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Research Article

Comparison of Antioxidant Components and Activities of Korean Black Soybeans

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Abstract Black soybeans are valued for their rich nutritional content and potential health benefits, attributed to their functional components that enhance antioxidant activity. In this study, we evaluate and compare the isoflavone and anthocyanin content, as well as the antioxidant potential, of seven Korean black soybean genotypes. Isoflavone content ranged from 2,032.8 to 3,536.8 μg/g, with Soman displaying the highest levels of both aglycones and glucosides, indicating notable bioactive potential. In terms of anthocyanins, Danheuk had the highest total content (24,080.6 μg/g), while Soman excelled in Pelargonidin-3-glucoside (Pg3glc). Soman also showed superior antioxidant activity across all measures, including total polyphenol, flavonoid content, as well as radical scavenging abilities (ABTS and DPPH). Strong correlations were found between total flavonoid content, total polyphenol content, genistin, total isoflavone content and antioxidant activity, while correlations with total anthocyanins were relatively weaker. These findings reveal significant genetic variability in isoflavone and anthocyanin content among soybean genotypes, with Soman showing particularly high antioxidant potential, suggesting its value for health-related applications and soybean breeding programs.

Key words Black soybean, Antioxidant, Isoflavone, Anthocyanin, Soman

Introduction

Soybean (*Glycine max*) is one of the most widely cultivated crops globally due to its extensive nutritional benefits and its unique seed composition, which includes high protein and oil content (Seo et al. 2019). Native to East Asia, soybeans have been integral to diets in many cultures for thousands of years, particularly in Asian countries where they are used in traditional dishes and medicinal preparations (Liu et al. 2022; Rizzo and Baroni 2018). Nutritionally, soybeans are a rich source of protein, making them a valuable plant-based protein option. They also provide essential amino acids, vitamins (including B vitamins), and minerals such as iron, calcium, and magnesium (Omoni et al. 2005; Qin et al. 2022). In addition to their nutritional value, soybeans are noted for their beneficial phytochemicals, including isoflavones and anthocyanins, which have been associated with various health benefits (Isanga & Zhang 2008). Soybeans have seed coats in a range of colors, such as yellow, black, brown, and green. Soybeans with a black seed coat are specifically known as black soybeans.

Black soybeans have a long history of use as folk medicine in China, Japan, and Korea, where they have been widely utilized for centuries (Dhungana et al. 2021; Kim et al. 2015; Xu & Chang 2008). Black soybeans contain higher levels of functional components such as anthocyanins compared to yellow soybeans, which has led to an increased use as ingredients in health-functional foods (Kumar et al. 2023; Yoon et al. 2018a). Research

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indicates that black soybeans contain higher concentrations of anthocyanins compared to other types of soybeans (Cho et al. 2013; Kim et al. 2015; Koh et al. 2014; Kumar et al. 2023).

The primary antioxidant components found in black soybeans are isoflavones and anthocyanins, which contribute significantly to their antioxidant activity (Choi et al. 2020; Lee et al. 2016). Anthocyanins are water-soluble natural pigments classified within the flavonoid group of polyphenols. The principal anthocyanins identified in the seed coat of black soybean include cyaniding-3-glucoside (Cy3glc), delphinidin-3-glucoside (Dp3glc), pelargonidin-3-glucoside (Pg3glc), and petunidin-3-glucoside (Pt3glc) (Choung et al. 2001; Lee et al. 2009). Additionally, several minor anthocyanins such as cyanidin-3-galactoside (Cy3gal), and peonidin-3-glucoside (Pn3glc) have been isolated (Ganesan & Xu 2017; Koh et al. 2014; Lee et al. 2009). Anthocyanins derived from black soybeans offer potential health benefits as complementary medicine and are incorporated into various formulations for their antioxidant, anti-inflammatory, nephroprotective, antidiabetic, anticancer, and anti-obesity effects, as demonstrated by many studies (Badshah et al. 2015; Jan et al. 2022; Kumar et al. 2023; Kurimoto et al. 2013; Kwon et al. 2007; Min et al. 2015).

Like other soybeans, black soybeans also contain isoflavones. Soy isoflavones are flavonoid compounds in 12 different chemical forms that act as phytoestrogens, potentially benefiting hormone balance, heart health, and bone density, and also reducing inflammation and oxidative stress (Akhlaghi et al. 2020; Alekel et al. 2015; Kim et al. 2019; Tit et al. 2018; Yu et al. 2016). These include aglycones like daidzein, glycitein, and genistein, as well as glucoside derivatives such as daidzin, glycitin, and genistin (Kim et al. 2019). Additionally, there are glucosides with malonyl-esters (e.g., 6″-O-malonyldaidzin) and glucosides with acetyl-esters (e.g., 6″-O-acetyldaidzin) (Yu & McGonigle 2005). While aglycones are present in smaller amounts, glucoside forms are more abundant, with malonylglucosides being the most common (Ahmad et al. 2017). Glucoside forms of isoflavones are inactive until they are converted into the active aglycone form by intestinal microbiota through hydrolysis in the human digestive system (Izumi et al. 2000).

These compounds have been demonstrated to have diverse and synergistic effects in preventing diseases such as diabetes, cancer, inflammation, and heart disease (Rimbach et al. 2008; Zhang et al. 2019). Additionally, they are believed to be among the most effective natural antioxidants due to their ability to neutralize free radicals and manage the excessive production of reactive oxygen species and reactive nitrogen species in the body (Kumar et al. 2023).

Research on soybean breeding for enhanced functional components has highlighted the significance of various nutrient-rich genotypes. Several studies have investigated anthocyanins and antioxidant potential in Korean black soybean landraces, as well as the isoflavone composition across different varieties (Choi et al. 2020; Kim et al. 2019; Lee et al. 2020). Additionally, other research has examined polyphenol content and antioxidant properties in colored soybeans (Cho et al. 2013; Malenčić et al. 2012). Broader analyses by have covered isoflavones, anthocyanins, and phenolics in black soybeans (Bursać et al. 2017; Choi et al. 2020; Dhungana et al. 2021; Koh et al. 2014). Elite Korean black soybean varieties, such as Cheongja3ho, Cheongja5ho, and Socheongja have been studied for their health benefits (Eum et al. 2020; Haque et al. 2016; Jeong et al. 2023; Kwon et al. 2007; Yoon et al. 2018b), providing a comprehensive understanding of how different genotypes contribute to improved health outcomes through their functional components. However, the relationship between antioxidant components and

their activity across different genetic backgrounds remains unclear.

The objectives of this study were to evaluate and compare the isoflavone and anthocyanin content, as well as the antioxidant potential, of seven Korean black soybean genotypes; to identify those with superior antioxidant properties and bioactive profiles; and to understand the relationship between antioxidant components and their potentials.

Materials and Methods

Plant materials

A total of seven Korean black soybean genotypes, Soman, Seoritae, Cheongja3ho, Cheongja5ho, Socheongja, Soriheuk, and Danheuk, including one landrace (Seoritae) and six cultivars, were used in this study. The isoflavone and anthocyanin content, as well as the antioxidant potential, were measured in soybean seeds grown in the field at the National Institute of Crop Science (Miryang, 35°29′32″ N, 128°44′35″ E) during the years 2019 and 2020. There were no replicated plots of each genotype within a year.

Isoflavone analysis

Standards of daidzein, glycitein, genistein, daidzin, glycitin, genistin, Malonyl Daizin, Malonyl Glycitin, Malonyl Genistin, Acetyl Daidzin, Acetyl Glycitin, and Acetyl Genistin were used and purchased from Sigma Aldrich (St. Louis, MO, USA). One gram of seed powder was subjected to extraction using 20 mL of 50% methanol, with continuous stirring at room temperature for 24 hours. After extraction, the mixture was centrifuged at 13,500 rpm for 10 minutes. The supernatant was then filtered through a 0.2 μm filter and collected into 1.5 mL vials. The isoflavone content in the extract was analyzed using an HPLC system (Ultimate 3000 HPLC, Dionex, Sunnyvale, CA, USA). The HPLC conditions were as follows: column - Lichrospher RPC18 (5 μm, 4 mm × 125 mm); solvent A - distilled water with 0.1% acetic acid; solvent B - acetonitrile with 0.1% acetic acid; flow rate - 1 mL/min; UV-Vis detector; sample injection volume - 10 μL (Dhungana et al. 2021).

Anthocyanin analysis

Standards of Dp3glc, Cy3gal, Cy3glc, Pt3glc, Pg3glc, and Pn3glc were used and purchased from Sigma Aldrich (St. Louis, MO, USA). The anthocyanin content in soybean seed coats was measured using the method outlined by Lee et al. (2009). For this, 0.1 g of hand-peeled seed coats were extracted with 30 mL of 20% methanol containing 1% (v/v) hydrochloric acid for 48 hours at refrigeration. After extraction, the mixture was centrifuged at 3,000 × g for 3 minutes at room temperature, and the supernatant was filtered through a 0.2 μm filter. The analysis of anthocyanins was performed using an HPLC system (Ultimate 3000 HPLC, Dionex, Sunnyvale, CA, USA) with a flow rate of 0.8 mL/min. The HPLC conditions were set as follows: column - YMC-Triart C18 (4.6 × 150 mm, 5 μm); solvent A - 0.1% trifluoroacetic acid in distilled water; solvent B - 0.1% trifluoroacetic

acid in methanol; detector - UV-Vis detector set to 530 nm; and the total analysis time was 45 minutes (Dhungana et al. 2021).

Preparation of extract for total polyphenol, total flavonoid, ABTS, and DPPH

Twenty grams of soybean seeds were ground into powder using a commercial grinder. Two grams of this powder were extracted with 10 mL of 80% ethanol for 24 hours in a shaking incubator set at 240 rpm. After the initial extraction, the process was repeated with an additional 10 mL of ethanol, and the old extract was transferred to a new Falcon tube. The first and second extracts were combined, followed by centrifugation at 3,000 × g for 3 minutes at room temperature. The resulting supernatant was then filtered through a 0.45 μm syringe filter (Dhungana et al. 2021).

ABTS and DPPH radical scavenging assay

The ABTS radical scavenging activity was assessed using the method outlined by Lee & Cho (2012) with minor modifications. An ABTS·+ stock solution was prepared by mixing a 7.4 mM ABTS·+ solution with a 2.6 mM potassium persulfate solution, both in ethanol, in a 1:1 ratio. This mixture was left in the dark at room temperature for approximately 14 hours. The stock solution was then diluted with ethanol to achieve an absorbance of 0.2-1.0 at 735 nm. In a 96-well plate, 200 μL of the ABTS·+ solution was combined with 20 μL of sample extracts and Trolox. The reaction was allowed to proceed for 30 minutes, after which absorbance was measured at 735 nm using a spectrophotometer (Thermo, Multiskan Spectrum, Vantaa, Finland). The scavenging activity was reported as Trolox equivalents (Dhungana et al. 2021).

For DPPH radical scavenging activity, the method described by Cho et al. (2013) was employed. Twenty microliters of sample extracts and Trolox were added to a 96-well plate. Following this, 200 μL of a 0.2 mM DPPH solution was added and mixed. The reaction mixture was kept in the dark for 30 minutes before measuring the absorbance at 520 nm using a spectrophotometer (Thermo, Multiskan Spectrum). The scavenging activity was calculated as Trolox equivalents (Dhungana et al. 2021).

Measurement of total polyphenol and flavonoid content

The total polyphenol content was measured using the Folin-Ciocalteu method, adapted from Celli et al. (2011). In a 96-well plate, 20 μL of sample extracts were combined with 100 μL of 10% Folin-Ciocalteu reagent and allowed to react for 5 minutes. Subsequently, 80 μL of 7.5% Na_2CO_3 was added, and the mixture was incubated in the dark for 30 minutes. A standard calibration curve was created using gallic acid (GA), with sample extracts replaced by GA concentrations of 0, 50, 100, 250, and 500 ppm. The absorbance of the reaction mixtures was then measured at 750 nm using a spectrophotometer (Thermo, Multiskan Spectrum) (Dhungana et al. 2021).

The total flavonoid content was determined using the method described by Celli et al. (2011) with some modifications. In 1.5 mL tubes, 100 μL of sample extracts were mixed with 400 μL of distilled water and 30 μL of 5% $NaNO_2$, then vortexed and left for 5 minutes. After this, 30 μL of 10% $AlCl_3$ was added, and the

mixture was allowed to stand for 6 minutes. Following this, 200 μL of 1 M NaOH and 240 μL of distilled water were added to the mixture, which was then vortexed. The absorbance was measured at 510 nm using a spectrophotometer (Thermo, Multiskan Spectrum). The calibration curve was plotted with catechin hydrate, and the total flavonoid content was reported as catechin equivalents (Dhungana et al. 2021).

Statistical analysis

Analysis of variance (ANOVA), Duncan's multiple range test (DMRT), and t-tests were used to compare antioxidant compounds and activities among the genotypes. Correlation coefficients were analyzed to examine the relationships between antioxidant compounds and their activities. The average values of three technical replicates were used for statistical analysis. All statistical analyses were performed using the R program (version 4.2.0).

Results

Analysis of variance (ANOVA) of antioxidant components and activity

The ANOVA analysis revealed highly significant differences among genotypes for isoflavone content, anthocyanin content, and antioxidant potentials ($p<0.001$), indicating substantial genetic effects on these traits. Environmental factors also significantly influenced isoflavone and anthocyanin levels ($p<0.001$), though genistein and Pt3glc showed no significant environmental effects. Additionally, there was a significant interaction between genotypes and experimental years ($p<0.001$), highlighting that the impact of genotype on these traits varies depending on the year. Overall, genotype plays a crucial role in determining these traits, with environmental conditions and year-specific factors also contributing to their variation (Supplementary Table S1).

Isoflavone content

The total isoflavone content and its various compositions differed significantly among the genotypes. The total isoflavone content ranged from 2032.8 to 3536.8 μg/g, with Soman having the highest level at 3536.8 μg/g, followed by Cheongja5ho (2724.0 μg/g), Danheuk (2460.4 μg/g), and Socheongja with the lowest at 2032.8 μg/g. Soman also had the highest total aglycone content, including daidzein, glycitein, and genistein, at 314.6 μg/g, while Socheongja had the lowest at 97.9 μg/g. The total glucoside content was highest in Soman at 576.7 μg/g, with Seoritae showing the lowest at 309.7 μg/g. Soman also exhibited the highest total malonylglucoside content at 2526.2 μg/g, largely due to high levels of malonyldaidzin and malonylgenistin. Soman's acetyl content is moderate among the varieties, with the highest concentration of acetyldaidzin. Notably, Seoritae had no detectable acetylglucosides (Fig. 1). Acetylgenistin was not detected in any of other varieties, whereas a minimal amount of it was detected in Soman (data not shown).

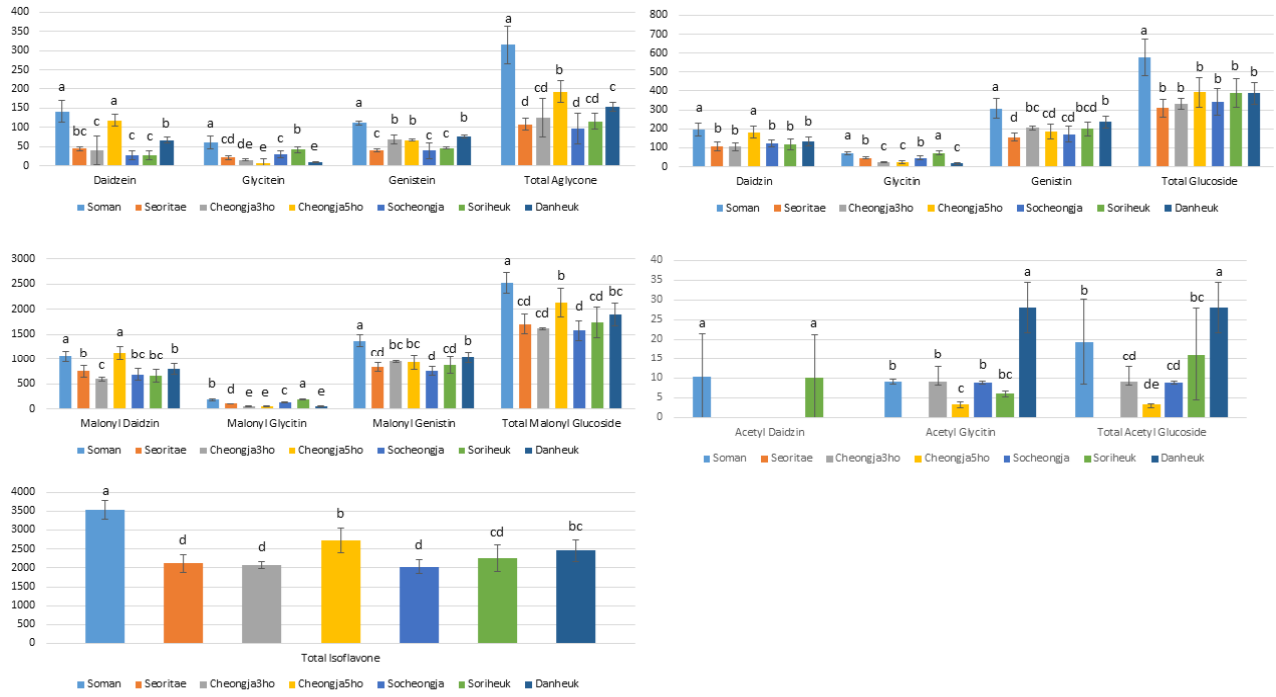

Fig. 1. Total isoflavone content and 12 compositions (μg/g) variations. Different letters indicate significantly different at $p<0.05$. Each Error bar indicates standard deviation (SD).

Daidzein and genistein content demonstrate very strong correlations total aglycone content (r=0.94**, 0.90**). In contrast, glycitein shows weaker correlations with the other compounds, with coefficients of 0.56** with total isoflavone content and 0.39** with genistein, and no significant relationship with daidzein content. Genistin and total glucoside content exhibit the strongest relationship, with a very high correlation (r=0.94**). Similarly, daidzin and total glucoside content also demonstrate a strong relationship (r=0.88**). The components with the highest correlations with total isoflavone are Malonylglucoside (r=0.99**), Malonylgenistin (r=0.93**), and Glucoside (r=0.90**). Conversely, components such as Malonylglycitin (r=0.27^{ns}) and Acetylglucoside (r=0.17^{ns}) have weaker correlations, indicating they contribute less to the overall isoflavone content (Table 1).

Table 1. Pearson's correlation among the total isoflavone and 12 compositions.

	De	Gle	Ge	TA	Di	Gly	Gi	TG	Mdi	Mgly	Mgi	TMG	AcDi	AcGly	AcGi	TAG	TIC
De	1	0.31	0.80	0.94	0.63	-0.02	0.38	0.46	0.71	0.02	0.55	0.68	0.32	-0.04	0.58	0.18	0.71
Gle	ns	1	0.39	0.56	0.07	0.72	0.25	0.33	-0.01	0.86	0.29	0.28	0.72	-0.18	0.64	0.33	0.35
Ge	**	**	1	0.90	0.51	0.00	0.63	0.55	0.52	0.04	0.79	0.70	0.29	0.28	0.54	0.41	0.75
TA	**	**	**	1	0.56	0.17	0.50	0.54	0.59	0.24	0.66	0.70	0.47	0.02	0.69	0.32	0.76
Di	**	ns	**	**	1	0.29	0.74	0.88	0.89	0.10	0.74	0.89	-0.09	0.02	0.17	-0.04	0.89
Gly	ns	**	ns	ns	ns	1	0.41	0.56	0.10	0.94	0.31	0.36	0.37	-0.34	0.26	-0.03	0.39
Gi	**	ns	**	**	**	**	1	0.94	0.49	0.24	0.89	0.77	0.03	0.37	0.24	0.31	0.82
TG	**	*	**	**	**	**	**	1	0.65	0.38	0.86	0.86	0.06	0.14	0.26	0.15	0.90
Mdi	**	ns	**	**	**	ns	**	**	1	-0.04	0.67	0.89	-0.11	-0.06	0.19	-0.12	0.85
Mgly	ns	**	ns	ns	ns	**	ns	*	ns	1	0.18	0.22	0.58	-0.29	0.38	0.15	0.27

Table 1. Continued.

	De	Gle	Ge	TA	Di	Gly	Gi	TG	Mdi	Mgly	Mgi	TMG	AcDi	AcGly	AcGi	TAG	TIC
Mgi	**	ns	**	**	**	**	**	**	**	ns	1	0.92	0.06	0.28	0.38	0.26	0.93
TMG	**	ns	**	**	**	**	**	**	**	ns	**	1	0.06	0.07	0.36	0.10	0.99
AcDi	*	**	ns	**	ns	*	ns	ns	ns	**	ns	ns	1	-0.07	0.66	0.60	0.13
AcGly	ns	ns	ns	ns	ns	*	*	ns	ns	ns	ns	ns	ns	1	-0.01	0.75	0.10
AcGi	**	**	**	**	ns	ns	ns	ns	ns	*	*	*	**	ns	1	0.43	0.41
TAG	ns	*	*	*	ns	ns	*	ns	ns	ns	ns	ns	**	**	**	1	0.17
TIC	**	*	**	**	**	*	**	**	**	ns	**	**	ns	ns	**	ns	1

De: daidzein, Gle: glycitein, Ge: genistein, TA: total aglycone, Di: daidzin, Gly: glycitin, Gi: genistin, TG: total glucoside, Mdi: malonyldaidzin, Mgly: malonylglycitin, Mgi: malonylgenistin, TMG: total malonylglucoside AcDi: acetyldaidzin, AcGly: acetylglycitin, AcGi: acetylgenistin, TAG: total acetylglucoside, TIC: total isoflavone content. ** and * mean there were significant difference at $p<0.01$ and 0.05, ns means no significantly different.

Anthocyanin content

Total anthocyanin content and the levels of six individual anthocyanins (Delphinidin-3-glucoside: Dp3glc, Cyanidin-3-galactoside: Cy3gal, Cyanidin-3-glucoside: Cy3glc, Petunidin-3-glucoside: Pt3glc, Pelargonidin-3-glucoside: Pg3glc, and Peonidin-3-glucoside: Pn3glc) varied significantly among the genotypes. Danheuk had the highest total anthocyanin content at 24,080.6 μg/g seed coat, followed by Socheongja with 20,613.1 μg/g seed coat, Cheongja5ho with 20,528.1 μg/g seed coat, and Seoritae with the lowest at 6,794.7 μg/g seed coat. Danheuk's total anthocyanin content was nearly 3.5 times higher than that of Seoritae (Fig. 2).

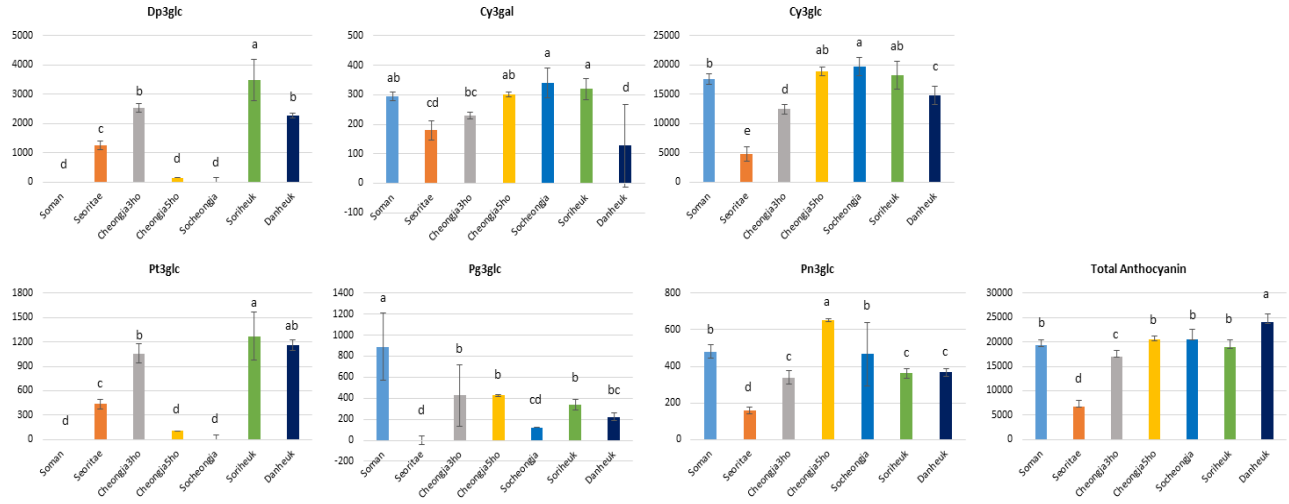

Fig. 2. Total anthocyanin content and 6 compositions (μg/g seed coat) variations. Different letters indicate significantly different at $p<0.05$. Each Error bar indicates standard deviation (SD).

The composition of individual anthocyanins also differed among the genotypes. Socheongja contained the highest levels of Cy3gal (340.1 μg/g seed coat) and Cy3glc (19,682.6 μg/g seed coat), which are major components of anthocyanins in black soybean seed coats. Soriheuk had the highest levels of Dp3glc (3,489.2 μg/g seed coat) and Pt3glc (1,271.6 μg/g seed coat). Pn3glc was most abundant in Cheongja5ho (651.6 μg/g seed coat), while

Soman had the highest Pg3glc content (891.2 μg/g seed coat). Pg3glc was not detected in Seoritae. Additionally, Dp3glc and Pt3glc were not detected in Soman and Socheongja (Fig. 2).

Total anthocyanin content has a very high positive correlation with Cy3glc (r=0.94**) and a strong positive correlation with Pn3glc (r=0.68**). Dp3glc has a very strong positive correlation with Pt3glc (r=0.97**), indicating that higher levels of Dp3glc are closely associated with higher levels of Pt3glc. However, Dp3glc shows negative correlations with other components, such as a moderate negative correlation with Cy3gal (r=-0.29^{ns}) and Pn3glc (r=-0.46**) (Table 2).

Table 2. Pearson's correlation among the total anthocyanin and 6 compositions.

	Dp3glc	Cy3gal	Cy3glc	Pt3glc	Pg3glc	Pn3glc	TAC
Dp3glc	1	-0.29	-0.24	0.97	-0.18	-0.46	0.10
Cy3gal	ns	1	0.66	-0.34	0.27	0.54	0.57
Cy3glc	ns	**	1	-0.21	0.39	0.80	0.94
Pt3glc	**	*	ns	1	-0.16	-0.41	0.12
Pg3glc	ns	ns	*	ns	1	0.45	0.39
Pn3glc	**	**	**	*	**	1	0.68
TAC	ns	**	**	ns	*	**	1

Dp3glc: delphinidin-3-glucoside, Cy3gal: cyaniding-3-galactoside, Cy3glc: cyaniding-3-glucoside, Pt3glc: petunidin-3-glucoside, Pg3glc: pelargonidin-3-glucoside, Pn3glc: peonidin-3-glucoside, TAC: total anthocyanin content.
** and * mean there were significant difference at p<0.01 and 0.05, ns means no significantly different.

Total polyphenol, flavonoid, ABTS, and DPPH

The antioxidant potentials varied among the genotypes. The range for total polyphenol content was 197.9 to 398.8 mg TE/100g, total flavonoid content ranged from 62.2 to 262.6 mg TE/100g, ABTS values ranged from 418.5 to 998.5 mg GAE/100g, and DPPH values ranged from 211.4 to 770.0 mg CAE/100g. Among the genotypes, Soman exhibited the highest levels for all measures: total polyphenol (398.8 mg TE/100g), total flavonoid (262.6 mg TE/100g), ABTS (998.5 mg GAE/100g), and DPPH (770.0 mg CAE/100g). In contrast, Seoritae had the lowest antioxidant potentials, with values of 197.9 mg TE/100g for polyphenols, 62.2 mg TE/100g for flavonoids, 418.5 mg GAE/100g for ABTS, and 211.4 mg CAE/100g for DPPH (Fig. 3).

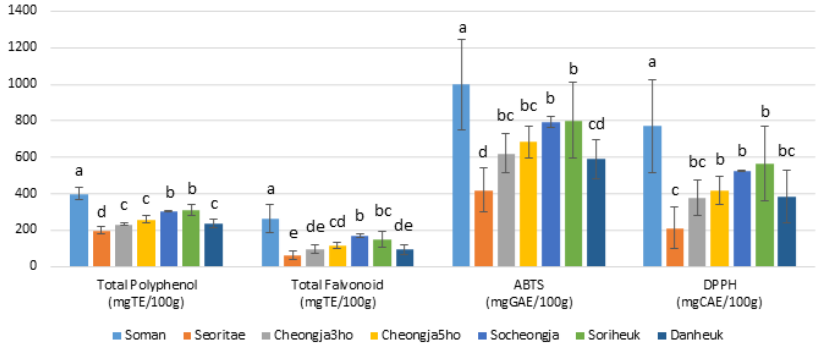

Fig. 3. Antioxidant potentials variations. Different letters indicate significantly different at p<0.05. Each Error bar indicates standard deviation (SD).

Total flavonoid is a particularly strong predictor of antioxidant activity, closely correlating with both ABTS and DPPH assays (r=0.95**, 0.95**). Additionally, total polyphenol also shows a significant correlation with these antioxidant measures (r=0.87**, 0.87**), although slightly weaker than total flavonoid. The near-perfect correlation between ABTS and DPPH (r=0.99**) indicates these two assays measure similar aspects of antioxidant activity (Table 3).

Table 3. Pearson's correlation among the antioxidant potentials.

	Total Polyphenol	Total Flavonoid	ABTS	DPPH
Total Polyphenol	1	0.94	0.87	0.87
Total Flavonoid	**	1	0.95	0.95
ABTS	**	**	1	0.99
DPPH	**	**	**	1

ABTS (2,2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid), DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity
** means there were significant difference at p<0.01.

Correlation between antioxidant components and activity

Isoflavone content was positively correlated with both ABTS and DPPH antioxidant activities. Specifically, daidzin (r=0.65**, 0.66**), glycitin (r=0.61**, 0.64**), genistin (r=0.70**, 0.76**), total glucoside (r=0.79**, 0.83**), malonylgenistin (r=0.64**, 0.68**), malonylglycitin (r=0.66**, 0.68**), and total isoflavone (r=0.67**, 0.69**) showed strong positive correlations. Regarding the correlation between anthocyanin content and antioxidant activity, positive correlations were observed with Cy3gal (r=0.59**, 0.56**), Cy3glc (r=0.67**, 0.62**), and total anthocyanin content (r=0.58**, 0.54**). Conversely, there were negative correlations between ABTS and DPPH with Dp3glc (r=-0.30, -0.26) and Pt3glc (r=-0.31, -0.27), although these were not statistically significant (Table 4).

Table 4. Pearson's correlation between the antioxidant components and activity.

	De	Gle	Ge	Di	Gly	Gi	Mdi	Mgly	Mgi	AcDi	AcGly	AcGi	TIC	Dp3glc	Cy3gal	Cy3glc	Pt3glc	Pg3glc	Pn3glc	TAC
ABTS	0.13^{ns}	0.37*	0.31*	0.65**	0.61**	0.70**	0.46**	0.48**	0.64**	-0.02^{ns}	0.08^{ns}	0.09^{ns}	0.67**	-0.30^{ns}	0.59**	0.67**	-0.31^{ns}	0.34*	0.43**	0.58**
DPPH	0.13^{ns}	0.39*	0.33*	0.66**	0.64**	0.76**	0.45**	0.50**	0.68**	-0.01^{ns}	0.13^{ns}	0.09^{ns}	0.69**	-0.26^{ns}	0.56**	0.62**	-0.27^{ns}	0.34*	0.35*	0.54**

De: daidzein, Gle: glycitein, Ge: genistein, Di: daidzin, Gly: glycitin, Gi: genistin, Mdi: malonyldaidzin, Mgly: malonylglycitin, Mgi: malonylgenistin, TMG: total malonylglucoside AcDi: acetyldaidzin, AcGly: acetylglycitin, AcGi: acetylgenistin, TAG: total acetylglucoside, TIC: total isoflavone content, Dp3glc: delphinidin-3-glucoside, Cy3gal: cyaniding-3-galactoside, Cy3glc: cyaniding-3-glucoside, Pt3glc: petunidin-3-glucoside, Pg3glc: pelargonidin-3-glucoside, Pn3glc: peonidin-3-glucoside, TAC: total anthocyanin content.
** and * mean there were significant difference at p<0.01 and 0.05, ns means no significantly different.

Discussion

In this study, we investigated the isoflavone and anthocyanin content, as well as the antioxidant potential, of seven Korean black soybean genotypes to identify those with superior antioxidant properties and bioactive profiles.

Isoflavone content

The significant variation in total isoflavone content and its composition across black soybean genotypes emphasize the genetic diversity that affects isoflavone profiles. In this study, total isoflavone content ranged from 2,032.8 to 3,536.8 μg/g. Soman, with its superior levels of both aglycones and glucosides, appears to have the greatest bioactive potential among the genotypes examined. In addition, total isoflavone in Soman shows a higher level than the domestic black soybean in reported researches (Dhungana et al. 2021; Kim et al. 2019; Yoon et al. 2021). Aglycones like daidzein, glycitein, and genistein are generally more effective than their glucoside counterparts due to their improved absorption in the human gut (Hsiao et al. 2020). The high aglycone content in Soman (314.6 μg/g) indicates that it may provide enhanced health benefits linked to these compounds. Nevertheless, glucosides remain important as they act as precursors to aglycones and can affect the absorption and metabolism of isoflavones (Kim et al. 2021). In addition, Soman's moderate acetyl content, featuring high levels of acetyldaidzin and minimal acetylgenistin, contrasts with the absence of acetylglucosides in Seoritae. This absence of acetylglucosides in Seoritae may limit its isoflavone profile's effectiveness compared to other genotypes. These genotype-specific differences demonstrate how varying genetic backgrounds can influence the overall bioactivity and potential health benefits of isoflavones.

Anthocyanin content

The variation in anthocyanin composition among black soybean genotypes reveals significant genetic and biochemical diversity. Danheuk stands out with the highest total anthocyanin content (24,080.6 μg/g), nearly 3.5 times greater than Seoritae (6,794.7 μg/g), indicating superior biosynthetic capacity or regulatory efficiency in Danheuk. Socheongja is notable for its high levels of Cy3gal (340.1 μg/g) and Cy3glc (19,682.6 μg/g), reflecting its dominant anthocyanin profile (Choi et al. 2020; Dhungana et al. 2021; Koh et al. 2014; Xu & Chang 2008). These components contribute significantly to the anthocyanin content in its seed coat. Cheongja5ho features high Pn3glc levels (651.6 μg/g), while Soman has the highest Pg3glc (891.2 μg/g). The absence of Pg3glc in Seoritae and the lack of Dp3glc and Pt3glc in Soman and Socheongja underscore genotype-specific differences in anthocyanin profiles. These variations imply specific genetic or enzymatic constraints affecting anthocyanin production in each genotype. The differences in individual anthocyanins among genotypes reveal diverse regulatory and metabolic pathways, which influence both the quantity and type of anthocyanins produced.

Total polyphenol, flavonoid, ABTS, and DPPH

The antioxidant potential of black soybean genotypes varied considerably, highlighting significant differences in their polyphenol and flavonoid contents, as well as their ability to scavenge free radicals. Soman emerged as the superior genotype in all antioxidant measures, including total polyphenol content (398.8 mg TE/100g), total flavonoid content (262.6 mg TE/100g), ABTS radical scavenging activity (998.5 mg GAE/100g), and DPPH radical scavenging activity (770.0 mg CAE/100g). These high values suggest that Soman has a robust antioxidant profile, potentially due to a higher concentration of polyphenolic compounds and a more effective free radical

scavenging mechanism (Lv et al. 2021; Olszowy 2019; Umeno et al. 2016). Conversely, Seoritae exhibited the lowest antioxidant potentials across all measures, with total polyphenol content at 197.9 mg TE/100g, total flavonoid content at 62.2 mg TE/100g, ABTS at 418.5 mg GAE/100g, and DPPH at 211.4 mg CAE/100g. This lower antioxidant activity implies that Seoritae has a reduced capacity for neutralizing oxidative stress compared to other genotypes. Total flavonoid content was found to be a particularly strong predictor of antioxidant activity, showing a high correlation with both ABTS (r=0.95**) and DPPH (r=0.95**).

This suggests that flavonoids play a crucial role in the antioxidant potential of soybeans, as they are effective in scavenging free radicals. Similarly, total polyphenol content also showed significant correlations with ABTS (r=0.87**) and DPPH (r=0.87**), though slightly weaker than flavonoid.

Correlation between antioxidant components and activity

The correlation analysis highlights that isoflavone content strongly correlates with antioxidant activities, with compounds such as genistin (r=0.70** for ABTS, 0.76** for DPPH) and total isoflavones (r=0.67** for ABTS, 0.69** for DPPH) showing particularly strong positive correlations. These results suggest that isoflavones significantly enhance antioxidant capacity, likely due to their effective free radical scavenging properties. In contrast, the relationship between anthocyanin content and antioxidant activities is less pronounced. While Cyanidin-3-galactoside (Cy3gal) and Cyanidin-3-glucoside (Cy3glc) show positive correlations (r=0.59** to 0.67**), the overall total anthocyanin content exhibits weaker correlations (r=0.58** for ABTS and 0.54** for DPPH). Additionally, the negative correlations observed with Delphinidin-3-glucoside (Dp3glc) and Petunidin-3-glucoside (Pt3glc) (r=-0.30 to -0.31) indicate that these anthocyanins may have a less significant impact on antioxidant activity, though these correlations are not statistically significant. Overall, isoflavones were more strongly associated with antioxidant activity compared to anthocyanins, suggesting their greater role in enhancing the antioxidant potential of the black soybeans used this study.

Soman's high antioxidant potentials

In this study, Soman stands out among black soybean genotypes due to its exceptional isoflavone content, featuring high levels of both aglycones and glucosides, which offer significant bioactive potential and improved health benefits due to better absorption (Hsiao et al. 2020; Izumi et al. 2000;). Its robust antioxidant profile is evidenced by superior measures in total polyphenol, flavonoid content, and radical scavenging activities (ABTS and DPPH), highlighting its effective free radical scavenging mechanisms (Lv et al. 2021; Olszowy 2019; Umeno et al. 2016). Furthermore, Soman is notable for its exceptionally high Pelargonidin-3-glucoside (Pg3glc) content, which distinguishes it with a unique anthocyanin profile among black soybean genotypes. This high level of Pg3glc suggests that Soman may offer specific health benefits associated with this anthocyanin, such as anti-inflammatory and chondroprotective effects previously reported in other crops (Amini et al. 2017; Chuntakaruk et al. 2021). The absence of Delphinidin-3-glucoside (Dp3glc) and Petunidin-3-glucoside (Pt3glc) in Soman further emphasizes its distinct anthocyanin composition (Park et al. 2015; Sundaramoorthy et al. 2015), potentially con-

tributing to its specialized nutritional and health attributes. This unique anthocyanin profile highlights Soman's potential for targeted applications in health and nutrition.

Conclusion

In summary, these findings highlight significant genetic variability in isoflavone and anthocyanin content among soybean genotypes, each impacting antioxidant potential. High levels of isoflavones and anthocyanins are linked to enhanced antioxidant activities, indicating the potential for selecting and breeding soybeans with optimized health benefits. Among the genotypes, Soman stands out as particularly promising due to its superior isoflavone content, impressive anthocyanin profile, and high antioxidant potential. This makes Soman especially valuable for health-related applications and breeding programs aimed at enhancing these traits. Understanding these genetic variations can facilitate the development of soybeans with tailored nutritional and therapeutic properties, thereby improving health outcomes and expanding their applications in food and medicine.

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Supplementary Table S1. ANOVA tables for isoflavone, anthocyanin compositions and antioxidant potentials.

Daidzein	Df	Sum Sq	Mean Sq	F value	Significance
Genotype (G)	6	64220	16055	1372	***
Year (Y)	1	5943	5943	507.8	***
G x Y	6	7117	1779	152	***
Residuals	28	234	12	-	-
Glycitein	Df	Sum Sq	Mean Sq	F value	Significance
Genotype (G)	6	10138	2534.6	193.22	***
Year (Y)	1	685	685.2	52.24	***
G x Y	6	1408	351.9	26.83	***
Residuals	28	262	13.1	-	-
Genistein	Df	Sum Sq	Mean Sq	F value	Significance
Genotype (G)	6	20658	5165	149.273	***
Year (Y)	1	22	22	0.634	ns
G x Y	6	2367	592	17.106	***
Residuals	28	692	35	-	-
Total aglycone	Df	Sum Sq	Mean Sq	F value	Significance
Genotype (G)	6	194980	48745	405.56	***
Year (Y)	1	11653	11653	96.95	***
G x Y	6	24157	6039	50.25	***
Residuals	28	2404	120	-	-
Daidzin	Df	Sum Sq	Mean Sq	F value	Significance
Genotype (G)	6	44646	11162	445.6	***
Year (Y)	1	2852	2852	113.9	***
G x Y	6	14131	3533	141.1	***
Residuals	28	501	25	-	-
Glycitin	Df	Sum Sq	Mean Sq	F value	Significance
Genotype (G)	6	10397	2599.2	469.79	***
Year (Y)	1	103	103.1	18.63	***
G x Y	6	1109	277.2	50.1	***
Residuals	28	111	5.5	-	-
Genistin	Df	Sum Sq	Mean Sq	F value	Significance
Genotype (G)	6	86322	21580	328.27	***
Year (Y)	1	3842	3842	58.44	***
G x Y	6	27303	6826	103.83	***
Residuals	28	1315	66	-	-
Total glucoside	Df	Sum Sq	Mean Sq	F value	Significance
Genotype (G)	6	281847	70462	321.11	***
Year (Y)	1	15761	15761	71.83	***
G x Y	6	95614	23903	108.93	***
Residuals	28	4389	219	-	-
Malonyldaidzin	Df	Sum Sq	Mean Sq	F value	Significance
Genotype (G)	6	1303703	325926	351.77	***
Year (Y)	1	173263	173263	187	***
G x Y	6	93350	23337	25.19	***
Residuals	28	18530	927	-	-

Supplementary Table S1. Continued.

Malonylglycitin	Df	Sum Sq	Mean Sq	F value	Significance
Genotype (G)	6	70633	17658	502.5	***
Year (Y)	1	786	786	22.366	***
G x Y	6	629	157	4.475	***
Residuals	28	703	35	-	-
Malonylgenistin	Df	Sum Sq	Mean S	F value	Significance
Genotype (G)	6	1364324	341081	366.95	***
Year (Y)	1	185168	185168	199.211	***
G x Y	6	39130	9783	10.533	***
Residuals	28	18590	929	-	-
Total malonylglucoside	Df	Sum Sq	Mean Sq	F value	Significance
Genotype (G)	6	4768690	1192172	281.51	***
Year (Y)	1	669985	669985	158.21	***
G x Y	6	218154	54538	12.88	***
Residuals	28	84697	4235	-	-
Acetyldaidzin	Df	Sum Sq	Mean Sq	F value	Significance
Genotype (G)	6	496.4	124.1	4489	***
Year (Y)	1	124.1	124.1	4489	***
G x Y	6	496.4	124.1	4489	***
Residuals	28	0.6	0.03	-	-
Acetylglycitin	Df	Sum Sq	Mean Sq	F value	Significance
Genotype (G)	6	418.4	104.61	826.2	***
Year (Y)	1	15.4	15.38	121.5	***
G x Y	6	54.6	13.66	107.9	***
Residuals	28	2.5	0.13	-	-
Acetylgenistin	Df	Sum Sq	Mean Sq	F value	Significance
Genotype (G)	6	0.020675	0.0051	6927	***
Year (Y)	1	0.005169	0.0051	6927	***
G x Y	6	0.020675	0.0051	6927	***
Residuals	28	0.003829	0.000191	-	-
Total acetylglucoside	Df	Sum Sq	Mean Sq	F value	Significance
Genotype (G)	6	1279.3	319.8	1428.5	***
Year (Y)	1	53.1	53.1	237.4	***
G x Y	6	600.9	150.2	671	***
Residuals	28	4.5	0.2	-	-
Total Isoflavone	Df	Sum Sq	Mean Sq	F value	Significance
Genotype (G)	6	9951893	2487973	337.11	***
Year (Y)	1	686958	686958	938	***
G x Y	6	477935	119484	16.19	***
Residuals	28	147604	7380	-	-
Dp3glc	Df	Sum Sq	Mean Sq	F value	Significance
Genotype (G)	6	29484595	7371149	2356.052	***
Year (Y)	1	23813	23813	7.611	***
G x Y	6	275943	68986	22.05	***
Residuals	28	62572	3129	-	-

Supplementary Table S1. Continued.

Cy3gal	Df	Sum Sq	Mean Sq	F value	Significance
Genotype (G)	6	98502	24626	387.64	***
Year (Y)	1	4430	4430	69.74	***
G x Y	6	15019	3755	59.1	***
Residuals	28	1271	64	-	-
Cy3glc	Df	Sum Sq	Mean Sq	F value	Significance
Genotype (G)	6	927658600	231914650	906.76	***
Year (Y)	1	4906211	4906211	19.18	***
G x Y	6	20687770	5171942	20.22	***
Residuals	28	5115217	255761	-	-
Pt3glc	Df	Sum Sq	Mean Sq	F value	Significance
Genotype (G)	6	4834284	1208571	6669.615	***
Year (Y)	1	66	66	0.364	ns
G x Y	6	98678	24670	136.141	***
Residuals	28	3624	181	-	-
Pg3glc	Df	Sum Sq	Mean Sq	F value	Significance
Genotype (G)	6	2855507	713877	82.96	***
Year (Y)	1	264525	264525	30.74	***
G x Y	6	517250	129313	15.03	***
Residuals	28	172099	8605	-	-
Pn3glc	Df	Sum Sq	Mean Sq	F value	Significance
Genotype (G)	6	806413	201603	893.7	***
Year (Y)	1	18701	18701	82.9	***
G x Y	6	143701	35925	159.3	***
Residuals	28	4511	226	-	-
Total anthocyanin	Df	Sum Sq	Mean Sq	F value	Significance
Genotype (G)	6	809023791	202255948	904.85	***
Year (Y)	1	3033003	3033003	13.57	***
G x Y	6	37440587	9360147	41.88	***
Residuals	28	4470505	223525	-	-
Total polyphenol	Df	Sum Sq	Mean Sq	F value	Significance
Genotype (G)	6	144798	36199	907.62	***
Year (Y)	1	653	653	16.38	***
G x Y	6	8743	2186	54.8	***
Residuals	28	798	40	-	-
Total flavonoid	Df	Sum Sq	Mean Sq	F value	Significance
Genotype (G)	6	145902	36475	2371.5	***
Year (Y)	1	21201	21201	1378.4	***
G x Y	6	16082	4020	261.4	***
Residuals	28	308	15	-	-
ABTS	Df	Sum Sq	Mean Sq	F value	Significance
Genotype (G)	6	1097863	274466	2491.6	***
Year (Y)	1	348476	348476	3163.4	***
G x Y	6	129253	32313	293.3	***
Residuals	28	2203	110	-	-

Supplementary Table S1. Continued.

DPPH	Df	Sum Sq	Mean Sq	F value	Significance
Genotype (G)	6	1023837	255959	1939.8	***
Year (Y)	1	287075	287075	2175.6	***
G x Y	6	172758	43189	327.3	***
Residuals	28	2639	132	-	-

*** means significantly different at $p<0.001$, ns means no significantly different.