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Research Article

Tissue-Specific Metabolic Profiling of Mungbean (*Vigna radiata* L.) Genotypes with Different Seed Coat Colors

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Mungbean (*Vigna radiata* L.) is one of the major legume crops containing high carbohydrate and protein contents. In this study, the total phenolic and flavonoid contents and ABTS/DPPH radical scavenging activity of whole sprouts and seed coats were evaluated by using 10 mungbean genotypes with diverse seed coat colors and origins. Qualitative/quantitative analysis of individual secondary metabolites was performed with ultrahigh performance liquid chromatography (UPLC). Overall, 23 polyphenols, including flavonoids, phenylpropanoids, and anthocyanins, were identified. Depending on genotypes, significant variations in the contents of each phytochemical were identified before/after germination. The results indicate that the specific pathways of phenolic compounds, including chlorogenic acid, coumestrol, genistein, and glycitein, are activated by sprouting in mungbean. The neo/chlorogenic acid contents had tissue specificity, even though it was isomeric. The anthocyanin contents were higher in green and yellow mungbeans than in black mungbeans. These findings in this study will provide valuable information to improve the food quality of mungbean sprouts with high polyphenolic contents.

1. Introduction

Mungbean (*Vigna radiata* L.) is one of the major legume crops with a high content of carbohydrates (50%–60%) and proteins (20%–24%) [1, 2]. Mungbeans contain high levels of antioxidant compounds, and the amount of functional substances varies depending on the genotype [3, 4]. In developing countries in Asia, mungbeans are one of the essential resources for starch and protein. Additionally, the consumption of mungbean sprouts has been increasing as fresh vegetables worldwide [5]. Mungbean sprouts grow fast and can be produced all year round, regardless of the environment. During germination, dynamic changes in phytochemical composition and antioxidant capacity have been reported in mungbean [6, 7]. The ethanol extract of mungbean sprouts has a higher amount of antioxidant compounds than that of seeds [8].

Most of the polyphenolic compounds of mungbeans are concentrated in the seed coats. Especially, the seed coat has high contents of vitexin (95.6%) and isovitexin (96.8%) [9, 10]. Vitexin and isovitexin have been reported to have beneficial effects on human health, such as antidiabetic, anti-inflammatory, and antioxidant effects [11]. The seed coat colors of mungbean vary from yellow, green, and brown to black. Differences in the content of functional substances lead to differences in seed coat colors in legumes [12]. Many studies have reported the diversities of functional substances and antioxidant effects in soybeans according to their seed coat colors. A high amount of anthocyanins was detected in the black seed coat, but no anthocyanins were detected in the yellow seed coat [13]. Black soybeans have been reported to have a higher antioxidant capacity than yellow soybeans [14].

Anthocyanins belong to the flavonoid family and are responsible for pigments in plant tissues, such as black, blue, and red [15, 16]. Cyanidin, delphinidin, malvidin, pelargonidin, peonidin, and petunidin have been detected as anthocyanins in legumes [10, 17]. They are known to positively affect high antioxidant capacity and anticancer activity in the human body [18, 19]. Black mungbean is known to have a higher antioxidant capacity than green mungbean [20]. The presence of anthocyanin has been proposed as

a possible reason for the higher antioxidant capacity, but metabolic profiling has not been conducted in mungbean seed coats [21].

Thanks to advances in techniques for metabolite analysis, metabolomics has been applied to physiology, pathology, biology, and food science [22, 23]. A metabolomics-based study has achieved great success in addressing a series of issues in biological, biomedical, and agricultural domains. Liquid chromatography (LC) for quantitative and qualitative analysis has become one of the most widely used approaches in metabolomics [22, 23]. In mungbean, there have been few studies of secondary metabolites, especially in the seed coat. In the current study, the antioxidant capacity of 10 mungbean genotypes with diverse seed coat colors was measured. Furthermore, individual polyphenol compounds of the seed coat and whole sprout were analyzed through ultrahigh performance liquid chromatography (UPLC). The results of this study will provide nutritional information on mungbean sprouts of different genotypes with diverse seed coat colors. Furthermore, LC-based metabolomic data can be powerful information to identify genetic factors mediating the biosynthetic pathways of phenolic compounds along with an omics approach. These findings will help improve the food quality of mungbean sprouts with a high amount of functional substances.

2. Materials and Methods

2.1. Sample Preparation. Mungbean sprouts were grown according to the method proposed by [24] with some modifications [24]. Fifty seeds were cultivated for each genotype. Mungbean seeds were rinsed three times with distilled water and soaked in distilled water using an incubator (JEIO TECH. ISS-4075R) under dark conditions at 37°C for 17 h. The germinated seeds were cultivated for 3 days using a plant growth chamber (Sundotcom, ST001A) at 28°C–30°C with an irrigation interval of 4 h and an irrigation time of 2 min (Figure S1). Mungbean sprouts of 10 genotypes cultivated for 3 days were dried at 70°C for 24 h in the incubator (JEIO TECH, ISS-4075R). Then, they were finely grounded. The grounded samples were extracted at 100 mg/mL (w/v) with 70% ethanol (Supelco, Cat. No. 1009831011) in the dark at room temperature (25°C) after 10 minutes of sonication. The extract was centrifuged at 13,000 rpm for 2 min after 24 h. The supernatant was filtered through a 0.22 μm syringe filter. Each extract was diluted with 70% ethanol to 1, 5, 20, and 50 mg/mL for the assay of antioxidant activity (ABTS and DPPH), total phenols, and total flavonoids, respectively.

The seed coat was physically separated from the 50 seeds, and 0.05 g of seed coats were used. Then, the seed coat was extracted at 50 mg/mL (w/v) with 70% ethanol in the dark at room temperature for 24 h. Each extract was diluted with 70% ethanol to 1, 1, 10, and 20 mg/mL for the assay of antioxidant activity (ABTS and DPPH), total phenols, and total flavonoids, respectively. All samples and compounds are weighed using an electronic microbalance (Ohaus, PX224KR, NJ, USA) (resolution: 0.1 mg).

2.2. Phenotype Measurement. Phenotypic traits of mungbean sprouts were measured on the third day after germination (DAG). Fresh and dry weights were measured using 30 sprouts. The yield of sprouts was measured as fresh weight/seed weight × 100 (%). The lengths of hypocotyl and roots and the thickness of hypocotyl were measured using ImageJ with the straight line and freehand line functions [25].

2.3. Content of Total Flavonoids. The total flavonoids content of extracts was determined according to the method proposed by [24] with some modifications [24]. The extract (500 μL) was mixed with 1 M potassium acetate (FUJIFILM, 169–21965) (100 μL) and 10% aluminum nitrate nonahydrate (JUNSEI, 37350–1201) (100 μL). Quercetin (Sigma-Aldrich®, Q4951) (0, 50, 100, and 200 mg/L) was used as the standard compound for the total flavonoid assay. After 40 min in the dark at room temperature, absorbance was measured at 405 nm using a spectrophotometer (Thermo Scientific MIB, Multiskan FC).

2.4. Content of Total Phenol. The total phenolic content of the extracts was determined according to the method proposed by [26] with some modifications [26]. Gallic acid (Sigma-Aldrich®, G7384) (0, 50, 100, and 200 mg/L) was used as a standard material. Samples (100 μL) were mixed with Folin-Ciocalteu (50 μL) and maintained at room temperature for 3 min. Then, a 20% Na_2CO_3 solution (300 μL) was added to the solution, which was left in the dark for 15 min at room temperature. The reaction solution volume was adjusted to 1 mL with distilled water after centrifugation at 13,000 rpm for 2 min. The absorbance of the obtained supernatant was measured at 738 nm.

2.5. ABTS Radical Scavenging Assay. The ABTS radical scavenging assay was conducted according to [27] with some modifications [27]. The ABTS radical cation (ABTS) was produced by reacting a 7 mM ABTS solution dissolved in distilled water with 2.45 mM potassium persulphate in a 1 : 1 ratio. The ABTS solution was diluted with phosphate-buffered saline to an absorbance of 0.7 (±0.03) at 734 nm. Ascorbic acid (0, 1, 5, 10, 25, 50, and 100 mg/L) was used as the standard material. All samples (20 μL) were mixed with ABTS solution (180 μL) and stored in a dark place at room temperature for 10 min. The absorbance at 738 nm was measured for each sample using a spectrophotometer.

2.6. DPPH Radical Scavenging Assay. A DPPH radical scavenging assay was performed to measure the antioxidant activity of the mungbean sprout and seed coat extract. Antioxidant capacity was evaluated using an OxiTec™ DPPH Antioxidant Assay Kit (BIOMAX, BO-DPH-500). Trolox (0, 40, 60, 80, and 100 mg/L) was used as a standard material. Assay buffer (80 μL) was added to Trolox (20 μL) and extraction (20 μL) of the mungbean sprout and seed coat. The solutions were mixed with DPPH solution (100 μL)

and stored in a dark place at room temperature for 30 min. The absorbance at 517 nm was measured for each sample using a spectrophotometer.

2.7. Analysis of Functional Substances by UPLC-PDA. The UPLC was conducted using a Shimadzu UPLC system (Nexera series equipped with MPM-40, SCL-40, SPD-M40, LC-40, SIL-40, and CTO-40 units from Shimadzu, Kyoto, Japan) with a photodiode array (PDA) detector. Phenolic and flavonoid compounds were separated on a ZORBAX SB-C18 column (3.5 μm, 4.6 mm × 150 mm; Agilent, PN 863953-902). The mobile phase gradients of ultrapure water–0.1% acetic acid solution (v/v; solvent A) and acetonitrile (solvent B) were flowed at 1 mL/min as follows: 0–10 min 95%–90% A, 10–11 min 90%–85% A, 11–15 min 85%–80% A, 15–16 min 80%–70% A, 16–25 min 70%–65% A, 25–28 min 65%–50% A, 28–32 min 50% A, and 32.1 min solvent A was increased from 50% to 95%, 32.1–40 min 95% A. Separation of anthocyanin was performed on a ZORBAX Eclipse XDB-C18 column (5 μm, 4.6 mm × 150 mm; Agilent, PN 993967-902). The mobile phase gradients of solvent A and solvent B were flowed at 1 mL/min as follows: 0–6 min 100%–95% A, 6–18 min: 95%–50% A, 18–23 min 50% A, 23–25 min: 50%–100% A, and 25–30 min: 100% A. The column oven temperature was set as 40°C, and the injection volume was 2 μL. The analysis results were determined based on standard calibration curves (10–100 mg/L) with three replicates. The photon wavelength of the detector scan range was set between 190 and 800 nm.

2.8. Statistical Analysis. Statistical comparisons were conducted by one-way ANOVA and Duncan's multiple range test using the SPSS Statistics 25 ($p < 0.05$). All analyses were performed in triplicate. The results were expressed as the mean ± standard deviation.

3. Results

3.1. Mungbean Genotypes. In total, 10 mungbean genotypes with various origins and seed coat colors were selected; brown (Figures 1(a) and 1(b)), black (Figures 1(c) and 1(d)), green (Figures 1(e)–1(h)), and yellow (Figures 1(i) and 1(j)) (Table 1). The 100 seed weights of the 10 genotypes ranged from 1.96 to 7.86 g (Table 1). Sprout yields varied from 897.99% to 1,836.28%. The total length of the mungbean sprout ranged from 1.82 ± 0.2 to 6.49 ± 0.31 cm on DAG 1, from 5.9 ± 1.14 to 11.26 ± 0.23 cm on DAG 2, and from 11.42 ± 1.55 to 20.23 ± 1.66 cm on DAG 3, respectively (Figures 2(a) and 2(b)). The 100 seed weight was the highest in V03720B-G (6) with 7.86 g, followed by V01946A-Y (10) and Vo1301 (5), with 7.8 and 6.1 g, respectively. JP103138-2 (4) and V01946A-Y (10) had the highest yields of 1,836.28% and 1,714.74%, respectively. There was no significant relationship between the weight of 100 seeds and yields. The thickness of hypocotyl ranged from 0.15 ± 0.01 to 0.26 ± 0.02 cm on DAG 1, from 0.19 ± 0.01 to 0.264 ± 0.01 cm on DAG 2, and from 0.2 ± 0.001 to 0.31 ± 0.01 cm on DAG 3, respectively (Figure 2(c)).

3.2. Contents of Biochemical Compounds and Antioxidant Capacity in the Seed Coats of Mungbeans. The seed coats were isolated from seeds of 10 genotypes to determine the antioxidant activity of the seed coat. The total flavonoid contents ranged from 1.14 ± 0.05 to 2.5 ± 0.13 mg/g, and total phenol content ranged from 8.87 ± 0.06 to 15.79 ± 0.01 mg/g (Figures 3(a) and 3(b)). ABTS radical scavenging activity had a minimum of 33% ± 0.02% and a maximum of 55% ± 0.002% antioxidant activity (Figure 3(c)). In the DPPH radical scavenging activity, the minimum and maximum values were 38% ± 0.03% and 62% ± 0.007%, respectively (Figure 3(d)).

The highest total flavonoid content was in JP229099 (7) (2.57 ± 0.13 mg/g). Total phenol content was highest in JP103138-2 (4) (15.79 ± 0.01 mg/g) and Vo5551 (8) (15.75 ± 0.03 mg/g). ABTS radical scavenging activity showed the highest values in Vo1301 (5) (53% ± 0.02%) and JP229099 (7) (55% ± 0.002%). Vo555 1(8) had the highest DPPH radical scavenging activity, reaching 62% ± 0.007%. V03720B-G (6) had the lowest antioxidant activity among the 10 genotypes analyzed.

3.3. Content of Biochemical Compounds and Antioxidant Capacity in Mungbean Sprouts. The total flavonoids, phenolic compounds, and antioxidant capacity in whole sprouts were measured in the 10 genotypes analyzed. The total flavonoid content varied from 0.54 ± 0.02 to 0.91 ± 0.05 mg/g, and the total phenol content varied from 11.68 ± 0.1 to 14.44 ± 0.13 mg/g (Figures 4(a) and 4(b)). ABTS radical scavenging activity ranged from 24% ± 0.004% to 35% ± 0.01% antioxidant activity, and the DPPH radical scavenging activity for measuring antioxidant activity ranged from 16% ± 4% to 25% ± 3% (Figures 4(c) and 4(d)).

The total flavonoid content was 0.86 ± 0.02 mg/g in Tecer-Hitam (2), 0.91 ± 0.05 mg/g in JP103138-2 (4), and 0.86 ± 0.03 mg/g in V03720B-G (6), which was significantly higher than those of the other genotypes. Tecer-Hitam (2) had the highest total phenol content of 14.44 ± 0.13 mg/g. The DPPH radical scavenging activity of JP103138-2 (4) was 25% ± 3%, and the ABTS radical scavenging activity of Tecer-Hitam (2) was 35% ± 0.01%, the highest value. Tecer-Hitam (2), a black mungbean, contained the largest amounts of biochemical compounds and has high antioxidant activity. Vo1301 (5) had the lowest flavonoid content and DPPH radical scavenging activity. It was shown in our results that antioxidant capacity had no significant association with the seed coat color, but there were significant variations among the 10 mungbean genotypes analyzed.

3.4. UPLC Analysis. The content of the biochemical components in the seed coat was measured using UPLC. In total, 23 secondary metabolites, including biochanin A, caffeic acid, catechin, chlorogenic acid, coumestrol, daidzein, daidzin, formononetin, gallic acid, genistein, genistin, glycitein, glycitin, isovitexin, kaempferol, myricetin, neochlorogenic acid, p-coumaric acid, quercetin, resveratrol, syringic acid, t-ferulic acid, and vitexin, were used as standard materials. Out of 23 polyphenols, flavonoids (e.g.,

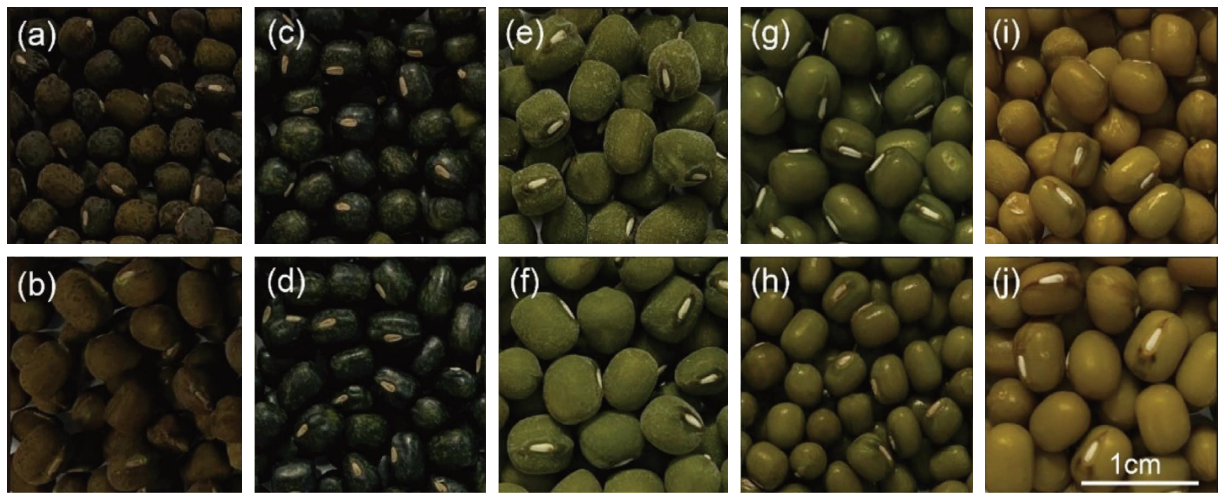

Figure 1: Seeds of 10 mungbean genotypes with different seed coat colors. (a) Damyang, Jeollanam-do-1994231, (b) Tecer-Hitam, (c) JP229177, (d) JP103138-2, (e) Vo1301, (f) V03720B-G, (g) JP229099, (h) Vo5551, (i) yellow gram, and (j) V01946A-Y.

Table 1: List of mungbean genotypes. The yield was measured at 100 seed weight and the fresh weight of the sprouts.

No.	Accession name	Origin*	Color	Seed luster	100 seeds weight (g)	Yield (%)
1	Damyang, Jeollanam-do-1994 3231	KOR	Black	Dull	1.96	939.80
2	Tecer-Hitam	IDN	Black	Dull	4.3	1,617.80
3	JP229177	IND	Black	Shine	3.22	1,699.90
4	JP103138-2	PAK	Black	Shine	3.48	1,836.28
5	Vo1301	CHN	Green	Dull	6.1	1,516.39
6	V03720B-G	USA	Green	Dull	7.86	1,633.59
7	JP229099	THA	Green	Shine	3.79	1,514.28
8	Vo5551	IRN	Green	Shine	2.96	897.99
9	Yellow gram	UNK	Yellow	Shine	5.14	1,693.75
10	V01946A-Y	PHL	Yellow	Shine	7.8	1,714.74

*CHN, China; IDN, Indonesia; IND, India; IRN, Iran; KOR, Korea; PAK, Pakistan; PHL, Philippines; THA, Thailand; UNK, United Kingdom; USA, United States of America.

catechin, isovitexin, myricetin, quercetin, and vitexin), isoflavonoids (e.g., biochanin A, coumestrol, daidzein, daidzin, genistein, genistin, glycitein, and glycitin), phenylpropanoids (e.g., caffeic acid, chlorogenic acid, neochlorogenic acid, p-coumaric acid, and t-ferulic acid), and other phenols (e.g., gallic acid, resveratrol, and syringic acid) were identified and quantified. Of the 21 polyphenols detected, syringic acid and glycitin were detected only in the seed coat, and glycitein, genistein, chlorogenic acid, t-ferulic acid, and coumestrol were detected only in sprouts. Vitexin, isovitexin, and myricetin were detected in much higher concentrations than the other secondary metabolites in the seed coat. The ranges of each compound detected in the seed coat were as follows: vitexin ($3{,}812.76 \pm 71.15$ –$8{,}390.86 \pm 96.96$ mg/100 g), isovitexin ($4{,}613.38 \pm 50.36$ –$9{,}572.42 \pm 25.19$ mg/100 g), and myricetin (454.93 ± 2.03 –$1{,}031.82 \pm 3$ mg/100 g) (Table S1). In whole sprouts, catechin and chlorogenic acid were the most abundant secondary metabolites detected. The ranges of each compound in the whole sprout were as follows: catechin (519.79 ± 2.45–$1{,}415.34 \pm 15.99$ mg/100 g) and chlorogenic acid (613.73 ± 2.08–$1{,}520.13 \pm 13.42$ mg/100 g) (Figures 5 and S2).

3.5. Anthocyanin Analysis. For the detection of anthocyanin components, peonidin-3-O-glucoside (Peo-3-G), delphinidin-3-O-glucoside (D-3-G), pelargonidin-3-O-glucoside (Pel-3-G), and cyanidin-3-O-glucoside (C-3-G) were used as standard materials. Only C-3-G and Pel-3-G were predominantly detected in all 10 genotypes analyzed. For C-3-G and Pel-3-G, the minimum values were 14.72 ± 0.36 and 79.75 ± 2.78 mg/100 g, respectively, and the maximum values were 44.79 ± 0.87 and 297.59 ± 8.79 mg/100 g, respectively (Figures 6(a) and 6(b)).

4. Discussion

Legumes are popular foods in daily diets consumed worldwide [24]. Seeds and legume sprouts are good sources of protein, dietary fiber, starch, vitamins, and antioxidant compounds, including phenols and flavonoids [26]. Flavonoids, powerful hydrogen-donating antioxidants, are the single group of phenolic phytochemicals. Flavonoids, such as daidzein and genistein, protect cells against oxidative damage and have anticancer activity [28–30]. Large amounts of vitexin and isovitexin have been reported in ethanol extracts of mungbean seeds, having high antioxidant and

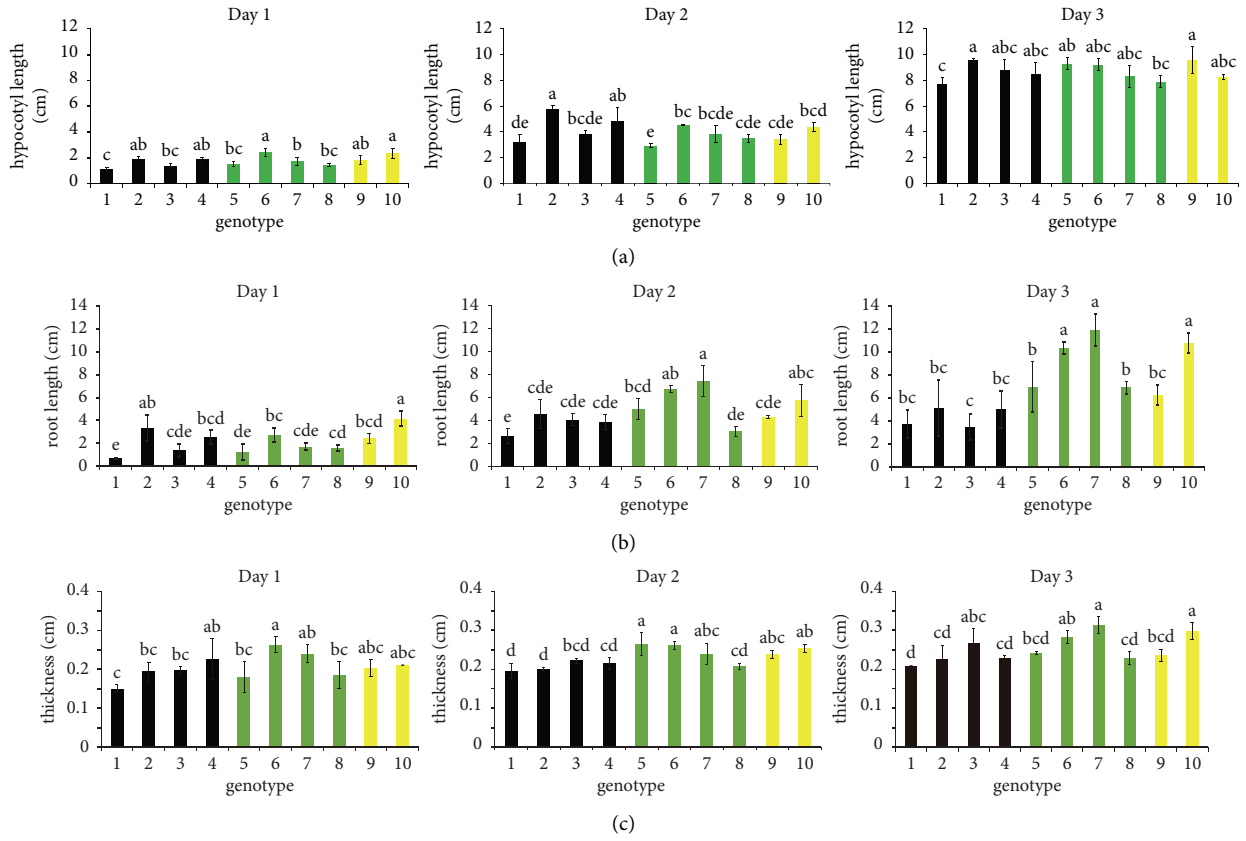

FIGURE 2: Phenotypic traits of 10 mungbean sprouts at 1, 2, and 3 DAGs. (a) The length of the hypocotyl; (b) the length of the root; (c) the thickness of the hypocotyl. The colors of each bar (black, green, and yellow) indicate the seed coat color. The error bar indicates the standard deviation. Lowercase letters above the bar indicate statistical significance ($p < 0.05$).

antiobesity effects [31]. DPPH radical scavenging activity was higher in mungbean sprouts than in soybean sprouts [27].

In the present study, the chemical properties of 10 mungbean genotypes with various seed coat colors were investigated. Several phenolic compounds were detected in the whole sprout and the seed coat, which have been well known to benefit human health, including catechin, chlorogenic acid, iso/vitexin, and resveratrol. Catechin has been reported to have antioxidant, anticancer, anti-inflammatory, and antiaging effects [32]. Resveratrol has been reported to have antiobesity effects, and chlorogenic acid could reduce the incidence of colorectal cancer by 70%–80% [33, 34].

In both ethanol extracts of the seed coat and whole sprout, the amount of individual phenolic compounds varied significantly depending on genotypes. The seed coat extracts had much higher total phenols and flavonoid contents than whole sprout extracts, leading to a higher antioxidant capacity. This result is consistent with previous findings that most phenolic compounds were concentrated in the mungbean seed coat [24, 35].

Among the 21 phytochemicals detected, glycitin, isovitexin, myricetin, neochlorogenic acid, syringic acid, and vitexin were identified mainly from the seed coat. In contrast, 10 chemicals were primarily detected from the whole sprout, including biochanin A, catechin, chlorogenic acid, coumestrol, daidzein, glycitein, genistein, genistin, t-ferulic acid, and quercetin. As studied previously, vitexin and isovitexin, the major antioxidants identified in mungbean, were identified only in the seed coat (Figure 5) [36]. Chlorogenic acid and neochlorogenic acid were mainly detected in the whole sprout and the seed coat, respectively, even though they were isomeric. Neo/chlorogenic acids have been well known to lead to the anticancer effect of coffee [33]. In, the contents of cytokinin isomers also vary depending on tissues [37]. These results indicate that isomers of secondary metabolites have tissue specificity in mungbean.

In the isoflavonoid biosynthetic pathway, glycitin was detected only in the seed coat, whereas glycitein, a precursor of glycitin, was identified only in the whole sprout [38]. In soybean, glycitin and glycitein had been reported to be decreased and increased, respectively, during germination [39]. Also, in mungbean, glycitin had been reported to be decreased after germination [40]. These findings indicate that the biosynthesis of glycitin may be blocked, which is catalyzed by isoflavone 7-O-glucosyltransferase (EC: 2.4.1.170), and glycitein accumulates since germination starts in mungbean (Figures 5 and 7) [38]. Coumestrol and daidzin share a precursor of daidzein, which is detected on both seed coats and sprouts. However, coumestrol was

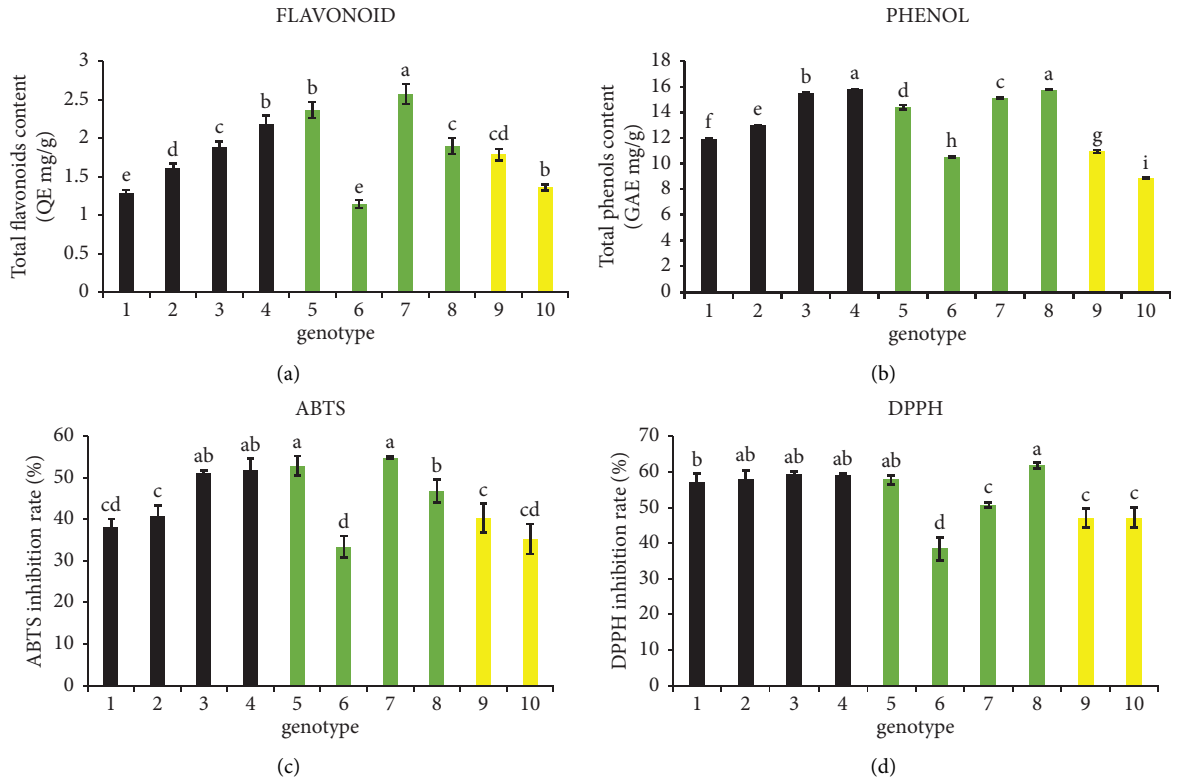

Figure 3: The content of biochemical compounds and antioxidant activity in the seed coats of mungbeans. (a) Total flavonoids, (b) total phenols, (c) ABTS radical scavenging activity, and (d) DPPH radical scavenging activity. The colors of each bar (black, green, and yellow) indicate the seed coat color. The error bar indicates the standard deviation. Lowercase letters above the bar indicate statistical significance ($p < 0.05$).

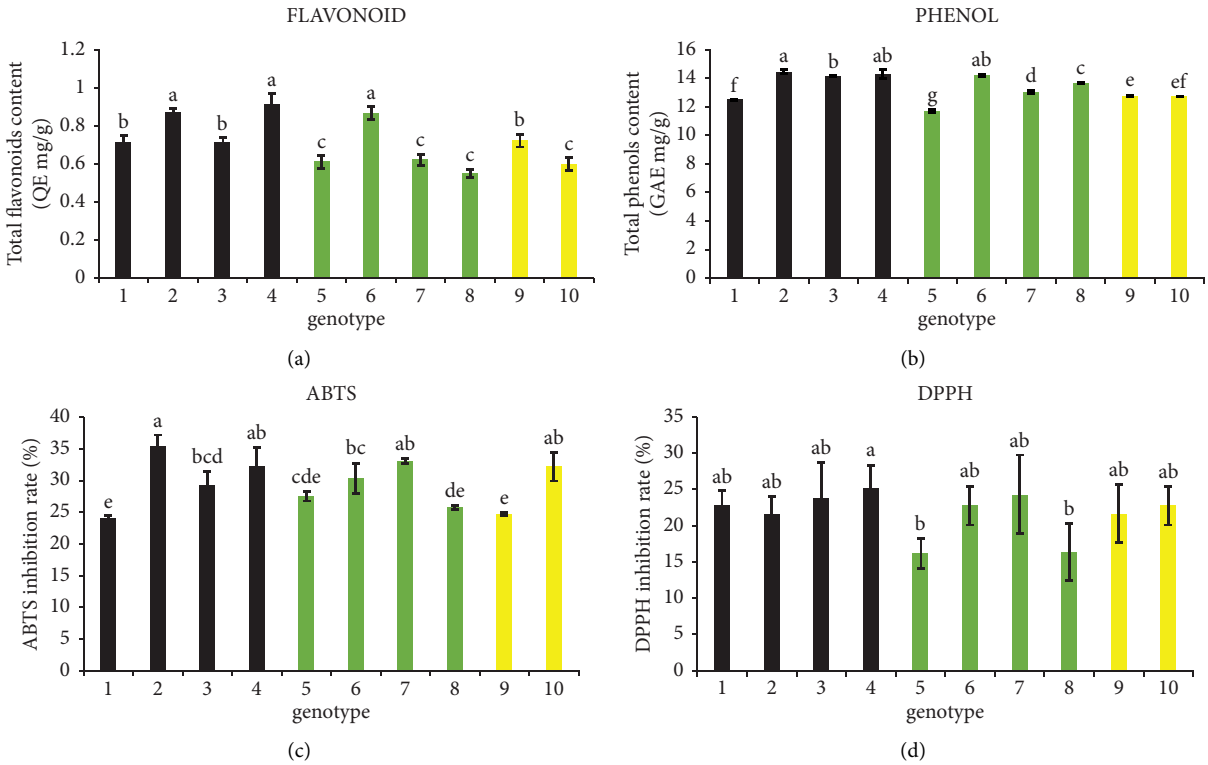

Figure 4: The content of biochemical compounds and antioxidant activity in mungbean sprouts. (a) Total flavonoids, (b) total phenols, (c) ABTS radical scavenging activity, and (d) DPPH radical scavenging activity. The colors of each bar (black, green, and yellow) indicate the seed coat color. The error bar indicates the standard deviation. Lowercase letters above the bar indicate statistical significance ($p < 0.05$).

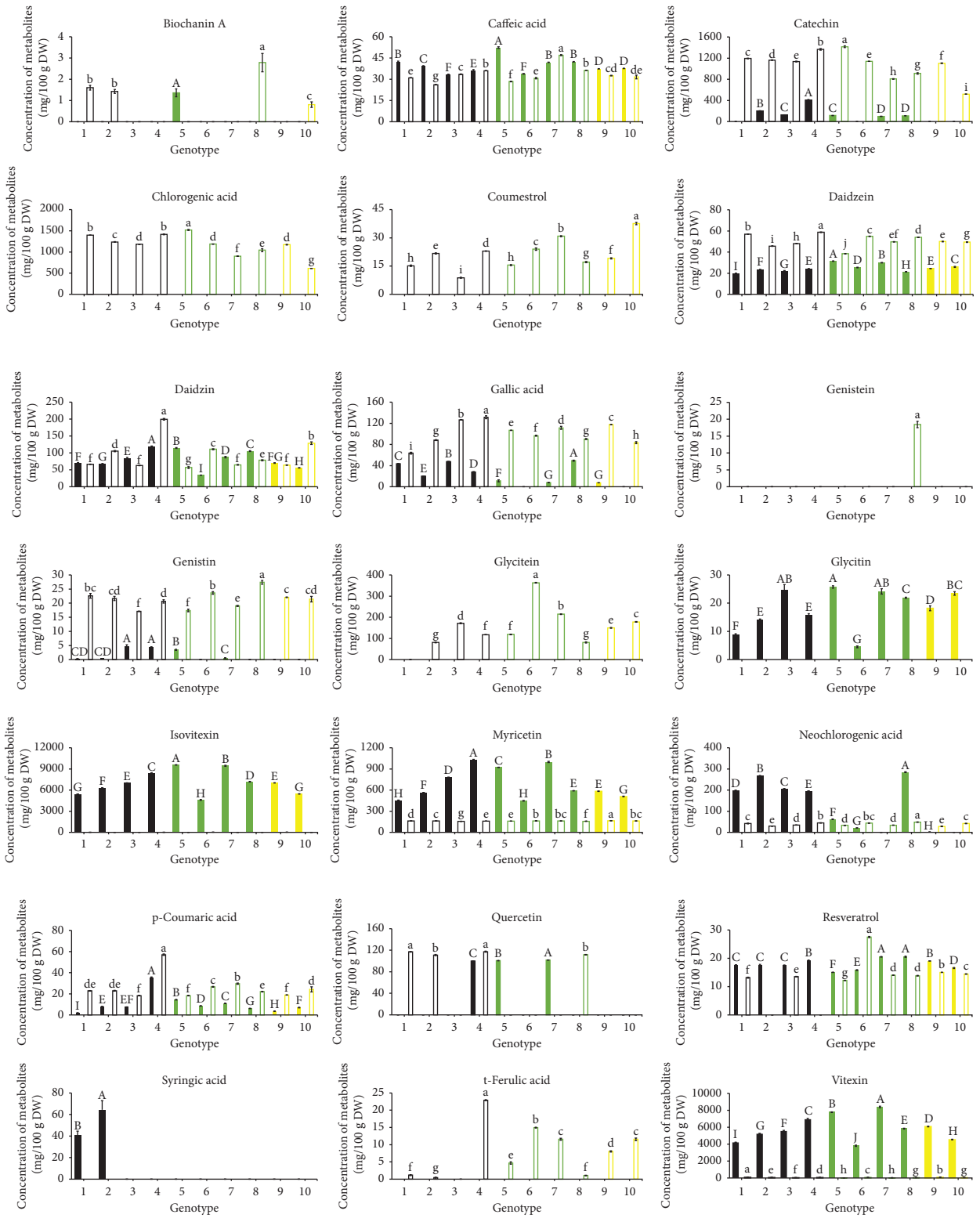

Figure 5: Metabolomic analysis of mungbean sprouts and seed coats. *X* and *y* axes indicate genotype and concentration of metabolites (mg/100 g DW), respectively. Capital and lowercase letters indicate significant differences among whole sprouts and the seed coat, respectively. The solid bar represents the seed coat, and the empty bar represents the whole sprout. Statistical analysis was conducted with a post hoc Duncan test ($p < 0.05$).

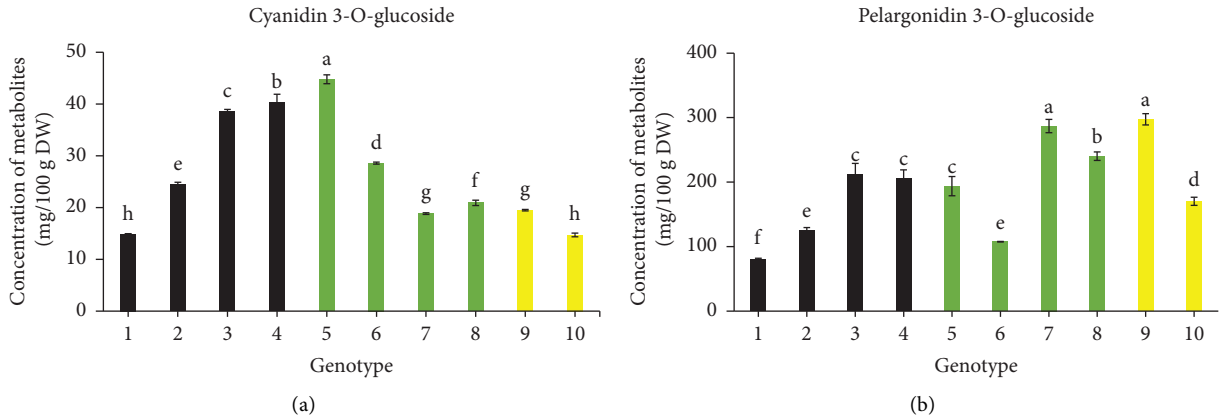

FIGURE 6: The contents of anthocyanins in mungbean seed coats. (a) Cyanidin 3-O-glucoside and (b) Pelargonidin 3-O-glucoside. *X* and *y* axes indicate genotype and concentration of metabolites (mg/100 g DW), respectively. The colors of each bar (black, green, and yellow) indicate the seed coat color. The error bar indicates the standard deviation. Lowercase letters above the bar indicate statistical significance ($p < 0.05$).

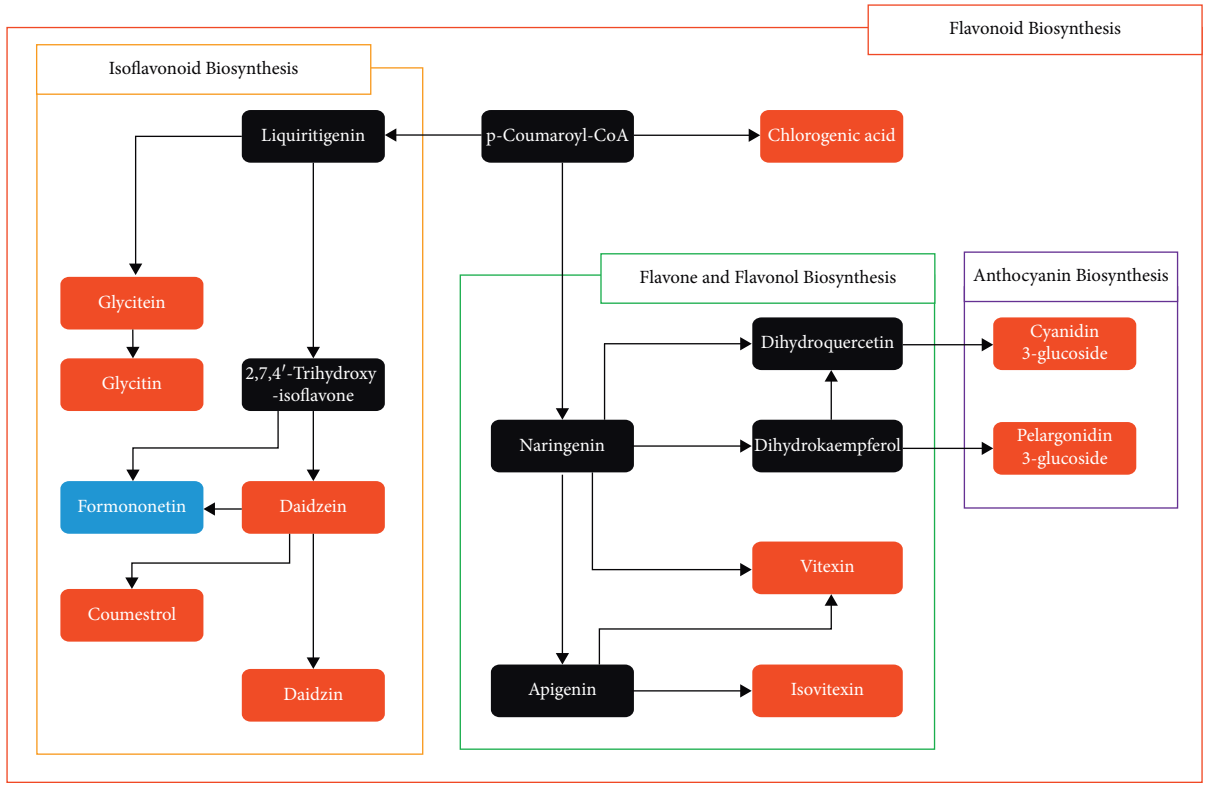

FIGURE 7: Biosynthesis pathway of secondary metabolites in the mungbean seed coat. The compounds used as standards for UPLC are indicated in red and blue. Red boxes indicate the compounds detected, and blue boxes indicate the compounds not detected in both extracts of the whole sprout and seed coat. Black boxes indicate the precursors of the target compounds (genome.jp/kegg/pathway.html).

identified only in the whole sprout. Additionally, the contents of daidzein were much higher in the whole sprouts than in the seeds. Mungbean sprouting may activate the biosynthesis of coumestrol, which has not been fully understood as one of the end products of isoflavonoid biosynthetic pathways [41]. Daidzin was detected on the seed coat and whole sprout and had no clear trend (Figures 5 and 6). These findings indicate that germination causes dynamic changes in the metabolic pathways in mungbean and provides information to understand the biosynthetic pathways of nutritionally valuable phytochemicals. For the validation of the findings in this study about the biosynthetic pathways of secondary metabolites, the expression levels of the candidate genes encoding key enzymes in the pathways are required to be measured through quantitative real-time PCR or RNA sequencing technology. There have been a few transcriptomic studies for secondary metabolites in mungbeans. Under salinity stress, the contents of secondary

metabolites and antioxidant capacity were improved in mungbean sprouts, and key genetic factors had been identified through RNA-sequencing technology [42]. Along with LC-based metabolomics data, such an integrated omics approach can be a powerful tool to identify genetic factors mediating the biosynthetic pathways of functional substances [43].

A qualitative-quantitative analysis of individual anthocyanins was conducted in the mungbean seed coat. In the seed coat of black soybean, five anthocyanins, including delphinidin 3-O-glucoside (Del-3-G), cyanidin 3-O-galactoside (C-3-Gal), cyanidin 3-O-glucoside (C-3-Glu), petunidin 3-O-glucoside (Pet-3-G), and peonidin 3-O-glucoside (Peo-3-G), were detected [44]. In the current study, only Pel-3-G and C-3-Glu were predominantly detected in all 10 genotypes (Table S1). In a previous study, C-3-Glu and Pel-3-G were detected only in black mungbean and not in green mungbean [18]. However, in the present study, the highest C-3-Glu and Pel-3-G were detected in Vo1301 (5) and yellow gram (9) genotypes with green and yellow seed coats, respectively. Unlike soybeans, our results indicate no significant correlation between the contents of anthocyanins and seed coat color in mungbean. Instead, syringic acid was quantified only in the seed coats of two genotypes, Damyang, Jeollanam-do-1994 3231 (1) and Tecer-Hitam (2), with brown seed coats (Table S1). Polymerization of gallic acid and syringic acid has been reported to confer brown pigmentation [45]. In general, the content of gallic acid in seed coats was also higher in brown or black seeds than in green or yellow seeds (Table S1). These results indicate that gallic acid and syringic acid might be the leading causes of seed coat color in mungbean and not anthocyanins.

The contents of total phenols, total flavonoids, and antioxidant activity had no significant correlation with seed coat color and origin. In contrast, the accumulation of individual polyphenolic compounds had tissue and genotype specificity in mungbeans. Our findings indicate that mungbean sprouts and seed coats should be consumed for the synergistic effect of mungbean intake. Although to identify candidate key genes regulating the contents of the target secondary metabolites, further transcriptomic analysis is required, the biochemical features identified in this study will help understand the biosynthetic pathways of phytochemicals and the nutritional values in mungbean sprouts and seed coats [42, 46]. These findings will provide valuable information for molecular breeding to develop mungbean cultivars for sprout production with high polyphenol content accompanying beneficial effects on human health.

5. Conclusions

Antioxidant activity and the contents of individual secondary metabolites of whole sprouts and seed coats were evaluated using 10 mungbean genotypes with diverse seed colors and origins in this study. This study was developed to provide nutritional information for mungbean sprouts from different genotypes with different seed coat colors. Depending on genotypes, significant variations in the contents of each phytochemical were identified before/after germination. Individual polyphenolic compounds had tissue and genotype specificity in mungbean. These findings will provide valuable information for molecular breeding to develop mungbean cultivars with high polyphenol content for sprout production.

Data Availability

The data that support the findings of this study are available in the supplementary material of this article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

S Jeon and BC Kim contributed equally to this work; S Jeon analyzed and interpreted the data and wrote the manuscript; S Jeon and BC Kim performed the experiment and interpreted the data; J Ha set up the research and designed the experiment.

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Supplementary Materials

This file contains Supplementary Figures S1-S2 and Tables S1-S2. https://downloads.hindawi.com/journals/jfq/2023/7555915.f1.zip (*Supplementary Materials*)

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