Effect of soil exchangeable potassium content on cesium absorption and partitioning in buckwheat grown in a radioactive cesium-contaminated field

Katashi Kubo, Shigeto Fujimura, Hiroyuki Kobayashi, Takeshi Ota and Takuro Shinano

Agricultural Radiation Research Center, National Agriculture and Food Research Organization (NARO) Tohoku Agricultural Research Center, Arai, Japan; Crop Production Systems Division, NARO Central Region Agricultural Research Center, Tsukuba, Japan

ABSTRACT

The effect of soil exchangeable (plant-available) potassium (ExK) content on cesium (Cs) absorption and translocation in buckwheat was evaluated in a field contaminated with radioactive Cs (134Cs and 137Cs, RCs) in 2013. The RCs concentration in buckwheat was significantly positively correlated with the naturally occurring stable Cs (133Cs, SCs) concentration, and was lower at higher soil ExK content. The RCs and SCs were actively absorbed by buckwheat until the flowering stage. The soil ExK content was significantly negatively correlated with soil exchangeable RCs and SCs (ExRCs and ExSCs) concentrations. Greater RCs and SCs absorption by buckwheat in soils with low ExK contents was mainly due to higher soil ExRCs and ExSCs concentrations. Reproductive organs showed the largest differences in SCs concentration between low-ExK and high-ExK plots. The root–shoot and shoot–reproductive organs translocations of SCs markedly decreased with increasing soil ExK content. In the root–shoot and shoot–reproductive organs translocations, the discrimination of SCs and K decreased with decreasing soil ExK content. Our main findings were as follows: (1) because RCs are mainly taken up at the earlier growth stage, potassium should be applied as a basal fertilizer to decrease the RCs concentration in buckwheat; (2) lower soil ExK content led to higher soil ExRCs concentrations, resulting in greater RCs absorption by buckwheat; (3) the high Cs absorption and translocation and weaker discrimination between Cs and K in low ExK content soil may be due to the expression of K transporter(s) with weak discrimination between Cs and K.

1. Introduction

An accident at the Tokyo Electric Power Company (TEPCO)’s Fukushima Dai-ichi (No. 1) nuclear power plant (FDNPP), caused by the Great East Japan Earthquake and tsunami on 11 March 2011, released radioactive cesium (134Cs and 137Cs, RCs) into the environment in eastern Japan (Yasunari et al., 2011). In the agricultural fields, there has been a focus on reducing the translocation of RCs from the soil to edible parts of crops. The overall aims are to reduce internal radiation exposure in humans consuming agricultural products containing RCs and to increase the food security of agricultural products.

Buckwheat (Fagopyrum esculentum Moench) is a functional food in the aspect of rich minerals, amino acid and organic acid (Giménez-Bastida & Zieliński, 2015; Katar et al., 2016; Wijngaard & Arendt, 2006), and an important field crop in eastern Japan. However, it is a crop which accumulates high RCs concentration (Broadley et al., 1999). Therefore, the decreasing of RCs concentration in buckwheat is a most important point in Japanese agriculture. In 2011 and 2012, 0.9 and 0.5% of grain samples collected from 330 and 2918 farmers’ fields in eastern Japan, respectively, exceeded 100 Bq kg−1 (the standard limit of radioactive nuclides for food materials from April 2012) (MAFF, 2013; MHLW, 2012). The Ministry of Agriculture, Forestry and Fisheries (MAFF) advised farmers and processors of several countermeasures to reduce the RCs concentrations in buckwheat (MAFF, 2014a). These countermeasures included the application of potassium (K) fertilizers to soil before buckwheat cultivation to increase the soil exchangeable (plant-available) potassium (ExK) content (Kubo et al., 2015), and polishing of buckwheat grains after harvest (Kubo et al., 2016a). Since these countermeasures were introduced, no grain samples exceeded 100 Bq kg−1 in 1394 and 848 farmers’ fields in 2013 and 2014, respectively (MAFF, 2015a, 2016), although one sample in 541 farmers’ fields in 2015 exceeded 100 Bq kg−1 (MAFF, 2016). Therefore, increasing the soil ExK content
by applying K fertilizers has largely contributed to the decreased accumulation of RCs in buckwheat as well as rice and soybean (MAFF, 2014b, 2015b).

The translocation of RCs from soil to the edible parts of buckwheat depends on how much is taken up from the soil and how it is partitioned among plant parts (and also on whether lodging has resulted in adhesion of soil particles with high RCs concentrations to the grain surface; Kubo et al., 2016a). Although buckwheat has relatively higher ability to accumulate RCs (Broadley et al., 1999), it is unclear how the absorption and partitioning of RCs are affected by the soil ExK content. In rice, accelerated translocation of RCs from the shoot to grain under K-deficient conditions has been observed in a hydroponic system (Nobori et al., 2014), and the relationship between soil ExK content and translocation of RCs and SCs may be explainable in terms of K transporter expression (Fujimura et al., 2014). Plants are thought to contain multiple K transporters (Grabov, 2007; Kim et al., 1998). Among them, the high affinity K+ transporter (HAK) has been analyzed in terms of its expression under low-K conditions and its role in Cs uptake/translocation. AtHAK5 and OsHAK5 are highly expressed under K starvation in Arabidopsis and rice, respectively. These transporters play major roles in K acquisition by roots in low external K conditions and in upward transport of K from roots to shoots in K-deficient plants (Gierth et al., 2005; Yang et al., 2014). Rubio et al. (2000) reported that the HAK transporter transports Cs in barley and Arabidopsis. Qi et al. (2008) showed that the AtHAK5 plays a physiological role at low K concentrations and provides a Cs-uptake pathway in Arabidopsis. Other experiments showed that ZIFL2 (Zinc-Induced Facilitator-Like 2) is a functional transporter that mediates K and Cs influx (Remy et al., 2015). As these crops, buckwheat also may have interactions in absorption and partition between Cs and K.

Therefore, in this study, we determined how the absorption and partition of RCs by buckwheat plants is affected by the soil ExK content, with an aim to establish the most effective method of K application. We particularly focused on the exchangeable RCs (ExRCs) concentration in soil, because this form is most readily absorbed by plants, and is therefore the most important form of RCs translocated from soil to plants (Endo et al., 2013). Partitioning of RCs in buckwheat plants was evaluated in detail by using the naturally occurring stable isotope Cs (133Cs, SCs), which is assumed to have a similar fate to that of RCs in plants (see review by Yamaguchi et al., 2012).

2. Materials and methods

A field experiment was conducted at a farmer’s field in Date City, Fukushima Prefecture, in 2013. This was the same site where we analyzed the effect of K application on the RCs concentration in buckwheat grain (Kubo et al., 2015) and the effect of grain polishing after harvest on the RCs concentration in lodged buckwheat grain (Kubo et al., 2016a). The characteristics of the field, fertilization, experimental treatments, sowing date and plot size were described in Kubo et al. (2015). The samples used in this study were obtained along with the experiment of Kubo et al. (2015). In the field, there were four levels of ExK content by the K application: no K2O application (7.9 mg K2O 100 g−1 dry soil; ExK7.9 plot), and 25, 45, and 65 mg K2O 100 g−1 dry soil (ExK25, ExK45, and ExK65 plots, respectively); and two levels of zeolite application; no application and 1.0 t ha−1. The experimental design was split-plot which had four levels of K application (main plot) and two levels of zeolite application (subplot) with three replications. Because zeolite application did not markedly affect buckwheat growth (132 kg 10 a−1 in no zeolite application and 130 kg 10 a−1 in zeolite application in grain yield) or RCs accumulation (58 Bq kg−1 in no zeolite application and 51 Bq kg−1 in zeolite application in grain RCs concentration) (Kubo et al., 2015), this study showed solely on the effect of K application as the result of split-plot statistical analysis. Plant density was 163 ± 3 plant m−2 (n = 24). Buckwheat plants and the soil just below the plants were sampled at the 17 days after seeding (DAS) (seedling stage), 30 DAS (flowering stage), 51 DAS (grain-filling stage), and 70 DAS (mature stages). Root was sampled from the area of 0.2 m depth and 0.75 m width (both 0.325 m from a center of row) with shovel (218 mm length, 180 mm width). Twenty centimeter depth of soil samples were collected from around the plant root with the round worm scoop (Fujiwara Scientific Company Co., Ltd., Tokyo Japan) at the same time as the plant sampling was taken. Soil collections by the scoop were conducted ten times for each plot, and they treated as a sample with mixing. The plants were divided into root and aerial parts (shoot) at the seedling stage; into root, stem, petiole, leaf, and reproductive organs (flower and/or grain) at the flowering and grain-filling stages; and into root, shoot, and reproductive organs at the mature stage. Separately, from the plants divided into several parts, plants of each plot were sampled from 1.5 m2 for the yield survey at mature stage. Grain was prepared by threshing, winnowing, and polishing before determining grain yield (based on 15% water content) and subsequent analysis as described in Kubo et al. (2015). Each part of plant was rinsed with distilled water after washing with tap water. Root samples were carefully separated from soil in the tap water with fingers and sponge brush (089-52-31-15, TGK Co. Ltd., Tokyo, Japan) on the 1.0 mm sieve.

Then, each part was cut into small pieces and dried for 48 h at 80 °C in a ventilated oven to determine dry weight (DW). Dried plant parts were ground into a powder using a blender (D3V-10, Osaka Chemical Co., Osaka,
Japan), and the RCs concentration was determined using a germanium semiconductor detector (GC2520-7500SL; Canberra Japan KK, Tokyo, Japan). The measurement of RCs concentrations had a 10% range error, and the results were decay-corrected to the day of sampling. To measure stable Cs (SCs) and K concentrations, 0.5 g ground material was digested in 5.0 mL HNO₃ (Ultrapur-100, Kanto Chemical Co., Inc., Tokyo, Japan) for 1 h at 100 °C using a hot block acid digestion system (DigiPREP LS, SCP Science, Baie D’Urfé, Quebec, Canada) (Baba & Goto, 2009), and the SCs and K concentrations were determined by inductively coupled plasma mass spectrometry (ICP-MS) (7700X, Agilent Technologies, Waldbronn, Germany; NexION 350S, PerkinElmer, Waltham, MA, USA). Soil samples collected at each growth stage of buckwheat were dried at 40 °C for 7 days, and prepared as described previously (Kubo et al., 2015). Soil ExK, ExRCs, and exchangeable stable Cs (ExSCs) were extracted from soil samples at a soil-to-solution ratio of 1:10 in 1 M ammonium acetate (H₃N₄OAc) with shaking for 1 h. The soil ExK content was determined by atomic absorption spectrophotometry (ZA3000, Hitachi High-Tech Science, Tokyo, Japan). The soil ExRCs concentration was measured using a germanium semiconductor detector as described above. The soil ExSCs concentration was measured by ICP-MS. To evaluate the ratio of concentration in SCs and K between plant part A and plant part B, SCs ratio and K ratio were calculated as follows:

SCs ratio = SCs (ng g⁻¹) in plant part A / SCs (ng g⁻¹) in plant part B

K ratio = K (mg g⁻¹) in plant part A / K (mg g⁻¹) in plant part B

We considered the higher ratio indicated higher concentration of SCs or K in plant part A compared to plant part B.

To evaluate the efficiency of SCs transport from plant part A to B compared with the efficiency of K transport, the SCs/K discrimination factor (DF, Ciuffo et al. (2003); Godyn et al. (2016); Middleton et al. (1960); Sanches et al. (2008)) between plant parts was estimated as follows:

DF = (SCs (ng g⁻¹) in plant part A / K (mg g⁻¹) in plant part A) / (SCs (ng g⁻¹) in plant part B / K (mg g⁻¹) in plant part B)

A DF value lower than 1.0 indicates that K is transported more efficiently than SCs.

SPSS software (IBM SPSS ver. 22 for Windows; IBM Japan) was used for the statistical analysis. Analysis of variance (ANOVA) using the general linear model (GLM) procedure and multiple comparisons with the Ryan-Einot-Gabriel-Welsch procedure were used to evaluate the differences among treatments. To evaluate the relationships between characteristics, Pearson’s correlation analysis was conducted.

3. Results

3.1. Soil ExK during cultivation, buckwheat growth, yield and RCs concentration in grain

The soil ExK content differed among the ExK7.9, ExK25, ExK45, and ExK65 plots at the mature stage (Figure 1), and the plots with higher K application kept higher soil ExK content. The largest decrease in soil ExK content during the growth period was in the ExK65 plot. The RCs concentration in soil at mature stage was 3776 ± 454 Bq kg⁻¹ (average ± standard deviation, data was not shown). The DW of buckwheat plant parts did not differ among treatments at all growth stages, except for the stem and reproductive organs at the grain-filling stage (Table 1). The average yield of buckwheat grain across all treatments was 131.2 g m⁻², and there was no difference in grain yield among the treatments (ExK7.9 125.5, ExK25 133.8, ExK45 133.2 and ExK65 132.9 g m⁻²). As in our previous study, the grain RCs concentration was significantly higher in the ExK7.9 plot than in the other K treatments, and was lower in plots with higher soil ExK contents (Kubo et al., 2015).

3.2. Relationships among Soil ExK, soil ExRCs, soil ExSCs, and RCs and SCs absorption by buckwheat

There were significant differences among treatments in the concentrations of RCs, SCs and K in whole buckwheat plants at all growth stages (Table 2). The highest concentrations of RCs and SCs in whole buckwheat plants were at the flowering stage except for RCs concentration in plots ExK25, ExK45 and ExK65. The RCs and SCs concentrations in plants in the ExK7.9 plot were significantly higher than those in plants in other plots at all growth stages, and were two to four times higher than those in plants in the ExK25.
The K concentration in whole plants tended to be lower in the ExK7.9 plot than in other plots, but the differences were smaller than those observed for RCs and SCs concentrations. The RCs concentration was significantly positively correlated with the SCs concentration in buckwheat shoots at all growth stages and in the grain at the mature stage (Table 2).

The soil ExK content was significantly negatively correlated with the soil ExRCs concentration at the mature stage (Figure 2(a)), and the buckwheat plants in plots with higher soil ExK content had lower RCs concentrations (Figure 2(b)). The buckwheat RCs concentration was significantly positively correlated with the soil ExRCs concentration (Figure 2(c)). The ratio of buckwheat RCs concentration to soil ExRCs concentration was not significantly correlated with soil ExK content, and the translocation of ExRCs from soil to buckwheat was not affected by soil ExK content (Figure 2(d)). The relationships among soil ExK content, soil ExSCs concentration, and buckwheat SCs concentration were similar to those observed for RCs (Figure 2(e)–(h)).

3.3. Partitioning of Cs and discrimination between Cs and K in buckwheat plants

The SCs and K concentrations in the different plant parts are shown in Table 3. The SCs concentration was the highest in ExK7.9 plot in all plant parts at all growth stages except for the root at the grain-filling stages. The largest differences in SCs concentrations in buckwheat plants were between the ExK7.9 and ExK25 plots. For example, the SCs concentration in the reproductive organs was six times higher in ExK7.9 than in ExK25 at flowering stage. The K concentrations in all plant parts also differed significantly among plots, except for the petiole and leaf at the grain-filling stage and the reproductive organs at the flowering and grain-filling stages. The K concentrations in plant parts were relatively higher in plots with higher soil ExK contents. The differences in K concentrations in each plant part between the ExK7.9 and ExK25 plots were relatively smaller than the differences in SCs concentrations.

The ratios of SCs and K between different plant parts are shown in Figure 3. At the seedling stage, the SCs shoot to root ratio was significantly higher in ExK7.9 than in other plots, and the ratio in ExK7.9 was greater than 1.0. The K shoot to root ratio was lower in ExK7.9 than in other plots, and it was greater than 1.0 in all plots. At the flowering stage, the SCs stem to root, petiole to stem, and flower to petiole ratios were the highest in ExK7.9, and decreased with increasing soil ExK contents. The SCs petiole to stem ratio in all plots and flower to petiole ratio in ExK7.9 were greater than 1.0. The K flower to petiole ratio was significantly higher in ExK7.9 than in other plots. The K stem to root and petiole to stem ratios were greater than 1.0 in all plots. At the grain-filling stage, the SCs stem to root, petiole to stem, and reproductive organs to petiole ratios were significantly higher in ExK7.9 than in other plots. The K reproductive organs to petiole ratios were higher in plots with lower soil ExK contents. At the mature stage, the K shoot to root was greater than 1.0 in all plots. The K grain to shoot ratios were higher in plots with lower soil ExK contents. Also, the SCs shoot to root and grain to shoot ratios were higher in plots with lower soil ExK contents.

The DF between different plant parts are shown in Figure 4. The DF between root and shoot (stem) were significantly higher in ExK7.9 than in the other plots at all growth stages. The DF between stem and petiole and between petiole and reproductive organs were highest in ExK7.9, and decreased with higher soil ExK contents at the flowering stage. DF between shoot and grain were greater than 1.0 in all the plots.

4. Discussion

4.1. Effect of soil ExK content on Cs accumulation in buckwheat grain

The RCs and SCs concentrations in buckwheat grain were higher in plots with lower soil ExK contents (Kubo et al., 2015), although the growth and yield did not differ.

Table 1. Dry weight (mg plant⁻¹) of different parts of buckwheat plants at different growth stages in each plot.

| Plant part | Root | Stem | Petiole | Leaf | Reproductive organs |
|------------|------|------|---------|------|---------------------|
| **Seedling stage** |      |      |         |      |                     |
| K7.9       | 15.4 | 141  |         |      |                     |
| K25        | 17.3 | 151  |         |      |                     |
| K45        | 17.1 | 149  |         |      |                     |
| K65        | 16.6 | 145  |         |      |                     |
| ANOVA      | ns   |      |         |      |                     |
| **Flowering stage** |      |      |         |      |                     |
| K7.9       | 139  | 792  | 55.6    | 401  | 106                |
| K25        | 113  | 724  | 44.5    | 315  | 85                 |
| K45        | 131  | 837  | 56.6    | 391  | 105                |
| K65        | 138  | 827  | 53.2    | 368  | 105                |
| ANOVA      | ns   | ns   | ns      | ns   | ns                 |
| **Grain-filling stage** |      |      |         |      |                     |
| K7.9       | 160  | 1047  | 36.3    | 213  | 1465               |
| K25        | 146  | 984   | 35.4    | 203  | 1464               |
| K45        | 182  | 1493  | 46.1    | 224  | 2042               |
| K65        | 142  | 1043  | 29.2    | 154  | 1457               |
| ANOVA      | ns   | *     | ns      | ns   | *                  |
| **Maturity stage** |      |      |         |      |                     |
| K7.9       | 188  | 1429  | 1933    |      |                     |
| K25        | 212  | 1522  | 2030    |      |                     |
| K45        | 225  | 1622  | 1855    |      |                     |
| K65        | 191  | 1451  | 1609    |      |                     |
| ANOVA      | ns   | ns    | ns      |      | ns                 |

1Flower and/or grain.
2Whole shoot.
3x Significant difference at 0.01 ≤ p < 0.05. ns – not significant.
4Different letters indicate significant differences (Ryan–Einot–Gabriel–Welsch test; p < 0.05).
5Whole shoot except for grain.
translocation has also been observed in various crops (Fowler & Christenson, 1959; Fujimura et al., 2013; Kato et al., 2015; Kondo et al., 2015; Sandeep & Manjaiah, 2008). However, the effect of soil ExK content on the behavior of RCs in soil and buckwheat plants is still unclear. The results of this study show that RCs and SCs behave similarly in soil and in buckwheat plants. Our results also illustrate: (1) the time course of RCs absorption in buckwheat; (2) the effect of soil ExK content on the absorption of RCs and SCs by buckwheat; and (3) the effect of soil ExK content on SCs translocation and discrimination between SCs and K in buckwheat plants.

4.2. RCs and SCs behavior in soil and buckwheat plants

The RCs concentrations in buckwheat plants and grain were significantly correlated with the SCs concentrations at all growth stages (Table 2). Also, the relationships among soil ExRCs concentration, soil ExK content, and buckwheat RCs concentration were similar to those observed among soil ExSCs concentration, soil ExK content, and buckwheat SCs concentration (Figure 2). These results suggested that naturally existing SCs and the RCs originating from the FDNPP accident in 2011 had similar behaviors in the field at 2013 after some RCs had become fixed in clay minerals, although there may have been different forms of RCs in soil shortly after the accident (Saito et al., 2014).

Table 2. Concentrations of RCs, SCs, and K in whole buckwheat plants and correlation coefficient between RCs and SCs in shoot and grain.

| RCs (Bq kg⁻¹) | Seedling | Flowering | Grain-filling | Maturity |
|--------------|----------|-----------|---------------|----------|
| K7.9         | 887.8a   | 924.9a    | 386.4b        | 329.7a   |
| K25          | 214.4b   | 199.6b    | 128.2b        | 122.9b   |
| K45          | 163.8b   | 132.8b    | 74.4b         | 78.1b    |
| K65          | 120.8b   | 100.4b    | 86.2b         | 61.5b    |
| ANOVA        | ***      | ***       | ***           | ***      |
| SCs (mg g⁻¹) |          |           |               |          |
| K7.9         | 187.0b   | 302.9a    | 108.0a        | 94.2a    |
| K25          | 46.0b    | 90.8b     | 46.9b         | 32.5b    |
| K45          | 35.4b    | 60.9b     | 29.0b         | 22.6b    |
| K65          | 27.6b    | 52.7b     | 33.6b         | 18.1b    |
| ANOVA        | ***      | ***       | ***           | ***      |

4.2. RCs and SCs behavior in soil and buckwheat plants

The RCs concentrations in buckwheat plants and grain were significantly correlated with the SCs concentrations at all growth stages (Table 2). Also, the relationships among soil ExRCs concentration, soil ExK content, and buckwheat RCs concentration were similar to those observed among soil ExSCs concentration, soil ExK content, and buckwheat SCs concentration (Figure 2). These results suggested that naturally existing SCs and the RCs originating from the FDNPP accident in 2011 had similar behaviors in the field at 2013 after some RCs had become fixed in clay minerals, although there may have been different forms of RCs in soil shortly after the accident (Saito et al., 2014).

Figure 2. Relationships between soil ExK content, soil ExRCs concentration, and RCs concentration in buckwheat (a–d) and between soil ExK content, soil ExSCs concentration, and SCs concentration in buckwheat (e–h). Cited and reprocessed from Kubo et al. (2015). Correlation coefficients were calculated with power approximation (a, b, d, e, f, h) and linear approximation (c, g), respectively.
From these results, we conclude that K fertilizer should be applied as a basal fertilizer before sowing to decrease the RCs concentration in buckwheat.

### 4.4. Effect of soil ExK content on soil ExRCs and ExSCs concentrations, and RCs and SCs absorption by buckwheat

Kamei-Ishikawa et al. (2008) reported that the transfer of RCs from soil to crops can be predicted from the transfer of SCs. Because smaller samples are required for SCs analysis than for RCs analysis, we considered that SCs would be useful to evaluate the behavior of RCs inside the plant body, and to evaluate the fate of RCs between the soil and plants. Therefore, we measured the SCs concentration in plant parts as a substitute for RCs to evaluate RCs translocation within buckwheat plants.

#### 4.3. RCs and SCs absorption by buckwheat during growth

The time-course analysis showed that buckwheat plants mainly absorbed RCs and SCs until the flowering stage, and more RCs and SCs were absorbed in the plots with lower soil ExK contents (Table 2). The soil ExK content decreased until the flowering stage (Figure 1). Our previous study showed that the absorption of soil ExK by buckwheat plants contributes to the decrease in RCs translocation from soil to shoot and grain in buckwheat (Kubo et al., 2015). Increasing the soil ExK content before sowing led to reduced RCs and SCs concentrations in buckwheat plants from the seedling stage until the mature stage (Table 2). Rice and broad bean have also been shown to absorb large amounts of RCs at the early growth stage (Nobori et al., 2014; Zhu et al., 2002). From these results, we conclude that K fertilizer should be applied as a basal fertilizer before sowing to decrease the RCs concentration in buckwheat.

The soil ExK content was always higher in plots with additional K during buckwheat cultivation (Figure 1). Lower soil ExK contents resulted in higher RCs and SCs concentrations in buckwheat (Figure 2(b) and (f)). We assumed that there were two factors in the relationship between soil ExK content and the concentrations of RCs and SCs in buckwheat: that is, (1) the effect of soil ExK content on soil ExRCs and ExSCs concentrations; and (2) the antagonistic interaction between K and Cs (RCs and SCs) in soil during the absorption of RCs and SCs by buckwheat. Soils with higher ExK contents had lower ExRCs and ExSCs concentrations (Figure 2(a) and (e)). The concentrations of RCs and SCs in buckwheat were lower in plants grown in soil with lower ExRCs and ExSCs concentrations (Figure 2(c) and (g)).

### Table 3. SCs and K concentrations in different parts of buckwheat plants at four growth stages in each plot.

| Seedling stage | Root       | Stem       | Petiole     | Leaf | Reproductive organs | Root       | Stem       | Petiole     | Leaf | Reproductive organs |
|----------------|------------|------------|-------------|------|---------------------|------------|------------|-------------|------|---------------------|
| K7.9           | 112.1 a    | 195.3 a    |             |      |                     | 20.0 b     | 29.4 b     |             |      |                     |
| K25            | 61.5 b     | 44.1 b     |             |      |                     | 24.7 c     | 40.8 c     |             |      |                     |
| K45            | 58.6 b     | 32.7 b     |             |      |                     | 24.9 c     | 42.6 c     |             |      |                     |
| K65            | 49.0 b     | 25.1 b     |             |      |                     | 26.3 c     | 45.1 c     |             |      |                     |
| ANOVA          | ***        | ***        | ***         | ***  | ***                 | ***        | ***        | ***         | ***  | ***                 |
| Flowering stage| K7.9       | 325.7      | 474.1 b     | 473.3 b | 334.4 c  | 17.1 c     | 47.3 b     | 97.4 c     | 23.8 b | 12.8                |
| K25            | 286.1      | 63.9 b     | 99.8 b      | 93.2 b | 54.3 b   | 22.9 b     | 69.6 b     | 135.2 c    | 31.6 b | 13.1                |
| K45            | 209.9      | 47.0 b     | 49.8 b      | 48.0 b | 26.7 b   | 25.4 b     | 70.9 b     | 140.2 b    | 32.3 b | 12.9                |
| K65            | 208.0      | 42.0 b     | 38.2 b      | 32.6 b | 15.4 b   | 26.0 b     | 77.5 b     | 149.2 b    | 33.3 b | 12.9                |
| ANOVA          | ns         | ***        | ***         | ***  | ***                 | ***        | ***        | ***         | ***  | ns                  |
| Grain-filling stage | K7.9      | 274.7      | 112.6 b     | 201.0 a | 207.9 a  | 68.1 a     | 16.8 b     | 40.0 c     | 61.6 b | 19.5                |
| K25            | 278.7      | 45.7 b     | 60.1 b      | 68.8 b | 20.9 b   | 21.4 b     | 53.0 b     | 64.7 b     | 21.2 b | 7.7                 |
| K45            | 185.9      | 31.3 b     | 34.4 b      | 41.1 b | 10.5 b   | 23.5 b     | 57.5 b     | 62.0 b     | 22.9 b | 7.5                 |
| K65            | 205.9      | 34.9 b     | 43.7 b      | 51.6 b | 11.5 b   | 23.7 b     | 62.7 b     | 64.8 b     | 23.6 b | 7.7                 |
| ANOVA          | ns         | ***        | ***         | ***  | ***                 | ***        | ***        | ***         | ***  | ns                  |
| Maturity stage | K7.9       | 205.2 a    | 102.8 a     | 53.0 b  | 14.7 b   | 23.3 a     | 4.5 b      | 4.5 b      | 4.5 b  | 4.5 b                |
| K25            | 170.3 b    | 25.4 b     | 10.0 b      | 16.3 b | 26.3 b   | 4.5 b      | 4.5 b      | 4.5 b      | 4.5 b  | 4.5 b                |
| K45            | 143.5 b    | 14.4 b     | 6.0 b       | 18.3 b | 30.0 b   | 4.6 a      | 4.6 a      | 4.6 a      | 4.6 a  | 4.6 a                |
| K65            | 103.3 b    | 14.2 b     | 4.8 b       | 20.2 b | 33.6 b   | 4.4 b      | 4.4 b      | 4.4 b      | 4.4 b  | 4.4 b                |
| ANOVA          | *          | ***        | ***         | ***  | ***                 | ***        | ***        | ***         | ***  | ***                 |

1Flower and/or grain.
2Different letters indicate significant differences (Ryan-Einot-Gabriel-Welsch test; p < 0.05).
3Whole shoot.
4***, ** and *, significant difference at p < 0.001, 0.001 ≤ p < 0.01 and 0.01 ≤ p < 0.05, respectively. ns – not significant.
5Whole shoot except for grain.
Figure 3. SCs and K ratios among parts of buckwheat plants at four growth stages in each plot. SCs ratio and K ratio of 'plant part A: plant part B' show ratios of SCs and K concentrations in plant part B to those in plant part A. Different letters indicate significant differences (Ryan-Einot-Gabriel-Welsch test; p < 0.05).

Figure 4. SCs/K discrimination factor in different parts of buckwheat plants at four growth stages in each plot. DF of 'plant part A: plant part B' show SCs/K discrimination factor between plant part A and B. Different letters indicate significant differences (Ryan-Einot-Gabriel-Welsch test; p < 0.05).
and (h)). These results suggested that the lower concentrations of RCs and SCs in buckwheat grown in soil with a high ExK content were mainly due to decreased concentrations of ExRCs and ExSCs in the soil. In terms of the mechanism, it is likely that the RCs and SCs absorbed and/or fixed to exchangeable sites and frayed-edge sites in the interlayers of clay minerals become difficult to exchange with ammonium because of reduced space between interlayers and/or blocking of exchangeable sites by K when the soil ExK content is high. Kruglov et al. (2005) reported that the decrease in the soil ExRCs concentration by adding K is because the extra K in soil increases the selectivity of RCs sorption by clay minerals, and decreases the selectivity of RCs uptake by plants compared with K. In a different perspective, Kraffczyk et al. (1984) reported that K treatment affects the composition of low molecular weight organic acids excreted by plant roots, and Chiang et al. (2011) showed that the low molecular weight organic acids excreted by plant roots play a critical role in releasing RCs from clay minerals. Thus, there is evidence that the soil ExK content affects both clay minerals and plant root traits, which is reflected by the absorbability of RCs in buckwheat. We detected small effects of the antagonistic interaction between soil K and Cs on the absorption of RCs and SCs in buckwheat in this study. This may have been due to the composition of clay minerals in the study field. Experiments in soils with different chemical properties (e.g., different clay minerals composition, organic matter content, and concentrations of other minerals) and with different crop species may produce different results from those of this study. Further research on the effects of clay mineral characteristics and soil chemical properties on the relationships between soil ExK content and soil ExRCs concentrations is required.

4.5. Effect of soil ExK content on SCs distribution among plant parts

The K concentration in each plant part at the seedling and flowering stages was lower in the ExK7.9 plot than in the other plots, except in the reproductive organs at the flowering stage (Table 3). At the grain-filling and mature stages, the K concentrations in the root, stem, and shoot were lower in ExK7.9 than in the other treatments. From these results, it was considered that buckwheat plants tended to be sort of K-deficient in the ExK7.9 plot, although the DW of each plant part did not differ significantly among plots (Table 1). In contrast, the SCs concentration was higher in the ExK7.9 plot than in other plots in the root at the seedling and mature stages and in shoot parts at all growth stages (Table 3). The SCs shoot to root ratio was significantly higher in ExK7.9 than in other plots at all growth stages (Figure 3). The SCs petiole to stem and reproductive organs to petiole ratios were higher in plots with lower soil ExK contents (Figure 3). These results indicated that low soil ExK content was related to low K concentration in buckwheat, and the increasing demand for K in buckwheat. Increasing demand for K in buckwheat seemed to result in high SCs translocation from root to stem, from stem to petiole, and from petiole to reproductive organs because K ions and Cs ions have similar chemical forms (White & Broadley, 2000). The relationship between soil ExK content and translocation of RCs and SCs in buckwheat plants may be explainable in terms of K transporters expression, as described in introduction. These K transporters may be functioning at Cs translocation among plant parts. In buckwheat, soil ExK-induced changes in the expression of such transporters might reduce discrimination between Cs and K (Figure 4) especially between root and shoot/stem, and could affect the RCs/SCs acquisition and partitioning in the plant. In this experiment, the DF between petiole and leaf, between petiole and reproductive organs and between shoot and reproductive organs were greater than 1.0 in all the plots (Figure 4). These results may indicate that Cs is well-transported to leaf and reproductive organs compared to K, and indicate that above ground plant parts in buckwheat do not have enough discrimination between Cs and K. Cs transport from the shoot to the newly-formed parts (leaf, grain etc.) would associate multiple K transporters and ion channels. Some K transport proteins may relate to the inadequate discrimination between Cs and K, although detailed evidence is not acquired in this study. For the better understanding of the Cs and K discrimination in plants, further research on the transporter proteins is needed. Genotypic variation in Cs partitioning and discrimination among buckwheat varieties may also exist, as is the case for cadmium partitioning in common wheat (Kubo et al., 2016b). These challenges would be useful to devise strategies to decrease RCs concentrations in crops.

5. Conclusion

The main results of this study were as follows:

(1) Buckwheat plants mainly absorbed RCs and SCs until the flowering stage. Therefore, it is important to apply basal K fertilizer before sowing to decrease the RCs concentration in buckwheat plants.

(2) Soil ExRCs and ExSCs concentrations were positively correlated with the RCs and SCs contents of buckwheat, but negatively correlated with soil ExK content. These findings indicated that greater absorptions of RCs and SCs by buckwheat in soils with low ExK contents were because of higher soil ExRCs and ExSCs concentrations.
(3) Low soil ExK content promoted RcS and ScS translocation from root to shoot and from shoot to reproductive organs in buckwheat, indicating that K transporters responsible for Cs transport may be more highly expressed in plants growing in soils with low ExK contents.

Acknowledgments

We thank Mr. Kazutoshi Gondo (Kurume Research Park Co., Ms. Michie Mimori, and Ms. Yurie Yoshida for sample preparation; and Ms. Yuki Sato, Ms. Tomoko Saito, Ms. Yukari Watanabe, Dr. Tetsuya Eguchi for assistance with the soil ExK analyses; and Ms. Yoshihiro Yashima, a farmer in Date, Fukushima, for his cooperation during these experiments. We thank the following staff at the Agricultural Radiation Research Center, NARO Tohoku Agricultural Research Center: Dr. Hisaya Matsunami and Mr. Yoshihiko Takahashi for their assistance with 134Cs and 137Cs analyses; Dr. Tetsuya Eguchi for assistance with the soil ExK analyses; and Ms. Yoshihiro Yashima, a farmer in Date, Fukushima, for his cooperation during these experiments. We thank the following staff at the Agricultural Radiation Research Center, NARO Tohoku Agricultural Research Center: Dr. Hisaya Matsunami and Mr. Yoshihiko Takahashi for their assistance with 134Cs and 137Cs analyses; Dr. Tetsuya Eguchi for assistance with the soil ExK analyses; and Ms. Yuki Sato, Ms. Tomoko Saito, Ms. Yukari Watanabe, Ms. Michie Mimori, and Ms. Yurie Yoshida for sample preparation. We also thank Mr. Kazutoshi Gondo (Kurume Research Park Co., Ltd.) for his support in the ICP-MS analyses.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This study was partially supported by Grants-in-Aid for Scientific Research JSPS, Japan [grant number 15H02438], [grant number 15K11961]; the ‘Development of Decontamination Technologies for Radioactive Substances in Agricultural Land’ project from MAFF, Japan.

References

Baba, Y., & Goto, I. (2009). Rapid determination of cadmium in brown rice by heating block digestion systems and ICP-MS. Japanese Journal of Soil Science and Plant Nutrition, 80, 271–274.

Broadley, M. R., Willey, N. J., & Mead, A. (1999). A method to assess taxonomic variation in shoot caesium concentration among flowering plants. Environmental Pollution, 106, 341–349. doi:10.1016/S0269-7491(99)00105-0

Chiang, P. N., Wang, M. K., Huang, P. M., & Wang, J. J. (2011). Effects of low molecular weight organic acids on 137Cs release from contaminated soils. Applied Radiation and Isotopes, 69, 844–851. doi:10.1016/j.apradiso.2011.02.043

Ciuffo, L., Velasco, H., Belli, M., & Sansone, U. (2003). 137Cs soil-to-plant transfer for individual species in a semi-natural grassland. Influence of potassium soil content. Journal of Radiation Research, 44, 277–283. doi:10.1269/jrr.44.277

Endo, R., Kadokura, H., Tanaka, K., Ubuakata, S., Tsubura, H., & Ozaki, Y. (2013). Analysis of factors involved in absorption of radioactive cesium for processing tomatoes. Radioisotopes, 62, 275–280. doi:10.3769/radioisotopes.62.275

Fowler, E. B., & Christenson, C. W. (1959). Cesium-137 uptake by plants, factors affecting uptake of radioactive cesium by lettuce, grass, and alfalfa. Journal of Agricultural and Food Chemistry, 7, 847–849. doi:10.1021/jf60106a008

Fujimura, S., Yoshioka, K., Saito, T., Sato, M., Sato, M., Sakuma, Y., & Muramatsu, Y. (2013). Effects of applying potassium, zeolite and vermiculite on the radiocesium uptake by rice plants grown in paddy fieldsoils collected from Fukushima prefecture. Plant Production Science, 16, 166–170. doi:10.1626/pps.16.166

Fujimura, S., Ishikawa, J., Sakuma, Y., Saito, T., & Sato, M. (2014). Theoretical model of the effect of potassium on the uptake of radiocesium by rice. Journal of Environmental Radioactivity, 138, 122–131. doi:10.1016/j.jenvrad.2014.08.017

Gierzth, M., Mäsér, P., & Schroeder, J. I. (2005). The potassium transporter AtHAK5 functions in K+ deprivation-induced high-affinity K+ uptake and AKT1K+ channel contribution to K+ uptake kinetics in Arabidopsis roots. Plant Physiology, 137, 1105–1114. doi:10.1104/pp.104.057216

Giménez-Bastida, J. A., & Ziléliński, H. (2015). Buckwheat as a functional food and its effects on health. Journal of Agricultural and Food Chemistry, 63, 7896–7913. doi:10.1021/acs.jafc.5b02498

Godyn, P., Dolhańczuk-Śródka, A., Ziembik, Z., & Moliszewska, E. (2016). Influence of K on the transport of Cs-137 in soil–plant root and root-leaf systems in sugar beet. Journal of Radioanalytical and Nuclear Chemistry, 307, 325–331. doi:10.1007/s10967-015-4270-7

Grabov, A. (2007). Plant KT/KUP/HAK potassium transporters: Single family – multiple functions. Annals of Botany, 99, 1035–1041. doi:10.1093/aob/mcm066

Kamei-Ishikawa, N., Tagami, K., & Uchida, S. (2008). Estimation of 137Cs plant root uptake using naturally existing 133Cs. Journal of Nuclear Science and Technology, 45, 146–151. doi:10.1080/00223131.2008.10875997

Katar, D., Olgun, M., & Turan, M. (2016). Analysis of morphological and biochemical characteristics of buckwheat (Fagopyrum esculentum Moench) in comparison with cereals. CyTA – Journal of Food, 14, 176–185. doi:10.1080/19476337.2015.1076522

Kato, N., Kihou, N., Fujimura, S., Ikeba, M., Miyazaki, N., Saito, Y., … Itoh, S. (2015). Potassium fertilizer and other materials as countermeasures to reduce radiocesium levels in rice: Results of urgent experiments in 2011 responding to the Fukushima Daiichi Nuclear Power Plant accident. Soil Science and Plant Nutrition, 61, 179–190. doi:10.1080/00380768.2014.995584

Kim, E. J., Kwak, J. M., Uozumi, N., & Schroeder, J. I. (1998). AtKUP1: An Arabidopsis gene encoding high-affinity potassium transport activity. Plant Cell, 10, 51–62. doi:10.1105/tpc.10.1.51

Kondo, M., Makino, T., Eguchi, T., Goto, A., Nakano, H., Takai, T., … Kimura, T. (2015). Comparative analysis of the relationship between Cs and K in soil and plant parts toward control of Cs accumulation in rice. Soil Science and Plant Nutrition, 61, 144–151. doi:10.1080/00380768.2014.973348

Kraftczyk, I., Trolldenier, G., & Beringer, H. (1984). Soluble root exudates of maize: Influence of potassium supply and rhizosphere microorganisms. Soil Biology and Biochemistry, 16, 315–322. doi:10.1016/0038-0717(84)90025-7

Krugo, V. S., Suslina, L. G., Anisimov, V. S., & Aleksakhin, R. M. (2005). Mechanism of the effect of K+ and NH4+ ions on 137Cs accumulation by two-week-old barley plants from soddy-podzolic soils. Eurasian Journal of Soil Science, 38, 1082–1090.

Kubo, K., Nemoto, K., Kobayashi, H., Kuriyama, Y., Harada, H., Matsunami, H., … Shinano, T. (2015). Analyses and countermeasures for decreasing radioactive cesium in buckwheat in areas affected by the nuclear accident in 2011.
Factors affecting to increase radioactive cesium concentration in rice and its countermeasures (2nd ed.). Retrieved December 5, 2016, from http://www.maff.go.jp/j/kanbo/joho/saigai/pdf/h25kome.pdf***

MAFF. (2016). Summary of the test results on radioactive cesium concentration in agricultural products produced from April 2014 onward. Retrieved December 5, 2016, from http://www.maff.go.jp/j/kanbo/joho/saigai/pdf/h26/since_140401.html#H26hinmoku***

MHLW. (2012). Radioactive materials in foods – current situation and protective measures. Retrieved December 5, 2016, from http://www.mhlw.go.jp/english/topics/2011_08/dl/food-130926_1.pdf

Middleton, L. J., Handley, R., & Overstreet, R. (1960). Relative uptake and translocation of potassium and cesium in barley. *Plant Physiology*, 35, 913–918.

Nobori, T., Kobayashi, N. I., Tanoi, K., & Nakashima, T. M. (2014). Effects of potassium in reducing the radiosodium translocation to grain in rice. *Soil Science and Plant Nutrition*, 60, 772–781. doi:10.1007/s00380-014-9476-1

Qi, Z., Hampton, C. R., Shin, R., Barkla, B. J., White, P. J., & Schachtman, D. P. (2008). The high affinity K+ transporter ATHAKS plays a physiological role in plants at very low K+ concentrations and provides a caesium uptake pathway in *Arabidopsis*. *Journal of Experimental Botany*, 59, 595–607. doi:10.1093/jxb/erm330

Remy, E., Cabrito, T. R., Batista, R. A., Teixeira, M. C., Sá-Corrêa, I., & Duque, P. (2015). The major facilitator superfamily transporter ZIFL2 modulates cesium and potassium homeostasis in Arabidopsis. *Plant and Cell Physiology*, 56, 148–162. doi:10.1093/pcp/pcu157

Rubio, F., Santa-María, G. E., & Rodriguez-Navarro, A. (2000). Cloning of *Arabidopsis* and barley cDNAs encoding HAK potassium transporters in root and shoot cells. *Physiologia Plantarum*, 109, 34–43. doi:10.1034/j.1399-3054.2000.100106.x

Saito, T., Makino, H., & Tanaka, S. (2014). Geochemical and grain-size distribution of radioactive and stable cesium in Fukushima soils: Implications for their long-term behavior. *Journal of Environmental Radioactivity*, 138, 11–18. doi:10.1016/j.jenvrad.2014.07.025

Sanches, N., Anjos, R. M., & Mosquera, B. (2008). 40K/137Cs discrimination ratios to the aboveground organs of tropical plants. *Journal of Environmental Radioactivity*, 99, 1127–1135. doi:10.1016/j.jenvrad.2008.01.003

Sandeep, S., & Manjahia, K. M. (2008). Transfer factors of 134Cs to crops from Typic Haplustept under tropical region as influenced by potassium application. *Journal of Environmental Radioactivity*, 99, 349–358. doi:10.1016/j.jenvrad.2007.08.011

White, P. J., & Broadley, M. R. (2000). Mechanisms of cesium uptake by plants. *New Phytologist*, 147, 241–256. doi:10.1046/j.1469-8137.2000.00704.x

Wijngaard, H. H., & Arendt, E. K. (2006). Buckwheat. *Cereal Chemistry*, 83, 391–401. doi:10.1094/CC-83-0391

Yamaguchi, N., Takata, Y., Hayashi, K., Ishikawa, S., Kuramata, M., Eguchi, S., … Hiradate, S. (2012). Behavior of radiocaesium in soil-plant systems and its controlling factor: A review. *Bulletin of National Institute for Agro-Environmental Sciences*, 31, 75–129.

Yang, T., Zhang, S., Hu, Y., Wu, F., Hu, Q., Chen, G., … Xu, G. (2014). The role of a potassium transporter OsHAK5 in potassium acquisition and transport from roots to shoots in rice at low potassium supply levels. *Plant Physiology*, 166, 945–959. doi:10.1104/pp.114.246520

Yasunari, T. J., Stohl, A., Hayano, R. S., Burkhart, J. F., Eckhardt, S., & Yasunari, T. (2011). Cesium-137 deposition and contamination of Japanese soils due to the Fukushima nuclear accident. *Proceedings of the National Academy of Sciences*, 108, 19530–19534. doi:10.1073/pnas.112058108

Zhu, Y. G., Shaw, G., Nisbet, A. F., & Wilkins, B. T. (2002). Effect of external potassium supply and plant age on the uptake of radiocaesium by broad bean: interpretation of results from a large-scale hydroponic study. *Environmental and Experimental Botany*, 47, 173–187. doi:10.1016/S0098-8472(01)00124-1

*In Japanese with English summary or abstract.

**In Japanese with English title.

***In Japanese.