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Comparison of Isoflavone Concentrations in Soybean (*Glycine max* (L.) Merrill) Sprouts Grown under Two Different Light Conditions

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We determined and compared the composition and content of isoflavones in the cotyledon, hypocotyl, and root of 17 soybean sprout varieties grown under dark and light conditions. The total average isoflavone concentrations in 17 soybean sprout varieties were 2167 $\mu g\ g^{-1}$ (green sprout) and 2538 $\mu g\ g^{-1}$ (yellow sprout) in cotyledons, 1169 $\mu g\ g^{-1}$ (green sprout) and 1132 $\mu g\ g^{-1}$ (yellow sprout) in hypocotyls, and 2399 $\mu g\ g^{-1}$ (green sprout) and 2852 $\mu g\ g^{-1}$ (yellow sprout) in roots. There were no significant differences in total isoflavone concentrations between the green and yellow sprouts. However, significant differences in total isoflavone amounts were observed among the three organs, with roots exhibiting the highest total isoflavone concentrations followed by cotyledons and hypocotyls. Total daidzin concentrations of green (775 $\mu g\ g^{-1}$) and yellow (897 $\mu g\ g^{-1}$) sprouts increased to more than 4 times that in seeds (187 $\mu g\ g^{-1}$). Yellow sprouts contained the highest (1122 $\mu g\ g^{-1}$) total genistin concentrations, and green (155 $\mu g\ g^{-1}$) and yellow (155 $\mu g\ g^{-1}$) sprouts had more total glycitin concentrations than seeds. In cotyledons of green and yellow sprouts, genistin, daidzen, and glycitin constituted more than 67%, more than 28%, and less than 4% of the total isoflavone contents, respectively. In hypocotyls, total daidzin represented more than 45% of the total isoflavones, and total glycitin was higher than in cotyledons and roots. Malonylglycoside concentrations were highest in cotyledons, whereas glycoside concentrations were highest in hypocotyls and roots. The high accumulation of isoflavones in roots is consistent with isoflavones serving as signal molecules in the induction of microbial genes involved in soybean (*Glycine max*) nodulation.

KEYWORDS: Soybean; green sprouts; yellow sprouts; isoflavones; HPLC; hypocotyls; cotyledons

INTRODUCTION

Research on the soybean [*Glycine max* (L.) Merrill] and its products has been receiving increased attention in recent years due to its potential beneficial effects on human health. Isoflavones in soybeans include daidzein, genistein, and glycitein along with their glycosides, and malonate conjugates are the main phenolic compounds that are essential for preventing menopausal symptoms and the incidences of osteoporosis, cardiovascular diseases, and certain types of cancers (*1*). In Korea, the soybean is an important crop and is utilized as a good source of oil and high-quality protein (*2–4*). Koreans traditionally have consumed large quantities of soybeans and soy foods. As such, soybeans and soy foods contain important ingredients that increasingly attract consumers (*5, 6*). A variety of soy foods is consumed in Asia, including tofu, miso, soymilk, soy sauce, soy flour, green or dried soybeans, soybean sprouts, fermented soy food called natto, soy meat patties, soy cheese, soy ice cream, and soy yogurt (*7*).

The soybean sprout (Kongnamool), which is a product 6–7 days after germination, has become an important traditional vegetable food in Korea. Despite its high nutritional value, the soybean is a low-cost crop that is relatively easy to produce (*8*). Owing to high demand and popularity throughout the year, mass production of soybean sprouts is an important agricultural business in Korea (*9*). Many studies on cultivation techniques, nutritional value, breeding, and the prevention of sprout rot have been conducted to improve the quality and quantity of soybean sprouts. The different levels of isoflavones in green and yellow soybean sprouts as well as the effect of light quality on the content of isoflavones have been reported previously (*10, 11*). Chi et al. (*10*) observed that the amount of nutrients, such as

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Table 1. Mean Isoflavone Concentrations (μg g^{-1}) in Soybean Seeds and Green and Yellow Soybean Sprouts (n = 3)

variety	total isoflavone concentrations		
	soybean seed	green sprout	yellow sprout
Anpyungkong	800	2229	1945
Bukwangkong	722	1626	2612
Dachaekong	807	2041	2673
Dawonkong	377	936	1355
Eunhakong	929	2282	1393
Hannamkong	531	1613	1630
Kwangankong	526	2904	2576
Myungjunamulkong	1017	1784	2174
Namhaekong	519	1414	2353
Orialtaekong	570	1190	2100
Pureunkong	919	2472	2063
Saebyulkong	722	2316	2590
Sobaeknamulkong	617	1034	1855
Sojinkong	994	2357	1816
Somyungkong	1080	2175	2396
Sorokkong	1303	1731	3119
Sowonkong	1241	2399	2302
mean	804	1912	2174
CV (%)	1.1	59	58
$LSD_{(0.05)}$	18	1866	2103

crude protein, crude fat, aspartic acid, crude fiber, vitamins, and minerals, is much higher in green soybean sprouts than in common yellow soybean sprouts. However, greening of the cotyledons resulting from light-dependent chlorophyll formation decreases the marketability of the sprouts because customers prefer yellow-colored cotyledons (*8, 12*). As a result, the preference for the yellow sprouts grown under dark conditions reduces the nutritional benefits of the soybeans.

Kim et al. (*13*) reported that total isoflavone concentrations in soybean sprouts of 30 varieties range from 768 to 3343 μg g^{-1} with mean values of 1321–2475 μg g^{-1}. In addition, total isoflavone levels of sprouts increased gradually during the cultivation period and were highest in roots, then cotyledons, and then hypocotyls (*14, 15*). In Korean soybean varieties, the contents of aglycone-type isoflavones such as daidzein and genistein were significantly elevated during germination (*14–16*). The difference in total isoflavone levels was also noted between soybean sprouts grown under light and dark conditions. Green soybean sprouts accumulated more total isoflavones than yellow soybean sprouts, and the higher the malonyldaidzin or malonylgenistin concentration, the higher the total isoflavone concentrations (*10, 11*).

The objectives of the present study were to further understand the effect of genetics on isoflavone concentrations among soybean varieties and to examine the variation and concentrations of isoflavones in different organs of soybean sprouts (cotyledons, hypocotyls, and roots) grown under dark and light conditions.

MATERIALS AND METHODS

Preparation of Soybeans and Soybean Sprouts. A total of 17 soybean varieties (**Table 1**) were obtained from the Wellbeing Nature Co., Ltd. (30 Songgyeri, Seolseongmyeon, Icheonsi, Gyeonggido) and were cultivated at Iksan (Honam Agricultural Research Institute, RDA, Korea) in 2005. After the soybean seeds were harvested and dried, they were stored at room temperature until used.

All seeds were germinated under two different light conditions in a greenhouse for 5 days using the method of Kim et al. (*11*). In brief, yellow soybean sprouts were produced in a dark room. For green soybean sprouts, seeds were germinated in the dark and then transferred to a yellow box (acryl film, 30 cm × 30 cm × 45 cm) under a yellow light source (550–590 nm wavelengths). This green sprout cultivation method for producing high levels of secondary metabolites has been patented by our research group in Korea (Patent No. 428997), China (Patent No. 194802), and Japan (Patent No. 3631648). Both yellow and green sprouts were grown at 25 °C with three replications and were separated into cotyledons, hypocotyls, and roots. All samples were freeze-dried at below –30 °C until dryness was achieved.

Quantitative High-Performance Liquid Chromatography (HPLC) Analysis of Isoflavones. Each seed or seedling organ sample was ground using a laboratory grinder. HPLC analysis was conducted according to the method of Wang and Murphy (*17*). The pulverized sample (2 g) was mixed with 2 mL of 0.1 N HCl and 10 mL of acetonitrile (ACN), stirred for 2 h at room temperature, and filtered through Whatman No. 42 filter paper. The filtrate was concentrated to dryness under a vacuum at –30 °C. The dried samples were redissolved in 10 mL of 80% HPLC-grade methyl alcohol (MeOH). Aliquots of the samples were filtered through a 0.45 μm filter unit (Titan syringe filter nylon membrane), transferred to 2 mL vials, and subjected to HPLC.

The Young-Lin HPLC system used was equipped with a model M-930 pump, a model M-720 UV detector, and a YMC-Pack ODS-AM-303 column (250 × 4.6 mm i.d.;Yong-Lin Inc., Korea). Isoflavones were detected at a wavelength of 254 nm.

We applied the modified HPLC method of Lee et al. (*18, 19*). A linear gradient was used with solvents A (0.1% glacial acetic acid in HPLC-grade distilled water) and B (0.1% glacial acetic acid in ACN). Following the injection of 20 μL, solvent B was increased from 15 to 35% in 50 min and was held at 35% for 10 min at a flow rate of 1 mL min^{-1}.

A total of 12 isoflavone standards, namely, daidzein, genistein, glycitein, daidzin, genistin, glycitin, acetyldaidzin, acetylgenistin, acetylglycitin, malonyldaidzin, malonylgenistin, and malonylglycitin, were purchased from LC Laboratories (U.S.A.) and were used for calibration curves. Each standard was dissolved in dimethylsulfoxide (DMSO). Standard curves were plotted at three concentrations of 1, 50, and 100 μg mL^{-1}, and a high linearity of $r^2 > 0.998$ was obtained from each calibration curve (data not shown). Each isoflavone in samples was dissolved in DMSO. All standards were identified by retention times of the authentic standards, and their concentrations were calculated by comparing their peak areas with those of the standard curves. DMSO was purchased from Merck (Germany). ACN, MeOH, acetic acid, and HPLC-grade water were obtained from J. T. Baker (U.S.A.).

Statistical Analysis. HPLC analyses of isoflavones were conducted with three replicates of each sample using a completely randomized design. The statistical analyses were undertaken using the general linear models procedure of the SAS software package program developed by the SAS Institute (*20*). The results were analyzed using an analysis of variance. Differences among the means of samples were determined by the least significant difference (LSD) test at the 0.05 probability level.

RESULTS AND DISCUSSION

In the cotyledons of green sprouts, the amounts of total isoflavones were highest in Sowonkong (4360 μg g^{-1}) and were lowest in Namhaekong (1057 μg g^{-1}). However, individual isoflavone levels widely varied depending on the varieties; the lowest was 16 μg g^{-1} genistein in Pureunkong, and the highest

Table 2. Mean Isoflavone Concentrations (μg g^{-1}) in Cotyledons, Hypocotyls, and Roots in Green and Yellow Soybean Sprouts ($n = 3$)

	cotyledon		hypocotyl		root	
variety	green sprout	yellow sprout	green sprout	yellow sprout	green sprout	yellow sprout
Anpyungkong	3026	2237	1240	673	2421	2939
Bukwangkong	1925	2270	1378	1603	1575	3964
Dachaekong	2572	3048	1306	1129	2245	3842
Dawonkong	1359	1379	663	322	786	2363
Eunhakong	1281	2000	706	490	4860	1688
Hannamkong	1782	1637	728	497	2330	2757
Kwangankong	1715	1823	1731	1207	5266	4698
Myungjunamulkong	3122	2747	951	840	1278	2936
Namhaekong	1057	1491	1230	1694	1954	3873
Orialtaekong	1388	2035	673	902	1510	3362
Pureunkong	2629	2990	2691	1930	2097	1271
Saebyulkong	1739	2318	1620	1904	3587	3548
Sobaeknamulkong	1137	1317	1294	969	670	3279
Sojinkong	2721	3301	655	1051	3696	1095
Somyungkong	2852	4095	1179	1467	2494	1626
Sorokkong	2181	4188	1147	1914	1864	3255
Sowonkong	4360	4262	686	655	2150	1990
mean	2167	2538	1169	1132	2399	2852
CV (%)	4.7	12	3.0	4.1	4.6	5.8
$LSD_{(0.05)}$	216	670	75	98	233	350

was 1715 μg g^{-1} malonylgenistin in Sowonkong. Malonylglycitin, acetylglycitin, and acetylgenistin were either undetectable or present in low or trace amounts. In the hypocotyls of green sprouts, the highest level of total isoflavones was 2691 μg g^{-1} in Pureunkong, and the lowest was 655 μg g^{-1} in Sojinkong. The levels of total isoflavones in the roots of green sprouts ranged from 670 μg g^{-1} (Sobaeknamulkong) to 5266 μg g^{-1} (Kwangankong). Individual isoflavone concentrations also greatly varied in the hypocotyls and roots of green sprouts in which malonylglycitin and acetylglycitin were undetectable in most varieties. For whole soybean sprouts, the total isoflavone concentrations were between 936 μg g^{-1} (Dawonkong) and 2904 μg g^{-1} (Kwangankong) in green sprouts and between 1355 μg g^{-1} (Dawonkong) and 3119 μg g^{-1} (Sorokkong) in yellow sprouts (**Table 1** and the Supporting Information).

As for the yellow sprouts, 12 individual isoflavones in cotyledons, hypocotyls, and roots exhibited a wide range of variation. In cotyledons, Sowonkong had the highest levels of daidzin (465 μg g^{-1}), acetyldaidzin (218 μg g^{-1}), and malonyldaidzin (1963 μg g^{-1}), whereas Somyungkong had the highest levels of genistin (1013 μg g^{-1}) and glycitein (31 μg g^{-1}). In yellow hypocotyls, the highest contents of glycitin (281 μg g^{-1}), malonyldaidzin (526 μg g^{-1}), daidzein (404 μg g^{-1}), glycitein (125 μg g^{-1}), and genistein (162 μg g^{-1}) were found in Bukwangkong, Pureunkong, Kwangankong, Anpyungkong, and Dachaekong, respectively. In the roots, daidzin (561 μg g^{-1}) and daidzein (1628 μg g^{-1}) were highest in Kwangankong, whereas glycitin (299 μg g^{-1}), malonyldaidzin (576 μg g^{-1}), acetyldaidzin (94 μg g^{-1}), and malonylgenistin (325 μg g^{-1}) were highest in Bukwangkong. Sorokkong, Namhaekong, and Orialtaekong had the highest concentrations of genistin (1721 μg g^{-1}), glycitein (110 μg g^{-1}), and genistein (125 μg g^{-1}), respectively. However, malonylglycitin, acetylglycitin, and acetylgenistin were not detected in yellow cotyledons and were either undetectable or detected in traces amounts in the hypocotyls of most varieties (**Table 2** and the Supporting Information).

Figure 1 shows the total means of isoflavone concentrations in cotyledons, hypocotyls, and roots in green and yellow sprouts of 17 soybean varieties. As compared to other tissues, hypocotyls contained at least 2-fold lower levels of isoflavones in both green (1169 μg g^{-1}) and yellow (1132 μg g^{-1}) sprouts. The highest isoflavone level was found in the roots of green and yellow sprouts (2399 and 2852 μg g^{-1}, respectively). Although no significant difference in total isoflavone concentrations was noted between green and yellow sprouts, there were significant differences between cotyledons and roots and between hypocotyls and roots ($p < 0.05$). Total isoflavone concentrations in roots were similar to those in hypocotyls. Overall, roots and hypocotyls contained the highest and lowest levels of isoflavones.

Our results agree with a previous report (*11*) in that Sowonkong and Pureunkong have the highest total isoflavones and Dawonkong has the lowest total isoflavones among the green and yellow sprouts. In fact, several earlier research reports (*18, 19, 21*) indicated that the differences in isoflavone concentrations in soybean sprouts are attributed to genotypes (varieties), cultivation locations, and environment (cropping years). Our results confirm the effect of genotype on isoflavone concentrations in soybean sprouts.

We compared the isoflavone contents in sprout soybean seeds and green and yellow soybean sprouts on the basis of the four groups, as shown in **Figure 2**. Glycosides (total sum of daidzin, glycitin, and genistin) and malonylglycosides (sum of malonyldaidzin, malonylglycitin, and malonylgenistin) were much

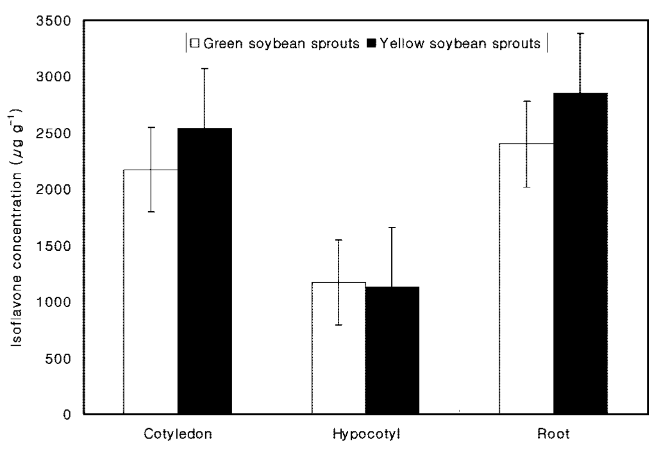

Figure 1. Mean total isoflavone concentrations in three different organs of green and yellow soybean sprouts. Bars indicate mean ± standard error, $n = 3$.

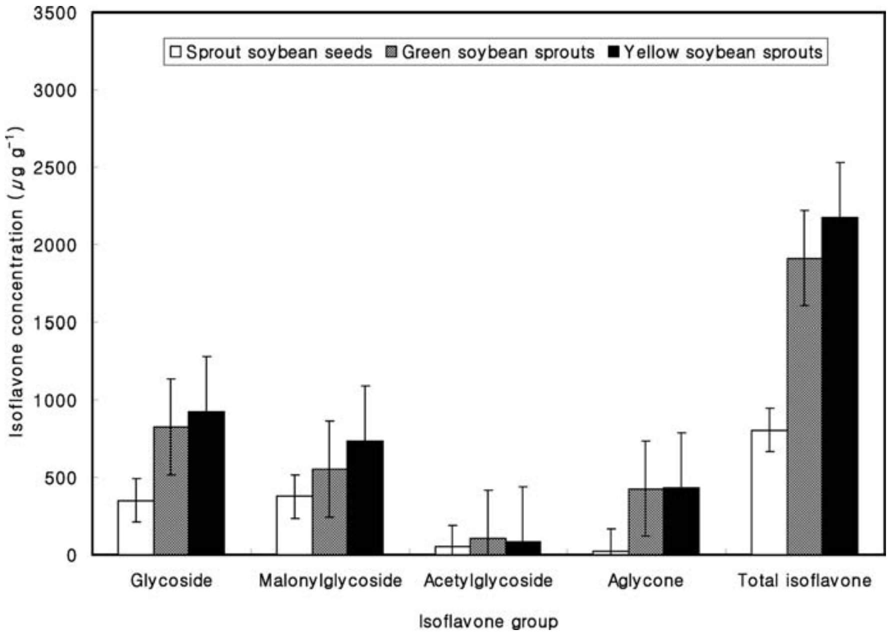

Figure 2. Mean isoflavone concentrations in soybean seeds and green and yellow soybean sprouts (glycoside, daidzin + genistin + glycitin; malonylglycoside, malonyldaidzin + malonylgenistin + malonylglycitin; acetylglycoside, acetyldaidzin + acetylgenistin + acetylglycitin; aglycone, daidzein + genistein + glycitein). Bars indicate mean ± standard error, $n = 3$.

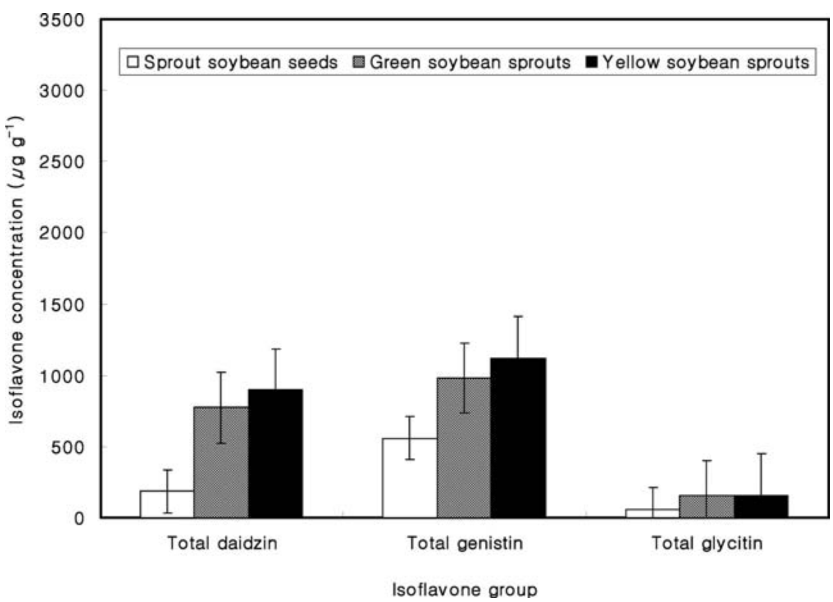

Figure 3. Mean total daidzin, genistin, and glycitin concentrations in soybean seeds and green and yellow soybean sprouts (total daidzin, daidzin + malonyldaidzin + acetyldaidzin + daidzein; total genistin, genistin + malonylgenistin + acetylgenistin + genistein; total glycitin, glycitin + malonylglycitin + acetylglycitin + glycitein). Bars indicate mean ± standard error, $n = 3$.

more abundant than aglycones (sum of daidzein, glycitein, and genistein) and acetylglycosides (sum of acetyldaidzin, acetylglycitin, and acetylgenistin). The differences in total isoflavones between green and yellow sprouts were not significant ($p < 0.05$). However, the differences in isoflavone contents between seeds and green and yellow sprouts were significant ($p < 0.05$). Interestingly, there were significantly more total isoflavones in green and yellow sprouts than in sprout seeds. It is similar to the report of Kirakosyan et al. (*22*) that levels of isoflavones in soybean seedlings were higher than in seeds. This is due to an enhanced synthesis of isoflavones and the release of glycones from isoflavone conjugates stored in the seeds as the soybean seedling develops (*23*). It appears that isoflavones accumulate in the roots of germinating seeds; isoflavones may excrete and participate in plant–microbe interactions as well as inhibit infections by plant pathogens (*24*). In this regard, our results are consistent with the fact that isoflavones are essential for soybean root nodulation by nitrogen-fixing rhizobacteria (*25*). The present study shows that the synthesis and accumulation of glycosides, malonylglycosides, and aglycones increase throughout sprout development, whereas those of acetylglycoside undergo only a negligible change.

Figure 3 shows a comparison of total daidzin (Tdin; sum of daidzin, malonyldaidzin, acetyldaidzin, and daidzein), total genistin (Tgin; sum of genistin, malonylgenistin, acetylgenistin, and genistein), and total glycitin (Tgly; sum of glycitin, malonylglycitin, acetylglycitin, and glycitein) in sprout soybean seeds and green and yellow soybean sprouts. Tdin concentrations in green (775 $\mu g\ g^{-1}$) and yellow (897 $\mu g\ g^{-1}$) sprouts increased to more than 4 times that in sprout seeds (187 $\mu g\ g^{-1}$). Tgin

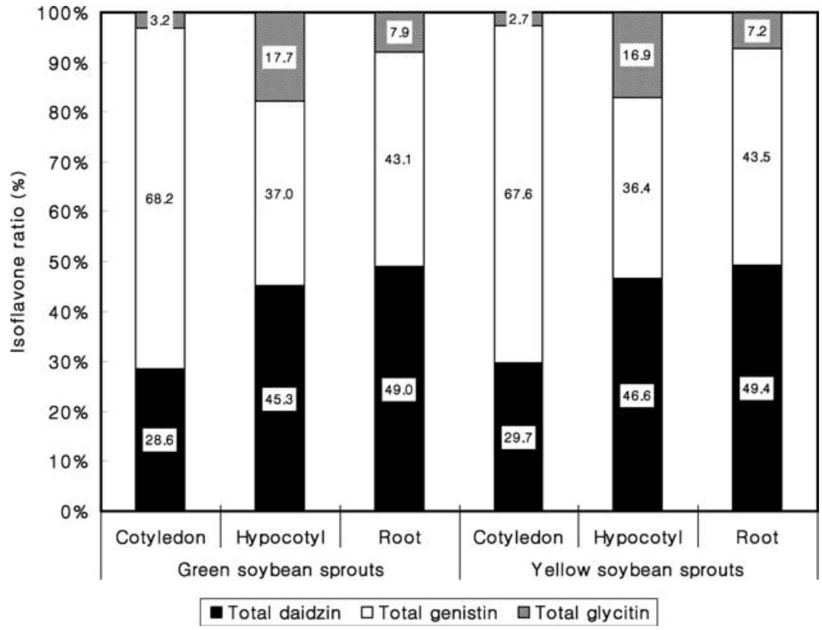

Figure 4. Mean percent daidzin, genistin, and glycitin in three different organs of green and yellow soybean sprouts (total daidzin, daidzin + malonyldaidzin + acetyldaidzin + daidzein; total genistin, genistin + malonylgenistin + acetylgenistin + genistein; total glycitin, glycitin + malonylglycitin + acetylglycitin + glycitein).

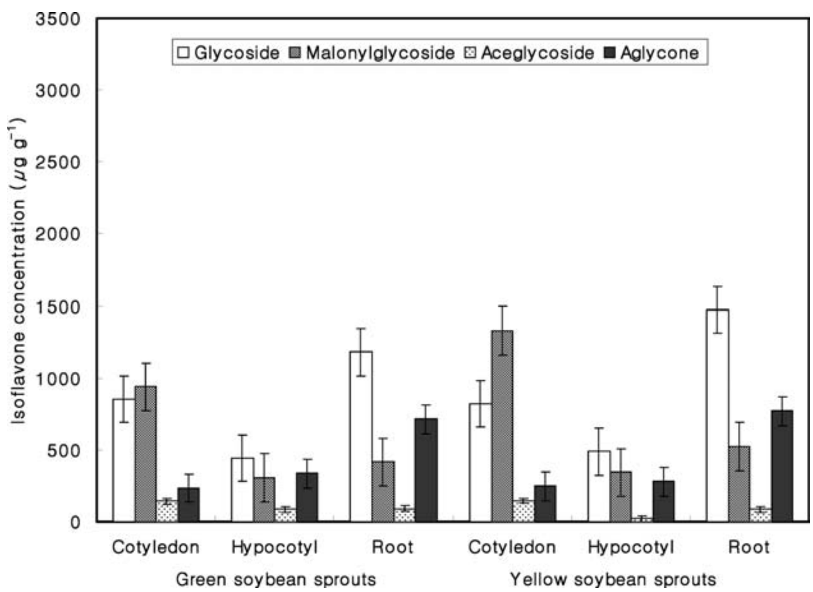

Figure 5. Mean isoflavone concentrations in cotyledons, hypocotyls, and roots of green and yellow soybean sprouts (glycoside, daidzin + genistin + glycitin; malonylglycoside, malonyldaidzin + malonylgenistin + malonylglycitin; acetylglycoside, acetyldaidzin + acetylgenistin + acetylglycitin; aglycone, daidzein + genistein + glycitein). Bars indicate mean ± standard error, $n = 3$.

concentrations were highest in yellow (1122 μg g^{-1}) and green (982 μg g^{-1}) sprouts. Tgly concentrations in green and yellow sprouts were about 154 μg g^{-1}.

Overall, the accumulation of isoflavones was ranked in the order Tgin > Tdin > Tgly in the green and yellow soybean sprouts and soybean seeds. In general, yellow sprouts accumulated more Tgin and Tdin than green sprouts or seeds. According to Kim et al. (*13*), the total isoflavone concentrations in soybean sprouts increase gradually during development, reaching a maximum level on the fifth day after germination. In addition, the highest accumulation of isoflavones occurred in roots followed by cotyledons and hypocotyls. Choi and Sohn (*26*) and Kim et al. (*27*) reported that the contents of total isoflavones and individual isoflavones increased to a large extent during seedling growth. Chi et al. (*10*) and Kim et al. (*11*) showed that the concentrations of isoflavones in sprouts were much higher than those in soybean seeds. All of the previous reports corroborate our present results.

The distribution of Tdin, Tgin, and Tgly in cotyledons, hypocotyls, and roots of green and yellow sprouts is presented in **Figure 4**. In the cotyledons of green and yellow sprouts, Tgin, Tdin, and Tgly comprised about 68%, 29%, and 3% of the total isoflavones, respectively. Tdin represented more than 45% of the total isoflavones in the hypocotyls of the two different sprout types, and hypocotyls contained about 2-fold and 6-fold higher levels of Tgly than cotyledons and roots, respectively. Kim et al. (*27*) reported that daidzein and genistein accumulated to the highest level in the roots and cotyledons, respectively. This result is consistent with our data.

As stated earlier, yellow and green soybeans had statistically equivalent mean total isoflavone concentrations. However, concentrations of the four isoflavone groups varied among cotyledons, hypocotyls, and roots (**Figure 5**). Cotyledons accumulated the highest level of malonylglycoside, whereas hypocotyls and roots accumulated the highest levels of glycoside and aglycone. Eldridge and Kwolek (*28*) reported that soybean seed hypocotyls and cotyledons contained 6120–16921 and 375–2393 $\mu g\ g^{-1}$ total isoflavones, respectively. More recently, Suzuki et al. (*24*) reported that roots of 5–7-day-old seedlings contained much more isoflavones than cotyledons and that the isoflavone conjugate-hydrolyzing β-glucosidase enzyme accumulated abundantly in the roots but negligibly in the hypocotyls and cotyledons. It follows that isoflavones accumulating in growing roots could lead to more nitrogen fixation and increased resistance to attack by pathogenic fungi (*23*). It is assumed that isoflavones synthesized in soybean seed hypocotyls is transferred from the cotyledons to the hypocotyls and roots since the isoflavones, such as genistein, are a primary receptor molecule for the formation of the nodule by nitrogen-fixing rhizobacteria in the roots of legume roots, including soybeans (*29*).

The quantity and quality of light is known to effect the nutritional composition and isoflavone concentrations of soybean sprouts, as green sprout showed a several-fold increase in daidzein and genistein compared to yellow sprouts and soybean seeds (*9*). Green soybean sprouts cultivated under colored light accumulated significantly more mean total isoflavones than yellow sprouts grown under dark conditions (*10*). However, we failed to find a significant difference in total isoflavone concentrations between green and yellow sprouts (**Figure 1**). It is possible that changes in the concentration and composition of isoflavones may be driven by light quality and exposure duration and dependent of the genotypes of the soybean samples selected. Recently, Kirakosyan et al. (*22*) demonstrated that isoflavone levels in soybean roots and shoots fluctuated significantly in response to exposure to different durations of phytochrome-mediated light treatments such as light, dark, red, and far-red wavelengths. The finding that a yellow light source (550–590 nm wavelength) affected isoflavone concentrations in green soybean sprouts in this study suggested that different wavelengths may either enhance or diminish isoflavone accumulation. Further studies are in progress to identify the optimum wavelengths that would lead to the enhancement of isoflavone amounts in growing soybean sprouts.

Supporting Information Available: Tables providing concentrations of 12 individual isoflavones in the different parts of green and yellow soybean sprouts. This material is available free of charge via the Internet at http://pubs.acs.org.

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