Title

Effects of improved sodium uptake ability on grain yields of rice plants under low potassium supply

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Abstract

Sodium uptake is a factor that determines potassium use efficiency in plants as sodium can partially replace potassium in plant cells. Rice (*Oryza sativa*) roots usually exclude sodium but actively take it up when the plant is deficient in potassium. In rice roots, a sodium transporter OsHKT2;1 mediates the active sodium uptake. We previously revealed that variation in the expression of *OsHKT2;1* underlie the variation in sodium uptake between a low-sodium-uptake *indica* cultivar, IR64, and a high-sodium-uptake *japonica* cultivar, Koshihikari. In the present study, we evaluated IR64 and its near-isogenic line IR64-K that carrying *OsHKT2;1* and neighboring genes inherited from Koshihikari for grain yields. IR64-K had a greater average grain yield and harvest index than IR64 in a pot culture experiment with three levels of potassium fertilizer. The differences were most significant under treatment without potassium fertilizer. IR64-K also showed a slightly higher grain yield than IR64 when grown in a paddy field without potassium fertilizer application. These results suggest that the enhanced ability of sodium uptake improves grain yields of rice plants under low-potassium-input conditions.

Keywords: beneficial element, potassium, QTL, rice, sodium

Introduction

Potassium (K^+) is one of the three most limiting nutrients in crop production along with nitrogen and phosphate. The soil supplies K^+ to plants; however, the supply is often inadequate for the K^+ requirement of crops. Thus, the addition of fertilizer K^+ is required for stable food production, and the world consumption of K^+ fertilizer has been growing (IFASTAT, https://www.ifastat.org/databases). Therefore, increasing the efficiency of K^+ fertilizer and reducing the loss of resources is an important issue in both costs of agricultural production and environmental conservation. The overall K^+ fertilizer use-efficiency is determined by multiple factors (White et al., 2021), among which our research focuses on the improvement of K^+ use efficiency in plants.

In plant cells, K^+ remains a free cation, and, as a mobile cation, K^+ regulates membrane electro-potential (Schroeder and Fang, 1991) and cell water potential (Mengel and Arneke, 1982). Therefore, K^+ nutrition is related to the water status and movement of other minerals in plants. It also contributes to phloem transport of assimilates (Mengel and Haeder, 1977; Deeken et al., 2002; Gajdanowicz et al., 2011; Dreyer et al., 2017). Moreover, K^+ in cytosol activates many K^+-dependent enzymes (Evans and Sorger, 1966, Gohara and Di Cela, 2016).

Sodium (Na^+) is unessential for most plants. Excessive accumulation of Na^+ in the cytosol is even harmful (Munns and Tester, 2008); however, a moderate amount of Na^+ is beneficial for many crop species, especially when deficient in K^+ (Lehr et al., 1953; Marschner, 1971; Takahahi & Maejima, 1998; Subbarao et al., 2003; Kronzucker et al., 2013). Na^+ shows many similarities to K^+ in its chemistry and can partly substitute for K^+ in plant cells. Therefore, using Na^+ as an alternative cation is a factor determining K^+ use efficiency in plants. The exact role of

61 Na^+ as an alternative nutrient is not fully proven yet, but it has been thought that Na^+ replaces
62 K^+ as an osmoticum in the vacuole (Marschner, 1971). This notion is because Na^+ is less
63 effective in activating K^+-dependent enzymes (Evans and Sorger, 1966; Page & Di Cera, 2006).
64 While Na^+ plays a role in vacuoles, liberated K^+ could work in the cytosol. Further, it seems
65 also difficult for Na^+ to replace the role of adjusting membrane potential as the ion selectivity of
66 K^+ channels is high (Dreyer and Uozumi, 2011). Meanwhile, an enhancement of Na^+ influx may
67 cause membrane depolarization and affects K^+ movements. Low to moderate Na^+ supply, for
68 instance, increases shoot K^+ content in wheat (*Triticum aestivum*) plants under K^+-deficient
69 conditions (Krishnasamy et al., 2014).
70 Rice (*Oryza sativa*) plants, the staple crop in most Asian countries, get a moderate benefit from
71 Na^+. Rice plants take up little Na^+ when they are sufficient with K^+. However, rice plants
72 actively take up Na^+ under K^+ deficiency (Akai et al., 2012; Hasegawa et al., 1990; Miyamoto et
73 al., 2012), and a sodium transporter, OsHKT2;1, mediates this uptake of Na^+ (Horie et al., 2007).
74 Specific temperate *japonica* cultivars take up more Na^+ than many other cultivars (Miyamoto et
75 al., 2012). We previously detected a major quantitative trait locus for shoot Na^+ concentration in
76 seedlings under K^+ deficiency, located at the distal end of chromosome 6 near *OsHKT2;1*
77 (Miyamoto et al., 2012). Using the map-based cloning method, we narrowed the candidate
78 region to 150-kbp and found that *OsHKT2;1* was still included in 21 genes predicted in that
79 region (Miyamoto et al., 2015). The deduced amino acid sequence of *OsHKT2;1* is identical
80 among rice cultivars with different Na^+ uptake abilities; alternatively, high Na^+ uptake cultivars
81 show higher *OsHKT2;1* expression (Miyamoto et al, 2015). This means that the expression
82 level of *OsHKT2;1* determines the Na^+ accumulation in K^+-deficient rice cultivars. Recently, a

genome-wide association study also indicates the significance of *OsHKT2;1* expression level for the variation in internal K^+ use efficiency among rice cultivars (Hartley et al., 2020). K^+-use efficiency of *indica* rice could be improved using this genetic variation. In the process of previous map-based cloning, we selected several near-isogenic lines that carry *OsHKT2;1* and neighboring genes inherited from a *japonica* cultivar Koshihikari in a genetic background of *indica* cultivar IR64. Young seedlings of those near-isogenic lines showed higher expression levels of *OsHKT2;1* and Na^+ uptake than parental IR64 (Miyamoto et al., 2015); however, we had not evaluated the grain yield of the near-isogenic line yet. Here, we evaluated the growth, grain yield, and uptake of Na^+ and K^+ of IR64 and one of the near-isogenic lines named IR64-K under low-K^+ input.

Materials and methods

Plant materials

Seeds of IR64, an *indica* high-yielding cultivar, were obtained from Rice Genome Resource Center, Tsukuba, Japan. IR64-K, renamed from 2031-15-87-71, is a near-isogenic line with higher Na^+ accumulation than IR64 and was selected in our previous study (Miyamoto et al., 2015) from BC_4F_2 seeds of line 12-4205 that has a recurrent parent of IR64 and a donor parent of Koshhikari (Nagata et al., 2015). The seeds were kindly provided by Dr. Masahiro Yano from the National Institute of Agrobiological Sciences, Tsukuba, Japan. In the parental BC_4F_1 plant of 12-4205, a part of chromosome 1, 6, and 11 were heterozygous. (Nagata et al., 2015). Using simple sequence repeat (SSR) markers (McCouch et al., 2002), we investigated genotypes of these regions in IR64-K. Two marker loci, RM8111 and RM10787, on

105 chromosome 1 were homozygous for the Koshihikari allele, and thus, approximately 6 to 10
106 Mbp region on chromosome 1 is inherited from Koshihikari in IR64-K. Two marker loci,
107 RM280 and RM1812, on chromosome 11 were homozygous for the IR64 allele. As for
108 chromosome 6, a region between two markers RM20657 and RM5814 are homozygous for the
109 Koshihikari allele. The length of this region was in the range of 100–488 kb, and eighty genes,
110 including *OsHKT2;1*, are predicted.

111 **Hydroponic experiment**

112 The culture solution used for the hydroponic experiment contained 0.75 mmol L^{-1} $(NH_4)_2SO_4$,
113 0.25 mmol L^{-1} $(NH_4)_2HPO_4$, 0.5 mmol L^{-1} $CaCl_2$, 0.5 mmol L^{-1} $MgCl_2$, 0.09 mmol L^{-1}
114 $FeC_6H_5O_7 \cdot nH_2O$ and Arnon's micronutrient (cited by Hewitt, 1966) besides varying
115 concentrations of KCl and NaCl. Plants were grown in a growth chamber (NS-280 FHW;
116 Takayama Seisakusyo, Kyoto, Japan) under the following conditions: temperature 30°C,
117 photoperiod 12h, and light intensity 350 μmol m^{-2} s^{-1}.
118 Twenty seeds of IR64 and IR64-K were imbibed in water with a fungicide (Torifumine, Nippon
119 soda co., Tokyo) for two days at 30°C. The imbibed seeds were sown on a nylon-mesh (18
120 mesh, 24 × 36 mm) supported by a plastic frame floating on 1 L of culture solution with 0.75
121 mmol L^{-1} KCl without NaCl. Then, seeds were subsequently sown in each float, and four floats
122 were in the container. The uniform size 7-day-old seedlings were pulled out from the mesh and
123 transplanted into three containers. The seedlings, three IR64 and three IR64-K per container,
124 were held in holes in a plastic plate on a 1-L container with a piece of urethane foam. The KCl
125 and NaCl concentrations in the culture solution were as follows: 0.08 mmol L^{-1} (low) KCl and 0
126 mmol L^{-1} NaCl, 0.08 mmol L^{-1} (low) KCl and 0.38 mmol L^{-1} NaCl, and 0.75 mmol L^{-1}

(sufficient) KCl and 0 mmol L^{-1} NaCl. The culture solutions were not aerated and renewed twice a week. Plants were harvested 14 days after transfer.

Seedling preparation for soil cultures

Imbibed seeds, approximately 500 seeds for each line, were sown on a fertilized granulated soil (Ryujo-Baido, Ibikawa Kogyo co., Ogaki) on May 9, 2018 and May 3, 2019. Seedlings were raised in a glass greenhouse located at the North Campus of Kyoto University, Kyoto, Japan.

Pot culture experiment with three levels of K^+ fertilizer

Dr. Naoki Moritsuka from Kyoto University, Kyoto, Japan, kindly provided the soil used for the pot culture. It was taken from a paddy in the former Experimental Farm of the Graduate School of Agriculture, Kyoto University, located in Takatsuki, Osaka, Japan (Moritsuka et al., 2019). The soil was air-dried, sieved through a 4-mm mesh and used for the culture experiment.

Each 12-kg batch of the soil was put into plastic pots with 0.05 m^2 soil surface area. A total of 18 pots were prepared, and fertilizers were applied to the pots five days before transplanting. Every pot received 2.36 g of $(NH_4)_2SO_4$ and 1.00 g of Na_2HPO_4. Three levels of K (0, 30, and 150 mg K kg^{-1} soil) were applied as KCl. The pots were flooded with deionized water and paddled.

Three seedlings as one hill was transplanted per pot on June 5, 2018. Pots were kept in the greenhouse until maturity, regularly watered with deionized water, and maintained under flooded conditions. During the growth period, plant heights and the number of tillers were determined every week. Above-ground parts of the plants were harvested at maturity on October 5, 2018.

Field experiments

149 Field experiments were conducted from June to September 2018 and 2019 in a farmer's paddy
150 field located in Shugakuin Imperial Villa, Kyoto, Japan. The field area was about 250 m^2. The
151 field was managed under regional farming practice, except for the fertilizer application, by
152 Kyoto Agriculture Research Institute. Each year, ammonium sulfate (60 kg N ha^{-1}) was applied
153 as basal fertilizer after paddling. Phosphate and potassium fertilizers were not applied. Urea (30
154 and 20 kg N ha^{-1} in 2018 and 2019, respectively) was top-dressed before heading.
155 On June 4, 2018 and May 31, 2019, IR64 and IR64-K seedlings, one seedling per hill, were
156 transplanted with spacings of 18 cm between hills and 30 cm between rows. The rows of IR64
157 and IR64-K, 20 rows for each genotype, were arranged alternately. During growing periods,
158 five pairs of adjoining IR64 and IR64-K plants were selected at random every two weeks, and
159 their plant height and number of tillers were measured; then, three of those five pairs were
160 pulled out from the field for measuring the dry weights and K^+ and Na^+ contents. At the harvest
161 stage, ten consecutive hills for each genotype were harvested from seven pairs of adjoining rows
162 evenly distributed throughout the field. Plants were air-dried for three weeks, and the weight of
163 the whole above-ground plants and grains of the 70 plants were determined. Values were
164 converted to weights per area based on the plant density.
165 **Analysis of Na^+ and K^+ in plant samples**
166 Harvested plants were washed with tap water, rinsed with distilled water, blotted dried, and
167 separated into shoots and roots. When necessary, shoots were further separated into panicles and
168 remaining. Samples were dried in an oven at 70°C for two days. After the determination of the
169 dry weight, plant samples were milled into fine powders using a cutter mill. Approximately

100-mg aliquots of samples were digested with HNO_3-H_2SO_4, and the digested samples were filled up to the final volume with 0.1 mol L^{-1} HCl.

K^+ and Na^+ concentrations were determined by flame photometry (AA-6300; Shimadzu, Kyoto, Japan).

Measurement of exchangeable Na^+ and K^+ in soil

Air-dried soil was passed through a 2-mm sieve. Exchangeable-K^+ and Na^+ were extracted with 1 mmol L^{-1} ammonium acetate at soil: solution ratio of 1:20 by shaking 1hr. K^+ and Na^+ concentrations in the filtrated extracts were determined by flame photometry (AA-6300; Shimadzu, Kyoto, Japan).

Results

Na^+ and K^+ uptake property of IR64-K seedling

First, we evaluated the Na^+ and K^+ uptake of IR64 and IR64-K at the seedling stage in hydroponics. The three treatments were 1) sufficient K^+ (0.75 mmol L^{-1}) with Na^+ supplementation (0.38 mmol L^{-1}), 2) low K^+ (0.08 mmol L^{-1}) without Na^+ supplementation, and 3) low K^+ (0.08 mmol L^{-1}) with Na^+ supplementation (0.38 mmol L^{-1}).

The 2-week low-K^+ treatment significantly decreased the shoot K^+ concentration in rice seedlings (Fig. 1a). Control rice plants supplied sufficient K^+ did not accumulate much Na^+ in shoots, even though 0.38 mmol L^{-1} NaCl was added to the culture solution (Fig. 1b). The shoot Na^+ concentration was markedly higher in plants under low-K^+ with Na^+ supplementation, and IR64-K plants accumulated more Na^+ than the IR64 under this treatment (Fig. 1b). K^+ concentrations in roots showed a similar tendency as shoots (Fig. 1c). Roots Na^+ concentration

192 under the low-K^+ with Na^+ was higher than those in other treatments and not significantly
193 different between IR64 and IR64-K plants (Fig. 1d). Consistent with our previous results
194 (Miyamoto et al., 2015), it was confirmed that IR64-K seedlings took up more Na^+ than the
195 IR64.
196
197 **Growth of IR64 and IR64-K plants under different K^+ supply**
198 A pot culture experiment with three K^+ fertilizer levels was performed using K^+-deficient soil.
199 The soil contained 47.3 mg exchangeable-K^+ and 20.0 mg exchangeable-Na^+ per kg of air-dried
200 soil. We would refer to the three K^+ fertilizer levels, none, 30 mg K^+ kg^{-1} soil, and 150 mg K^+
201 kg^{-1} soil, as K0, K30, and K150, respectively. From the early stage after transplanting, it was
202 obvious that K^+-fertilizer application promoted the growth of rice plants (Fig. 2). This activity
203 indicated that plants in K0 pots were in a shortage of K^+. In K0 pots, IR64-K plants started to
204 grow taller than IR64 from the booting stage. A similar trend, though to a lesser extent, was also
205 observed for plants in K30 pots; alternatively, the plant height change over time was similar
206 between the two genotypes in K150 pots (Fig. 2a). The number of tillers was higher in IR64 in
207 K0, and K30 pots and higher in IR64-K in K150 pots, but these differences were not statistically
208 significant (Fig. 2b).
209 The whole above-ground dry weight of plants in full maturity, on average, was not different
210 between IR64 and IR64-K (Fig. 3a, 3g), and alternatively, the dry grain weight was higher in
211 IR64-K plants (Fig. 3b, 3g), indicating that IR64-K had a larger harvest index than IR64 (Fig.
212 3c, 3g). No significant interaction between the K^+ treatment and the genotype was detected for
213 these parameters; however, the largest difference in the grain dry weight between IR64 and

IR64-K was observed in K0 plants. The lower grain yield of IR64-K under K0 treatment was caused mainly by the significant reduction in the filling ratio (Fig. 3d).

The K^+ content in mature rice plants increased with increasing K^+ fertilizer levels (Fig. 3e, 3g). In 150K plants, most of the K^+ was distributed to straw; however, K0 and K30 treatments markedly reduced the ratio of K^+ remained in straw. Under the K0 condition, 47% of K^+ in IR64 and 62% in IR64-K were transported to the grain.

The Na^+ content in rice plants decreased with increasing K^+ fertilizer levels (Fig. 3f, 3g), even though the amount of available Na^+, 560 mg per pot, was the same for all treatments. The Na^+ content in IR64-K plants was higher than in IR64 plants (Fig. 3f, 3g). In both the genotype, little Na^+ was distributed to the grain (Fig. 3f).

Growth and cation uptake of IR64 and IR64-K plants in a paddy field

IR64 and IR64-K plants were grown in a paddy field without K^+ fertilizer application. The field soil contained a moderately low amount of K^+. The exchangeable-K^+ content measured before planting was 103-mg kg^{-1} soil in 2018 and 83-mg kg^{-1} soil in 2019. The exchangeable-Na^+ content was 9-mg kg^{-1} soil in 2018 and 17 mg kg^{-1} soil in 2019. Water in the irrigation canal measured in May 2018 contained 1.5 mg L^{-1} K^+ and 5.1 mg L^{-1} Na^+.

In the two-year experiment, changes in plant growth over time were similar between IR64 and IR64-K (Fig. 4a). A significant interaction in two-way ANOVA with time and genotype as factors were not detected for the shoot, root, and panicle dry weights (Fig. 4d). Any of these parameters, on average, were not significantly different between IR64 and IR64-K; furthermore, the plant height was also not different between IR64 and IR64-K (Supplemental Fig. S1a).

Finally, the number of tillers on average was not different between genotypes in 2018 and higher in IR64-K in 2019 (Supplemental Fig. S1b).

IR64 and IR64-K did not differ in K^+ concentration in straws, grains, and roots (Fig. 4b). The concentration of Na^+ in straw was higher in IR64-K in both years (Fig. 4c). Root Na^+ concentration was not different between IR64 and IR64-K (Fig. 4c). A significant interaction between time and genotype was not detected for cation concentrations, except for the panicle Na^+ concentration in 2019 (Fig. 4d).

In both years, the rice grain yield was slightly higher in IR64-K than in IR64 (Fig. 7).

Discussion

Our pot culture experiment showed that IR64-K plants had a higher average grain yield than IR64 (Fig. 3). Naturally, Koshihikari-derived genes other than *OsHKT2;1* on chromosome 1, chromosome 6, or even other chromosomes might contribute to this yield increment. To elucidate the contribution of *OsHKT2;1*, it is necessary to prepare and examine plants with lesser Koshihikari-derived region. However, the most marked difference in the grain yield between IR64-K and IR64 arose under the K0 treatment, and the filling ratio in K0 plants was significantly higher in IR64-K. Further, IR64-K plants showed a higher Na^+ accumulation. These results indicate that IR64-K and IR64 differently respond to low-K^+ conditions seemingly through the enhanced ability of Na^+ uptake.

The tendency of higher grain yield in IR64-K even under the K150 treatment may be because of the growing condition with restricted root zone and limited supply of K^+ from the environment. On the average of two genotypes, the K^+ content in the whole shoot at harvest was 370, 710,

and 2000 mg for K0, K30, and K150, respectively. The sum of exchangeable and fertilizer K^+ in a pot before transplanting was 570, 930, and 2,400 mg. This means that 65%, 76%, and 83% of such K^+ was accumulated in the above-ground parts at harvest. Readily available K^+ in pots was nearly exhausted during the growing period.

Furthermore, the K^+ requirement of plants varies with the growth stage. In rice plants, the K^+ uptake rate per unit area is maximum at panicle initiation (Hasegawa and Sasaki, 2009). Transient deficiency of K^+ might arise during the peak of K^+ demand, even under K150 treatment. To confirm that the higher grain yield in IR64-K is brought about through the enhanced expression of *OsHKT2;1*, we plan to examine the expression level of *OsHKT2;1* in several growth stages.

A characteristic of IR64-K plants was their larger harvest index than IR64 (Fig. 3c, 3g). Although the amount of Na^+ translocated into grains was significantly higher in IR64-K, Na^+ in grains was little even in IR64-K (Fig. 3g, 3f); therefore, it is unlikely that the difference in Na^+ translocation itself contributes to the difference in the harvest index. Under K0 treatment, the total amount of K^+ was not different between two genotypes, but K^+ in grains was larger in IR64-K plants than in IR64 plants (Fig. 3e). The result indicates that more K^+ was translocated from the leaves to the grains in IR64-K than IR64 under the K0 condition. Since K^+ is necessary for phloem transport of assimilates (Mengel and Haeder, 1977; Deeken et al., 2002; Gajdanowicz et al., 2011; Dreyer et al., 2017), increased Na^+ uptake and consequent more K^+ loading into phloem vessels may be a cause of the high harvest index.

Field-grown IR64-K also had a relatively higher grain yield than IR64 (Fig. 5). The change in dry weights and K^+ concentration during the growing period was similar in IR64-K and IR64,

but Na^+ concentration in shoots was higher in IR64-K (Fig. 4). These results, consistent with pot culture experiment results, suggest that an increase in the ratio of translocation of assimilates to grains contributed to increasing grain yield. In fields, there can be a small but continuous K^+ input from irrigation water and soil (Mikkelsen and Roberts, 2021); therefore, our field trial also suggests that the introduced Koshihikari genes can improve grain yield of IR64 under a moderate shortage of K^+. However, content of K^+ and Na^+ available to plants in soils varies from field to field. As our hydroponic experiment shows, IR64-K plants cannot take up Na^+ when the Na^+ concentration around roots is too low (Fig. 1). We are currently carrying experiments to estimate the level of Na^+ in soils and plants that can effectively mitigate the yield decrease under K^+ deficiency.

Finally, it should also be noted that harvesting is a pathway by which K^+ is removed from agricultural soils (Mikkelsen and Roberts, 2021) and that K^+ replenishment to soils is essential in the long term. We propose that increasing the Na^+ uptake ability of rice plants, combined with proper soil management, will contribute to increase K^+ fertilizer use-efficiency.

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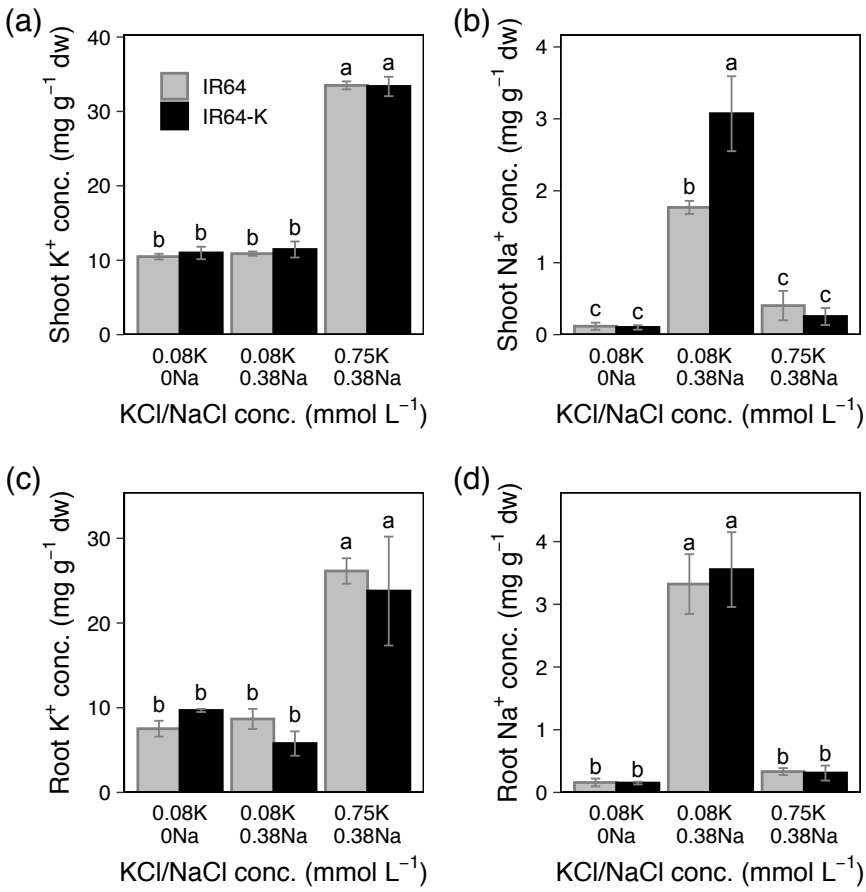
423 **Figure legend**

424 **Figure 1.** K^+ and Na^+ concentrations in 21-day-old IR64 and IR64-K seedlings hydroponically
425 grown under different K^+ and Na^+ supplies. (a) K^+ concentration in shoots, (b) Na^+
426 concentration in shoots, (c) K^+ concentration in roots, and (d) Na^+ concentration in roots. Seeds
427 were sown on a culture solution containing 0.75-mmol L^{-1} KCl. K^+ and Na^+ treatments started
428 seven days after sowing. Three combinations of supplied KCl and NaCl concentrations were as
429 follows: 0.08 and 0, 0.08 and 0.35, and 0.75 and 0.35 mmol L^{-1}. Gray boxes indicate IR64
430 plants, and black boxes indicate IR64-K plants. The data represent means ± SD (n = 3).
431 Different alphabets indicate significant differences among groups ($p < 0.05$, Tukey's test).
432
433 **Figure 2.** Plant height (a) and the number of tillers (b) of IR64 and IR64-K plants over the
434 growth period in pot culture using K^+-deficient soil under various K^+ fertilizer supplies. Three
435 levels of K^+ fertilizer, none (K0), 30 mg (K30), and 150 mg (K150) K^+ per kg soil, were applied
436 before transplanting as KCl. Gray and black circles indicate IR64 and IR64-K plants,
437 respectively. The data represent means ± SD (n = 3). Statistical significance was tested using
438 two-way repeated-measures ANOVA. (c) Plants at the maturing stage. The photos were taken
439 on Sep 2, 2018.
440
441 **Figure 3.** Dry weights, yield components, and concentrations of cations of matured IR64 and
442 IR64-K plants in the pot culture experiment. (a) The total above-ground weight that is expressed
443 as the sum of the weights of straw (solid bar) and grain (empty bar), (b) grain dry weight, and
444 (c) harvest index. (d) Yield components. From left to right: number of panicles per pot, number

445 of spikelets per panicle, percentage of the filling spikelets, and 1000-grain weight. (e) K^+
446 content in straw (solid bar) and grains (empty bar). (f) Na^+ content in straw (solid bar) and
447 grains (empty bar; it is hard to recognize because the values are too small). In panels (a) to (f),
448 gray bars indicate IR64, and black bars indicate IR64-K. Values are expressed as means ± SD (n
449 = 3). Different alphabets indicate significant differences among groups ($p < 0.05$, Tukey's test).
450 (g) Statistical significances tested in the two-way ANOVA with the genotype and K^+-treatment
451 as factors. $^{*}p < .05$; $^{**}p < .01$; $^{***}p < .001$; ns: not significant.
452
453 **Figure 4.** Changes in dry weight and cation concentrations over time in IR64 and IR64-K plants
454 grown in a paddy field without K^+ fertilizer application. (a) dry weight, (b) K^+ concentration,
455 and (c) Na^+ concentration. (d) Statistical significances tested using the two-way ANOVA with
456 the genotype and time as factors. Gray and black symbols indicate IR64 and IR64-K plants,
457 respectively.
458
459 **Figure 5.** Grain yield (a) and whole shoot dry weight (b) of IR64 and IR64-K plants grown in a
460 paddy field without K^+ fertilizer application. Weights per area are calculated based on the
461 weights of 70 hills. Numbers above bars indicate a percentage with IR64 as 100.



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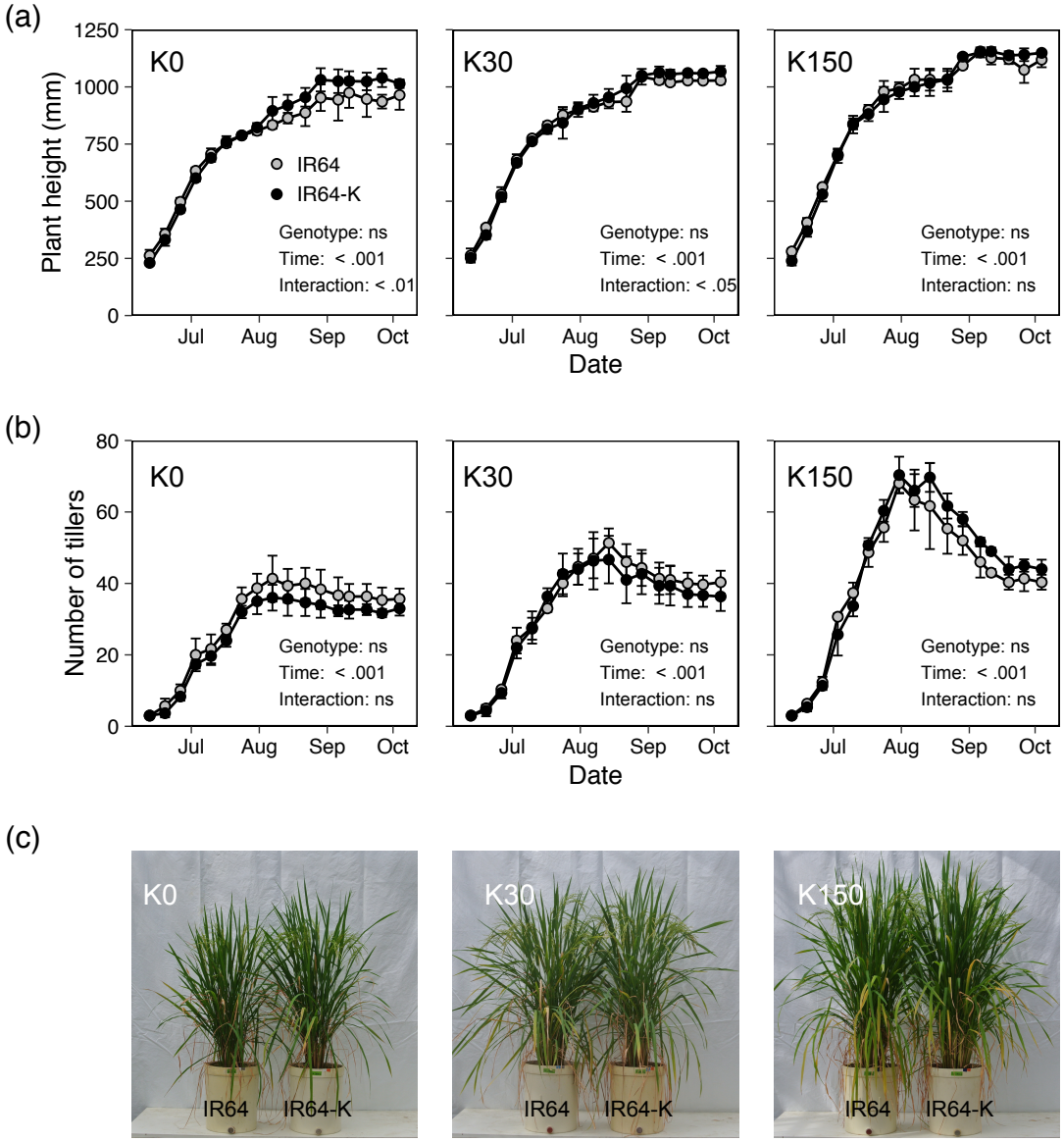

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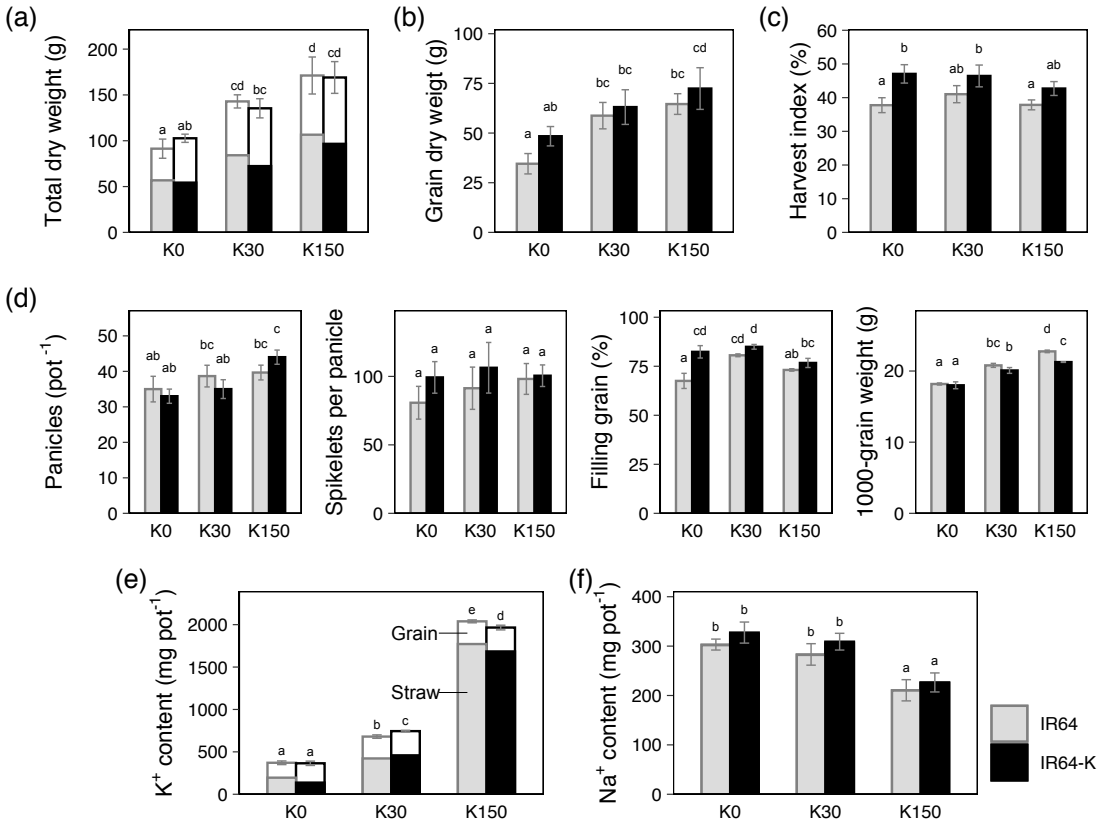
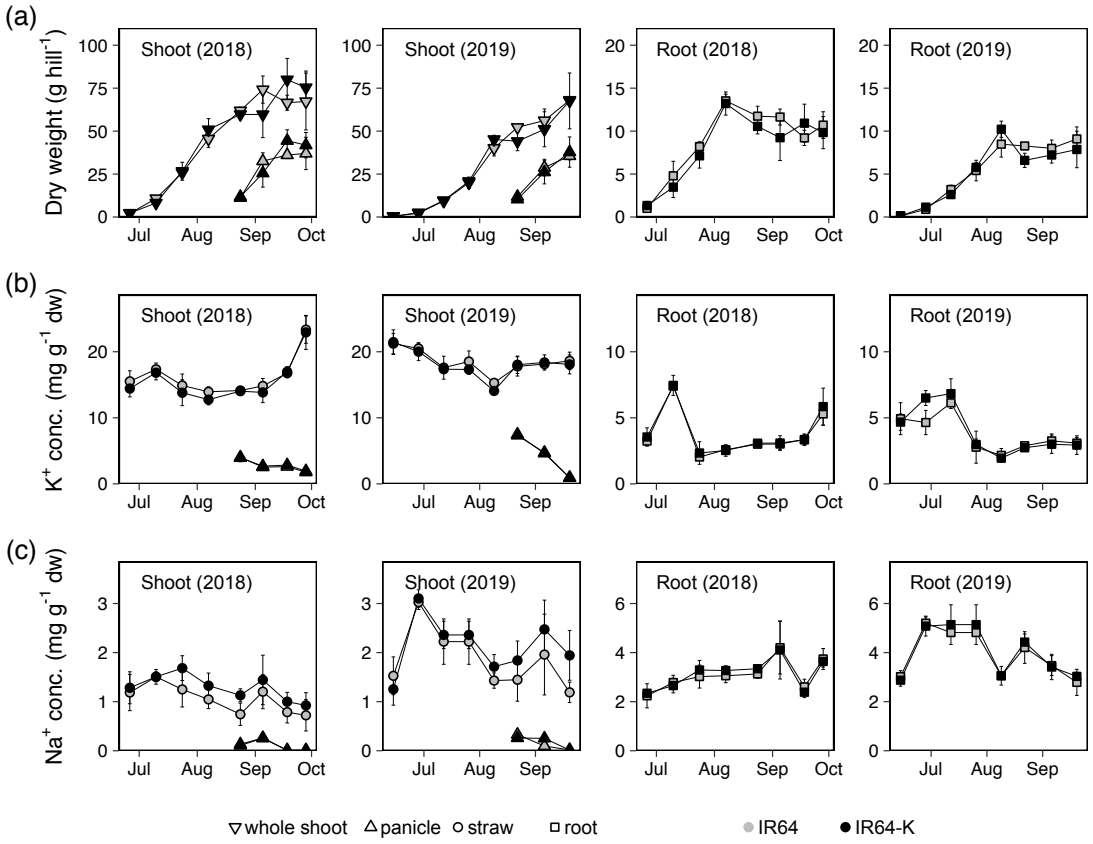


(g)

Two-way ANOVA

	DW_total	DW_straw	DW_grain	Harvest_index	Panicles	Spikelets	Filling_ratio	Thousand_grain	K_total	K_straw	K_grain	Na_total	Na_straw	Na_grain
Treatment	***	***	***	ns	***	ns	***	***	***	***	**	***	***	ns
Genotype	ns	*	*	***	ns	ns	***	***	ns	ns	ns	*	*	*
Interaction	ns	ns	ns	ns	ns	ns	**	**	***	*	ns	ns	ns	ns

Figure 3. Dry weights, yield components, and concentrations of cations of matured IR64 and IR64-K plants in the pot culture experiment. (a) The total above-ground weight that is expressed as the sum of the weights of straw (solid bar) and grain (empty bar), (b) grain dry weight, and (c) harvest index. (d) Yield components. From left to right: number of panicles per pot, number of spikelets per panicle, percentage of the filling spikelets, and 1000-grain weight. (e) K^+ content in straw (solid bar) and grains (empty bar). (f) Na^+ content in straw (solid bar) and grains (empty bar; it is hard to recognize because the values are too small). In panels (a) to (f), gray bars indicate IR64, and black bars indicate IR64-K. Values are expressed as means ± SD (n = 3). Different alphabets indicate significant differences among groups ($p < 0.05$, Tukey's test). (g) Statistical significances tested in the two-way ANOVA with the genotype and K^+-treatment as factors. $^{*}p < .05$; $^{**}p < .01$; $^{***}p < .001$; ns: not significant.

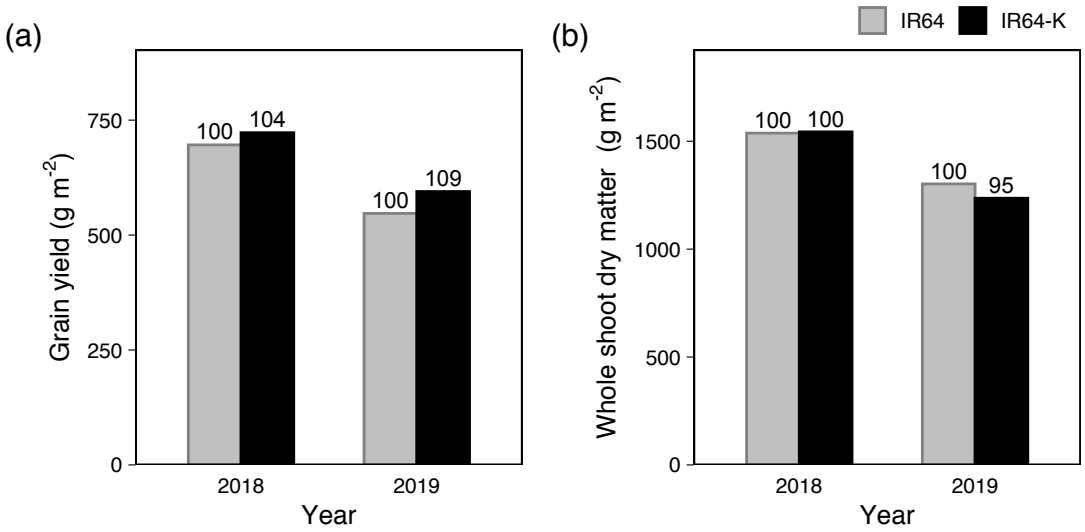


(d)

Two-way ANOVA

	DW_shoot	DW_panicle	DW_root	K_straw	K_panicle	K_root	Na_straw	Na_panicle	Na_root
2018									
Genotype	ns	ns	ns	ns	ns	ns	**	ns	ns
Time	***	***	***	***	***	***	***	***	***
Interaction	ns	ns	ns	ns	ns	ns	ns	ns	ns
2019									
Genotype	ns	ns	ns	ns	ns	ns	*	*	ns
Time	***	***	***	***	***	***	***	***	***
Interaction	ns	ns	ns	ns	ns	ns	ns	**	ns

Figure 4. Changes in dry weight and cation concentrations over time in IR64 and IR64-K plants grown in a paddy field without K^+ fertilizer application. (a) dry weight, (b) K^+ concentration, and (c) Na^+ concentration. (d) Statistical significances tested using the two-way ANOVA with the genotype and time as factors. Gray and black symbols indicate IR64 and IR64-K plants, respectively.



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