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Differential abilities of Korean soybean varieties to biosynthesize glyceollins by biotic and abiotic elicitors

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Abstract Glyceollins synthesized in soybeans that are exposed to biotic or abiotic stress have been reported to have health benefits. Considering that glyceollins are *de novo* synthesized from daidzein via several enzymatic steps and that isoflavone concentration widely varies among soybean varieties, the abilities of 60 soybean cultivars to synthesize glyceollins were compared under different elicitation conditions. Soybeans accumulated glyceollins differentially depending upon the cultivar when elicited with *Aspergillus sojae*. Contrary to our hypothesis that high isoflavone varieties may accumulate glyceollins more efficiently upon elicitation, glyceollin accumulation in response to fungal elicitation was not related with the concentration of either total isoflavones or daidzein in soybeans. Rather the glyceollin levels were significantly affected by soybean cultivar and most effectively increased by fungal infection. The data suggest that the selection of a strong fungal elicitor and a soybean cultivar with genotype that highly expresses the genes involved in glyceollin biosynthesis is essential for efficient glyceollin production.

Keywords: glyceollins, soybean cultivar, elicitor, phytoalexins, isoflavones

Introduction

Glyceollins are a class of phytoalexins produced in soybeans that are exposed to biotic or abiotic stress (1). Previous studies demonstrated that these prenylated pterocarpans have antioxidant, anti-fungal, anti-inflammatory, anti-tumorigenic, and several other biological activities (2–6). As glyceollins are derived from daidzein, a soybean isoflavone, there is a high possibility that soybean varieties with high isoflavone content produce glyceollins more efficiently than those with low isoflavone content upon elicitation (7), i.e., the accumulation of glyceollins in elicited soybeans is expected to be dependent upon the isoflavone content of each variety. To date, it has been reported that glyceollin accumulation is affected by the fungal strain and type of stress (8,9). The other important potential determinant for glyceollin biosynthesis could be the expression levels of genes involved in glyceollin synthesis, starting from phenylalanine to daidzein and glyceollin isomers (7,10,11). In particular, the genes involved in the glyceollin synthesis pathway may be differently expressed according to the soybean variety. Furthermore, gene expression may be influenced by diverse abiotic elicitors such as ultraviolet (UV) light, metals, and jasmonate (12,13).

Therefore, this study attempted to compare the abilities of soybean varieties to synthesize glyceollins upon abiotic or biotic stress.

Materials and Methods

Soybean samples The high isoflavone variety Aga 3 was provided by Soyventure (Daegu, Korea), and other varieties were obtained from Department of Southern Area, National Institute of Crop Science, Rural Development Administration.

Elicitation of glyceollins *De novo* synthesis of glyceollins was induced as reported previously (14). In brief, *Aspergillus* (*A.*) *sojae* or *Rhizopus* (*R.*) *oligosporous* cultures were inoculated at 25°C in the dark on potato dextrose agar. Inoculums were prepared by harvesting the fungal spores after 5 days of incubation. Moreover, soybean seeds were sterilized by soaking in 70% (v/v) ethanol for 3 min, followed by quickly rinsing with deionized water. Then, the soybean seeds were soaked in autoclaved deionized water for 4 h, sliced into half, and placed into petri-dishes layered with a filter paper moistened with distilled water. The fungal spore suspension was dropped on the cut surface of each seed. Soybean seeds inoculated with spores of either *A. sojae* or *R. oligosporous* were stored in a dark chamber at 26°C for 3 days, cleaned from fungi, and freeze-dried. The soybean powder (5 g) was then homogenized in 80% (v/v) aqueous ethanol (15 mL), incubated at 50°C for 1 h, cooled, and centrifuged at 14,000×*g* for 10 min. The extracts were filtered through a sterile syringe filter with a 0.45 μm pore size (Sartorius

Biotech GmbH, Goettingen, Germany) and collected for glyceollin analysis using HPLC.

Furthermore, the selected soybean varieties cut into halves were subjected to one of the following elicitations: UV light, aluminum chloride, or methyl jasmonate. For the UV treatment, the seeds were irradiated under UV (312 nm) for 15–60 min (15 min, 483 J/cm^2; 30 min, 1495 J/cm^2; 60 min, 8970 J/cm^2) per day; UV intensity was measured by a UV intensity meter (308; OAI, San Jose, CA, USA). Then, the soybean sprouts were incubated under dark conditions for 3 days, followed by analysis for glyceollins by HPLC (PU-1580; Jasco, Tokyo, Japan). In the case of treatment with aluminum or methyl jasmonate, soybeans were incubated at the designated concentrations of elicitor solution for 3 days prior to glyceollin analysis.

Analysis of glyceollins using HPLC Analyses of glyceollins in the samples were conducted using a Jasco System Controller equipped with a Jasco UV2077 detector and a PU-1580 pump, as described previously (15). Glyceollins in the sample extracts were directly analyzed by HPLC equipped with a Gemini C18 (150×2.0 mm; 5 μm; Phenomenex, Torrance, CA, USA) reverse phase column. The concentrations of glyceollins were measured by monitoring at a wavelength of 280 nm and comparing with the standard (purity >90%), which was isolated and identified by Professor Soon Sung Lim at Hallym University (Chucheon, Korea) (14). Elution was conducted at a flow rate of 0.8 mL/min using the following solvent system: A=0.1% acetic acid/water; B=acetonitrile; 10% B to 35% B in 40 min, then 35% B to 10% B in 5 min, followed by holding at 10% B for 10 min. Retention times for glyceollins were 35.1 min (glyceollin III) and 36.0 min (glyceollin II/I). All HPLC analyses were run in duplicate.

Statistical analysis Data were expressed as mean±standard deviation of duplicate analyses.

Results and Discussion

Comparison of the glyceollin producing abilities of 60 soybean varieties elicited with fungal infection Sixty soybean varieties cultivated in the Korean peninsula were elicited with *A. sojae* for 3 days and subjected to glyceollin analysis by HPLC. The levels of glyceollins in soybean sprouts exposed to *A. sojae* varied widely among the 60 varieties (Table 1). Most of the soybean varieties contained, at most, negligible levels of glyceollins in the intact state before elicitation (data not shown). However, the infection of half-cut soybean with *A. sojae* spores during incubation for 3 days caused a significant induction in glyceollin synthesis. The glyceollin concentrations of the samples ranged from less than 100 to higher than 9,000 μg per gram. The Sunam, Kwangan, Anpyeong, Aga 3, and Danbaeg soybean varieties showed higher than 7,000 μg of glyceollins per gram of dry sample. Interestingly, the glyceollin content of each soybean variety was not correlated with the

Table 1. Glyceollin contents of 60 varieties of soybeans exposed to *A. sojae* and *R. oligosporous*

Variety	Glyceollins (mg/g)	
	*A. sojae**[1)]	*R. oligosporous***[2)]
Aga 3	7.247±0.544	0.296
Alchan	4.387±0.598	1.022
Anpyeong	7.467±0.088	0.129
Baegcheon	2.086±0.156	0.415
Baegun	3.996±0.325	0.338
Bosug	2.784±0.056	0.047
Cheongdu #1	0.526±0.509	3.507
Dachae	1.145±0.390	0.010
Daewon	3.724±0.300	4.543
Dagi	5.610±0.712	0.310
Dajang	1.282±0.354	3.152
Danbaeg	7.538±0.102	1.550
Dankyeong	2.981±0.095	3.380
Danmi	2.997±0.083	0.016
Doremi	1.372±0.273	0.024
Duyou	4.064±0.258	0.091
Eunha	4.590±0.674	0.555
Geomjeong #1	4.684±0.625	0.082
Geomjeong #3	1.087±0.344	0.369
Geomjeongol	2.929±0.187	0.147
Geumgang	0.995±0.382	0.810
Hannam	1.669±0.178	0.519
Hojang	1.099±0.417	0.064
Hwangkeum	3.910±0.277	1.128
Iksannamul	5.121±0.504	0.008
Jangki	3.244±0.075	0.035
Jangkyong	2.022±0.169	0.454
Jangmi	3.244±0.184	0.168
Jangsu	2.199±0.084	5.732
Jangwon	2.063±0.136	0.060
Jangyeob	1.423±0.251	0.022
Jinmi	0.575±0.541	0.372
Jinpum	2.095±0.151	0.045
Jinyul	3.183±0.364	0.509
Jonam	4.056±0.359	0.523
Kwangan	8.357±0.059	0.196
Malli	1.801±0.260	0.110
Mansu	0.972±0.366	0.051
Milyang	1.911±0.192	0.445
Mirang	2.564±0.092	3.774
Muhan	4.912±0.680	0.246
Myeongjunamul	1.526±0.274	3.780
Namweon	1.043±0.122	1.460
Nogchae	1.102±0.465	0.552
Paldo	6.082±0.351	0.014
Pangsa	6.245±0.152	0.549
Pungsannamul	4.210±0.177	0.836
Pureun	2.625±0.177	0.071
Saeal	3.035±0.087	2.650
Samnam	1.045±0.377	0.453
Seonyu	0.576±0.489	0.272
Shingi	0.938±0.433	0.959
Shinpaldal	1.012±0.422	0.015

Table 1. Continued

Variety	Glyceollins (mg/g)	
	*A. sojae**[1)]	*R. oligosporous***[2)]
Sobaegnamul	3.387±0.163	1.626
Socheong	1.386±0.379	0.044
Soho	5.390±0.120	8.129
Sokang	2.236±0.223	1.539
Somyeong	2.536±0.090	3.761
Sorog	3.327±0.063	2.644
Sunam	8.310±0.776	0.406
Wonhwang	4.192±0.646	0.268

[1)]*Values are means of duplicate analysis.
[2)]**Values obtained by a single measurement.

isoflavone level, although some high isoflavone varieties accumulated more glyceollins as observed in the highest isoflavone variety Aga 3 (Table 2). Some varieties, including Cheongdu #1, Miryang, Seonyu, and Jinmi, showed extremely low ability to synthesize glyceollins upon exposure to biotic stress, suggesting the null or negligible expression of one or more genes involved in glyceollin biosynthesis.

Elicitation of soybeans with *R. oligosporous* resulted in different patterns of glyceollin accumulation among the 60 soybean varieties. Some of the varieties of soybean that accumulated glyceollins upon exposure to *A. sojae* did not respond to *R. oligosporous*, as shown in Table 1. When stimulated by *R. oligosporous*, glyceollin synthesis was most significantly induced in the soybean varieties that were moderately elicited by *A. sojae*, including Soho, Jangsu, and Daewon.

Glyceollins are synthesized from phenylalanine in raw soybeans elicited with external stresses such as an insect attack, fungal infection, and physical damage. Phenylalanine is, in turn, converted into daidzein via a branch of the general phenylpropanoid pathway that produces flavonoid compounds in higher plants, finally producing pterocarpan and glyceollins (16). Glyceollins belong to a class of phytoalexins, which are induced upon biotic or abiotic stress, and are responsible for the defense of plants from infectious agents such as fungi. The pathway for glyceollin biosynthesis has been relatively well characterized, and most of the genes encoding enzymes involved in isoflavonoid biosynthesis have been cloned. However, further genes need to be identified for the later biosynthetic steps (10). According to a recent study by Asaki and colleagues, dimethylallyl diphosphate: (6αS,11αS)-3,9,6α-trihydroxypterocarpan [(2)-glycinol] 4-dimethyl-allyltransferase is likely to be involved in glyceollin I synthesis from glycinol, a direct precursor of glyceollins (10).

As shown in Fig. 1, the accumulation of glyceollins in soybeans did not appear to be proportional to the isoflavone content, indicating that glyceollin content was rather dependent on the responsiveness of genes involved in the glyceollin synthesis to biotic stress. The biosynthesis pathways of the major classes of phenylpropanoid compounds are now well established, and many of the corresponding genes have been identified. However, the regulatory genes that are responsible for fast, coordinated biosynthesis of phenylpropanoid compounds under biotic or abiotic stress have not been well understood (11). The candidate genes involved in the regulation of isoflavone biosynthesis upon biotic stress are phenylalanine ammonia-lyase, chalcone synthase, chalcone isomerase, and isoflavone synthase (11). Moreover, the genes that play major regulatory roles in glyceollin synthesis under stress conditions need to be determined.

Induction of glyceollin biosynthesis by UV light Glyceollin synthesis was induced by fungal infection and exposure to UV light. Using Aga 3 variety of soybean showed the highest isoflavone content among the 60 varieties of soybean; UV light applied to presoaked half-cut soybean for 15 min per day resulted in maximal glyceollin accumulation, whereas lengthened exposure beyond 15 min did not result in further increase in the levels of glyceollins in soybean sprout, as shown in Fig. 2A. Pre-soaked and half-cut soybeans accumulated glyceollins during incubation in a time-dependent manner. In particular, glyceollin content showed dramatic increases on the 2nd and 4th days after incubation (Fig. 2A).

There were also wide differences in the ability to accumulate glyceollins upon UV exposure among the soybean varieties. That is, the levels of soybean glyceollins on the 4th day after incubation were 500, 1,500, 2,000, and 2,500 μg/g for Sunam, Soho, Kwangan, and Danbaeg, respectively, which were much lower than those elicited by fungal infection (*A. sojae*) (Fig. 2B).

Induction of glyceollin biosynthesis by aluminum Glyceollin synthesis in Aga 3 soybean was stimulated by aluminum chloride in a dose-dependent manner from 0 to 50 μM (Fig. 3A). Accumulation of glyceollins in soybeans was relatively small compared to the level induced by either fungal infection or UV light.

Induction of glyceollin biosynthesis by jasmonate Upon exposure to methyl jasmonate, Aga 3 variety showed a dose-dependent stimulation of glyceollin biosynthesis (Fig. 3B). This soybean variety showed increased glyceollin synthesis up to ~2,200 μg/g upon exposure to 12 μM methyl jasmonate.

Biosynthesis of phytoalexins such as glyceollins is reportedly stimulated by a biotic elicitor, including fungal infection, and abiotic elicitors such as UV, iodoacetate, Triton X-100, and metal ions (e.g., iron, copper, mercury, and silver) (17). This study showed that glyceollins were most effectively induced by fungal infection, UV treatment, and methyl jasmonate, whereas aluminum chloride had only a limited effect.

Bisynthesis of glyceollins was also reported to be induced by glucan, a fungal cell wall component. *Phytophthora megasperma* f. sp. glycinea wall glucan elicited glyceollin to levels as high as ~600 μg/g of tissue but only in the uppermost cell layers of treated cotyledons (18). However, we could not observe glyceollin production in soybean exposed to fungal homogenate containing cell wall

Table 2. Isoflavone contents of 60 varieties of soybean

Variety	Total Isoflavones (mg/g)	Glycoside (mg/g)			Malonyl (mg/g)			Acetyl (mg/g)			Aglycone (mg/g)		
		daidzin	genistin	glycitin	daidzin	genistin	glycitin	daidzin	genistin	glycitin	daidzein	genistein	glycitein
Aga3	14.95±0.50[1)]	1.59±0.58	2.31±0.81	0.23±0.15	4.89±0.39	2.55±3.60	0.35±0.03	0.12±0.02	0.01±0.02	1.40±0.03	0.04±0.06	tr	1.46±2.06
Alchan	2.23±0.36	0.13±0.00	0.41±0.00	0.03±0.03	0.58±0.45	0.51±0.65	0.10±0.12	0.01±0.01	tr	0.11±0.06	tr	0.03±0.00	0.32±0.46
Anpyeong	2.92±0.54	0.17±0.00	0.31±0.00	0.11±0.00	0.91±0.42	0.59±0.84	0.25±0.24	0.08±0.01	tr	0.10±0.14	tr	tr	0.40±0.57
Baegcheon	2.20±0.56	0.42±0.00	0.19±0.17	0.01±0.00	0.64±0.91	0.48±0.66	tr	0.03±0.04	tr	0.10±0.07	tr	tr	0.32±0.45
Baegun	1.99±0.07	0.14±0.00	0.10±0.14	0.05±0.07	0.91±0.00	tr	0.05±0.00	0.03±0.00	tr	0.11±0.00	tr	tr	0.59±0.00
Bosug	2.54±0.50	0.44±0.00	0.31±0.00	0.14±0.00	0.75±0.52	0.39±0.50	0.14±0.17	0.02±0.01	tr	0.08±0.06	tr	0.03±0.00	0.25±0.35
Cheongdu#1	1.44±0.19	0.07±0.00	0.14±0.19	0.07±0.00	0.48±0.00	0.01±0.00	0.13±0.00	0.03±0.00	tr	0.10±0.00	tr	tr	0.39±0.00
Dachae	2.03±0.46	0.12±0.00	0.30±0.00	0.06±0.00	0.70±0.53	0.37±0.52	0.12±0.15	0.04±0.03	tr	0.04±0.00	0.01±0.02	0.03±0.00	0.25±0.35
Daewon	1.38±0.24	0.10±0.05	0.13±0.14	0.01±0.01	0.46±0.36	0.34±0.44	0.07±0.10	0.04±0.00	tr	0.01±0.02	tr	0.01±0.00	0.22±0.31
Dagi	2.20±0.26	0.10±0.14	0.11±0.14	0.16±0.09	0.88±0.00	0.38±0.53	0.26±0.00	tr	tr	0.01±0.02	tr	tr	0.26±0.36
Dajang	1.96±0.29	0.06±0.08	0.19±0.27	0.07±0.05	0.74±0.47	0.42±0.60	0.15±0.18	tr	tr	tr	tr	tr	0.29±0.40
Danbaeg	0.88±0.08	0.08±0.00	tr[2)]	0.02±0.00	0.30±0.00	0.02±0.00	0.06±0.00	tr	tr	0.06±0.08	tr	tr	0.31±0.00
Danmi	0.61±0.00	0.06±0.00	tr	tr	0.32±0.00	0.01±0.00	0.02±0.00	0.02±0.03	tr	0.01±0.00	0.16±0.23	tr	0.19±0.00
Dankyeong	2.19±0.60	0.10±0.14	0.09±0.00	0.04±0.05	0.82±0.70	0.51±0.73	0.14±0.12	0.09±0.00	tr	0.12±0.17	0.10±0.14	0.03±0.00	0.35±0.49
Doremi	1.60±0.14	0.13±0.00	0.10±0.14	0.09±0.00	0.65±0.00	tr	0.09±0.00	tr	tr	0.09±0.00	0.10±0.15	tr	0.44±0.00
Duyou	2.46±0.21	0.19±0.00	0.37±0.00	0.01±0.00	0.99±0.00	0.38±0.54	0.17±0.00	tr	tr	0.04±0.00	tr	0.02±0.00	0.26±0.36
Eunha	4.57±0.21	0.37±0.00	0.15±0.21	0.31±0.00	1.99±0.00	0.02±0.00	0.49±0.00	0.49±0.00	tr	0.20±0.00	0.17±0.24	tr	0.94±0.00
Geomjeong#1	1.23±0.12	0.08±0.00	0.09±0.12	0.02±0.00	0.54±0.00	0.01±0.00	0.13±0.00	0.04±0.03	tr	0.05±0.00	tr	tr	0.30±0.00
Geomjeong#3	2.07±0.23	0.22±0.00	0.17±0.23	0.04±0.00	0.91±0.00	0.02±0.00	0.23±0.00	0.02±0.00	tr	0.08±0.00	tr	tr	0.42±0.00
Geomjeongol	2.28±0.20	0.09±0.00	tr	0.3±0.00	1.04±0.00	0.26±0.00	0.09±0.11	tr	0.14±0.20	Tr	tr	tr	tr
Geumgang	1.80±0.10	0.13±0.00	0.17±0.24	tr	0.60±0.50	0.40±0.56	0.15±0.00	0.03±0.00	0.09±0.07	0.042	tr	0.01±0.02	0.27±0.38
Hannam	1.51±0.00	0.10±0.00	tr	0.03±0.00	0.75±0.00	0.07±0.00	0.12±0.00	0.03±0.00	0.07±0.00	0.067	tr	tr	0.33±0.00
Hojang	1.07±0.00	0.06±0.00	tr	tr	0.53±0.00	0.01±0.00	0.11±0.00	0.020	0.07±0.00	0.069	tr	tr	0.25±0.00
Hwangkeum	1.43±0.17	0.06±0.000	0.12±0.17	tr	0.57±0.00	0.01±0.00	0.38±0.00	0.06±0.00	0.11±0.00	0.106	tr	tr	0.46±0.00
Iksannamul	3.48±0.25	0.46±0.00	0.18±0.25	0.13±0.00	1.59±0.00	0.05±0.00	0.28±0.00	tr	0.12±0.00	0.122	tr	tr	0.54±0.00
Jangki	2.78±0.51	0.19±0.00	0.51±0.00	0.2±0.00	1.18±0.00	0.04±0.00	0.28±0.00	tr	0.07±0.00	0.066	tr	tr	0.37±0.52
Jangkyong	4.49±0.43	0.64±0.00	0.20±0.29	0.20±0.00	1.94±0.00	0.02±0.00	0.44±0.00	0.01±0.00	0.10±0.14	0.204	tr	tr	0.90±0.00
Jangmi	3.04±0.20	0.20±0.00	0.14±0.20	0.06±0.00	1.57±0.00	tr	0.29±0.00	0.02±0.00	0.14±0.00	0.137	tr	tr	0.63±0.00
Jangsu	2.30±0.20	0.25±0.00	0.31±0.00	0.12±0.00	0.95±0.00	0.03±0.00	0.15±0.00	0.06±0.00	0.08±0.09	0.143	tr	tr	0.24±0.34
Jangwon	1.03±0.07	0.10±0.00	0.14±0.00	0.07±0.00	0.29±0.00	0.03±0.00	0.05±0.00	0.03±0.01	0.09±0.00	0.093	tr	tr	0.15±0.22
Jangyeob	0.94±0.02	0.05±0.00	0.06±0.09	0.01±0.00	0.39±0.00	0.01±0.00	0.11±0.00	0.02±0.00	0.04±0.00	0.035	tr	tr	0.16±0.22

Table 2. Continued

Variety	Total Isoflavones (mg/g)	Glycoside (mg/g)			Malonyl (mg/g)			Acetyl (mg/g)			Aglycone (mg/g)		
		daidzin	genistin	glycitin	daidzin	genistin	glycitin	daidzin	genistin	glycitin	daidzein	genistein	glycitein
Jinmi	2.95±0.26	0.32±0.00	0.29±0.00	0.09±0.00	0.81±0.51	0.57±0.71	0.15±0.21	tr	0.15±0.09	0.091	tr	0.02±0.00	0.49±0.33
Jinpum	2.19±0.16	0.13±0.00	0.28±0.00	0.07±0.00	0.94±0.00	0.05±0.00	0.22±0.00	0.02±0.00	0.04±0.00	0.036	tr	tr	0.26±0.37
Jinyul	1.10±0.12	0.10±0.00	0.08±0.12	0.05±0.00	0.40±0.00	tr	0.10±0.00	tr	0.04±0.00	0.038	tr	tr	0.32±0.00
Jonam	2.02±0.56	0.19±0.01	0.08±0.12	0.09±0.08	0.87±0.54	0.35±0.49	0.18±0.22	0.02±0.00	tr	tr	tr	tr	0.23±0.33
Kwangan	1.59±0.10	0.07±0.00	0.07±0.10	0.04±0.00	0.86±0.00	0.01±0.01	0.20±0.00	0.01±0.01	0.06±0.00	0.062	tr	tr	0.25±0.00
Malli	2.99±0.32	0.54±0.00	0.23±0.32	0.15±0.00	1.13±0.00	0.03±0.00	0.24±0.00	tr	0.14±0.00	0.137	tr	tr	0.54±0.00
Mansu	0.94±0.15	0.07±0.00	0.14±0.00	0.01±0.00	0.45±0.00	0.02±0.00	0.10±0.00	tr	0.03±0.00	0.029	tr	tr	0.10±0.15
Milyang	1.43±0.17	0.10±0.00	0.12±0.17	0.03±0.00	0.55±0.00	0.02±0.00	0.01±0.00	0.04±0.05	0.08±0.00	0.076	tr	tr	0.43±0.00
Mirang	0.59±0.11	0.04±0.00	0.08±0.11	tr	0.14±0.00	tr	tr	0.01±0.00	0.07±0.00	0.073	tr	tr	0.24±0.00
Muhan	0.04±0.00	0.00±0.00	tr	tr	0.02±0.00	tr	0.08±0.07	0.04±0.02	tr	tr	tr	tr	0.02±0.00
Myeongjunamul	2.01±0.47	0.12±0.08	0.35±0.00	0.04±0.04	0.55±0.49	0.44±0.59	0.04±0.00	0.03±0.02	0.08±0.02	0.098	0.01±0.02	0.023	0.29±0.41
Namwon	1.86±0.00	0.18±0.00	tr	0.03±0.00	0.87±0.00	0.04±0.00	0.17±0.20	0.04±0.00	0.15±0.00	0.148	tr	tr	0.54±0.00
Nogchae	3.25±0.78	0.65±0.00	0.37±0.00	0.14±0.00	1.00±0.78	0.44±0.53	0.07±0.08	0.02±0.00	0.16±0.03	0.140	tr	tr	0.27±0.39
Paldo	1.37±0.39	0.15±0.00	0.20±0.00	0.11±0.00	0.54±0.23	0.03±0.00	0.21±0.00	0.03±0.03	0.04±0.00	0.038	0.14±0.20	0.02±0.00	0.21±0.30
Pangsa	2.59±0.00	0.27±0.00	0.39±0.00	0.20±0.00	0.98±0.00	0.02±0.00	0.21±0.00	tr	0.04±0.00	0.040	0.22±0.31	tr	0.21±0.31
Pungsannamul	1.72±0.09	0.11±0.00	0.23±0.00	0.01±0.00	0.76±0.00	0.02±0.00	0.15±0.00	0.05±0.01	0.09±0.00	0.091	0.13±0.18	tr	0.19±0.27
Pureun	2.24±0.49	0.18±0.00	0.35±0.00	0.04±0.05	0.72±0.51	0.43±0.54	0.13±0.17	0.03±0.01	0.09±0.06	0.053	tr	tr	0.27±0.38
Saeal	3.05±0.25	0.27±0.27	0.18±0.25	0.08±0.00	1.34±0.00	0.02±0.00	0.23±0.00	0.03±0.00	0.12±0.00	0.124	tr	tr	0.63±0.00
Samnam	2.20±0.31	0.24±0.16	0.17±0.09	0.12±0.15	0.67±0.24	0.59±0.80	0.08±0.10	tr	0.09±0.13	0.184	tr	tr	0.26±0.37
Seonyu	0.72±0.04	0.57±0.72	0.05±0.07	0.01±0.00	0.14±0.15	0.24±0.34	0.02±0.00	0.02±0.00	tr	tr	tr	tr	0.16±0.23
Shingi	2.05±0.00	0.57±0.72	0.18±0.00	0.20±0.00	0.83±0.00	0.02±0.00	0.10±0.00	tr	0.13±0.00	0.126	tr	tr	0.38±0.00
Shinpaldal	3.17±0.20	0.22±0.03	0.14±0.20	0.27±0.00	1.37±0.00	tr	0.33±0.00	0.02±0.00	0.15±0.00	0.146	tr	tr	0.56±0.00
Sobaegnamul	1.85±0.20	0.18±0.24	0.15±0.20	0.07±0.00	0.67±0.00	0.01±0.00	0.20±0.00	tr	0.13±0.00	0.133	tr	tr	0.54±0.00
Socheong	3.25±0.63	0.06±0.01	0.19±0.27	0.14±0.01	1.58±0.00	0.02±0.02	0.30±0.00	0.02±0.03	0.15±0.00	0.149	tr	tr	0.61±0.00
Soho	3.45±0.22	0.37±0.45	0.43±0.00	0.14±0.02	1.15±0.00	0.52±0.00	0.22±0.00	0.04±0.00	0.04±0.00	0.038	0.03±0.04	0.04±0.00	0.17±0.25
Sokang	2.47±0.31	0.33±0.29	0.16±0.22	0.06±0.00	1.17±0.00	0.02±0.00	0.30±0.00	0.037	0.06±0.09	0.126	tr	tr	0.54±0.00
Somyeong	3.91±0.37	0.14±0.19	0.26±0.37	0.12±0.00	1.61±0.00	0.05±0.00	0.50±0.00	0.08±0.00	0.19±0.00	0.193	tr	tr	0.82±0.00
Sorog	4.75±0.10	0.28±0.39	0.24±0.34	0.13±0.11	2.06±0.00	0.78±0.97	0.34±0.00	0.02±0.00	0.12±0.00	0.119	0.02±0.03	0.01±0.02	0.49±0.70
Sunam	2.67±0.40	0.1±0.03	tr	0.13±0.14	1.20±0.51	0.39±0.56	0.25±0.13	0.02±0.01	0.09±0.13	tr	0.01±0.01	tr	0.39±0.55
Wonhwang	2.11±0.25	0.11±0.00	0.17±0.25	0.10±0.00	0.87±0.00	0.03±0.00	0.23±0.00	0.06±0.00	0.13±0.00	0.129	tr	tr	0.42±0.00

*[1)]Values are means of duplicate analysis.
[2)]tr, trace

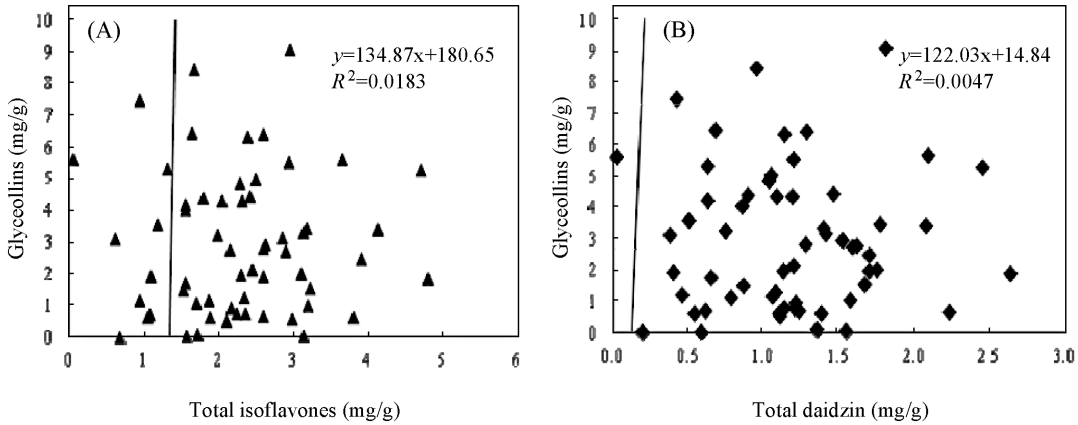

Fig. 1. Correlation between the isoflavone content and glyceollin biosynthesis in 60 varieties of soybean. (A) total isoflavones and glyceollins. (B) total daidzin and glyceollins. R^2 represents regression coefficient.

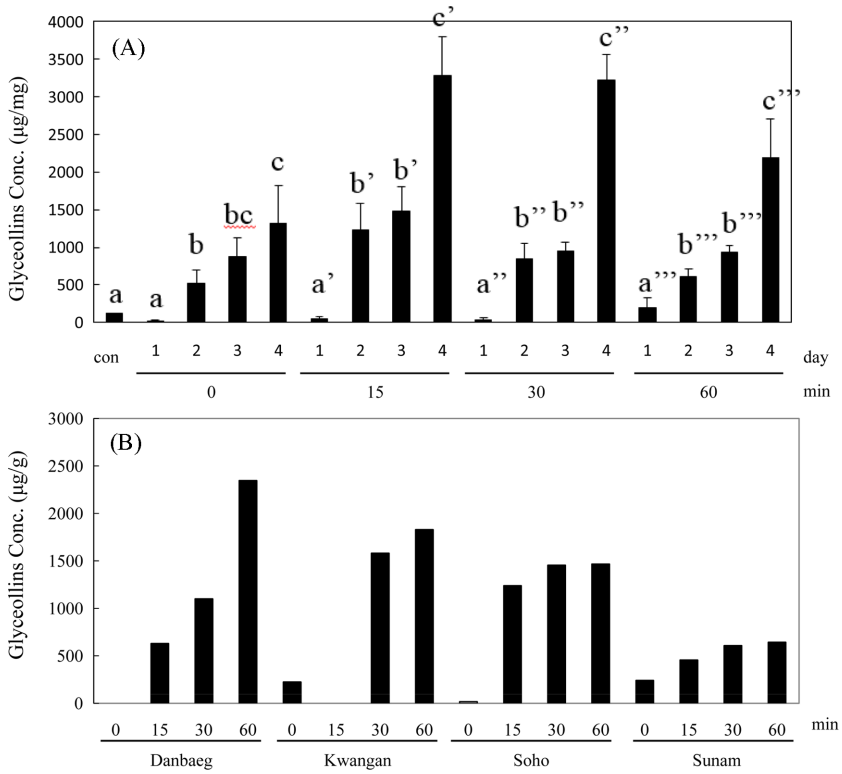

Fig. 2. Induction of glyceollin biosynthesis by UV light in various soybean varieties. A: Aga 3 variety of soybean was soaked in distilled water for 4 h, half-cut, and exposed to UV-B (312 nm) light for different times (min) per day for different incubation periods (day) for glyceollin induction. B: Various soybean varieties were soaked in distilled water for 4 h, half-cut, and irradiated under UV light for 15, 30, and 60 min every day for 4 days, followed by glyceollin analysis by HPLC. Values not sharing common letter are significantly different from each other (p<0.05) as tested by Duncan's multiple rage test.

components (data not shown). In addition, the treatment of soybean tissue with UV (254 nm, 660 μW/cm^2) for 30 min resulted in glyceollin accumulation at the levels of 3 to 385 μg per g tissue depending on sections in soybean, which is a significantly lower level than that observed in our study (18). This discrepancy might be caused by the differences in soybean variety and UV wavelength and intensity used in the study.

The mechanisms by which biotic and abiotic elicitors promote isoflavonoid phytoalexin production in soybean remain unclear. Since elicitors do not have apparently common structural features, it is most likely that they trigger the biosynthesis of phytoalexins through a simple cell damage in the host plants. In addition, the possibility that elicitors may induce phytoalexin formation via the release of an endogenous signal molecule(s) in plant cannot be excluded (17,19).

Recent studies have shown that glyceollins possess several

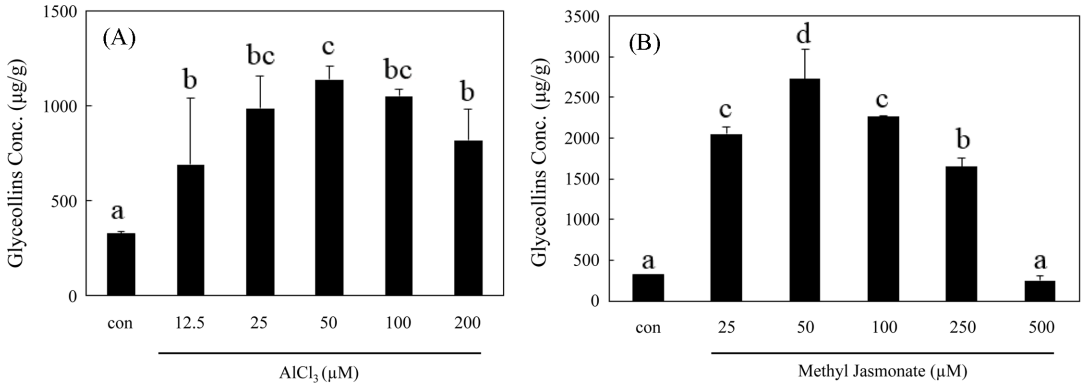

Fig. 3. Induction of glyceollin biosynthesis by $AlCl_3$ or methyl jasmonate in Aga 3 variety of soybean. Soybeans were soaked in distilled water for 4 h, half-cut, treated with various concentrations of $AlCl_3$ (A) or methyl jasmonate (B), and incubated at 25℃ for 3 days. Values not sharing common letter are significantly different from each other (p<0.05) as tested by Duncan's multiple rage test.

biological activities, including antioxidative, anti-inflammatory, anti-fungal, and anti-cancer activities (4,6,14,19,20). Furthermore, our previous studies demonstrated that glyceollins induce antioxidant enzymes via the Nrf2-mediated pathway, protecting cells from oxidative stress as well as inhibiting PDGF-induced smooth muscle cell proliferation (19,21).

In conclusion, glyceollin biosynthesis was varied widely according to the soybean variety and type of elicitor, and it was independent of the total isoflavone or daidzein level. This suggests that glyceollin biosynthesis is mainly governed by the expression of genes encoding the daidzein biosynthesis pathway from phenylalanine. Accordingly, careful optimization of the various factors involved is required for the efficient production of glyceollins with health benefits, and the genes encoding the rate-limiting step(s) of glyceollin biosynthesis remain to be identified.

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Disclosure The authors declare no conflict of interest.

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