



Effect of different planting times on the quantitative variation of total seed isoflavone content and composition in Korean soybean cultivars (*Glycine max* (L.) Merr.)

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Abstract

There is great interest in the enhancement of isoflavones as one of the functional ingredients in soybean. This study aimed to investigate the effects of changes in the ecological environment over different planting times on isoflavone content. A total of 28 Korean soybean cultivars were grown at different planting times in late May, mid-June, and early July and their isoflavone content was measured over 2 years (2017 and 2018). Analyses of variance revealed significant effects of genotypes, planting times, years, and their interactions on isoflavone content. The average content of total isoflavone, as well as its component groups of malonylglucoside and aglycon, increased significantly as the seed planting time was delayed from late May to early July. The accumulation of each isoflavone component varied with changes in the planting time. The isoflavone content of the soybean cultivars for soy-sprout and soy-paste and tofu were higher for plantings in early July than for those in late May and/or mid-June, except for the black soybean cultivars. Despite significant correlations of the isoflavone content of the 28 cultivars among the three planting times, the responses of individual cultivars varied in isoflavone content by planting time. When planting was delayed, the time to flowering and maturity was also delayed and the number of days of growth from planting or flowering to maturity decreased; however, this was not related to isoflavone content. When planting was delayed, the temperature during the ripening period from flowering to maturity was lower, which was inversely related to the isoflavone content.

Keywords Soybean · Isoflavone · Aglycone · Glucoside · Malonylglucoside · Acetylglucoside · Planting time · Environment

Introduction

Isoflavone, together with coumestran and lignan, has a similar structure and efficacy to the female hormone estrogen; therefore, it is called phytoestrogen and is especially abundant in soybean. Isoflavone in soybeans is a polyphenolic component that acts as an antioxidant in the defense system of the plant against abiotic and biotic external stresses

(Aliferis et al. 2014; Gutierrez-Gonzalez et al. 2010; Phillips and Kapulnik 1995). The efficacy of bone health that helps in the bioactive functioning of the human body has been proven previously (Lee et al. 2019; Messina 2000); thus, soybean isoflavone has been used as a functional raw material for healthy functional foods. Soybean is not only a major source of plant protein, but also attracts attention as a raw material for healthy food. The soybean is the second most important food crop after rice and is the main ingredient of the traditional Korean diet, including soy-paste, tofu, and soy-sprout. Therefore, the possibility of ingesting and utilizing soybean functional ingredients such as isoflavone is very high (Kim et al. 2001).

It is known that soybean (*Glycine max* (L.) Merr.) contains approximately 0.1–0.4% isoflavone, with the content and composition differing significantly based on the cultivars and cultivation environment, such as the location and cultivation year (Britz et al. 2011; Carrera et al. 2011; Hoeck

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et al. 2000; Murphy et al. 2009). Previous studies have verified highly significant variations in isoflavone content among soybean cultivars with different genetic backgrounds. Kim et al. (2019) reported that the variation effect in isoflavone content due to genetic factors was large, with the broad-sense heritability values for each component group of isoflavones found to be as high as 0.8–0.92, except for the acetylglucoside group (0.12). The same cultivar can have different isoflavone contents, which is influenced by environmental factors. In particular, different levels of isoflavone content have been reported based on the cultivation environments such as planting time and fertilization level, different weather conditions, and the altitude of the cultivation area (Hasanah et al. 2015; Lee et al. 2003; Yi and Yoon 2012; Yun et al. 2006).

Although there are various functional ingredients in soybean, limited studies have been conducted regarding their enhancement. Soybean cultivars must be developed with high isoflavone content for their industrialization end-uses, which include as general food and in functional diets and cosmetics. Determining the effects of the cultivation environment and methods on the isoflavone content is also important for their practical use in soybean production sites. Soybean cultivars may not be always planted on time owing to atypical weather and/or field conditions; thus, they are often sown intentionally early or late to avoid poor environmental conditions. This change in cultivation time causes various physiological reactions of soybean cultivars to the ecological environment, such as temperature and photoperiod, for their flowering and maturation. The present study aimed to investigate the effects of changes in ecological environments with different planting times on soybean isoflavone content and compositional variation.

Materials and methods

Plant materials and field experiments

A total of 28 soybean cultivars that were grown mainly in Korea or had been recently developed were included in the present study. These cultivars were classified based on their end-uses, i.e., 11 cultivars for soy-paste and tofu, 11 cultivars for soy-sprouts, and six cultivars for cooking with rice (Table 1). They were grown over 2 years, 2017 and 2018, at the Daegu experimental field site at the Department of Southern Area Crop Science of the National Institute of Crop Science, RDA in Habin-myeon, Dalseong-gun, Gyeongsangbuk-do, Republic of Korea. The planting dates occurred at three different times of 3-week intervals for each year; the first planting was in late May (May 29, 2017 and May 23, 2018), the second was in mid-June (June 14, 2017 and June 12, 2018), and the third was in early July (July 10, 2017 and July 4, 2018). The design

of the field experiment, planting of seeds, and management of the experimental field were conducted according to the methodology described by Kim et al. (2019). The numbers of days to flowering and/or maturity from planting were measured for the individual cultivars in each year. The harvested seeds were dried for 3 days, threshed, and then selected for subsequent analysis. The 100-seed weight trait of individual cultivars was measured using three repetitions. To determine the effect of the climatic conditions on the isoflavone accumulation, meteorological data such as average temperature and precipitation during the ripening period from flowering to maturity were investigated for the individual cultivars. The weather data in Daegu were obtained from the Korea Meteorological Administration website (<https://www.weather.go.kr/>).

Isoflavone analysis

Seed samples of the 28 soybean cultivars in the three sowing time experiments were crushed using a 100-mesh size without breaking the seed coat using a vibrating sample mill (T1-100 model, CMT Co Ltd., Tokyo, Japan) and stored in a 4 °C refrigerator until subsequent isoflavone analysis. For calibration, isoflavone standards of daidzein, glycitein, genistein, daidzin, glycitin, and genistin were purchased from Sigma (St. Louis, MO, USA). Other standards of 6"-*O*-malonyldaidzin, 6"-*O*-malonylglycitin, 6"-*O*-malonylgenistin, 6"-*O*-acetyldaidzin, 6"-*O*-acetylglycitin, and 6"-*O*-acetylgenistin from Nacalai Tesque Inc. (Kyoto, Japan) were used. Seed sample preparations for soy isoflavone extraction and quantification using high-performance liquid chromatography for total isoflavone and its components were undertaken following the procedures described by Kim et al. (2019).

Statistical analysis

Analyses of variance were performed using the general linear model procedure (PROC GLM) in SAS 9.4 (SAS Institute Inc., USA). This linear model used the effect of genotypes, planting dates, replication, years, and their interactions among the components. Comparisons between the means of cultivars for each isoflavone component were made using the least significant difference at a 0.05 probability level. Pearson's correlation was calculated using PROC CORR in SAS 9.4 (SAS Institute Inc., USA) to determine the relationship among the pairs of variables of agronomic traits or meteorological data and the isoflavone content in the 28 soybean cultivars for the various planting dates.

Table 1 List of the 28 Korean soybean cultivars classified by their end-uses

No	Name	End-use	Year	Flower color ^a	Seed-coat color ^b	100-seed wt.(g)
1	Taekwangkong	Soy-paste and Tofu	1991	P	Y	25.3
2	Daewonkong	Soy-paste and Tofu	1997	W	Y	25.6
3	Saedanbaek	Soy-paste and Tofu	2010	W	Y	20.7
4	Uram	Soy-paste and Tofu	2010	W	Y	25.8
5	Neulchan	Soy-paste and Tofu	2011	W	Y	21.7
6	Jinpung	Soy-paste and Tofu	2012	P	Y	23.0
7	Taeseon	Soy-paste and Tofu	2013	P	Y	22.2
8	Seonpung	Soy-paste and Tofu	2013	W	Y	25.9
9	Daepung2ho	Soy-paste and Tofu	2014	W	Y	20.9
10	Daechan	Soy-paste and Tofu	2014	W	Y	24.5
11	Miso	Soy-paste and Tofu	2014	W	Y	28.2
12	Pungsannamulkong	Soy-sprout	1996	P	Y	10.1
13	Sorog	Soy-sprout	2001	P	PG	11.9
14	Bosug	Soy-sprout	2003	P	Y	8.6
15	Pungwon	Soy-sprout	2006	W	Y	10.9
16	Sinhwa	Soy-sprout	2007	P	Y	12.1
17	Wongwang	Soy-sprout	2007	P	Y	10.9
18	Sohwang	Soy-sprout	2008	P	Y	8.5
19	Haepum	Soy-sprout	2012	W	Y	10.4
20	Haewon	Soy-sprout	2014	P	Y	8.1
21	Soyeon	Soy-sprout	2015	W	Y	10.1
22	Aram	Soy-sprout	2016	P	Y	9.9
23	Cheongja #3	Cooked w/ rice and others	2004	P	B	32.1
24	Ilpumgeomjeong #2	Cooked w/ rice and others	2005	P	B	25.0
25	Socheong	Cooked w/ rice and others	2006	W	B	15.7
26	Socheong2	Cooked w/ rice and others	2009	W	B	12.2
27	Socheongja	Cooked w/ rice and others	2014	W	B	12.0
28	Cheongja4	Cooked w/ rice and others	2015	P	B	30.1

^aFlower color: P purple, W white^bSeedcoat color: Y yellow, B black, PG pale green

Results and discussion

Quantitative variation of isoflavone and its components based on planting time

For the 28 domestically grown Korean soybean cultivars, the amount of total isoflavone and its component groups were analyzed for the three planting times over 2 years from 2017 to 2018. The results of the analyses of variance of the isoflavone and component content showed significant effects of genotypes, planting times, years and their interactions (Table 2).

The average isoflavone content increased overall as the seed planting time was delayed from late May to early July (Fig. 1). The average total isoflavone content over the 2 years was 2934 $\mu\text{g/g}$ for the third planting in early July, which was higher than that for the first planting in late May and

the second planting in mid-June (2564 $\mu\text{g/g}$ and 2790 $\mu\text{g/g}$, respectively). Similar patterns of increases in isoflavone content were observed in the order of late May < mid-June < early July for both years. The average isoflavone content of the harvested samples in 2017 (3224 $\mu\text{g/g}$) was higher than that of the samples in 2018 (2303 $\mu\text{g/g}$).

The quantitative variations of the isoflavone components based on the seed planting times are shown in Figs. 1 and 2. Similar to the total isoflavone content, the malonyl-based glucoside component, which accounted for the highest proportion of soybean isoflavone composition, increased significantly as the planting time was delayed. Despite the low composition ratio of isoflavones, the non-glycosylated aglycon group showed similar tendencies in content variation with that of the malonyl-based isoflavone group at the different planting times. In contrast, there was no specific trend in content variation for the glucoside-based isoflavone

Table 2 Analyses of variance for total isoflavone and its component group contents of the 28 soybean cultivars grown at three different planting times over 2 years (2017 and 2018)

SV	T_Aglycon	T_Glucoside	T_M_Glucoside	T_Ac_Glucoside	Total
Genotype (G)	182.6****	146.4****	664****	316.4****	665.3****
Planting time (P)	166.5****	25.3****	514.4****	319.3****	310.4****
Year (Y)	57.5****	0.2 ^{ns}	9400.6****	5663.9****	5645.0****
G × P	10.2****	21.6****	29.4****	40.5****	28.0****
G × Y	14.1****	13.8****	65.4****	67.3****	51.0****
G × P × Y	10.2****	12.9****	21.1****	36.2****	21.6****
P × Y	133.5****	83.9****	9.1****	666.4****	25.6****
C.V	13.4	11.1	4.9	13.4	5.0
LSD(0.05)	32.8	52.1	187.3	3.1	220.3
Mean (ug/g)	152.4	293.3	2362.4	14.5	2762.7

T_Aglycon: Daidzein + Glycitein + Genistein, T_Glucoside: Daidzin + Glycitin + Genistin

T_M_Glucoside: 6''-O-malonyldaidzin + 6''-O-malonylgenistin + 6''-O-malonylglycitin

T_Ac_Glucoside: 6''-O-acetyldaidzin + 6''-O-acetylgenistin + 6''-O-acetylglycitin

Total: T_Aglycon + T_Glucoside + T_M_Glucoside + T_Ac_Glucoside

**** $p < 0.0001$, *ns* not significant

group based on the planting time. Because the composition of the aglycon and glucoside groups made up a low proportion of the total isoflavone content, they had little effect on the variation in total isoflavone content.

As shown in Fig. 2, the quantitative variation of the isoflavone components that had relatively low content ratios, such as glycitein, glycitin, and malonylglycitin, was not large and was stable regardless of the planting time. Conversely, components such as genistein and daidzein in the aglycon group and malonylgenistin and malonyldaidzin in the malonyl group consistently increased as the planting time was delayed to early July; however, genistin and daidzin in the glucoside group did not increase after planting in mid-June. The content of the acetyl glucoside group was too low (15–22 $\mu\text{g/g}$) to determine the content variation associated with the change in planting times. The annual variation in isoflavone content was significant except for the glucoside group and the interaction between cultivation year and planting time was significant for all component groups. Therefore, the accumulation of each isoflavone component varied differently to changes in the planting time owing to the variance in climatic and ecological environments over 2 years.

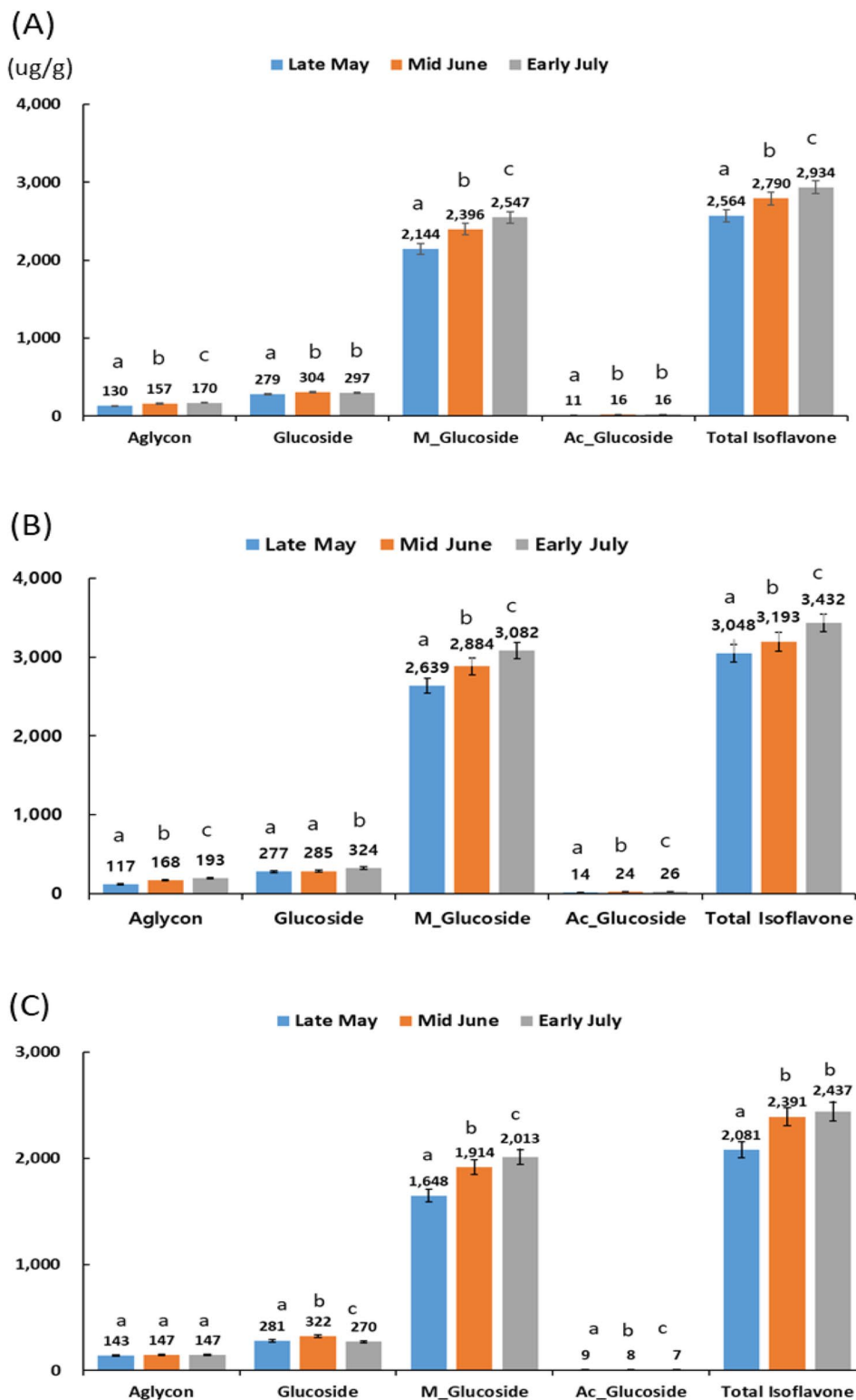
Variation in isoflavone content of soybean cultivar groups at different planting times

In the present study, the 28 soybean cultivars were grouped by end-use and their content variations of total isoflavone and component groups based on planting times are shown in Fig. 3. The average levels of isoflavone content were higher in the order of soybean cultivar groups for soy-sprout > soy-paste and tofu > cooking with rice without regard to planting times. This finding for the isoflavone levels of soybean groups by end-use was consistent with those of previous

studies (Kim et al. 2019, 2018; Park et al. 2012). The isoflavone content was higher at planting in early July than that in late May and/or mid-June for the cultivars for soy-sprout and soy-paste and tofu, although not for the black soybean cultivars. The average isoflavone content of 11 cultivars for soy-sprout was 2992 $\mu\text{g/g}$ for the late May planting, 3335 $\mu\text{g/g}$ in mid-June, and 3372 $\mu\text{g/g}$ in early July; however, a significant difference was not observed between the mid-June and early July plantings. The average isoflavone content of the 11 cultivars for soy-paste and tofu also varied depending on planting time, i.e., 2270 $\mu\text{g/g}$ for the late May planting, 2514 $\mu\text{g/g}$ in mid-June, and 2826 $\mu\text{g/g}$ in early July, and their variation in isoflavone content was significant Yun et al. (2006) reported that isoflavone content increased as the planting time was delayed regardless of the ecological type of soybean studied as seen in the early-matured or mid- to late-matured cultivars. In a study by Sakai et al. (2005), the isoflavone content of the soybean was not related to seed shape (i.e., seed color and size) but was significantly correlated with the planting period. Therefore, the isoflavone content of soybean seeds is affected by environmental factors during different planting periods regardless of the maturity of the cultivars.

Correlations of the average isoflavone contents of the 28 soybean cultivars grown at the three different planting times over 2 years were in the range of 0.81–0.93 with statistical significance ($p < 0.0001$) (Fig. 4). Correlations of the 28 soybean cultivars among the planting time treatments were 0.8–0.93 in 2017 and 0.62–0.78 in 2018. Therefore, the relative ranks of the 28 soybean cultivars changed with variation in their isoflavone content over the planting periods. Despite the significant interactions of cultivars with planting time and years, some soybean cultivars more stably maintained high or low levels of isoflavone content

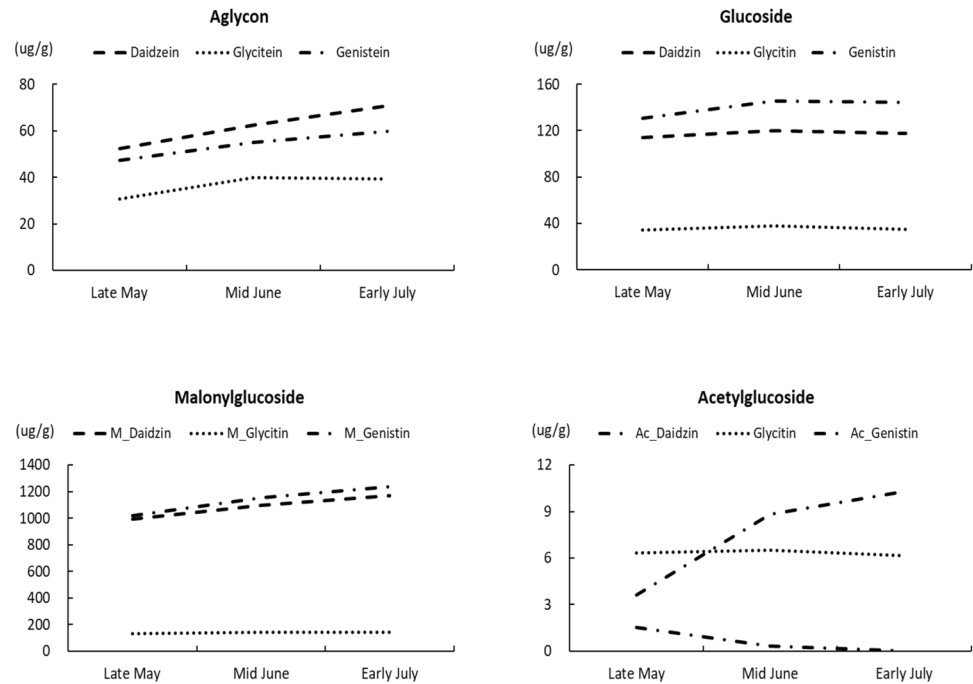
Fig. 1 Variation in the contents of total isoflavone and the four component groups of aglycon, glucoside, malonylglucoside, and acetylglucoside at three different planting times for the 28 soybean cultivars: **A** over 2 years of 2017–2018, **B** in 2017, and **C** in 2018



than others. Based on the average isoflavone content over 2 years, the soybean cultivars with high values were “Jinpung” (3715 ug/g), “Uram” (3685 ug/g), and “Daepung2ho” (3659 ug/g) for soy-paste and tofu, and “Sorok” (4474 ug/g),

“Haewon” (4074 ug/g), “Wonkwang” (3626 ug/g), and “Sin-hwa” (3361 ug/g) for soy-sprout. In contrast, the soybean cultivars with low isoflavone content were “Taekwangkong” (1326 ug/g) and “Saedanbaek” (1398 ug/g) for soy-paste and

Fig. 2 Changes in the contents of the 12 isoflavone components in the aglycon, glucoside, malonylglucoside, and acetylglucoside groups at three planting times for the 28 soybean cultivars over 2 years (2017–2018)



tofu and “Sohwang” (1684 ug/g) for soy-sprout. The average isoflavone contents of the six black soybean cultivars for cooking with rice were in the range of 1765 ug/g (“Cheongja 4”)–2,906 ug/g (“Socheong”). The quantitatively stable variations of soybean cultivars identified with high or low isoflavone contents over different planting times in the present study were consistent with the results of a previous study (Kim et al. 2019).

Table 3 shows the distribution of the 28 soybean cultivars by isoflavone content range averaged over 2017–2018 based on the planting period from late May to early July. Responses such as a trend of increase or decrease concerning the variation of the isoflavone content were different for each cultivar. Except for some cultivars such as “Sorok”, “Sohwang”, “Socheongja”, and “Socheong”, most cultivars including “Daepung2ho”, “Jinpung”, and “Shinhwa” showed an increased isoflavone content at planting in mid-June and early July rather than in late May. As shown in Fig. 5, cultivars including “Daepung2ho”, “Sinhwa”, and “Cheongja4” showed a significant increase in isoflavone content that was 7–16% higher when planted in mid-June than that in late May and was 5–8% higher when planted in mid-June than that in early July. Conversely, for “Uram”, there was no change in the content until planting in mid-June, but it increased when planting in early July. The isoflavone content of “Haewon” increased after planting in late May; however, there was little change in the content when planting occurred in mid-June and early July. “Socheongja” was a non-consistent cultivar whose isoflavone content decreased even after planting in early July. These results suggest that diverse interactions of individual soybean cultivars with the

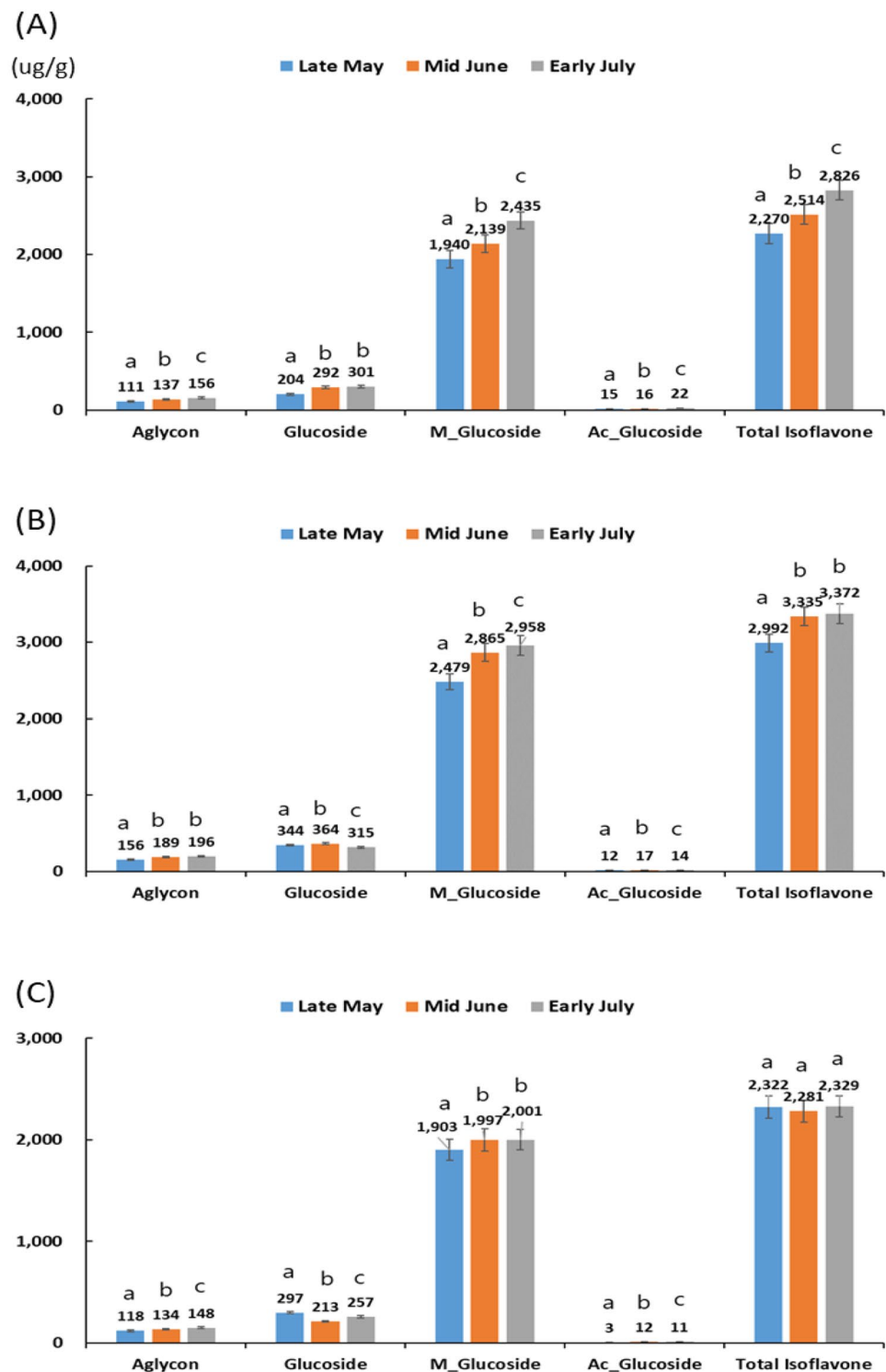
ecological environment during the ripening period based on the planting time might influence the synthesis and accumulation of isoflavone. Yi and Yoon (2012) found that the variations in the time and speed of isoflavone accumulation were different based on the soybean cultivars or planting time when monitored until maturation after flowering of black soybean cultivars.

Effect of the growth environment and climatic change after different planting times on isoflavone content

As the planting time of the cultivars was late in the present study, the flowering and maturity of the soybean cultivars were delayed and the number of days of growth from planting or flowering to maturity decreased. This was because the time required for vegetative growth was shortened and the conversion to reproductive growth was faster as the soybean plants responded to shorter day length conditions due to late planting. The ecological responses of soybean cultivars during the growth period varied based on the weather environmental differences in the different cultivation years.

In 2018, the planting time was 2–6 days earlier than in 2017; however, maturity was delayed by approximately 1–6 days. Therefore, the average number of days from flowering to maturity was approximately 4–8 days higher in 2018 than that in 2017. In the present study, the change in the number of growth days by planting time had little effect on isoflavone content. There was no significant correlation between the number of days of growth from flowering to maturity and isoflavone content in the 28 soybean cultivars

Fig. 3 Variation in the contents of total isoflavone and the 4 component groups of aglycon, glucoside, malonylglucoside, and acetylglucoside at three different planting times for the 28 soybean cultivars grouped by their end-uses over 2 years (2017–2018): **A** 11 cultivars for soy-paste and tofu, **B** 11 cultivars for soy-sprout, and **C** six cultivars for cooking with rice



in 2017 and 2018 (data not shown). Early-matured soybean cultivars with fewer numbers of growth days have a lower content of isoflavones than mid- or late-matured cultivars (Kim et al. 2019; Sakai et al. 2005; Yun et al. 2006). However, the isoflavone content of the soybean cultivars with similar ranges of early- or late-maturity was not significantly

affected by the number of days of growth from planting or flowering to maturity (Kim et al. 2019).

If planted later than the proper planting time, the seed weight of some soybean cultivars might decrease because of the shortening of the maturity period not being adequate for seed ripening and enlargement. The correlation between

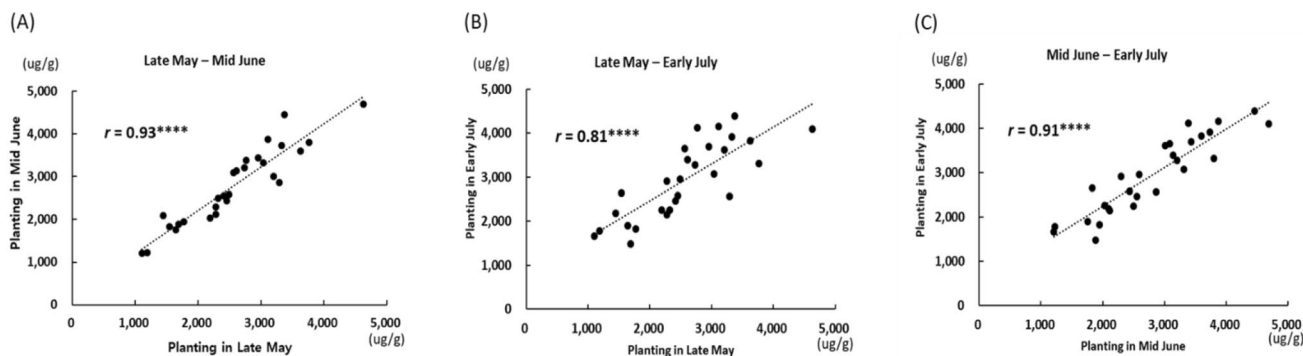


Fig. 4 Correlations of total isoflavone contents of the 28 soybean cultivars at three different planting times over 2 years (2017–2018): **A** plantings in late May and mid-June, **B** plantings in late May and early July, and **C** plantings in mid-June and early July

Table 3 Distribution of the 28 soybean cultivars by average content of total isoflavone at three different planting times from 2017 to 2018

Isoflavone content (ug/g)	End-use	Planting time		
		Late May	Mid-June	Early July
4500–5000	Soy-paste and Tofu	–	–	–
	Soy-sprout	Sorog	Sorog	–
	Cooking w/ rice	–	–	–
4000–4500	Soy-paste and Tofu	–	–	Jinpung
	Soy-sprout	–	Haewon	Haewon, Pungwon, Sorog
	Cooking w/ rice	–	–	–
3500–4000	Soy-paste and Tofu	Uram	Jinpung, Daepung2ho, Uram	Daepung2ho, Uram
	Soy-sprout	Wongwang	Wongwang	Sinhwa, Soyeon, Haepum
	Cooking w/ rice	–	–	–
3000–3500	Soy-paste and Tofu	Daepung2ho, Jinpung	Seonpung	Seonpung
	Soy-sprout	Haewon, Haepum, Aram	Sinhwa, Pungwon, Aram, Bosug, Soyeon, Haepum	Bosug, Wongwang, Aram
	Cooking w/ rice	Socheong	–	–
2500–3000	Soy-paste and Tofu	Seonpung	Miso	Daechan, Taeseon
	Soy-sprout	Sinhwa, Pungwon, Bosug, Soyeon	–	–
	Cooking w/ rice	–	Socheong, Ilpumgeomjeong #2	Ilpumgeomjeong #2, Socheong2, Socheong
2000–2500	Soy-paste and Tofu	Miso, Daechan, Daewonkong	Daechan, Neulchan, Daewonkong	Miso, Daewonkong, Neulchan
	Soy-sprout	Pungsannamulkong	Pungsannamulkong	Pungsannamulkong
	Cooking w/ rice	Ilpumgeomjeong #2, Socheong2, Cheongja #3	Socheong2, Cheongja #3	Cheongja #3
1500–2000	Soy-paste and Tofu	Taeseon	Taeseon	Saedanbaek, Taekwangkong
	Soy-sprout	Sohwang	Sohwang	–
	Cooking w/ rice	Socheongja, Cheongja4	Socheongja, Cheongja4	Cheongja4, Socheongja
< 1500	Soy-paste and Tofu	Neulchan, Saedanbaek, Taekwangkong	Saedanbaek, Taekwangkong	–
	Soy-sprout	–	–	Sohwang
	Cooking w/ rice	–	–	–

the number of growth days from flowering to maturity and the 100-seed weight of the 28 cultivars for all planting times in the present study was 0.53 ($p < 0.01$) in 2018 and 0.23 ($p > 0.05$) in 2017. As suggested in previous studies (Kim et al. 2019; Yun et al. 2006), the trend was that

the larger the seed size, the lower the isoflavone content of the soybean cultivars. In our study, the correlation was not statistically significant except for those at the plantings of late May ($r = -0.41$, $p < 0.05$) and mid-June ($r = -0.44$, $p < 0.05$) in 2018, respectively (Fig. 6). Regardless of the

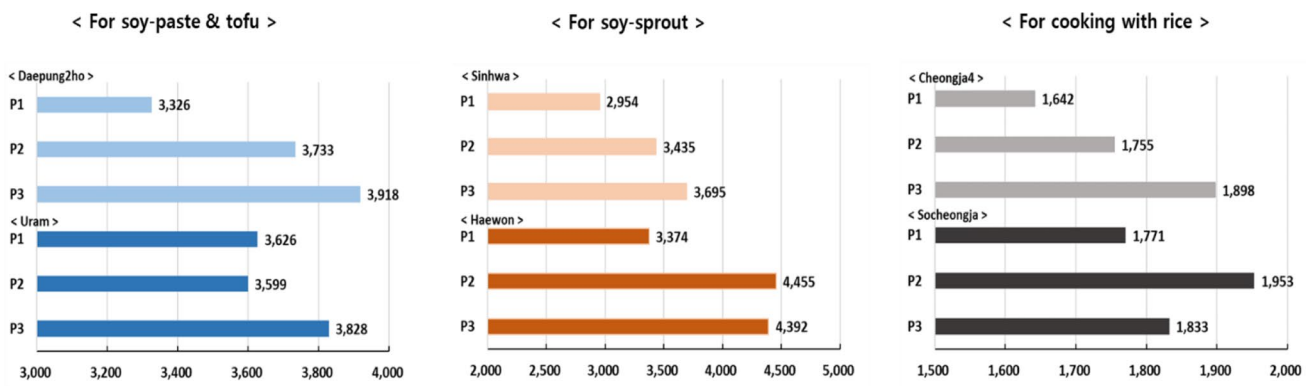


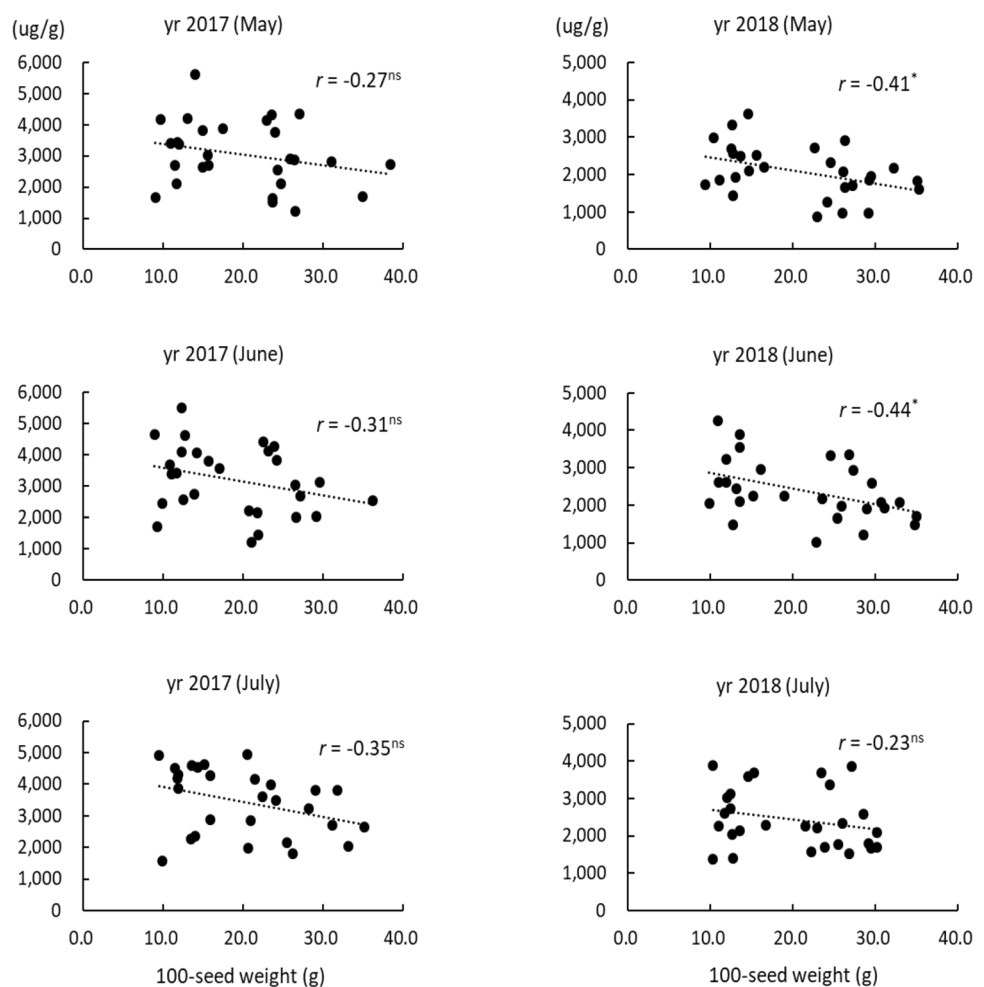
Fig. 5 Variation in the total isoflavone contents of some soybean cultivars grouped by their end-uses for three different planting times of late May (P1), mid-June (P2), and early July (P3) over 2 years (2017–2018)

planting period, the soybean cultivars for soy-sprout had a smaller seed size and higher isoflavone content than those for soy-paste and tofu and for cooking with rice. Black soybean cultivars with large seed sizes have relatively lower isoflavone content than that of other cultivars. The traits of seed size and isoflavone content of the soybean cultivars

classified by their end-uses might have some influence on their correlation.

As the flowering and maturity of the soybean cultivars were delayed owing to late planting, the daily average temperature and the highest and lowest temperatures during the flowering and maturity periods of each cultivar were

Fig. 6 Correlation of 100-seed weight with total isoflavone content for the 28 soybean cultivars grown at three different planting times in 2017 and 2018



lowered, as shown in Table 4. In particular, a decrease in the average temperature of approximately 2 °C at the planting in July compared to that in June was greater than the decrease of approximately 1 °C at the planting in June compared to that in May. There was an inverse correlation between the average temperature in the flowering-maturity period and isoflavone content regardless of the planting time; however, no statistical significance was observed (Fig. 7). The range of average temperature during the flowering-maturity period of the 28 soybean cultivars was greater owing to an abnormal heatwave and large temperature variation in July–September

in 2018 compared to 2017. Yearly differences in climatic conditions, especially during the ripening stage of soybeans, might affect the accumulation of isoflavone. According to previous studies, the synthesis and accumulation of soybean isoflavone components are sensitive to temperature changes in cultivation environments (Britz et al. 2011; Chennupati et al. 2012; Hong et al. 2018; Tsukamoto et al. 1995). In a study by Britz et al. (2011), the variation in isoflavone content of early-matured soybean cultivars was sensitive to changes in temperature and precipitation and the isoflavone content was decreased by 50% under warm conditions.

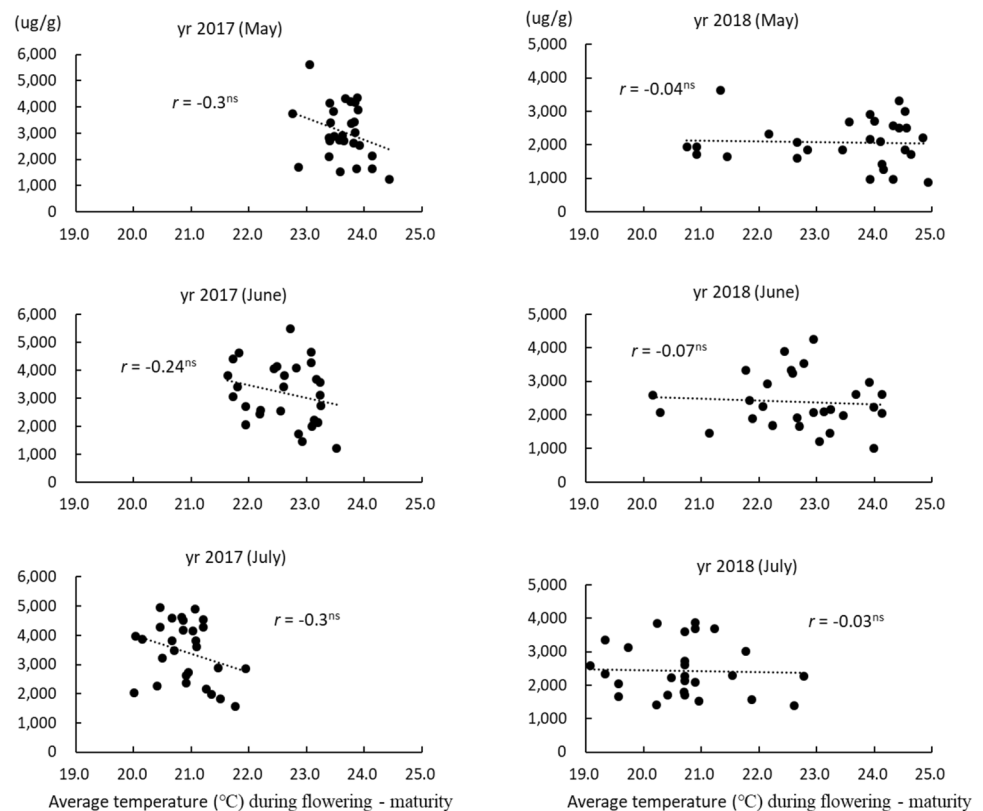
Table 4 Average values of the 28 soybean cultivars by flowering and maturing dates, number of growth days, 100-seed weight, and air temperatures (mean, highest, and lowest) in 2017 and 2018 at 3 planting times

Planting Time ^a	Year	Flowering date	Maturing date	No. of days (planting-maturity)	No. of days (flowering-maturity)	100-Seed weight (g)	Mean temp ^b (°C)	High temp ^b (°C)	Low temp ^b (°C)
Late May	2017	7.22	10.10	133.9	80.8	20.0	23.7	28.8	19.4
	2018	7.23	10.16	146.5	85.1	21.0	23.6	28.4	19.5
Mid June	2017	8.4	10.14	121.8	71.4	18.8	22.8	27.8	18.5
	2018	7.30	10.16	126.9	78.2	21.4	22.7	27.6	18.6
Early July	2017	8.19	10.22	103.8	64.4	20.1	21.1	26.2	16.6
	2018	8.11	10.23	112.0	73.0	19.9	20.8	25.7	16.6

^aPlanting times (month.date): Late May [(2017) 5.29, (2018) 5.23], Mid June (6.14, 6.12), Early July (7.10, 7.4)

^bAir temperature (mean, highest, lowest) values at different planting times were average calculated in the period from flowering to maturity for 28 soybean cultivars in each year

Fig. 7 Correlation of average temperature during the flowering to maturity periods with total isoflavone content for the 28 soybean cultivars grown at three different planting times in 2017 and 2018



According to Hong et al. (2018), the higher the cultivation area altitude, the higher the isoflavone content of soybeans produced in the highlands where isoflavone content was affected by the low temperature at maturity. According to Chennupati et al. (2012), the inhibition of the accumulation of isoflavones in seeds under high temperature conditions was particularly remarkable during the late growth period of soybeans from seed development to maturity. In addition, the expression level of the related genes of isoflavone synthesis was different for each growth period.

In the present study, we found that the changes in the ecological environmental factors such as low temperature during the ripening period in association with changes in the planting period influenced the isoflavone content of the soybean. Because the isoflavone content varied depending on the soybean cultivars and planting periods, it is necessary to examine the possibility of improving the soybean isoflavone content using the planting time while considering the environmental factors of the cultivation area. Despite the interaction between the soybean cultivars and planting period, it would be advantageous to select cultivars with consistently high levels of isoflavone content regardless of the planting time. As demand for higher soy isoflavones grows, the production of soybeans with enhanced functional ingredients is important as an industrial material, as well as for human diets. The development of agricultural production technology for optimal varieties and the improvement of useful ingredients will result in an increase in soybean production and consumption via the creation of new values and demand for food crops.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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