A potassium (K)-transporter, OsHAK3, is required for K-homeostasis in rice under low-K and high-salinity conditions

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Abstract

Potassium (K⁺) is a macronutrient for plant growth and development. Although a number of K-transporters are encoded in various plant genomes, functional characterization of these transport genes have lagged behind of genomic information in crop plants. In rice genome, a large expansion of high-affinity K-transporters (HAKs/KUPs/KTs) has been identified as compared to those in Arabidopsis genome suggesting a functional diversity of these transporters in cereals. We report here the functional characterization of a HAK member, OsHAK3, in rice using CRISPR-assisted genetic analysis. Expression of OsHAK3 was mainly found in roots and its protein was targeted to the plasma membrane. Loss of function of OsHAK3 led to a reduction of K⁺ uptake rate and K⁺ content, consistent with the finding that mutant plants became stunted under low-K⁺ conditions. In addition, the growth of Oshak3 mutants was more sensitive to salt stress due to altered K/Na ratio in the plants. Together, our data demonstrate that OsHAK3 plays a crucial role in K⁺ homeostasis, especially under K⁺-limited conditions and when plants face salinity stress.

KEYWORDS

Potassium; rice; OsHAK3; K⁺ uptake; K⁺ homeostasis; salinity stress

1 INTRODUCTION

Potassium (K⁺) is the most abundant cation in living plant cells and plays crucial roles in many physiological processes such as enzyme activation, osmoregulation, and stomatal movement (Clarkson & Hanson, 1980; Leigh & Wyn Jones, 1984). Although the K⁺ concentration in the cytoplasm often reaches 100 mM, the K⁺ concentration in the soil is much lower, usually below 1 mM (Schroeder et al., 1994; Luan et al., 2009; Li et al., 2017; Luan et al., 2017). As low-K⁺ status in the soil is a common abiotic stress that plants have to face, they have evolved a series of mechanisms that can sense low-K⁺ stress and increase K⁺ utilization efficiency under K⁺-deficient conditions (Schachtman & Shin, 2007; Luan et al., 2009; Maathuis, 2009; Wang et al., 2013; Li et al., 2017; Wang et al., 2017; Tang et al., 2020).

K⁺ is not metabolized in plant cells and K⁺ nutrition is mainly determined by K⁺ uptake and translocation/remobilization in plants. A large number of genes encoding K⁺ transporters and channels have been identified in various plant genomes and the function of some of those has been studied in model plant Arabidopsis (Gierth & Mäser, 2007; Lebaudy et al., 2007; Ward et al., 2009; Isabelle et al., 2018; Tang et al., 2020). For example, AtAKT1 encodes an inward-rectifying Shaker K⁺ channel expressed in epidermis

and cortex of Arabidopsis root where it mediates K^+ uptake into root cells over a wide range of external K^+ concentrations (Lagarde et al., 1996; Hirsch et al., 1998; Spalding et al., 1999). Under low-K conditions, AtAKT1 is activated by calcium sensor CBL1/9 and their interacting protein kinase CIPK23 (Li et al., 2014; Xu et al., 2006). Moreover, AtHAK5 from the HAK/KUP/KT family is characterized as a high-affinity K^+ transporter in Arabidopsis roots, whose transcription is induced under K^+ deficient conditions (Rubio et al., 2000; Gierth et al., 2005) and its activity is also enhanced by CBL1/9-CIPK23-dependent phosphorylation (Martinez et al., 2015; Ragel et al., 2015). AtHAK5 and AtAKT1 are considered as two major components that contribute to K^+ uptake in Arabidopsis roots (Gierth et al., 2005; Pyo et al., 2010; Nieves-Cordones et al., 2014).

In plants, the HAK/KUP/KT family is the largest K transporter family. In Arabidopsis there are 13 members of K-transporters in the HAK/KUP/KT family and they appear to function in various physiological processes, such as K^+ uptake (Ahn et al., 2004; Gierth & Maser, 2007; Gierth et al., 2005), cell expansion (Elumalai et al., 2002; Osakabe et al., 2013), root hair growth (Rigas et al., 2001), auxin distribution (Vicente-Agullo et al., 2004; Rigas et al., 2013), and osmotic/drought responses (Osakabe et al., 2013). These diverse functions of HAK/KUP/KT transporters may all result from their critical roles in maintaining cellular K^+ homeostasis. Interestingly, rice genome features a large gene expansion in the HAK/KUP/KT family with 27 members (Yang et al., 2009). Among them, OsHAK2 exhibits higher Na^+ over K^+ transport activities and may play a role in salt transport (Horie et al., 2011). OsHAK1, OsHAK5 and OsHAK16 appear to function under low-K and high salinity to maintain K-homeostasis (Chen et al., 2015; Yang et al., 2014; Feng et al., 2019). OsHAK21 play crucial roles in the maintenance of the Na^+/K^+ homeostasis in rice under salt stress (Shen et al., 2015).

In this study, we reveal that OsHAK3, one member of the rice HAK/KUP/KT family mediates K^+ uptake under low- K^+ conditions as well as in K^+ -regulated salt tolerance.

2 MATERIALS AND METHODS

2.1 Plant Growth Conditions

Rice (*Oryza sativa* ssp. japonica ‘Nipponbare’) was used as the wild type in this study and for the development of all transgenic plant lines. Hoagland hydroponic cultivation of rice was performed according to an earlier procedure (Li et al., 2014) with modification in the concentrations of K^+ as specified in the figure legends. Nutrient solutions were replaced once per week or as needed by experimental treatments.

2.2 Yeast Complementation Assay

For the constructs used in functional complementation in CY162 cells, the coding sequences of *OsHAK3* was amplified using the specific primers listed in Table S1 from rice genomic cDNA. The PCR product was digested with *Hind* III and *Xba* I and ligated into pYES2 vector, and transformed into yeast strain CY162, a K^+ uptake-deficient strain of *Saccharomyces cerevisiae* (*MATa*, $\Delta trk1$, *trk2* ::pCK64, *his3*, *leu2*, *ura3*, *trp1* and *ade2*) (Anderson et al., 1992). We performed yeast transformations using the LiAC/ssDNA/PEG method, and the growth assays on arginine phosphate (AP) medium were performed as described previously (Horie et al., 2011).

2.3 qRT-PCR Analysis

We examined the growth of the two *Oshak3* mutants under salt. Total RNA was extracted using the TRIzol reagent (Invitrogen) following the manufacturer’s instructions. Samples were treated with DNase (Promega) to eliminate genomic DNA contamination. Two micrograms of DNA-free RNA was used for reverse transcription by Moloney murine leukemia virus reverse transcriptase (Promega) with anchored oligo (dT₁₈). qRT-PCR was performed on an ABI PRISM 7500 real-time PCR instrument (Applied Biosystems) using Power SYBR Green (Qiagen; CFX96 system). All primer pairs for expression assays or subcloning are listed in Table S1. Primer specificity was confirmed by analysis of the melting curves. The 2^{-T} method (Livak and Schmittgen, 2001) was used to quantify the value of every sample using *Actin* as an internal reference.

2.4 GUS Assays

The 1606-bp fragment before the ATG codon of *OsHAK3* was amplified from rice genomic DNA (cv Nipponbare) with the specific primers listed in Table S1. This fragment was used as *OsHAK3* promoter in this study and was digested with *Hind* III and *Eco*R I and constructed into pCAMBIA1301 vector. The plasmid was transformed into Nipponbare rice via callus transformation as described previously (Upadhyaya et al., 2000), which was mediated by *Agrobacterium* EHA105. The transgenic lines were initially screened by hygromycin resistance and GUS staining. The histochemical analysis of GUS staining in different tissues of rice was performed as described previously (Ai et al., 2009). The samples were examined and photographed with an Olympus SZX12 microscope equipped with a camera. Quantification of GUS activity was performed using the Image J software provided by the microscope manufacturer.

2.5 Subcellular Localization Analysis

For subcellular localization analysis, the *OsHAK3* cDNA was amplified without its stop codon using the specific primers listed in Table S1, subsequently digested with *Xba* I, and then inserted into the multi-cloning sites in the plant binary vector pCAMBIA1390 to make the 35S:*OsHAK3-GFP* fusion construct. Transient expression in rice of *OsHAK3 :GFP* in the pCAMBIA1390 vector and *SP1:RFP* in the modified pCAMBIA1300 vector were conducted using the polyethyleneglycol method (Li et al., 2009). For the plasma membrane localization of *OsHAK3*, we used a confocal laser scanning microscope (LSM880, Carl Zeiss) to detect.

2.6 Generation of *OsHAK3* CRISPR/Cas9 Knockout Transgenic Plants

CRISPR/Cas9 system (the Cas9 vector pYLCRISPR/Cas9Pubi-H provided by Professor Yaoguang Liu) was applied to generate *Oshak3-1* mutants. The pYLCRISPR/Cas9Pubi-H is a plant binary vector in which Cas9p was driven by the maize ubiquitin promoter (pUBI) with hygromycin selectable marker gene having two *Bsa* I sites flanked by a toxic negative selectable marker (*ccdB* gene) for the cloning of sgRNA expression cassette. The sgRNA1 sequences (GCCTCCCTCGTCATCGGCGACGG), which targeted exons 2, of the *OsHAK3* locus (Figure S3) were designed with the web tool CRISPR Plant (Xie et al., 2014). The 20-bp target sequence has to be immediately followed by a NGG motif (PAM), which is essential for Cas9 binding to the target DNA. sgRNA1 cloning into pYLCRISPR/Cas9Pubi-H was performed as described (Usman et al., 2020).

CRISPR/Cas9 system (the Cas9 vector pH-Ubi-cas9-7 provided by Professor Lijia Qu) was applied to generate *Oshak3-2* mutants. The sgRNA2 sequences (GGTGCTGGTGATCGCGA CGCTGG), which targeted exons 6, of the *OsHAK3* locus (Figure S3) were designed with the web tool CRISPR Plant (Xie et al., 2014). The 20-bp target sequence has to be immediately followed by a NGG motif (PAM), which is essential for Cas9 binding to the target DNA. sgRNA2 cloning into the pOs-sg RNA plasmid and subsequent transfer to pH-Ubi-cas9-7 was performed as described (Miao et al., 2013).

Transgenic plants were obtained by co-culture of seed embryo-derived calli (*Oryza sativa* L. cv. Nipponbare) with *A. tumefaciens* strain EHA105 (harbouring the sgRNAs in the pYLCRISPR/Cas9Pubi-H and pH-Ubi-cas9-7 plasmids) following the procedure of Upadhyaya et al (2000). To identify mutations in regenerated plants. Genomic DNA was amplified by using mutation detection primers which designed to flank the designated target site. Then, the sequence chromatograms were analyzed by DSDecode tool (<http://dsdecode.scgene.com/>) to check the genotype of the transgenic plants. The two independent homozygous mutant lines from the T1 generation which were named *Oshak3-1* and *Oshak3-2* used for further study. The primers used for CRISPR/Cas9 construction and mutants detection are listed in Table S1.

2.7 Measurement of chlorophyll

We used equations developed by Porra et al. (1989) to estimate chlorophyll contents. Chlorophyll was extracted from the rice shoots with 10 mL 80% (v/v) acetone containing 2.5 mM sodium phosphate buffer (pH 7.8) and incubated in darkness for 30 h at room temperature. After centrifugation (4°C, 15 min, 13000

$\times$ g), we measured the absorbances $A_{663.6}$ and $A_{646.6}$ with a spectrophotometer (UV-1700, Shimadzu, Kyoto, Japan).

2.8 Ion Content Analysis

For ion content analysis (K^+ , Na^+), samples were washed thoroughly with double-distilled water three times, and dried at 80 for 48 h to constant weight. The samples were treated in a muffle furnace at 300 for 1 h, 575 for 7 h, and finally dissolved in 0.1 N HNO_3 . K^+ or Na^+ concentrations were then measured by ICP-AES (Varian 715-ES) at the 766.49nm (for K^+) or 589.592 nm (for Na^+) wave length.

2.9 Measurement of root K^+ net uptake rate

The measurement of K^+ uptake rate was followed previously described procedures (Nieves-Cordones et al., 2010; Yang et al., 2014), with some modifications. 7-d-old rice seedlings were grown in the IRRI solution for 21 d and then transferred to 0 mM K^+ solutions for 10 d. For the K^+ uptake assay, K^+ -starved plants were incubated in the medium containing 0.01 or 10 mM K^+ for 7 d, and the roots and shoots were collected for K^+ content measurements using inductively coupled plasma analysis. The net K uptake rate into the plant was calculated according to the equation (Nieves-Cordones et al., 2010): Net uptake rate = $(C_2 - C_1)/[(t_2 - t_1) \times (R_2 + R_1)/2]$, where C is the total K^+ content, R is the root dry weight and t is the time; the subscripts 1 and 2 indicate the start and end of the period for which the uptake rate is calculated: $t_2 - t_1 = 7$ d, and $(R_2 + R_1)/2 =$ the mean root dry weight.

2.10 Measurement of K^+ concentration and K^+ export rate in xylem sap

7-d-old rice seedlings were grown in the IRRI solution for 2 weeks and then transferred to 10 or 0.01 mM K^+ solutions for 4 weeks before the sampling. The detailed protocols for the shoot excision, collecting the xylem sap by cotton ball, determining K^+ concentration by ICP and calculating the xylem K^+ export rate were described previously by Yang et al. (2014).

3 RESULTS

3.1 Phylogenetic position, expression pattern, and subcellular localization of *OsHAK3*

According to an earlier study (Yang et al., 2009) and the so far up to date genomics information, we have confirmed that Nipponbare rice subspecies genome encode 27 HAK/KUP/KT family members. To better understand the phylogenetic relationship of the HAK/KUP/KT family proteins, a phylogenetic tree was constructed based on the alignment of the full-length protein sequences of 27 rice *OsHAKs* and 13 *Arabidopsis* *AtHAK/KUP/KTs*. The *Arabidopsis* *AtHAK/KUP/KTs* family was divided into four major clades (I-IV). However, the rice HAK/KUP/KT proteins were expanded to five clades (I-V). Among them, clusters I, II and IV were the most abundant in rice. The IV clade includes only four rice HAK/KUP/KT transporters. The singleton of HAK5 clade (clade I) in *Arabidopsis* is expanded to eight members in rice. Clusters I, II and IV comprise 88.89%, 60% and 100% rice HAK/KUP/KT proteins, respectively. The increased cluster and members of the rice HAK/KUP/KT family showed a dramatic gene expansion as compared to the same family in *Arabidopsis* genome (with 13 genes) (Figure S1).

The cluster II is the largest HAK/KUP/KT clade in plant, including 15 members (with nine members of rice and six members of *Arabidopsis*). The HAK/KUP/KT cluster II members are greatly diverse in their sequences and functions, and participated in different regulatory processes such as root hairs development and cell expansion (Rigas et al., 2001; Elumalai et al., 2002). But the functions of HAK/KUP/KT cluster II transporters in rice were much less known. The predicted unknown *OsHAK3* protein in rice was categorized into cluster II and shares 60.42% amino acid identity with the same cluster member *AtKUP3* which transcript levels being strongly induced by K^+ starvation (Eugene et al., 1998), which suggested that *OsHAK3* may also be involved in K^+ nutrition. We therefore decided to characterize the physiological function of *OsHAK3* in rice.

We first determined the expression pattern of *OsHAK3* gene in different tissues of rice plants and subcellular localization of the *OsHAK3* protein. The qRT-PCR analysis of RNA samples isolated from various organs

of Nipponbare (Nip) plants showed that *OsHAK3* was expressed mainly in the roots and mature anthers. Transcripts of *OsHAK3* were also present in lower amounts in stems, leaves, and glumes (Figure 1A). The expression of *OsHAK3* was not affected by external K^+ level but up-regulated under salt stress (Figure 1B; Figure S2A, S2B, S2C; Figure 1C). To investigate the expression pattern of *OsHAK3* in more detail, we performed GUS activity staining of transgenic rice plants harboring the *OsHAK3*promoter-GUS fusion construct. Consistent with the qRT-PCR results, strong GUS signals were detected in the roots (Figure 1Di, 1Dii). Cross sections of GUS-stained roots showed that *OsHAK3* was strongly expressed almost in all cell types, including exodermis, cortex, endodermis, and xylem parenchyma cells bordering the vessels (Figure 1Dvi, 1Diii). In addition, GUS activity was also detected in mature anthers (Figure 1Div, 1Dv) and mesophyll cells (Figure 1Dvii).

To test the subcellular localization of OshAK3, a green fluorescence protein (GFP) reporter construct was generated to express a OshAK3-GFP fusion protein driven by the cauliflower mosaic virus 35S promoter. The same vector expressing GFP only was used as a control. The GFP-only control and the OshAK3-GFP fusion construct were each introduced into the protoplasts from rice stem cells, and GFP signals were examined using confocal microscopy. While GFP-only signal was observed mainly in the cytoplasm and nucleus, OshAK3-GFP fluorescence was predominantly localized at the plasma membrane. To confirm the plasma membrane localization of OshAK3, we co-transformed the OshAK3-GFP construct with a construct containing a known plasma membrane protein fused to red fluorescent protein (SP1-RFP)(Li et al., 2009) into the protoplasts from rice stem cells. We found that GFP signals produced by the OshAK3-GFP fusion overlapped well with RFP signals generated by SP1-RFP (Figure 2). Based on these results, we concluded that OshAK3 is localized to the plasma membrane in rice cells.

3.2 OshAK3 mediates K^+ uptake and salt tolerance in yeast

To investigate the transport activity of OshAK3, we performed complementation tests using the yeast strain CY162 (*MATa*, $\Delta trk1$, *trk2* ::pCK64,*his3*, *leu2*, *ura3*, *trp1* and *ade2*) which lacks the high-affinity K^+ -transporters Trk1/2 and thus defective in K^+ uptake (Anderson et al., 1992). When grown on AP medium containing 10 mM KCl, no significant differences were scored among transformants harboring either the empty vector or OshAK3 (Figure 3A). When the concentration of K^+ in AP medium was decreased to 0.01 mM, yeast mutant transformed with OshAK3 grew better as compared to that transformed with the empty pYES2 vector (Figure 3A). This indicates that OshAK3 mediates K^+ uptake into yeast cells.

The K^+ uptake-deficient CY162 strain shows Na^+ hypersensitivity, a property that can be used to determine whether the exogenous gene is involved in both K^+ uptake and salt tolerance in yeast cells (Horie et al., 2011b). Thus, we plated the transformants on AP medium containing 10 mM KCl in combination with 0, 150 mM, 250 mM or 300 mM NaCl. There were no significant differences among transformants when NaCl was absent in the medium. However, yeast cells transformed with OshAK3 grew better than that of the vector-transformed cells when extracellular Na^+ concentrations were above 150 mM (Figure 3B). Thus, OshAK3-mediated K^+ transport likely enhances the salt tolerance of CY162 cells.

3.3 *OsHAK3* Is Required for Growth under Low- K^+ Conditions

Considering its expression pattern (in roots and anther) and subcellular localization (in plasma membrane), we hypothesize that OshAK3, as a typical K^+ transporter, may be involved in K^+ uptake and translocation in roots (and possibly in reproductive tissues). To explore the possible role of *OsHAK3* in K^+ uptake or translocation, we generated several gene-edited loss-of-function lines of *OsHAK3* with CRISPR/Cas9 system in Nipponbare (Nip) background. Two mutants (*Oshak3-1*, *Oshak3-2*) of *OsHAK3* were characterized in details in the current study. The *Oshak3-1* had a 1-bp insertion in the second exon of LOC_Os01g27170, resulting in a frameshift mutations from 183 amino acid, *Oshak3-2* mutant had a 2-bp insertion in the sixth exon of LOC_Os01g27170, resulting in a frameshift mutations from 364 amino acid and premature translation termination at 375 amino acid eventually (Figure S3). No potential off-target cleavage was detected using the web-based tool CRISPR-P (<http://cbi.hzau.edu.cn/cgi-bin/CRISPR>; Liu et al., 2017). We examined the growth of the two independent *Oshak3* mutants (*Oshak3-1*, *Oshak3-2*) under K^+ -sufficient and K^+ -

deficient conditions. When grown in K^+ -sufficient hydroponic solution (10 mM K^+), the *Oshak3* mutants and the wild-type (Nip) grew well without discernible differences (Figure 4A). When grown in K^+ -deficient hydroponic conditions (0 mM K^+), however, the *Oshak3* mutants showed more stunted stature than Nip. Along with the increase of external K^+ concentrations, the differences between the *Oshak3* mutants and Nip became smaller and eventually disappeared at 10 mM K^+ concentration (Figure 4A).

As a more quantitative measure, we assayed root and shoot growth in Nip and mutant plants. The *Oshak3* mutants were more severely inhibited compared with Nip when grown in hydroponic conditions containing less than 10 mM K^+ (Figure 4Ba, 4Bb). Consistent with the observed size difference, the fresh weights of the *Oshak3* mutants were significantly lower compared with Nip (Figure 4Bc). In addition, the *Oshak3* mutants had significantly lower levels of chlorophyll than Nip (Figure 4Bd).

Our data above suggest that knockout *OsHAK3* did not appear to affect seed germination or the initial phase of seedling establishment but rather affected further plant growth under the K^+ -deficient condition. To examine this further, we grew Nip, the *Oshak3* mutants on K^+ -sufficient hydroponic solution (10 mM K^+) for 4 d and then transferred the seedlings to the K^+ -deficient hydroponic solution (0 mM K^+) and monitored plant growth for 14 d after transfer. The *Oshak3* mutants became visibly smaller than Nip 14 d after the transfer (Figure 5A). The root length, shoot length and fresh weight of the *Oshak3* mutants were less than those of Nip (Figure 5B, 5C, 5D). In addition, we examined the growth of the *Oshak3* mutants in soil-culture conditions (with 0.63 mg/g external K) (Figure 6A). The *Oshak3* mutants showed brown spots on older leaves, a typical K^+ -deficient symptom of rice plants (Figure 6B). In addition, the roots and shoots in the mutants were shorter than those in Nip (Figure 6C, 6E, 6F). The seed setting rate and 1000-grain weight in the mutants were also reduced than that in Nip (Figure 6D, 6G, 6H). These results suggest that knockout the *OsHAK3* gene affected plant growth and development under low- K^+ conditions, possibly due to changes in K^+ accumulation in the mutant plants.

3.4 *OsHAK3* Functions in K^+ Accumulation in Plants

To test whether the growth retardation phenotype of *Oshak3* was due to the reduction of K^+ content, we measured the K^+ content in Nip and the *Oshak3* mutants. As shown in Figure 4C, there was no obvious difference in K^+ content among different plants in K^+ -sufficient hydroponic solution. However, the K^+ content in *Oshak3* root and shoot were significantly lower than that of Nip under K^+ -deficient conditions (Figure 4C).

When plants were grown in the K^+ -sufficient hydroponic solution (10 mM K^+) for 4 d and then transferred to the K^+ -deficient hydroponic conditions (0 mM K^+), the K^+ content decreased in both *Oshak3* mutants and Nip during the time course in low- K^+ hydroponic conditions (Figure 5E). However, the *Oshak3* mutants displayed a more drastic decrease in their K^+ content compared with Nip. Fourteen days after the transfer, the *Oshak3* mutants appeared stunted growth, and the K^+ content in these plants represented 25% decrease compared with Nip (Figure 5E).

We conclude from the above data that knockout of *OsHAK3* impaired K^+ accumulation in the plants under the low- K^+ condition.

3.5 *OsHAK3* Is a Key Transporter for K^+ Uptake in Plants under Low K^+ conditions

The expression analysis indicated that *OsHAK3* showed strong expression in root tissues (Figure 1D). Direct K^+ measurements indicated that, under low K^+ conditions, the roots and shoot of the two *Oshak3* mutants both accumulated less K^+ than that of Nip (Figure 4C). These results suggest that *OsHAK3* may be involved in K^+ acquisition and/or root-to-shoot translocation in root tissues.

To test whether *OsHAK3* participated in K^+ uptake or translocation, we performed the K^+ uptake and translocation assay. The seeds of the Nip and *Oshak3* mutants were germinated in water for 7 d, transferred to the hydroponic solutions containing 10 mM K^+ for 21 d, then transferred to the hydroponic solutions containing 0 mM K^+ for 10 d. For the K^+ uptake and translocation assay, K^+ -starved plants were incubated in the medium containing 0.01 or 10 mM K^+ for 7 d, and the roots and shoots were collected separately

for K^+ content measurements using inductively coupled plasma (ICP) analysis. As shown in Figure 7A and Figure 7B, the K^+ contents in both the roots and shoots were significantly decreased in *Oshak3* mutant under 0.01 mM K^+ condition, the K^+ uptake rate which calculated as described as methods was reduced in *Oshak3* mutant compared with Nip (Figure 7C). The above results showed that the knockout of *OsHAK3* impaired K^+ uptake in rice root.

Then, we calculated the shoot/root ratio of K^+ content to determine the K^+ distribution between shoot and root. The shoot/root K^+ ratio in the *Oshak3* mutants were similar to that of Nip under low- K^+ condition (0.01 mM K^+) or K^+ -sufficient condition (10 mM K^+) (Figure 7D), which indicated that the K^+ translocation from root to shoot was not affected in *Oshak3* mutants. In addition, the K^+ concentrations of xylem sap and K^+ secretion rate into xylem from different plants were measured. Under K^+ -sufficient condition (10 mM K^+), there was no difference in xylem sap K^+ concentrations and K^+ secretion rate into xylem among various plant (Figure 7E, 7F). Under low- K^+ condition (0.01 mM K^+), though the xylem sap K^+ concentrations in *Oshak3* were reduced (Figure 7E), but the K^+ secretion rate into xylem in *Oshak3* are similar to the K^+ secretion rate in Nip (Figure 7F), which suggested that the root-to-shoot K^+ transport is not affected in *Oshak3* mutants. The above datas showed that *OsHAK3* is not involved in root-to-shoot K^+ transport.

Taken together, these results demonstrated that *OsHAK3* is involved in K^+ uptake in root and is required for rice adaptation to the low- K^+ environment.

3.6 *OsHAK3* functions in mediating Na^+/K^+ homeostasis under salt stress

The expression levels of *OsHAK3* was up-regulated by salt stress (Figure 1C), which implied that *OsHAK3* may be involved in the response to salt stress.

We examined the growth of the *Oshak3* mutants (*Oshak3-1*, *Oshak3-2*) under salt stress as follows. The 14-d-old plants of *Oshak3* and Nip grown in 10 mM K^+ condition were transferred to hydroponic solutions containing 10 mM K^+ and different Na^+ concentrations (0 mM, 50 mM, 100 mM, 150 mM Na^+). After 7 d in these hydroponic solutions, the growth of *Oshak3* was inhibited to a greater extent as compared to Nip and was accompanied by chlorosis in the old leaves (Figure 8A). To quantify the phenotypes, we compared the *Oshak3* mutants and the Nip plants by measuring fresh weight and chlorophyll contents under salt stress. The fresh weights of both roots and shoots in *Oshak3* mutants were substantially lower than that of Nip (Figure 8B). The *Oshak3* mutant plants also contained less chlorophyll than Nip (Figure 8B), consistent with the chlorotic phenotype. Thus, the *Oshak3* mutants were more sensitive to salinity stress than the Nip plants.

The effect of salinity stress on plants is partitioned into two major phases: osmotic stress and Na^+ toxicity (Munns & Tester 2008; Hasegawa 2013). We observed no significant differences between the *Oshak3* mutants and Nip when seedlings were treated with 20% PEG6000 (polyethylene glycol with an average molecular weight of 6000 Da) (Figure S4). Thus, we hypothesized that the salt sensitivity of the *Oshak3* mutants may be attributable to Na^+ toxicity. We measured Na^+ and K^+ contents in shoots and roots of diverse plant materials, including Nip and *Oshak3* mutants. Under control condition (10 mM K plus 0 mM Na^+), we observed no substantial differences in either Na^+ or K^+ contents among the different plant materials (Figure 8C, 8D). Seven days after treatment with NaCl (50 mM, 100 mM, 150 mM Na^+), the *Oshak3* mutants accumulated more Na^+ and less K^+ than Nip in both shoots and roots (Figure 8C, 8D). The Na^+/K^+ ratios in both shoots and roots of the *Oshak3* mutants were significantly higher than the corresponding values for Nip (Figure 8E). Thus, *OsHAK3* enhanced rice salt tolerance by elevating the K^+/Na^+ ratio in the rice root and shoot.

Taken together, these data showed that *OsHAK3* function in Na^+/K^+ homeostasis under salt stress, which is essential for the salt tolerance in rice

4 DISCUSSION

Plant roots absorb K^+ through root hairs, epidermis, and cortex cells that harbor a number of K-transporters and channels. We show here that *OsHAK3*, a high-affinity K-transporter, plays a crucial role in K-uptake

in rice roots. Because OsHAK3 is ubiquitously expressed in epidermis, root hairs, and cortex of rice roots, it may serve as a house-keeping transport system for acquiring K^+ in rice plants.

It is interesting to find that rice OsHAK family features a large expansion as compared to the same family in Arabidopsis. In particular, the singleton of HAK5 clade (clade I) in Arabidopsis is expanded to 8 members in rice. Among the HAK5-clade members, several genes have been functionally characterized, including OsHAK5, OsHAK1, OsHAK16 and OsHAK21 (Yang et al., 2014; Chen et al., 2015; Feng et al., 2019; Shen et al., 2015). These studies suggested that increased number of genes in HAK family in rice may be functionally diverged according to different anatomy of rice plants (Yang et al., 2014; Chen et al., 2015; Chen et al., 2017; Feng et al., 2019; Shen et al., 2015). The cluster II is the largest HAK/KUP/KT clade in plant and the cluster II members are greatly diverse in their sequences and functions, participating root hairs development and cell expansion regulatory processes and so on (Rigas et al., 2001; Elumalai et al., 2002). But the functions of the cluster II HAK/KUP/KT transporters in rice were much less characterized. The predicted unknown OsHAK3 protein of the cluster II shares 60.42% amino acid identity with the same cluster member AtKUP3 which transcript levels being strongly induced by K^+ starvation (Eugene et al., 1998), which suggested that OsHAK3 may also be involved in K^+ nutrition.

We observed that knockout of *OsHAK3* resulted in very severe inhibition of rice growth and development (Figure 4), resulting in reduced seed setting rate and grain yield at the end (Figure 6G, 6H). It is remarkable that such growth retardation of the mutants in comparison to Nip was mainly caused by severe K^+ deficiency. As shown in Figure 4C, contents in both roots and shoots of the mutants were less than that in Nip. These results indicated that OsHAK3 is critical for rice to maintain adequate K^+ nutrition. OsHAK1 and OsHAK5 are two major root K^+ acquisition transporters in rice under low- K^+ conditions. K^+ deficiency sustainably up-regulated the expression of *OsHAK1* and *OsHAK5* (Yang et al., 2014; Chen et al., 2015). We found that *OsHAK1* and *OsHAK5* transcription were up-regulated in the *Oshak3* mutant after low K^+ treatment (Figure S5). In addition, we also observed that *OsHAK1* and *OsHAK5* transcripts level in the *Oshak3* mutant were much higher than that in Nip, even under K^+ sufficient conditions (Figure S5). The above data showed that the *OsHAK1* and *OsHAK5* transcriptional regulation in the *Oshak3* mutant is somehow affected, which indicated *OsHAK3*, *OsHAK1* and *OsHAK5* play some non-redundant roles in K^+ acquisition and K^+ -mediated rice growth.

Maintaining adequate K^+ and homeostasis of K^+/Na^+ ratio at salt stress condition is important for plant survival and growth (Horie et al., 2009; Shin & Adams 2014). Therefore, enhancing the expression of HAK transporters is an attractive target for the improvement of K^+ acquisition and plant growth under saline conditions (Nieves-Cordones et al., 2010). Under salt stress, the expression is enhanced (Figure 1C) and the K^+ contents in shoots and roots of *Oshak3* mutants were lower than those in Nip (Figure 8D). In contrast, the Na^+ contents of *Oshak3* mutants were significantly higher than the corresponding values of Nip (Figure 8C). The calculated Na^+/K^+ ratios in both shoots and roots of the *Oshak3* mutants were significantly higher than the corresponding values for Nip (Figure 8E). These results suggest that knockout of the *OsHAK3* leads to a decreased K^+/Na^+ ratio in roots and shoots and to salt sensitivity. Similar phenotypes have been observed in the *oshak1*, *oshak5*, *oshak16*, *oshak21* mutants (Chen et al., 2015; Yang et al., 2014; Feng et al., 2019; Shen et al., 2015), implying that similar to OsHAK1, OsHAK5, OsHAK16 and OsHAK21, OsHAK3 also plays an important role in enhancing rice tolerance to salt stress. The decreased K^+ content that accompanied the increased Na^+ content in the *Oshak3* mutants suggests that OsHAK3 may function as a K^+/Na^+ antiporter, which simultaneously mediates K^+ uptake and Na^+ extrusion, future studies could conduct the complementation tests using the yeast AXT3K strain in which the genes encoding Na^+ export pumps are disrupted to verify whether OsHAK3 mediate Na^+ flux in yeast systems. In addition, the elevation of Na^+ contents in *Oshak3* mutants may be an indirect result due to the disruption of OsHAK3.

Transcriptional regulation of K^+ transporters is a universal mechanism for different plant species in response to K^+ -deficient stress (Wang et al., 2013). The transcription of the HAK/KUP/KT cluster I members such as *AtHAK5* (Gierth et al., 2005), *OsHAK1* (Chen et al., 2015) and *OsHAK5* (Yang et al., 2014), all respond to K^+ deficiency, so that K^+ uptake in rice root is enhanced. The phenotype assays showed that

OsHAK3 activity is more important under K⁺-deficient conditions. However, the real-time PCR results and GUS assays showed that *OsHAK3* transcript was not changed under low-K⁺ treatment (Figure 1B, Figure S2), which indicated *OsHAK3* may be regulated at post-transcription or posttranslational level. A growing number of reports showed that post-translational regulation is important for both K⁺ channels and transporters (Wang & Wu., 2013). For instance, the AtAKT1 and OsAKT1 channel are phosphorylated by AtCIPK23 and OsCIPK23, respectively, whose activity are enhanced under K⁺-deficient conditions (Xu et al., 2006; Li et al., 2014). The K⁺ transporter AtHAK5 requires post-transcriptional regulation (Rubio et al., 2014), whose activity is regulated by protein kinase AtCIPK23 via phosphorylation (Martinez et al., 2015; Ragel et al., 2015). We speculated that *Oshak3* might be also regulated by phosphorylation. Using Scansite 4.0 software, seven phosphorylation sites (S30, Y47, S66, S172, S311, T514 and S767) have been predicted to exist on *Oshak3* protein sequence (808 amino acids). Further studies will conduct the point mutations in *Oshak3* to test whether these sites contributed to the K⁺ transport activity of *Oshak3* in yeast system and rice.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

L.Z., D.M. L.C., and S.L. conceived and designed the experiments. L.Z., X.C., M.H. performed experiments. X.S., Y.M. assisted in the inductively coupled plasma assay. Y.L., Z.S. assisted in some of the subcellular localization. T.X., Y.P., S.G., Z.C. assisted in some of the molecular experiments. L.Z., D.M., L.C., S.L. analyzed data. L.Z., D.M. and S.L. wrote the manuscript.

REFERENCES

- Ahn S.J., Shin R., & Schachtman D.P. (2004). Expression of KT/KUP genes in Arabidopsis and the role of root hairs in K⁺ uptake. *Plant Physiol* 134: 1135-1145.
- Ai P., Sun S., Zhao J., Fan X., Xin W., Guo Q., Yu L., Shen Q., Wu P., & Miller A.J. (2009). Two rice phosphate transporters, OsPht1;2 and OsPht1;6, have different functions and kinetic properties in uptake and translocation. *Plant Journal* 57: 798-809.
- Anderson J.A., Huprikar S.S., Kochian L.V., Lucas W.J. & Gaber R.F. (1992). Functional expression of a probable Arabidopsis thaliana potassium channel in *Saccharomyces cerevisiae*. *Proceedings of the National Academy of Sciences* 89: 3736-3740.
- Chen G., Hu Q.D., Luo L., Yang T.Y., Zhang S., Hu Y.B., Yu L., & Xu G.H. (2015). Rice potassium transporter *OsHAK1* is essential for maintaining potassium-mediated growth and functions in salt tolerance over low and high potassium concentration ranges. *Plant, Cell and Environment*, 38: 2747-2765.
- Clarkson D.T., & Hanson J.B. (1980). The mineral nutrition of higher plants. *Annu Rev Plant Physiol* 31: 239-298.
- Elumalai R.P., Nagpal P., & Reed J.W. (2002). A mutation in the Arabidopsis KT2/KUP2 potassium transporter gene affects shoot cell expansion. *Plant Cell* 14: 119-131.
- Eugene J.K., June M. K., Nobuyuki U., & Julian I. S. (1998). AtKUP1: An Arabidopsis Gene Encoding High-Affinity Potassium Transport Activity. *Plant Cell* 10: 51-62.

- Feng H.M., Tang Q., Cai J., Xu B.C., Xu G.H., & Yu L. (2019). Rice OsHAK16 functions in potassium uptake and translocation in shoot, maintaining potassium homeostasis and salt tolerance. *Planta* 250: 549-561.
- Gierth M., Mäser P., & Schroeder J.I. (2005). The potassium transporter HAK5 functions in K⁺ deprivation-induced high-affinity K⁺ uptake and AKT1 K⁺ channel contribution to K⁺ uptake kinetics in Arabidopsis roots. *Plant Physiol.* 137:1105-1114.
- Gierth M., & Maser P. (2007). Potassium transporters in plants-involvement in K⁺ acquisition, redistribution and homeostasis. *FEBS Lett.* 581: 2348-2356.
- Hasegawa PM (2013). Sodium (Na⁺) homeostasis and salt tolerance of plants. *Environmental and Experimental Botany* 92:19-31.
- Hirsch R.E., Lewis B.D., Spalding E.P., & Sussman M.R. (1998). A role for the AKT1 potassium channel in plant nutrition. *Science* 280: 918-921.
- Horie T., Hauser F., & Schroeder J.I. (2009). HKT transporter-mediated salinity resistance mechanisms in Arabidopsis and monocot crop plants. *Trends in Plant Science* 14: 660-668.
- Horie T., Sugawara M., Okada T., Taira K., Nakayama P. K., Katsuhara M., Shinmyo A., & Nakayama H. (2011). Rice sodium-insensitive potassium transporter, OsHAK5, confers increased salt tolerance in tobacco BY2 cells. *J Biosci Bioeng* 111(3): 346-56.
- Horie T., Sugawara M., Okada T., Taira K., Kaothien N. P., Katsuhara M., & Nakayama H. (2011b). Rice sodium-insensitive potassium transporter, OsHAK5, confers increased salt tolerance in tobacco BY2 cells. *Journal of Bioscience and Bioengineering* 111: 346-356.
- Isabelle C., & Isabelle G. (2018). The Complex Fine-Tuning of K⁺ Fluxes in Plants in Relation to Osmotic and Ionic Abiotic Stresses. *Int J Mol Sci.* 20: 715.
- Lagarde D., Basset M., Lepetit M., Conejero G., Gaymard F., Astruc S., & Grignon C. (1996). Tissue-specific expression of Arabidopsis AKT1 gene is consistent with a role in K⁺ nutrition. *Plant Journal* 9:195-203.
- Lebaudy A., Very A.A., & Sentenac H. (2007). K⁺ channel activity in plants: genes, regulations and functions. *FEBS Lett* 581: 2357-2366.
- Leigh R.A., & Wyn J.R.G. (1984). A hypothesis relating critical potassium concentrations for growth to the distribution and functions of this ion in the plant cell. *New Phytol.* 97: 1-13.
- Li J., Yu L., Guo N. Q., Li J., Xu Z.J., Wu W.H., & Wang Y. (2014). Os-AKT1 channel is critical for K⁺ uptake in rice roots and is modulated by the rice CBL1-CIPK23 complex. *Plant Cell* 26, 3387-3402.
- Li S.B., Qian Q., Fu Z.M., Zeng D.L., Meng X.B., Kyoizuka J., Maekawa M., Zhu X.D., Zhang J., Li J.Y., & Wang Y.H. (2009). Short panicle1 encodes a putative PTR family transporter and determines rice panicle size. *Plant Journal* 58: 592-605.
- Liu H., Ding Y., Jin W., Xie K., & Chen L.L. (2017). CRISPR-P 2.0: An improved CRISPR-Cas9 tool for genome editing in plants. *Mol Plant* 10: 530-532.
- Livak K.J., & Schmittgen T.D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2^{-ΔΔC_T} method. *Methods* 25: 402-408.
- Li W.H., Xu G. H., Abdel A., & Yu L. (2017). Plant HAK/KUP/KT K⁺ transporters: function and regulation. *Seminars in Cell & Developmental Biology* S1084 -9521(16): 30368-8.
- Luan M., Tang R.J., Tang Y., Tian W., Hou C., Zhao F., Lan W., & Luan S. (2017). Transport and homeostasis of potassium and phosphate: limiting factors for sustainable crop production. *J Exp Bot* 68 (12): 3091-3105.

- Luan S., Lan W.Z., & Lee S.C. (2009). Potassium nutrition, sodium toxicity, and calcium signaling: connections through the CBL-CIPK network. *Curr Opin Plant Biol* 12: 339-346.
- Maathuis F.J.M. (2009). Physiological functions of mineral macronutrients. *Curr Opin Plant Biol* 12: 250-258.
- Martinez V., Pardo J.M., & Quintero F.J. (2015). The CBL-Interacting Protein Kinase CIPK23 regulates HAK5-mediated high-affinity K^+ uptake in Arabidopsis roots. *Plant Physiol* 169:2863-2873.
- Miao J., Guo D., Zhang J., Huang Q., Qin G., Zhang X., Wan J., Gu H., & Qu L.J. (2013). Targeted mutagenesis in rice using CRISPR-Cas system. *Cell Res* 23: 1233-1236.
- Munns R & Tester M(2008). Mechanisms of salinity tolerance. *Annual Review of Plant Biology* 59: 651-681.
- Nieves C.M., Aleman F., Martinez V., & Rubio F. (2014). K^+ uptake in plant roots. The systems involved, their regulation and parallels in other organisms. *J Plant Physiol* 171: 688-695.
- Osakabe Y., Arinaga N., Umezawa T., Katsura S., Nagamachi K., Tanaka H., Ohiraki H., Yamada K., Seo S.U., & Abo M. (2013). Osmotic stress responses and plant growth controlled by potassium transporters in Arabidopsis. *Plant Cell* 25: 609-624.
- Porra R.J., Thompson W.A., & Kriedemann P.E. (1989). Determination of accurate extinction coefficients and simultaneous-equations for assaying chlorophyll-B extracted with 4 different solvents-verification of the concentration of chlorophyll standards by atomic-absorption spectroscopy. *Biochimica et Biophysica Acta* 975: 384-394.
- Pyo Y.J., Gierth M., Schroeder J.I., & Cho M.H. (2010). High-affinity K^+ transport in Arabidopsis: HAK5 and AKT1 are vital for seedling establishment and postgermination growth under low-potassium conditions. *Plant Physiol* 153: 863-875.
- Ragel P., Rodenas R., Garcia M.E., Andres Z., Villalta I., Nieves C.M., Rivero R.M., Martinez V., Pardo J.M., & Quintero F.J. (2015). The CBL-Interacting Protein Kinase CIPK23 regulates HAK5-mediated high-affinity K^+ uptake in Arabidopsis roots. *Plant Physiol* 169: 2863-2873.
- Rigas S., Debrosses G., Haralampidis K., Vicente A.F., Feldmann K.A., Grabov A., Dolan L., & Hatzopoulos P. (2001). TRH1 encodes a potassium transporter required for tip growth in Arabidopsis root hairs. *Plant Cell* 13:139-151.
- Rigas S., Ditegou F.A., Ljung K., Daras G., Tietz O., Palme K., & Hatzopoulos P. (2013). Root gravitropism and root hair development constitute coupled developmental responses regulated by auxin homeostasis in the Arabidopsis root apex. *New Phytol* 197: 1130-1141.
- Rubio F., Santa M.G.E., & Rodriguez N.A. (2000). Cloning of Arabidopsis and barley cDNAs encoding HAK potassium transporters in root and shoot cells. *Physiol Plant* 109: 34-43.
- Rubio F., Fon M., Rodenas R., Nieves C. M., Aleman F., Rivero R.M., & Martinez V. (2014). A low K^+ signal is required for functional high-affinity K^+ uptake through HAK5 transporters. *Physiol Plant* 152: 558-570.
- Schroeder J.I., Ward J.M., & Gassmann W. (1994). Perspectives on the physiology and structure of inward-rectifying K^+ channels in higher plants: biophysical implications for K^+ uptake. *Annu Rev Biophys Biomol. Struct* 23: 441-471.
- Schachtman D.P., & Shin R. (2007). Nutrient sensing and signaling: NPKS. *Annu Rev Plant Biol* 58: 47-69.
- Shen Y., Shen L., Shen Z.X., Jing W., Ge H.L., Zhao J.Z., & Zhang W.H. (2015). The potassium transporter OSHAK21 functions in the maintenance of ion homeostasis and tolerance to salt stress in rice. *Plant, Cell and Environment* 38: 2766-2779.

- Shin R., & Adams E. (2014). Transport, signalling and homeostasis of potassium and sodium in plants. *Journal of Integrative Plant Biology* 56: 231-249.
- Spalding E.P., Hirsch R.E., Lewis D.R., Qi Z., Sussman M.R., & Lewis B.D. (1999). Potassium uptake supporting plant growth in the absence of AKT1 channel activity: inhibition by ammonium and stimulation by sodium. *J Gen Physiol* 113: 909-918.
- Tang R.J., Wang C., Li K., & Luan S. (2020). CBL-CIPK calcium signaling network: united paradigm from 20 years of discoveries. *Trends Plant Sci* 25(6): 604-617.
- Tang R.J., Zhao F.G., Yang Y., Wang C., Li K.L., Kleist T.J., Lemaux P.G., & Luan S. (2020). A calcium signalling network activates vacuolar K⁺ remobilization to enable plant adaptation to low-K environments. *Nature plants* 6: 384-393.
- Upadhyaya N., Surin B., Ramm K., Gaudron J., Schunmann P.H.D., Taylor W., Waterhouse P.M., & Wang M.B. (2000). Agrobacterium-mediated transformation of Australian rice cultivars Jarrah and Amaro using modified promoters and selectable markers. *Aust J Plant Physiol* 27: 201-210.
- Usman B., Nawaz G., Zhao N., Liu Y.g., & Li R.b. (2020). Generation of High Yielding and Fragrant Rice (*Oryza sativa* L.) Lines by CRISPR/Cas9 Targeted Mutagenesis of Three Homoeologs of Cytochrome P450 Gene Family and OsBADH2 and Transcriptome and Proteome Profiling of Revealed Changes Triggered by Mutations. *Plants* 9: 788.
- Vicente A.F., Rigas S., Desbrosses G., Dolan L., Hatzopoulos P., & Grabov A. (2004). Potassium carrier TRH1 is required for auxin transport in Arabidopsis roots. *Plant Journal* 40: 523-535.
- Wang Y., & Wu W.H. (2013). Potassium transport and signaling in higher plants. *Annu. Rev. Plant Biol* 64: 451-476.
- Wang Y., & Wu W.H. (2017). Regulation of potassium transport and signaling in plants. *Current Opinion in Plant Biology* 39: 123-128.
- Ward J.M., Maser P., & Schroeder J.I. (2009). Plant ion channels: gene families, physiology, and functional genomics analyses. *Annu Rev Physiol* 71: 59-82.
- Xie K., Zhang J., & Yang Y. (2014). Genome-wide prediction of highly specific guide RNA spacers for CRISPR-Cas9-mediated genome editing in model plants and major crops. *Mol Plant* 7: 923-926.
- Xu J., Li H.D., Chen L.Q., Wang Y., Liu L.L., He L., & Wu W.H. (2006). A protein kinase, interacting with two calcineurin B-like proteins, regulates K⁺ transporter AKT1 in Arabidopsis. *Cell* 125: 1347-1360.
- Yang T., Zhang S., Hu Y., Wu F., Hu Q., Chen G., & Xu G. (2014). The role of a potassium transporter OSHAK5 in potassium acquisition and transport from roots to shoots in rice at low potassium supply levels. *Plant Physiology* 166: 945-959.
- Yang Z.F., Gao Q.S., Sun C.S., Li W.J., Gu S.L., & Xu C.W. (2009). Molecular evolution and functional divergence of HAK potassium transporter gene family in rice (*Oryza sativa* L.). *J Genet Genomics* 36: 161-172.

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