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ORIGINAL RESEARCH

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 active sodium uptake. We previously revealed that variation in the expression of *OsHKT2;1* underlies the variation in sodium accumulation between a low-sodium-accumulating *indica* cultivar, IR64, and a high-sodium-accumulating *japonica* cultivar, Koshihikari. In the present study, we evaluated IR64 and its near-isogenic line IR64-K carrying *OsHKT2;1* and neighboring genes inherited from Koshihikari for grain yield. 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 the potassium fertilizer. IR64-K also showed a slightly higher grain yield than IR64 when grown in a paddy field without applying the potassium fertilizer. These results suggest that enhanced sodium uptake ability improves the grain yield of rice plants under low-potassium-input conditions.

KEYWORDS
beneficial element, *Oryza sativa*, potassium, QTL, rice, sodium

1 | 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 mineral and energy 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 & Fang, 1991) and cell water potential (Mengel & 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 (Deeken et al., 2002; Dreyer et al., 2017; Gajdanowicz et al., 2011; Mengel & Haeder, 1977). Moreover, K^+ in cytosol activates many K^+-dependent enzymes (Evans & Sorger, 1966; Gohara & Di Cera, 2016).

Kumiko Ochiai and Kousuke Oba equally contributed to this work.

Sodium (Na^+) is unessential for most plants. Excessive accumulation of Na^+ in the cytosol is even harmful (Munns & Tester, 2008); however, a moderate amount of Na^+ is beneficial for many crop species, especially when deficient in K^+ (Kronzucker et al., 2013; Lehr, 1953; Marschner, 1971; Subbarao et al., 2003; Takahashi & Maejima, 1998). 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 Na^+ as an alternative nutrient is not yet fully proven, but it has been thought that Na^+ replaces K^+ as an osmoticum in the vacuole (Marschner, 1971). This notion is because Na^+ is less effective in activating K^+-dependent enzymes (Evans & Sorger, 1966; Page & Di Cera, 2006). Whereas Na^+ plays a role in vacuoles, liberated K^+ functions in the cytosol. Furthermore, it seems difficult for Na^+ to replace the role of adjusting membrane potential as the ion selectivity of K^+ channels is high (Dreyer & Uozumi, 2011). Notably, Na^+ influx enhancement may cause membrane depolarization and affects K^+ movements. Low to moderate Na^+ supply, for instance, increases shoot K^+ content in wheat (*Triticum aestivum*) plants under K^+-deficient conditions (Krishnasamy et al., 2014).

Rice (*Oryza sativa*) plants, the staple crop in most Asian countries, get a moderate benefit from Na^+. Rice plants take up little Na^+ when they are sufficient with K^+. However, rice plants actively take up Na^+ under K^+ deficiency (Akai et al., 2012; Hasegawa et al., 1987, 1990; Miyamoto et al., 2012), and a sodium transporter, OsHKT2;1, mediates this uptake of Na^+ (Horie et al., 2007). Specific temperate *japonica* cultivars take up more Na^+ than many other cultivars (Miyamoto et al., 2012). We previously detected a quantitative trait locus (QTL) for shoot Na^+ concentration in rice seedlings under K^+ deficiency. The QTL explained 74% of the phenotypic variance located at the distal end of chromosome 6 near *OsHKT2;1* (Miyamoto et al., 2012). Using the map-based cloning method, we narrowed the candidate region to 150 kbp and found that *OsHKT2;1* was still included in 21 genes predicted in that region (Miyamoto et al., 2015). The deduced amino acid sequence of *OsHKT2;1* is identical among rice cultivars with different Na^+-accumulating abilities; alternatively, high Na^+-accumulating cultivars show higher *OsHKT2;1* expression (Miyamoto et al., 2015). This means that the expression 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. However, the effects of Na^+ uptake on rice at the reproductive stage require further clarification because previous studies have only used rice plants at the vegetative stage. Here, we investigated the effect of increased Na^+ uptake ability on the grain yields of rice plants under low-K^+ conditions using a near-isogenic line carrying *OsHKT2;1* and neighboring genes inherited from a high-Na^+-accumulating *japonica* cultivar Koshihikari in a genetic background of low-Na^+-accumulating *indica* cultivar IR64.

2 | MATERIALS AND METHODS

2.1 | 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 *OsHKT2;1* expression and 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 Koshihikari (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 chromosomes 1, 6, and 11 was heterozygous (Nagata et al., 2015). Using simple sequence repeat markers (McCouch et al., 2002), we investigated the genotypes of these regions in IR64-K. Two marker loci, RM8111 and RM10787, on chromosome 1 were homozygous for the Koshihikari allele; thus, an approximately 6–10 Mbp region on chromosome 1 is inherited from Koshihikari in IR64-K. Two marker loci, RM280 and RM1812, on chromosome 11 were homozygous for the IR64 allele. For chromosome 6, a region between the two markers RM20657 and RM5814 was homozygous for the Koshihikari allele. The length of this region was in the range of 100–488 kb, and 80 genes, including *OsHKT2;1*, are predicted.

2.2 | Hydroponic experiment

Culture solution for a hydroponic experiment was prepared with distilled water. The solution contained 0.75 mmol L^{-1} $(NH_4)_2SO_4$, 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} $FeC_6H_5O_7{\cdot}nH_2O$, and Arnon's micronutrient (cited by Hewitt, 1966) besides varying concentrations of KCl and NaCl. Plants were grown in a growth chamber (NS-280 FHW; Takayama Seisakusyo, Kyoto, Japan) under the following conditions: temperature 30°C, photoperiod 12 h, and light intensity 350 μmol m^{-2} s^{-1}.

Twenty seeds of IR64 and IR64-K were imbibed in water with a fungicide (Torifumine, Nippon Soda Co., Tokyo, Japan) for 2 days at 30°C. The imbibed seeds were sown on a nylon mesh (18 mesh, 24 × 36 mm) supported by a plastic frame floating on 1 L of culture solution with 0.75 mmol L^{-1} KCl without NaCl. Ten seeds were sown in each float, and four floats were in the container. The uniform size 7-day-old seedlings were pulled out from the mesh and transplanted into three containers. The seedlings, three IR64 and three IR64-K per container, were held in holes in a plastic plate on a 1-L container with a piece of urethane foam. The KCl and NaCl concentrations in the culture solution were as follows: 0.08 mmol L^{-1} (low) KCl and 0 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.

2.3 | 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, Japan) 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.

2.4 | 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 5 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 were 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.

2.5 | Field experiments

Field experiments were conducted from June to September 2018 and 2019 in a farmer's paddy field located in Shugakuin Imperial Villa, Kyoto, Japan. The field area was about 250 m^2. The field was managed under regional farming practice, except for the fertilizer application, by Kyoto Agriculture Research Institute. Each year, ammonium sulfate (60 kg N ha^{-1}) was applied as basal fertilizer after paddling. Phosphate and potassium fertilizers were not applied. Urea (30 and 20 kg N ha^{-1} in 2018 and 2019, respectively) was top-dressed before heading.

On June 4, 2018, and May 31, 2019, IR64 and IR64-K seedlings, one seedling per hill, were transplanted with spacings of 18 cm between hills and 30 cm between rows. The rows of IR64 and IR64-K, 20 rows for each genotype, were arranged alternately. During growing periods, five pairs of adjoining IR64 and IR64-K plants were selected at random every 2 weeks, and their plant height and number of tillers were measured; then, three of those five pairs were pulled out from the field for measuring the dry weights and K^+ and Na^+ contents. At the harvest stage, 10 consecutive hills for each genotype were harvested from seven pairs of adjoining rows evenly distributed throughout the field. Plants were air-dried for 3 weeks, and the weight of the whole above-ground plants and grains of the 70 plants were determined. Values were converted to weights per area based on the plant density.

2.6 | Analysis of Na^+ and K^+ in plant samples

Harvested plants were washed with tap water, rinsed with distilled water, blotted dried, and separated into shoots and roots. When necessary, shoots were further separated into panicles and remaining. Samples were dried in an oven at 70°C for 2 days. After the determination of the 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-6200; Shimadzu, Kyoto, Japan).

2.7 | 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 mol L^{-1} ammonium acetate at soil:solution ratio of 1:20 by shaking 1 h. K^+ and Na^+ concentrations in the filtrated extracts were determined by flame photometry (AA-6200; Shimadzu, Kyoto, Japan).

3 | RESULTS

3.1 | Na^+ and K^+ accumulation property of IR64-K seedling

First, we evaluated the Na^+ and K^+ accumulation 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 (Figure 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 (Figure 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 (Figure 1b). K^+ concentrations in roots showed a similar tendency as shoots (Figure 1c). Roots Na^+ concentration under the low K^+ with Na^+ was higher than that in roots under other treatments. It was not significantly different between IR64 and IR64-K plants (Figure 1d). Consistent with our previous results (Miyamoto et al., 2015), it was confirmed that IR64-K seedlings took up more Na^+ than the IR64.

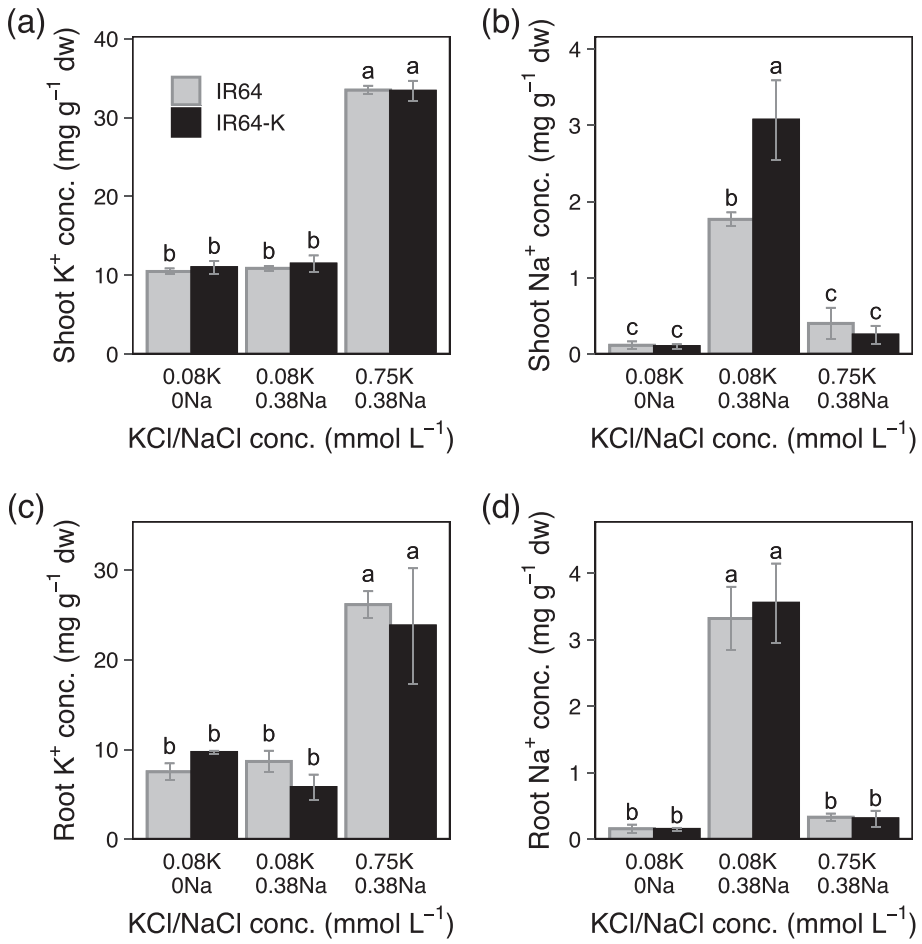


FIGURE 1 K^+ and Na^+ concentrations in 21-day-old IR64 and IR64-K seedlings hydroponically grown under different K^+ and Na^+ supplies. (a) K^+ concentration in shoots, (b) Na^+ concentration in shoots, (c) K^+ concentration in roots, and (d) Na^+ concentration in roots. Seeds were sown on a culture solution containing 0.75 mmol L^{-1} KCl. K^+ and Na^+ treatments started 7 days after sowing. Three combinations of supplied KCl and NaCl concentrations were as follows: 0.08 and 0, 0.08 and 0.38, and 0.75 and 0.38 mmol L^{-1}. Gray boxes indicate IR64 plants, and black boxes indicate IR64-K plants. The data represent means ± SD ($n = 3$). Different alphabets indicate significant differences among groups ($p < 0.05$, Tukey's test)

3.2 | Growth of IR64 and IR64-K plants under different K^+ supply

A pot culture experiment with three K^+ fertilizer levels was performed using K^+-deficient soil. The soil contained 47.3 mg exchangeable K^+ and 20.0 mg exchangeable Na^+ per kg of air-dried soil. We would refer to the three K^+ fertilizer levels, none, 30 mg K^+ kg^{-1} soil, and 150 mg K^+ kg^{-1} soil, as K0, K30, and K150, respectively. From the early stage after transplanting, it was obvious that K^+ fertilizer application promoted the growth of rice plants (Figure 2). This activity indicated that plants in K0 pots were in a shortage of K^+. Visual observations showed that IR64-K plants in K0 pots started to grow taller than IR64 from the booting stage, and plant height measurements showed a significant interaction between time and genotype in K0 treatment (Figure 2a). A similar trend, though to a lesser extent, was also observed for plants in K30 pots; alternatively, the plant height change over time was similar between the two genotypes in K150 pots (Figure 2a). The number of tillers was higher in IR64 in K0 and K30 pots and higher in IR64-K in K150 pots, but these differences were not statistically significant (Figure 2b).

The whole above-ground dry weight of plants in full maturity, on average, was not different between IR64 and IR64-K (Figure 3a,g), and alternatively, the dry grain weight was higher in IR64-K plants (Figure 3b,g), indicating that IR64-K had a larger harvest index than IR64 (Figure 3c,g). No significant interaction between the K^+ treatment and the genotype was detected for 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 under K0 treatment was caused mainly by the significant reduction in the filling ratio (Figure 3d).

The K^+ content in mature rice plants increased with increasing K^+ fertilizer levels (Figure 3e,g). In K150 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 (Figure 3f,g), even though the amount of available Na^+, 560 mg per pot, was the same for all treatments. For Na^+ accumulation, the main effect of genotype was significant at the 5% level (Figure 3g) and the Na^+ content in IR64-K plants was higher than in IR64 plants. In both the genotypes, little Na^+ was distributed to the grain (Figure 3f).

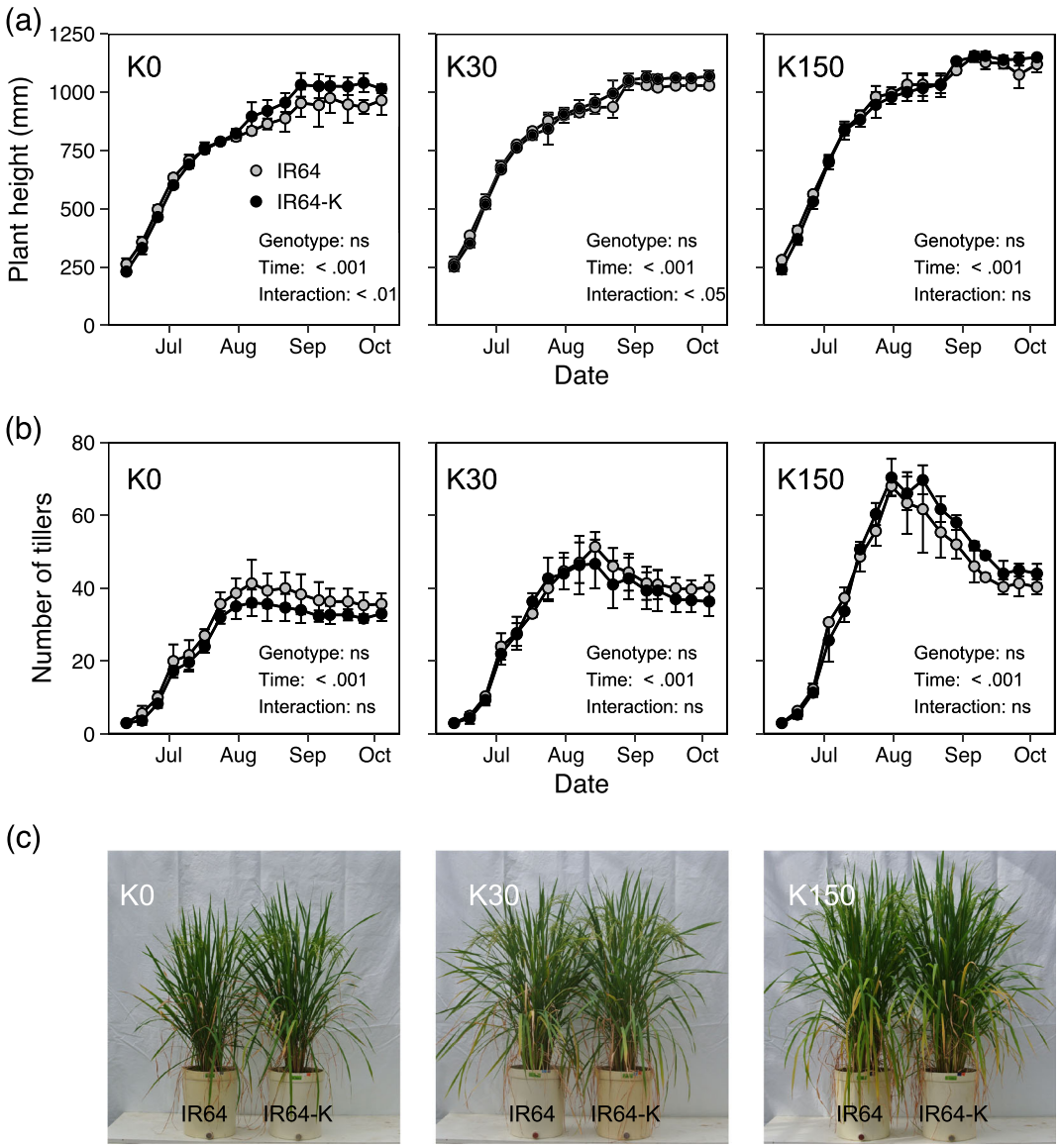


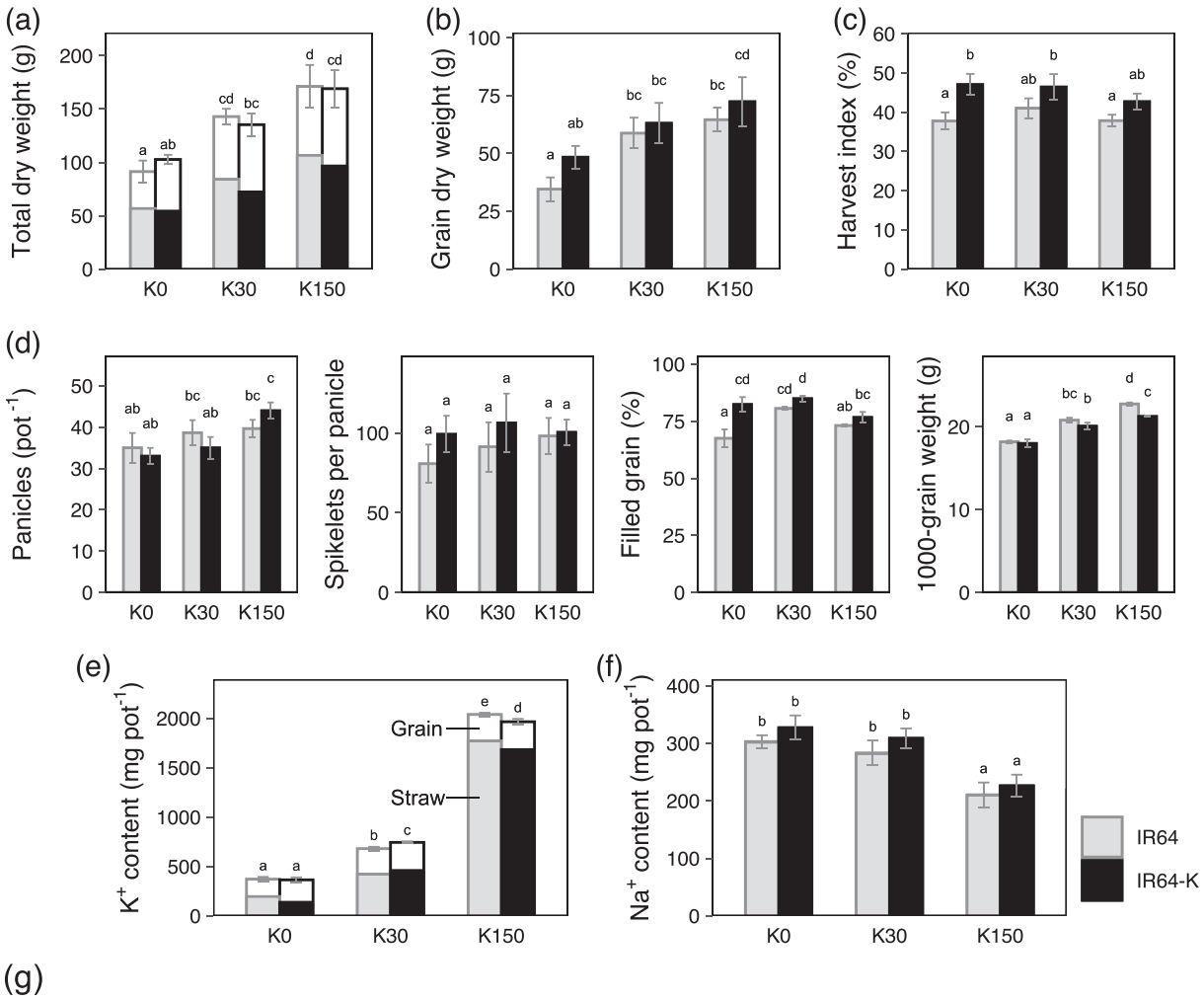
FIGURE 2 Plant height (a) and the number of tillers (b) of IR64 and IR64-K plants over the growth period in pot culture using K^+-deficient soil under various K^+ fertilizer supplies. Three levels of K^+ fertilizer, none (K0), 30 mg (K30), and 150 mg (K150) K^+ per kg of soil, were applied before transplanting as KCl. Gray and black circles indicate IR64 and IR64-K plants, respectively. The data represent means ± SD ($n = 3$). Statistical significance was tested using two-way repeated-measures ANOVA. (c) Plants at the maturing stage. The photos were taken on September 2, 2018

3.3 | Growth and cation accumulation 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 2-year experiment, changes in plant growth over time were similar between IR64 and IR64-K (Figure 4a). A significant interaction in two-way ANOVA with time and genotype as factors was not detected for the shoot, root, and panicle dry weights (Figure 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 (Figure S1a). Finally, the number of tillers on average was not different between genotypes in 2018 and higher in IR64-K in 2019 (Figure S1b).

IR64 and IR64-K did not differ in K^+ concentration in straws, grains, and roots (Figure 4b). The concentration of Na^+ in straw was higher in IR64-K in both years (Figure 4c). Root Na^+ concentration was not different between IR64 and IR64-K (Figure 4c). A significant



(g)

Two-way ANOVA

	Dry weight				Yield components				K^+ content			Na^+ content		
	total	straw	grain	HI	Panicle	Spikelet	Filled grain	1000-grain	total	straw	grain	total	straw	grain
Treatment	***	***	***	ns	***	ns	***	***	***	***	**	***	***	ns
Genotype	ns	*	*	***	ns	ns	***	***	ns	ns	ns	*	*	*
Interaction	ns	ns	ns	ns	ns	ns	**	**	***	*	ns	ns	ns	ns

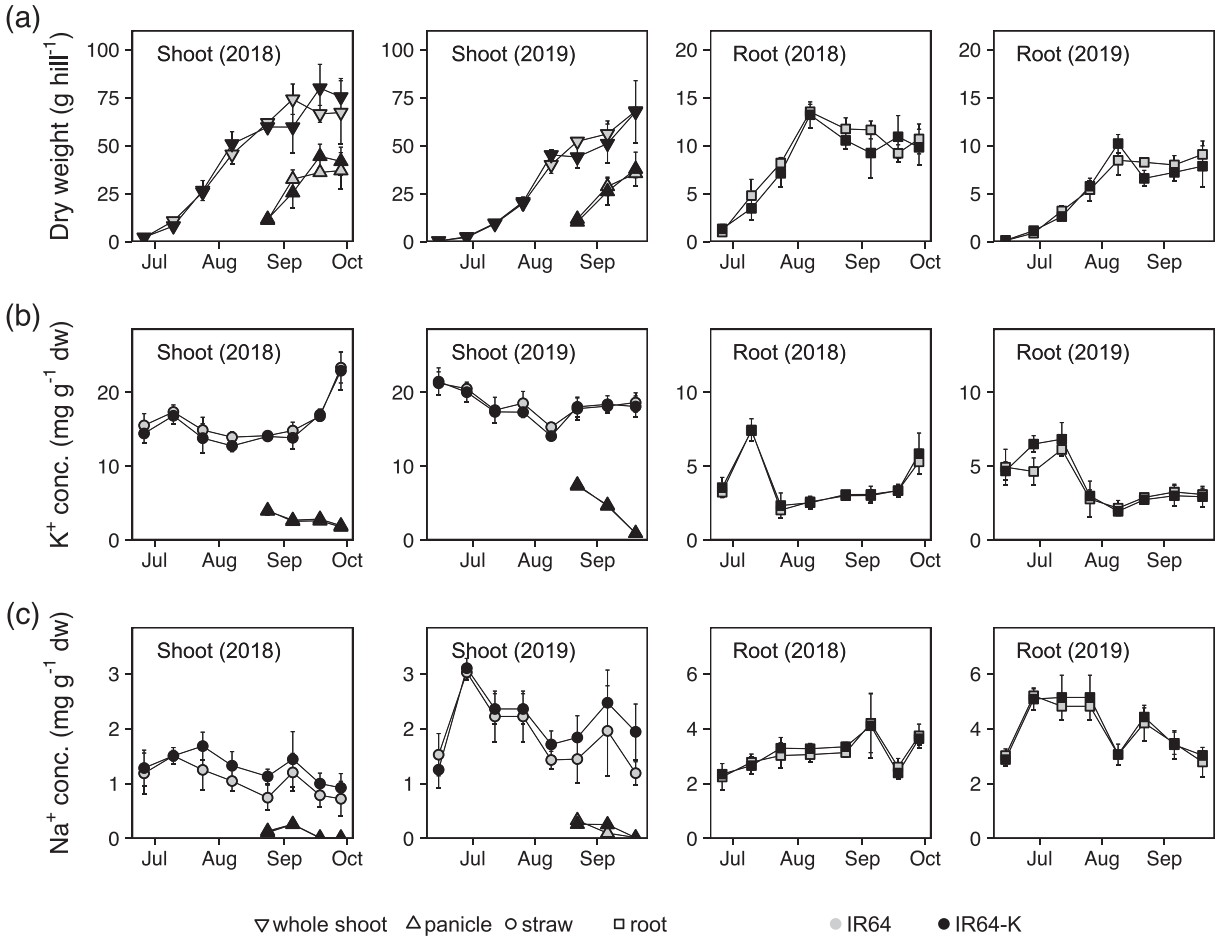
FIGURE 3 Dry weights, yield components, and contents of cations of matured IR64 and IR64-K plants in the pot culture experiment. (a) The total above-ground weight that is expressed as the sum of the weights of straw (solid bar) and grain (empty bar), (b) grain dry weight, and (c) harvest index. (d) Yield components. From left to right: Number of panicles per pot, number of spikelets per panicle, percentage of the filling spikelets, and 1,000-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. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ns, not significant

interaction between time and genotype was not detected for cation concentrations, except for the panicle Na^+ concentration in 2019 (Figure 4d).

In both years, the rice grain yield was slightly higher in IR64-K than in IR64, although the difference was not statistically significant (Figure 5). As a 2-year average, the grain yield of IR64-K increased to 106% of that of IR64.

4 | DISCUSSION

Our pot culture experiment showed that IR64-K plants had a higher average grain yield than IR64 (Figure 3). The most marked difference between IR64-K and IR64 arose under the K0 treatment. Further, in plants under the K0 treatment, there was a significant difference in grain filling rate between the two genotypes. Therefore, our results



(d)

Two-way ANOVA

	Dry weight			K^+ conc.			Na^+ conc.		
	Shoot	Panicle	Root	Straw	Panicle	Root	Straw	Panicle	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

indicate that IR64 and IR64-K have different tolerance to K^+ deficiency when evaluated based on their grain yields.

K^+ deficiency may reduce leaf development, tillering, and each yield component of rice plants (De Datta & Mikkelsen, 1985). Decreased fertility, which is observed in K0 plants but alleviated in IR64-K compared with IR64 (Figure 3d), also has been reported for K^+-deficient rice plants (Chen et al., 2018; De Datta & Mikkelsen, 1985; Hasegawa et al., 1987). Inadequate transport of assimilates from source to sink organs is one cause of sterility under K^+ deficiency (Chen et al., 2018). This is because K^+ in phloem cells is necessary for stabilizing the membrane potential and thus for loading and long-distance transport of assimilates (Deeken et al., 2002; Dreyer et al., 2017; Gajdanowicz et al., 2011; Tian et al., 2021). In K0 plants, K^+ content in grains was higher in IR64-K than in IR64 (Figure 3e). The total K^+ content in whole shoots did not differ between the two rice lines, which suggests that more K^+ was

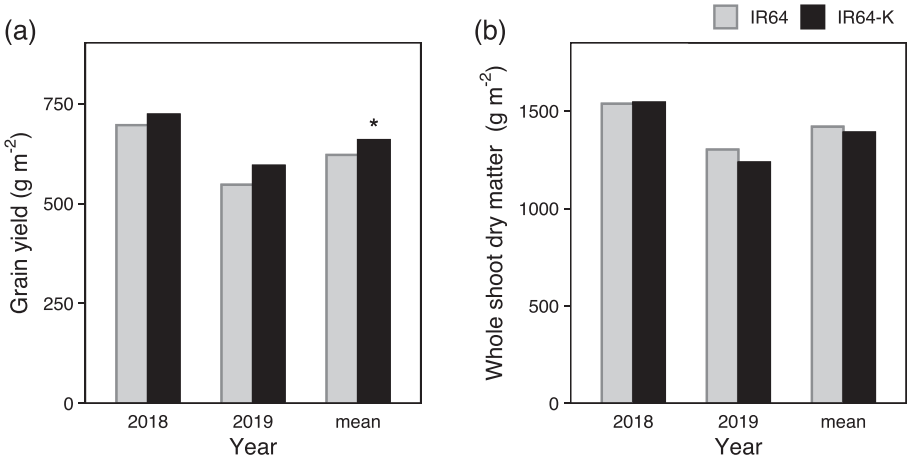


FIGURE 5 Grain yield (a) and whole shoot dry weight (b) of IR64 and IR64-K plants grown in a paddy field without K^+ fertilizer application. Weights per area are calculated based on the weights of 70 hills, 10 hills per block. *$p < 0.05$ was considered significant (one-way ANOVA with blocking)

translocated to the grains from the leaves in IR64-K than IR64. The improvement in grain yield and harvest index of IR64-K may have arisen due to the increased K^+ amount loaded into phloem vessels.

The molar concentration of K^+ in the straw of K150 plants, as an average of both lines, was 0.44 mmol g^{-1} dw, which decreased to as low as 0.077 mmol g^{-1} in K0 plants. In contrast, the total concentration of K^+ and Na^+ was maintained at 0.32 mmol g^{-1} in K0 plants compared with 0.55 mmol g^{-1} in K150 plants (Table S1). The generally accepted role of Na^+ as a substitute for K^+ is that it acts as an osmotic solute in vacuoles (Marschner, 1971); quantitatively, Na^+ is likely to partially compensate for the decrease in K^+ concentration in both lines. The difference in straw Na^+ concentration between the lines was about 10%–20% of the Na^+ concentration in IR64 plants. Although it did not improve vegetative growth (Figure 2), the difference was not negligible.

The extra Na^+ in the straw of IR64-K may have increased K^+ distributed in grains by liberating K^+ from leaf vacuoles. Alternatively, increased Na^+ accumulation may have affected membrane potential. Tian et al. (2021) reported that the K^+ concentration in phloem exudate was higher in salt-stressed rice plants than in control plants and that the phloem K^+ concentration was higher in wild-type plants than in OsAKT2—a phloem localized K^+ channel—knock-out mutant plants. They proposed that accumulated Na^+ in the shoot apoplast may have caused hyperpolarization of the phloem cell membrane and promoted OsAKT2-mediated K^+ loading (Tian et al., 2021). In K^+-deficient rice plants, although the absolute cation concentration is not as high as that in salt-stressed plants, the ratio of Na^+ to K^+ in plants increases. Thus, Na^+ may have some impact on the membrane potential of phloem cells.

The tendency of higher grain yields in IR64-K was observed even under K150 treatment (Figure 3b). This 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 aboveground parts at harvest. This indicates that readily available K^+ in pots was nearly exhausted during the growing period.

Additionally, the shoot Na^+ concentrations of IR64 and IR64-K under K150 treatment—1.98 and 2.33 mg g^{-1} dw, respectively—were considerably higher than previously reported values of 0.6 mg g^{-1} dw for rice plants grown in a field under K^+-sufficient conditions and 1.8 mg g^{-1} dw for plants grown in a K^+-deficient environment (Akai et al., 2012). Such high Na^+ concentrations suggest that K150 plants suffered from K^+ deficiency during at least some stage of the growing period. 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 et al., 1990). Transient K^+ deficiency may more likely to occur during the peak of K^+ demand.

Although IR64 and IR64-K plants did not differ in K^+ accumulation (Figure 3e,g), they differed in Na^+ accumulation (Figure 3f,g) under all three levels of K^+ fertilizer application. These results suggest that the increased yield of IR64-K is due to the improved internal K^+ utilization efficiency brought about by the enhanced Na^+ accumulation. Naturally, Koshihikari-derived genes other than *OsHKT2;1* on chromosomes 1 and 6, or even other chromosomes, might contribute to yield increment. To elucidate the contribution of *OsHKT2;1*, we are preparing new lines with fewer Koshihikari-derived regions. We also plan to examine the expression level of *OsHKT2;1* in several growth stages.

Field-grown IR64-K also had a higher grain yield than IR64 (Figure 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 (Figure 4). The results indicate that IR64-K plants took up and accumulated more Na^+ than IR64 in the field as well as in the pot experiment. Our findings thus suggest that rice cultivars with enhanced Na^+ uptake would be advantageous in reducing K^+ fertilizer use. However, it should be noted that the K^+ and Na^+ content available to plants in the soil varies from field to field and that harvesting is the pathway by which K^+ is removed from agricultural soils (Mikkelsen & Roberts, 2021), so replenishing land with K^+ is essential in the long term.

In conclusion, enhancement of Na^+ uptake ability contributes to yield increment in rice plants under low-K^+ environments. The harvest

index of IR64-K was higher than that of IR64, suggesting that a greater K^+ distribution rate contributes to a higher grain yield. Further research is needed to clarify the mechanism through which Na^+ substitutes for K^+ in plant cells.

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CONFLICT OF INTEREST

The authors did not report any conflict of interest.

AUTHOR CONTRIBUTIONS

KOc and TMa conceived and designed the research. KOb performed most of the experiments. KOc and KOb analyzed the data. KOd carried preliminary experiments. TMi contributed to previous studies that served as the basis of this study and selected IR64-K. KOc wrote the manuscript with input from the authors.

DATA AVAILABILITY STATEMENT

The datasets analyzed in this study are available from the corresponding author upon reasonable request.

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