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1 Potassium applications reduced cesium uptake and altered strontium

2 translocation in soybean plants

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Abstract 13

After the Tokyo Electric Power Company's Fukushima Dai-ichi Nuclear Power Plant accident 14 15 in 2011, radioactive cesium (RCs) was released in greater concentrations than radioactive strontium (RSr) in the surrounding environment. Most of the countermeasures were developed 16 17 to mitigate the RCs transfer from the soil to plants. However, to avoid what has happened after 18 the Chernobyl and Mayak accidents, preventing the transfer of RSr from soil to plants should 19 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 20 21 implementation increases the cost and labor requirements. Considering the preparedness for 22 nuclear accidents, it remains unclear how this countermeasure will be affected if RCs and RSr 23 are released simultaneously. We aimed to explore the effect of K applications on cesium (Cs) 24 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, 25 using different K applications (i.e., no, normal, and high K applications). The dry weight and 26 mineral concentrations of K, Cs, Sr, calcium (Ca), magnesium (Mg), and nitrogen (N) 27 28 concentration in plants and exchangeable K (ExK), exchangeable Cs (ExCs), exchangeable Sr (ExSr), exchangeable Ca (ExCa), exchangeable Mg (ExMg), NH₄⁺ (ammonium), and NO₃⁻ 29 30 (nitrate) concentrations in the soils were evaluated. This study revealed that K application 31 reduced Cs, Ca, and Mg uptake but did not affect the ExSr, ExCa, and ExMg concentrations in 32 the soil and did not change the uptake of Sr. On the other hand, K concentration of the plant 33 especially at later growth stage, which indicates re-translocation of Sr was negatively regulated 34 by K concentration.

- Keywords: Soybean; cesium; strontium; countermeasure; potassium. 35 36

37 **1. Introduction**

The earthquake and subsequent tsunami that occurred at the Tokyo Electric Power Company's 38 39 Fukushima Dai-ichi Nuclear Power Plant (FDNPP) in 2011 released several radionuclides including radioactive cesium (RCs) and radioactive strontium (RSr) to eastern Japan (Morino 40 et al. 2011). RCs have relatively long half-lives (e.g., 30.2 years for ¹³⁷Cs and 2.06 years for 41 ¹³⁴Cs) and high-energy emissions of β and γ radiation. ⁹⁰Sr is a β -ray emitting radionuclide 42 with a high fission yield, relatively half-life (29 years), and high transferability to plants 43 44 (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 45 46 soil to plants also needs to be prevented to avoid what has happened in the previous Chernobyl 47 and Mayak accidents (Chu et al. 2015).

After the FDNPP accident, the application of potassium (K) fertilizers was the most 48 49 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; 50 51 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. 52 53 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 54 55 evaluated. This may help support and validate the application of K to reduce the absorption of 56 RCs and RSr in the event of a future incident.

57 The addition of K application to the soil may also change the soil's nutrient balance, which impacts on plant growth. Nitrogen (N) is a determinant element in soybean growth 58 59 (Osaki et al. 1992). Previous reports have shown that cesium (Cs) uptake can increase with increasing nitrogen (N) concentration and decreasing K concentration in plants (Evans and 60 Dekker 1969; Belli et al. 1995). The uptake of elements such as Cs, Sr, K, and calcium (Ca) 61 are positively correlated with each other (Chu et al. 2015) but their interaction with N remains 62 63 poorly understood. Thus, a study of the role of these various nutrients in response to the effect 64 of K application to reduce the rate of radionuclide transmission from soil to plants is necessary 65 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. 66

In this study, we 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.

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2. Material and methods

2.1. Experimental design

79 The experiment was conducted at Date City in Fukushima Prefecture, Japan in 2019 and 2020. 80 The soil type is gray lowland soil, based on the classification of cultivated soils in Japan. Each field was fertilized before cultivation with the following nutrients: 20 kg N ha⁻¹ ((NH₄)₂SO₄) 81 before the seedling stage, and 60 kg N ha⁻¹ after the flowering stages, and 100 kg P_2O_5 ha⁻¹ 82 $(Ca_3(PO_4)_2)$ with 1000 kg ha⁻¹ magnesium lime applied on the same day. The experimental 83 treatments comprised three application levels of K: no K application, normal K application 84 (increasing the level of exchangeable K (ExK) up to 25 mg K₂O 100 g⁻¹ and applying 100 kg 85 K_2O ha⁻¹), and high K application (increasing the level of ExK up to 45 mg K_2O 100 g⁻¹ and 86 applying 100 kg K₂O ha⁻¹). The amount of K (100 kg K₂O ha⁻¹) was applied as potassium 87 sulfate (K₂SO₄, 50.0% K₂O). In 2019, 132 g/22.5 m² of K was applied to increase the level as 88 normal K application and 551 g/22.5 m² of K was applied to increase the level as high K 89 application. In 2020, 0 mg/22.5 m² of K was applied to increase the level as a normal K 90 application, and 664 mg/22.5 m² to increase the level as a high K application. A randomized 91 block design with three replications in each field was used. Soybeans (Glycine max (L.) Merr. 92 93 var. Tachinagaha) were sown on May 27 and harvested on October 28, 2019; and sown on June 94 23 and harvested on October 26, 2020.

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2.2. Sample collection

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

101 Soil samples were collected from a depth of 15 cm around the plant roots using a worm 102 scoop (Fujiwara Scientific Co., Ltd.). Soil samples were air-dried at 40 °C for 1 week and then 103 passed through a 2.0 mm sieve, for chemical analysis. 104 105

2.3. Mineral element measurements

106 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 incubated 107 108 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 109 110 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 111 112 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: 113 114 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 115 H₂SO₄ (98%)-H₂O₂. 116

For elemental analysis of the soil, ExK, exchangable Cs (ExCs), exchangeable Sr 117 118 (ExSr), exchangeable Ca (ExCa), and exchangeable Mg (ExMg) were extracted from dried soil 119 samples at a soil:solution ratio of 1:10 in 1 M ammonium acetate (NH₄OAc) with shaking for 120 1 h. Soil ExK, ExCs, ExSr, ExCa, and ExMg concentrations were measured using ICP-MS. 121 For the analysis of inorganic N, ammonium (NH_4^+) and nitrate (NO_3^-) in the soil, the dried soil 122 samples (4 g) were shaken with 2 M KCl (40 mL) in 50 mL polycarbonate tubes on and end-123 to-end shaker (150 rpm, 60 min). The soil was filtered using filter paper, and the concentrations in the supernatant were determined by colorimetric assays at 630 and 538 nm with a microplate 124 125 reader (BioTek EPOCH² Microplate Reader) for ammonium and nitrate, respectively.

For measurement of ammonium, we used the Indophenol method according to 126 127 (Scheiner 1976), with minor modifications. The supernatant was prepared by adding 1 mL of 128 the sample solution, 2 mL of distilled water, and 1 mL of a combination of A and B color-129 developing liquids (0.4 mL of A and 0.6 mL of B color-developing liquids). The A colordeveloping liquids are containing 15 g of phenol dissolved in 200 mL buffer solution and 130 adjusted to 250 mL by adding 0.05 g of sodium nitroprusside. The B color-developing liquids 131 are containing 40 mL of 1 M NaOH to 1 mL of sodium hypochlorite solution and adjust to 100 132 133 mL with distilled water. Before measuring the absorbance, the supernatant was well stirred and 134 left for 60 minutes.

For the measurement of nitrate, we used the Ando and Ogata method (Ando and Ogata 136 1980). Prepare for 2.5 M ammonia solution by adjusting 16.7 mL of 28% special grade 137 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 138 water (28%), then adjusts to 1 L with distilled water. The first color former solution was 139 prepared by adding 100 mL of distilled water and 100 mL of concentrated HCl to 500 mg of 140 sulfanilamide to dissolve and then adjust to 1 L with distilled water. The second color former 141 142 solution was prepared by dissolving 50 mg of N-1-Naphthylethylenediamine dihydrochloride 143 $(C_{12}H_{16}Cl_2N_2)$ with distilled water and adjusting to 1 L. The reduction auxiliary stock solution 144 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 15 145 146 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 147 148 was well stirred and left for 10 minutes before measuring the absorbance.

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2.4. Statistical analyses

Data were statistically analyzed using SPSS Statistics 25 (SPSS Inc., Chicago, IL, USA). Oneway 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.

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3. Results 159

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 S1. 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.

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3.2. Effect of K application on mineral elements in plant

167 The concentrations of K, Cs, Sr, Ca, Mg, and N in various parts of the soybean plants in 168 response to K applications are shown in Figures 1 and 2 for 2019 and 2020, respectively. The 169 K concentration in all plant organs (stems, leaves, pods, and seeds) differed significantly 170 among K applications in both years, except for pods and seeds at maturity, and stems, leaves, 171 and seeds at harvest in 2019. The 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.

179 Except for the leaves at the pod formation and maturity stages in 2019 and the flowering 180 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. For example, the 181 182 largest decrease in the Sr concentration of leaves was observed in high-K applications at the maturation stage in 2020. However, the Ca concentration in leaves and stems relatively 183 184 decreased with increasing K application levels at all growth stages in both years, except for the 185 vegetative and harvest stages in 2019, and the vegetative stage in 2020. The largest decrease in Ca concentration was observed in pods at the harvest stage in 2020. In contrast, the Ca 186 187 concentration of the stems increased with increasing K application level at the harvest stage in 188 2019. Moreover, Mg concentration in all plant organs differed significantly among K 189 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 190 191 application has generally led to decreased Mg concentrations in the plant parts. For example, the Mg concentration of leaves at the maturation stage in 2020 was 76 times lower with high 192 193 K application than without K application.

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.

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3.3. Effect of K application on mineral elements in soil

The ExK, ExCs, ExSr, ExCa, ExMg, ammonium, and nitrate concentrations of the soil in response to K applications are shown in Figure 3. 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.

216 The ammonium concentration in the soil generally decreased with increasing the K 217 application levels at the harvest stage in 2019 and from flowering to harvest stages in 2020. 218 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 219 220 vegetative to maturity stages in 2019 and the vegetative stage in 2020. However, the nitrate 221 concentration in the soil was reduced by increasing the K application from pod formation to 222 harvest in 2019 and from flowering to harvest in 2020. A large decrease in nitrate concentration 223 in the soil was observed during the pod formation stage in 2019 with high K application. The 224 reduction in nitrate in the soil was two times higher with K application than without K 225 application at the pod formation stage in 2019. In contrast, the difference in nitrate 226 concentration was relatively small during the vegetative and flowering stages in 2019 and the 227 vegetative stage in 2020. Moreover, the nitrate concentration in 2020 increases from flowering 228 to pod formation.

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3.4. Correlations among mineral elements in plant and soil

The Pearson correlation analysis was performed to establish the correlation between the 231 concentration of each pair of elements in plants (K-Cs, K-Sr, K-Mg, Sr-Ca, Sr-N, and Sr-Mg) 232 and soil (ExK-ExCs, ExK-ExSr, ExK-ExMg, ExSr-ExCa, ExSr-ExMg, ExSr-ammonium, and 233 234 ExSr-nitrate) under K application (Table 1). The Pearson correlation analysis of mineral 235 elements in plants showed that K concentration was significantly negatively correlated with Cs 236 and Sr concentrations in both years, and but only with Mg concentrations in 2020 (Figure S2). 237 The Sr concentration was significantly positively correlated with Ca and Mg concentration in 238 both years and significantly inversely correlated with N concentration in 2020 (Figure S3). In order to further elucidate the relationship between K and Sr, we explored its relationship in 239

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 4).

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 (Figure S4). The ExSr concentration in the soil was significantly positively correlated with the ExCa and ExMg concentrations in the soil in both years (Figure S5). 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 (Figure S5).

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4. Discussion

252 Since the nuclear incident in the FDNPP, the practice of applying fertilizer in cultivation 253 activities has been recommended as a preventive measure to reduce RCs transmission to the 254 edible parts of plants. The application of K in cultivation has successfully reduced the rate of 255 RCs translocation (e.g., Kato et al. 2015). However, there is no explanation for how this 256 countermeasure relates to Sr. It is necessary to consider the simultaneous release of RCs and 257 RSr into the environment should another nuclear accident occur. This implementation may also 258 affect the nutritional balance and the state of other elements such as K, Cs, Sr, Ca, Mg and N, 259 and the interrelationships of these elements are poorly understood.

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

262 Except for the pods and seeds at the mature stage and stems, leaves, and seeds at the harvest 263 stage in 2019, the K concentration in all plant parts differed significantly among K application 264 in both years (Figures 1 and 2). The largest difference in K concentration in soybean plants was 265 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 266 concentration in the soil, although the dry weight does not generally vary among the K 267 268 treatments (Figure S1), 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 269 270 decrease in stable Cs concentrations (Figures 1 and 2) in all plant organs. The largest decrease 271 in stable Cs concentration was observed in the seeds at the harvest stage in 2020 with high K 272 application. A similar tendency was also observed in the analysis of RCs uptake behavior using 273 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 274 application effectively reduces RCs (Kato et al. 2015) and stabilizes Cs (Tsukada et al. 2002) 275 in rice. K application might be inhibiting the translocation of Cs from soil to plant regardless 276 of radioactive or stable status due to competition between K and Cs at the root absorption site. 277 278 Plant root cell membranes appear to be mainly involved in Cs uptake via two transport 279 pathways, namely the K transporter and the K channel pathway (Zhu and Smolders 2000). Cs 280 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 281 282 of K transporters with a high affinity for Cs.

The increased K application led to decreased Sr concentration in leaves, Ca 283 284 concentration in the leaves and stems (Figure 1 and 2) as confirmed by previous studies (Neilsen and Edwards 1982; Melnitchouchk and Hodson 2004; Tuma et al. 2004). Additionally, 285 286 Mg concentrations in plant parts were reduced relatively by increased K application (Figure 1 287 and 2) as confirmed by previous studies (Lanyon and Heald 1983; Kang et al. 2019). Our results 288 showed that with a high K application, the leaves at maturation stages in 2020 displayed the 289 largest drop in Sr and Mg concentrations. However, the largest decrease in Ca concentration 290 appeared with high K application in the pods at the harvest stage in 2020. Sr and Ca or Mg are 291 chemically similar and compete for the same receptor sites on biological membranes, but Sr 292 cannot replace Ca or Mg in plants. More than 50% of Sr is absorbed by plants and accumulates 293 in cell walls, similar to Ca (Anupama et al. 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 294 295 Ca applied (Knez and Stangoulis 2021). Ca is involved in the response to external stimuli in 296 major cellular processes, but the response depends on the movement of K across membranes 297 (Sardans and Penuelas 2021) while Sr is transported from the roots via a K plasma membrane 298 transporter (Burger and Lichtscheidl 2019). Moreover, the presence of high Ca concentrations 299 can also lead to Mg deficiency (Hansen and Munns, 1988; Plaut and Grieve, 1988). In case of 300 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 301 302 chlorophyll synthesis, ion transport, and cation balance regulation, in addition to activating 303 more than 300 enzymes (He et al., 2012). As in this study, the decreased Ca concentrations 304 were similar to the decreases in Sr concentrations due to the K application. The Sr and Ca 305 concentrations and also Sr and Mg concentrations had a similar pattern in leaves, i.e., 306 decreasing with increasing K application at the pod formation and maturity in 2019, and at the flowering and maturity stage in 2020 (Figures 1 and 2). Sr may use the same cell entry 307

308 mechanisms as Ca and Mg, such as plasma membrane transporters, due to its physicochemical 309 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 310 K in soil solution might influence the presence of other ions (including K, Ca, Mg and Sr) 311 312 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 313 314 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 315 316 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 317 318 et al. 2016). While these findings suggest that K application in the soils suppresses Sr transport 319 to the leaves but does not affect the soybean's dry weight it might be due to ExMg 320 concentration in the soils (Figure 3) affected to Mg uptake then dry weights as confirmed by a 321 previous study (Hailes et al. 1997). The Mg element is instrumental in forming dry matter and 322 partitioning carbon to sink organs since carbohydrate accumulation in source leaves is reduced by Mg deficiency (Gransee and Fuhrs 2013) as confirmed by our results (Figure 1-2). There is 323 324 a similar pattern between Sr and Mg concentrations in leaves that decreased with increasing K. 325 Despite its small amount in this study, Sr is not detrimental to plant growth, and its uptake is a 326 side effect of divalent cation absorption. Nevertheless, the K level in this study was adequate 327 for seed production.

328 329

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

331 The ExK concentrations in the soil were relatively higher in application with higher K levels 332 in both years, except for the harvest stage in 2020 (Figure 3). This is expected owing to the 333 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 334 stages (harvest) in both years. It might be due to the characteristics of K uptake which is slowed 335 336 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 337 338 they were excluded from this study. Uzoho and Ekeh (2014) reported that soil K status affects 339 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). 340

341 K application generally led to decreased ExCs concentrations in the soil in both years (Figure 3). Increasing ExK in the soil tends to decrease ExCs, which has also been observed in 342 various crops (Kato et al. 2015; Kubo et al. 2015, 2017, 2018; Matsunami et al. 2021). ExK 343 344 concentrations in the soil increased following K fertilizer application and suppressed RCs 345 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 et al. 2013). The 346 347 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 348 349 soil (Figure 3) 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 350 351 Sr) share the same soil particle binding site (Bonomelli et al. 2019). Consequently, excess or deficiency of K can affect the availability of the other cations in the soil, including Sr and Ca 352 353 or Sr and Mg. For example, K application to the soil might decrease the ease of replacing the 354 clay fraction Mg, and consequently, less Mg is available for uptake (Hovland and Caldwell 355 1960). Tuma et al. (2004) revealed that K and Ca could not be handled separately from other 356 nutrients because antagonistic relationships arise. Though we have found that Sr uptake was decreased in the shoot of soybean by the application of K, we did not analyze the root, so the 357 358 possibility of root-to-shoot transfer was decreased by K application is remained. In order to 359 determine whether K application has caused Sr uptake and/or distribution among plants, a 360 confirmation test should be conducted.

The ammonium and nitrate concentrations in the soil (Figure 3) generally decreased 361 362 with increasing K application, as confirmed by previous reports (Beauchamp 1982; Nguyen et 363 al. 2001; Ajazi et al. 2013). Increased K application might result in saturation in the interlayer 364 space in the soil, which decreases ammonium fixation or impairs ammonium release (Scherer 1982; Nieder et al. 2011) and decreases nitrate concentrations in the soil. If plants do not absorb 365 nitrate in the soil solution, it can easily leach because nitrate and soil are negatively charged 366 (Ito 2018). Moreover, nitrate levels in the soil increased from flowering to pod formation in 367 368 2020, might be due to the additional application of N fertilizer in this study that applied after 369 370 flowering stages then influences nitrate concentration in the soil.

4.3. The relationship of Sr with K with the reduction of Cs concentration

371 Correlation analysis showed that the K concentration in the plant organs was significantly 372 negatively correlated with stable Cs and Sr concentrations in the plant organs in both years, 373 and but only with Mg concentrations in 2020 (Figure S2). However, the effect of K application 374 on Sr concentration was not observed unlike Cs or Mg (Figures 1 and 2). These results reveal 375 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 K's relationship with Sr. Though it 376 was also indicated that higher K concentrations in the plant organs were accompanied by lower 377 Cs, Sr, and Mg concentrations in the plant organs after K application, as confirmed by previous 378 379 studies (Kabata-Pendias and Szteke 2015; Myrvang et al. 2016), we have further analyzed the 380 relationship between K and Sr relationship divided by the growth stages and plant organs 381 (Figure 4). 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 382 383 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 384 385 movement in the plant is different. Plants can rapidly transfer K throughout their entire organ system, but Ca becomes largely immobile, and Sr behaves similarly to Ca (Creger and Allen 386 1969; Mengel 1985). It is further confirmed by the differences between plant organs (Figure 387 388 4). Significant negative correlations were observed except for seeds, and the relationship was 389 most obvious in the leaf, and it was also confirmed that the K and Sr decreased with the growth. 390 Since our results only showed a reduction in Sr concentration in the leaves while reductions in 391 Mg concentrations relatively occurred in all plant organs after K application (Figure 1-2), also 392 the Sr concentration was significantly positively correlated with Ca and Mg concentration in 393 both years (Figure S3), it was estimated that the absorption of Sr was not disturbed by the K 394 application while the re-translocation of Sr was disturbed, while it is further requested to 395 investigate how Sr was stored and distributed with the change of K levels in the organs. When 396 Sr was absorbed into plants and their cells using K and Ca channels (Burger and Lichtscheidl 397 2018, 2019), the K effect may have altered the behavior of these channels.

398 Moreover, the ExK concentration was significantly negatively correlated with the ExCs 399 concentration in both years (Figure S4). The ExK concentration in the soil was unlikely to be 400 correlated with the ExSr concentration in the soil and ExMg concentrations in the soil (Figure 401 S4). 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) 402 403 reported on the effects of soil K and Ca on RCs and RSr transfer to lettuce plants, but the 404 relationship of K to Sr has not yet been clarified. Our results revealed that K application directly 405 decreased Cs uptake, and indirectly decreased Sr and Mg uptake by the soybean plants. During 406 root absorption, it may be not only the involvement and competition between K and Cs that 407 influence Cs translocation from soil to plants, 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 408

409 competitively inhibited Mg uptake and resulting in reduced protein synthesis (Guo et al., 2016).
410 An indirect relationship between K suppress the uptake rate of Sr needs further investigation.
411 It is particularly important to examine the mineral balance of the related elements that might
412 support the application of K for reducing RCs and RSr translocation from soil to plants in a
413 preparedness scenario for nuclear accidents.

414 There was significantly positively correlated between Sr with Ca and Sr with Mg 415 concentration occurred in this study, either in plant or soil. However, the role of K in the soilplant interaction between Sr and Ca is not yet clear. The results of this recent study illustrate 416 417 that (1) K application did not affect Sr transport, but it might affect re-translocation of Sr; (2) 418 the link between K and Sr clearly changed with different growth phases, and it was shown that 419 throughout the early growth stages, the Sr concentration was largely steady regardless of the 420 quantity of K in the plant; (3) it is likely that K interacts with both Sr and Ca or Mg when 421 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 422 423 application to the soil, these cations (Ca, Mg and Sr) compete through competition in the apoplast of the root cortex (Smolders et al. 1997). The Sr concentration was small and there 424 425 was no actual reduction was observed by K application, which indicate that K application does 426 not support the idea to reduces RSr. However, as the re-translocation of Sr was reduced when 427 K concentration is low, it is important to investigate whether this mechanism could occur in 428 429 other species and/or in the combination of other minerals in the future.

5. Conclusions

430 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 431 432 Mg concentrations in plant organs but did not affect dry weight. In addition, increased K 433 application typically leads to decreased Sr concentrations in the leaves. K application increased 434 ExK and decreased ExCs, ammonium, and nitrate concentrations in the soil but did not affect ExSr, ExCa, and ExMg. Our findings imply that increasing ExK concentration in the soil 435 suppressed only ExCs and not ExSr concentration, but it might have affected Sr re-436 437 translocation into plant tissues. Though we 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 438 hand, re-translocation of Sr in the plant was negatively affected by K concentration. Research 439 440 on the impact of the exclusive use of K fertilizer on Sr translocation and the involvement of 441 other related mineral elements is required.

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653 Figures and Tables





Figure 1. The 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).



659

Figure 2. The effect of K applications on K, Cs, Sr, Ca, N and Mg concentrations in soybeanplants at Date City in 2020. The vertical bars indicate the standard error of three replicates. The

662 different letters (a, b, ab, and c) represent significant differences (p < 0.05) according to

663 Tukey's test following a one-way ANOVA (n = 3).

664



665

Figure 3. The 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).

670





v



673 applications at Date City in the different growth stages and plant organs.

Parameters	2019		2020	
	Pearson's r	<i>p</i> -value	Pearson's r	<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*

674 **Table 1.** Tabulated summary of Pearson correlation analysis among elements in plant and soil.

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

676 Supplementary online material



677

Figure S1. The 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).





683 Figure S2. Pearson's correlation of K with Cs, Sr, and Mg concentration in soybean plants

684 after K applications at Date City in 2019 and 2020.



685 Treatment ○ No K ● Normal K ◎ High K Stage ○ Vegetative □ Flowering ◇ Pod Formation △ Maturity ▽ Harvest

Figure S3. Pearson's correlation of Sr with Ca, Mg, and N concentration in soybean plantsafter K applications at Date City in 2019 and 2020.

688





690 **Figure S4.** Pearson's correlation of ExK with ExCs, ExSr, and ExMg concentration in the soil

691 after K applications at Date City in 2019 and 2020.



692

Figure S5. 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.

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