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Research Article

Metabolic and Developmental Changes in Germination Process of Mung Bean (*Vigna radiata* (L.) R. Wilczek) Sprouts under Different Water Spraying Interval and Duration

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Mung bean is one of the world's most important legume crops and is a major protein source, particularly in developing countries. Various polyphenolic compounds and nutrients accumulate in mung bean sprouts during germination. Mung bean sprouts are consumed globally as an excellent food source of bioactive phenolic compounds. The contents of phenols and flavonoids and antioxidant activity were monitored for four days after germination under four different spraying conditions using three mung bean cultivars. On the third day after germination, the sprout extract showed the highest antioxidant capacity. The length and thickness of hypocotyl of mung bean sprouts appeared to be the most suitable for consumption on the third day after germination. Using high-performance liquid chromatography analysis, eight phytochemicals were identified, and neochlorogenic acid was identified for the first time in mung bean sprouts. End products (neochlorogenic acid, chlorogenic acid, vitexin, and isovitexin) showed certain trends in their contents for four days, while intermediates (caffeic acid, catechin, syringic acid, and p-coumaric acid) were highly responsive to watering condition and cultivars. Watering interval significantly affected the length of root and lateral root development. Both cultivars and watering conditions and/or their interaction significantly affected the biochemical and physical traits of mung bean sprouts. The results suggest that watering conditions need to be considered as an important factor to improve food quality of mung bean sprouts. Our phenotypic and metabolic profiling would provide potential information for production of mung bean sprouts that fit consumers' preferences.

1. Introduction

Mung bean (*Vigna radiata* (L.) R. Wilczek) has been cultivated as a major resource of protein and carbohydrate in many developing counties in Asia [1]. Mung bean sprout has been reported to contain high concentrations in ascorbic acid and key polyphenols, which are directly related to antioxidant scavenging activity [2, 3]. In addition to these bioactive compounds, they are rich in essential amino acids, dietary fiber, and vitamins to improve health benefits for humans [4]. With these nutritional values, consumption of mung bean sprouts has increased according to dietary trends worldwide; they are usually sprinkled on the top of noodles to promote the texture of foods and are used as ingredients for soup, bread, and stirred-fried foods [1].

Sprouting is a complex process inducing dynamic metabolic and physical changes in mature seeds, which begin with imbibition and end up with the development of seeds into seedlings [5]. In soybean, a reference legume species, the contents of phenolics and isoflavone at sprouting stage have been intensively investigated under various germination conditions such as temperature, light, and water spraying interval and duration [6–10]. The level of antioxidant and sprout development were primarily affected by the spraying interval and duration [10]. In various plant species, the effects of the spraying interval and duration on the morphological and biochemical changes have been reported earlier [11–13]. In onion, total phenolic contents decreased as spraying duration increased, while total flavonoid contents generally increased

[11]. Another research showed that the root length of better-head lettuce increased when the spraying interval is relatively short [13].

Polyphenols are one of the secondary metabolites that have a wide range of biological effects including anti-inflammatory, antidiabetic, and anticancer effects beyond antioxidant capacity [14]. Phenolic acids and flavonoids are important phenolic compounds abundant in legume species and have powerful antioxidant scavenging activities. Regular consumption of legume crops can reduce the risk of chronic diseases [15, 16] such as Alzheimer's, cardiovascular, cancer, cataract, diabetes, and coronary artery diseases [17–19]. In mung bean, various polyphenols had been reported to be significantly accumulated in their sprouts during the germination stage [2, 20]. These compounds (caffeic acid, ferulic acid, gallic acid, *p*-coumaric acid, catechin, and rutin) are mainly observed to be increased time-dependently, and antioxidant activities were also enhanced as bioactive compounds increased in mung bean sprouts [2]. The polyphenol accumulation of mung bean sprouts was highly influenced by environmental conditions [21]. However, the effects of water spraying interval and duration on the contents of secondary metabolites and antioxidant activities have not been reported in mung bean sprouts yet, which had been reported as one of the most important factors affecting antioxidant levels in soybean sprout [10]. Therefore, we hypothesize that water spraying conditions can enhance the polyphenols in mung bean sprout to produce high-quality sprout vegetables.

The aims of our study are to investigate the effects of spraying conditions on physical developments, total phenols and flavonoid contents, antioxidant activity, and polyphenolic compounds of mung bean sprouts. The polyphenolic compounds were qualified and quantified to reveal the changes in polyphenol composition during germination using three mung bean cultivars (Dahyeon, Samgang, and Sunhwa). Dahyeon has relatively high polyphenolic compounds in its sprouts, Samgang has high sprout yield and is commonly used for sprout production, and Sunhwa is a reference cultivar of mung bean [22–25].

2. Materials and Methods

2.1. Plants Sample Preparation. Three mung bean cultivars, "Dahyeon," "Samgang," and "Sunhwa" (provided by Crops Genomics Lab in Seoul National University, Seoul, South Korea), were used in this study. The seeds were harvested at the Gangneung-Wonju National University Experimental Farm in Gangneung, South Korea (37.77°N, 128.86°E). The seeds were sterilized with 75% ethanol for 1 min and washed thoroughly with distilled water three times. The sterilized seeds were soaked in distilled water for 16 h at 37°C by using an incubator (ISS-4075R, Jeiotech) for germination. Then, the seeds were transplanted into the plant growth chamber (ST-001A, Sundotcom) set at 28–30°C with ~90% relative humidity and cultivated for 1, 2, 3, and 4 days under dark conditions (Figure S1). During cultivation, automatic water supply was ensured, and the spraying condition was comprised of two spraying intervals (2 hours, 4 hours) and two spraying durations (2 minutes, 4 minutes): 2-hour spraying interval with 2 mins spraying duration (2 h/2 m), 2-hour spraying interval with 4 mins spraying duration (2 h/4 m), 4-hour spraying interval with 2 mins spraying duration (4 h/2 m), and 4-hour spraying interval with 4 mins spraying duration (4 h/4 m).

2.2. Phenotype Measurements. Total 50 mung bean seeds were cultured for each experimental group, and measurements were conducted using 30 randomly collected seeds. The thickness of hypocotyl and the length of epicotyl, hypocotyl, and root were measured using ImageJ [26], and the thickness was measured at the thickest part in hypocotyl. The number of lateral roots with over 1 mm of length was counted.

2.3. Extraction Procedure. 30 fresh mung bean sprouts randomly collected were completely dried at 70°C for 24 hours using the incubator (ISS-4075R, Jeiotech) [27]. Samples were ground into fine powder and dissolved in 70% ethanol (w/v, 1 : 10). After 24 h in the dark, the mixture was sonicated at room temperature for 10 min and centrifuged at 13,000 rpm for 5 min. After centrifugation, the supernatant was filtered through a 0.22 μm syringe filter and was diluted with distilled water to 1,000, 10,000, 10,000, 50,000, and 100,000 ppm for assay of ABTS, DPPH, phenol, flavonoid, and HPLC, respectively.

2.4. Determination of Total Phenolic Contents. Total phenolic contents were measured using Folin-Ciocalteu colorimetric method with slight modification [28]. The supernatant of each mung bean sprout extract (100 μL) was mixed with Folin-Ciocalteu Reagent (50 μL). After five minutes at room temperature, 20% sodium carbonate (300 μL) was added and kept in dark for 15 min at room temperature. Then, the mixtures were diluted with distilled water (1 mL). Total phenolic compounds were detected using a spectrophotometer (Thermo Scientific MIB, Multiskan FC, 738 nm). Gallic acid was used to construct the standard curve (0–200 μg/mL). The contents were measured as milligrams gallic acid equivalent (GAE) per 1 g of dry weight (GAE mg/g). The total phenolic contents determinations were performed in triplicate.

2.5. Determination of Total Flavonoid Contents. Total flavonoid contents were measured using the aluminum nitrate colorimetric method with slight modifications [29]. Mung bean sprouts extract (400 μL) was mixed with 10% aluminum nitrate (80 μL) and 1 M aqueous potassium acetate (80 μL). After 30 min, absorbance of the mixtures was determined using the spectrophotometer at 405 nm. The flavonoids content was measured as quercetin equivalent from the calibration curve of quercetin standard solution (0–200 μg/mL), and the results were expressed as milligrams quercetin equivalent (QAE) per 100 g of dry weight (QAE mg/100 g). The total flavonoids determinations were conducted in triplicate.

2.6. Antioxidant Ability Assay. Antioxidant capacity was measured by ABTS (2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) and DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging assay. The scavenging effect of samples for ABTS radicals was evaluated based on the method described by Yen [30]. The ABTS working solution was prepared by mixing 7.4 mM ABTS stock solutions with 2.6 mM potassium persulfate at a ratio of 1 : 1. After 24 h in the dark at room temperature, the samples were diluted with phosphate-buffered saline to an absorbance of about 0.7 at 734 nm. The supernatant (20 μL) was mixed with prepared ABTS solutions (180 μL). Then, the samples were kept in the dark for 10 min, and their absorbance was read at 734 nm with a spectrophotometer against a blank. Experiments for each sample were carried out in triplicate, and the result was expressed as a percentage of scavenging achieved by the ABTS.

Scavenging of DPPH radical was measured using an antioxidant status kit (Dojindo, DPPH Antioxidant Assay Kit) according to the protocol provided by the manufacturer with slight modification. Briefly, each sample (20 μL) was mixed with assay buffer (80), and DPPH solution (100 mg/mL) was added. The scavenging was detected using the spectrophotometer at 517 nm. Experiments for each sample were carried out in triplicate, and the result was expressed as a percentage of scavenging achieved by the DPPH.

2.7. Profile of Polyphenolic Compounds Using HPLC Analysis. The analysis of polyphenolic compounds was performed using the High-Performance Liquid Chromatography-system (Shimadzu, Kyoto, Japan) equipped with a diode array UV-vis detector. The compounds were separated using a C18 column (Shimadzu, Kyoto, Japan; 250 × 4.6 mm, 5 μm). Solvent A was water and solvent B was acetonitrile. The flow rate was 0.7 mL/min, and the column temperature was set as 30°C. Initially, the ratio of solvent B begins at 12%, increased to 36% until 20 min, decreased to 5% until 25 min, and was maintained until 30 min. The analysis was conducted at least three replicates. In total, 11 metabolites were used as standard compounds including quercetin (CFN99272, Chem faces, China), neochlorogenic acid (CFN97472), catechin (CFN99646), chlorogenic acid (CFN99116), caffeic acid (CFN99190), trans-ferulic acid (CFN92394), vitexin (CFN98601), myricetin (CFN98877), kaempferol (CFN98838), syringic acid (CFN98884), and isovitexin (CFN98620).

2.8. Statistical Analysis. Statistical analysis was performed in *R* [31]. The significant differences were calculated by one-way and two-way analysis of variance (ANOVA) followed by Duncan's multiple tests. Correlation analyses were identified using Pearson's correlation. P value < 0.05 was defined as statistical significance.

3. Results

3.1. General Growth in Mung Bean Sprouts. Mung bean sprouts were cultivated under different water spraying intervals and durations using three cultivars, Dahyeon, Samgang, and Sunhwa. The developments of three cultivars were monitored for four days by measuring their lengths of epicotyl (Figure 1(a)), hypocotyl (B), and root (C), the thickness of hypocotyl (D), and the number of lateral roots (E). The length of hypocotyl increased rapidly from DAG (days after germination) 1 to DAG 3 and then increased slightly up to DAG 4 with the emergence of epicotyl (Figure 1(b)). Epicotyl emerged on DAG 3 (Figure 1(a)). The length of root increased until DAG 4 (Figure 1(c)). There were no remarkable differences in the thickness of hypocotyl (Figure 1(d)). As cultivation time increases, the emergence of lateral roots became abundant (Figure 1(e)). The length of root ($P < 0.01$) and the number of lateral root ($P < 0.05$) significantly differed depending on the watering conditions. Under the condition of 2 h interval, the length of root was significantly longer than that of samples grown under the condition of 4 h interval. The lateral roots were most abundant at 2 h/2 m condition. Four different watering conditions (4 h/2 m, 4 h/4 m, 2 h/2 m, and 2 h/4 m) were applied during the cultivation. The length of epicotyl, the length of hypocotyl, the length of root, the thickness of hypocotyl, and the number of lateral roots are shown in Table 1.

3.2. Dynamic Changes in Total Phenol and Flavonoid Contents and Antioxidant Activities during Germination. The polyphenolic contents and antioxidant activities in mung bean sprouts were measured for four days (Figure 2). Clustering analysis was conducted to identify the general trends in bioactivities during mung bean sprouts growth, and dynamic changes in the contents of polyphenolic compounds and the antioxidant activities were observed for the first three days after germination (Figure 2).

In general, total phenolic and flavonoid contents gradually increased for the first three days (Figure 2). Their contents reached the highest level on DAG 3, and then, their contents slightly decreased (no significance) (Figures 2(a) and 2(b)). The antioxidant activities showed a similar pattern to the contents of polyphenolics, and the highest activities of both ABTS and DPPH were observed on DAG 3 (Figures 2(c) and 2(d)).

3.3. Phenolics and Flavonoids and Antioxidant Activities in Mung Bean Sprouts. We measured polyphenolic contents and antioxidant capacities in mung bean sprouts on DAG 3, when physical traits were the most suitable to be consumed as sprouts and the overall bioactivity peaked (Figure 2). Under the same watering condition, significant differences were detected among the mung bean cultivars (Figure 3). In total phenolic contents, Dahyeon, Samgang, and Sunhwa had the highest contents at 2 h/4 m, 4 h/4 m, and, 2 h/2 m conditions, respectively (Figure 3(a)). The contents of flavonoids were similar to those of phenolics in general, and notably, the cultivar Sunhwa contained much higher contents than the Samgang and Dahyeon cultivars under 2 h/2 m and 4 h/4 m conditions (Figure 3(b)).

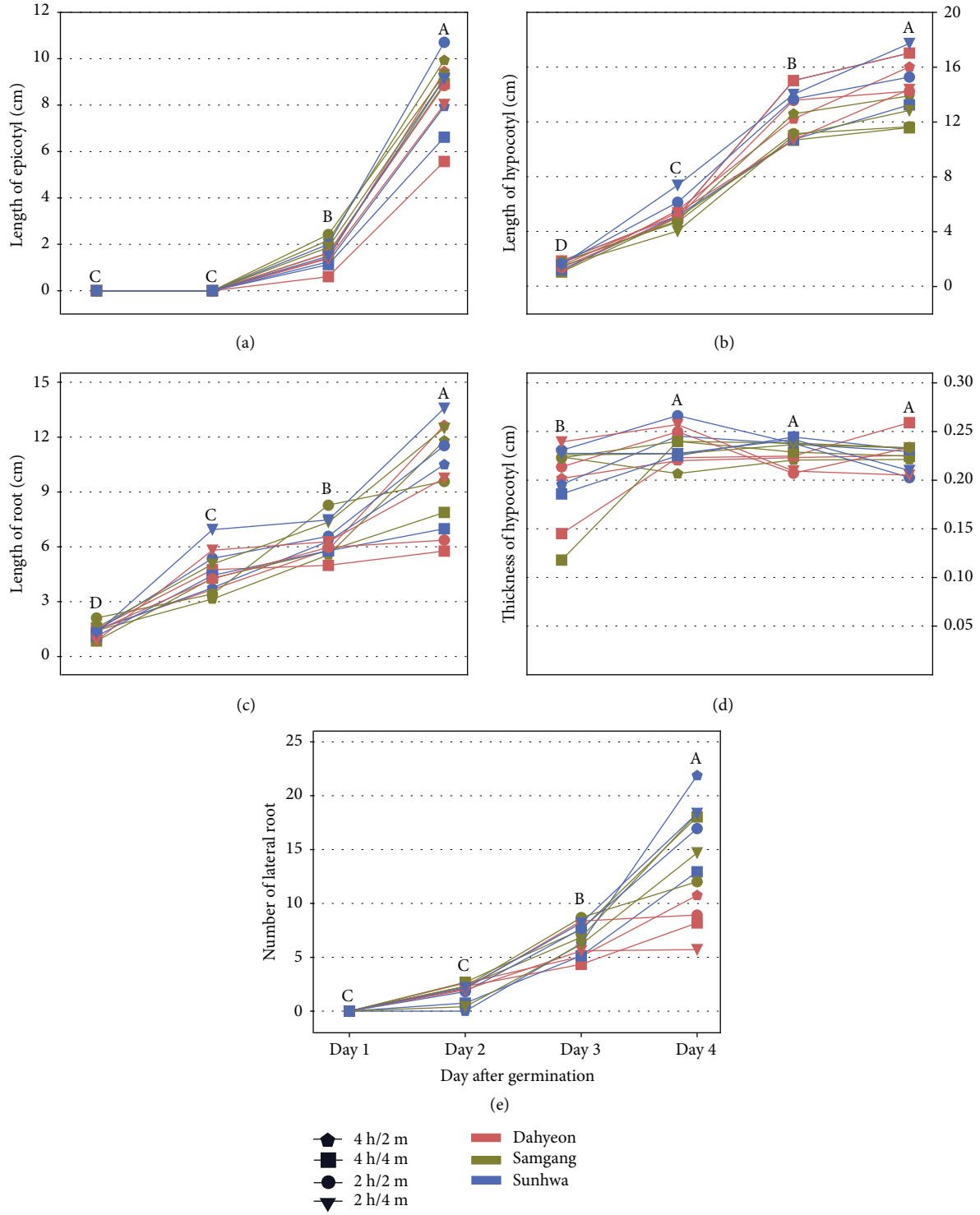

FIGURE 1: Developments of mung bean sprouts for four days after germination. (a) The length of epicotyl, (b) the length of hypocotyl, (c) the length of root, (d) the thickness of hypocotyl, and (e) the number of the lateral roots. Line color and marker indicate mung bean cultivars and watering conditions, respectively. Results with different uppercase letters indicate statistical significance ($P < 0.05$).

Antioxidant activities detected through ABTS and DPPH assays all agreed well with each other (Figures 3(c) and 3(d)). The cultivars Sunhwa and Samgang showed the highest and the lowest antioxidant activity in general, respectively (Figures 3(c) and 3(d)). The patterns of antioxidant activities were positively correlated with the pattern of the contents of phenolics and flavonoids (Figure 4), The significant positive correlations were observed between the phenol and flavonoid ($r = 0.89$, $P < 0.01$), phenol and ABTS ($r = 0.96$, $P < 0.01$), phenol and DPPH ($r = 0.91$,

Table 1: The effects of watering condition on morphological changes of mung bean sprout for four days after germination.

Organ	Day	Dahyeon				Samgang				Sunhwa			
		42	44	22	24	42	44	22	24	42	44	22	24
Length of epicotyl (cm)	1	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0
	3	1.4 ± 0.4	0.6 ± 0.4	1.6 ± 0.7	1.4 ± 0.5	2.2 ± 0.8	1.6 ± 0.6	2.4 ± 0.9	1.9 ± 1.2	1.3 ± 0.5	1.1 ± 0.5	2 ± 0.6	1.5 ± 0.6
	4	9.5 ± 1.9	5.6 ± 1.9	8.8 ± 2.5	8 ± 2.5	9.9 ± 1.5	8.9 ± 1.5	9.3 ± 2.3	9.2 ± 2.2	7.9 ± 2	6.6 ± 2.3	10.7 ± 3.3	9.1 ± 1.9
Length of hypocotyl (cm)	1	1.1 ± 0.3	1.9 ± 0.3	1.4 ± 0.4	1.5 ± 0.3	1.7 ± 0.4	1.1 ± 0.2	1.8 ± 0.3	1.5 ± 0.4	1.9 ± 0.4	1.2 ± 0.4	1.7 ± 0.4	1.5 ± 0.5
	2	5.6 ± 1	5.2 ± 1.5	5 ± 0.8	5.4 ± 1.9	4.8 ± 1.6	5 ± 0.9	4.7 ± 1	4 ± 1.1	5.2 ± 1.6	5.2 ± 1.1	6.1 ± 1.2	7.4 ± 1.3
	3	12.2 ± 2.8	15 ± 2.7	13.6 ± 3.3	10.7 ± 3.3	12.6 ± 2.8	10.7 ± 1.4	11.1 ± 2.1	11 ± 3.5	15 ± 3.2	10.7 ± 2.3	13.7 ± 2.9	14 ± 2.7
	4	16 ± 2.5	17 ± 1.7	14.2 ± 1.6	14.4 ± 2	13.9 ± 1.2	11.6 ± 0.8	11.7 ± 2.3	12.8 ± 1.1	17 ± 2.6	13.2 ± 2.4	15.3 ± 2.2	17.7 ± 2.3
Length of root (cm)	1	1.5 ± 0.5	1.5 ± 0.5	1.4 ± 0.9	0.9 ± 0.7	1.4 ± 0.9	0.8 ± 0.3	2.1 ± 0.8	1.6 ± 1.1	1.4 ± 0.7	1.1 ± 0.5	1.4 ± 0.9	1.3 ± 0.8
	2	3.7 ± 1.2	4.7 ± 1.3	4.3 ± 1.7	5.8 ± 3	3.1 ± 1.8	4.3 ± 1.2	3.4 ± 1.9	5 ± 1.8	3.8 ± 2	4.4 ± 1.3	5.4 ± 1.6	6.9 ± 1.7
	3	5.8 ± 2.2	5 ± 1.5	6 ± 2.4	6.3 ± 2.5	5.6 ± 3.4	5.8 ± 1.8	8.3 ± 1.5	7.3 ± 3.3	6.3 ± 2.8	5.8 ± 2.1	6.6 ± 2.5	7.5 ± 3
	4	12.6 ± 3.1	5.8 ± 2.4	6.4 ± 1.9	9.8 ± 2.8	11.8 ± 2.4	7.9 ± 3.3	9.6 ± 2.6	12.5 ± 2.8	10.5 ± 3.2	7 ± 3.5	11.5 ± 1.6	13.6 ± 3
Thickness of hypocotyl (cm)	1	0.2 ± 0.1	0.1 ± 0	0.2 ± 0.1	0.2 ± 0	0.2 ± 0.1	0.1 ± 0	0.2 ± 0	0.2 ± 0.1	0.2 ± 0	0.2 ± 0	0.2 ± 0	0.2 ± 0.1
	2	0.2 ± 0	0.2 ± 0	0.2 ± 0.1	0.3 ± 0	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0	0.2 ± 0	0.2 ± 0.1	0.3 ± 0	0.2 ± 0
	3	0.2 ± 0	0.2 ± 0	0.2 ± 0	0.2 ± 0	0.2 ± 0	0.2 ± 0.1	0.2 ± 0	0.2 ± 0	0.2 ± 0	0.2 ± 0.1	0.2 ± 0	0.2 ± 0
	4	0.2 ± 0	0.3 ± 0.1	0.2 ± 0	0.2 ± 0	0.2 ± 0	0.2 ± 0	0.2 ± 0	0.2 ± 0	0.2 ± 0	0.2 ± 0	0.2 ± 0	0.2 ± 0
Number of lateral roots	1	0	0	0	0	0	0	0	0	0	0	0	0
	2	2.6 ± 0.9	2.3 ± 0.6	2.1 ± 1.2	2 ± 1	2.3 ± 1.3	2.7 ± 1.3	2.3 ± 1.2	0.4 ± 0.3	0 ± 0	0.8 ± 0.4	1.8 ± 0.5	2.2 ± 1.3
	3	5.1 ± 0.6	4.4 ± 1.4	8.4 ± 1.4	5.6 ± 1.2	6.9 ± 1.1	7.6 ± 1.3	8.7 ± 1.3	6.2 ± 1.5	6.3 ± 1.9	5.1 ± 1.2	7.7 ± 1.3	8.2 ± 1.6
	4	10.8 ± 1.2	8.2 ± 1	7.8 ± 1.4	5.7 ± 1.7	18.3 ± 1.8	18 ± 2	12 ± 1.4	14.7 ± 2.3	21.9 ± 2.4	12.9 ± 2.4	17 ± 2.4	18.4 ± 2.5

Note: Four different watering conditions (4 h/2 m, 4 h/4 m, 2 h/2 m, and 2 h/4 m) were applied during the cultivation. The length of epicotyl, the length of hypocotyl, the length of root, the thickness of hypocotyl, and the number of lateral roots are given.

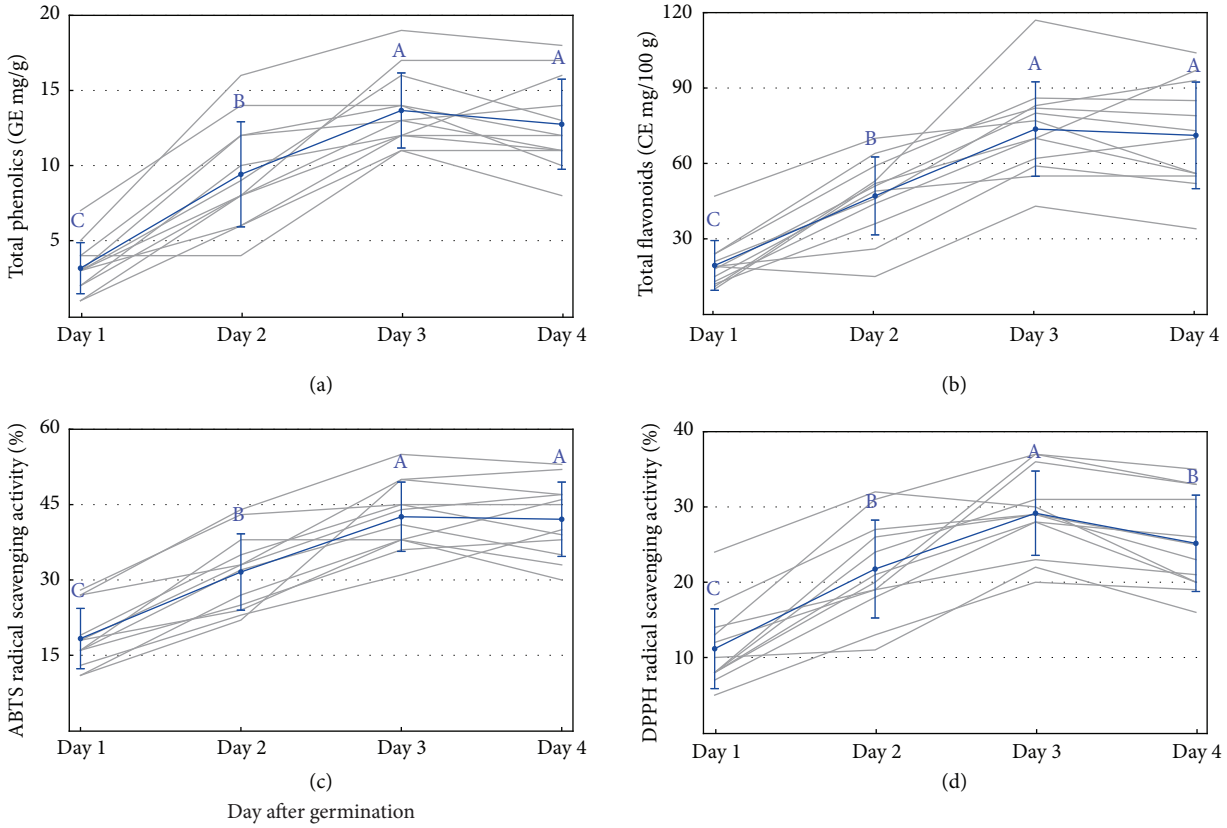

Figure 2: Changes of total phenolic and flavonoid contents, and antioxidant activity in mung bean sprouts. (a) Total phenolics, (b) total flavonoids, (c) ABTS radical scavenging activity, and (d) DPPH radical scavenging activity. The cluster analysis was performed by the K-means method. The blue lines are representative of trends in each assay, and results with different uppercase letters indicate statistical significance ($P < 0.05$).

$P < 0.01$), flavonoid and ABTS ($r = 0.91$, $P < 0.01$), flavonoid and DPPH ($r = 0.92$, $P < 0.01$), and ABTS and DPPH ($r = 0.9$, $P < 0.01$) (Figure 4).

To investigate the effect of watering conditions on the bioactivities of mung bean sprouts, we compared the contents of bioactive compounds under different watering conditions in each cultivar (Figure S2). In most cases, the antioxidant capacity and the contents of phenolics and flavonoids of each cultivar were significantly affected by the watering conditions (Figure S2). The highest contents of phenolic compounds were recorded in Dahyeon, Samgang, and Sunhwa under the watering conditions of 2 h/4 m, 4 h/4 m, and 2 h/2 m, respectively (Figure S2(a)). The contents of flavonoids showed significant differences depending on the watering conditions in each cultivar (Figure S2(b)). Their antioxidant activities were generally consistent with the contents of polyphenolics (Figure S2). Notably, only Dahyeon showed nonsignificant variations regarding the antioxidant capacity under different watering conditions, indicating the antioxidant capacity of Dahyeon cultivar was less affected by the environmental factors (Figures S2(c) and S2(d)).

In two-way ANOVA, on the flavonoid contents and antioxidant activity detected by DPPH, the main effects of cultivars and watering conditions and the interactions between the cultivars and watering conditions were highly significant (Table 2). On the phenolic contents, only the interaction between the cultivars and watering conditions had significant effect. On the oxidant activity detected by ABTS, the main effect of watering condition had no significant effect.

3.4. Polyphenolics Profile Using HPLC Analysis. Out of 11 substances that had been reported to be abundant in mung bean seeds and their sprouts, eight metabolites, including neochlorogenic acid, chlorogenic acid, catechin, caffeic acid, syringic acid, isovitexin, vitexin, and *p*-coumaric acid, were qualified and quantified by HPLC in the present study (Figure 5).

Out of eight polyphenolic compounds identified in this study, four compounds, neochlorogenic acid, caffeic acid, vitexin, and isovitexin, showed certain trends regardless of cultivars and watering conditions (Figures 5(a), 5(d), 5(f), and 5(g)). The neochlorogenic acid content kept an increasing trend after germination, and the caffeic acid increased for the first three days after germination, but the contents slightly decreased after DAG 3 (Figure 5(d)), and also the vitexin and isovitexin were declined as mung bean sprouts grew (Figures 5(f) and 5(g)). In contrast, the contents of chlorogenic acid, catechin, syringic acid, and *p*-coumaric acid varied regardless of cultivars and watering conditions (Figure 5).

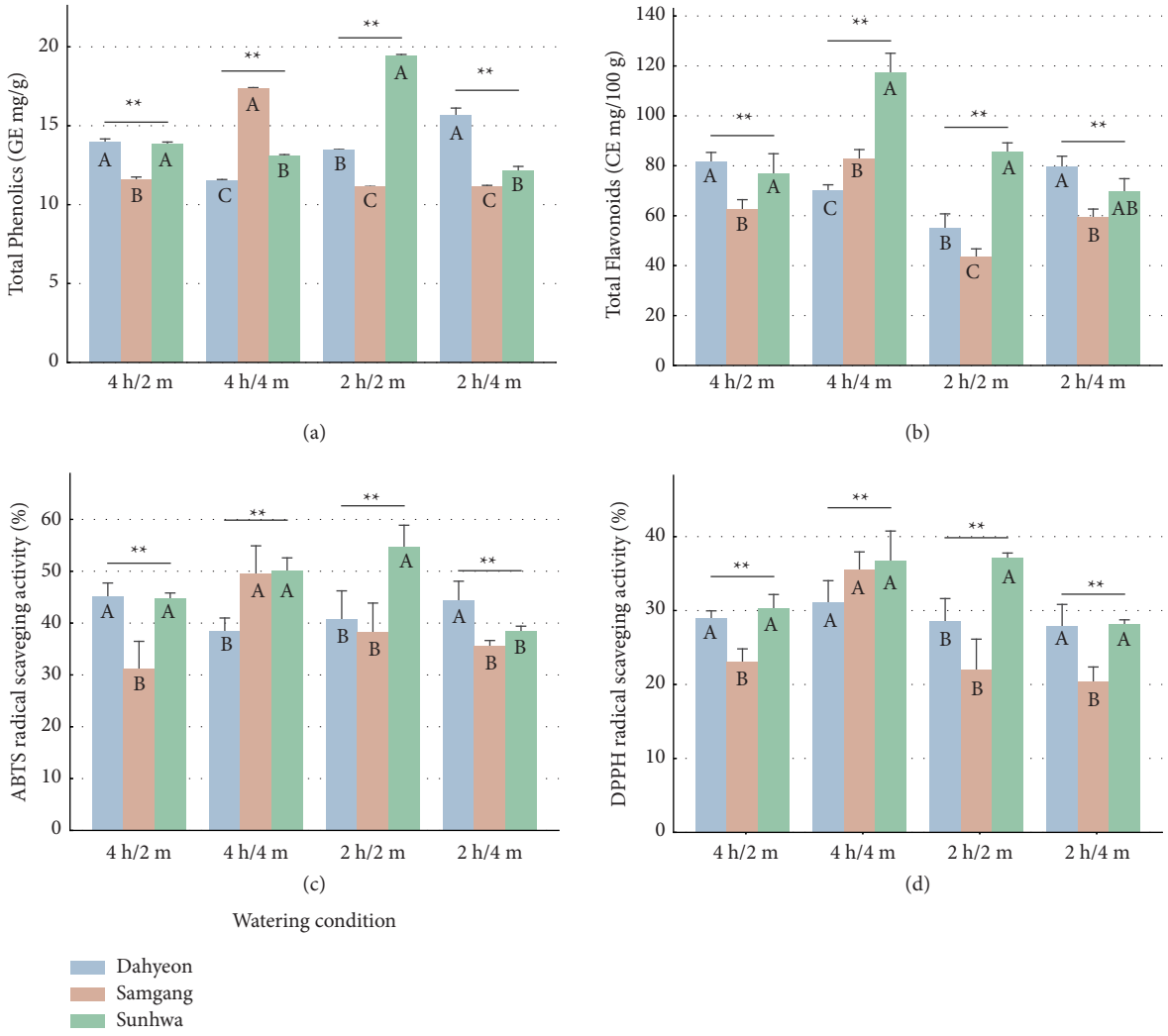

Figure 3: Comparison of (a) total phenolic contents, (b) total flavonoid contents, (c) antioxidant scavenging activity of ABTS, and (d) antioxidant scavenging activity of DPPH in three mung bean cultivars cultivated on different watering conditions. The comparison was conducted using mung bean sprouts after day 3 germination. The results were expressed with a standard error bar, and different lowercase letters indicate statistical significance ($P < 0.05$). The asterisks indicate P value of statistical significance ($P < 0.05^{*}$, $P < 0.01^{**}$, $P < 0.001^{***}$).

On DAG3, when the sprout extracts had the highest antioxidant capacity, neochlorogenic acid was the most abundant secondary metabolite in mung bean sprouts with 87.5 mg/100 g for mean value, followed by vitexin and *p*-coumaric acid with 10.9 mg/100 g and 6.4 mg/100 g, respectively. The contents of the other polyphenolics detected were chlorogenic acid (3.2 mg/100 g), catechin (4.2 mg/100 g), caffeic acid (4.3 mg/100 g), syringic acid (3.4 mg/100 g), and isovitexin (2.8 mg/100 g).

4. Discussion

In mung bean sprout, a thicker hypocotyl and lower ratio of root to hypocotyl are preferred for consumers, while epicotyl and lateral roots are considered as the negative feature by reducing texture [10, 32, 33]. In our observation, the length of roots and the number of lateral roots were increased when spraying interval was 2 hours compared to 4 hours (Figures 1(c) and 1(e)). This agrees well with previous report in soybean sprout that the length of roots was more affected by the spraying interval than the spraying duration, and the length of roots significantly increased when spraying occurred more frequently [10]. This result showed that spraying interval is one of the most important factors to improve food quality of mung bean sprouts. On DAG 3, all cultivars showed the most preferable traits to be consumed as sprouts with longer hypocotyls, shorter epicotyls and roots, and less lateral roots (Figure 1), which is consistent with the study that mung bean sprouts had the highest sensory quality at DAG 3 [34]. In addition, mung bean sprouts had the values of polyphenolic compounds with the highest bioactivities on DAG 3. The correlation analysis presented a strong positive correlation between antioxidant activities and polyphenolic compounds (Figure 4). On the fourth day, the antioxidant activities appeared to be similar to or lower than the third day (Figure 2), indicating mung bean sprouts are the most suitable to be consumed on the third day after germination.

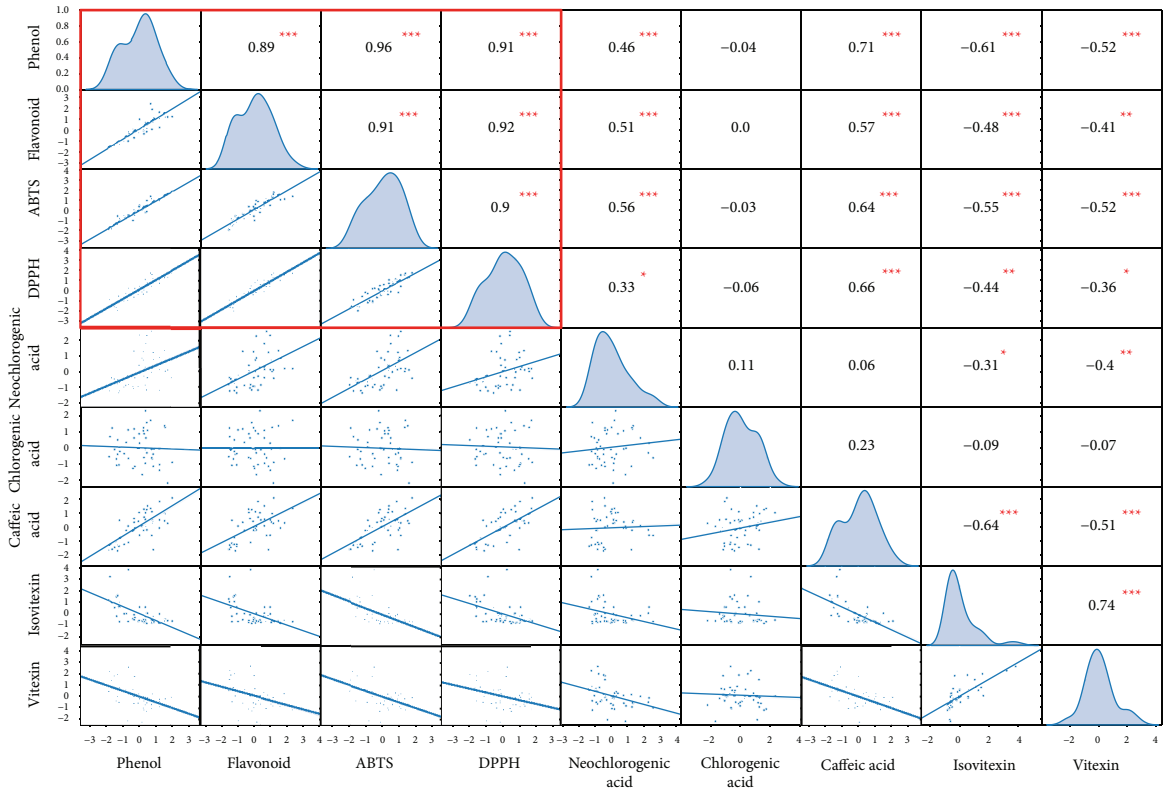

Figure 4: Correlation among four assays (phenol, flavonoid, ABTS, and DPPH) and five metabolites (neochlorogenic acid, chlorogenic acid, caffeic acid, isovitexin, and vitexin). Numeric values in the upper map are the correlation coefficient between two assays, and the stars indicate the statistical significance of the value. The lower map shows a scatter plot with a trend line for the correlation coefficient value, and the diagonal line shows a density plot of the value.

Table 2: Two-way analysis of variance results.

Parameters	Effects	Df	SS	MS	F	P
Phenol	Cultivar	2	20.21	10.105	3.109	0.060918
	Watering	2	3.96	1.981	0.609	0.550978
	Cultivar:watering	4	115.59	28.897	8.892	0.000103***
	Residuals	27	87.75	3.25		
Flavonoid	Cultivar	2	3922	1961.2	20.721	0.00000352***
	Watering	2	3665	1832.7	19.363	0.00000608***
	Cultivar:watering	4	2351	587.6	6.209	0.00112**
	Residuals	27	2555	94.6		
DPPH	Cultivar	2	366.6	183.29	13.871	0.0000718***
	Watering	2	337.6	168.8	12.775	0.000125***
	Cultivar:watering	4	173.5	43.37	3.282	0.025733*
	Residuals	27	356.8	13.21		
ABTS	Cultivar	2	422.7	211.36	6.073	0.00664**
	Watering	2	155.8	77.91	2.239	0.126030
	Cultivar:watering	4	505	126.25	3.628	0.0172*
	Residuals	27	939.7	34.8		

The effects of watering conditions and cultivars on the total phenolic and flavonoid contents and antioxidant activities of ABTS and DPPH are given. DF, degrees of freedom; SS, sum of squares; MS, mean squares. The asterisks indicate P value of statistical significance ($P < 0.05^*$, $P < 0.01^{**}$, $P < 0.001^{***}$).

In our approach, the antioxidant activities were measured by two colorimetric methods using ABTS and DPPH [35, 36]. Both are the most widely used approaches to detect the antioxidant capacity of plant extracts because they deliver fast and reproducible results and are easy to perform [35, 37–43]. In comparison with other assays such as CUPric Reducing Antioxidant Capacity (CUPRAC) and Ferric Reducing Antioxidant Power (FRAP) assay methods, previous studies reported that the Trolox® equivalent antioxidant capacity values found with CUPRAC are correlated

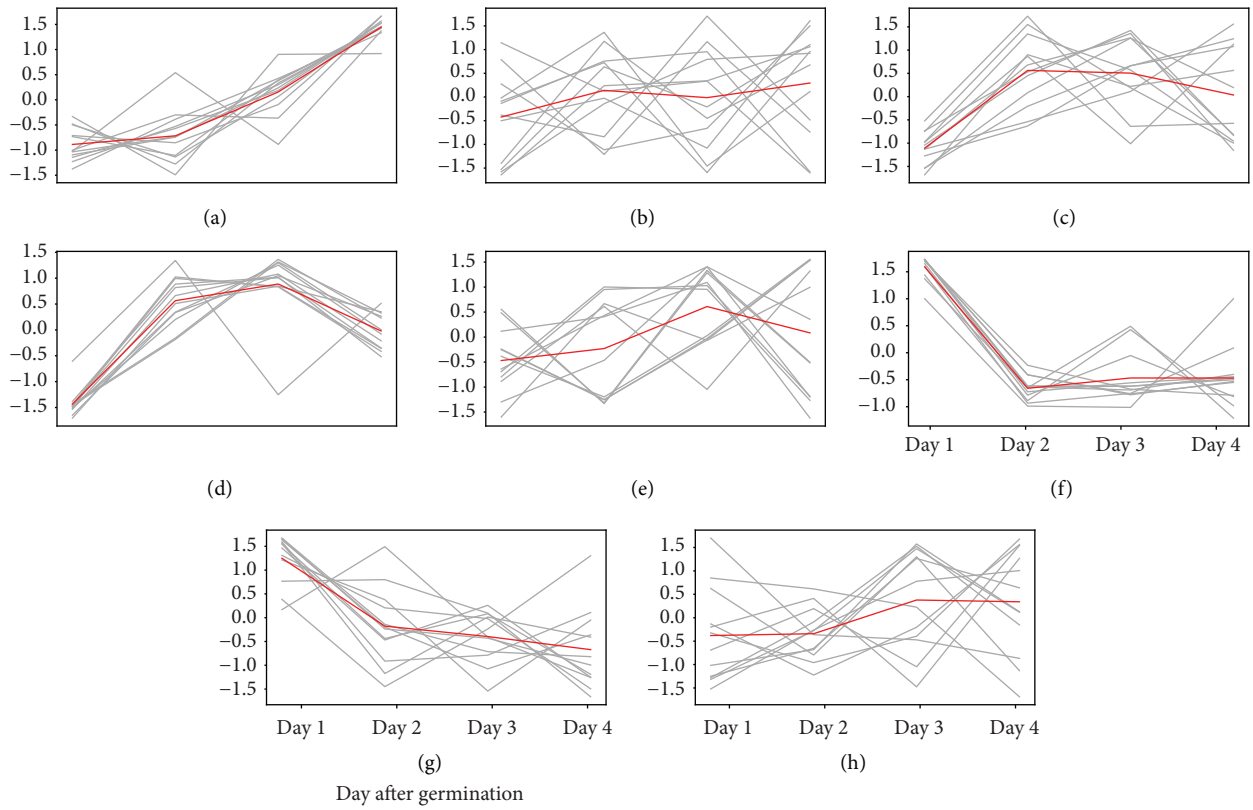

Figure 5: Changes of metabolites in mung bean sprouts for 4 days. (a) Neochlorogenic acid, (b) chlorogenic acid, (c) catechin, (d) caffeic acid, (e) syringic acid, (f) isovitexin, (g) vitexin, and (h) p-coumaric acid. The cluster analysis was performed by the K-means method. The red lines are representative of trends in each metabolite.

linearly ($r = 0.8$) to those of ABTS [44], and DPPH and ABTS had a higher correlation with total phenol contents than FRAP in various plant extracts [37]. However, ABTS and DPPH show several differences in their response to antioxidants [36]. ABTS can be solubilized in aqueous and organic media, which can detect both lipophilic and hydrophilic compounds in the sample, while DPPH can only be dissolved by nonpolar media such as alcohols [36]. In our study, DPPH and ABTS assays showed different results to detect antioxidant capacity. The main effect of watering condition on antioxidant capacity detected by DPPH was highly significant ($P < 0.001$), while antioxidant capacity detected by ABTS had no significance on watering condition for the same samples (Table 2), indicating antioxidants mainly affected by watering conditions might be polar phytochemicals in mung bean sprout.

To further explore changes in metabolic composition, HPLC analysis was conducted for the mung bean sprout extracts (Figures 5 and 6). The neochlorogenic acid is an isomer of chlorogenic acid and has been reported to be present in peaches, almonds, and coffee beans with many pharmacological benefits including anticancer, antitumor, and anti-inflammatory beyond antioxidant capacity [45–48]. In our study, neochlorogenic acid was the most abundant polyphenolic compound, although it had not been reported in mung bean sprout (Figure 5(a)). The content of neochlorogenic acid had a significantly positive correlation with antioxidant capacity (Figure 4). It indicates that metabolic profiling of mung bean sprout is needed to discover phytochemicals that have not been reported and to fully reveal the nutritional benefits of mung bean sprout.

Vitexin and isovitexin, an isomer of vitexin, are bioactive compounds that had been mainly found in seed coats of mung bean with potent antioxidant capacity [49]. In our study, the content of isovitexin had a strong positive correlation with that of vitexin (Figure 4). In general, the secondary metabolites of mung bean sprouts increased as mung bean sprouts grew, whereas the isovitexin and vitexin were significantly declined after germination (Figure 5). Thus, to obtain specific compounds such as isovitexin and vitexin, the germination time of mung bean sprouts needs to be regulated according to target metabolites with specific functions.

Caffeic acid is one of the major hydroxycinnamic acids present in wine and coffee [50–52] and has a potent antioxidant activity [53]. In our analysis, the caffeic acid has shown a significant positive correlation with antioxidant activity detected by DPPH and ABTS (Figure 4). According to the Kyoto Encyclopedia of Genes and Genomes (KEGG) database, caffeic acids are converted into ferulic acids by caffeic acid 3-O-methyltransferase (COMT) (Figure 6). A previous study had observed that both caffeic acids and ferulic acid increased during the sprouting stage of mung bean [2]. However, ferulic acids were not detected in our

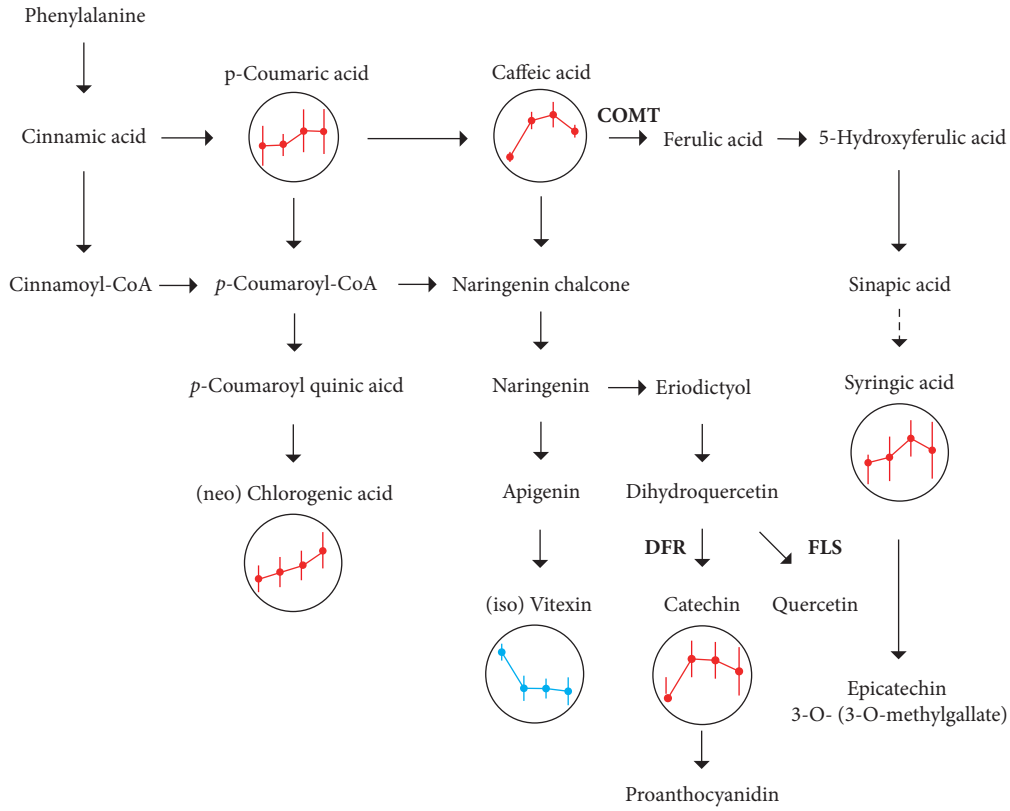

Figure 6: Schematic illustration of metabolic evolution of mung bean sprouts on the biosynthetic pathway during germination. Graphs in the circle show the changes of metabolites from day 1 to day 4 after germination. The dotted line indicates enzymic reaction that has not been fully identified in plants. COMT, DFR, and FLS are the caffeic acid 3-O-methyltransferase, dihydroflavonol, and flavonol synthase, respectively.

study, and, instead, syringic acid was detected, which was synthesized in the downstream of ferulic acid (Figure 6). The synthetic pathways from ferulic acid to syringic acid had not been fully identified, and our result may help to elucidate the pathway for synthesizing syringic acid.

Catechin is a flavonoid that shares the upstream biosynthetic pathway with quercetin [54]. In the flavonoid pathway, dihydroquercetin can diverge into catechin or quercetin by dihydroflavonol (DFR) or flavonol synthase (FLS), respectively [54]. Although an abundant level of catechin was detected in present study, quercetin was barely identified, which is inconsistent with previous report that two flavonoids were both steadily detected in mung bean sprouts [2, 55]. Because catechin and quercetin have a common precursor, the regulatory mechanism of the competition between these parallel pathways might decide which end products would be synthesized [56].

In the phenylalanine pathway, caffeic acid, catechin, *p*-coumaric acids, and syringic acids serve as intermediate metabolites in the middle of the chemical reaction to produce end products [57]. These intermediates can be synthesized into two or more metabolites by different enzymatic reactions (Figure 6), which are regulated by transcription factors (TFs) highly sensitive to environmental factors such as temperature, drought, and cold stress [58–61]. The flavonoid pathway has been reported to be highly regulated by various MYB TF family proteins [62]. Under drought dress, MYB111 and MYB1D TFs were upregulated, leading to an increase in the expression of FLS and COMT genes in arabidopsis [63]and wheat [64]. Enhanced expression of PAP1 and TT8 under low-temperature conditions resulted in increased expression level of DFR gene, causing an accumulation of anthocyanin contents in arabidopsis [65–67]. It indicates that the activities of the key enzymes such as COMT, DFR, and FLS might be affected by watering conditions, resulting in fluctuant contents of intermediates in the phenylalanine pathway (Figure 6).

5. Conclusion

For four days after the germination, we have observed dynamic changes in developments, total polyphenolic contents, antioxidant activities, and phytochemical composition of mung bean sprouts. According to watering conditions, significant differences were detected in both physical and biochemical traits even in the same cultivar. Our results show that watering conditions should be considered as an important factor to cultivate mung bean sprouts. Compared to other legume species, mung bean sprout had been less studied including breeding programs for a cultivar for sprout production. The physical and biochemical features identified in this study will help to

understand the composition of phytochemicals in mung bean sprouts and provide valuable information to breed a cultivar with a desired purpose.

Data Availability

The data used to support the findings of this study are included in the supplementary material of this article.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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Supplementary Materials

This file contains Supplementary Figures S1-S2 and Table S1. (*Supplementary Materials*)

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