Research Paper

Diversity of seed cesium accumulation in soybean mini-core collections

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Radiocesium is an extremely harmful radionuclide because of its long half-life; it is important to reduce its transfer from contaminated soil into crops. Here we surveyed genetic variation for seed cesium (Cs) concentration in soybean mini-core collections representing large genetic diversity. The collections grown over 3 years in rotational paddy fields exhibited varying seed Cs concentrations with significant year-to-year correlations, although the phenotypic stability of Cs concentration was lower than that of the congeners potassium (K) and rubidium (Rb). Although Cs is supposedly accumulated in plants via the K transport system, there was no apparent relationship between Cs and K concentrations, whereas a clear positive correlation was observed between Cs and Rb concentrations. Cs and K concentrations in seed showed slightly positive and negative correlations, respectively, with days to flowering. We selected several high or low Cs accumulator candidates on the basis of the 3 years of seed concentration data. These two groups showed significantly different seed Cs concentrations in another field. The differences could not be explained by flowering time alone. These results suggest that genetic variation for seed Cs concentration is present in soybean germplasm and would be useful for breeding low Cs-accumulating varieties.

Key Words: *Glycine max*, cesium, rubidium, potassium, core collection, genetic diversity.

Introduction

The accident at the Tokyo Electric Power Company's Fukushima Daiichi nuclear power plant in March 2011 released large amounts of radionuclides over a wide area around the nuclear power plant, leading to serious damage to agriculture in Japan (Nakanishi and Tanoi 2013). Among the emitted radionuclides, radiocesium isotopes have relatively long half-lives (2.06 years for ^{134}Cs , 30.2 years for ^{137}Cs). It is thus important to quickly establish a way to prevent the transfer of radiocesium into the food chain via the soil-to-plant pathway. Given that it is difficult to remove the radionuclides completely from contaminated soil, developing "safer" crops that accumulate little or no radiocesium is a recommended strategy for reducing food contamination. To this end, it is essential to understand the genetic variation in and molecular mechanisms of Cs accumulation in plants.

Cs is not an essential element for plants and causes toxicity observed as growth inhibition at high concentrations although its natural abundance in soil is too low to affect plant

growth (White and Broadley 2000). Because Cs is an alkali metal element with chemical properties similar to those of potassium (K), which is an essential plant nutrient and a major component of fertilizers, uptake and distribution of Cs are expected to occur mainly through the transport mechanism of K. In accord with this hypothesis, Cs absorption is inhibited by K application in many plant species (reviewed by Zhu and Smolders 2000). As many K^+ transporters and channels have been identified based on the analyses of genome sequence and knockout mutants in *Arabidopsis thaliana*, their functions in Cs uptake could also be investigated (White *et al.* 2010). For example, *AtHAK5*, a high-affinity K^+ transporter belonging to the *KUP/HAK/KT* family, has been shown to participate in Cs absorption by roots under conditions of low K^+ availability (Qi *et al.* 2008). In *Arabidopsis*, the inward-rectifying K^+ channel *AtAKT1* appears to play a major role in K^+ uptake by roots. However, its knockout mutant does not show reduced Cs^+ influx or accumulation, suggesting that *AtAKT1* is not a main Cs uptake pathway (Broadley *et al.* 2001, Qi *et al.* 2008). Cyclic nucleotide-gated cation channels (CNGCs) are implicated in numerous signaling pathways and permit diffusion of divalent and monovalent cations including Ca^{2+} and K^+ (Zelman *et al.* 2012). *Arabidopsis* contains 20

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putative CNGC genes on the genome. The mutants lacking some *AtCNGCs* showed reduced Cs accumulation although mutants lacking other *AtCNGCs* accumulated more Cs in their shoot than the wild type (Hampton *et al.* 2005). This information based on reverse-genetic approaches would be of great help in identifying genes associated with Cs uptake in other plant species and in identifying the mechanisms of Cs accumulation in the whole plant.

Surveys of genetic variation are also a valuable approach to identifying genes associated with Cs accumulation. For example, Kanter *et al.* (2010) reported genetic variation for shoot Cs concentration among 86 worldwide accessions of *Arabidopsis* and succeeded in detecting several QTLs for Cs accumulation using an F₂ population from a cross between high and low Cs-accumulating accessions. Based on sequencing analysis of candidate genes located at QTL positions, one of the detected QTLs likely corresponded to one of the *AtCNGCs*. Payne *et al.* (2004) also reported natural genetic variation and QTLs for shoot Cs concentration in *Arabidopsis*, although specific candidate genes for each QTL were not identified. Natural genetic resources are also very useful as breeding material for developing “safer” crops. Recently, 85 rice cultivars were compared with respect to Cs concentration and some whose concentrations in brown rice were lower over 2 years than those of other cultivars could be selected (Ohmori *et al.* 2014).

Fukushima and its surrounding prefectures are important soybean [*Glycine max* (L.) Merr.]-producing regions in Japan. Although “safer” varieties are needed, there is little information about the genetic variation and molecular mechanisms of Cs accumulation in soybean. The National Institute of Agrobiological Sciences (NIAS) Genebank in Japan has collected and conserved more than 10,000 soybean accessions including improved varieties, local landraces, and wild accessions in Japan and overseas. Although the large number of available accessions often causes difficulty of handling by researchers, two mini-core collections were recently selected from the NIAS Genebank soybean accessions (Kaga *et al.* 2012). Each of the two collections contains 96 accessions from Japanese and “exotic” germplasm, and these were selected to represent a large portion of the genetic diversity among the germplasm entries conserved in the NIAS Genebank, based on single-nucleotide

polymorphism variation and on morpho-agronomic trait variation, population structure, and geographic origin. These collections are expected to provide a platform for effectively exploiting genetic diversity and identifying novel traits for crop improvement.

In this study, we surveyed genetic variation for seed Cs concentration in soybean using the NIAS mini-core collections as a first step toward developing “safer” varieties. Although the phenotypic stability of seed Cs concentration appeared to be lower than that of seed K concentration and other agronomic traits such as seed weight and days to flowering, we selected candidate accessions with high or low seed Cs accumulation on the basis of 3 years of seed Cs concentrations and confirmed phenotypic stability beyond planting year and field. These results suggest that the soybean germplasm carries genetic diversity for seed Cs accumulation and that this diversity could be exploited for the development of “safer” varieties.

Materials and Methods

Plant materials and field conditions

The available 78 accessions of the NIAS Japanese Soybean Core Collection (here abbreviated JMC) and the available 79 accessions of the NIAS World Soybean Core Collection (WMC) were obtained from the NIAS Genebank and planted over 3 years, 2011–2013, in the Yawara experimental field (a rotational paddy field) at NARO (National Agriculture and Food Research Organization) Institute of Crop Science at Tsukuba-mirai, Ibaraki, Japan (36°00'N, 140°02'E) and in the Tsukuba experimental field (an upland field) of the National Institute of Agrobiological Sciences at Tsukuba, Ibaraki, Japan (36°02'N, 140°11'E). In the Yawara field, the soil is classified as a gray lowland soil, and the experimental plot in 2011 was approximately 0.7 km from that used in 2012 and 2013. Twelve plants per accession were sown in a single row of 70 cm width and with single plants at 13 cm intervals at the beginning of July (July 12, July 10, and July 10 in 2011, 2012, and 2013, respectively; **Table 1**). N, P₂O₅, and K₂O fertilizers were applied at 30, 100, and 100 kg/ha, respectively, as basal fertilizer. All plants for each accession were harvested and threshed together, and the seeds of all accessions were subjected to mineral analysis as

Table 1. Mean, maximum, and minimum values for the five traits in the two mini-core collections grown in the Yawara field

Year (sowing date)	Mini-core collection	No. of accessions ¹⁾	Concentration in seed (mg/kg DW)									Days to flowering			Seed weight (mg)		
			Cs			Rb			K								
			Mean	Max	Min.	Mean	Max	Min.	Mean	Max	Min.	Mean	Max	Min.	Mean	Max	Min.
2011 (July 12)	JMC	78	0.036	0.078	0.018	13.8	19.3	9.4	20700	25000	17600	43	57	30	253	563	74
	WMC	79	0.038	0.111	0.017	14.7	33.7	9.4	20200	23600	16100	45	80	27	153	308	49
2012 (July 10)	JMC	78	0.074	0.325	0.030	12.9	22.3	6.3	19900	24400	16900	43	59	30	249	496	78
	WMC	76	0.079	0.213	0.025	12.9	22.7	5.7	19500	22400	16300	44	73	26	154	331	51
2013 (July 10)	JMC	77	0.055	0.227	0.020	10.4	16.7	5.0	19200	22600	16400	43	60	30	275	611	85
	WMC	73	0.054	0.245	0.016	10.2	21.0	4.1	18900	21400	15500	45	67	25	164	323	66

¹⁾In 2012 and 2013, several accessions were excluded because of poor growth.

described below. In the Tsukuba field, the soil type was classified as an Andosol, and the experimental plots were rotated every year in the same field. Three plants per accession were sown in a single row of 80 cm width and with single plants at 30 cm intervals in late June (June 24, June 27, and June 19 in 2011, 2012, and 2013, respectively). N, P₂O₅, and K₂O fertilizers were applied at 18, 132, and 60 kg/ha, respectively, as basal fertilizer. All plants for each accession were harvested individually, and seeds from a representative single plant for 17 accessions, which were selected as high or low Cs accumulator candidates based on 3 years of seed concentration in the Yawara field, were subjected to mineral analysis.

Analysis of exchangeable ions in soil

Soils in the Yawara and Tsukuba fields after cultivation in 2013 were taken at depth of 0–15 cm from five places (four corners and center) and mixed. The air-dried soil samples were sieved to <2 mm. After 2 h extraction using 1 M NH₄OAc at a soil:solution ratio of 1:10, exchangeable potassium (³⁹K), rubidium (⁸⁵Rb), and cesium (¹³³Cs) concentrations (mg/kg DW) in the soils were determined by inductively coupled plasma–mass spectrometry (ICP-MS; Agilent 7700x, Agilent Technologies, Tokyo, Japan) using 5 µg/L indium (¹¹⁵In) as an internal standard.

Mineral analysis of soybean seed

A sample of 10 seeds from each accession was dried at 105°C for 20–24 h. The dried seeds were weighed and then ground into fine powder with a Multi-Beads Shocker (Yasui Kikai, Osaka, Japan). Ground seed samples of 20 mg were weighed into 10 ml Teflon® (Nalgene) centrifuge tubes and digested in 300 µl concentrated nitric acid at 105°C for 2 h. The digests were then made to 10 ml with ultrapure water. Concentrations of ³⁹K, ⁸⁵Rb, and ¹³³Cs were determined by ICP-MS using 5 µg/L ¹¹⁵In as an internal standard. The concentration of each sample with a single determination was converted into the amount of mineral elements (mg/kg DW) in each sample.

Statistical analysis

Statistical analysis was performed using JMP 9.0 software (SAS Institute, Cary, NC).

Results

Phenotypic variation and stability of seed Cs concentration and other traits

To investigate the variation and stability of seed Cs concentration in soybean germplasm, we analyzed seeds of two NIAS mini-core collections, JMC and WMC, which were planted over 3 years in the Yawara rotational paddy field, given that most soybean is cultivated in converted paddy fields in Japan. Because continuous soybean cropping often leads to detrimental effects on growth and yield, we changed the experimental plot between 2011 and 2012 but

used the same plot in 2012 and 2013. The exchangeable ion concentrations (mg/kg DW) of Cs, Rb, and K in the soil of the Yawara field after cultivation in 2013 were 0.27, 0.54, and 360, respectively. When the mini-core collections were sown in early July, a wide range of days to flowering was observed (Table 1, Fig. 1A). The range of days to flowering in JMC was narrower than that in WMC in agreement with the results of Kaga *et al.* (2012). Also, the distribution pattern for seed weight was very similar to that in the previous report; the range of seed weight in JMC was approximately twice that in WMC (Table 1, Fig. 1B). The correlation coefficient between years was extremely high ($r \geq 0.95$) for both days to flowering and seed weight (Table 2).

In the analysis of seed Cs concentration, we determined nonradioactive Cs (¹³³Cs), which is the only natural isotope of Cs. Given that there is no convincing evidence of discrimination by biological systems among ¹³³Cs, ¹³⁴Cs, and ¹³⁷Cs (Avery 1995), ¹³³Cs is expected to behave exactly like radiocesium in the soybean plant. In fact, Tsukada *et al.* (2002a) reported that soil-to-plant transfer factors (Cs

Fig. 1. Frequency distributions of days to flowering (A) and seed weight (B) in the mini-core collections grown in the Yawara field in 2013. Black and white bars represent JMC and WMC, respectively. JMC, Japanese Soybean Core Collection; WMC, World Soybean Core Collection.

Table 2. Correlations between the years in two mini-core collections grown in the Yawara field.

Year-Year	Concentration in seed (mg/kg DW)			Days to flowering	Seed weight (mg DW)
	Cs	Rb	K		
2011–2012	0.33**	0.46**	0.79**	0.95**	0.97**
2011–2013	0.27**	0.46**	0.73**	0.95**	0.96**
2012–2013	0.71**	0.78**	0.71**	0.98**	0.96**

** indicate significant differences at the 1% level.

A: 2011 **B: 2012** **C: 2013** **Fig. 2.** Frequency distributions of seed Cs concentration in the mini-core collections grown in the Yawara field in 2011 (A), 2012 (B), and 2013 (C). Black and white bars represent JMC and WMC, respectively. JMC, Japanese Soybean Core Collection; WMC, World Soybean Core Collection.

concentration in plant/Cs concentration in soil) of ^{137}Cs and ^{133}Cs were well correlated in polished rice. Variation of approximately 10-fold in Cs concentration was observed in each year, although the frequency distribution in 2011 was shifted below those in 2012 and 2013 (Table 1, Fig. 2). The mean concentrations (mg/kg DW) of Cs for all accessions analyzed were 0.037, 0.076, and 0.054 in 2011, 2012, and 2013, respectively. We determined the concentrations of

other homologous elements such as rubidium (^{85}Rb) and ^{39}K using the same seed samples (Table 1, Supplemental Fig. 1). The distribution ranges were similar between years for Rb and K (Supplemental Fig. 1). For the Rb concentration (mg/kg DW), the mean values were 14.2, 12.9, and 10.3 in 2011, 2012, and 2013, respectively, and variations of approximately fivefold were detected among accessions. For K concentration (mg/kg DW), the mean values were 20,500, 19,700, and 19,000 in 2011, 2012, and 2013, respectively, and variations among accessions were approximately 1.5-fold. In the correlation analysis between years, high correlations ($r > 0.7$) could be detected between 2012 and 2013 in all three alkali metals (Table 2). However, the correlation coefficients between 2011 and the other two years were much lower than that between 2012 and 2013 in Cs, although significant positive correlations ($p < 0.01$) could be detected in all combinations (Table 2, Fig. 3A). Similar relationships were also detected for Rb concentration, although its phenotypic stability between years appeared to be higher than that of Cs (Table 2). In contrast, we found high correlations ($r > 0.7$) between all pairs of years for seed K concentration (Table 2, Fig. 3B). As described above, the experimental plots were changed between 2011 and the other two years, possibly leading to differences in the experimental environment such as in soil conditions. We could not find specific differences between JMC and WMC in the patterns of frequency distribution and correlation for the three mineral contents. Analysis of variance (ANOVA) for the three-year datasets revealed significant effects of core collection accession (Genotype) as well as cultivation year (Environment) at the $p < 0.001$ level for the seed concentration of Cs, Rb, and K. These results suggest that there is genetic variation for seed Cs concentration in both Japanese and exotic soybean germplasm, although it may be more susceptible to environmental conditions than are flowering time, seed weight, and seed K concentration.

Relationships between seed Cs concentrations and other traits

Given that Cs, Rb, and K are congeners with similar chemical properties, these minerals could be accumulated in the soybean seed via a common uptake and transport pathway. We accordingly investigated the relationships among the three mineral concentrations in the seeds of the mini-core collections (Table 3, Fig. 4). We found no apparent relationship between K and the other two alkali metals, although we detected weak negative correlations in several cases (Table 3, Fig. 4A). By contrast, there were significant positive correlations between Cs and Rb in all three years (Table 3, Fig. 4B). In particular, the correlation coefficient was very high ($r = 0.9$) in 2011. We also investigated correlations between these three alkali metals and some agronomic traits. There were significant positive correlations between Cs concentration and days to flowering, although the correlation between them appears to be weak, with R^2 values of 0.26, 0.09, and 0.09 in 2011, 2012, and 2013,

A: Cs concentration

B: K concentration

Fig. 3. Year-to-year correlation for seed concentration (mg/kg DW) of Cs (A) and K (B) in the mini-core collections grown in the Yawara field. Closed and open circles indicate JMC and WMC, respectively. Cs, cesium; JMC, Japanese Soybean Core Collection; K, potassium; WMC, World Soybean Core Collection.

respectively (Table 3, Supplemental Fig. 2A). In contrast, negative correlations ($r < -0.4$) were observed between K concentrations and the days to flowering, whereas relatively strong positive correlations ($r > 0.6$) were observed between Rb concentration and days to flowering (Table 3, Supplemental Fig. 2B, 2C). We found significant positive correlations between K concentration and seed weight in WMC, but no such relationship in JMC (Table 3). Given that the range of seed weight was much wider in JMC than in WMC, as described above (Fig. 1B), seed weight is not expected to be related with K concentration in soybean. We found no correlations between seed weight and seed concentration of Cs or Rb (Table 3). We also investigated the effect of seed coat color on seed Cs concentration, given that seed coat color is an important agronomic trait in soybean. There was no difference in Cs concentration among accessions with yellow, brown, black, or green seed color (Supplemental Fig. 3). These results together suggest that the mechanism generating variation in seed Cs concentrations in soybean germplasm cannot be simply explained by differences in seed K concentrations and that Cs and Rb may be accumulated in soybean seed in a similar manner. In addition, seed Cs concentration in the soybean germplasm

appeared not to be directly associated with seed qualities such as seed weight and seed coat color, although it may be somewhat influenced by days to flowering, suggesting the possibility of changing seed Cs accumulation without changing agronomically important traits.

Selection of candidate accessions as high or low Cs accumulators

Given that genetic variation for seed Cs concentration appeared to be present in soybean germplasm based on the results of mini-core collections grown at the Yawara rotational paddy field, we next attempted to select high and low Cs accumulators from among the accessions. When we sorted accessions by seed Cs concentration, eight and nine accessions for the top or bottom 20% in every year over the 3 years were selected as candidates for high and low Cs accumulators, respectively (Supplemental Table 1). These selected 17 accessions showed diverse seed coat color, days to flowering, and seed weight. To determine whether their characteristics of seed Cs accumulation are stable under different cultivation conditions, we analyzed seeds of the selected accessions grown in the Tsukuba upland field in 2011–2013. Although the geographical location of the

Table 3. Correlations among the five traits measured in the two mini-core collections

	Year	Concentration in seed (mg/kg DW)									Days to flowering		
		Cs			Rb			K					
		Total	JMC	WMC	Total	JMC	WMC	Total	JMC	WMC	Total	JMC	WMC
Concentration in seed (mg/kg DW)	Rb	2011	0.90**	0.85**	0.93**								
		2012	0.62**	0.62**	0.63**								
		2013	0.67**	0.68**	0.67**								
	K	2011	-0.25**	-0.07	-0.36**	-0.25**	0.00	-0.37**					
		2012	-0.03	-0.01	-0.04	-0.21**	-0.27*	-0.16					
		2013	-0.11	-0.22	0.00	-0.10	-0.23*	0.00					
Days to flowering	2011	0.51**	0.31**	0.59**	0.61**	0.42**	0.66**	-0.63**	-0.61**	-0.66**			
	2012	0.30**	0.32	0.31**	0.65**	0.69**	0.66**	-0.58**	-0.56**	-0.61**			
	2013	0.30**	0.39**	0.25*	0.65**	0.68**	0.66**	-0.46**	-0.51**	-0.45**			
Seed weight (mg DW)	2011	0.00	0.30**	-0.21	-0.03	0.35**	-0.20	0.18*	-0.16	0.52**	-0.24**	-0.01	-0.40**
	2012	-0.04	-0.04	0.04	-0.04	-0.03	-0.07	0.33**	0.07	0.65**	-0.22**	-0.05	-0.40**
	2013	-0.01	-0.01	-0.05	-0.01	0.02	-0.13	0.27**	0.07	0.56**	-0.22**	-0.04	-0.43**

** and * indicate significant differences at the 1% and 5% levels, respectively.

Fig. 4. Correlations between the concentrations of Cs and other two alkali metals (mg/kg DW) in seeds of mini-core collections grown in the Yawara field; Cs-K (A) and Cs-Rb (B). Upper, middle and lower panels represent populations grown in 2011, 2012, and 2013, respectively. Closed and open circles indicate the JMC and WMC, respectively. Cs, cesium; JMC, Japanese Soybean Core Collection; K, potassium; Rb, rubidium; WMC, World Soybean Core Collection.

Tsukuba field (36°02'N, 140°11'E) is close to that of the Yawara field (36°00'N, 140°02'E), the soil type, gray lowland soil, of the Yawara rotational paddy field differed considerably from the Andosol in the Tsukuba upland field. The

exchangeable Cs, Rb, and K in soil (mg/kg DW) of the Tsukuba fields after cultivation in 2013 were 0.11, 1.1, and 510, respectively, meanwhile Cs, Rb, and K of the Yawara fields were 0.27, 0.54, and 360, respectively, as described

above. Thus, the Tsukuba field soil appeared to contain lower exchangeable Cs and higher exchangeable Rb and K than the Yawara field soil. In addition, the sowing dates in the Tsukuba field were approximately 20 days earlier than those in the Yawara field, leading to a change in days to flowering (**Supplemental Table 1**). Under these conditions, the distribution ranges of seed concentration for Rb and K differed only slightly between the Tsukuba and Yawara fields (data not shown). However, the frequency distributions of the Cs concentration in the Tsukuba field were shifted downward and narrower in comparison with those in the Yawara field (**Supplemental Table 1, Fig. 5**). The mean values of seed Cs concentration (mg/kg DW) among the 17 accessions grown in the Tsukuba field were 0.018, 0.023, and 0.016 in 2011, 2012, and 2013, respectively, and variations of only approximately fourfold were detected in all years. However, there were significant differences ($p < 0.01$) in Cs concentration between the two accession groups categorized as high or low Cs accumulator candidates grown in both Yawara and Tsukuba fields (**Supplemental Table 1, Fig. 5**). These results suggest that although the accumulated amounts of Cs may change depending on the cultivation condition, the characteristics of seed Cs accumulation are stable in each accession.

Discussion

Radiocesium isotopes threaten human health and living conditions over long periods, owing to their long half-lives. After release of radiocesium from nuclear weapon tests and accidents at nuclear power plants, many researchers have investigated physiological and genetic factors for reducing Cs accumulation in plants. However, most of the molecular mechanisms of Cs accumulation remain unknown. In this study, we surveyed genetic variation in seed Cs concentration among diverse soybean germplasm as a first step toward developing “safer” varieties. As a result of analyzing the NIAS mini-core collections grown in rotational paddy fields, approximately 10-fold variation was observed in the Cs concentrations (**Table 1, Fig. 2**). We have data showing that coefficient of variation (CV) for the exchangeable ^{133}Cs among six soil samples from a plot (30 m²) of the Yawara field is 11.4%, which suggests that variations in the exchangeable ^{133}Cs in the field probably had an insignificant impact on those in the seed Cs concentrations among accessions. The considerable variations were also observed in the concentrations of Rb and K, which are congeners of Cs (**Table 1, Supplemental Fig. 1**). However, variation in seed Cs concentration appeared to be uncorrelated with those in seed K concentration, whereas clear positive correlations were detected between the seed concentrations of Cs and Rb (**Table 3, Fig. 4**). Although Cs, Rb, and K are thought to accumulate via common uptake and transport pathways based on the similarity of their chemical properties (reviewed by White and Broadley 2000), it has often been reported that the distribution of K in plants is different from

that of Cs. Tsukada *et al.* (2002b) measured nonradioactive Cs and K in rice plant components including polished rice, rice bran, hull, straw, and root at harvest time and found that their distributions in the plant parts were clearly different. Menzel and Heald (1955) also showed that the Cs:K ratio varied between different plant parts in millet, oats, buckwheat, sweet clover, and sunflower. These phenomena could be explained by uptake and transportation mechanisms shared or overlapping between Cs and K. In higher plants, a large number of transporters and channels constitute the complex K⁺ transport system. In *Arabidopsis*, more than 20 genes encoding K⁺ transporters and channels have been identified (Wang and Wu 2013, Ward *et al.* 2009). They have different energetic coupling, affinity, and selectivity for K⁺, and different combinations of them are expressed in each tissue in complex signaling and physiological regulatory networks. Given that soybean is known as a paleopolyploid species and most genes are duplicated (Schmutz *et al.* 2010), many more K⁺ transporters and channels should be present and be functionally diversified in the soybean genome. It is thus highly possible that the difference of seed K concentration in the mini-core collection could be governed by multiple alleles of the multiple K⁺ transporter and channel genes, obscuring the relationship between Cs and K concentrations in soybean germplasm. However, the strong positive correlation between Cs and Rb concentrations suggests that they are accumulated in soybean seed via nearly identical transport pathways. In fact, Shinonaga *et al.* (1999) investigated uptake and distribution of Cs and Rb in maturing soybean plants and reported that the distributions of Cs in leaves, stems, pods, and seeds were very similar to those of Rb. Given that Cs and Rb are nonessential elements for plants and that specific transporters for these ions probably have not evolved, they are expected to be accidentally absorbed and accumulated in plants by transport systems for essential elements such as K. The ionic radius of Cs is closer to that of Rb than to that of K, suggesting the hypothesis that the accumulations of Cs and Rb in plants are mediated by a common and relatively small number of genes. If this is the case, we might use Rb as a reliable indicator in comparing Cs accumulation between plants, given that in most cases Rb is more abundant than Cs in soil (Kabata-Pendias 2001).

Correlation analysis showed no relationship of seed Cs concentration with seed weight (**Table 3, Supplemental Table 1**) or seed coat color (**Supplemental Fig. 3**). Also, seed weight appeared to have no or weak correlation with seed concentrations of Rb and K (**Table 3**). Several studies have investigated the relationship between seed weight and seed mineral concentration in legumes. Moraghan and Grafton (2001) analyzed seed concentrations of nine minerals in eight common bean (*Phaseolus vulgaris* L.) cultivars grown at five field locations and reported that the concentrations of calcium, manganese, and magnesium were negatively correlated with seed weight, whereas the phosphorus concentration was positive correlated with seed weight. Sankaran *et al.* (2009) attempted QTL analysis of the seed

Fig. 5. Box-plot of seed Cs concentration in two experimental fields, Yawara (A) and Tsukuba (B). Filled box (left) and open box (right) indicate the two accession groups categorized as low or high Cs accumulator candidates, respectively, which were selected based on the Cs concentrations in the Yawara field. Boxes represent quartiles 25–75%, and horizontal line within the box represents the median of the distribution (quartile 50%). Bars indicate quartiles 1–25% (above) and 75–100% (below). ** indicates a significant difference between low and high Cs-accumulating groups at the 1% level using Welch's *t*-test. Cs, cesium.

concentrations of eight minerals and seed weight in *Medicago truncatula* and reported that several QTLs for essential minerals overlapped with those for seed weight, suggesting that seed size is an important determinant of seed mineral concentration. However, clear correlations between seed K concentration and seed weight were not detected in these two studies, suggesting that seed K concentration is regulated independently of seed weight in legumes. In contrast, we found correlations between flowering date and seed concentrations of the three alkali metals. Although the seed concentrations of Cs and Rb appeared to be positively correlated with days to flowering, seed K concentration showed an inverse (negative) correlation (Table 3, Supplemental Fig. 2). The mechanisms causing these relationships remain unclear. However, one of the possible contributory factors is fluctuation of K content in soil of the experimental field during the soybean growing period. Soybean accumulates about 4 to 10 times more K in seed than other major crops such as rice, maize, and wheat (Ministry of Education, Culture, Sports, Science and Technology, Japan 2005). Soybean absorbs K^+ continuously until near plant maturity, with the most rapid rate of K accumulation observed during the period of rapid growth of vegetative parts (Hanway and Johnson 1985). Thus, the K-supplying capacity in soil drastically decreases with the growth of soybean plants, increasing the proportion of Cs and Rb to K in the soil. Given that late flowering time generally results in late maturation, late-flowering accessions must grow in soil with lower K content than do early-flowering accessions during the reproductive stage, possibly leading to low uptake of K and high uptake of Cs and Rb. However, the correlation between seed Cs concentration and the days to flowering appeared to be weak, and we found considerable variation in seed Cs concentration irrespective of flowering time (Table 3, Supplemental Fig. 2). Thus, it is possible that seed Cs accumulation could be changed without change in agronomically significant traits such as seed weight, seed

coat color, and the days to flowering. This prospect is essential information for breeding “safer” varieties.

In this study, we compared soybean germplasm grown in different years and fields, finding that the phenotypic stability of seed Cs concentration is lower than that of seed K concentration. The rotation of experimental plots in the Yawara paddy field between 2011 and 2012 appeared to cause the alteration of Cs accumulation in each accession (Table 2, Fig. 3). Seed Cs concentrations showed a tendency to be higher in the Yawara rotational paddy field than in the Tsukuba upland field (Supplemental Table 1, Fig. 5). Although we could not identify all factors affecting the phenotypic stability, K contents in soil are expected to have strong effects on Cs accumulation in plants, as described above. Kondo *et al.* (2015) recently reported that Cs concentration in rice plants showed a significant positive correlation with the exchangeable Cs/K ratio in the soil. The exchangeable Cs/K ratio in the soil of Yawara field appeared to be higher than that in the Tsukuba field, at least in 2013, a situation that could have increased the seed Cs concentration. Still, we identified 17 accessions whose characteristics of seed Cs accumulation were stable regardless of planted year and field (Supplemental Table 1, Fig. 5). The low Cs accumulator candidates have a tendency toward early flowering, whereas the high Cs accumulator candidates are inclined to late flowering. However, some accessions such as OUDU (NIAS Core Collection ID: GmWMC118, high Cs) and E C 112828 (GmWMC188, low Cs) appeared not to show such an association between days to flowering and seed Cs concentration (Supplemental Table 1). These results highly suggest again that there is genetic variation for seed Cs concentration in soybean germplasm and that we could improve seed Cs concentration independently of flowering time. Detailed examination of Cs accumulation in the selected accessions as high or low Cs accumulator candidates and further search for low Cs accumulators in soybean germplasm would lead to elucidating the molecular

mechanisms of Cs accumulation and developing “safer” varieties of soybean.

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