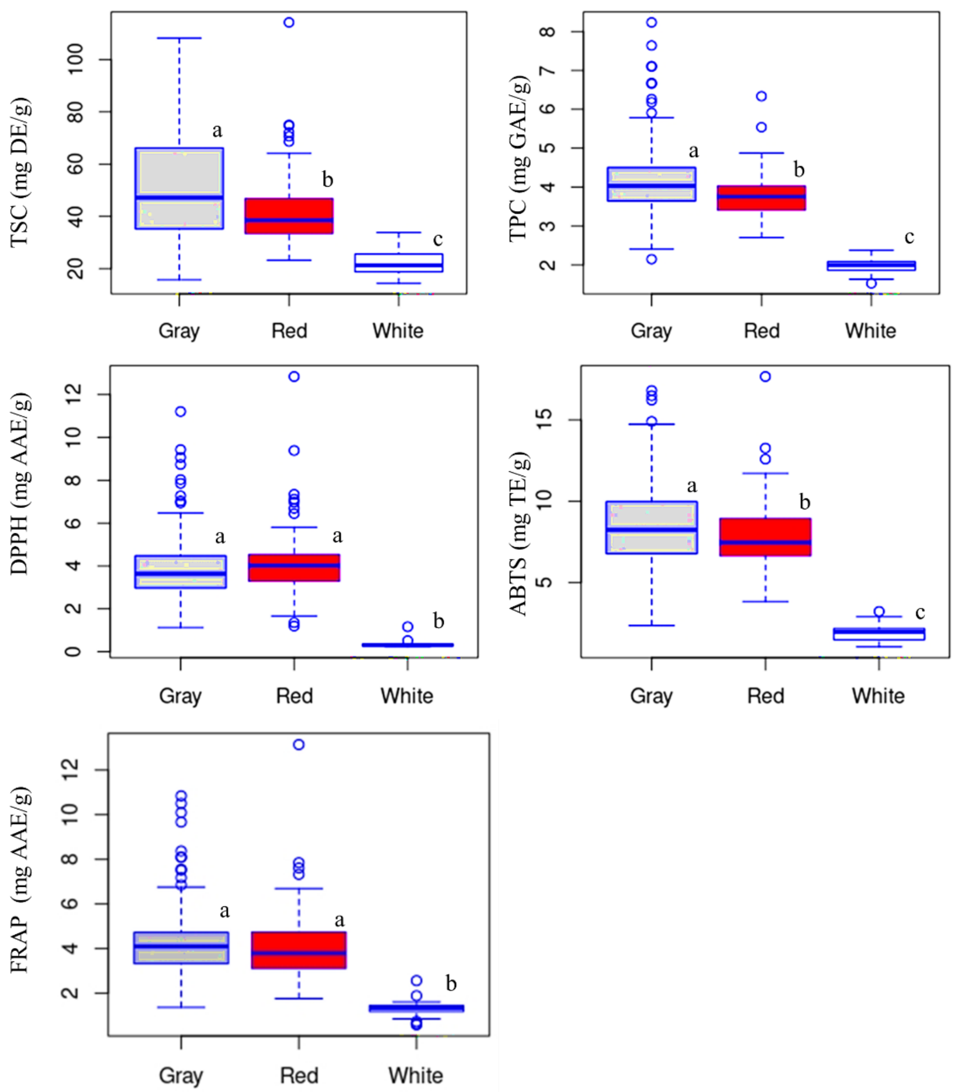

Fig. 1. Boxplots showing the variations of total phenolic content, total saponin content, and antioxidant activities between gray, red, and white adzuki beans. AAE: Ascorbic acid equivalent; ABTS: ABTS$^{•+}$ scavenging activity; DE: Diosgenin equivalent; DPPH: DPPH$^{•}$ scavenging activity; FRAP: Ferric reducing antioxidant power; GAE: Gallic acid equivalent; TE: Trolox equivalent; TPC: Total phenolic content; TSC: Total saponin content. Different letters on boxplots in a category indicate significantly different means ($p < 0.05$).

al., 2011). Johnson *et al*. (2022) recently discovered a TPC in the range of 53~152 mg GAE/100g in eight adzuki bean genotypes grown in Australia. Several factors, including post-harvest treatment conditions, growing conditions, and analysis techniques, are documented to alter TPC levels in adzuki beans. Therefore, the observed TPC variances in the previous studies could be attributed to these factors (Ha *et al*., 2021; Szakiel *et al*., 2011). Overall, the TPC values found in our investigation are within previously reported ranges.

In legumes, seed color has been utilized to discriminate between metabolite variations. However, there has not been much research on adzuki beans that evaluate the effect of seed color on metabolite levels utilizing a wide population of genotypes (Siah *et al*., 2014; Wang *et al*., 2008; Xu *et al*., 2007). In this study, the TPC variation among the different seed coat colors was statistically evaluated. The box plots in Fig. 1 depict the level of TPC in the adzuki bean accessions according to their seed coat color. Table 1 contains both numerical values and statistical data. TPC ranged from 2.14 to 8.24 mg GAE/g in gray adzuki beans, from 2.70 to 6.34 mg GAE/g in red adzuki beans, and from 1.52-2.37 mg GAE/g in white adzuki beans. The average TPC decreased in the order of gray (4.16 mg GAE/g) > red (3.79 mg GAE/g) > white 2.37 (mg GAE/g), with significant variation between each group ($p < 0.05$). Among the accessions with a TPC above the total mean (3.75 mg GAE/g), 67.74% were gray while the remaining 32.26% were red adzuki beans. The TPC of all the white adzuki beans was less than the total mean. Previously, Hori *et al*. (2006) found a much lower TPC in white adzuki beans compared to black, red, and green adzuki beans, which our findings agree with. A recent study also showed white adzuki beans to have a much lower TPC level compared to other colored genotypes (Nagao *et al*., 2023). In general, our findings along with these previous studies demonstrate that white adzuki beans might not be good sources of polyphenols compared to other colored genotypes.

Variation of TSC and effect of seed color difference

Saponins, found in high concentrations in Adzuki beans, work in tandem with polyphenols to prevent disease and improve health (Liu *et al*., 2017b). In this study, the TSC of all accessions was determined using the vanillin-H_2SO_4 assay as described before. With a mean of 44.27 mg DE/g, the TSC in the entire population was in the range of 14.36~114.22 mg DE/g, indicating an approximately 8-fold variation across the adzuki bean accessions (CV = 44.27%) (Table S1, Table 1). Around 40.88% of the adzuki bean accessions had a TSC greater than the total mean. Accession IT-194575 had the highest TSC, while accession IT-294630 had the lowest. Many studies have shown that saponin concentrations differ amongst adzuki bean genotypes, and genetic variations, post-harvest management, extraction procedures, and growing conditions can all impact TSC levels. Previously, TSC levels in two adzuki bean genotypes ranged from 10.33 to 19.40 mg/g after different sonication treatments (Chiu, 2021). In other studies, Luo *et al*. (2016) and Liu & Xu (2015) separately reported TSC of 45.60 mg DE/g and 10.82 mg SBaE/g in two different Chinese adzuki beans. The TSC value in the former study corresponded to the total mean value in our investigation.

Saponin levels in legumes are also affected by seed color variations. However only a few studies were published in adzuki beans (Siah *et al*., 2014; Wang *et al*., 2008; Xu *et al*., 2007). In this study, the effect of seed color variation on TSC was statistically examined, and significant variation was found (Fig. 1, Table 1). TSC in gray, red, and white adzuki beans ranged from 15.74 to 108.24, 23.46 to 114.22, and 14.36 to 33.85 mg DE/g, respectively. The average TSC level followed a similar pattern as TPC, being the highest in gray adzuki beans (50.94 mg DE/g) and the lowest in white adzuki beans (22.09 mg DE/g). Statistically, the average TSC varied significantly among the different seed coat colors ($p < 0.05$). Moreover, gray adzuki beans accounted for 72.73% of the accessions with a TSC greater than the total mean, whereas red adzuki beans accounted for the remaining 27.27%. Again, none of the white adzuki beans had a TSC greater than the total mean value. To the best of our knowledge, there are no previous studies that investigated the influence of seed coat color on total saponin content in adzuki beans. Overall, our research found that white adzuki beans are low in saponin content when compared to gray and red adzuki beans.

Variations of antioxidant activities and effect of seed color difference

In vitro tests are widely accepted as the most convenient and cost-effective assays for determining the total antioxidant activity of plant and food extracts. However, due to variances in the scavenging mechanism, a single experiment alone cannot be utilized to evaluate the total antioxidant activity of such extracts (Martins *et al*., 2022). In this study, three separate assays were utilized to evaluate the antioxidant activity of each adzuki bean accession (Table 1, Table S1). The $DPPH^{\bullet}$ scavenging activity, $ABTS^{\bullet+}$ scavenging activity, and FRAP were in the ranges of 0.23~12.84 mg AAE/g, 1.05~17.66 mg TE/g, and 0.59~13.14 mg AAE/g, respectively signifying a wide range of variations among the adzuki bean accessions. Accession IT-194575 displayed the highest $DPPH^{\bullet}$ scavenging activity, $ABTS^{\bullet+}$ scavenging activity, and FRAP at the same time. This accession had the highest TSC as well as a TPC of 6.34 mg GAE/g, which is the 7th highest level of all the 296 accessions. On the other hand, accessions IT-236259, IT-105890, and IT-196872 displayed the lowest $DPPH^{\bullet}$ scavenging activity, $ABTS^{\bullet+}$ scavenging activity, and FRAP, respectively. Compared to our study, much lower $ABTS^{\bullet+}$ scavenging activity (1.64~8.15 mg TE/g) and FRAP (0.41~5.44 mg AAE/g) ranges were reported, and the variations could be due to the difference in the number of genotypes investigated (Lee *et al*., 2017a; Sung *et al*., 2020). In addition to the studies mentioned above, other researchers have also revealed the antioxidant activities of a variety of adzuki bean genotypes. However, differences in extraction techniques, reporting methodologies, and tests used made comparing published values to our findings difficult (Gohara *et al*., 2016; Han *et al*., 2022). In general, our findings demonstrated wide variations of antioxidant activities among the different adzuki beans. Furthermore, accessions with greater levels of TPC and TSC displayed stronger antioxidant activities than those with lower levels of TPC and TSC (Table S1). Such findings have been reported in numerous prior research, indicating the roles of polyphenols and saponins in the regulation of reactive radicals (Johnson *et al*., 2022; Lee *et al*., 2017a).

The effect of seed coat color variations on each of the antioxidant activities was also statistically evaluated (Table 1, Fig. 1). The $DPPH^{\bullet}$ scavenging activity, $ABTS^{\bullet+}$ scavenging activity, and FRAP were in the ranges of 1.13~11.21 mg AAE/g, 2.36~16.80 mg TE/g, and 1.37~10.84 mg AAE/g in gray, 1.20~12.84 mg AAE/g, 3.84~17.66 mg TE/g, and 1.76~13.14 mg AAE/g in red, and 0.23~1.64 mg AAE/g, 1.05~3.23 mg TE/g, and 0.59~2.56 mg AAE/g in white adzuki beans, respectively. The average $ABTS^{\bullet+}$ scavenging activity and FRAP each decreased in the order of gray > red > white, while $DPPH^{\bullet}$ scavenging activity decreased in the order of red (4.14 mg AAE/g) > gray (3.91 mg AAE/g) > white (0.34 mg AAE/g) adzuki beans (Table 1, Fig. 1). $ABTS^{\bullet+}$ scavenging activity differed significantly between the three seed coat colors. Likewise, $DPPH^{\bullet}$ scavenging activity and FRAP of gray and red adzuki beans differed significantly from those of white adzuki beans but not from each other. Furthermore, gray adzuki beans accounted for 56.38, 72.85, and 65.96% of the accessions with values above the total means of $DPPH^{\bullet}$ scavenging activity, $ABTS^{\bullet+}$ scavenging activity, and FRAP, respectively, whereas red adzuki beans accounted for the remaining 43.62, 27.15, and 34.04%, respectively. For any of the antioxidant activities, no white adzuki bean exceeded the total mean values. Little research has been done on the effects of seed color on antioxidant activity in adzuki beans. In line with our findings, a recent investigation showed much lower $DPPH^{\bullet}$ scavenging activity in white adzuki beans as compared to other colored genotypes, including black and red adzuki beans (Nagao *et al*., 2023). In another study, Kim *et al*. (2015) observed that black adzuki beans had higher $DPPH^{\bullet}$ scavenging activity and $ABTS^{\bullet+}$ scavenging activity than red adzuki beans, further signifying the influence of seed color. In general, our findings demonstrated that seed coat color variation had a significant effect on adzuki bean antioxidant activities. As a result, it can be concluded that white adzuki beans do not have the same antioxidant content as gray and red adzuki beans.

Multivariate analysis

To gain a better understanding of the changes in total metabolite contents and antioxidant activity among the adzuki beans studied, hierarchical cluster analysis (HCA), principal component analysis (PCA), and Pearson's correlation analysis were conducted. The HCA in Fig. 2A clearly shows the low levels of TPC, TSC, and antioxidant activities in white adzuki beans. This result was reinforced further by the PCA, which was computed along the first two principal components (PC1 and PC2), which together contributed more than 90% of the total variance. As shown in the score plot, white adzuki beans were differentiated from gray and red adzuki beans (Fig. 2B). The gray and red landraces, on the other hand, were widely scattered with one another. As indicated in Fig. 2C and Table 2, TPC (20.44%), $DPPH^{\bullet}$ scavenging activity (20.67%), $ABTS^{\bullet+}$ scavenging activity (21.85%), and FRAP (21.03%) contributed the most to the variations observed along PC1, while TSC (74.12%) contributed the most along PC2. This was consistent with the HCA, which showed that TPC, $ABTS^{\bullet+}$ scavenging activity, $DPPH^{\bullet}$ scavenging activity, and FRAP were all clustered together (Fig. 2A). In general, the PCA supported by HCA in our study clearly separated white adzuki beans from gray and red accessions. As a result, TSC, TPC, $ABTS^{\bullet+}$ scavenging activity, $DPPH^{\bullet}$ scavenging activity, and FRAP could be important traits for distinguishing a diverse population of adzuki bean genetic materials with varying seed coat colors.

Pearson's correlation analysis was performed to compute the correlation between the analyzed parameters. Positive and strong correlations were found between the examined variables in all the adzuki beans. However, the correlation coefficient (*r*) values were greater in gray (Fig. 3A) and red (Fig. 3B) adzuki beans than in white (Fig. 3C) adzuki beans. In gray adzuki beans, for example, TPC had $r = 0.81$ with $DPPH^{\bullet}$ scavenging activity, $r = 0.76$ with $ABTS^{\bullet+}$ scavenging activity, and $r = 0.63$ with FRAP, all of which were significant at $p < 0.001$. However, in white adzuki beans, the correlation of TPC with $DPPH^{\bullet}$ scavenging activity, $ABTS^{\bullet+}$ scavenging activity, and FRAP was considerably weaker, with *r* of 0.58, 0.26, and 0.48,

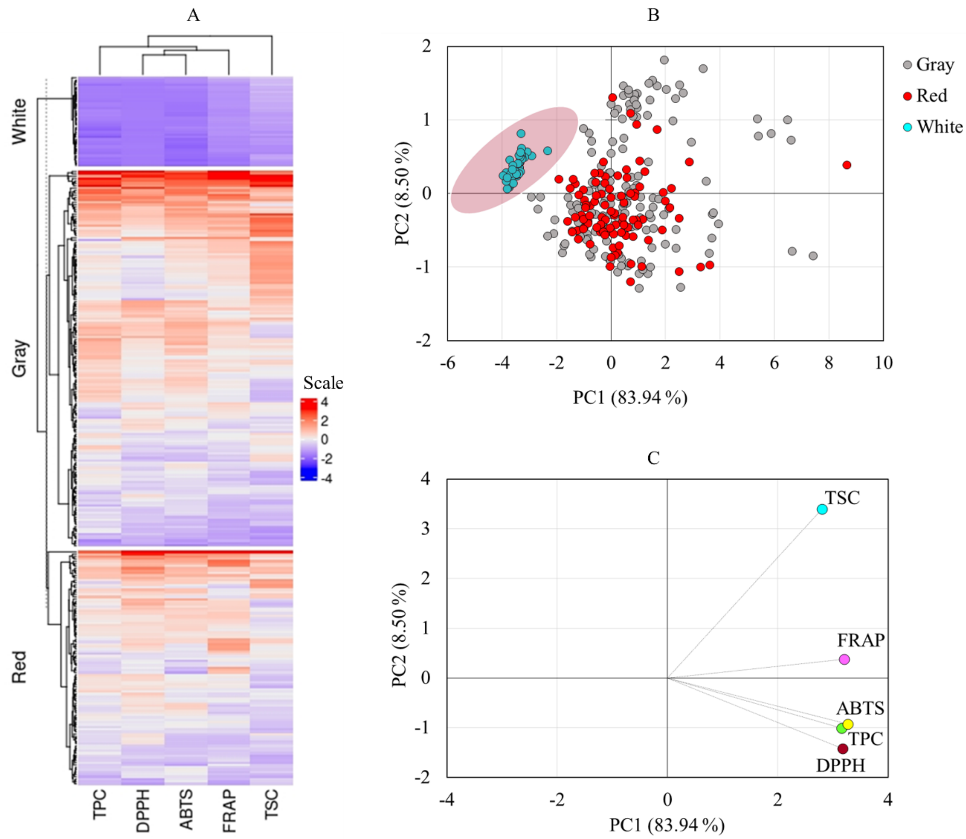

Fig. 2. Hierarchical cluster analysis (A), score plot of adzuki beans (B), and loading plot of variables (C) from principal component analysis. ABTS, $ABTS^{\bullet+}$ scavenging activity; DPPH, $DPPH^{\bullet}$ scavenging activity; FRAP: Ferric reducing antioxidant power; TPC: Total phenolic content; TSC: Total saponin content.

Table 2. Total phenolic content, total saponin content, and antioxidant activity of top-performing adzuki bean accessions.

TSC (mg DE/g)			TPC (mg GAE/g)			DPPH (mg AAE/g)			ABTS (mg TE/g)			FRAP (mg AAE/g)		
IT No.	Color	Content	IT No.	Color	Content	IT No.	Color	Activity	IT No.	Color	Activity	IT No.	Color	Activity
194575	Red	114.22	216286	Gray	8.24	194575	Red	12.84	194575	Red	17.66	194575	Red	13.14
216285	Gray	108.24	216306	Gray	7.64	216286	Gray	11.21	216306	Gray	16.80	216285	Gray	10.84
216305	Gray	107.21	216305	Gray	7.11	216285	Gray	9.43	216305	Gray	16.48	216286	Gray	10.50
216303	Gray	106.44	216303	Gray	7.10	103973	Red	9.39	216285	Gray	16.21	216306	Gray	10.09
216298	Gray	106.16	216299	Gray	6.68	103060	Gray	9.06	216298	Gray	14.90	216305	Gray	9.67
216299	Gray	100.91	216298	Gray	6.66	216306	Gray	8.74	216299	Gray	14.73	216298	Gray	8.37
240343	Gray	100.55	194575	Red	6.34	216298	Gray	8.03	216286	Gray	14.54	196895	Gray	8.10
211854	Gray	91.36	178391	Gray	6.26	216305	Gray	7.86	178391	Gray	14.27	216299	Gray	8.09
283300	Gray	90.28	216300	Gray	6.18	105643	Red	7.34	216303	Gray	14.21	178458	Red	7.85
208913	Gray	89.64	216289	Gray	5.91	216291	Gray	7.28	216300	Gray	14.12	178527	Red	7.61
216286	Gray	87.61	216291	Gray	5.79	104171	Red	7.12	186309	Red	13.27	216291	Gray	7.55
252929	Gray	85.38	196895	Gray	5.55	104855	Gray	7.04	196895	Gray	13.17	216303	Gray	7.51
280309	Gray	84.16	186309	Red	5.54	186309	Red	6.98	216295	Gray	12.82	196870	Red	7.32
195537	Gray	82.80	186322	Gray	5.52	216299	Gray	6.94	103973	Red	12.59	216300	Gray	7.18
208790	Gray	82.46	121459	Gray	5.37	105632	Red	6.69	186322	Gray	12.54	216295	Gray	6.85

AAE, ascorbic acid equivalent; ABTS, $ABTS^{\bullet+}$ scavenging activity; DE: Diosgenin equivalent; DPPH, $DPPH^{\bullet}$ scavenging activity; FRAP: Ferric reducing antioxidant power; GAE: Gallic acid equivalent; TE, Trolox equivalent; TPC: Total phenolic content; TSC: Total saponin content.

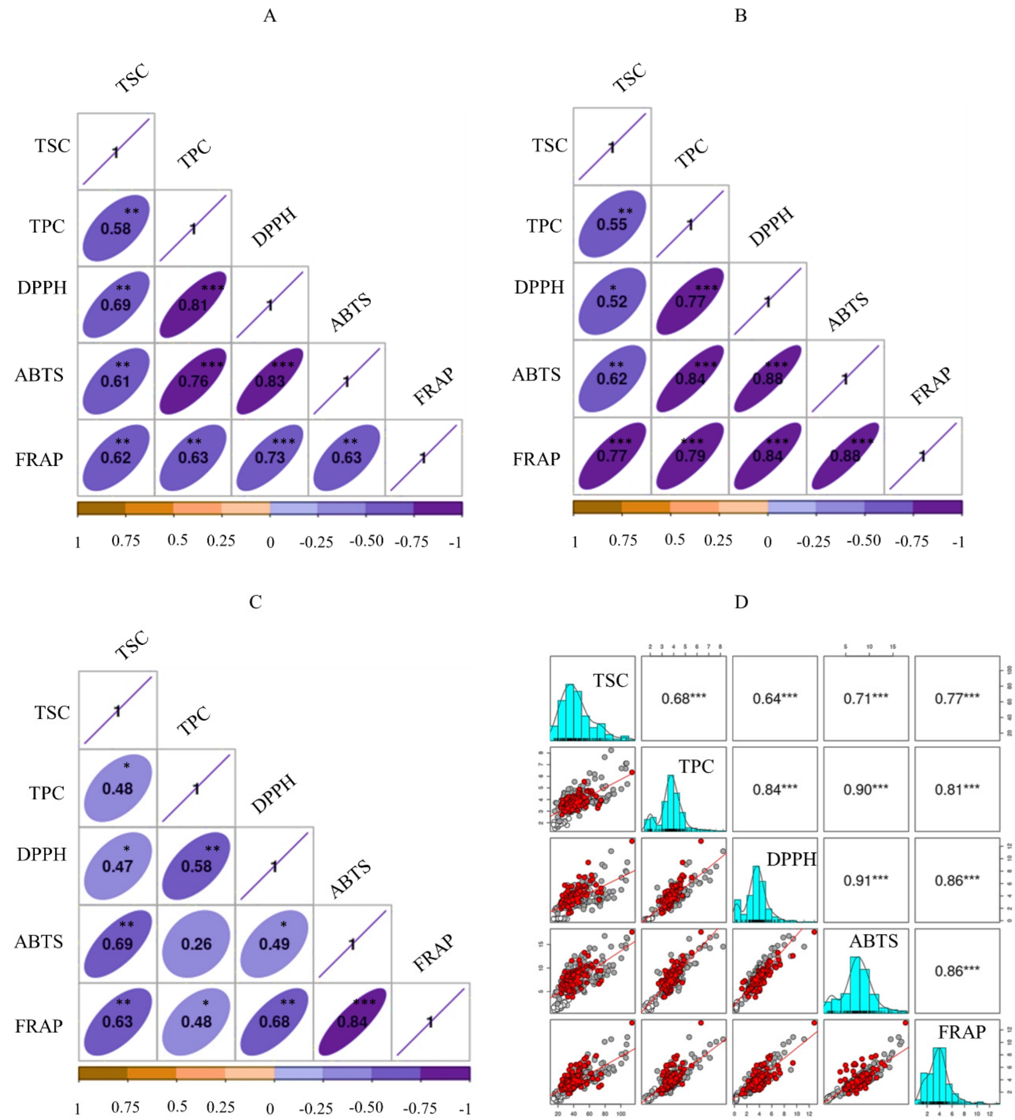

Fig. 3. Pearson correlation matrix of metabolite contents and antioxidant activities in gray (A), red (B), and white (C) adzuki beans, and the entire dataset (D). ABTS, $ABTS^{\bullet+}$ scavenging activity; DPPH, $DPPH^{\bullet}$ scavenging activity; FRAP: Ferric reducing antioxidant power; TPC: Total phenolic content; TSC: Total saponin content. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

respectively. TSC also correlated with antioxidant activities in a similar way as TPC, with $r \geq 0.61$ in gray, $r \geq 0.52$ in red, and $r \geq 0.47$ in white adzuki beans. Generally, TSC and TPC demonstrated positive associations with all antioxidant activities and with each other regardless of seed coat color (Fig. 3D), with all except the TPC link with $ABTS^{\bullet+}$ scavenging activity in white adzuki beans being significant. The observed strong and positive correlations were consistent with several prior research (Han *et al*., 2022; Lee *et al*., 2017a).

To summarize, this study investigated the variations of total phenolic content, total saponin content and antioxidant activities among a large population of adzuki bean accessions cultivated in Korea. All the variables studied including total phenolic content, total saponin content, $DPPH^{\bullet}$ scavenging activity, $ABTS^{\bullet+}$ scavenging activity, and ferric-reducing antioxidant power demonstrated a large range of variability among the adzuki beans. Furthermore, seed coat color variation had a significant effect on all of the parameters, with gray adzuki beans having the highest average values of all except $DPPH^{\bullet}$ scavenging activity and white landraces having the lowest levels. As previously indicated, adzuki beans with high levels of TPC and TSC are useful due to their multiple health benefits (Li *et al*., 2022). This

Table 3. Eigenvalues and individual and cumulative contributions of variables in the first five principal components.

Variables	PC1	PC2	PC3	PC4	PC5
TSC	16.020	74.117	2.062	6.558	1.243
TPC	20.438	6.557	47.661	14.271	11.073
DPPH	20.666	12.874	15.995	25.046	25.419
ABTS	21.848	5.525	2.335	9.776	60.516
FRAP	21.027	0.928	31.948	44.348	1.749
Eigenvalue	4.197	0.425	0.188	0.118	0.072
Variability (%)	83.936	8.505	3.759	2.358	1.443
Cumulative (%)	83.936	92.440	96.199	98.557	100.000

ABTS, $ABTS^{•+}$ scavenging activity; DPPH, $DPPH^{•}$ scavenging activity; FRAP: Ferric reducing antioxidant power; TPC: Total phenolic content; TSC: Total saponin content. PC1: First principal component; PC2: Second principal component; PC3: Third principal component; PC4: Fourth principal component; PC5: Fifth principal component.

study also identified the top 15 superior adzuki beans showing the highest levels of each parameter (Table 3). In particular, accessions IT-194575, IT-216286, IT-216298, IT-216299, and IT-216305 contained high levels of total phenolic content, total saponin content, $DPPH^{•}$ scavenging activity, $ABTS^{•+}$ scavenging activity, and ferric-reducing antioxidant power all at the same time. Accession IT-216303, on the other hand, showed high levels of all parameters except $DPPH^{•}$ scavenging activity, whereas accession IT-216306 showed high levels of all parameters except total saponin content. Accession IT-216300 likewise had high levels of all parameters except total saponin content and $DPPH^{•}$ scavenging activity. As a result, these accessions have the potential to be valuable sources of health-promoting metabolites. Furthermore, the findings of this study may inspire further molecular research in the future. Identifying specific saponins and polyphenol components in gray, red, and white adzuki beans, on the other hand, would also be a good future research endeavor.

ACKNOWLEDGMENT

This research was funded by the Research Program for Agricultural Science and Technology Development (Project No. PJ015788) of the National Institute of Agricultural Sciences, Rural Development Administration (Jeonju, Korea).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Table 1. General information and average total metabolite content and antioxidant activity of 296 Adzuki bean accessions grown in Korea.

General information				Metabolite contents and antioxidant activities				
IT-No.	Origin	Genotype	Seed coat color	TPC (mg GAE/g)	TSC (mg DE/g)	DPPH (mg AAE/g)	ABTS (mg TE/g)	FRAP (mg AAE/g)
25924	JPN	Unknown	Red	4.00	41.68	4.23	7.96	4.32
100899	KOR	Landrace	Red	4.00	34.84	3.46	7.96	3.30
100916	KOR	Landrace	Red	3.84	35.37	3.06	7.24	3.60
100961	KOR	Landrace	Gray	2.76	23.50	2.52	4.48	1.78
100997	KOR	Landrace	Red	4.27	45.35	4.56	9.00	4.56
101052	KOR	Landrace	Red	4.03	42.15	4.10	8.95	3.72
101083	KOR	Landrace	Red	4.04	40.75	4.09	8.93	4.14
101098	KOR	Landrace	Red	3.38	23.26	2.70	6.14	2.95
101355	KOR	Landrace	Gray	3.11	26.69	3.53	6.26	2.58
102814	KOR	Landrace	Red	3.72	46.72	4.09	8.25	4.16
102856	KOR	Landrace	Red	4.59	48.50	5.23	10.83	5.82
102970	KOR	Landrace	Red	3.79	30.25	2.37	5.37	2.88
102977	KOR	Landrace	Red	3.50	43.79	3.07	7.33	3.91
103037	KOR	Landrace	Red	3.56	24.41	1.95	4.64	2.59
103054	KOR	Landrace	Red	3.94	45.13	4.02	8.17	4.42
103060	KOR	Landrace	Gray	5.08	76.34	9.06	12.53	5.58
103329	KOR	Landrace	Red	3.63	34.39	2.37	5.64	3.11
103372	KOR	Landrace	Red	4.02	37.01	3.75	7.76	4.20
103420	KOR	Landrace	Gray	3.37	33.36	4.28	6.64	2.81
103427	KOR	Landrace	Gray	3.63	42.70	4.50	7.63	3.01
103581	KOR	Landrace	Gray	3.48	38.12	4.27	7.50	2.89
103602	KOR	Landrace	Gray	2.82	33.62	3.29	6.49	2.33
103603	KOR	Landrace	Red	3.45	34.57	2.25	5.49	2.79
103719	KOR	Landrace	Red	4.32	40.23	3.46	7.29	3.74
103751	KOR	Landrace	Red	3.83	47.04	3.02	6.74	3.54
103771	KOR	Landrace	Red	3.62	37.98	2.55	5.22	2.80
103937	KOR	Landrace	Red	3.84	28.68	5.72	9.17	4.69
103953	KOR	Landrace	Red	4.07	30.22	4.42	7.44	4.15
103962	KOR	Landrace	Red	3.95	32.93	5.39	8.86	4.69
103972	KOR	Landrace	Red	3.95	25.62	4.07	7.95	4.03
103973	KOR	Landrace	Red	4.77	58.52	9.39	12.59	6.69
104019	KOR	Landrace	Red	3.91	44.49	5.08	9.51	4.77
104158	KOR	Landrace	Red	3.81	33.17	3.38	6.80	3.60
104171	KOR	Landrace	Red	4.88	46.94	7.12	11.71	5.64
104203	KOR	Landrace	White	1.75	20.74	0.34	1.57	1.37
104275	KOR	Landrace	Gray	2.40	20.46	1.67	3.80	1.37
104301	KOR	Landrace	White	1.80	31.54	0.26	2.08	1.39
104309	KOR	Landrace	Gray	3.32	32.44	2.54	5.17	1.93
104400	KOR	Landrace	Gray	3.71	26.80	2.47	5.43	1.86
104439	KOR	Landrace	Red	3.35	31.93	2.93	7.65	2.60

Supplementary Table 1. General information and average total metabolite content and antioxidant activity of 296 Adzuki bean accessions grown in Korea (Continued).

General information				Metabolite contents and antioxidant activities				
IT-No.	Origin	Genotype	Seed coat color	TPC (mg GAE/g)	TSC (mg DE/g)	DPPH (mg AAE/g)	ABTS (mg TE/g)	FRAP (mg AAE/g)
104459	KOR	Landrace	Red	3.37	34.57	3.89	9.08	3.07
104530	KOR	Landrace	Red	3.85	37.91	4.07	8.93	3.15
104547	KOR	Landrace	Red	3.58	36.38	3.90	8.06	2.63
104549	KOR	Landrace	Red	4.28	52.02	5.47	9.81	3.63
104552	KOR	Landrace	Red	4.15	47.13	4.58	9.84	3.70
104753	KOR	Landrace	Red	3.42	38.97	4.05	5.34	2.50
104814	KOR	Landrace	Red	3.42	31.61	3.96	5.29	2.39
104825	KOR	Landrace	Red	3.28	33.52	4.03	5.14	2.40
104833	KOR	Landrace	Red	3.40	37.72	3.81	5.15	2.43
104855	KOR	Landrace	Gray	4.63	54.72	7.04	12.32	4.70
104868	KOR	Landrace	Red	3.74	43.56	4.72	6.81	3.19
104914	KOR	Landrace	White	2.01	25.58	0.30	2.14	1.35
105196	KOR	Landrace	Gray	3.30	20.50	2.28	5.92	1.91
105281	KOR	Landrace	Gray	3.83	37.10	4.02	7.65	3.24
105318	KOR	Landrace	Gray	3.92	39.44	4.23	7.83	3.44
105343	KOR	Landrace	Gray	3.34	36.14	3.38	7.13	2.70
105403	KOR	Landrace	Gray	4.17	53.04	5.14	9.58	3.83
105427	KOR	Landrace	Gray	3.97	55.71	4.79	9.10	3.66
105573	KOR	Landrace	Red	3.52	42.10	4.29	6.70	3.23
105591	KOR	Landrace	Red	4.65	61.25	6.44	10.42	4.95
105592	KOR	Landrace	Gray	4.06	47.14	5.39	9.35	3.91
105632	KOR	Landrace	Red	4.52	64.10	6.69	10.11	4.21
105643	KOR	Landrace	Red	4.74	62.66	7.34	10.66	5.04
105698	KOR	Landrace	White	1.52	16.37	0.27	2.02	1.18
105890	KOR	Landrace	White	1.63	19.66	0.23	2.28	1.32
105959	KOR	Landrace	Gray	3.39	26.29	3.06	6.32	2.38
108674	KOR	Landrace	Gray	4.18	60.99	5.97	10.90	4.27
108770	KOR	Landrace	Gray	4.22	32.04	5.73	10.62	4.10
108791	KOR	Landrace	Gray	3.81	29.11	3.17	6.54	2.50
108821	KOR	Landrace	Red	3.91	75.02	4.52	7.28	3.12
108865	KOR	Landrace	Gray	4.56	52.12	5.90	10.02	4.20
108880	KOR	Landrace	Red	2.94	35.43	1.67	7.28	1.84
108932	KOR	Landrace	Gray	4.37	57.51	5.87	9.87	4.16
108935	KOR	Landrace	Red	3.12	36.59	1.36	6.94	2.41
108952	KOR	Landrace	Red	2.97	33.59	1.20	5.62	1.76
109112	KOR	Landrace	Gray	3.42	32.43	2.01	4.90	2.04
110996	KOR	Landrace	Gray	3.40	28.33	2.48	6.34	2.60
111131	KOR	Landrace	Red	2.70	25.30	3.15	6.71	2.29
111146	KOR	Landrace	Red	2.98	29.02	3.29	7.34	2.67
111148	KOR	Landrace	Red	3.86	37.45	5.57	11.54	4.33

Supplementary Table 1. General information and average total metabolite content and antioxidant activity of 296 Adzuki bean accessions grown in Korea (Continued).

General information				Metabolite contents and antioxidant activities				
IT-No.	Origin	Genotype	Seed coat color	TPC (mg GAE/g)	TSC (mg DE/g)	DPPH (mg AAE/g)	ABTS (mg TE/g)	FRAP (mg AAE/g)
111160	KOR	Landrace	Red	3.09	35.91	3.14	7.30	2.38
111165	KOR	Landrace	Gray	3.63	30.01	2.32	6.74	2.57
112783	KOR	Landrace	Gray	3.92	35.20	2.50	7.14	2.74
112824	KOR	Landrace	Gray	4.27	45.22	3.41	8.95	3.82
112871	KOR	Landrace	White	1.86	20.34	0.27	2.13	1.30
112935	KOR	Landrace	Gray	4.78	46.62	4.03	11.10	4.33
113119	KOR	Landrace	Gray	3.56	35.33	2.50	6.50	2.58
113139	KOR	Landrace	White	1.73	19.60	0.33	2.59	1.46
113215	KOR	Landrace	Red	3.41	30.33	2.95	6.57	3.23
113249	KOR	Landrace	White	2.01	25.15	0.28	2.31	1.52
113259	KOR	Landrace	Gray	4.10	41.33	3.34	9.46	3.54
113383	KOR	Landrace	Red	3.41	35.44	4.18	8.12	3.00
113419	KOR	Landrace	Red	3.34	31.38	3.41	7.54	3.55
113423	KOR	Landrace	White	2.10	24.20	0.33	1.90	1.41
113429	KOR	Landrace	Red	3.51	40.20	4.33	9.23	2.65
113436	KOR	Landrace	Gray	4.09	37.44	2.63	8.13	3.09
113437	KOR	Landrace	Red	3.27	33.44	3.12	7.21	2.97
113455	KOR	Landrace	Gray	3.30	32.84	2.23	6.48	2.38
119917	KOR	Landrace	Gray	3.59	40.43	3.09	8.25	3.24
119922	KOR	Landrace	Gray	4.45	43.76	3.29	8.81	3.54
121444	KOR	Landrace	Gray	4.75	52.47	4.02	8.81	4.51
121459	KOR	Landrace	Gray	5.37	57.97	4.77	10.69	5.21
134952	KOR	Landrace	Gray	4.79	50.23	3.70	7.54	4.11
134972	KOR	Landrace	Gray	4.41	44.63	2.86	5.17	3.31
138107	KOR	Landrace	White	1.91	15.61	0.27	1.50	1.24
138157	KOR	Landrace	White	2.01	26.54	0.29	2.17	1.41
138160	KOR	Landrace	Red	3.28	25.78	2.29	5.36	3.35
142423	JPN	Unknown	Red	3.88	35.81	3.94	8.67	4.66
142424	JPN	Unknown	Red	3.96	48.11	3.90	9.37	5.04
142425	JPN	Unknown	Red	3.97	38.52	3.96	9.20	4.92
142426	JPN	Unknown	Red	3.56	31.63	3.16	7.40	4.09
142427	JPN	Unknown	Red	4.14	38.15	4.49	8.88	5.06
142461	JPN	Unknown	Red	3.75	26.48	3.30	6.33	3.11
142462	JPN	Unknown	Red	4.25	33.25	4.60	7.46	3.81
142463	JPN	Unknown	Red	4.57	46.07	5.81	9.10	4.60
142707	JPN	Unknown	Red	4.01	29.86	4.38	7.54	3.65
158257	KOR	Landrace	Gray	4.77	41.08	3.55	7.16	4.03
158261	KOR	Landrace	Gray	4.71	45.97	3.71	10.16	3.81
162747	KOR	Landrace	White	2.02	28.47	0.28	2.13	1.34
178388	KOR	Landrace	White	2.03	20.61	0.32	2.14	1.50

Supplementary Table 1. General information and average total metabolite content and antioxidant activity of 296 Adzuki bean accessions grown in Korea (Continued).

General information				Metabolite contents and antioxidant activities				
IT-No.	Origin	Genotype	Seed coat color	TPC (mg GAE/g)	TSC (mg DE/g)	DPPH (mg AAE/g)	ABTS (mg TE/g)	FRAP (mg AAE/g)
178389	KOR	Landrace	Gray	4.82	51.72	4.17	10.74	4.37
178390	KOR	Landrace	White	2.16	28.55	0.52	2.90	1.89
178391	KOR	Landrace	Gray	6.26	60.31	6.11	14.27	5.98
178458	KOR	Landrace	Red	4.29	68.62	5.76	11.37	7.85
178461	KOR	Landrace	Gray	5.14	49.11	4.79	12.34	4.82
178518	KOR	Landrace	Gray	4.62	43.73	3.82	9.51	3.85
178519	KOR	Landrace	Gray	4.76	48.42	3.92	10.08	4.08
178521	KOR	Landrace	Red	3.04	45.37	2.96	6.84	4.75
178527	KOR	Landrace	Red	4.03	56.13	5.41	10.46	7.61
178528	KOR	Landrace	Gray	5.18	51.69	4.14	10.17	4.33
180464	KOR	Landrace	White	2.37	33.85	1.16	3.23	2.56
180468	KOR	Landrace	Red	3.35	55.34	4.44	8.69	6.17
180478	KOR	Landrace	Red	3.68	54.36	4.51	8.82	6.30
180498	KOR	Landrace	Gray	3.08	18.86	1.95	4.73	1.84
180537	KOR	Landrace	Gray	2.99	15.74	1.72	4.20	1.82
180601	KOR	Landrace	Gray	3.72	17.20	2.02	4.93	1.90
180627	KOR	Landrace	Gray	3.96	24.29	3.12	6.98	2.85
180879	KOR	Landrace	White	2.05	23.79	0.33	1.86	1.45
180885	KOR	Landrace	Gray	4.25	29.74	4.03	9.36	3.98
180906	KOR	Landrace	Red	3.81	57.15	4.33	8.28	3.70
180907	KOR	Landrace	Gray	3.86	23.75	2.87	7.19	2.92
180911	KOR	Landrace	White	1.97	25.48	0.32	1.82	1.39
180912	KOR	Landrace	Red	4.73	56.58	4.02	6.90	3.30
180913	KOR	Landrace	Red	3.26	70.61	4.57	9.43	4.10
180915	KOR	Landrace	White	2.08	21.86	0.34	2.27	1.44
180916	KOR	Landrace	Red	3.54	72.13	3.37	6.64	2.84
181898	KOR	Landrace	Red	3.48	49.40	4.05	5.64	2.84
181902	KOR	Landrace	White	2.03	25.75	0.33	2.22	1.45
181958	KOR	Landrace	White	2.04	26.54	0.34	2.59	1.52
186247	KOR	Landrace	Gray	4.59	27.89	3.77	8.61	3.69
186248	KOR	Landrace	Gray	4.71	35.71	4.86	10.24	4.52
186253	KOR	Landrace	Gray	4.42	33.79	4.34	9.78	3.94
186257	KOR	Landrace	Gray	3.70	18.54	1.94	4.94	2.16
186262	KOR	Landrace	White	2.08	28.55	0.34	2.70	1.61
186281	KOR	Landrace	Gray	3.86	26.81	2.43	6.51	3.14
186291	KOR	Landrace	White	1.93	22.95	0.28	2.06	1.40
186294	KOR	Landrace	White	1.86	16.45	0.30	2.05	1.39
186298	KOR	Landrace	Red	4.05	31.33	3.75	8.28	3.93
186299	KOR	Landrace	Red	4.22	38.61	4.23	8.75	4.22
186300	KOR	Landrace	Gray	3.64	25.40	3.20	7.38	3.34

Supplementary Table 1. General information and average total metabolite content and antioxidant activity of 296 Adzuki bean accessions grown in Korea (Continued).

General information				Metabolite contents and antioxidant activities				
IT-No.	Origin	Genotype	Seed coat color	TPC (mg GAE/g)	TSC (mg DE/g)	DPPH (mg AAE/g)	ABTS (mg TE/g)	FRAP (mg AAE/g)
186306	KOR	Landrace	Gray	4.64	36.59	4.58	10.20	4.68
186307	KOR	Landrace	Gray	4.29	27.17	3.29	7.55	3.60
186309	KOR	Landrace	Red	5.54	53.56	6.98	13.27	6.26
186310	KOR	Landrace	Red	3.66	31.25	3.30	7.57	3.59
186313	KOR	Landrace	Gray	3.79	26.07	2.75	6.47	3.01
186314	KOR	Landrace	White	1.95	26.59	0.29	2.12	1.42
186322	KOR	Landrace	Gray	5.52	41.10	5.79	12.54	5.97
186324	KOR	Landrace	Gray	4.64	37.23	4.91	11.14	5.06
186325	KOR	Landrace	Gray	4.43	31.40	3.84	8.86	4.07
186326	KOR	Landrace	Gray	4.49	28.23	3.34	7.88	3.54
186327	KOR	Landrace	Gray	4.94	35.98	4.74	10.75	4.70
186328	KOR	Landrace	Gray	4.90	33.40	4.46	9.92	4.46
186330	KOR	Landrace	White	1.93	19.38	0.29	1.06	0.60
186331	KOR	Landrace	Gray	4.24	27.43	3.66	7.92	3.82
186333	KOR	Landrace	White	1.88	23.59	0.27	1.42	0.67
189358	JPN	Unknown	Red	3.53	32.46	4.80	8.19	4.72
189359	JPN	Unknown	Red	3.76	39.57	4.44	8.60	4.53
189360	JPN	Unknown	Red	3.26	24.49	2.82	5.55	3.17
189361	JPN	Unknown	Red	3.59	36.40	3.96	7.05	3.76
189362	JPN	Unknown	Red	3.94	74.76	5.24	9.45	4.94
191131	KOR	Landrace	Gray	4.31	32.54	4.04	8.47	3.83
191175	KOR	Landrace	Red	4.10	42.58	4.24	6.93	3.54
191178	KOR	Landrace	Gray	4.48	32.19	4.38	9.23	4.15
191248	KOR	Landrace	Gray	4.13	46.62	3.02	6.60	3.82
194572	KOR	Landrace	Red	3.32	36.72	3.25	6.46	3.63
194574	KOR	Landrace	Red	3.54	43.17	3.94	7.28	3.96
194575	KOR	Landrace	Red	6.34	114.22	12.84	17.66	13.14
194577	KOR	Landrace	Red	3.25	39.26	3.54	6.35	3.56
195036	KOR	Landrace	Gray	4.05	43.65	3.63	8.25	4.66
195050	KOR	Landrace	Gray	3.52	40.60	2.45	6.35	3.57
195131	KOR	Landrace	White	2.08	22.50	0.36	1.49	0.85
195132	KOR	Landrace	Gray	4.46	43.01	2.48	5.86	3.34
195142	KOR	Landrace	Gray	3.90	70.69	3.03	7.77	4.39
195224	KOR	Landrace	Gray	4.96	77.65	5.13	11.58	6.76
195325	KOR	Landrace	Gray	3.67	70.17	2.88	7.11	4.45
195356	KOR	Landrace	Gray	3.52	70.56	3.35	8.73	5.14
195363	KOR	Landrace	Gray	3.90	73.05	2.60	6.87	4.28
195384	KOR	Landrace	Gray	3.50	47.56	2.35	5.45	3.51
195537	KOR	Landrace	Gray	4.03	82.80	4.17	10.07	6.11
195594	KOR	Landrace	Gray	3.86	80.87	3.79	8.69	5.18

Supplementary Table 1. General information and average total metabolite content and antioxidant activity of 296 Adzuki bean accessions grown in Korea (Continued).

General information				Metabolite contents and antioxidant activities				
IT-No.	Origin	Genotype	Seed coat color	TPC (mg GAE/g)	TSC (mg DE/g)	DPPH (mg AAE/g)	ABTS (mg TE/g)	FRAP (mg AAE/g)
196868	KOR	Landrace	Gray	3.82	68.06	3.41	8.34	5.07
196869	KOR	Landrace	Gray	4.16	53.37	2.65	6.30	3.86
196870	KOR	Landrace	Red	4.18	53.53	5.62	8.46	7.32
196871	KOR	Landrace	Red	3.48	46.25	3.82	6.61	5.36
196872	KOR	Landrace	White	1.75	17.50	0.27	1.11	0.59
196894	KOR	Landrace	Red	3.66	42.92	4.21	7.10	6.43
196895	KOR	Landrace	Gray	5.55	57.96	6.47	13.17	8.10
196897	KOR	Landrace	Gray	4.10	77.05	3.71	8.91	5.18
204149	KOR	Landrace	Red	4.03	45.20	4.37	6.66	6.38
204158	KOR	Landrace	White	1.83	16.71	0.29	1.47	0.73
208533	KOR	Landrace	Red	3.40	40.69	3.45	4.88	4.05
208534	KOR	Landrace	Red	3.38	36.41	3.56	4.97	6.32
208535	KOR	Landrace	Red	3.37	34.26	3.14	5.17	5.58
208790	KOR	Landrace	Gray	4.24	82.46	3.55	8.51	5.29
208791	KOR	Landrace	Red	3.89	48.15	4.78	6.89	6.59
208810	KOR	Landrace	Gray	3.63	72.66	3.13	7.76	4.53
208846	KOR	Landrace	Red	3.13	38.39	3.36	3.84	5.27
208890	KOR	Landrace	Red	3.24	32.66	2.23	3.90	3.80
208913	KOR	Landrace	Gray	4.52	89.64	4.64	10.32	6.06
208944	KOR	Landrace	White	1.96	18.17	0.29	1.51	1.12
209326	KOR	Landrace	Gray	3.62	72.62	2.93	7.72	4.71
211854	KOR	Landrace	Gray	4.48	91.36	3.65	9.02	5.45
213272	KOR	Landrace	Gray	3.70	56.59	2.96	6.91	4.03
216285	JPN	Landrace	Gray	5.33	108.24	9.43	16.21	10.84
216286	JPN	Landrace	Gray	8.24	87.61	11.21	14.54	10.50
216289	JPN	Landrace	Gray	5.91	75.35	6.39	10.97	6.60
216290	JPN	Landrace	Gray	4.28	64.65	4.31	10.18	4.31
216291	JPN	Landrace	Gray	5.79	67.10	7.28	10.96	7.55
216295	JPN	Landrace	Gray	3.78	50.84	6.33	12.82	6.85
216298	JPN	Landrace	Gray	6.66	106.16	8.03	14.90	8.37
216299	JPN	Landrace	Gray	6.68	100.91	6.94	14.73	8.09
216300	JPN	Landrace	Gray	6.18	66.62	5.97	14.12	7.18
216303	JPN	Landrace	Gray	7.10	106.44	6.48	14.21	7.51
216305	JPN	Landrace	Gray	7.11	107.21	7.86	16.48	9.67
216306	JPN	Landrace	Gray	7.64	79.42	8.74	16.80	10.09
218257	KOR	Landrace	Gray	3.87	55.58	4.04	9.78	4.74
219512	KOR	Landrace	Gray	3.65	49.18	2.84	6.87	3.16
229635	KOR	Landrace	Gray	4.53	41.47	4.76	11.06	5.36
229704	KOR	Landrace	Gray	3.77	49.13	3.14	7.26	3.50
229706	KOR	Landrace	Gray	4.71	53.51	4.89	11.25	5.50

Supplementary Table 1. General information and average total metabolite content and antioxidant activity of 296 Adzuki bean accessions grown in Korea (Continued).

General information				Metabolite contents and antioxidant activities				
IT-No.	Origin	Genotype	Seed coat color	TPC (mg GAE/g)	TSC (mg DE/g)	DPPH (mg AAE/g)	ABTS (mg TE/g)	FRAP (mg AAE/g)
229962	KOR	Landrace	Gray	4.28	53.81	3.81	9.41	4.41
235359	KOR	Landrace	Gray	4.14	47.46	3.74	9.24	3.93
236188	KOR	Landrace	White	1.98	15.59	0.31	1.38	1.20
236196	KOR	Landrace	Gray	4.28	55.25	3.75	8.70	4.06
236199	KOR	Landrace	White	2.04	19.21	0.28	1.28	1.22
236206	KOR	Landrace	Gray	2.79	32.79	3.74	8.89	4.15
236251	KOR	Landrace	White	2.15	20.13	0.35	1.69	1.27
236259	KOR	Landrace	White	2.08	15.23	0.34	1.05	1.10
236268	KOR	Landrace	Gray	3.52	42.01	2.73	6.72	3.17
236283	KOR	Landrace	Gray	4.25	42.55	3.48	8.82	4.31
236286	KOR	Landrace	Gray	3.81	41.29	3.13	7.57	3.39
236287	KOR	Landrace	Gray	4.03	43.06	2.81	6.31	3.10
236292	KOR	Landrace	White	1.92	18.79	0.32	1.26	1.19
236304	KOR	Landrace	White	2.11	20.34	0.35	1.71	1.32
236596	CHN	Landrace	Gray	4.18	31.28	3.21	7.72	3.59
236597	CHN	Landrace	Gray	3.25	53.73	2.35	4.83	2.56
238022	KOR	Landrace	Gray	3.66	65.80	3.15	6.55	3.66
239918	KOR	Landrace	White	2.15	23.34	0.37	1.93	1.20
239998	KOR	Landrace	Gray	3.68	67.73	3.14	6.94	3.70
239999	KOR	Landrace	Gray	3.69	69.82	3.76	8.31	4.36
240001	KOR	Landrace	Gray	3.69	52.68	2.41	5.14	2.88
240085	KOR	Landrace	Gray	3.87	65.60	3.15	7.09	4.13
240087	KOR	Landrace	Gray	3.69	48.63	3.37	6.90	3.90
240113	KOR	Landrace	Gray	3.79	69.59	3.17	8.16	4.45
240343	KOR	Landrace	Gray	4.90	100.55	4.97	12.09	6.60
240374	KOR	Landrace	Gray	3.67	52.55	1.85	5.21	3.05
242323	KOR	Landrace	Gray	4.10	72.26	3.56	9.28	5.13
242395	KOR	Landrace	Gray	4.03	74.15	2.93	8.29	4.50
252929	KOR	Landrace	Gray	4.40	85.38	4.66	10.57	5.83
252938	KOR	Landrace	Gray	4.03	59.24	2.97	8.49	3.79
252941	KOR	Landrace	Gray	3.88	75.33	3.37	7.89	4.41
263894	KOR	Landrace	Gray	3.83	73.54	3.38	8.05	4.49
263906	KOR	Landrace	Gray	3.82	60.53	3.13	7.98	4.36
280293	CHN	Landrace	Gray	3.99	72.70	3.45	8.29	4.52
280295	CHN	Landrace	Gray	5.16	80.91	4.25	10.09	5.21
280306	CHN	Landrace	Gray	4.05	73.31	3.28	8.46	4.32
280309	CHN	Landrace	Gray	4.36	84.16	3.73	9.38	4.95
283300	KOR	Landrace	Gray	4.85	90.28	4.72	10.63	5.67
286492	KOR	Landrace	Gray	4.12	73.48	2.36	8.80	4.95
290138	KOR	Landrace	Gray	3.77	63.40	1.69	7.15	4.15

Supplementary Table 1. General information and average total metabolite content and antioxidant activity of 296 Adzuki bean accessions grown in Korea (Continued).

General information				Metabolite contents and antioxidant activities				
IT-No.	Origin	Genotype	Seed coat color	TPC (mg GAE/g)	TSC (mg DE/g)	DPPH (mg AAE/g)	ABTS (mg TE/g)	FRAP (mg AAE/g)
292991	KOR	Landrace	Gray	3.47	28.76	2.38	4.68	2.71
293064	KOR	Landrace	Gray	2.60	47.12	5.34	9.21	5.40
293065	KOR	Landrace	Gray	3.71	41.43	3.94	6.86	4.05
293132	JPN	Landrace	Gray	2.50	19.56	1.13	2.36	1.69
293155	CHN	Landrace	Gray	2.14	21.78	1.81	3.41	2.19
293444	KOR	Landrace	Gray	3.39	45.21	4.70	7.23	4.48
293450	KOR	Unknown	Gray	4.40	55.74	5.50	8.76	5.21
293759	PRK	Unknown	Gray	3.17	47.23	3.68	7.29	4.40
293760	PRK	Unknown	Gray	2.92	37.21	3.20	6.31	3.64
293929	KOR	Landrace	Gray	3.59	35.05	3.68	5.77	3.49
293930	KOR	Landrace	Gray	3.64	45.39	4.15	8.13	4.62
294630	JPN	Breeding line	White	1.85	14.36	0.28	1.60	0.98
294637	JPN	Landrace	Gray	3.24	42.28	3.57	6.82	4.18
296738	KOR	Landrace	Gray	3.08	41.34	3.71	6.72	4.14
296739	KOR	Landrace	Gray	3.59	48.40	4.31	7.91	4.92
318778	KOR	Landrace	Gray	3.14	40.08	3.06	6.10	3.75
Minimum				1.52	14.36	0.23	1.05	0.59
Maximum				8.24	114.22	12.84	17.66	13.14
Mean				3.75	44.27	3.53	7.43	3.84
SD				1.03	19.72	1.91	3.11	1.80
CV (%)				27.47	44.54	54.04	41.88	46.79

AAE, ascorbic acid equivalent; ABTS, $ABTS^{\bullet+}$ scavenging activity; CHN: China, CV: Coefficient of variation; DE: Diosgenin equivalent; DPPH, $DPPH^{\bullet}$ scavenging activity; FRAP: Ferric reducing antioxidant power; GAE: Gallic acid equivalent; JPN: Japan, KOR: South Korea, PRK: North Korea SD: Standard deviation; TE: Trolox equivalent; TPC: Total phenolic content; TSC: Total saponin content.