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Note

Wide genetic variation in phenolic compound content of seed coats among black soybean cultivars

Siviengkhek Phommalath[1)], Masayoshi Teraishi[1)], Takanori Yoshikawa[1,2)], Hiroki Saito[1)], Takuji Tsukiyama[1)], Tetsuya Nakazaki[1)], Takatoshi Tanisaka[1,2)] and Yutaka Okumoto*[1)]

[1)] *Graduate School of Agriculture, Kyoto University*, Kitashirakawa, Sakyo, Kyoto 606-8502, Japan

[2)] *Present address: Department of Agriculture for Regional Reclamation, Kibi International University*, Minami-Awaji, Hyogo 656-0484, Japan

Black soybeans have been used as a food source and also in traditional medicine because their seed coats contain natural phenolic compounds such as proanthocyanidin and anthocyanin. The objective of this research is to reveal the genetic variation in the phenolic compound contents (PCCs) of seed coats in 227 black soybean cultivars, most of which were Japanese landraces and cultivars. Total phenolics were extracted from seed coats using an acidic acetone reagent and the proanthocyanidin content, monomeric anthocyanin content, total flavonoids content, total phenolics content, and radical scavenging activity were measured. The cultivars showed wide genetic variation in PCCs. Each of the contents was highly correlated with one another, and was closely associated with radical scavenging activity. PCCs were also moderately associated by flowering date but not associated by seed weight. Cultivars with purple flowers had a tendency to produce higher PCCs compared with cultivars with white flowers, suggesting that the *W1* locus for flower color can affect phenolic compound composition and content. Our results suggest that developing black soybean cultivars with high functional phenolic compounds activity is feasible.

Key Words: seed coat, black soybean, proanthocyanidin, anthocyanin, antioxidant.

Introduction

Soybean (*Glycine max* (L.) Merr.) is one of the most popular crops in the world. Many traditional soy-foods that are currently consumed in Asian countries, including tofu, tempeh, and natto, are known to be healthy foods. Worldwide soy consumption has continually increased due to soy's nutritional properties and the functional characteristics of the compounds it contains. The black soybean, a type of soybean with a black seed coat, has been used as a medicinal food for centuries. Black soybeans have also become more popular as a food source in recent years, presumably because they contain rich nutritional resources and healthy functional substances that yellow soybeans lack (Ross and Kasum 2002, Tsoyi *et al.* 2008).

Proanthocyanidins, also called condensed tannins, are oligomeric or polymeric flavonoid molecules (Beecher 2004). They are widely distributed throughout the plant kingdom but are found predominantly in fruits, legume seeds, and certain kinds of plant-derived beverages, such as juice, wine, tea, and cocoa (Santos-Buelga and Scalbert 2000). Proanthocyanidin functionally affects plant, animal, and human health. The main function of proanthocyanidin in plants is to protect against pathogens such as fungi and insect pests as well as against UV radiation (Dixon *et al.* 2005). Its role in human health is to protect against cancer activity, potentially enhance the cardioprotective system, protect skin from sun damage, and boost anti-inflammatory activity (Santos-Buelga and Scalbert 2000, Scalbert *et al.* 2005).

Anthocyanins are an important class of phenolic compounds belonging to the flavonoid class of plant secondary metabolites (Kong *et al.* 2003). Anthocyanins are soluble in polar solvents and are stored in the vacuoles of cells in flowers, fruits, and seed coats with red to black pigmentation (Koes *et al.* 2005, Tanaka *et al.* 2008). Because they are pigments, anthocyanins make plants attractive to insects and other animals and thus encourage pollination and seed dispersal in nature. They also protect themselves against stressful conditions such as insect damage, phytoalexins, antibacterial agents, and high doses of radiation (Gonzali *et al.* 2009, Harbone and Williams 2001, Kong *et al.* 2003). In addition, anthocyanins have been used in pharmaceuticals for human therapy in anti-inflammatory, anti-edema (Kong

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*Corresponding author (e-mail: okumoto3@kais.kyoto-u.ac.jp)

et al. 2003), and anti-tumor roles (Kamei *et al.* 1998), and they have also been used against diabetes (Takikawa *et al.* 2010).

The seed coats of black soybeans exhibit high levels of antioxidant activity because of their high concentration of flavonoids, especially proanthocyanidin and anthocyanin (Kim *et al.* 2006). Seed coat polyphenol levels are higher in black soybeans than in green or yellow soybeans (Kumar *et al.* 2010, Xu and Chang 2008b). Several reports have suggested the possibility of wide genetic diversity in the phenolic content of black soybean cultivar seed coats, but these studies have focused on only a limited number of cultivars (Choung *et al.* 2001, Furuta *et al.* 2003, Malencic *et al.* 2007, 2008). Recently, Zhang *et al.* (2011) analyzed the phenolic compounds of 60 cultivars, but all were derived from China. The aim of this report is to determine the genetic diversity of phenolic compound contents (PCCs) in the seed coats of black soybean cultivars that were mainly derived from Japan. Kaga *et al.* (2012) classified soybean genetic resources using 191 single nucleotide polymorphism (SNP) markers and revealed that Japanese soybean accessions belonged to different clusters than Chinese landraces. Therefore, it is important to know the variation of PCCs in the Japanese population. Correlations among PCCs and agronomic traits were also investigated.

The *W1* gene regulates flower colors (purple and white) (Zabala and Vodkin 2007). It also regulates anthocyanin components in seed coats. Anthocyanins in seed coats with purple flowers are hydrated at the B ring but seed coats with white flowers are not (Takahashi *et al.* 2010). We then compared PCCs between cultivars with different flower colors to investigate the effects of the *W1* gene on PCCs.

Materials and Methods

Plant materials

Two hundred and twenty-seven black soybean cultivars were provided from the National Institute of Agrobiological Sciences (NIAS) Genebank and the Nagano Chushin Agricultural Experiment Station in Japan. The cultivars mainly originated from Japan, but a few are from Korea, the USA, India, and China (Supplemental Table 1). Ten plants for each cultivar were grown and harvested in 2003 and 2010 at the experimental farm of Kyoto University, Kyoto, Japan. The sowing dates were 18 June, 2003 and 25 June, 2010, respectively. N, P_2O_5, and K_2O were applied as basal fertilizers in quantities of 20, 60, and 70 kg/ha, respectively. Plant spacing was 10 × 30 cm. Seeds were harvested as a bulk, and were kept in a seed storage room at 10°C with relative humidity of 20% until use.

Extraction of total phenolics from seed coats

Total phenol compounds were extracted according to the method of Xu and Chang (2008a), because of its high extraction efficiency. Briefly, seed coats were peeled from seeds. The seed coats (20 mg) were then immersed in a buffer consisting of acetone/water/acetic acid (70 : 29.5 : 0.5, v/v/v). They were extracted twice, first with 1 ml of the buffer for 16 h and then with 0.5 ml for 6 h, at room temperature under dark conditions. The combined extract was stored at 4°C in the dark until use. The extractions were conducted in triplicate for each individual line.

Determination of proanthocyanidin (condensed tannin) content

One hundred microliters of the extraction sample were added to 6 ml of 0.4% (w/v) vanillin in methanol and 3 ml of 4% (v/v) hydrochloric acid, and allowed to stand for 15 min (Broadhurst and Jones 1978). The absorbance was measured at 500 nm against methanol as a blank in a spectrophotometer (BioSpec-1600, Shimadzu, Japan). Proanthocyanidin (condensed tannin) content (PAC) was expressed as milligrams of (+)-catechin equivalents (mg CAE/g sample) using a (+)-catechin calibration curve. The linear range of the calibration curve was 10 to 1000 μg/ml.

Determination of monomeric anthocyanin content

Monomeric anthocyanin content (MAC) was determined using a pH differential method (Lee *et al.* 2005). One hundred microliters of the extraction sample was added to 1 ml of 0.025 M potassium chloride (pH 1.0) and another 100 μl was added to 1 ml of 0.4 M sodium acetate (pH 4.5). Each of these was allowed to stand for 20 min and was then measured at 520 and 700 nm against water as a blank in a spectrophotometer. MAC was calculated according to the formula described in Lee *et al.* (2005), and was expressed as milligrams of cyanidin-3-glucoside equivalents (mg of CGE/g sample) using the calibration curve of cyanidin-3-glucoside from 50 to 1000 μg/ml.

Determination of total flavonoid content

Total flavonoid content (TFC) was determined using a colorimetric method (Zhishen *et al.* 1999). One hundred microliters of the extraction sample was mixed with 500 μl of distilled water and 30 μl of 5% sodium nitrite. Sixty microliters of 10% aluminum trichloride was added after 6 min, and the mixture was allowed to stand for an additional 5 min. Next, 200 μl of 1 M sodium hydroxide and 1 ml of distilled water were added. The absorbance was measured at 510 nm against a blank (the same mixture without the extraction sample) in a spectrophotometer. TFC was expressed as milligrams of (+)-catechin equivalents (mg CAE/g sample) using the (+)-catechin calibration curve. The linear range of the calibration curve was 10 to 1000 μg/ml.

Determination of total phenolic content

The total phenolic content (TPC) was determined using the Folin–Ciocalteu assay (Xu and Chang 2008b). One hundred microliters of the extraction sample was mixed with 6 ml of distilled water and 500 μl Folin–Ciocalteu's reagents solution and allowed to stand for 3 min. Next, 1.5 ml of 7% sodium carbonate was added. Eight minutes later,

1.9 ml distilled water was added and allowed to stand for 120 min at room temperature. The absorbance was measured at 765 nm against distilled water as a blank in a spectrophotometer. TPC was expressed as milligrams of gallic acid equivalents (mg of GAE/g sample) using the calibration curve of gallic acid from 50 to 1000 μg/ml.

Radical scavenging activity

The radical scavenging activity (RSA) was determined using a DPPH assay, as described by Chen and Ho (1995). One hundred microliters of the extracted sample was mixed with 0.5 mM DPPH solution and allowed to stand for 90 min at room temperature under dark conditions. The absorbance (A_{sample}) was measured at 517 nm against ethanol as a blank in a spectrophotometer. A negative control ($A_{control}$) consisted of extraction solvent mixed with 0.5 mM DPPH solution. The percent of DPPH discoloration of the sample was calculated according to the following equation:

$$\text{Percent discoloration (\%)} = (1\text{-}A_{sample}/A_{control}) \times 100.$$

Statistical analysis

Three replicate samples from each variety were analyzed. Analysis of variance (ANOVA) was performed to test for significance differences between cultivars, between years, and for their interactions. Correlation analysis was used to characterize the relationships among the four contents and RSA, and between these traits and seed weight or flowering time.

Results and Discussion

The variation in the traits tested in the seed coats of black soybean cultivars is shown in Table 1 and Fig. 1. All of the traits showed significant differences ($p < 0.01$) among the 227 black soybean cultivars. Cultivation year was significant ($p < 0.01$) for PAC, TFC, TPC, and RSA but not for MAC ($p > 0.05$). Additionally, there was significant interaction ($p < 0.01$) between cultivars and cultivation year for all of the traits tested (Table 1).

The cultivars with the highest and lowest PAC values in 2010 are shown in Table 2. The PAC values in 2010 ranged from 9.8 to 311.3 mg/g CAE. The MAC, TFC, TPC, and RSA values in 2010 ranged from 0.1 to 16.0 mg/g CGE, 1.0 to 62.1 mg/g CAE, 19.3 to 389.3 mg/g GAE, and 0.8 to 88.7%, respectively (Table 1). When the values from 2003 and 2010 were compared, we found that some cultivars appeared to produce PCCs stably across the years tested, but others did not (Table 2).

The highest values of PAC (311.3 mg/g CAE) and TPC (388.2 mg/g GAE) measured in this experiment were approximately 20 times higher than the highest values of PAC (17.4 mg/g CAE) and TPC (60.6 mg/g GAE), which were reported in another study that analyzed the seed coats of 60 Chinese black soybean varieties (Zhang *et al.* 2011). We used different genetic resources from theirs, but this discrepancy was probably due to the different extraction solvents used in the studies. We used acidic acetone (acetone/water/acetic acid (70 : 29.5 : 0.5, v/v/v) as our extraction solvent because of its higher extraction efficiency (Xu and Chang 2007).

The anthocyanin content was measured based on cyanidin-3-glucoside content in this study, according to the method of Lee *et al.* (2005). There are six monomeric anthocyanins (3-glucoside of pelargonidin, cyanidin, peonidin, delphinidin, petunidin, and malvidin), of which, cyanidin-3-glucoside is the most prevalent in the soybean seed coat (Zhang *et al.* 2011). The range of MAC values observed in this study was similar to those in previous reports, in which monomeric anthocyanins in soybean seed coats were determined by means of high-performance liquid chromatography (HPLC) (Choung *et al.* 2001, Yoshida *et al.* 1996, Zhang *et al.* 2011). However, Yoshida *et al.* (1996) found a wild soybean (*Glycine soja*) accession with a high cyanidin-3-glucoside content (20.4 mg/g), indicating that plants with a higher MAC values could be present in the wild soybean population.

PCCs had a moderately positive relationship with flowering time (Table 3). On average, cultivars derived from the southern area of Japan tended to have a higher PAC than cultivars from the northern area, although a few cultivars with a late flowering date over 60 days after sowing showed

Table 1. Ranges and analysis of variance of PCCs in seed coats of black soybean cultivars

Source of variation	PAC[a]	MAC	TFC	TPC	RSA
	Mean Square				
Years	4770.7 **[b]	0.5	11069.6 **	868103.2 **	57.3 **
Cultivars	17463.9 **	63.0 **	976.8 **	32076.2 **	1788.1 **
C × Y[c]	3838.0 **	12.1 **	241.1 **	7385.0 **	655.6 **
Error	326.4	0.5	9.9	480.4	15.5
Range	(mgCAE/g)	(mgCGE/g)	(mgCAE/g)	(mgGAE/g)	(%)
2003	8.5–262.3	0.1–15.1	1.3–73.6	27.2–438.5	3.2–84.3
2010	9.8–311.3	0.1–16.0	1.0–62.1	19.3–389.3	0.8–88.7

[a] Trait abbreviations: Phenolic compound contents (PCCs), Proanthocyanidin content (PAC), Monomeric anthocyanin content (MAC), Total flavonoid content (TFC), Total phenolic content (TPC), Radical scavenging activity (RSA).

[b] *,** = Significant difference at $p < 0.05$ and $p < 0.01$ levels, respectively.

[c] C × Y = Interaction between cultivars and years.

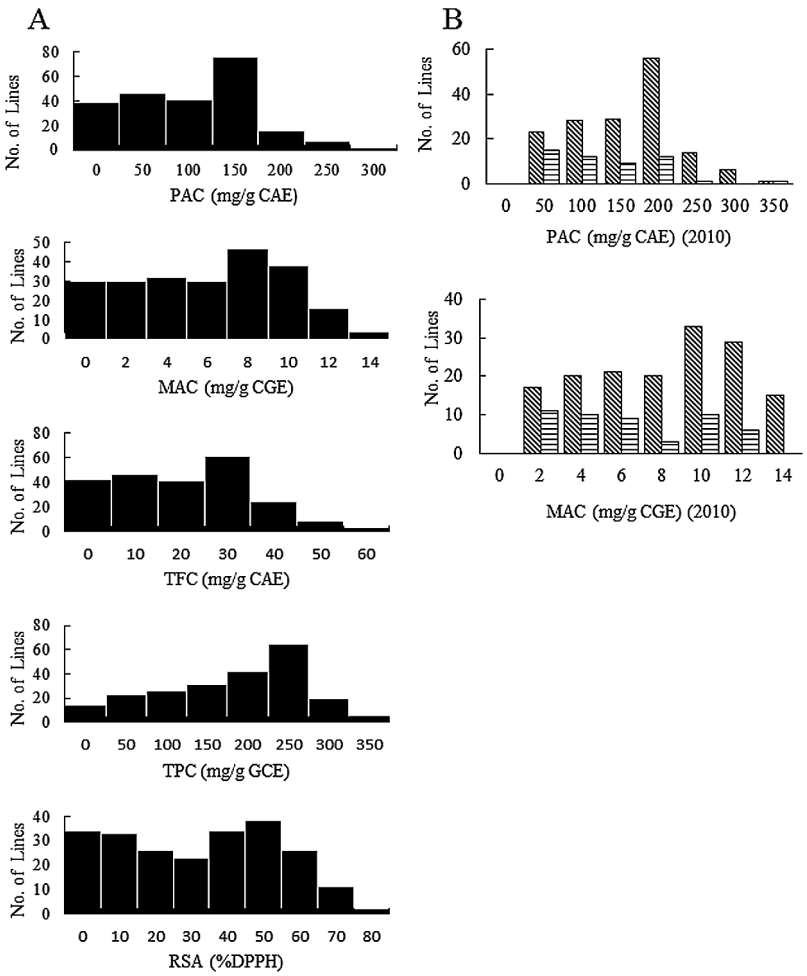

Fig. 1. A. Distribution of PAC, MAC, TFC, TPC, and RSA in seed coats of various black soybean cultivars in 2010. B. Comparison of PAC and MAC between purple- and white-flowered cultivars. Trait abbreviations: phenolic compound contents (PCCs), proanthocyanidin content (PAC), monomeric anthocyanin content (MAC), total flavonoid content (TFC), total phenolic content (TPC), radical scavenging activity (RSA). ▨: Cultivars with purple flowers ▤: Cultivars with white flowers.

a lower PACs (Fig. 2). Because northern cultivars generally begin to flower earlier than southern cultivars, temperature during maturation of northern cultivars is probably higher than southern cultivars in Kyoto. High temperature was reported to suppress the accumulation of PCCs in soybean and grape berry. Tsukamoto *et al.* (1995) found that the isoflavone content was lower in seeds that developed under high temperature conditions than in seeds that developed under lower temperatures. The accumulation of PCCs in grape berry is also associated with temperature. Higher temperature inhibited the expression of flavonoid pathway genes and resulted in lower phenolic accumulation (Cohen *et al.* 2008, 2012, Yamane *et al.* 2006). Our findings in this research may be attributed to the same mechanisms. Further research is needed to determine the relationships between PCCs and temperature during maturation in accurately-controlled environments, such as in a phytotron. Seed weight was not associated with PCCs (Table 3).

We classified the cultivars by flower color according to evaluation data from the NIAS genebank. There were 49 cultivars with white flowers and 157 with purple flowers. Ranges of PACs and MACs in cultivars with white flowers were similar to those in cultivars with purple flowers (Fig. 2), but many cultivars with white flowers had a tendency to produce lower values compared with those with purple flowers. The average PAC of cultivars with white flowers and purple flowers was 100 and 138 mg/g CAE, respectively. The average MAC of cultivars with white flowers and purple flowers was 5.3 and 7.4 mg/g CGE, respectively. The difference in the average between the two groups was significant at a 5% level in PAC ($t = 3.4$, $p < 0.05$) and in MAC ($t = 3.4$, $p < 0.05$). The *W1* locus for flower color encodes a flavonoid 3′,5′-hydroxylase protein that can affect PCCs: the seed coats of black soybean with purple flowers contain delphinidin 3-*O*-glucoside and cyanidin 3-*O*-glucoside, whereas the seed coats of black soybean with white flowers only contain cyanidin 3-*O*-glucoside (Buzzell *et al.* 1987). The comparison between cultivars with different-colored flowers thus suggests that the *W1* locus partly influences PCCs, although the other

Table 2. PCCs, RSA, seed size and flowering time of cultivars with highest and lowest PAC in 2010

	JP number	PAC[a] (mg CAE/g)		MAC (mg CGE/g)		TFC (mg CAE/g)		TPC (mg GAE/g)		RSA (%)		100 Seed Weight (g)	Flowering date (days)[b]
		2003	2010	2003	2010	2003	2010	2003	2010	2003	2010	2010	2010
Highest	JP90782	262.3 ± 8.2	311.3 ± 17.5	13.5 ± 1.5	16.0 ± 1.1	59.4 ± 4.5	62.1 ± 4.5	379.8 ± 11.0	388.2 ± 10.8	81.2 ± 1.6	88.7 ± 3.0	14.1 ± 1.4	63
	JP76320	187.7 ± 8.5	310.2 ± 35.3	12.9 ± 4	14.9 ± 0.2	44.6 ± 3.2	61.7 ± 3.2	331.5 ± 1.5	388.7 ± 15.5	49.4 ± 2.7	79.4 ± 1.0	29.6 ± 1.0	59
	JP110336	110.5 ± 12.7	298.5 ± 22.6	7.8 ± 0.8	14.6 ± 0.5	26.6 ± 3.7	46.1 ± 3.7	224.7 ± 16.1	338.0 ± 20.4	26.0 ± 3.8	80.6 ± 3.4	55.3 ± 0.9	50
	JP29296	162.3 ± 26.2	277.3 ± 13.8	11.8 ± 0.6	14.4 ± 1.2	33.2 ± 8.8	56.7 ± 4.5	300.3 ± 46.3	370.2 ± 2.4	58.4 ± 0.6	79.9 ± 6.2	11.5 ± 0.6	65
	JP29627	217.0 ± 36.5	271.3 ± 11.9	10.9 ± 0.5	13.5 ± 0.3	53.3 ± 0.8	56.2 ± 2.5	356.3 ± 14.2	382.3 ± 14.5	57.9 ± 5.4	79.9 ± 3.6	12.3 ± 0.7	64
	JP49593	157.2 ± 13.2	271.3 ± 27.2	10.9 ± 2.2	12.0 ± 0.6	47.9 ± 10.5	46.2 ± 3.0	324.2 ± 40.6	294.8 ± 19.4	53.5 ± 2.9	69.7 ± 5.1	61.7 ± 2.9	47
	JP76317	156.2 ± 20.6	260.8 ± 11.1	7.7 ± 0.4	12.7 ± 0.3	37.8 ± 1.0	42.3 ± 1.0	271.3 ± 13.8	320.0 ± 9.4	40.8 ± 3.1	66.1 ± 6.0	43.1 ± 1.4	47
	JP76327	204.0 ± 4.4	260.3 ± 9.5	11.8 ± 0.2	13.9 ± 0.3	45.5 ± 2.6	41.0 ± 2.6	338.2 ± 10.4	315.3 ± 10.1	58.5 ± 7.2	68.9 ± 2.5	46.6 ± 1.7	48
	JP35378	203.7 ± 21.8	260.0 ± 14.8	9.5 ± 0.4	10.0 ± 0.2	72.0 ± 2.7	58.2 ± 2.4	386.5 ± 14.3	327.5 ± 13.2	72.9 ± 4.1	72.4 ± 5.4	17.1 ± 0.4	47
	JP28360	206.0 ± 34.0	249.5 ± 4.1	14.9 ± 1.5	13.9 ± 0.3	35.9 ± 1.8	55.7 ± 2.1	351.7 ± 17.9	389.3 ± 7.5	69.4 ± 1.4	77.6 ± 2.4	34.4 ± 2.8	48
Lowest	JP76515	96.3 ± 2.4	22.7 ± 7.9	3.5 ± 0.1	0.3 ± 0.2	25.4 ± 2.2	2.0 ± 1.5	220.5 ± 5.2	44.7 ± 6.3	28.8 ± 2.7	5.2 ± 0.3	43.9 ± 2.8	37
	JP73129	95.3 ± 19.8	22.2 ± 18.1	5.0 ± 0.8	0.5 ± 0.5	34.4 ± 1.3	1.8 ± 1.2	265.2 ± 11.4	39.8 ± 7.8	38.9 ± 5.6	5.3 ± 0.7	44.9 ± 4.8	40
	JP76517	113.8 ± 13.8	22.0 ± 2.7	5.7 ± 1.0	0.2 ± 0.1	34.1 ± 1.3	1.8 ± 0.1	256.5 ± 31.8	37.8 ± 1.2	39.8 ± 4.8	3.0 ± 0.4	41.9 ± 1.7	37
	JP76557	107.3 ± 1.1	21.8 ± 10.3	3.4 ± 0.5	1.0 ± 0.6	29.0 ± 1.8	2.5 ± 0.9	245.3 ± 17.8	52.3 ± 13.6	30.5 ± 2.7	3.6 ± 0.9	42.4 ± 3.0	40
	JP35276	82.0 ± 10.6	21.8 ± 3.2	4.6 ± 0.8	0.7 ± 0.2	24.8 ± 4.3	4.7 ± 0.9	221.3 ± 33.0	50.3 ± 4.1	ND[c]	5.2 ± 0.6	ND	34
	JP53356	27.7 ± 9.2	20.5 ± 3.1	0.3 ± 0.1	0.8 ± 0.2	9.2 ± 2.7	3.1 ± 1.3	86.3 ± 9.6	19.3 ± 4.2	6.2 ± 4.3	5.0 ± 0.6	22.2 ± 0.7	34
	JP35135	118.8 ± 7.7	18.8 ± 6.0	7.0 ± 0.4	0.8 ± 0.6	34.4 ± 1.7	2.4 ± 0.6	279.7 ± 15.5	38.5 ± 12.5	ND	4.9 ± 2.2	24.0 ± 1.4	40
	JP28229	29.5 ± 8.2	14.0 ± 5.2	2.0 ± 1.0	0.4 ± 0.3	6.0 ± 2.1	3.1 ± 0.5	98.7 ± 19.4	34.2 ± 3.9	14.5 ± 2.9	4.7 ± 7.4	22.3 ± 2.0	34
	JP28698	25.5 ± 2.3	13.2 ± 5.7	1.7 ± 0.2	0.3 ± 0.1	5.8 ± 0.9	1.6 ± 1.2	75.0 ± 7.1	55.5 ± 8.2	8.6 ± 0.3	5.2 ± 0.4	22.9 ± 3.7	39
	JP76583	75.8 ± 9.1	9.8 ± 4.9	0.5 ± 0.6	0.3 ± 0.3	24.0 ± 2.1	1.0 ± 0.5	193.8 ± 18.8	32.8 ± 9.1	13.1 ± 2.0	0.8 ± 0.2	46.8 ± 4.0	37

Data are expressed as means ± standard deviations (n = 3) on a dry-weight basis.

[a] Trait abbreviations: Phenolic compound contents (PCCs), Proanthocyanidin content (PAC), Monomeric anthocyanin content (MAC), Total flavonoid content (TFC), Total phenolic content (TPC), Radical scavenging activity (RSA).

[b] Days after sowing.

[c] ND: No data.

Table 3. Correlations between agronomic traits and PCCs in seed coats among black soybean cultivars

	MAC[a]	TFC	TPC	RSA	Seed weight	Flowering Date
PAC	0.90 ***[b]	0.92 ***	0.94 ***	0.93 ***	0.06	0.58 ***
MAC		0.76 ***	0.87 ***	0.83 ***	0.27 ***	0.43 ***
TFC			0.95 ***	0.94 ***	–0.13	0.64 ***
TPC				0.96 ***	0.02	0.58 ***
RSA					0.00	0.54 ***
Seed weight						–0.22 *

[a] Trait abbreviations: Phenolic compound contents (PCCs), Proanthocyanidin content (PAC), Monomeric anthocyanin content (MAC), Total flavonoid content (TFC), Total phenolic content (TPC), Radical scavenging activity (RSA).
[b] *,**,*** Significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

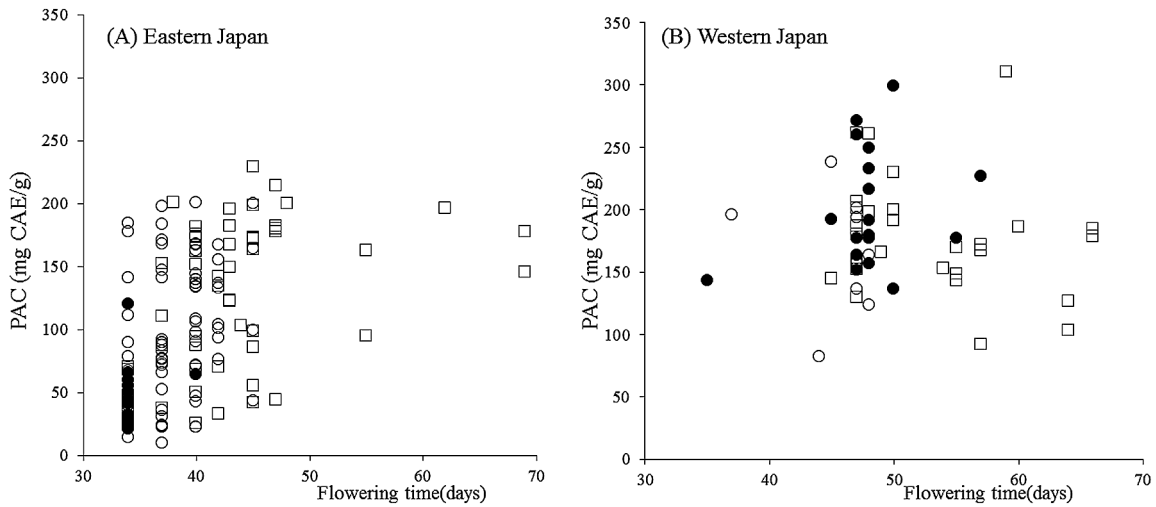

Fig. 2. Correlations between PACs and flowering times in 2010. (A) ●: Hokkaido ○: Tohoku □: Kanto-Koshinetsu. (B) ●: Tokai-Kinki ○: Chugoku-Shikoku □: Kyushu.

genetic factors may also be involved in the control of PCCs, as indicated by the overlapping variation between the two groups (Fig. 2).

Proanthocyanidin and anthocyanin are known as important phenolic compounds. Significant positive correlations were observed among PCCs (Table 3). Our results further indicated that high levels of the proanthocyanidin and anthocyanin pigments provided a significant contribution to RSA in the black soybean seed coats (Table 3). The synchronized accumulation of the phenolic compounds may indicate that all of the phenolic compounds may be regulated by the same genetic factors. Therefore, developing black soybean cultivars with high phenolic compound functional activity is feasible.

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