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Strontium behavior from soil to plants
(土壌から植物へのストロンチウムの動態)

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Chapter 1. General Introduction

1.1 Radio-cesium and radio-strontium

Radio-cesium (RCs) and radio-strontium (RSr) are common radionuclides produced by nuclear fission that pose serious health and environmental hazards. RCs and RSr were possibly released into the environment by the nuclear weapons testing around the 1950s and 1960s (Jekovský et al., 2020), and nuclear power accidents such as Chernobyl Nuclear Power Plant (CNPP) in 1986 and Fukushima Daichii Nuclear Power Plant (FDNPP) in 2011. Major radionuclides that have been known to contaminate the land after nuclear power plant accidents are RCs (^{134}Cs and ^{137}Cs , whose half-lives are 2.07 and 30.2 years, respectively) and RSr (^{90}Sr , whose half-life is 28.5 years) (David et al., 2001; Dubchak, 2017; Sharma, 2020).

Cesium (Cs) is an alkali metal with chemical properties related to potassium (K). ^{134}Cs and ^{137}Cs , are commonly found after nuclear power plant accidents. Both emit beta and gamma radiation. With a mean energy of 0.157 MeV, ^{134}Cs produces one beta particle per transformation (Chen et al., 2015). It also emits 2.23 gamma rays on average per transformation, with a mean energy of 0.698 MeV (Chen et al., 2015). However, the pollution from ^{134}Cs were no longer detectable due to the short half-life, especially for brown rice and cereals in the case of FDNPP accident (Fukushima Prefecture, 2023). ^{137}Cs undergoes β -decay (94.6% E_{max} 0.512 MeV β^-) to metastable barium ($^{137\text{m}}\text{Ba}$, $t_{1/2}$ = 2.6 min) which in turn decays to stable ^{137}Ba through gamma emission (0.662 MeV γ) and directly to stable barium (5.4% E_{max} 1.174 MeV β^-) (Lehto and Hou, 2010a; Mehmood, 2018).

Strontium (Sr) is an alkaline earth metal with chemical properties related to calcium (Ca). The substantial isotopes of ecological concern are ^{90}Sr (E_{max} 0.546 MeV β^-) and its daughter, Yttrium (^{90}Y). Yttrium has a half-life of about 60 h; however, its beta decay (E_{max} 2.274 MeV) intensifies the beta emission of ^{90}Sr (Lehto and Hou, 2010b; Mehmood, 2018). ^{90}Sr and its daughter, ^{90}Y are the high energy beta radiation sources (Vajda and Kim, 2010; Lehto and Hou, 2010b; Mehmood, 2018).

Through external and internal pathways, beta and gamma radiation emitted by RCs and RSr can have profound consequences for human health. According to IAEA (2004), beta radiation (β) consists of electrons emitted by unstable nuclei, smaller and more penetrating than alpha particles. It can be absorbed by materials like plastic or glass, typically not penetrating beyond the skin's top layer. High-energy beta emitters, however, can cause skin burns and pose

hazards if inhaled or ingested. Gamma radiation (γ), high-energy photons emitted from unstable nuclei, causes ionization as it passes through matter. It is highly penetrating, requiring dense materials such as steel or lead for effective shielding. Gamma radiation can deliver significant doses to internal organs without the need for inhalation or ingestion.

1.2 Transport of radio-caesium and radio-strontium from soil to crops

Agricultural concerns following RCs and RSr fallout from nuclear power plant accidents are significant and can have long-lasting impacts on the environment and food supply. Once they are released into the atmosphere, they can settle onto the soil through fallout. The radionuclides can bind to soil particles, leading to their accumulation in the topsoil. It is known that crops grown in soil contaminated with radionuclides can take up them through the roots and then transport them throughout their organs, posing a direct threat to consumers.

Sr is not highly mobile in most soils, and when deposited from the atmosphere onto the soil surface, it does not penetrate very deeply (Romney et al., 1960). The removal of the soil's surface will eliminate the majority of the RSr. Because Cs is irreversibly sorbed on frayed-edge sites (FESs) of illitic minerals, its mobility after deposition onto surface soil is quite low (Takeda, 2012). Therefore, soil management practices and remediation efforts may need to address.

After the FDNPP accident, till March 2012, RCs concentration levels over 500 Bq/kg (provisional regulation level in 2011 for general food) were identified in rice in the north and west of FDNPP. Nevertheless, after the introduction of remediation procedures were introduced almost no rice exceeded 100 Bq/kg (Fukushima Prefecture, 2023). In Ukraine, 34 years after the CNPP accident, grain samples from fields in the Ivankiv district (50 km from CNPP) contained ^{90}Sr at concentrations above permissible levels for human consumption. The combination of ^{90}Sr and ^{137}Cs concentrations was detected and exceeded in almost half of all the 116-grain samples (Labunska et al., 2021). In fact, despite being thousands of kilometers from the nearest bombsite, radioactive fallout still cycles through plants and animals more than fifty years after the bomb fell (Kaste et al., 2021). As a result of the nuclear accident, monitoring food and food-related research has received high priority.

Concerns have been raised about agricultural products being directly impacted by radioactive fallout following the nuclear power plant disaster. The Transfer Factor (TF) was introduced as a critical parameter in radiological impact assessment and environmental safety assessments, especially when evaluating the transfer of RCs and RSr from soil to crops. In the soil-to-plant TF, the radioactive concentration in a plant (usually measured in dry weight units,

but it is normally used the fresh weight in most of official reports from local governments) is compared to the radionuclide concentration in the soil (also usually measured in dry weight units) (IAEA, 1994). The TF were calculated on dry weight basis for crops using the following equation:

$$TF = \frac{\text{Radionuclide concentration in plants (Bq kg}^{-1} \text{ dry weight)}}{\text{Radionuclide concentration in soil (Bq kg}^{-1} \text{ dry weight)}}$$

Study using their stable form to simulate the transport of RCs and RSr from soil to plants has grown steadily. It is because of their identical chemical properties, biological characteristics, safety, environmental concerns, and ease of analysis (Moyen & Roblin, 2010; Kuke et al., 2016; Vacula et al., 2020; Yan et al., 2020). Consequently, stable ions chemically analogous to radionuclides can act as good suppressors via root uptake competition (Choi et al., 2008). However, stable isotopes pose challenges due to their fixed time scale. That is newly attached radioactive nuclides tend to be located outer part of soil particles. Unlike radioactive isotopes, their lack of dynamic decay affects behavior and migration, shaping mitigation strategies.

1.3 Potassium and calcium fertilizer in reducing radio-caesium and radio-strontium transport from soil to crops

K and Ca fertilizers are crucial in reducing the transport of RCs and RSr from soil to crops, respectively. The underlying chemistry is a key factor, as both RCs and RSr belong to the same group in the periodic table as K and Ca, respectively. This chemical similarity prompts plants to absorb RCs through K transporter and K channel, while RSr through Ca channel, even though these radioactive elements have no beneficial functions in plants. Root uptake serves as the primary pathway for RCs and RSr to enter crops from the soil. An elevated concentration of K or Ca in the soil intensifies the competition with RCs and RSr, hindering their uptake. Notably, applying K and Ca fertilizers has proven a highly effective countermeasure in reducing RCs and RSr transport (Romney et al., 1960; Myhre et al., 1964; Nisbet 1993; Shaw 1993; Zhu and Smolders 2000; Fujimura et al., 2013; Kato et al., 2015). Supplying plants with adequate K and Ca fertilizer encourages the absorption of these essential nutrients while diminishing the uptake of RCs and RSr, thereby enhancing food safety. IAEA (2020) has well documented that RCs are transferred to crops grown on soil that is treated with extra K. For example, transfer to brown rice, soybean, buckwheat, leafy vegetables, fruit vegetables, and root crops. On the other hand, ⁹⁰Sr concentration decreased 30% by Ca application in corn grain and soybean grain in Watkinsville, Georgia, US (Myhre et al., 1964). Moreover, the

combination of K and Ca application reduced the root uptake of both ^{137}Cs and ^{85}Sr by Chinese cabbage (Choi et al., 2008) and rice (Choi et al., 2012). However, there is information available about the effect of Ca application on Cs uptake (Kihana & Yamagami, 2022), but not much is known about the effect of K application on Sr uptake even though K is mentioned to inhibit Sr uptake by plants (Kabata-Pendias & Szteke, 2015). Therefore, the possible influence of K application on Sr needs to be explored. Nevertheless, other precautions may be necessary, particularly when it comes to different plant types, soil conditions, or even environmental conditions.

1.4 Interactions of cesium and strontium with other elements

It is essential to consider the interactions of Cs and Sr with other elements in plant-soil systems, particularly for food safety and environmental protection. Cs is an alkali metal, and Sr is an alkaline-earth metal. They share chemical similarities with K and Ca, essential nutrients for plant growth. Cs and Sr can enter plant root cells with K and Ca through the same or similar ion transport mechanisms (Maathuis & Sanders, 1997; Burger & Lichtscheidl, 2018; 2019). Also, K transporters show other elements' permeability. They are most likely engaged when K transporters are used to enter plants and their cells. However, it is known that the transport of Cs and Sr also becomes influenced by other elements (Burger & Lichtscheidl, 2018; 2019), especially elements in alkali and alkaline-earth metals. A study in *Amaranthus*, Chu et al. (2015) reported that significant positive correlation among As, Rb, Al, Fe, Ni, and Cs in shoot and among Ca, Mg, Mn, Zn, B, Ba, Cd, Sr in shoot. Menzel et al. (1954) reported that inverse proportions were observed between the uptake of Sr and Ba with exchangeable soil Ca, and Rb and Cs with exchangeable soil K. In *Acacia*, Mg, Sr, and Ba precipitated together with Ca, mainly in their phyllodes (He et al., 2012). Moreover, their precipitation was biologically induced under various soil types, plants, and tissues within each plant; precipitation also depended on the tissue age. Understanding the relationship among mineral elements can better pinpoint the mechanism that governs Cs and Sr absorption and distribution, which still needs to be clarified.

1.5 Research objectives

In this series of studies, my hypotheses and questions are: (1) what is the behavior of Sr transport from the soil to soybean plants after applying K fertilizer? (2) how is the transport behavior of Sr from different soil types to Komatsuna plants after K fertilizer is applied? (3) what is the transport behavior of Sr and Cs after K and Ca fertilizers are applied to soybean

plants? To answer the first question, in Chapter 2, the different levels of K fertilizer applied to soybeans grown in K-deficient soil. For the second question, in Chapter 3, the different levels of K fertilizer applied to Komatsuna plants grown in two different soil types. Finally, to answer the third question, in Chapter 4, K and Ca fertilizers are applied simultaneously on soybean plants, focusing on the transport of Cs and Sr behavior and their partitioning within plant organs. Furthermore, the transport behavior of Sr from soil to plants is explained in this study. The mechanisms of Sr and the interactions with other elements in plant-soil systems are discussed. This knowledge will provide valuable information for optimizing fertilization practices, enhancing food safety, and managing Sr and Cs contamination in agricultural systems. The impacts may extend to environmental and public health contexts, making the study relevant and significant.

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Chapter 2. Effect of Potassium Application on Cesium and Strontium Uptake of Soybean Plants

2.1. Introduction

The earthquake and subsequent tsunami that occurred at the Tokyo Electric Power Company's Fukushima Dai-ichi Nuclear Power Plant (FDNPP) in 2011 released several radionuclides including radioactive cesium (RCs) and radioactive strontium (RSr) to eastern Japan (Morino, Ohara, and Nishizawa 2011). RCs has relatively long half-lives (e.g., 30.2 years for ^{137}Cs and 2.06 years for ^{134}Cs) and high-energy emissions of β and γ radiation. ^{90}Sr is a β -ray emitting radionuclide with a high fission yield, relatively half-life (29 years), and high transferability to plants (Konno and Takagai 2018; Tsukada et al. 2005). RCs has been released in greater concentrations than RSr in the case of the FDNPP accident. However, the transfer of RSr from soil to plants also needs to be prevented to avoid what has happened in the previous Chernobyl and Mayak accidents (Chu et al. 2015).

After the FDNPP accident, the application of potassium (K) fertilizers was the most effective method and practical countermeasure for preventing agricultural crops from absorbing RCs in contaminated fields (Fujimura et al. 2014; Kato et al. 2015; Kubo et al. 2015; Matsunami et al. 2021). However, the result of this implementation may also influence strontium (Sr) behavior, even though it is less of an issue in the case of the FDNPP accident. Nuclear accident preparedness must include countermeasures, especially if RCs and RSr will be handled simultaneously. How K application for RCs mitigation alters RSr uptake should be evaluated. This may help support and validate the application of K to reduce the absorption of RCs and RSr in the event of a future incident.

The addition of K application to the soil may also change the soil's nutrient balance, which impacts on plant growth. Previous reports have shown that cesium (Cs) uptake can increase with increasing nitrogen (N) concentration and decreasing K concentration in plants (Evans and Dekker 1969; Belli et al. 1995). The uptake of elements such as Cs, Sr, K, and calcium (Ca) are positively correlated with each other (Chu et al. 2015) but their interaction with other elements are not well studied yet. Thus, a study of the role of these various nutrients in response to the effect of K application to reduce the rate of radionuclide transmission from soil to plants is necessary to ensure a harmonious balance between soil and plants. This nutrient balance is the key to increasing nutrient use efficiency to maintain soil fertility and plant productivity.

In this study, I conducted field experiments on soybean plants treated with K applications in Date City, Fukushima Prefecture, for two growing seasons (2019–2020). Despite studies that have found soil K and Ca affect Cs and Sr uptake in lettuce (Roca and Vallejo, 1995), their interrelationship has not been elucidated especially between K and Sr. The K applications have been shown to reduce Cs transport from soil to plants, but it has not been determined if K also reduces Sr transport. And if it occurs, K application for the remediation of agriculture after nuclear accidents can be used not only for radioactive Cs but also for Sr. The purpose of this study is to determine how K application affects the uptake of Cs and Sr simultaneously by soybean plants, one of Fukushima's most important agricultural products.

2.2. Material and methods

2.2.1. Experimental design

The experiment was conducted at Date City in Fukushima Prefecture, Japan in 2019 and 2020. The soil type is gray lowland soil, based on the classification of cultivated soils in Japan (Amano, 1985). Each field was fertilized before cultivation with the following nutrients: 20 kg N ha⁻¹ ((NH₄)₂SO₄) before the seedling stage, and 60 kg N ha⁻¹ after the flowering stages, and 100 kg P₂O₅ ha⁻¹ (Ca₃(PO₄)₂) with 1000 kg ha⁻¹ magnesium lime applied on the same day. The experimental treatments comprised three application levels of K: no K application, normal K application (increasing the level of exchangeable K (ExK) up to 25 mg K₂O 100 g⁻¹ and applying 100 kg K₂O ha⁻¹), and high K application (increasing the level of ExK up to 45 mg K₂O 100 g⁻¹ and applying 100 kg K₂O ha⁻¹). The amount of K (100 kg K₂O ha⁻¹) was applied as potassium sulfate (K₂SO₄, 50.0% K₂O). In 2019, 58.67 kg ha⁻¹ of K was applied to increase the level as normal K application and 244.89 kg ha⁻¹ of K was applied to increase the level as high K application. In 2020, 0 kg ha⁻¹ of K was applied to increase the level as a normal K application, and 295.11 kg ha⁻¹ to increase the level as a high K application. A randomized block design with three replications in each field was used. Soybeans (*Glycine max* (L.) Merr. var. Tachinagaha) were sown on May 27 and harvested on October 28, 2019; and sown on June 23 and harvested on October 26, 2020.

2.2.2. Sample collection

Plant and soil samples were collected from each field at each growth stage (vegetative, flowering, pod formation, maturity, and harvest). Plant samples were divided into the stems (including petiole), leaves, pods, and seeds. They were then dried at 80 °C for two days, weighed, and homogenized for subsequent elemental analysis.

Soil samples were collected from a depth of 15 cm around the plant roots using a worm scoop (Fujiwara Scientific Co., Ltd.). Soil samples were air-dried at 40°C for 1 week and then passed through a 2.0 mm sieve, for chemical analysis.

2.2.3. Mineral element measurements

The elemental composition of the plants and soil was determined as previously described (Watanabe et al. 2021). Dried plant samples (stems, leaves, pods, and seeds) were digested with 2 mL of 61% (w/v) HNO₃ at 110°C in a DigiPREP apparatus (SCP Science, Quebec, Canada) for 2 h until the solution had almost disappeared. After cooling, 0.5 mL of hydrogen peroxide (H₂O₂) was added, and the samples were incubated at 110°C for 20 min. Once digestion was complete, the tubes were cooled, and the volume was adjusted to 10 mL by adding 2% (w/v) HNO₃ to ultrapure water. The concentrations of K, Cs, Sr, Ca and Mg in the digests were analyzed using an inductively coupled plasma mass spectrometry (ICP-MS: ELAN DRC-e; PerkinElmer, Waltham, MA, USA). To measure N concentrations in the plants using the Kjeldahl method (Watanabe et al. 2015), the dried plant samples were digested with H₂SO₄ (98%)-H₂O₂.

For elemental analysis of the soil, ExK, exchangeable Cs (ExCs), exchangeable Sr (ExSr), exchangeable Ca (ExCa), and exchangeable Mg (ExMg) were extracted from dried soil samples at a soil:solution ratio of 1:10 in 1 M ammonium acetate (NH₄OAc) with shaking for 1 h. Soil ExK, ExCs, ExSr, ExCa, and ExMg concentrations were measured using ICP-MS. For the analysis of inorganic N, ammonium (NH₄⁺) and nitrate (NO₃⁻) in the soil, the dried soil samples (4 g) were shaken with 2 M KCl (40 mL) in 50 mL polycarbonate tubes on an end-to-end shaker (150 rpm, 60 min). The soil was filtered using ADVANTEC No.6 filter paper (ADVANTEC TOYO KAISHA, Tokyo, Japan), and the concentrations in the supernatant were determined by colorimetric assays at 630 and 538 nm with a microplate reader (BioTek EPOCH² Microplate Reader) for ammonium and nitrate, respectively.

For the measurement of ammonium, I used the Indophenol method according to Scheiner (1976), with minor modifications. The supernatant was prepared by adding 1 mL of the sample solution, 2 mL of distilled water, and 1 mL of a combination of A and B color-developing liquids (0.4 mL of A and 0.6 mL of B color-developing liquids). The A color-developing liquids are containing 15 g of phenol dissolved in 200 mL buffer solution and adjusted to 250 mL by adding 0.05 g of sodium nitroprusside. The B color-developing liquids are containing 40 mL of 1 M NaOH to 1 mL of sodium hypochlorite solution and adjust to 100 mL with distilled water. Before measuring the absorbance, the supernatant was well stirred and left for 60 minutes.

For the measurement of nitrate, I used the Ando and Ogata method (Ando and Ogata 1980). Prepare for 2.5 M ammonia solution by adjusting 16.7 mL of 28% special grade ammonia to 100 mL with 0.25 M KCl solution. Prepare for reduction auxiliary stock solution by dissolving 186.4 g of KCl in 0.8 L of distilled water, add 167 mL of special grade ammonia water (28%), then adjust to 1 L with distilled water. The first color former solution was prepared by adding 100 mL of distilled water and 100 mL of concentrated HCl to 500 mg of sulfanilamide to dissolve and then adjust to 1 L with distilled water. The second color former solution was prepared by dissolving 50 mg of N-1-Naphthylethylenediamine dihydrochloride ($C_{12}H_{16}Cl_2N_2$) with distilled water and adjusting to 1 L. The reduction auxiliary stock solution was diluted 10-fold and took 4 mL then placed in a test tube with adding 1 mL of sample solution, 0.75 g of metallic zinc, and plugged. The test tubes were immediately shaken for 15 minutes and took 2 mL of the supernatant into another test tube. The supernatant was left for 20 minutes and add 2 mL of each of the first and second color former solutions. The supernatant was well stirred and left for 10 minutes before measuring the absorbance.

2.2.4. Statistical analyses

Data were statistically analyzed using SPSS Statistics 25 (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) was used to evaluate the results at $p < 0.05$ probability level. Tukey's test was calculated only when the ANOVA F-test indicated significant treatment effects at the significant level ($p < 0.05$). Values are reported as mean $\pm$ SE of three replicates. Pearson's correlation analysis was conducted to evaluate the relationship between the mineral elements in the plant and soil. The figures were visualized using R Studio.

2.3. Results

2.3.1. Dry weight

The effect of K applications on the dry weight of the stems, leaves, pods, and seeds of soybean plants is shown in Figure 2.1. The dry weight did not differ in all growth stages during the two years with increasing K application, except for the stems and leaves at the vegetative stage, stems, and pods at harvest in 2019, and stems and leaves at maturity and stems at harvest in 2020. The dry weight was relatively lower without the application of K.

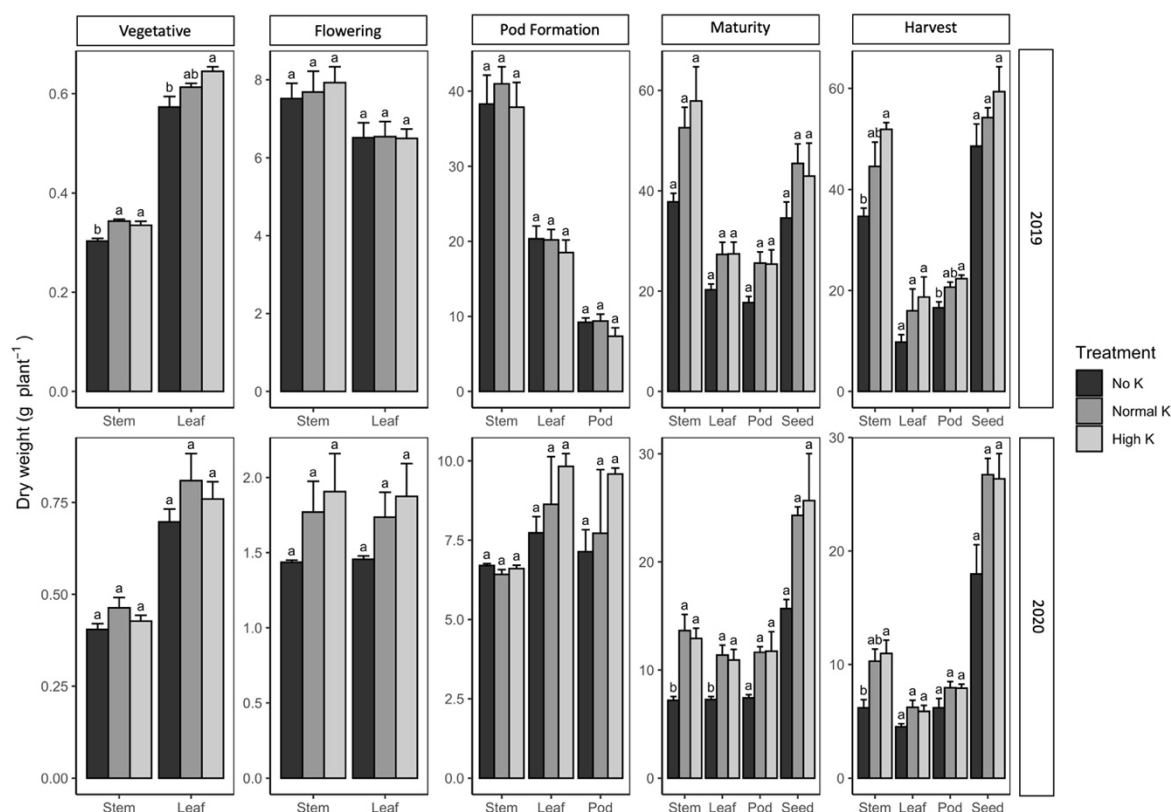


Figure 2.1. Effect of K applications on the dry weight of soybean plants at Date City in 2019 and 2020. The vertical bars indicate standard error of three replicates. Different letters (a, b, and ab) represent significant differences ($p < 0.05$) according to Tukey's test following a one-way ANOVA ($n = 3$).

2.3.2. Effect of K application on availability of mineral elements in plant

The concentrations of K, Cs, Sr, Ca, Mg, and N in various parts of the soybean plants in response to K applications are shown in Figures 2.2 and 2.3 for 2019 and 2020, respectively. The K concentration in all plant organs (stems, leaves, pods, and seeds) differed significantly among K applications in both years, except for pods and seeds at maturity, and stems, leaves, and seeds at harvest in 2019. K concentration in the plant organs was relatively higher in applications with higher K levels. For example, the K concentration in pods was ten times higher in the high K application than in the no K application at the harvest stage in 2020. However, the stable Cs in all plant parts differed significantly among K applications in both years, except for stems and leaves at vegetative and harvest stages in 2019. Increased K application has generally led to decreased stable Cs concentrations in the plant parts. For example, the stable Cs concentration of seeds at the harvest stage in 2020 was 46 times lower with high K application than without K application.

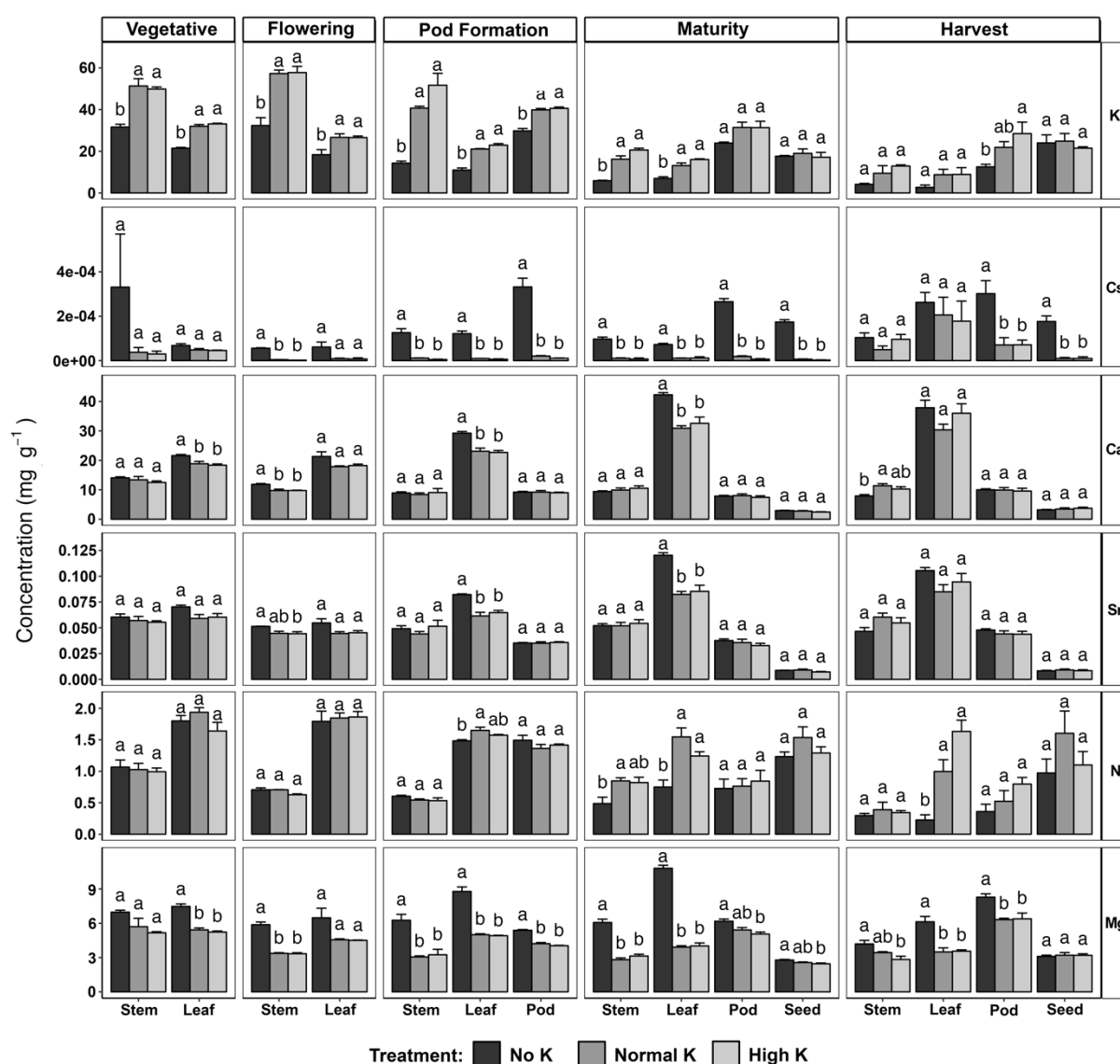


Figure 2.2. Effect of K applications on K, Cs, Sr, Ca, N and Mg concentrations in soybean plants at Date City in 2019. The vertical bars indicate the standard error of three replicates. The different letters (a, b, and ab) represent significant differences ($p < 0.05$) according to Tukey's test following a one-way ANOVA ($n = 3$).

Except for the leaves at the pod formation and maturity stages in 2019 and the flowering to harvest stages in 2020, the Sr concentration did not differ among K applications. Increased K application has generally led to decreased Sr concentrations in the leaves at pod formation and maturity stages in 2019, and flowering to harvest stages in 2020. For example, the largest decrease in the Sr concentration of leaves was observed in high-K applications at the maturity stage in 2020. However, in 2019, the Ca concentration decreased with increasing K application levels in stem at flowering stage and leaves at vegetative, pod formation, and harvest stages. In 2020, the Ca concentration decreased with increasing K application levels in stem at

flowering stage, leaves at flowering to maturity stages, and pod at harvest stage. The largest decrease in Ca concentration was observed in pods at the harvest stage in 2020. In contrast, the Ca concentration of the stems increased with increasing K application level at the harvest stage in 2019. Moreover, Mg concentration in all plant organs differed significantly among K applications in both years, except for stems at vegetative, leaves at flowering, and seeds at harvest in 2019, and pods at pod formation and seeds at maturity in 2020. Increased K application has generally led to decreased Mg concentrations in the plant parts. For example, the Mg concentration of leaves at the maturity stage in 2020 was 76 percent lower with high K application than without K application.

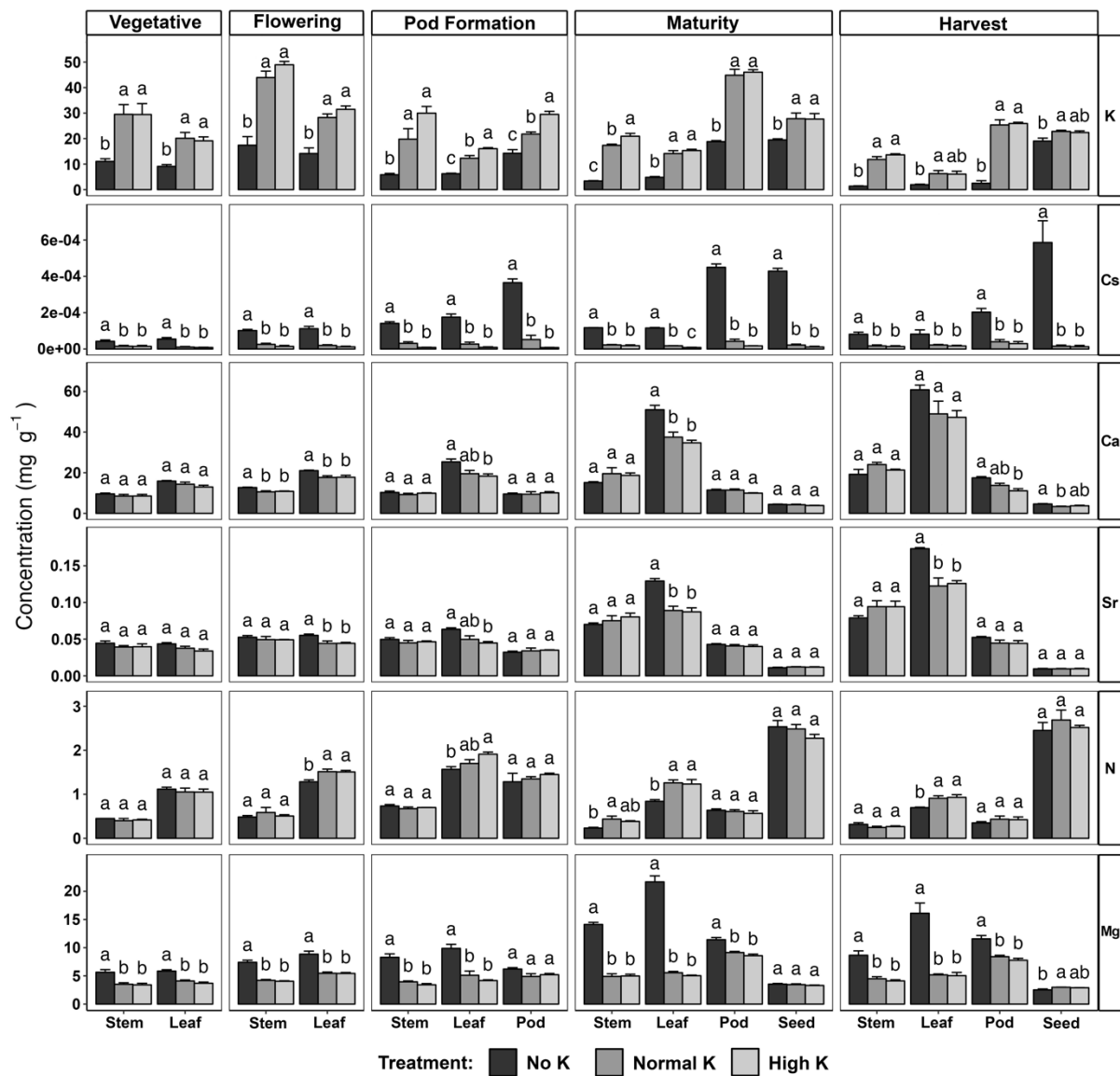


Figure 2.3. Effect of K applications on K, Cs, Sr, Ca, N and Mg concentrations in soybean plants at Date City in 2020. The vertical bars indicate the standard error of three replicates. The

different letters (a, b, ab, and c) represent significant differences ($p < 0.05$) according to Tukey's test following a one-way ANOVA ($n = 3$).

The N concentration of stems and leaves generally increased with increasing the K application levels from pod formation to harvest stages in 2019 and flowering to harvest stages in 2020. The largest differences in N concentration of soybean plants were between the high K application and the no K applications. For example, at the harvest stage in 2019, the leaf N concentration was seven times higher with the high K application than with no K application. Conversely, the differences were smaller at early stages in both years, such as during vegetative growth and flowering in 2019 and vegetative growth in 2020.

2.3.3. Effect of K application on mineral elements availability in soil

The ExK, ExCs, ExSr, ExCa, ExMg, ammonium, and nitrate concentrations of the soil in response to K applications are shown in Figure 2.4. The ExK concentration in the soil differed significantly among K applications in both years, except for the harvest stage in 2020. The ExK concentrations in the soil was relatively higher in applications with higher K levels. For example, ExK concentrations at the pod formation stage in 2019 were four times higher with high K application than without K application.

However, the ExCs concentrations at all stages differed significantly among K applications in both years. Moreover, increased K application has generally led to decreased ExCs concentrations in the soil. For example, the highest reduction in ExCs concentrations in the soil was at the flowering stage in 2020, which decreased six -fold with high K application. However, the ExSr, ExCa and ExMg concentrations in all stages did not differ among the K applications at any growth stages in either year, except at vegetative and harvest in 2020 of ExMg concentrations.

The ammonium concentration in the soil generally decreased with increasing the K application levels at the harvest stage in 2019 and from flowering to harvest stages in 2020. The largest decrease in ammonium concentration in the soil was observed in the harvest stage with high K application in 2019. On the other hand, the difference was small during the vegetative to maturity stages in 2019 and the vegetative stage in 2020. However, the nitrate concentration in the soil was reduced by increasing the K application from pod formation to harvest in 2019 and from flowering to harvest in 2020. A large decrease in nitrate concentration in the soil was observed during the pod formation stage in 2019 with high K application. The reduction in nitrate in the soil was two times higher with K application than without K application at the pod formation stage in 2019. In contrast, the difference in nitrate

concentration was relatively small during the vegetative and flowering stages in 2019 and the vegetative stage in 2020. Moreover, the nitrate concentration in 2020 increases from flowering to pod formation.

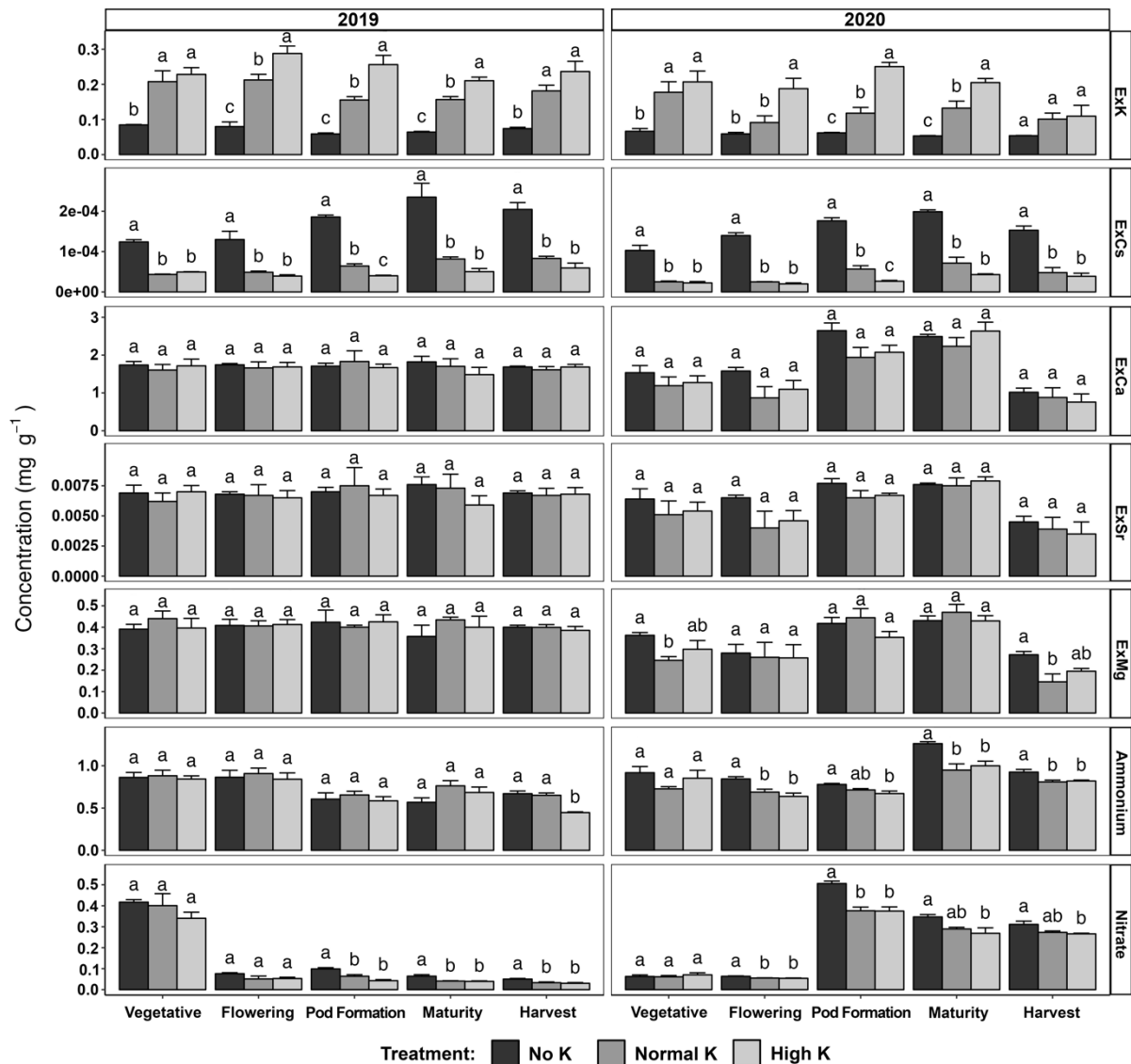


Figure 2.4. Effect of K applications on ExK, ExCs, ExSr, ExCa, ExMg, ammonium and nitrate concentrations in the soil at Date City in 2019 and 2020. The vertical bars indicate the standard error of three replicates. Different letters (a, b, c, and ab) represent significant differences ($p < 0.05$) according to Tukey's test following a one-way ANOVA ($n = 3$).

2.3.4. Correlations among mineral elements in plant and soil

The Pearson correlation analysis was performed to establish the correlation between the concentration of each pair of elements in plants (K-Cs, K-Sr, K-Mg, Sr-Ca, Sr-N, and Sr-Mg) and soil (ExK-ExCs, ExK-ExSr, ExK-ExMg, ExSr-ExCa, ExSr-ExMg, ExSr-ammonium, and ExSr-nitrate) under K application (Table 2.1). The Pearson correlation analysis of mineral

elements in plants showed that K concentration was significantly negatively correlated with Cs and Sr concentrations in both years, and but only with Mg concentrations in 2020 (Appendix 1). The Sr concentration was significantly positively correlated with Ca and Mg concentration in both years and significantly inversely correlated with N concentration in 2020 (Appendix 2). In order to further elucidate the relationship between K and Sr, I explored its relationship in growth stages and plant organs. The K concentration was significantly negatively correlated with Sr concentration in all growth stages except vegetative and in all plant organs except seed (Figure 2.5).

Table 2.1. Tabulated summary of Pearson correlation analysis among elements in plant and soil.

Parameters	2019		2020	
	Pearson's <i>r</i>	<i>p</i> -value	Pearson's <i>r</i>	<i>p</i> -value
K vs Cs (plant)	−0.29	0.05*	−0.22	0.05*
K vs Sr (Plant)	−0.28	0.05*	−0.49	0.05*
K vs Mg (plant)	−0.17	0.05	−0.39	0.05*
Sr vs Ca (plant)	0.92	0.05*	0.93	0.05*
Sr vs N (plant)	0	0.95	−0.47	0.05*
Sr vs Mg (plant)	0.5	0.05*	0.52	0.05*
ExK vs ExCs (soil)	−0.83	0.05*	−0.7	0.05*
ExK vs ExSr (soil)	−0.13	0.4	0.16	0.3
ExK vs ExMg (soil)	−0.07	0.7	0.17	0.3
ExSr vs ExCa (soil)	0.92	0.05*	0.93	0.05*
ExSr vs ExMg (soil)	0.88	0.05*	0.96	0.05*
ExSr vs ammonium (soil)	−0.11	0.5	0.4	0.05*
ExSr vs nitrate (soil)	−0.04	0.8	0.34	0.05*

Significant modules are denoted with * ($p < 0.05$).

The Pearson correlation analysis of mineral elements in soil showed that the ExK concentration in the soil was significantly negatively correlated with ExCs concentrations in the soil in both years but was not likely to be correlated with ExSr and ExMg concentrations in the soil (Appendix 3). The ExSr concentration in the soil was significantly positively correlated with the ExCa and ExMg concentrations in the soil in both years (Appendix 4). The ExSr concentration in the soil was unlikely to be correlated with ammonium and nitrate concentrations in the soil in 2019 but significantly in 2020 (Appendix 4).

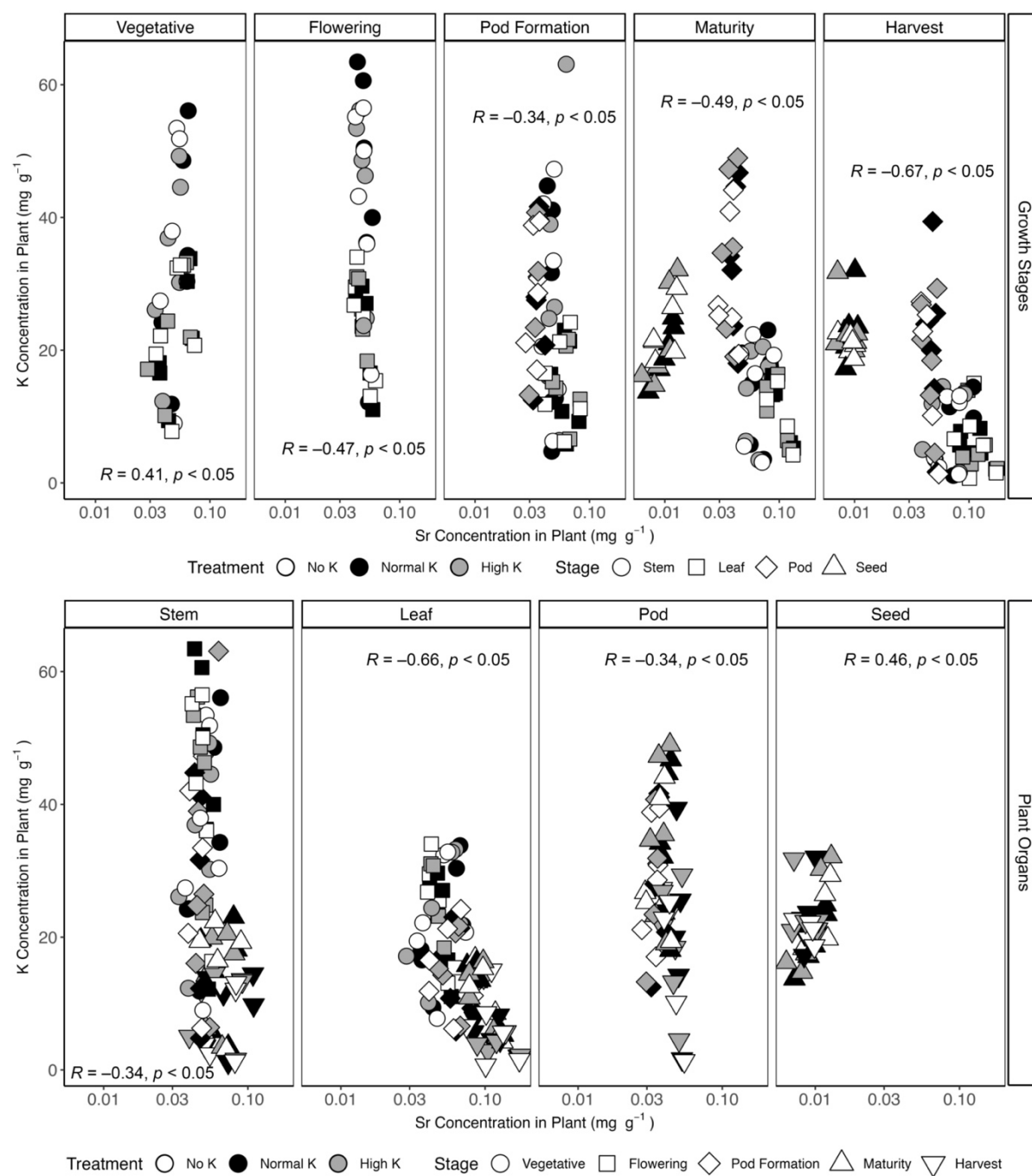


Figure 2.5. Pearson's correlation between K and Sr concentration in soybean plants after K applications at Date City in the different growth stages and plant organs.

2.4. Discussion

Since the nuclear incident in the FDNPP, the practice of applying fertilizer in cultivation activities has been recommended as a preventive measure to reduce RCs transmission to the edible parts of plants. The application of K in cultivation has successfully reduced the rate of RCs translocation (e.g., Kato et al. 2015). However, there is no explanation for how this

countermeasure relates to Sr. It is necessary to consider the simultaneous release of RCs and RSr into the environment if another nuclear accident occur. This implementation may also affect the nutritional balance and the state of other elements such as K, Cs, Sr, Ca, Mg and N, and the interrelationships of these elements are poorly understood.

2.4.1. Effect of K application on K, Cs, Sr, Ca, Mg, and N concentration in plant

Except for the pods and seeds at the mature stage and stems, leaves, and seeds at the harvest stage in 2019, the K concentration in all plant parts differed significantly among K application in both years (Figures 2.2 and 2.3). The largest difference in K concentration in soybean plants was in the pods at the harvest stage in 2020, when high K application was ten times higher than no K application. A higher K concentration in plant organs can be attributed to a higher K concentration in the soil, although the dry weight does not generally vary among the K treatments (Figure 2.1), as confirmed by previous studies on buckwheat (Kubo et al. 2017) and soybean (Matsunami et al. 2021). However, increased K application has generally led to a decrease in stable Cs concentrations (Figures 2.2 and 2.3) in all plant organs. The largest decrease in stable Cs concentration was observed in the seeds at the harvest stage in 2020 with high K application. A similar tendency was also observed in the analysis of RCs uptake behavior using the same field (Suzuki et al., 2023), which indicate that a similar behavior of RCs and Cs was observed nine years after the accident. These results are consistent with other findings that K application effectively reduces RCs (Kato et al. 2015) and stabilizes Cs (Tsukada et al. 2002) in rice. K application might be inhibiting the translocation of Cs from soil to plant regardless of radioactive or stable status due to competition between K and Cs at the root absorption site. Plant root cell membranes appear to be mainly involved in Cs uptake via two transport pathways, namely the K transporter and the K channel pathway (Zhu and Smolders 2000). Cs and K have similar chemical properties and compete for the binding sites in proteins (Avery 1995). According to (Fujimura et al. 2014), adequate external K levels inhibit the expression of K transporters with a high affinity for Cs.

The increased K application led to decreased Sr concentration in leaves, Ca concentration in the leaves and stems (Figures 2.2 and 2.3) as confirmed by previous studies (Neilsen and Edwards 1982; Melnitchoukh and Hodson 2004; Tuma et al. 2004). Additionally, Mg concentrations in plant parts were reduced relatively by increased K application (Figures 2.2 and 2.3) as confirmed by previous studies (Lanyon, Heald, and Page 1983; Kang, Seo, and Ishii 2019). Our results showed that with a high K application, the leaves at maturity stages in 2020 displayed the largest drop in Sr and Mg concentrations. However, the largest decrease in

Ca concentration appeared with high K application in the pods at the harvest stage in 2020. Sr and Ca or Mg are chemically similar and compete for the same receptor sites on biological membranes, but Sr cannot replace Ca or Mg in plants. More than 50% of Sr is absorbed by plants and accumulates in cell walls, similar to Ca (Anupama, Ashok, and Naveena 2016; Gupta et al. 2018; Qiu et al. 2021). Ca is relatively immobile in plant cells and is not readily circulated to tissue parts despite the high Ca applied (Knez and Stangoulis 2021). Ca is involved in the response to external stimuli in major cellular processes, but the response depends on the movement of K across membranes (Sardans and Penuelas 2021) while Sr is transported from the roots via a K plasma membrane transporter (Burger and Lichtscheidl 2019). Moreover, the presence of high Ca concentrations can also lead to Mg deficiency (Hansen and Munns, 1988; Plaut and Grieve, 1988). In case of Mg, the physiological role of this divalent cation is different from Ca. There are many fundamental functions of Ca in cellular metabolism, while Mg plays a significant role in chlorophyll synthesis, ion transport, and cation balance regulation, in addition to activating more than 300 enzymes (He et al., 2012). As in this study, the decreased Ca concentrations were similar to the decreases in Sr concentrations due to the K application. Sr and Ca concentrations and also Sr and Mg concentrations had a similar pattern in leaves, i.e., decreasing with increasing K application at the pod formation and maturity in 2019, and at the flowering and maturity stage in 2020 (Figures 2.2 and 2.3). Sr may use the same cell entry mechanisms as Ca and Mg, such as plasma membrane transporters, due to its physicochemical similarities (Burger and Lichtscheidl, 2019). Ca can be replaced by Sr, thereby lowering Mg and Ca content in plants (Burger and Lichtscheidl, 2019; Moyen and Roblin, 2010). Increasing K in soil solution might influence the presence of other ions (including K, Ca, Mg and Sr) which is played a role in the adsorption and release of Sr and RSR due to competition for exchange sites, thus influencing Sr uptake (Burger and Lichtscheidl 2019). Also, the involvement of other elements, such as Sr, interferes with Ca uptake (Rato et al. 2010) and Mg uptake (Moyen and Roblin, 2010). The decreased Sr and Ca levels in the leaves in this study as confirmed by previous reports (Moyen and Roblin 2010; Chen et al. 2012; Zhang et al. 2020) might be due to the indirect involvement of Mg after K was applied (Trankner et al. 2018; Ding et al. 2016). While these findings suggest that K application in the soils suppresses Sr transport to the leaves but does not affect the soybean's dry weight it might be due to ExMg concentration in the soils (Figure 2.4) affected to Mg uptake then dry weights as confirmed by a previous study (Hailes et al. 1997). Mg is instrumental in forming dry matter and partitioning carbon to sink organs since carbohydrate accumulation in source leaves is reduced by Mg deficiency (Gransee and Fuhrs 2013) as confirmed by this study (Figures 2.2

and 2.3). There is a similar pattern between Sr and Mg concentrations in leaves that decreased with increasing K. Despite its small amount in this study, Sr is not detrimental to plant growth, and its uptake is a side effect of divalent cation absorption. Nevertheless, the K level in this study was adequate for seed production.

2.4.2. Effect of K application on ExK, ExCs, ExSr, ExCa, ExMg, ammonium, and nitrate concentration in soil

The ExK concentrations in the soil were relatively higher in application with higher K levels in both years, except for the harvest stage in 2020 (Figure 2.4). This is expected owing to the application of K in this study. However, there is no difference between normal and high K application to ExK concentrations in the soil at beginning (vegetative) and at the end of growth stages (harvest) in both years. It might be due to the characteristics of K uptake which is slowed in the vegetative stage but increases rapidly during flowering maturity stages (Halevy, 1976; Mullins and Burmester, 1990). In addition, it could be caused by soil properties, even though they were excluded from this study. Uzoho and Ekeh (2014) reported that soil K status affects by sand, silt, clay, silt/clay ratio, organic matter, pH, total N, P, Ca, Mg, cation exchange capacity, base saturation, sodium (Na), and hydrogen (H).

K application generally led to decreased ExCs concentrations in the soil in both years (Figure 2.4). Increasing ExK in the soil tends to decrease ExCs, which has also been observed in various crops (Kato et al. 2015; Kubo et al. 2015, 2017, 2018; Matsunami et al. 2021). ExK concentrations in the soil increased following K fertilizer application and suppressed RCs uptake during the growing season (Komatsu et al. 2017), which is also expected for stable Cs. Generally, Cs ions exchange with K ions at frayed edge sites (Okumura, Nakamura, and Machida 2013). The reduction in ExCs might be due to formation of the frayed edge sites on the clay minerals after K applied (Kubo et al. 2017). In contrast, the ExSr and ExCa, and ExMg concentrations in the soil (Figure 2.4) did not differ among K applications in either year, except at vegetative and harvest in 2020 of ExMg concentrations. K, Ca and Mg (which have similar characteristics to Sr) share the same soil particle binding site (Bonomelli, Gil, and Schaffer 2019). Consequently, excess or deficiency of K can affect the availability of the other cations in the soil, including Sr and Ca or Sr and Mg. Hovland and Caldwell (1960) reported that adding K to the soil system increases loss of Mg as K out-competes Mg for exchangeable sites on plant roots. Tuma et al. (2004) revealed that K and Ca could not be handled separately from other nutrients because antagonistic relationships arise. Though I have found that Sr uptake was decreased in the shoot of soybean by the application of K, I did not analyze the root, so

the possibility of root-to-shoot transfer was decreased by K application is remained. In order to determine whether K application has caused Sr uptake and/or distribution among plants, a confirmation test should be conducted.

The ammonium and nitrate concentrations in the soil (Figure 2.4) generally decreased with increasing K application, as confirmed by previous reports (Beauchamp 1982; Nguyen et al. 2001; Ajazi et al. 2013). Increased K application might result in saturation in the interlayer space in the soil, which decreases ammonium fixation or impairs ammonium release (Scherer 1982; Nieder, Benbi, and Scherer 2011) and decreases nitrate concentrations in the soil. If plants do not absorb nitrate in the soil solution, it can easily leach because nitrate and soil are negatively charged (Ito 2018). Moreover, nitrate levels in the soil increased from flowering to pod formation in 2020, might be due to the additional application of N fertilizer in this study that applied after flowering stages then influences nitrate concentration in the soil.

2.4.3. Relationship of Sr with K with the reduction of Cs concentration

Correlation analysis showed that the K concentration in the plant organs was significantly negatively correlated with stable Cs and Sr concentrations in the plant organs in both years, and but only with Mg concentrations in 2020 (Appendix 1). However, the effect of K application on Sr concentration was not observed unlike Cs or Mg (Figures 2.2 and 2.3). These results reveal that K application reduces the transfer of Cs and Mg from soil to plant organs. However, the different mechanisms should be considered in the case of the relationship between K Sr. Though it was also indicated that higher K concentrations in the plant organs were accompanied by lower Cs, Sr, and Mg concentrations in the plant organs after K application, as confirmed by previous studies (Kabata-Pendias and Szteke 2015; Myrvang et al. 2016), I have further analyzed the relationship between K and Sr relationship divided by the growth stages and plant organs (Figure 2.5). The relationship between K and Sr clearly changed with growth stages, and it was demonstrated that during the early growth stages, the Sr concentration was rather stable regardless of the K concentration level in the plant while there was a clear negative relationship was observed during the maturity and harvest stages. This is indicating that the K and Sr movement in the plant is different. Plants can rapidly transfer K throughout their entire organ system, but Ca becomes largely immobile and re-translocation is low, and Sr behaves similarly to Ca (Creger and Allen 1969; Mengel 1985). It is further confirmed by the differences between plant organs (Figure 2.5). Significant negative correlations were observed except for seeds, and the relationship was most obvious in the leaf, and it was also confirmed that the K and Sr decreased with the growth. Since our results only

showed a reduction in Sr concentration in the leaves while reductions in Mg concentrations relatively occurred in all plant organs after K application (Figures 2.2 and 2.3), also the Sr concentration was significantly positively correlated with Ca and Mg concentration in both years (Appendix 2), it was estimated that the absorption of Sr was not disturbed by the K application while the re-translocation of Sr was disturbed, while it is further requested to investigate how Sr was stored and distributed with the change of K levels in the organs. In the absorption of Sr into plants and their cells, no specific transporters for Sr have been identified; instead, it has been mentioned that Sr utilizes Ca and K channels (Burger and Lichtscheidl, 2018; 2019), potentially altering Sr translocation.

Moreover, the ExK concentration was significantly negatively correlated with the ExCs concentration in both years (Appendix 3). The ExK concentration in the soil was unlikely to be correlated with the ExSr concentration in the soil and ExMg concentrations in the soil (Appendix 3). It can be explained that increasing the ExK concentration in the soil suppressed ExCs and not ExSr, ExCa and ExMg concentrations in the soil. Previously, Roca and Vallejo (1995) reported on the effects of soil K and Ca on RCs and RSr transfer to lettuce plants, but the relationship of K to Sr has not yet been clarified. Our results revealed that K application directly decreased Cs uptake, and indirectly decreased Sr and Mg uptake by the soybean plants. During root absorption, it may be not only the involvement and competition between K and Cs that influence Cs uptake, but there may also be the effect of related elements (such as Mg) on Sr translocation from soil to plants. The large amounts of K competitively inhibited Mg uptake and resulting in reduced protein synthesis (Guo et al., 2016). An indirect relationship between K suppress the uptake rate of Sr needs further investigation. It is particularly important to examine the mineral balance of the related elements that might support the application of K for reducing RCs and RSr translocation from soil to plants in a preparedness scenario for nuclear accidents.

There was significantly positively correlation between Sr with Ca and Sr with Mg concentration found in this study, either in plant or soil. However, the role of K in the soil-plant interaction between Sr and Ca is not yet clear. The results of this recent study illustrate that (1) K application did not affect Sr transport, but it might affect re-translocation of Sr; (2) the link between K and Sr clearly changed with different growth phases, and it was shown that throughout the early growth stages, Sr concentration was largely steady regardless of the quantity of K in the plant; (3) it is likely that K interacts with both Sr and Ca or Mg when entering the plant's cells, since K and Ca or Mg share channels during transport to plant organs with Sr (Burger and Lichtscheidl 2019); or (4) during the reduction of Cs uptake using K

application to the soil, these cations (Ca, Mg and Sr) compete through competition in the apoplast of the root cortex (Smolders et al. 1997). Sr concentration was small and there was no actual reduction was observed by K application, which indicate that K application does not support the idea to reduces R_{Sr}. However, as the re-translocation of Sr was reduced when K concentration is low, it is important to investigate whether this mechanism could occur in other species and/or in the combination of other minerals in the future.

2.5. Conclusions

Our results indicate that K application to soybean plants influenced the variables in this study. K application generally increased the K and N concentrations and decreased the Cs, Ca, and Mg concentrations in plant organs but did not affect dry weight. In addition, increased K application typically leads to decreased Sr concentrations in the leaves. K application increased ExK and decreased ExCs, ammonium, and nitrate concentrations in the soil but did not affect ExSr, ExCa, and ExMg. These findings imply that increasing ExK concentration in the soil suppressed only ExCs and not ExSr concentration, but it might have affected Sr re-translocation in the plant tissues. Though I have hypothesized that Sr uptake was also reduced by the application of K fertilizer, however the results did not support the idea, on the other hand, re-translocation of Sr in the plant was negatively affected by K concentration. Research on the impact of the exclusive use of K fertilizer on Sr translocation and the involvement of other related mineral elements is required.

2.6. References

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Chapter 3. Effect of Potassium Application on Cesium and Strontium Transport of Komatsuna Plant Grown in Two Different Soil Types

3.1 Introduction

After the nuclear power plant accidents, some radionuclides became a common problem, i.e., RCs and RSr in the Chernobyl Nuclear Power Plant (CNPP) in 1986, and ^{134}Cs and ^{137}Cs in the Tokyo Electrical Power Company's Fukushima Dai-ichi Nuclear Power Plant (FDNPP) in 2011 (McLaughlin et al., 2012). ^{134}Cs and ^{137}Cs , whose half-lives are 2.07 and 30.2 years, respectively, while ^{90}Sr has a radioactive decay half-life of 28.5 years (David et al., 2001; Dubchak, 2017; Sharma, 2020). They fall out and then contaminate the environment, including agricultural fields. In the context of food security, their translocation from soil to edible parts of crops is one of the most critical factors in ensuring human health.

Various countermeasures were performed to combat the transport of radionuclides from contaminated farmland to plants. In the case of the FDNPP accident, several studies report that using potassium (K) fertilizer effectively reduced ^{134}Cs and ^{137}Cs in rice, soybean, buckwheat, peanut (Kato et al., 2015; Kubo et al., 2015, 2017, 2021), and ^{137}Cs in soybean (Matsunami et al., 2021) and some vegetables (Shinano et al., 2023). The importance of K fertilizer was also reported after the CNPP accident. For example, Rosén (1996) reported that the K-fertilization seems to have decreased the RCs transfer from soil to grass. In addition, fertilizer application is a soil-based method for suppressing their transport to crops in the case of RSr contamination in agricultural fields. It has been well-studied in previous reports that calcium (Ca) application can be used to counteract RSr transport from soil to plants (Fowler and Christenson, 1959; Al-Oudat et al., 2006, 2021; Wang et al., 2017).

Despite extensive studies of each radioactive ion transport from soil to plants, it has yet to be extensively studied for treating RCs and RSr, which are often released simultaneously after nuclear incidents. The implementation of a countermeasure to address one specific radionuclide has demonstrated effectiveness, yet it raises concerns about potential impacts on other elemental minerals. It would have adverse effects by increasing the availability of other radionuclides. Our previous report found that despite the K application reducing Cs uptake by suppressing exchangeable Cs (ExCs) and K application also alters Sr translocation in soybean but not exchangeable Sr (ExSr) concentration in the soil (Chapter 2 in this study). Therefore,

other factors may be involved in Sr translocation within the plant that is not explained in the previous chapter.

K application might change Sr re-translocation in various plant organs as reported in the previous chapter. Considering the phenomenon that occurs in soybeans, where seeds are the main harvesting organ, this research needs to be extended to Komatsuna (*Brassica rapa* L. var. *perviridis*), a leafy vegetable, because of its different harvesting characteristics. Notably, the edible part of Komatsuna consists of the entire shoot, very different from the seed-centric structure of soybeans. Therefore, it is necessary to investigate how RSr behaves in Komatsuna to ensure food safety. In this study, I used stable instead of radioactive forms, given that stable Sr is considered to possess chemical and biological characteristics identical to RSr (Zhang et al., 2020). This study will facilitate us to deeply understand the effect of K fertilizer on the behavior of Cs and Sr as well as in the soil-plant system and further explain the factors that may affect the transport of Cs and Sr from soil to plants and translocation within the plant after K fertilizer applied.

3.2. Material and methods

3.2.1. Experimental materials and treatments

The pot experiments were conducted in a greenhouse at the Research Faculty of Agriculture, Hokkaido University (43.414°N, 141.2024°E), Japan, from September 11th to October 13th, 2022. The temperature was kept at 25°C with the light period in 16 h and the dark period in 8 h. I used two different types of soil in this study. The first soil (Hokudai soil) was obtained from the K deficient (NP fertilized) plot of Hokkaido University's long-term experimental fields, developed in 1914. The second soil (Ryozen soil) was collected from the K deficient (NP fertilized) plot in Date City, Fukushima Prefecture, Japan. The soil type is brown lowland (Hokudai soil) and gray lowland soil (Ryozen soil), based on Japan's classification of cultivated soils (Amano, 1985). Soil physicochemical properties are shown in Table 3.1. Air-dried soil was passed through a 5-mm sieve, filling each pot with 3.3 kg of soil. The soil was fertilized on the day before cultivation as follows: 150 kg N ha⁻¹ NH₄Cl, 100 kg P₂O₅ ha⁻¹ (CaHPO₄), and 1000 kg ha⁻¹ magnesium lime applied one day before basal fertilizer application. For K fertilization was applied as potassium sulfate (K₂SO₄, 50.0% K₂O) in different level treatments, i.e., no K application, normal K application (100 kg K₂O ha⁻¹), and high K application (200 kg K₂O ha⁻¹). Five Komatsuna seeds were sown in a pot, and plants were thinned to three seed (14 days after sowing). Each treatment was replicated three times with a total of 24 pots. The pots

were distributed in a randomized design. All pots were equally irrigated with water over the whole experiment (50-150 mL of tap water), depending on the growth.

3.2.2. Sample collection

Komatsuna plants were harvested at four weeks after sowing. The plant and soil samples were collected from each pot. Plant samples were harvested (including leaves and stems, except roots). When collecting samples, shoot part was carefully taken from the pots, washed with distilled water, and the shoot fresh weight was measured. Then dried at 80 °C for two days and homogenized for subsequent elemental analysis.

Three hundred gram of soil samples were taken from each pot using a worm scoop. Soil samples were air-dried at 40 °C for one week and then passed through a 2.0 mm sieve for chemical analysis.

3.2.3. Sample analysis

Plant and soil element concentrations were determined in the same way as in Chapter 2. Plant element concentrations in the digests were analyzed by using an inductively coupled plasma mass spectrometry (ICP-MS: ELAN DRC-e; PerkinElmer, Waltham, MA, USA). Plant element concentrations in this study include K, Cs, Ca, Sr, Mg, Li (lithium), Na (sodium), Rb (rubidium), Fe (iron), Mn (manganese), Zn (zinc), Cu (copper), Al (aluminum), Co (cobalt), Ni (Nickel), Cd (cadmium), and As (Arsenic). For soil elements analysis, elements were extracted from dried soil samples at a soil:solution ratio of 1:10 in 1 M ammonium acetate (NH₄OAc) with shaking for 1 h at 150 rpm. Then the concentrations were measured using ICP-MS. Soil element analysis in this study designated as ExK, ExCs, ExCa, ExSr, ExMg, exchangeable Li (ExLi), exchangeable Na (ExNa), exchangeable Rb (ExRb), exchangeable Fe (ExFe), exchangeable Mn (ExMn), exchangeable Zn (ExZn), exchangeable Cu (ExCu), exchangeable Al (ExAl), exchangeable Co (ExCo), exchangeable Ni (ExNi), exchangeable Cd (ExCd), and exchangeable As (ExAs).

Soil pH was measured by mixing air-dried soil with deionized water (soil:solution ratio, 1:5), then stirred with a shaker machine for 1 h, left for 1 h, and eventually measured with a pH meter (Watanabe et al., 2015). Humus content was determined by Kumata method (Kato, 2012). Using a microplate reader (BioTek EPOCH² Microplate Reader, USA), two grams of soil was colorimetrically by 20 mL of 0.05 M Na₄P₂O₇ / 0.25 M NaOH and determined at 530 nm. Particle size distribution was determined by Pipette method (Gee & Bauder, 1986).

3.2.4. Calculation of concentration ratio values

The radionuclides transfer from soil to plant is often parameterized by the transfer factor or TF (Gupta et al., 2017). This is the activity concentration of the radionuclide per unit dry mass in the plants (Bq kg⁻¹) derived by the one in the soil (Bq kg⁻¹). On the other hand, concentration ratio (CR) is used to investigate the direct effect of the ratio of plant nutrient concentration to nutrient concentration (available fraction). CR is used to explain the ratio of plant nutrient concentration to exchangeable fraction concentration. The CR was calculated as follows:

$$CR = \frac{\text{Plant element concentration (mg g}^{-1}\text{)}}{\text{Soil exchangeable concentration (mg g}^{-1}\text{)}}$$

3.2.5. Statistical analyses

Statistical analysis was done using SPSS Statistics 25 (SPSS Inc., Chicago, IL, USA). To evaluate the results at $p < 0.05$ probability level, I used a One-way analysis of variance (ANOVA). Tukey's test was calculated only when the ANOVA F-test indicated significant treatment effects at the significant level ($p < 0.05$). Values are reported as mean $\pm$ SE of three replicates. Pearson's correlation analysis was conducted to evaluate the relationship between the mineral elements in the plant and soil. The figures were visualized using R Studio.

3.3. Results

3.3.1. Characterizations of soil types

In this study, Ryozen soil had sand, silt, and clay, as 44.62%, 21.44%, and 33.95%, respectively. Hokudai soil, on the other hand, had sand, silt, and clay content as 33.64%, 44.47%, and 21.88%, respectively. According to USDA textural classes of soils, Ryozen soil (gray lowland soil) indicated as sandy clay loam textural class and Hokudai soil (brown lowland soil) indicated as loam textural class. Both soils in this study indicated K deficient soil with low pHs. Hokudai soil had higher EC, ExK, ExCa, and humus content in comparison to Ryozen soil. The details of physical and chemical characterizations of both studied soils are shown in Table 3.1.

3.3.2. Effect of K application on mineral elements in soil

Soil ExK, ExCs, ExCa, ExSr, and ExMg concentrations in response to K applications are shown in Table 3.2. Other soil exchangeable element concentrations (ExLi, ExNa, ExRb, ExBa, ExFe, ExMn, ExZn, ExCu, ExAl, ExCo, ExNi, ExCd, and ExAs) in response to K applications are shown in Appendix 5. Soil ExK increased significantly with the increase of K applications in Ryozen soil, as well as ExBa and ExCu in Hokudai soil. The increasing pattern was observed

after increasing K application in ExK in Hokudai soils, but not significantly confirmed. Soil ExRb in Hokudai soil decreased significantly with increasing K application, as well as ExBa in Ryozen soil. However, the decreasing pattern was observed after increasing K application in ExCs and ExSr in both soils, but not significantly confirmed. In addition, the increasing K application had no significant effect on soil ExCa, ExCd, ExLi, ExMg, ExNa, ExFe, ExMn, ExZn, ExAl, ExCo, ExNi, and ExAs in both soils. The increasing K application had no significant effect on soil ExCs, ExRb, and ExCu in Ryozen soil.

Table 3.1. Physical and chemical characterizations of both studied soils.

Parameter	Ryozen Soil	Hokudai Soil
Soil types	Gray lowland	Brown lowland
pH	5.16	5.12
EC mS/m	5.44	6.92
Exchangeable K (mg g ⁻¹)	0.057	0.086
Exchangeable Ca (mg g ⁻¹)	2.20	3.11
Humus %	1.19	7.91
Sand %	44.62	33.64
Silt %	21.44	44.47
Clay %	33.95	21.88

3.3.3. Fresh and dry weight

Effect of K applications on fresh weight, dry weight, leaves number, shoot length, and leaf length of Komatsuna are shown in Table 3.3. As I expected, owing to application of K in this study, Komatsuna's fresh weight and dry weight increased with the increase of K application in both soils. However, further increment of K application had no significant effect on Komatsuna's leaves number, shoot length, and leaf length in both soils.

Table 3.2. Effect of K applications on element concentration in plant and soil, and their concentration ratio of Komatsuna plants grown in two different soil types.

Location	Treatment	K	Cs	Ca	Sr	Mg
		mg g ⁻¹	μg g ⁻¹	mg g ⁻¹	μg g ⁻¹	mg g ⁻¹
Ryozen soil	No K	20.87 ± 0.84 c	0.27 ± 0.03 a	30.01 ± 2.76 a	71.52 ± 7.12 a	6.55 ± 0.91 a
	Normal K	35.84 ± 2.14 b	0.18 ± 0.01 b	22.32 ± 1.83 ab	50.09 ± 4.45 ab	4.99 ± 0.36 a
	High K	52.90 ± 1.87 a	0.10 ± 0.01 b	19.80 ± 0.90 b	41.88 ± 2.32 b	4.70 ± 0.23 a
Hokudai soil	No K	16.40 ± 0.20 c	42.90 ± 1.93 a	25.21 ± 0.39 a	72.20 ± 1.23 a	5.31 ± 0.38 a
	Normal K	33.25 ± 3.08 b	31.20 ± 1.46 b	20.57 ± 1.27 b	56.30 ± 3.33 b	4.63 ± 0.21 a
	High K	58.39 ± 0.35 a	9.70 ± 0.76 c	15.62 ± 1.21 c	40.30 ± 2.34 c	4.01 ± 0.45 a
Location	Treatment	ExK	ExCs	ExCa	ExSr	ExMg
		mg g ⁻¹	μg g ⁻¹	mg g ⁻¹	μg g ⁻¹	mg g ⁻¹
Ryozen soil	No K	0.054 ± 0.002 b	0.086 ± 0.004 a	2.35 ± 0.04 a	7.38 ± 0.29 a	0.61 ± 0.01 a
	Normal K	0.073 ± 0.002 b	0.085 ± 0.004 a	2.24 ± 0.07 a	6.54 ± 0.09 a	0.57 ± 0.02 a
	High K	0.114 ± 0.008 a	0.079 ± 0.005 a	2.53 ± 0.08 a	6.86 ± 0.11 a	0.62 ± 0.02 a
Hokudai soil	No K	0.095 ± 0.002 a	5.45 ± 0.02 a	3.92 ± 0.17 a	11.90 ± 0.29 a	0.27 ± 0.03 a
	Normal K	0.107 ± 0.014 a	5.40 ± 0.14 a	3.94 ± 0.07 a	11.85 ± 0.09 a	0.23 ± 0.02 a
	High K	0.129 ± 0.009 a	4.81 ± 0.23 a	3.61 ± 0.07 a	11.21 ± 0.11 a	0.22 ± 0.01 a
Location	Treatment	CRK	CRCs	CRCa	CRSr	CRMg
Ryozen soil	No K	393.1 ± 29.5 a	2.95 ± 0.21 a	12.78 ± 0.95 a	9.67 ± 0.68 a	10.75 ± 1.23 a
	Normal K	484.3 ± 22.2 a	1.99 ± 0.16 b	10.05 ± 0.87 ab	7.72 ± 0.67 ab	8.71 ± 0.46 a
	High K	474.1 ± 46.5 a	1.25 ± 0.15 b	7.85 ± 0.39 b	6.12 ± 0.33 b	7.57 ± 0.31 a
Hokudai soil	No K	172.4 ± 3.8 b	7.87 ± 0.26 a	6.46 ± 0.27 a	6.09 ± 0.22 a	19.80 ± 1.75 a
	Normal K	322.1 ± 33.5 a	5.79 ± 0.18 b	5.23 ± 0.34 ab	4.76 ± 0.26 b	20.37 ± 2.14 a
	High K	458.5 ± 32.6 a	2.03 ± 0.16 c	4.31 ± 0.20 b	3.59 ± 0.15 b	18.53 ± 0.80 a

Number after ± denote the standard error. Different letters represent significant differences according to the Tukey's test ($p < 0.05$) following a one-way ANOVA ($n = 3$).

Table 3.3. Effect of K applications on yield components of Komatsuna grown in two different soil types.

Location	Treatment	Fresh Weight	Dry Weight	Leaves Number	Shoot Length	Leaf Length
		g pot ⁻¹	g pot ⁻¹	pot ⁻¹	plant ⁻¹	plant ⁻¹
Ryozen soil	No K	56.49 ± 3.84 b	4.90 ± 0.35 b	22.00 ± 1.73 a	25.60 ± 0.84 a	14.17 ± 0.61 a
	Normal K	78.68 ± 2.97 a	7.12 ± 0.20 a	25.33 ± 2.19 a	25.40 ± 1.56 a	15.53 ± 0.85 a
	High K	89.74 ± 5.25 a	7.58 ± 0.11 a	24.67 ± 1.20 a	25.97 ± 1.30 a	15.83 ± 0.59 a
Hokudai soil	No K	64.31 ± 4.76 b	5.36 ± 0.38 b	21.67 ± 1.45 a	24.40 ± 0.49 a	16.27 ± 0.12 a
	Normal K	113.59 ± 8.13 a	9.28 ± 0.80 a	22.33 ± 0.33 a	26.63 ± 0.50 a	18.63 ± 0.97 a
	High K	107.11 ± 10.39 a	9.40 ± 0.84 a	23.00 ± 1.53 a	27.33 ± 1.13 a	17.50 ± 1.04 a

Number after ± denote the standard error. Different letters represent significant differences according to the Tukey's test ($p < 0.05$) following a one-way ANOVA ($n = 3$).

3.3.4. Effect of K application on concentration and uptake of mineral elements in plant

Effect of K applications on K, Cs, Ca, Sr, and Mg concentrations of Komatsuna grown in two different soil types are shown in Table 3.2. Effect of K application on other element

concentrations (Li, Na, Rb, Ba, Fe, Mn, Zn, Cu, Al, Co, Ni, Cd, and As) of Komatsuna grown in two different soil types are shown in Appendix 6. Plant K concentration of Komatsuna increased significantly with increasing K applications in both soils. Plant Cs, Ca, Sr, Rb, Ba, and Mn concentrations of Komatsuna decreased significantly with increasing K application in both soils. Similar pattern occurred in plant Li, Na, and Zn concentrations of Komatsuna decreased significantly with increasing K application in Ryozen soil. Plant Ba, Fe, Al, Co, Ni, and As concentrations of Komatsuna decreased significantly with increasing K application in Hokudai soil. However, the decreasing pattern was observed after increasing K application in plant Mg concentration in both soils, but not significantly confirmed. The increasing K application had no significant effect on concentration of Fe, Cu, Al, Co, Ni, Cd, and As in plant in Ryozen soil. Increasing K application had no significant effect on plant Mg, Li, Na, Zn, Cu, and Cd in Hokudai soil.

Table 3.4. Effect of K applications on the amount of accumulated elements of Komatsuna grown in two different soil types.

Location	Treatment	K	Cs	Ca	Sr	Mg
		mg pot ⁻¹	µg pot ⁻¹	mg pot ⁻¹	µg pot ⁻¹	mg pot ⁻¹
Ryozen soil	No K	101.67 ± 3.54 c	1.4 ± 0.22 a	148.94 ± 23.07 a	0.36 ± 0.06 a	32.72 ± 6.46 a
	Normal K	252.04 ± 9.71 b	1.3 ± 0.06 ab	158.32 ± 9.02 a	0.36 ± 0.02 a	35.42 ± 1.67 a
	High K	400.79 ± 14.73 a	0.7 ± 0.09 b	149.88 ± 5.43 a	0.32 ± 0.01 a	35.57 ± 1.52 a
Hokudai soil	No K	87.79 ± 5.19 c	230.2 ± 20.52 a	135.41 ± 10.98 a	0.39 ± 0.03 a	28.6 ± 3.34 a
	Normal K	303.71 ± 3.35 b	288.8 ± 22.06 a	190.62 ± 18.79 a	0.52 ± 0.05 a	42.6 ± 1.74 a
	High K	548.37 ± 46.08 a	92.3 ± 14.92 b	148.13 ± 21.32 a	0.38 ± 0.05 a	38.04 ± 6.19 a

Number after ± denote the standard error. Different letters represent significant differences according to the Tukey's test ($p < 0.05$) following a one-way ANOVA ($n = 3$).

Effect of K applications on amount of accumulated elements K, Cs, Ca, Sr, and Mg of Komatsuna grown in two different soil types are shown in Table 3.4. Effect of K application on other amount of accumulated elements (Li, Na, Rb, Ba, Fe, Mn, Zn, Cu, Al, Co, Ni, Cd, Mo, and As) of Komatsuna grown in two different soil types are shown in Appendix 7. Amount of accumulated K increased significantly with increasing K applications in both soils. Amount of accumulated As increased significantly with increasing K applications in Ryozen soil. Amount of accumulated Rb increased significantly with increasing K applications in Hokudai soil. Amount of accumulated Cs decreased significantly with increasing K application in both soils. Amount of accumulated Na and As decreased significantly with increasing K application in Ryozen soil. Amount of accumulated Ba decreased significantly with increasing K application in Hokudai soil. The increasing K application had no significant effect on amount of

accumulated Ca, Sr, Mg, Li, Fe, Mn, Zn, Cu, Al, Co, Ni, and Cd in both soils. The increasing K application had no significant effect on amount of accumulated Ba in Ryozen soil. The increasing K application had no significant effect on amount of accumulated Na and As in Hokudai soil.

3.3.5. Concentration ratio of plant elements to soil exchangeable elements

The concentration ratio (CR) of plant elements to soil exchangeable elements of Komatsuna grown in different soil types are shown in Table 3.2 (K, Cs, Ca, Sr, and Mg) and Appendix 8 (Li, Na, Rb, Ba, Fe, Mn, Zn, Cu, Al, Co, Ni, Cd, and As). CR of K significantly increased with increasing K application levels in Ryozen soil. However, the increasing pattern was observed after increasing K application in CR of K in Hokudai soil, but not statistically significant. CR of Cs, Ca, Sr, and Ni decreased significantly with increasing K application levels in both soils. The decreasing pattern was observed in the CR of Mg in both soils, but not statistically significant. The increasing K application had no significant effect on CR of Ba, Fe, Mn, Zn, Cu, Al, Co, Cd, and As in Ryozen soil. The increasing K application had no significant effect on CR of Li, Na, Rb, Zn, and Co in Hokudai soil.

3.3.6. Relationship among mineral elements in plant and soil

Pearson correlation analysis was performed to establish the correlation between the concentration of each pair of elements in soil and plants (Table 3.5). Soil ExK was significantly negatively correlated with soil ExCs in Hokudai soil but not statistically significant in Ryozen soil (Appendix 9a). Soil ExK was negatively correlated with soil ExSr in both soils but not statistically significant (Appendix 9b). Correlation between soil ExK and ExCa was significantly positively correlated in Ryozen and negatively correlated in Hokudai soil (Appendix 9c). Correlation between soil ExK and ExMg were positively correlated in Ryozen and negatively correlated in Hokudai soil (Appendix 9d). Soil ExSr was significantly positively correlated with ExCa in Hokudai soil, but not statistically significant in Ryozen soil (Appendix 9e). Soil ExSr was significantly positively correlated with ExMg in both soils (Appendix 9f).

Plant K was significantly negatively correlated with plant Cs, Sr, and Ca in both soils (Appendix 10a-c). Plant K was significantly negatively correlated with plant Mg in Hokudai soils but not statistically significant in Ryozen soil (Appendix 10d). Plant Sr was significantly positively correlated with plant Ca and plant Mg in both soils (Appendix 10e-f). In addition, Soil ExK was significantly negatively correlated with the CR of Cs in Ryozen soil but not statistically significant in Hokudai soil (Appendix 11a). Soil ExK was significantly negatively

correlated with the CR of Sr in both soils (Appendix 11b). In Ryozen soil, Soil ExCa was negatively correlated with CR of Sr (Appendix 11d) and CR of Cs (Appendix 11c). In Hokudai soil, soil ExCa was positively correlated with CR of Sr (Appendix 11d) and CR of Cs (Appendix 11c).

Table 3.5. Tabulated summary of Pearson correlation analysis among elements in plant and soil.

Parameters	Ryozen soil		Hokudai soil	
	Pearson's <i>r</i>	<i>p</i> -value	Pearson's <i>r</i>	<i>p</i> -value
Soil ExK vs Soil ExCs	−0.4	0.3	−0.91	0.05*
Soil ExK vs Soil ExSr	−0.14	0.7	−0.39	0.3
Soil ExK vs Soil ExCa	0.71	0.05*	−0.25	0.5
Soil ExK vs Soil ExMg	0.41	0.3	−0.27	0.5
Soil ExSr vs Soil ExCa	0.56	0.1	0.96	0.05*
Soil ExSr vs Soil ExMg	0.79	0.05*	0.78	0.05*
Plant K vs plant Cs	−0.92	0.05*	−0.97	0.05*
Plant K vs plant Sr	−0.82	0.05*	−0.94	0.05*
Plant K vs plant Ca	−0.79	0.05*	−0.92	0.05*
Plant K vs plant Mg	−0.64	0.05	−0.68	0.05*
Plant Sr vs plant Ca	1	0.05*	0.99	0.05*
Plant Sr vs plant Mg	0.94	0.05*	0.81	0.05*
Soil ExK vs CR of Cs	−0.81	0.05*	−0.61	0.1
Soil ExK vs CR of Sr	−0.77	0.05*	−0.77	0.05*
Soil ExCa vs CR of Cs	−0.43	0.03	0.61	0.1
Soil ExCa vs CR of Sr	−0.52	0.2	0.31	0.4

Significant modules are denoted with * ($p < 0.05$).

3.4. Discussion

3.4.1. ExCs, ExSr, and other soil exchangeable elements after K applied

Increasing K application levels in soil led to increased soil ExK but decreased soil ExCs concentration in both soils (Table 3.2). Similarly, correlation analysis revealed that soil ExK concentration was negatively correlated with soil ExCs concentration in both soils (Table 3.5). This study suggests that soil ExCs decreases as soil ExK increases. The relationship between soil ExK and ExCs is often influenced by competition for sorption sites, particularly at frayed edge sites (FES) on clay minerals (Sawhney, 1972; Brouwer et al., 1983). When the soil ExK decreases, Cs fixed in the FES should release, and vice versa (Gommers et al., 2005; Thiry et

al., 2005). The K application has also been observed to reduce soil exchangeable ^{134}Cs and ^{137}Cs of buckwheat (Kubo et al., 2015), exchangeable stable Cs and ^{134}Cs and ^{137}Cs of buckwheat (Kubo et al., 2017), exchangeable ^{137}Cs of soybean (Matsunami et al., 2021; Suzuki et al., 2023). However, increasing K application levels did not change soil ExCa, ExSr, ExMg, ExNa, ExFe, ExMn, ExZn, ExAl, ExCo, ExNi, and ExAs concentrations in both soils. Previous results also observed a similar pattern: soil ExCa, ExSr, and ExMg concentrations were not changed by K applications for two years of field experiments (Chapter 2). The absence of any effect of increasing K levels on soil ExCa, ExSr, and ExMg concentrations could be attributed to their sharing the same soil particle binding site (Bonomelli et al., 2019), affecting their availability in the soils. Consequently, the K application has a more significant impact on soil ExK than soil ExCs, ExCa, ExSr, and ExMg concentrations (Table 3.2). The correlation analysis between soil ExK with ExCs and ExSr was negatively correlated in both soils. However, the correlation between soil ExK with ExMg and ExCa was different among the soils (Table 3.5). The soil ExK was positively correlated with soil ExMg and ExCa in Ryozen soil but showed a negatively correlated in Hokudai soil (Table 3.5). Thus, it might be due to the higher clay content in Ryozen soil than in Hokudai soil (Table 3.1). Clay soils contain more Ca and Mg than sandy soils (Loide, 2004).

3.4.2. Cs, Sr, and other element uptakes after K applied

Increasing K application tends to increase plant K concentration (Table 3.2) and amount of accumulated K (Table 3.4) then affect the fresh and dry weight of Komatsuna (Table 3.3) which indicates the initial soil K level was low enough to check the impact of K application. Plant Cs concentration (Table 3.2) and uptake (Table 3.4) decreased with increasing K application in both soils. It might be due to the chemical competition that K appears to compete with Cs to inhibit the uptake and their transport (Shaw & Bell, 1991). It well-known that K fertilizer reduces the Cs concentration in K-deficient soils. As K mobilizes through cation exchange reactions, adding more K fertilizer inhibits Cs ions uptake by roots, which in turn lowers the availability of Cs (Smolders & Tsukada, 2011). As mentioned in the introduction part, K fertilizer successfully decreases the transfer of RCs from soil to plants as in the case of FDNPP and CNPP, but whether K application decreases RCs and RSr simultaneously remains to be explored. In this study, plant Sr concentration decreased with increasing K application (Table 3.2). It is well known that high-affinity K transporter transports K especially under low K condition which other monovalent cations (Cs, Na, Rb) uptake can be competed (Macleod & Snell, 1950; Zhu & Smolders, 2000). On the other hand, divalent Ca channel seems to have a

role to uptake Cs in soybean (Nihei & Saito, 2020). However, it is still not cleared whether it is also functioned in Komatsuna.

In the case of other elements, such as plant Na and Rb concentration in Royzen soil and plant Ba concentration in Hokudai soil (Appendix 6), their uptake (Appendix 7) was reduced from soil to plants might also be involved in the K or Ca transport mechanism after K applied in the soil. There is a possibility that Sr, Na, Rb, and Ba uses K transporter, or K and Ca channels for their transport to the plants. For example, the family of high-affinity K transporter, the KUP/HAK/KT family has been identified to participate in Cs absorption (White & Broadley, 2000). Hypac 1 and IP₃-Dependent in the Beet root (*Beta vulgaris*) have been identified to participate in Sr absorption. Hypac 2 in the Beet root and *Vicia faba* guard cells have been identified to participate in Ca, Ba, Sr, Mg, and K absorption (White, 2000). VDCC2 in the root cells of Rye (*Secale cereale* L.) have been identified to participate in K, Cs, Rb, Na, Ba, Ca absorption (White, 2000). Maxi in the root cells of Rye (*Secale cereale* L.) have been identified to participate in K, Cs, Rb, Na, Li, Ba, Sr, Ca, Mg, Co, and Mn absorption (White, 2000). The *rca* channel in root cells of wheat have been identified to participate in Na, Cs, K, Li, and Rb absorption (Piñeros & Tester, 1997). Several previous studies have confirmed the reduction of Cs (Komatsu et al., 2017), Ca and Mg (Rhodes et al., 2018; Antonangelo et al., 2019; Volf et al., 2022) in plant organs after K was applied. However, the K application decreased plant Li, Ba, and Zn concentration in Ryozen soil, and decreased plant Rb, Be, Fe, Al, Co, Ni, and As in Hokudai soil (Appendix 5). This result might indicate that K application had variations on different elements in different soil conditions, which affect how plants respond to nutrient applications.

In previous study (Chapter 2) reported that plant Sr, Ca, and Mg concentrations had a similar pattern in soybean leaves that decreased by K application. Nevertheless, the correlation between plant K and Sr concentrations in soybean suggested that K application might alter the translocation of Sr among plant organs instead of reducing it (Chapter 2). In this study, the K application decreased plant Sr and Ca (including Mn) concentrations of Komatsuna in both soils (Table 3.2 and Appendix 6). However, the amount of accumulated Ca and Sr (including Mn) by Komatsuna remains unchanged after application of K in both soils (Table 3.4 and Appendix 7). These results indicated that K application decreases in Sr and Ca (including Mn), but the actual uptake of these elements may not be significantly changed, suggesting that the overall nutrient uptake or transport mechanism in Komatsuna might be regulated in a way that compensates for the changes in soil nutrient availability. Sr and Ca correlation was negatively correlated even when grown in different soils (Table 3.5), indicated that the relationship

between Sr and Ca is not solely dependent on specific soil conditions. It suggests that this correlation is inherent to the plant's physiology or nutrient regulation mechanisms. Moreover, since the root part was not the target in this study because it is not an edible part of Komatsuna, the competition between Sr and K might occur at the root absorption site. Moreover, K and Sr are both cations, but plants tend to preferentially take up K over Sr might be due to essential for their growth and physiological functions. Further studies need to explore whether Sr may be stored at the root of Komatsuna (Moyen and Roblin, 2010, 2013) and how this is reflected in their partitioning inside plant tissues.

3.4.3. Differences in Cs and Sr transport from two soil types to plant after K applied

I used two different soil types to explore the effect of K application on Cs and Sr transport from soil to plants. I want to know whether the transport of Cs and Sr to plants is different if they grow in different soils. In this study, two soils have similarities in that they are K-deficient soils with relatively low pH (range 5.12 to 5.16). First soil (designated as Ryozen soil) belongs to gray lowland soil, having sand, silt, and clay, as 44.62%, 21.44%, and 33.95%, respectively. According to USDA textural classes of soils, Ryozen soil is indicated as sandy clay loam textural class. Second soil (designated as Hokudai soil) is brown lowland soil, having sand, silt, and clay content of 33.64%, 44.47%, and 21.88%, respectively. According to USDA textural classes of soils, Hokudai soil is indicated as loam textural class. However, Hokudai soil has higher EC, ExK, ExCa, and humus content than Ryozen soil (Table 3.1).

After K fertilizer was applied to Ryozen soil, the soil kept showing less ExK and ExCa concentrations than in Hokudai soil (Table 3.2). Furthermore, the presence of humus in the soil can alter the surface charge and ion exchange capacity of clay mineral, which further influences Cs adsorption. If soil organic matter is present at frayed edge sites, mineral clays weakly absorb Cs, so large amounts of Cs is considered to remain mobile (Berns et al., 2018). Organic matter blocked Cs from accessing the strong affinity sites of clay minerals (Manaka et al., 2021). In a previous study, soil organic matter was shown to adsorb significantly fewer Cs than solid soil, so this source is likely to contribute the greatest amount of mobile Cs (Van Bergeijk et al., 1992; Nakamaru et al., 2007; Manaka et al., 2021). However, Cs uptake is strictly regulated by K availability. Suzuki et al. (2024) has investigated the TF of RCs and ExK at the same field with or without manure application in the field for five years and also investigated by pot experiment. Suzuki et al. (2024) have concluded that the application of organic materials significantly changed the relationship between ExK and TF and at the same ExK level, that is the TF become lower if the organic materials were applied. As a result, Ryozen soil has a lower

humus content than Hokudai soil (Table 3.1) then influences the soil ExCs concentration and their transport to Komatsuna (Table 3.2). In addition, a higher concentration of RCs activity was found in the clay-sized soil particles compared to the larger-sized soil particles in the surface layers of undisturbed soils by the observations followed by atmospheric nuclear weapons testing and the Chernobyl NPP accident (Livens & Baxter, 1988; He & Walling, 1996; Spezzano, 2005). It was also confirmed in the case of the FDNPP accident, the activity concentrations of RCs were higher in clay-sized soil particles than those in sand-sized soil particles (Saito et al., 2014; Koarashi et al., 2018). Sr can also be adsorbed onto the surface of clay particles due to its positively charged nature in the soil, like Cs (Khaleghpanah et al., 2010), but does not fix to the FES. As mentioned in Chapter 1, Sr is not highly mobile in most soils, and when deposited from the atmosphere onto the soil surface, it does not penetrate very deeply (Romney et al., 1960). In this study, Ryozen soil seems to have a higher clay content than Hokudai soil (Table 3.1), which would be more affect to Cs and Sr activities after K is applied.

As I mentioned earlier, K application did not change soil ExCa, ExSr, and ExMg concentrations in both soils (Table 3.2). This result indicates a competition or interaction between these elements for specific sites in the soil, potentially limiting the impact of K application on their availability. However, K application significantly decreased plant Cs, Sr, Ca, and their concentration ratio in both soils (Table 3.2). Even though soil concentrations of Cs, Sr, and Ca were stable, plant roots showed significant decreases in their concentrations as well as their concentration ratios. This suggests that plant roots were selectively uptake and transport these elements. K application may have influenced the plant's ability to absorb Cs, Sr, and Ca, leading to reduced concentrations in plant tissues. Soil ExK was negatively correlated with soil ExCs, soil ExSr, CR of Cs, and CR of Sr in both soils, as well as soil ExCa was negatively correlated with CR of Sr and CR of Ca in Ryozen soil (Table 3.5). It may be attributed to the inhibition of other cation uptake by plant roots due to increased mobilization of K in cation exchange reactions resulting from adding K fertilizer (Smolders & Tsukada, 2011). It should be noted that Cs uptake might differ from Sr uptake (including Ca and Mg uptake). Nihei & Saito (2020) suggest potential inhibitory effect of Ca on cesium (Cs) uptake in soybean. The specific interaction between K and Sr remains an area requiring further investigation to determine whether a similar inhibitory effect of K on Sr uptake exists. As soil K concentrations increase, any subsequent reduction in Cs and Sr influx into crop roots involves the mechanisms with complexity and potential effectiveness (Shaw, 1993). In this study, K application reduced the amount of accumulated Cs instead of Sr of Komatsuna (Table 3.4). It might be the differential response in Cs and Sr uptake indicates that the effects of K

application are not uniform across all elements. It suggests that the influence of K on nutrient uptake is ion-specific, and the mechanisms governing the uptake of Cs and Sr by Komatsuna may be distinct. Nevertheless, Ca and Sr are immobile in the phloem and only mobile in the xylem (Feller, 1989). As a result, their translocation within plant tissues is limited, and the K fertilizer application has less effect on the amount of accumulated Sr (Table 3.2) than the amount of accumulated Cs of Komatsuna in this study.

3.5. Conclusion

This study showed that K application increased soil ExK, and decreased soil ExCs, which leads a negative correlation between ExK and ExCs. Despite K application's limited effect by K application on soil ExCs, ExSr, and ExCa concentrations, it significantly reduced plant Cs, Sr, and Ca concentrations, implying selective nutrient uptake by plant roots. The different response between Cs and Sr uptake by Komatsuna suggests that ion-specific influences of K application on nutrient uptake mechanisms. Soil composition, particularly clay content, may change Cs and Sr activities after K application, emphasizing the role of soil characteristics in nutrient dynamics. Importantly, this study concluded that the effect of K on Sr absorption varied among crop species.

The study highlighted the necessity for further exploration into the complex interactions between K and other elements (Cs, Ca, Sr, and Mg) to better understand their specific effects on nutrient transport in plants. This holds particular relevance when using K as a countermeasure for RCs, requiring consideration of interactions in diverse varieties and soil types. Such research contributes to enhancing food safety and environmental protection.

3.6. References

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Chapter 4. Co-application effect of Potassium and Calcium on Cesium and Strontium Transport from Soil to Plants

4.1 Introduction

Radionuclide fallout from nuclear accidents has led to increased research on reducing the transport of radionuclides from soil to plants. For most accident scenarios, RCs and RSr are the most important contributors to the ingestion dose (Wilkins et al., 1993). The transfer of these radionuclides from soil to plants is an essential route of food contamination for humans. Therefore, human health must be protected against exposure to radionuclides, mainly through internal food consumption pathways.

K and Ca fertilizers are well-known as effective soil-based fertilizer treatments for reducing RCs and RSr uptake in plants (Nisbet et al., 1993). It is because K and Cs, and Ca and Sr belong to the same chemical groups and share some similar properties, respectively. This allows K and Ca to compete successfully for uptake by roots with Cs and Sr. For example, the use of K fertilizer for reducing RCs was successful in rice, soybean, peanut, buckwheat, and some vegetables (Kato et al., 2015; Kubo et al., 2015; 2017; 2021; Suzuki et al., 2023; Shinano et al., 2023). For Ca fertilizer, this countermeasure was successful for reducing RSr in lettuce, grass, alfalfa, and oats (Fowler & Christenson 1959; Evans & Dekker 1962). However, not only one radionuclide is generally released to contaminate the terrestrial ecosystem after nuclear incidents. For example, in the case of the Chernobyl nuclear power plant accident in 1986 releases several radionuclides, i.e., ^{134}Cs , ^{136}Cs , ^{137}Cs , ^{89}Sr ^{90}Sr , and others (IAEA, 2006). Therefore, countermeasures against mixed deposition of radionuclides may be necessary to preparedness in the future.

In the case of mixed depositions of radionuclides, e.g., RCs and RSr, the use of co-application of K and Ca fertilizer may be a candidate for an effective countermeasure. Previous studies reported that combining KCl and $\text{Ca}(\text{OH})_2$ reduces 60% of ^{137}Cs and ^{85}Sr in Chinese cabbage and rice (Choi et al., 2008; 2012). However, it remains poorly understood how this countermeasure affect soybean which is one of the traditional sources of protein for the Japanese people. The co-application of K and Ca to soil containing mixed radionuclide deposits may also have undesirable impacts. It examines the impact of co-application of K and Ca on the behavior of Cs and Sr (which are interchangeable with RCs and RSr, respectively) from soil to soybean plant. This study will provide valuable insights that can help agricultural

systems optimize fertilization practices, enhance food safety, and manage Cs and Sr contamination in agricultural systems.

4.2. Material and methods

4.2.1. Experimental design

A pot experiment was conducted at a controlled greenhouse of the Research Faculty of Agriculture, Hokkaido University (43.414°N, 141.2024°E), Japan, from December 2022 to April 2023. The temperature was kept at 25°C with the light period in 16 h and the dark period in 8 h. Soybean seeds (*Glycine max* (L.) Merr. var. Yukihome) were sown in Wagner pots (diameter, 16 cm; height 20 cm; Fujiwara Scientific Company Co., Ltd., Tokyo Japan) filled with 8 kg air-dried soil (gray lowland soil, based on Japan's classification of cultivated soils). The soil was collected from the K deficient (NP fertilized) plot in Date City, Fukushima Prefecture, Japan. The air-dried soil was passed through a 5-mm sieve before filling the pots. The soil chemical properties are shown in Table 4.1.

Table 4.1. Physical and chemical characterizations of soils.

Parameter	Ryozen Soil
Soil types	Gray lowland
pH	5.16
EC mS/m	5.44
Exchangeable K (mg g ⁻¹)	0.057
Exchangeable Ca (mg g ⁻¹)	2.20
Humus %	1.19
Sand %	44.62
Silt %	21.44
Clay %	33.95

The experiment was set up into five treatments with three replicates (a total of 15 pots), including control (without K and Ca application), Ca, K, K with Ca, and K with high Ca application. In each replicate, the soil was fertilized on the day before cultivation as follows: 19.77 kg N ha⁻¹ (NH₄)₂SO₄ and 19.77 kg P₂O₅ ha⁻¹ Mg₃(PO₄)₂·8H₂O. For normal K fertilization was applied as potassium chloride (KCl, 100 kg K₂O ha⁻¹). For Ca fertilization was applied as calcium carbonate (CaCO₃, 40.0% Ca) in different levels, i.e., normal Ca (247.11 kg Ca ha⁻¹) and high Ca (494.21 kg Ca ha⁻¹) application.

The soybean seeds were sown in each pot and the seedlings were thinned to one per pot. The pots were distributed in a randomized design. All pots were equally irrigated with deionized water over the whole experiment.

4.2.2. Sample collection

Soybean plants were harvested at physiological maturity (i.e., four months after sowing). Plant and soil samples were collected from each pot. The dried plant samples were divided into root, stem, petiole, pod, and seed. When collecting samples, each plant was carefully taken from the pots and washed with distilled water. They were then dried at 80 °C for two days, weighed for dry weight, and homogenized for subsequent elemental analysis. Soil samples (300 gr) were taken from each pot using a scoop. Soil samples were air-dried at 40 °C for one week and then passed through a 2.0 mm sieve for chemical analysis.

4.2.3. Mineral element measurements

Plant and exchangeable soil element concentrations were measured in the same way as in Chapter 2 and 3. Plant element concentrations in this study include K, Cs, Ca, Sr, and Mg. Soil element concentrations in this study include soil ExK, ExCs, ExCa, ExSr, and ExMg. Plant element and soil exchangeable were determined using an inductively coupled plasma mass spectrometry (ICP-MS: ELAN DRC-e; PerkinElmer, Waltham, MA, U.S.A). Soil pH and humus was measured in the same way as in Chapter 3. Soil electrical conductivity (EC) was observed by measuring the soil solution (1:5 soil to deionized water w/w) with EC meter. The soil EC was measured using the CM-30V conductivity meter (TOA, Japan).

4.2.4. Calculation of concentration ratio values

To evaluate the ratio of concentration in each element (Sr, Ca, Cs, K, and Mg) between plant organ A and plant organ B, the ratio of each element was calculated as follows (Kubo et al., 2017):

$$\text{Element ratio} = \frac{\text{Element in plant organ A (mg g}^{-1}\text{)}}{\text{Element in plant organ B (mg g}^{-1}\text{)}}$$

The higher ratio indicated higher concentration of each element in plant organ A compared to plant organ B.

To evaluate the ratio of ExSr/ExCa and ExCs/ExK in soil, the ratio was calculated as follows:

$$\text{Element ratio} = \frac{\text{ExSr or ExCs in soil (mg g}^{-1}\text{)}}{\text{ExCa or ExK in soil (mg g}^{-1}\text{)}}$$

The higher ratio indicated higher concentration of elements among plant to plant and soil to soil.

I also conducted the concentration ratio (CR) of each element (Sr, Ca, Cs, K, and Mg) in plant to soil was calculated in the same way as Chapter 3.

4.2.5. Discrimination factor

To evaluate the Sr and Cs transport from plant organ A to B compared with the efficiency of Ca and K transport, the discrimination factor (DF) of Sr/Ca and Cs/K among plant organs was estimated as follows:

$$DF \text{ of } Sr/Ca = \frac{Sr \text{ in plant organ A} / Ca \text{ in plant organ A}}{Sr \text{ in plant organ B} / Ca \text{ in plant organ B}}$$

$$DF \text{ of } Cs/K = \frac{Cs \text{ in plant organ A} / K \text{ in plant organ A}}{Cs \text{ in plant organ B} / K \text{ in plant organ B}}$$

A DF value lower than 1.0 indicates that K or Ca is transported more efficiently than Sr or Cs from plant organ A to plant organ B.

4.2.6. Statistical analyses

I used SPSS Statistics 25 (SPSS Inc., Chicago, IL, USA) for statistical analysis. To evaluate the results at $p < 0.05$ probability level, I used a One-way analysis of variance (ANOVA). Tukey's test was calculated only when the ANOVA F-test indicated significant treatment effects at the significant level ($p < 0.05$). Values are reported as mean $\pm$ SE of three replicates. To evaluate the relationship between the mineral in the plant and soil, Pearson's correlation analysis was conducted. The figures were visualized by R Studio and procreate.

4.3. Results

4.3.1. Soil exchangeable elements

Soil ExSr, ExCa, ExCs, ExK, and ExMg concentrations in response to co-application of K and Ca are shown in Figure 4.1. K alone and combined with Ca application significantly decreased soil ExSr concentration compared to Ca alone application. However, combination of K with Ca or high Ca application did not significantly affect soil ExSr concentration compared to control and K alone application. Combination of K with high Ca application significantly increased soil ExCa concentration compared to control and K alone application. However, combination of K with Ca or high Ca application did not significantly affect the soil ExCa concentration compared to Ca alone application. Soil ExCs, ExK, and ExMg concentrations showed that there were no remarkable differences among the five treatments.

Soil electrical conductivity (EC) and soil pH in response to co-application of K and Ca are shown in Table 4.2. K alone and combined with Ca or high Ca application significantly

increased soil EC compared to control and Ca alone application. The co-application of K and Ca influenced soil pH but was not statistically significant.

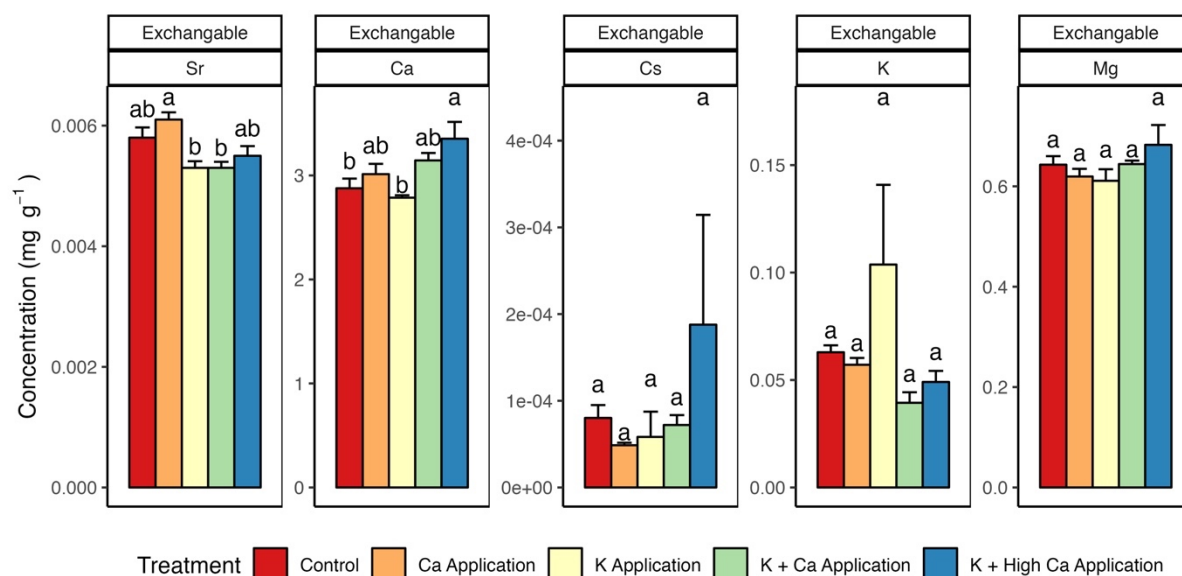


Figure 4.1. Soil ExSr, ExCa, ExCs, ExK, and ExMg concentrations in response to co-application of K and Ca in soybean. Vertical bars indicate the standard error of three replicates. Different letters (a, b, and c) represent significant differences ($p < 0.05$) according to Tukey's test following a one-way ANOVA ($n = 3$).

Table 4.2. Soil electrical conductivity (EC) and soil pH in response to co-application of K and Ca in soybean.

Treatment	Soil EC	Soil pH
Control	17.61 ± 2.55 b	5.59 ± 0.37 a
Ca application	14.28 ± 1.67 b	6.46 ± 0.19 a
K application	34.28 ± 1.67 a	5.36 ± 0.11 a
K + C application	36.43 ± 2.88 a	5.57 ± 0.35 a
K + high Ca application	35.33 ± 5.27 a	6.04 ± 0.04 a

Number after $\pm$ denote the standard error. Different letters represent significant differences according to the Tukey's test ($p < 0.05$) following a one-way ANOVA ($n = 3$).

4.3.2. Plant mineral elements

Plant Sr, Ca, Cs, K, and Mg concentration of soybean in response to co-application of K and Ca are shown in Figure 4.2. Combination of K with high Ca application significantly decreased Sr concentration in root compared to control. Combination of K and Ca or high Ca application

significantly decreased Sr concentration in pod compared to Ca alone application. Sr concentration in stem, petiole, and seed did not differ among all treatments. Sr uptake in root, stem, pod, and seed did not differ among all treatments. K alone and combination of K and Ca application significantly decreased pod Ca concentration compared to Ca alone application. However, Ca concentration in the root, stem, petiole, and seed did not differ among all treatments. Co-application of K and Ca had no effect on the Cs, K and Mg concentration among plant organs.

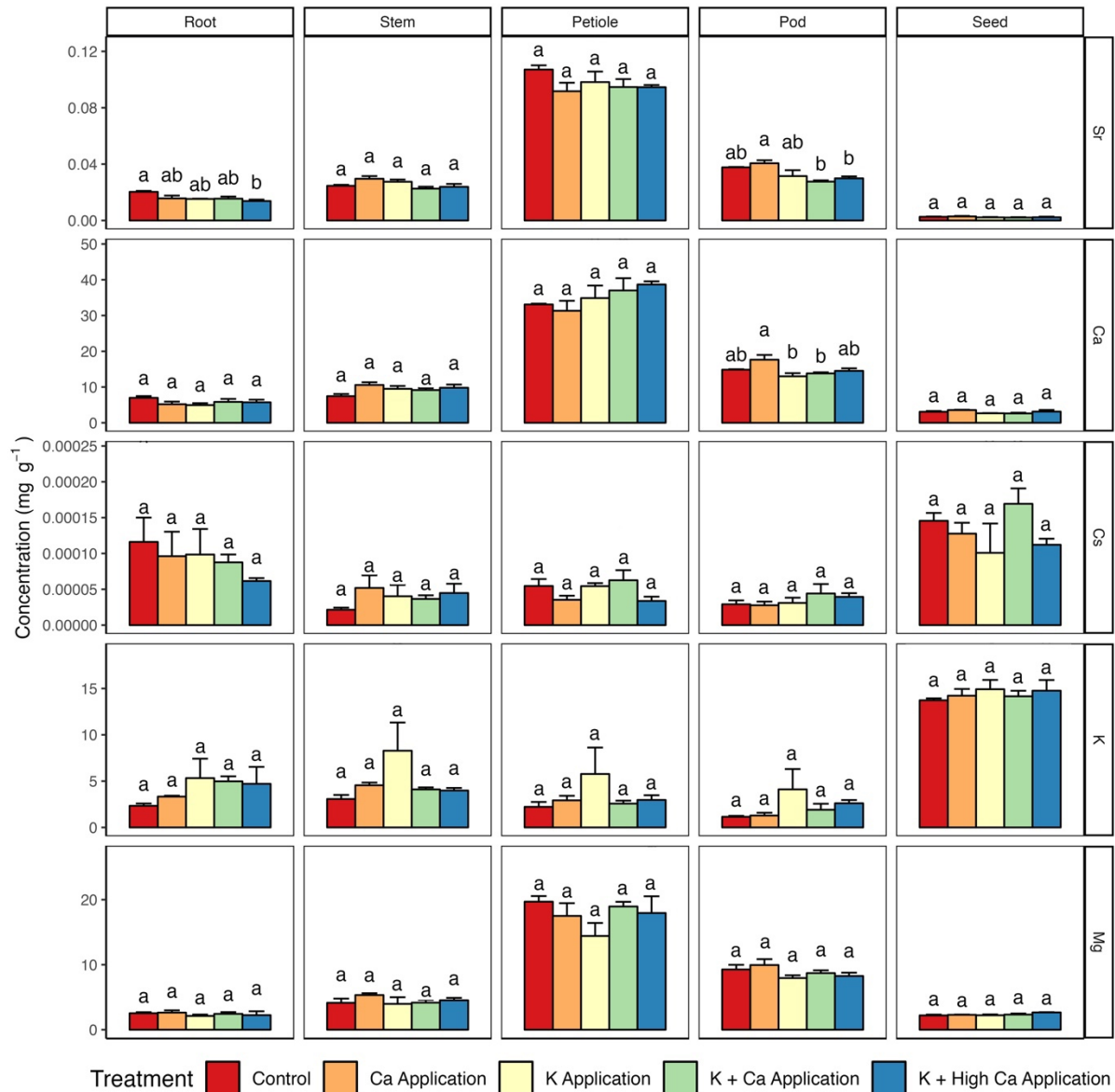


Figure 4.2. Sr, Ca, Cs, K, and Mg concentrations of plant organs in response to co-application of K and Ca in soybean. Vertical bars indicate the standard error of three replicates. Different letters (a, b, and c) represent significant differences ($p < 0.05$) according to Tukey's test following a one-way ANOVA ($n = 3$).

Sr, Ca, Cs, K, and Mg uptake of soybean in response to co-application of K and Ca are shown in Table 4.3. K and combined with high Ca application significantly decreased Sr uptake in petiole compared to control. Sr uptake in root, stem, pod, and seed did not differ among all treatments. K application was significantly increased K uptake in seed compared to control. K uptake in root, stem, petiole, and pod did not differ among all treatments. Ca, Cs, and Mg uptake in all plant organs did not differ among all treatments.

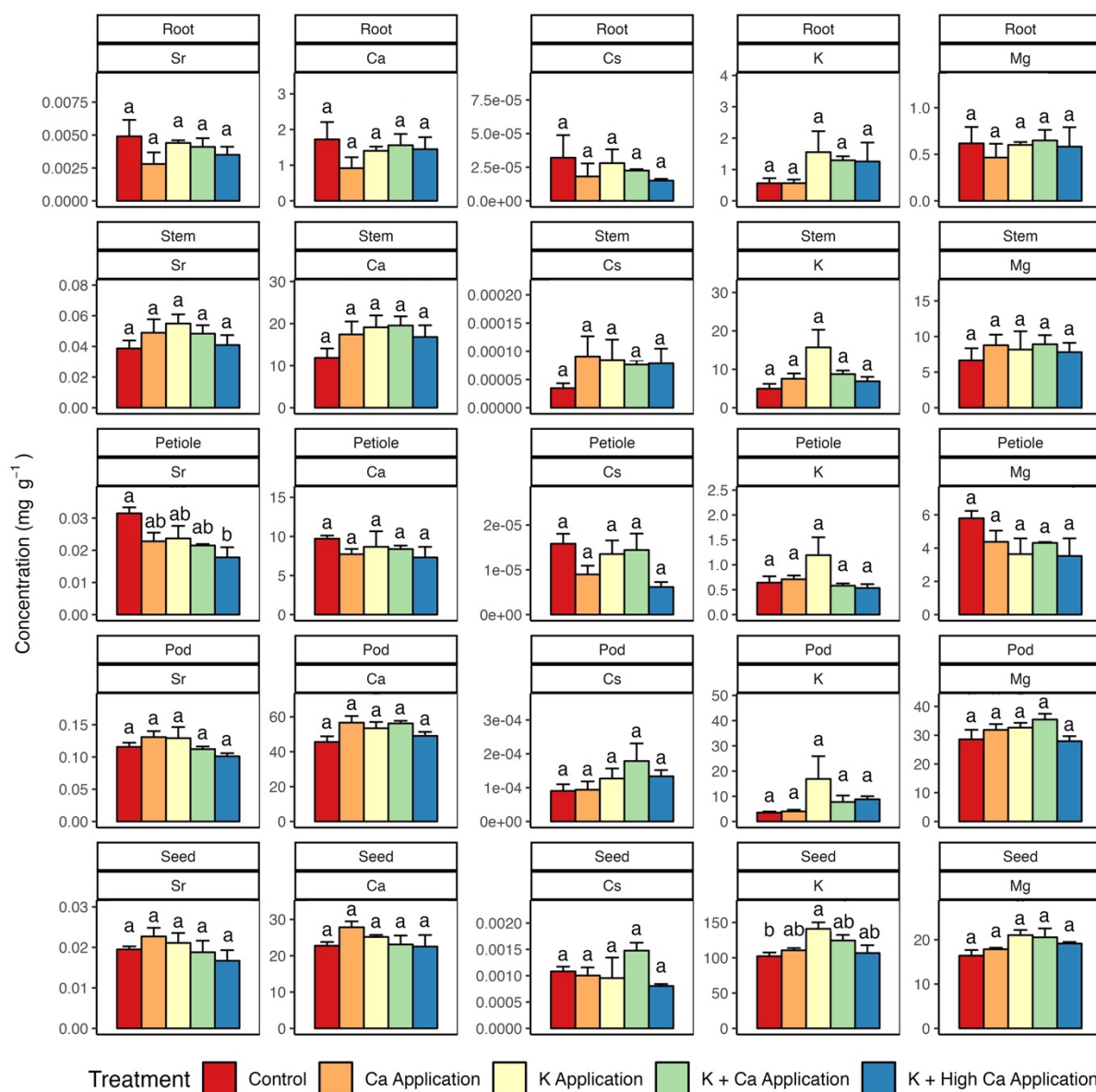


Figure 4.3. Uptake of Sr, Ca, Cs, K, and Mg in plant organs in response to co-application of K and Ca in soybean. Vertical bars indicate the standard error of three replicates. Different letters (a, b, and c) represent significant differences ($p < 0.05$) according to Tukey's test following a one-way ANOVA ($n = 3$).

Mineral elements distribution among plant organs of soybean in response to co-application of K and Ca are shown in Figure 4.4. The distribution of Sr, Ca, and Mg within plant tend to be accumulated in pod, while Cs and K accumulated in seed of soybean.

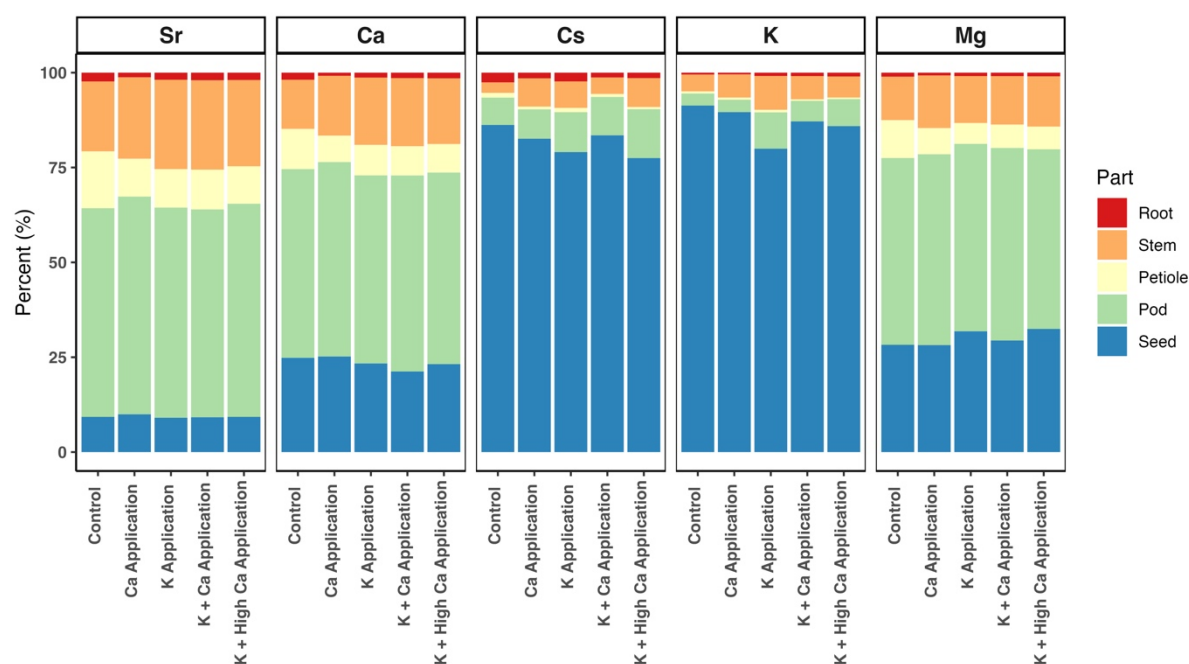


Figure 4.4. Percentage of the accumulation of Sr, Ca, Cs, K, and Mg in plant organs in response to co-application of K and Ca in soybean. Vertical bars indicate the standard error of three replicates. Different letters (a, b, and c) represent significant differences ($p < 0.05$) according to Tukey's test following a one-way ANOVA ($n = 3$).

4.3.3. Dry weight

Dry weight of soybean in response to co-application of K and Ca are shown in Figure 4.5. Root, stem, and petiole dry weights did not change among all treatments. K alone and combination of K with Ca application significantly increased pod dry weight compared to control. K alone and combination of K with Ca application significantly increased the seed dry weight compared to control and combination of K with high Ca application.

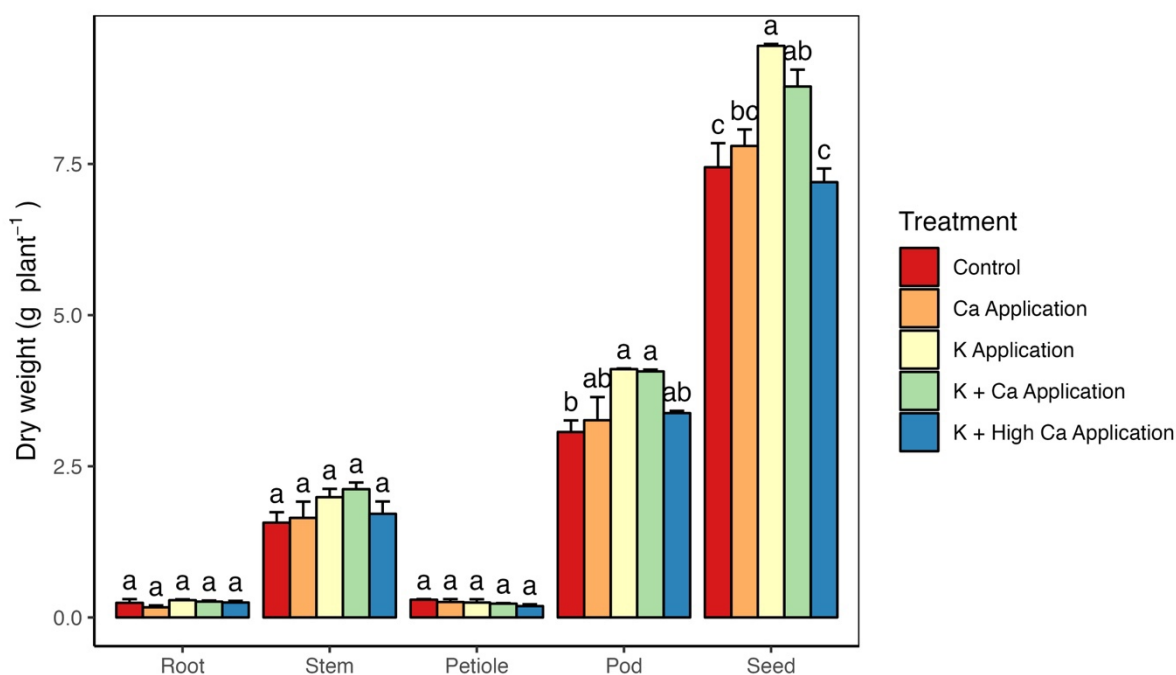


Figure 4.5. Dry weight in response to co-application of K and Ca in soybean. Vertical bars indicate the standard error of three replicates. Different letters (a, b, and c) represent significant differences ($p < 0.05$) according to Tukey's test following a one-way ANOVA ($n = 3$).

4.3.4. Concentration ratio of elements

Ratio of Sr, Ca, Cs, K, and Mg among plant organs in response to co-application of K and Ca are shown in Figure 4.6. Combination of K with Ca application significantly decreased Sr stem-to-petiole ratio compared to Ca alone application. Ca alone application significantly decreased Sr petiole to pod ratio compared to combination of K with Ca application. Co-application of K and Ca had no significant effect on Sr root-to-stem and pod-to-seed ratio. Ca root-to-stem ratio significantly decreased by Ca alone application compared to control. Ca stem-to-petiole ratio significantly decreased by combination of K with Ca or high Ca application compared to Ca application. Co-application of K and Ca had no significant effect on Ca petiole-to-pod and pod-to-seed ratio. Cs root-to-stem ratio significantly decreased by combination of K with high Ca application compared to control. Co-application of K and Ca had no significant effect on Cs stem-to-petiole, petiole-to-pod, and pod-to-seed ratio. Ratio of K and Mg among plant organs did not differ among all treatments.

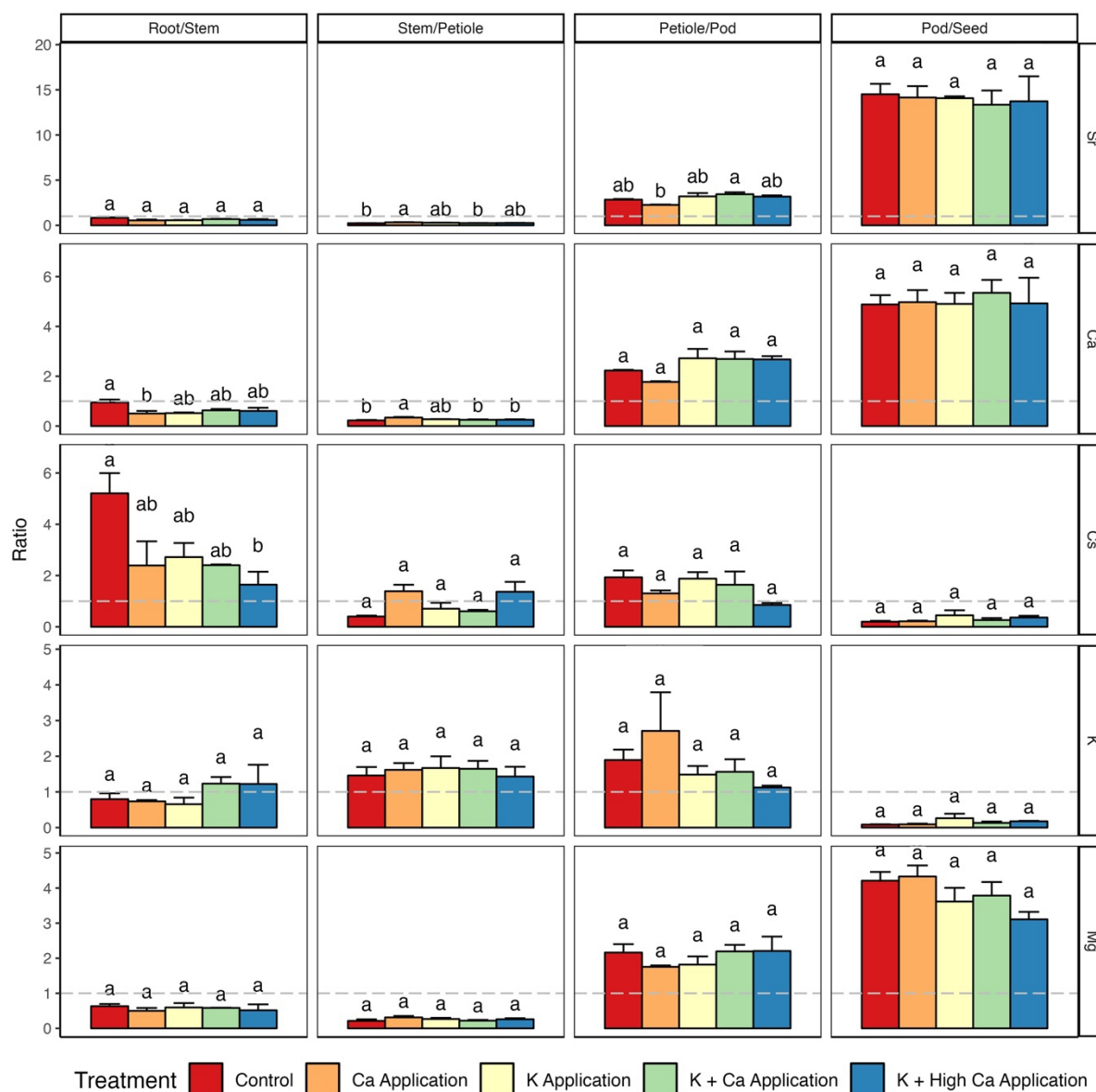


Figure 4.6. Ratio of Sr, Ca, Cs, K, and Mg in plant organs in response to co-application of K and Ca in soybean. Vertical bars indicate the standard error of three replicates. Different letters (a, b, and c) represent significant differences ($p < 0.05$) according to Tukey's test following a one-way ANOVA ($n = 3$).

Concentration ratio (CR) of Sr, Ca, Cs, K, and Mg of soybean plants in response to co-application of K and Ca are shown in Table 4.3. Combination of K with Ca application was significantly increased CR-K compared to control and Ca alone application. Co-application of K and Ca had no effect on CR of Sr, Ca, Cs, and Mg.

Table 4.3. Concentration ratio (CR) of plant elements to soil elements in response to co-application of K and Ca in soybean.

Treatment	CR-Sr	CR-Ca	CR-Cs	CR-K	CR-Mg
Control	2.87 ± 0.06 a	1.32 ± 0.21 a	2.54 ± 0.08 a	141.76 ± 5.85 b	7.12 ± 0.14 a
Ca application	2.85 ± 0.08 a	1.90 ± 0.22 a	2.81 ± 0.15 a	167.48 ± 13.19 b	7.82 ± 0.30 a
K application	2.71 ± 0.11 a	4.38 ± 2.64 a	2.41 ± 0.03 a	123.39 ± 22.32 ab	6.73 ± 0.17 a
K + C application	2.48 ± 0.04 a	1.64 ± 0.18 a	1.17 ± 0.07 a	238.97 ± 16.76 a	7.01 ± 0.13 a
K + high Ca application	2.59 ± 0.13 a	0.97 ± 0.33 a	2.30 ± 0.16 a	200.27 ± 15.44 ab	6.84 ± 0.38 a

Number after $\pm$ denote standard error. Different letters represent significant differences according to the Tukey's test ($p < 0.05$) following a one-way ANOVA ($n = 3$).

Ratio of ExSr/ExCa and ExCs/ExK in response to co-application of K and Ca are shown in Figure 4.7. Combination of K with Ca or high Ca application was significantly decreased the ratio of ExSr/ExCa compared to control, Ca alone, and K alone application. The co-application of K and Ca had no significant effect on ExCs/ExK ratio.

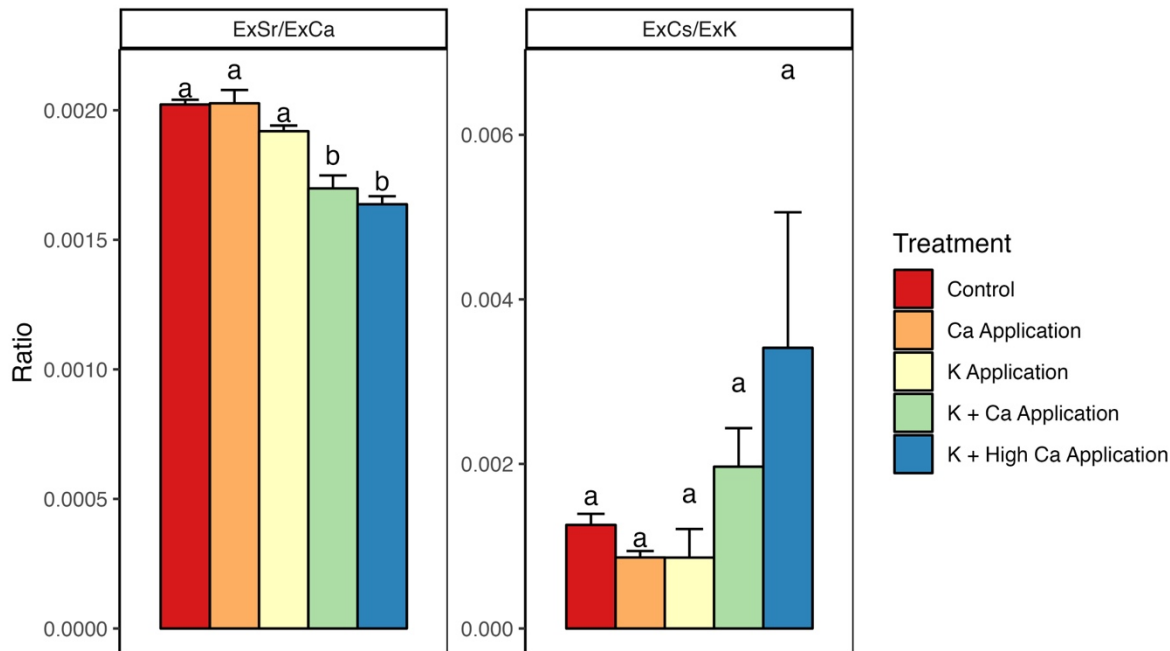


Figure 4.7. Ratio of ExSr/ExCa and ExCs/ExK in response to co-application of K and Ca. Vertical bars indicate the standard error of three replicates. Different letters (a, b, and c) represent significant differences ($p < 0.05$) according to Tukey's test following a one-way ANOVA ($n = 3$).

4.3.5. Discrimination factor of elements among plant organs

Discrimination factor (DF) of plant Cs/K and Sr/Ca within plant organs in response to co-application of K and Ca are shown in Figure 4.8. Co-application of K and Ca had no effect on DF of plant Cs/K and Sr/Ca.

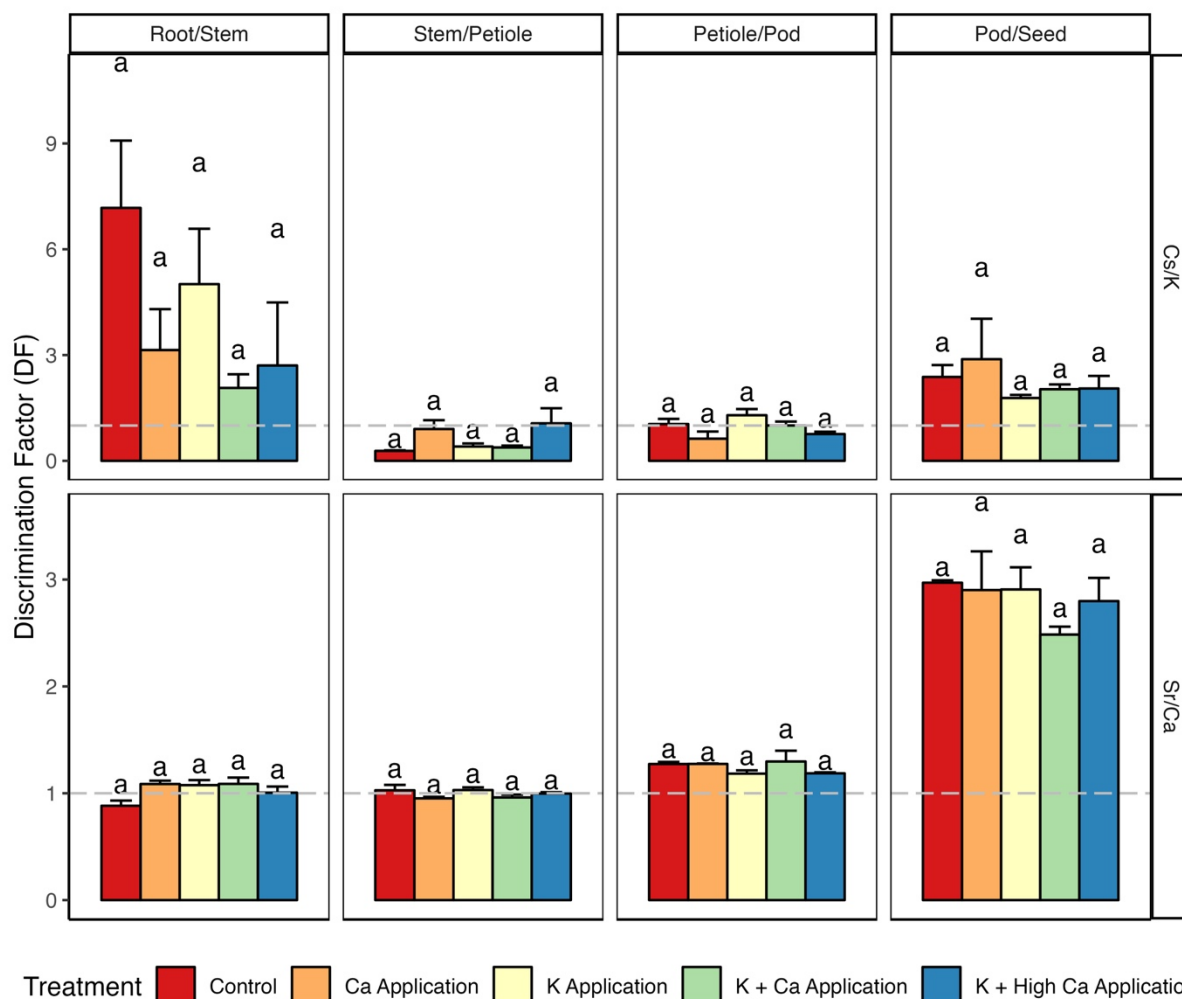


Figure 4.8. DF of plant Cs/K and Sr/Ca within plant organs in response to co-application of K and Ca. Vertical bars indicate the standard error of three replicates. Different letters (a, b, and c) represent significant differences ($p < 0.05$) according to Tukey's test following a one-way ANOVA ($n = 3$).

4.3.6. Relationship among mineral elements in plant and soil

Pearson correlation analysis was performed to establish correlation between concentration of each pair of elements in soil and plants (Table 4.4). Plant K was significantly negatively correlated with plant Mg, but not significantly correlated with plant Sr, Cs, and Ca. Plant Ca

was significantly positively correlated with plant Mg, but not significantly correlated with plant Sr and Cs. Plant Mg was not significantly correlated with plant Sr and Cs.

Soil ExK was significantly negatively correlated with soil ExMg, but not significantly correlated with soil ExSr, ExCa, and ExCs. Soil ExCa was not significantly correlated with soil ExSr, ExCs, and ExMg. Soil ExMg was not significantly correlated with soil ExSr and ExCs. Soil ExK, ExCa, and ExMg were not significantly correlated with soil ExSr and ExCs.

Table 4.4. Tabulated summary of Pearson correlation analysis among elements in plant and soil.

Parameters	Pearson's r	p-value
Plant vs Plant		
K vs Sr	-0.01	0.95
K vs Cs	-0.51	0.05
K vs Ca	-0.29	0.3
K vs Mg	-0.74	0.05*
Ca vs Sr	0.2	0.5
Ca vs Cs	0.02	0.9
Ca vs Mg	0.6	0.05*
Mg vs Sr	0.36	0.2
Mg vs Cs	0.29	0.3
Soil vs Soil		
ExK vs ExSr	0.04	0.9
ExK vs ExCs	-0.17	0.5
ExK vs ExCa	-0.38	0.2
ExK vs ExMg	-0.56	0.05*
ExCa vs ExSr	0.08	0.8
ExCa vs ExCs	0.48	0.05
ExCa vs ExMg	0.25	0.4
ExMg vs ExSr	-0.31	0.3
ExMg vs ExCs	0.12	0.7
Soil vs Plant		
ExK vs Sr	0.42	0.1
ExK vs Cs	-0.44	0.1
ExCa vs Sr	-0.47	0.1
ExCa vs Cs	0.04	0.9
ExMg vs Cs	0.28	0.3
ExMg vs Sr	-0.35	0.2

Significant modules are denoted with * ($p < 0.05$).

4.4. Discussion

4.4.1. Co-application of K and Ca effect on plant mineral elements

Concentration and uptake of plant mineral elements were influenced by Co-application of K and Ca, as shown in Figures 4.2 and 4.3. Combination of K and high Ca application decreased the root Sr concentration and Sr uptake in the petiole compared to the control. K alone and a combination of K and increasing Ca application decreased pod Sr concentration compared to Ca alone application. This result indicated that K combined with increasing Ca application effectively decreased root Sr concentration and reduced their uptake in petiole of soybean. The lower concentration of Sr was detected in pod when combination of K with increasing Ca application than Ca application alone. Apparently, Ca fertilizer effectively reduces Sr uptake due to competition of Sr uptake with Ca for cation channel access (Demidchik et al., 2002). Before they translocated to the shoots, Ca and Sr compete for adsorption sites in root and binding sites on membrane transport systems in root (Moyen & Roblin, 2010). These results are consistent with previous studies that reduction of Sr by increase of Ca application in maize (Moyen & Roblin, 2010), bush bean (Wallace, 1971), tomato (Bowen & Dymond, 1956), and the R_{Sr} was decreased by Ca application in common beans (Romney et al., 1960). Although co-application of K and Ca significantly impacted the concentration and uptake of Sr, their combined application did not alter the concentrations and uptake of Cs and Mg. For K, uptake by the seed was significantly increased by K alone application than control. These results suggested that increasing Ca application decreases Sr concentration in the root which promotes their reduction to the shoot without affecting other elements (Cs and Mg).

4.4.2. Co-application of K and Ca effect on soil exchangeable elements

Soil exchangeable elements were influenced by the co-application of K and Ca, as shown in Figure 4.1. Soil Ex_{Sr} concentration was lower in soil treated with K alone and combination of K and Ca than Ca alone application. However, soil Ex_{Ca} concentration was higher in soil treated with combination of K and high Ca application than control and K alone treatment. This result indicated that combination of K and increasing Ca application can lead to an increased soil Ex_{Ca} concentration. As a result, the displacement of Sr from exchange sites due to compete with Ca (Ahmad, 1995; Chiang et al., 2010), then reducing soil Ex_{Sr} concentration. An immediate interaction might occur in terms of cation exchange and consequence that Ca gets sorbed onto the soil surface by replacing dissimilar cations of relatively lesser valence from exchangeable sites present in soil (Cherian & Arnepalli, 2015). Although additional studies are required to understand the adsorption and desorption of Sr in the agricultural field when co-application of K and Ca is applied to combat mixed radionuclides deposition (R_{Cs} and R_{Sr}),

Sr may substitute with Ca or K at exchangeable sites (Figure 4.9b). Moreover, there is a possibility of Mg involved in their substitution in soil minerals, as confirmed by a negative correlation of soil ExK with ExMg in this study (Table 4.4). Soil minerals, such as clay minerals and organic matter can attract and retain positively charged ions including K, Cs, Ca, Sr, and Mg (Cherian and Arnepalli, 2015). After combination of K and Ca applied, its likely increasing the soil EC (Table 4.2) which may promote nutrients availability for plants (Vyavahare et al., 2023). A schematic of competition between K, Cs, Ca, Sr, and Mg at exchangeable sites is shown in Figure 4.9. In this study, soil ExCs, ExK, and ExMg concentration did not differ among the treatments, which suggested that increasing Ca application, especially affected soil ExSr concentration without significantly impacting other exchangeable elements (ExCs, ExK, and ExMg).

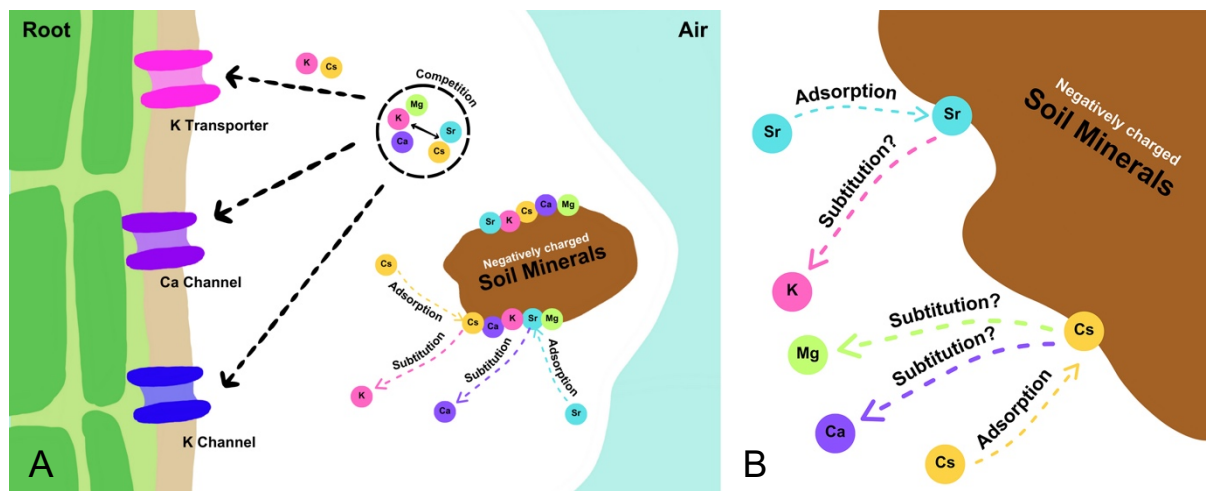


Figure 4.9. Co-application of K and Ca may promote the complex ion dynamics in soil-plant systems: A competitive interaction at exchangeable sites and cation channels (A); and the adsorption and desorption of elements in the soil minerals when co-application of K and Ca is applied to combat mixed deposition (RCs and RSr) at exchangeable sites (B).

4.4.3. Co-application effect of K and Ca on Cs and Sr distribution among plant organs

Understanding how RCs and RSr are distributed in crops is essential for ensuring food safety. After RCs and RSr fall out into the environment and contaminate crops, plants that accumulate high concentrations of RCs and RSr may pose risks to human and animal health if these plants are part of the food chain. Many previous studies used stable instead of radioactive forms because they have identical chemical properties and biological characteristics (Kuke et al., 2016; Yan et al., 2020). In this study, I considered that a stable form would be useful to evaluate the behavior of RCs and RSr inside the plant body and their fate between the soil and plants especially when considering long-term effect of these radionuclides in the agricultural field.

Ratio of Sr, Ca, Cs, K, and Mg in plant organs were influenced by co-application of K and Ca, as shown in Figure 4.6. Sr stem-to-petiole ratio was significantly lower in combination of K and Ca application than in Ca alone application. However, Sr petiole-to-pod ratio was higher in combination of K and Ca application than in Ca alone application. These results indicated that the addition of K may influence the movement of Sr within plant organs. For Ca, the movement from root-to-stem ratio was significantly higher in Ca alone application than control. Ca stem-to-petiole ratio was significantly lower in combination of K with Ca or high Ca application than Ca alone application. Based on these results, the increase in K contributes to re-translocation of Sr and Ca in soybeans. As described in Chapter 2 the increase of K contributes to re-translocation of Sr (along with Ca and Mg) in soybeans, however, specific mechanism of K facilitates this re-translocation are still unclear and require further research. Moreover, percentage of the Sr accumulation (including Ca and Mg) in this study was confirmed in soybean pods (Figure 4.4), indicating a focus on understanding the final storage of these elements. For Cs (and K), they were found to accumulate more in seed (Figure 4.4). These findings are in agreement with previous reports that Cs tend to accumulate more in seed (Nihei et al., 2018) and Sr accumulates in soybean pods (Sudia & Ahlgren, 1964). Cs and Sr had difference in their typical behavior within the vascular system of plants. Cs is indeed highly mobile within both the xylem and phloem, but Sr tends to be less mobile within the xylem (Zeller & Feller, 2000; Marschner 2011). Therefore, concerning the competition between cationic minerals within soybean plants, this study suggested that increasing K application may affect the translocation of Sr, along with Ca and Mg translocation.

Combination of K and high Ca application significantly reduced Cs root-to-stem ratio compared to control (Figure 4.6), suggesting possibility of an absorption pathway in Cs involving Ca. These results coincide with findings of Nihei and Saito (2020), who reported that high Ca concentration led to a reduction of ^{133}Cs concentration in soybean leaves. Massas et al. (2010) reported that Ca application significantly reduced the ^{134}Cs uptake by various plants, including radish, cucumber, soybean, and sunflower plants. They suggested that increase of Ca in the soil matrix, apart from directly competing with Cs for root uptake, also contributed to the immobilization of Cs in soil, reducing its availability to plants for uptake. These results are consistent with Kihana & Yamagami (2022) reported that Ca potentially inhibit Cs absorption. Based on these results, combination of K and increasing Ca application may influence absorption and translocation of Cs in soybean plants. An involvement of Ca in Cs dynamics may have implications for understanding and potentially controlling Cs uptake in plants.

Interactions between K, Cs, Ca, Sr, and Mg within plant organs are complex. Although competition may occur between Cs with K and Sr with Ca, the discrimination factor between Cs with K and Sr with Ca within plant organs did not change by co-application of K and Ca (Figure 4.8). However, correlation analysis has shown that plant Mg was significantly negatively correlated with plant K, while plant Mg significantly positively correlated with plant Ca (Table 4.4). These results indicated that an increase in K may be associated with a reduction in Mg along with Ca and Sr within plant organs. Kang et al. (2019) reported that high levels of Cs or K translocation in plant organs inhibit Ca and Mg translocation and lead to their reduction. When their competition occurs in plant, excess K competes with Mg to reduce protein synthesis function because about 75% of leaf Mg is involved in protein synthesis (Guo et al., 2016).

In addition, pod dry weight is higher in K alone and combination of K and Ca application than control (Figure 4.5). Seed dry weight decreased by combination of K and high Ca application than K alone application (Figure 4.5). These results suggested that increasing Ca may reduce dry weight due to excessive Ca which inhibits Mg uptake (Yan & Hou, 2018). An adequate of Mg is required for plant growth to allow biomass formation and carbohydrate partitioning (Farhat et al., 2016). Based on these results, co-application of K and Ca may promote competition among cations including K, Ca, and Mg in the plant. The K transporter and the K or Ca channel may likely be responsible for the absorption pathway within plant organs as well, suggesting a connection between transporters or channels for K and Ca in influencing the uptake of these elements, along with Cs and Sr (Burger and Lichtscheidl, 2018, 2019). A schematic of competition between K, Cs, Ca, Sr, and Mg at root absorption site is shown in Figure 4.9a.

4.4.4. Transport of Cs and Sr from soil to plants affected by co-application of K and Ca

Chemically analogous ions are known to compete for plant uptake. In light of the potential mixed deposition of RCs and RSr following nuclear accidents, I investigated the co-application of K and Ca as a strategy to mitigate Cs and Sr uptake by plants. Correlation analysis among soil ExCa with plant Sr was negatively correlated, but not statistically significant (Table 4.4). Co-application of K and Ca had no significant effect on CR-Sr (Table 4.3). However, Combination of K with a high Ca application effectively reduced the concentration and uptake of Sr in this study (Figures 4.2 and 4.3). It seems that co-application of K and Ca increased soil ExCa and decreased soil ExSr (Figure 4.1), thus altering Sr translocation in plant organs (Figure 4.4). An increase in soil ExCa may reduce the transport of Sr from soil to plants. These results are in harmony with the ratio among elements in soil which are influenced by the co-application of K and Ca (Figure 4.7). Ratio of ExSr/ExCa was lower in combination of K with

Ca or high Ca application than control, Ca alone, and K alone application, suggesting that proportion of soil ExSr relative to soil ExCa decreased by combination of K and increasing Ca application. These results indicated that a reduction in their ratio may suggest changes in availability in the soil or competition for uptake at root absorption site between Sr and Ca. Combination of K and increasing Ca application may promote Ca to be more efficiently absorbed by root from soil and translocate easily to plant body than Sr.

Co-application of K and Ca had no significant effect on concentration and uptake of Cs (Figures 4.2 and 4.3). Soil ExCs/ExK ratio did not change after co-application of K and Ca applied (Figure 4.7). These results indicated that the co-application of K and Ca did not significantly impact on Cs transport from soil to plant. In contrast, correlation analysis among soil ExK with plant Cs was negatively correlated, but not statistically significant (Table 4.4). Combination of K and high Ca application significantly reduced Cs root-to-stem ratio compared to control (Figure 4.6). Although CR-Cs did not change by co-application of K and Ca, reduction of Cs from root to stem might be because CR-K increased by co-application of K and Ca (Table 4.3). Although additional studies are required to understand the complex mechanism behind these phenomena, this study suggested that combination of K and Ca did not promote Cs transport from soil to plants.

4.5. Conclusion

This study revealed that co-application of K and Ca was augmenting soil ExCa may reduce the transport of Sr without concurrently affecting Cs transport from soil to plants. The distribution of Sr and Ca in soybean plant organs is altered by the addition of K, indicating its role in the re-translocation of Sr (along with Ca and Mg) within plants. This phenomenon is attributed to the competitive interaction between elements at exchangeable sites and then competition for cation channel access at the root absorption site before transport to the plants. Furthermore, it is important to clarify the complex mechanisms of Cs, Sr, and their chemical analogs in the soil-plant system to use a combination of K and Ca application as a strategy for post-accident countermeasures of mixed deposition (RCs and RSr).

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Chapter 5. General Discussion

5.1 Discussion

While individual studies extensively examine the transport of radioactive ions from soil to plants, there is a notable gap in understanding their simultaneous treatment, particularly for RCs and RSr released concurrently in nuclear incidents. Countermeasures targeting specific radionuclides have proven effective but introduce concerns regarding unintended consequences on other elemental minerals. This approach may inadvertently increase the availability of other radionuclides, leading to adverse effects. It attempts to investigate the treatment of RCs with K fertilizer against Sr, which is assumed to be similar to RSr. To understand how Sr behaves from soil to plants, I divided it into three scenarios.

In Chapter 2, I applied different level of K to soybean grown in K-deficient soil. It was observed that an increase in soil ExK concentration selectively suppressed ExCs without influencing ExSr concentration. Notably, a significant positive correlation between Sr, Ca, and Mg concentrations was identified in both plant and soil. K application did not alter the transport of Sr but exhibited a potential influence on its re-translocation. Correlation analysis between plant Sr and K was significantly negative after vegetative stage indicating that Sr concentrations remained relatively stable at early growth stages, irrespective of varying levels of K concentration. The study suggests a likely interaction between K and both Sr and Ca or Mg during their uptake. Additionally, during the reduction of Cs uptake with K application to the soil, competition among cations (Ca, Mg, and Sr) in the apoplast of the root cortex was assumed.

In Chapter 3, I administered varying levels of K fertilizer to Komatsuna cultivated in two distinct soil types characterized by K deficiency. The investigation revealed that K application in the soil led to an increase in soil ExK while concurrently reducing soil ExCs concentration. The inverse correlation between ExK and ExCs is ascribed to the competition for sorption sites, particularly at frayed edge sites (FES) on clay minerals. However, the impact of K application on other soil exchangeable elements, including Ca, Sr, Mg, Na, and various metals, was limited. While the application of K had a restricted impact on soil concentrations of ExCs, ExSr, and ExCa, it notably decreased the concentrations of Cs, Sr, and Ca within the plants. This points to a discernible selectivity in nutrient uptake by plant roots. K application reduces Cs uptake of Komatsuna instead of Sr uptake, indicating the ion-specific effects of K

application on nutrient uptake mechanisms, although Sr is not an essential element for plants. The changes in Cs and Sr activities after K application, particularly influenced by soil composition, especially clay content, underscores the significant role of soil characteristics in nutrient dynamics. Notably, the study concludes that the effect of K on Sr absorption varied among crop species.

In Chapter 4, I implemented the co-application of K and Ca to soybeans cultivated in K-deficient soils. The findings suggested that the combined application of K and Ca leads to a reduction in soybean Sr concentration, affecting their translocation within the plant due to an increase in soil ExCa. Application of this combination initiates competition and interaction among them and their chemical counterparts (Cs, Sr, and Mg) at exchange sites and cation channels. Additionally, the role of K in facilitating re-translocation within plant organs adds complexity to the ion dynamics within soil-plant systems. This intricate phenomenon is attributed to the competitive interaction between elements at exchangeable sites and subsequent competition for access to cation channels at the root absorption site before their transport to the plants. Unraveling these complex mechanisms governing the behavior of Cs, Sr and their chemical analogs in soil-plant systems is crucial for comprehending the efficacy of employing a combined application of K and Ca as a strategy for post-accident countermeasures against mixed deposition of RCs and RSr.

5.2 Conclusion

In **Chapter 2**, soybean plants were subjected to different K applications in Fukushima Prefecture, Japan. The study revealed that K application reduced plant Cs, Ca, and Mg but did not affect soil Sr concentrations. The concentration of K in the plant, especially during later growth stages, negatively regulated Sr concentration, indicating potential re-translocation of Sr influenced by K. **Chapter 3** explored the effects of K application on Cs and Sr transport in Komatsuna plants grown in pots with two soil types. K application enhanced Komatsuna growth, increased soil exchangeable K, and reduced exchangeable Cs, suggesting selective nutrient uptake. Despite decreasing plant Sr and Ca concentrations, the actual uptake of these elements remained unchanged, highlighting the plant's compensatory nutrient transport mechanisms. Although K reduces Cs, Sr, and Ca in plants, it does not enhance Sr uptake. In **Chapter 4**, the co-application of K and Ca in soybeans cultivated in K-deficient soil was investigated. The combined application of K and Ca led to a reduction in soybean Sr concentration, influencing its translocation within the plant due to increased soil ExCa. The study revealed competitive interactions and complex dynamics among K, Cs, Sr, and Mg at

exchange sites and cation channels. The co-application of K and Ca effectively reduced Sr absorption without concurrently affecting Cs transport from soil to plants.

Summary

Chapter 2. Effect of Potassium Application on Cesium and Strontium Uptake of Soybean Plants

After the Tokyo Electric Power Company's Fukushima Dai-ichi Nuclear Power Plant accident in 2011, radioactive cesium (RCs) was released in greater concentrations than radioactive strontium (RSr) in the surrounding environment. Most of the countermeasures were developed to mitigate the RCs transfer from the soil to plants. However, to avoid what has happened after the Chernobyl and Mayak accidents, preventing the transfer of RSr from soil to plants should be a priority. Although the application of potassium (K) fertilizers is the most effective method for preventing agricultural crops from absorbing RCs in contaminated fields, this implementation increases the cost and labor requirements. Considering the preparedness for nuclear accidents, it remains unclear how this countermeasure will be affected if RCs and RSr are released simultaneously. I aimed to explore the effect of K applications on cesium (Cs) and strontium (Sr) uptake and their interaction with and correlation to other elements in the soybean plants and soil. The field experiments were conducted in Fukushima Prefecture, Japan, using different K applications (i.e., no, normal, and high K applications). The dry weight and mineral concentrations of K, Cs, Sr, calcium (Ca), magnesium (Mg), and nitrogen (N) concentration in plants and exchangeable K (ExK), exchangeable Cs (ExCs), exchangeable Sr (ExSr), exchangeable Ca (ExCa), exchangeable Mg (ExMg), NH_4^+ (ammonium), and NO_3^- (nitrate) concentrations in the soils were evaluated. This study revealed that K application reduced Cs, Ca, and Mg uptake but did not affect the ExSr, ExCa, and ExMg concentrations in the soil and did not change the uptake of Sr. On the other hand, K concentration of the plant especially at later growth stage, which indicates re-translocation of Sr was negatively regulated by K concentration.

Chapter 3. Effect of Potassium Application on Cesium and Strontium Transport of Komatsuna Plant Grown in Two Different Soil Types

The benefits of potassium (K) fertilizer have yet to be explored in treating radio-cesium (RCs) and radio-strontium (RSr) simultaneously. However, addressing a specific radionuclide through countermeasures, while effective, raises concerns about unintended consequences, potentially increasing the availability of other radionuclides. This study explores the

effectiveness of K fertilizer on cesium (Cs) and strontium (Sr) transport from soil to plants. Komatsuna (*Brassica rapa* L. var. *perviridis*) used in this study with three treatments of K fertilizer (no, normal, and high K application) grown in pots with two different soil types, i.e., Hokudai soil and Ryozen soil. The fresh weight, dry weight, plant K, Cs, calcium (Ca), Sr, magnesium (Mg), lithium (Li), sodium (Na), rubidium (Rb), iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), aluminum (Al), cobalt (Co), nickel (Ni), cadmium (Cd), arsenic (As), their soil exchangeable and correlation were evaluated. K application enhances Komatsuna growth, increases soil ExK, and reduces ExCs, signifying selective nutrient uptake. Despite limited effects on ExCs, ExSr, and ExCa, K significantly decreases plant Cs, Sr, and Ca concentrations. The study reveals K-induced variations in different elements under diverse soil conditions. While K application reduces Sr and Ca concentrations in Komatsuna, uptake remains unchanged, suggesting compensatory nutrient transport. The negative Sr-Ca correlation across soils suggests inherent physiological or regulatory plant mechanisms. Although K reduces Cs, Sr, and Ca in plants, it does not enhance Sr uptake. This emphasizes the necessity for further research to unravel complex K interactions with elements, crucial for ensuring food safety and environmental protection.

Chapter 4. Effect Co-application of Potassium and Calcium on Cesium and Strontium Transport from Soil to Plants

Combination of potassium (K) and calcium (Ca) application in treating mixed deposition of radio-caesium (RCs) and radio-strontium (RSr) may be an effective countermeasure. Still, it may also have undesirable impacts on soil-plant systems. This study explores the co-application of K and Ca on cesium (Cs) and strontium (Sr) transport from soil to soybean plants. I used soybean (*Glycine max* (L.) Merr. var. Yukihomare) grown in K-deficiency soil subjected to five treatments (control as without K + Ca, Ca, K, K + Ca, and K + High Ca application). The dry weight, plant K, Cs, Ca, Sr, magnesium (Mg), and the soil exchangeable K (ExK), exchangeable Cs (ExCs), exchangeable Ca (ExCa), exchangeable Sr (ExSr), and exchangeable Mg (ExMg), soil electrical conductivity (EC), and soil pH were evaluated. The results indicated that a combination of K and Ca reduces soybean Sr concentration and influences their translocation due to increase soil ExCa. When a combination of K and Ca is applied, they and their chemical analogues compete (Cs, Sr, and Mg) and interact at exchange sites and cation channels, along with the role of K in re-translocation within plant organs, thereby revealing complex ion dynamics in soil-plant systems. This study suggested that a combination of K and

Ca application effectively reduce Sr absorption without concurrently affecting Cs transport from soil to plants.

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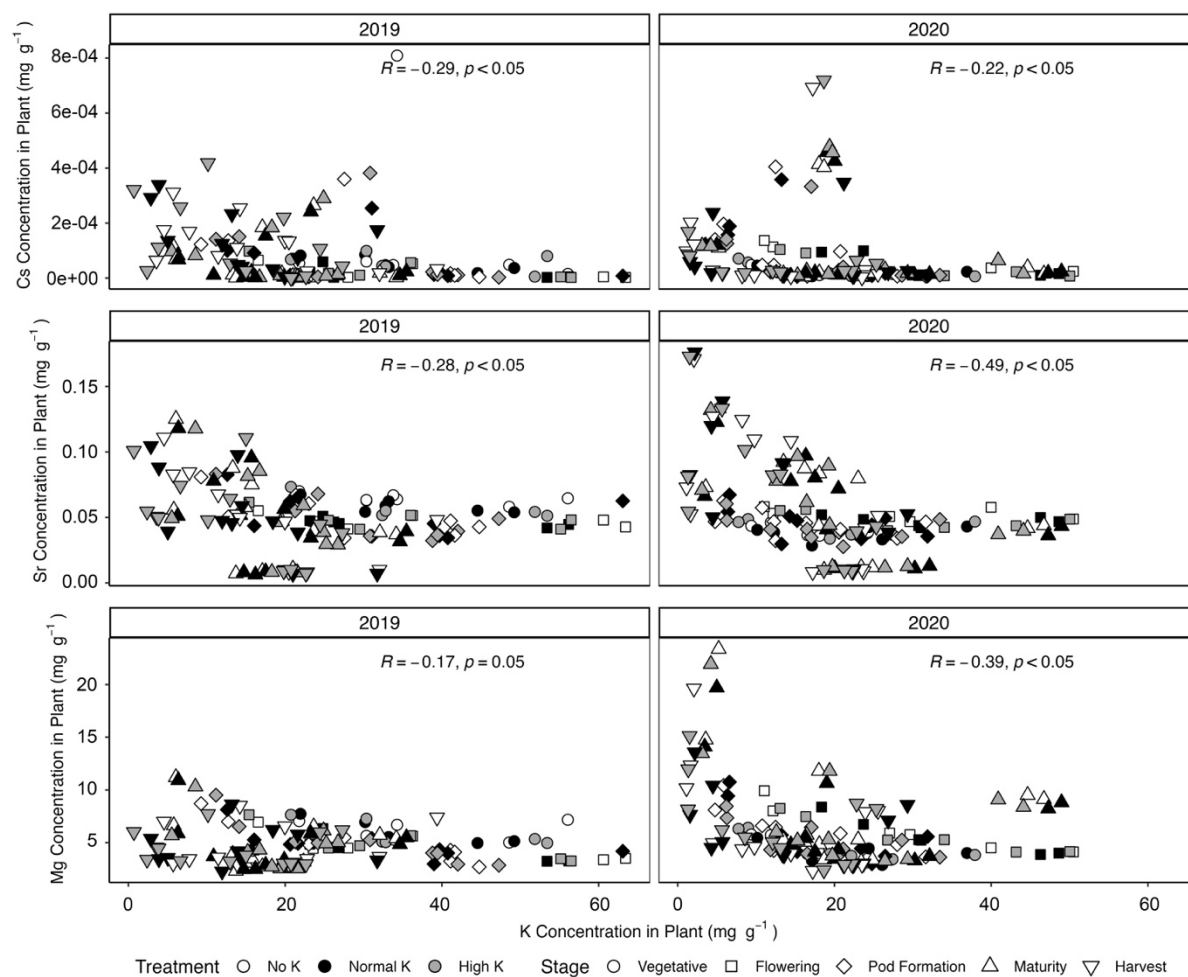
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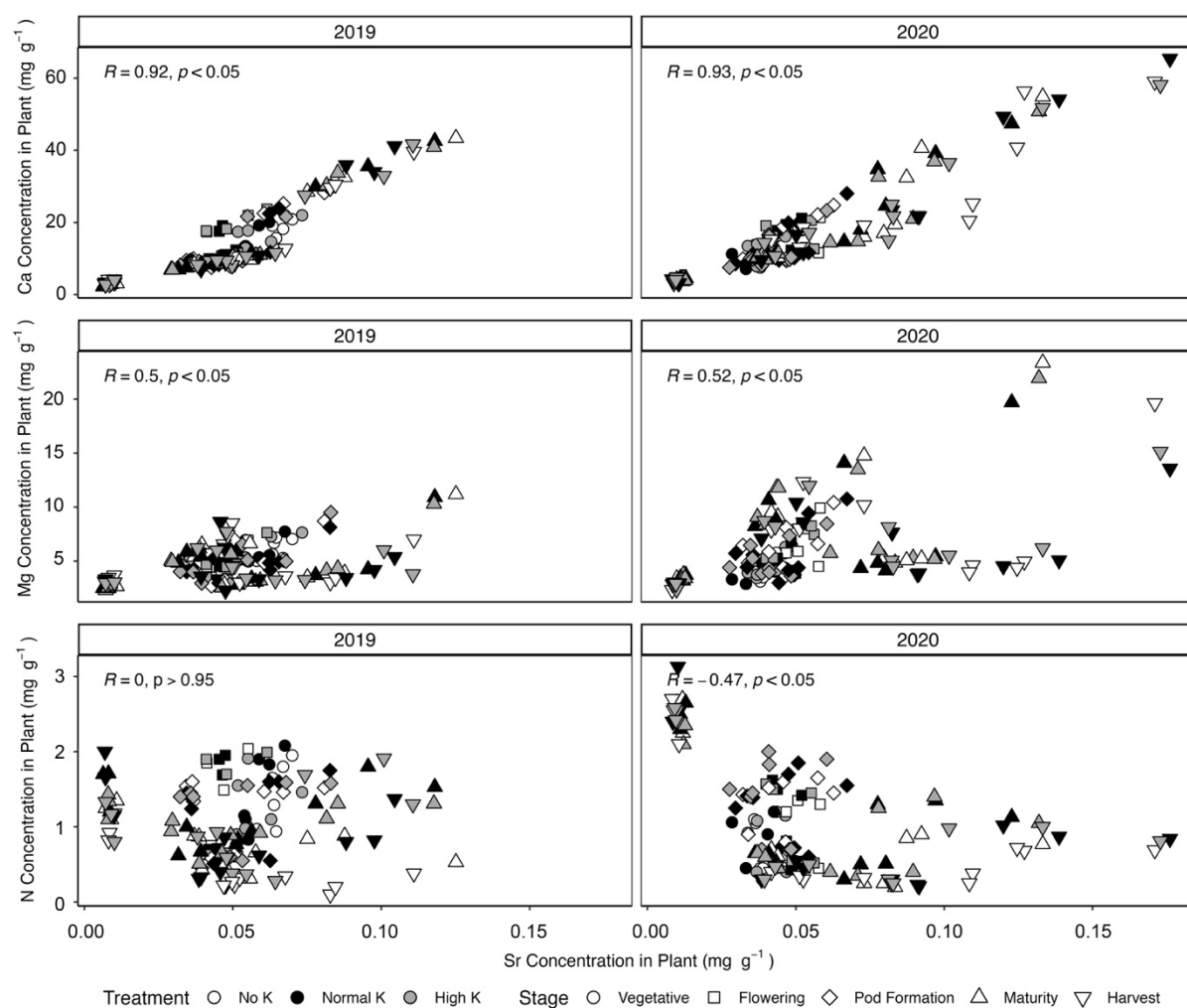
Last but not least, I offer my gratitude to my parents and family members whose prayers and inspirations are the torch to my destination. Whatever I am today, could never have been

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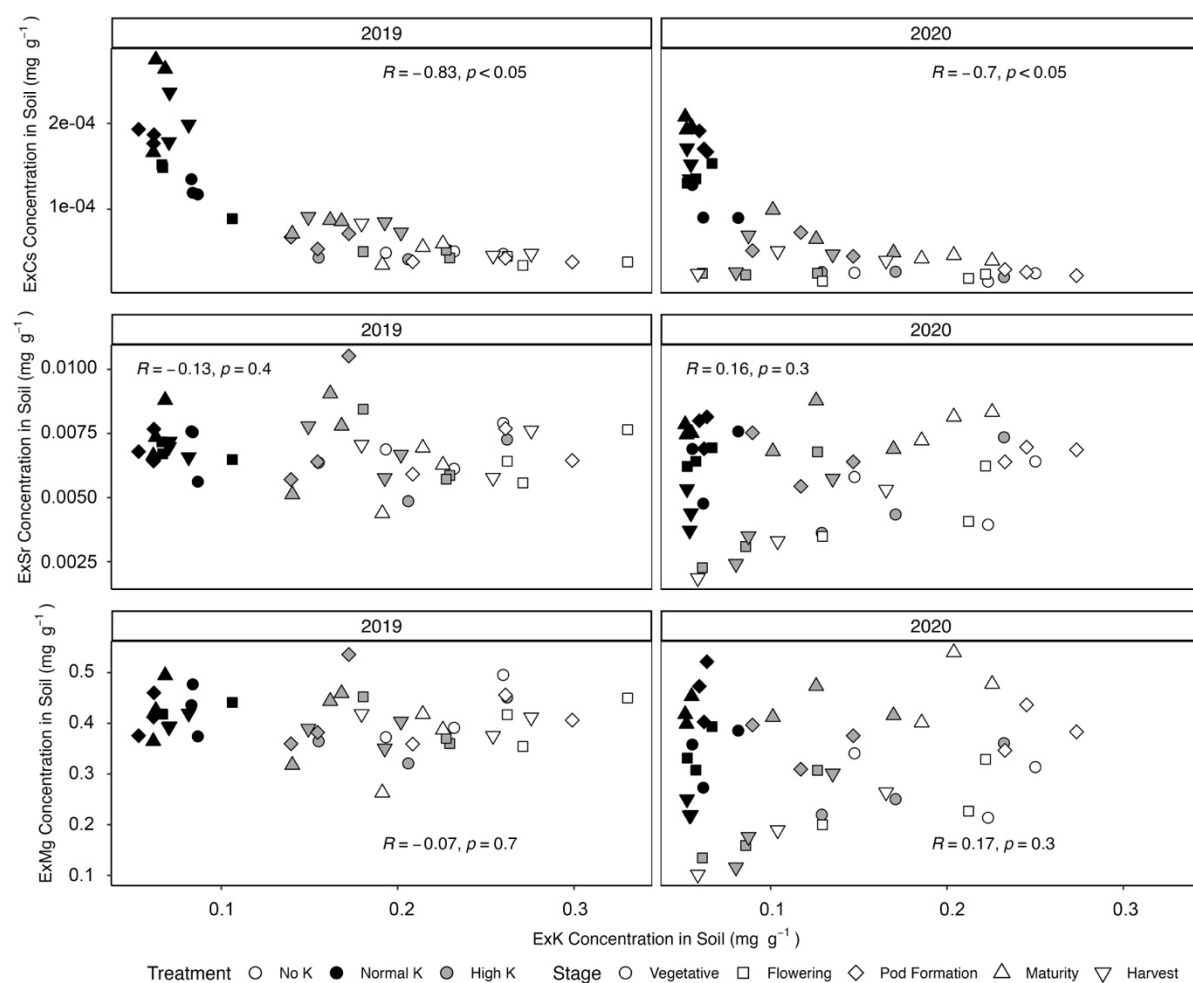
Appendices



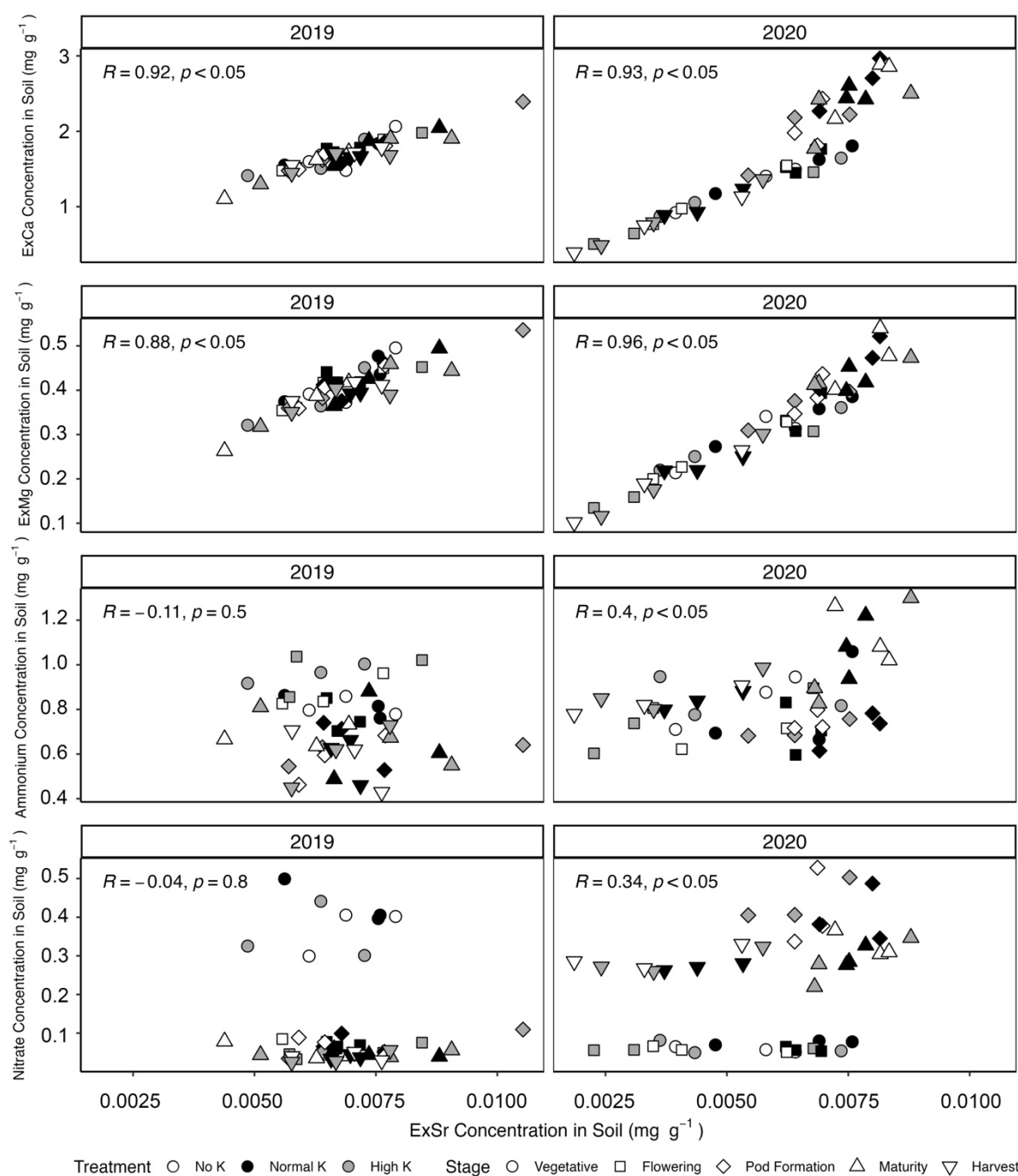
Appendix 1. Pearson's correlation of K with Cs, Sr, and Mg concentration in soybean plants after K applications at Date City in 2019 and 2020.



Appendix 2. Pearson's correlation of Sr with Ca, Mg, and N concentration in soybean plants after K applications at Date City in 2019 and 2020.



Appendix 3. Pearson's correlation of ExK with ExCs, ExSr, and ExMg concentration in the soil after K applications at Date City in 2019 and 2020.



Appendix 4. Pearson's correlation of ExSr with ExCa, ExMg, Ammonium, and Nitrate concentration in the soil after K applications at Date City in 2019 and 2020.

Appendix 5. The effect of K applications on other soil exchangeable elements of Komatsuna plants grown in two different soil types.

Location	Treatment	ExLi*	ExNa*	ExRb*	ExBa**
		$\mu\text{g g}^{-1}$	$\mu\text{g g}^{-1}$	$\mu\text{g g}^{-1}$	$\mu\text{g g}^{-1}$
Ryozen soil	No K	0.12 ± 0.002 a	42.26 ± 3.16 a	0.84 ± 0.06 a	0.024 ± 3.05 a
	Normal K	0.11 ± 0.009 a	125.69 ± 58.33 a	0.87 ± 0.04 a	0.022 ± 2.13 b
	High K	0.14 ± 0.005 a	64.39 ± 4.38 a	0.83 ± 0.03 a	0.022 ± 0.28 b
Hokudai soil	No K	0.29 ± 0.02 a	82.30 ± 2.14 a	1.92 ± 0.03 a	12.10 ± 0.12 b
	Normal K	0.36 ± 0.01 a	66.82 ± 8.93 a	1.72 ± 0.01 b	12.92 ± 0.29 ab
	High K	0.34 ± 0.01 a	82.99 ± 6.75 a	1.59 ± 0.05 b	14.31 ± 0.50 a
Location	Treatment	ExAs***	ExFe***	ExMn***	ExZn***
		$\mu\text{g g}^{-1}$	$\mu\text{g g}^{-1}$	$\mu\text{g g}^{-1}$	$\mu\text{g g}^{-1}$
Ryozen soil	No K	0.0244 ± 0.0013 a	5.06 ± 0.58 a	23.94 ± 2.00 a	0.19 ± 0.12 a
	Normal K	0.0218 ± 0.0009 a	4.75 ± 0.26 a	19.09 ± 0.83 a	0.37 ± 0.13 a
	High K	0.0215 ± 0.0004 a	3.97 ± 0.12 a	17.75 ± 0.98 a	0.30 ± 0.08 a
Hokudai soil	No K	0.051 ± 0.001 a	6.25 ± 0.25 a	24.65 ± 3.47 a	0.89 ± 0.06 a
	Normal K	0.048 ± 0.001 a	5.27 ± 0.15 a	27.81 ± 0.43 a	0.92 ± 0.02 a
	High K	0.047 ± 0.001 a	4.60 ± 0.53 a	29.05 ± 1.27 a	0.92 ± 0.05 a
Location	Treatment	ExCu***	ExAl***	ExCd***	ExCo***
		$\mu\text{g g}^{-1}$	$\mu\text{g g}^{-1}$	$\mu\text{g g}^{-1}$	$\mu\text{g g}^{-1}$
Ryozen soil	No K	0.15 ± 0.011 a	0.58 ± 0.12 a	0.016 ± 0.0004 a	0.024 ± 0.002 a
	Normal K	0.11 ± 0.006 a	1.24 ± 0.44 a	0.031 ± 0.0139 a	0.019 ± 0.001 a
	High K	0.13 ± 0.008 a	0.59 ± 0.14 a	0.015 ± 0.0003 a	0.022 ± 0.002 a
Hokudai soil	No K	0.07 ± 0.005 b	2.16 ± 0.04 a	0.066 ± 0.0011 a	0.037 ± 0.008 a
	Normal K	0.14 ± 0.006 a	3.60 ± 0.78 a	0.068 ± 0.0001 a	0.043 ± 0.002 a
	High K	0.11 ± 0.016 ab	2.58 ± 0.10 a	0.066 ± 0.0011 a	0.046 ± 0.002 a
Location	Treatment	ExNi***			
		$\mu\text{g g}^{-1}$			
Ryozen soil	No K	0.075 ± 0.0028 a			
	Normal K	0.099 ± 0.0139 a			
	High K	0.065 ± 0.0002 a			
Hokudai soil	No K	0.128 ± 0.014 a			
	Normal K	0.126 ± 0.002 a			
	High K	0.131 ± 0.006 a			

Number after $\pm$ denote the standard error. Different letters represent significant differences according to the Tukey's test ($p < 0.05$) following a one-way ANOVA ($n = 3$). *Alkali metals; **alkali-earth metals; ***transition-metals element.

Appendix 6. The effect of K applications on other element concentration of Komatsuna plants grown in two different soil types.

Location	Treatment	Li*	Na*	Rb*	Ba**
		mg kg ⁻¹	mg g ⁻¹	mg g ⁻¹	mg kg ⁻¹
Ryozen soil	No K	0.7 ± 0.07 a	7.71 ± 0.09 a	0.117 ± 0.006 a	44.2 ± 2.88 a
	Normal K	0.5 ± 0.03 b	4.67 ± 0.17 b	0.083 ± 0.003 b	21.9 ± 6.14 ab
	High K	0.6 ± 0.03 ab	2.58 ± 0.32 c	0.055 ± 0.005 c	19.1 ± 6.03 b
Hokudai soil	No K	5.1 ± 0.34 a	0.23 ± 0.02 a	0.195 ± 0.01 a	48.4 ± 3.76 a
	Normal K	4 ± 0.67 a	10.29 ± 5.06 a	0.164 ± 0.08 ab	10.2 ± 0.53 b
	High K	3 ± 0.48 a	7.43 ± 0.94 a	0.138 ± 0.003 b	7.3 ± 0.16 b
Location	Treatment	As***	Fe***	Mn***	Zn***
		mg kg ⁻¹	mg g ⁻¹	mg g ⁻¹	mg g ⁻¹
Ryozen soil	No K	0.335 ± 0.02 a	0.52 ± 0.005 a	0.304 ± 0.013 a	0.053 ± 0.001 a
	Normal K	0.336 ± 0.03 a	0.32 ± 0.17 a	0.223 ± 0.022 b	0.041 ± 0.002 b
	High K	0.340 ± 0.03 a	0.51 ± 0.02 a	0.22 ± 0.012 b	0.040 ± 0.002 b
Hokudai soil	No K	1.8 ± 0.42 a	1.719 ± 0.474 a	0.518 ± 0.021 a	0.132 ± 0.012 a
	Normal K	0.5 ± 0.06 b	0.343 ± 0.054 b	0.406 ± 0.025 ab	0.142 ± 0.009 a
	High K	0.6 ± 0.04 b	0.44 ± 0.048 b	0.37 ± 0.035 b	0.127 ± 0.010 a
Location	Treatment	Cu**	Al***	Cd***	Co***
		mg kg ⁻¹	mg g ⁻¹	mg kg ⁻¹	mg kg ⁻¹
Ryozen soil	No K	7.9 ± 0.48 a	0.16 ± 0.02 a	1.5 ± 0.19 a	0.47 ± 0.02 a
	Normal K	6.7 ± 0.15 a	0.12 ± 0.02 a	1.1 ± 0.06 a	0.44 ± 0.05 a
	High K	6.9 ± 0.35 a	0.17 ± 0.01 a	1 ± 0.04 a	0.44 ± 0.03 a
Hokudai soil	No K	7 ± 0.25 a	0.841 ± 0.274 a	1.4 ± 0.12 a	0.96 ± 0.05 a
	Normal K	5.6 ± 0.36 a	0.130 ± 0.033 b	1.9 ± 0.33 a	0.64 ± 0.08 b
	High K	5.8 ± 0.5 a	0.183 ± 0.029 b	1.4 ± 0.21 a	0.6 ± 0.03 b
Location	Treatment	Ni***			
		mg kg ⁻¹			
Ryozen soil	No K	2.9 ± 0.19 a			
	Normal K	2.4 ± 0.29 a			
	High K	2.6 ± 0.02 a			
Hokudai soil	No K	9.3 ± 2.11 a			
	Normal K	2.9 ± 0.27 b			
	High K	3.2 ± 0.25 b			

Number after ± denote the standard error. Different letters represent significant differences according to the Tukey's test ($p < 0.05$) following a one-way ANOVA ($n = 3$). *Alkali metals; **alkali-earth metals; ***transition-metals element.

Appendix 7. The effect of K applications on other element uptake of Komatsuna plants grown in two different soil types.

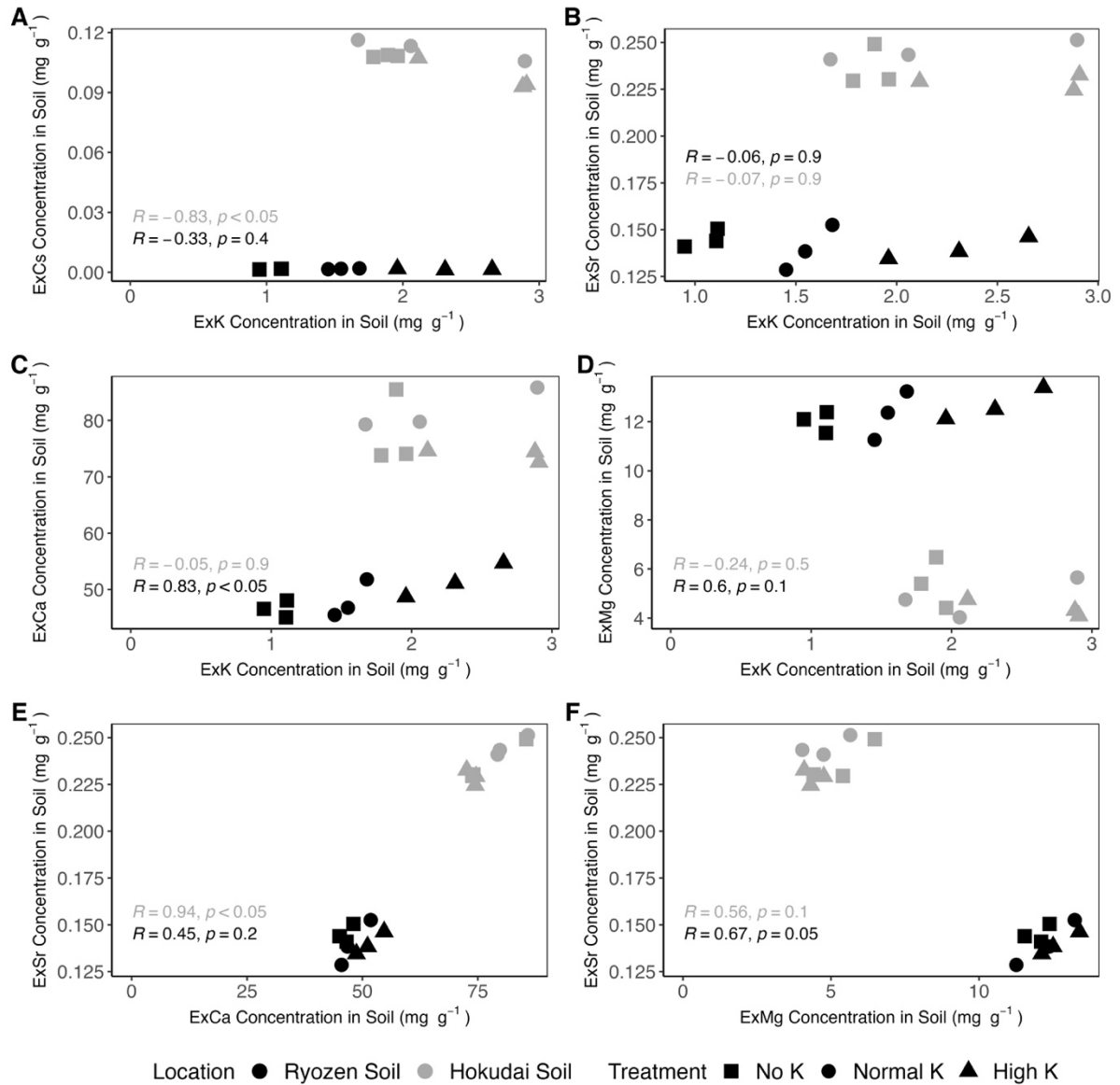
Location	Treatment	Li*	Na*	Rb*	Ba**
		$\mu\text{g pot}^{-1}$	mg pot^{-1}	mg pot^{-1}	$\mu\text{g pot}^{-1}$
Ryozen soil	No K	3.7 ± 0.53 a	37.81 ± 2.83 a	0.572 ± 0.028 a	0.219 ± 0.028 a
	Normal K	3.4 ± 0.3 a	33.29 ± 1.77 a	0.587 ± 0.006 a	0.154 ± 0.042 a
	High K	4.4 ± 0.21 a	19.49 ± 2.14 b	0.414 ± 0.029 b	0.144 ± 0.044 a
Hokudai soil	No K	27.4 ± 3.38 a	1.22 ± 0.08 a	1.037 ± 0.036 b	0.257 ± 0.005 a
	Normal K	37.2 ± 8.01 a	102.25 ± 52.6 a	1.509 ± 0.057 a	0.094 ± 0.03 b
	High K	28.5 ± 5.84 a	69.78 ± 10.85 a	1.304 ± 0.138 ab	0.069 ± 0.005 c
Location	Treatment	As***	Fe***	Mn***	Zn***
		$\mu\text{g pot}^{-1}$	mg pot^{-1}	mg pot^{-1}	mg pot^{-1}
Ryozen soil	No K	1.7 ± 0.22 b	2.56 ± 0.37 a	1.49 ± 0.15 a	0.26 ± 0.02 a
	Normal K	2.4 ± 0.17 ab	2.27 ± 1.23 a	1.58 ± 0.12 a	0.29 ± 0.01 a
	High K	2.6 ± 0.23 a	3.88 ± 0.12 a	1.67 ± 0.011 a	0.30 ± 0.02 a
Hokudai soil	No K	10 ± 2.77 a	9.58 ± 3.04 a	2.76 ± 0.10 a	0.70 ± 0.01 a
	Normal K	4.8 ± 0.44 a	3.1 ± 0.27 a	3.77 ± 0.42 a	1.33 ± 0.17 a
	High K	5.2 ± 0.73 a	4.22 ± 0.79 a	3.48 ± 0.45 a	1.20 ± 0.16 a
Location	Treatment	Cu**	Al***	Cd***	Co***
		$\mu\text{g pot}^{-1}$	mg pot^{-1}	$\mu\text{g pot}^{-1}$	$\mu\text{g pot}^{-1}$
Ryozen soil	No K	39 ± 4.91 a	0.77 ± 0.15 a	7.5 ± 1.38 a	2.3 ± 0.17 a
	Normal K	47.9 ± 1.51 a	0.85 ± 0.16 a	7.9 ± 0.41 a	3.1 ± 0.28 a
	High K	52.3 ± 3.44 a	1.27 ± 0.05 a	7.9 ± 0.45 a	3.3 ± 0.29 a
Hokudai soil	No K	37.5 ± 2.3 a	4.7 ± 1.58 a	7.3 ± 0.32 a	5.2 ± 0.62 a
	Normal K	51.8 ± 5.4 a	1.16 ± 0.21 a	17.7 ± 4.25 a	5.9 ± 0.71 a
	High K	55 ± 8.1 a	1.77 ± 0.42 a	13.7 ± 2.47 a	5.7 ± 0.59 a
Location	Treatment	Ni***			
		$\mu\text{g pot}^{-1}$			
Ryozen soil	No K	14.4 ± 1.86 a			
	Normal K	17.1 ± 2.31 a			
	High K	19.8 ± 0.31 a			
Hokudai soil	No K	51.5 ± 14.1 a			
	Normal K	26.7 ± 1.08 a			
	High K	30.3 ± 4.86 a			

Number after $\pm$ denote the standard error. Different letters represent significant differences according to the Tukey's test ($p < 0.05$) following a one-way ANOVA ($n = 3$). *Alkali metals; **alkali-earth metals; ***transition-metals element.

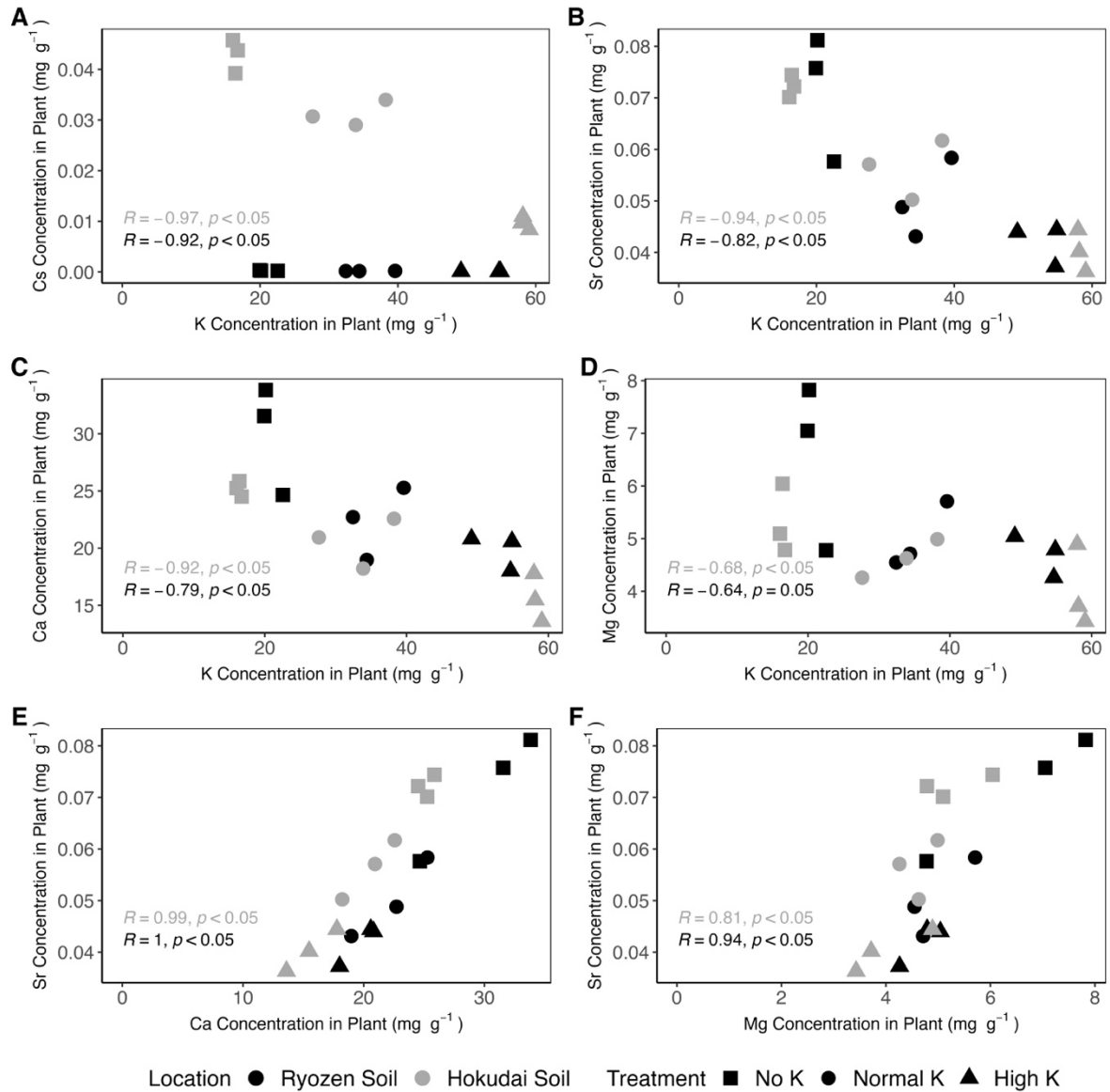
Appendix 8. The effect of K applications on CR other elements of komatsuna plants grown in two different soil types.

Location	Treatment	Li*	Na*	Rb*	Ba**
Ryozen soil	No K	6.36 ± 0.33 a	185.76 ± 14.42 a	143.20 ± 16.56 a	0.90 ± 0.01 a
	Normal K	4.55 ± 0.25 b	62.24 ± 18.20 b	95.65 ± 5.02 ab	0.70 ± 0.19 a
	High K	4.29 ± 0.21 b	41.20 ± 6.34 b	66.28 ± 3.75 b	0.74 ± 0.19 a
Hokudai soil	No K	17.80 ± 2.32 a	2.79 ± 0.16 a	101.59 ± 5.11 a	4.01 ± 0.017 a
	Normal K	11.17 ± 1.82 a	167.93 ± 79.12 a	95.31 ± 3.69 a	0.79 ± 0.002 b
	High K	8.83 ± 0.85 a	93.86 ± 17.94 a	86.97 ± 2.95 a	0.52 ± 0.001 b
Location	Treatment	As***	Fe***	Mn***	Zn***
Ryozen soil	No K	13.99 ± 1.51 a	106.97 ± 15.23 a	13.09 ± 1.59 a	763.33 ± 266.12 a
	Normal K	15.50 ± 1.45 a	73.13 ± 33.71 a	11.81 ± 1.39 a	184.10 ± 76.57 a
	High K	15.78 ± 0.93 a	129.47 ± 6.08 a	12.54 ± 1.09 a	168.56 ± 50.44 a
Hokudai soil	No K	35.90 ± 7.37 a	269.03 ± 53.82 a	21.89 ± 2.07 a	149.47 ± 5.84 a
	Normal K	10.87 ± 1.37 b	64.52 ± 6.47 b	14.59 ± 0.62 ab	154.10 ± 7.13 a
	High K	11.58 ± 0.34 b	96.94 ± 7.36 b	12.83 ± 1.16 b	136.98 ± 3.45 a
Location	Treatment	Cu**	Al***	Cd***	Co***
Ryozen soil	No K	53.05 ± 2.61 a	345.25 ± 131.76 a	93.43 ± 11.10 a	20.10 ± 1.90 a
	Normal K	59.24 ± 3.73 a	195.47 ± 95.18 a	61.86 ± 19.07 a	23.71 ± 3.64 a
	High K	54.27 ± 4.25 a	355.12 ± 100.94 a	68.26 ± 3.73 a	20.63 ± 1.42 a
Hokudai soil	No K	95.19 ± 7.17 a	395.98 ± 99.97 a	20.74 ± 1.50 a	29.43 ± 0.35 a
	Normal K	40.56 ± 2.79 b	42.47 ± 12.58 b	27.42 ± 3.96 a	14.55 ± 0.06 a
	High K	57.38 ± 7.17 b	72.19 ± 12.08 b	21.71 ± 2.22 a	13.16 ± 0.06 a
Location	Treatment	Ni***			
Ryozen soil	No K	38.83 ± 1.72 a			
	Normal K	25.52 ± 3.86 b			
	High K	40.05 ± 0.18 a			
Hokudai soil	No K	73.93 ± 14.47 a			
	Normal K	23.13 ± 1.64 b			
	High K	24.36 ± 1.49 b			

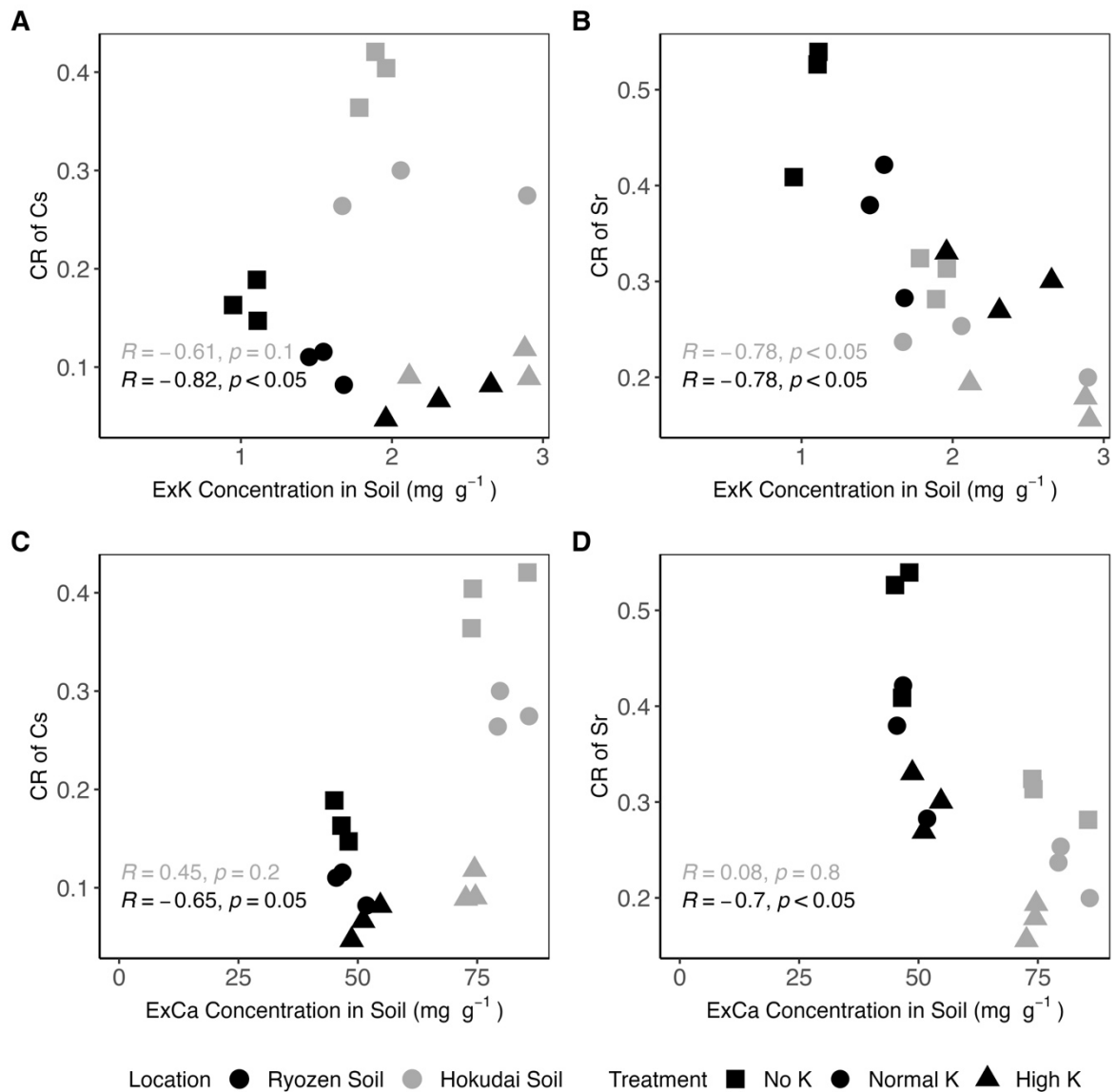
Number after ± denote the standard error. Different letters represent significant differences according to the Tukey's test ($p < 0.05$) following a one-way ANOVA ($n = 3$). * Alkali metals; ** alkali-earth metals; *** transition-metals element.



Appendix 9. Pearson's correlation between element concentrations on Komatsuna after K applied in two different soil types: (a) soil ExK and soil ExCs, (b) soil ExK and soil ExSr, (c) soil ExK and soil ExCa, (d) soil ExK and soil ExMg, (e) soil ExSr and soil ExCa, and (f) soil ExSr and soil ExMg.



Appendix 10. Pearson's correlation between element concentrations on Komatsuna after K applied in two different soil types: (a) plant K and plant Cs, (b) plant K and plant Sr, (c) plant K and plant Cs, (d) plant K and plant Mg, (e) plant Sr and plant Ca, and (f) plant Sr and plant Mg.



Appendix 11. Pearson's correlation between (a) soil ExK and concentration ratio of Cs, (b) soil ExK and concentration ratio of Sr, (c) soil ExCa and concentration ratio of Cs, and (d) soil ExCa and concentration ratio of Sr.