Title: The role of OsHAK5 in potassium acquisition and transport from roots to shoots in rice at low potassium supply levels

Authors: Tianyuan Yang, Song Zhang, Yibing Hu, Fachi Wu, Qingdi Hu, Guang Chen, Jing Cai, Ting Wu, Nava Moran, Ling Yu, Guohua Xu

SUPPLEMENTARY DATA

Table S1. The primers for Semi-quantitative RT-PCR and Real-time quantitative PCR of *OsHAK5*

Gene (DNA Accession No.)	Primers sequences (5' to 3')	Product length (bp)
OsHAK5 (AK241580)	Semi-F: GGCATCCCACCCATACTTCCTCA	614
	Semi-R: TACGTCATTCCTACCCGCAACAG	
	Real-time-F: CATTGTGGACTATTTTGAAAGAA	149
	Real-time-R: GGAGAACTACAGAAAAGCCAATC	
OsACT (*OsRac1*) (AB047313)	Semi-F: GGAACTGGTATGGTCAAGGC	750
	Semi-R: AGTCTCATGGATAACCGCAG	
	Real-time-F: TTATGGTTGGGATGGGACA	197
	Real-time-R: AGCACGGCTTGAATAGCG	

Table S2. The primers for promoter of *OsHAK5*

Gene(DNA Accession No.)	Primers sequences (5' to 3')	Product length (bp)
OsHAK5 (AK241580)	F: **GCG**TTAATTAACCATAGTTGCCAGACTGTTAG	1776
	R:**AGT**GGCGCGCCTCTCAGTGTATGGAATTTGCT	

Notes: 5´ spacer sequences are indicated in overstriking. The incorporated two restriction sitessequences of AscI(TTAATTAA) and PacI(GGCGCGCC) are underlined.

Table S3. The primers for*OsHAK5*-cDNA for construction of overexpression

Gene (DNA Accession No.)	Primers sequences (5' to 3')	Product length (bp)
OsHAK5(AK241580)	F:**ATT**GGTACCATGACCGAGCCTCTGCACAC R:**ATA**GCTAGCAGATTTCCAAGAACATCACCATC	2346

Notes: 5´ spacer sequences are indicated in overstriking. Incorporated two restrictionsites sequences ofKpnI (GGTACC) and NheI (GCTAGC) are underlined.

Table S4. The primers for identification two homozygous mutant lines of *oshak5*

Gene (DNA Accession No.)	Primers sequences (5' to 3')
OsHAK5 (AK241580)	DJ-3A09138 F: TGCATTTCCTCACTCAGCAC DJ-3A09138 R: GCAAGGATGGACATGATCTG HY-2A30318 F: TGGTTGCCTGAAGTTCTTCC HY-2A30318 R: GCCCAAATCTATCAGGCAAG
T-DNA Vector2715	2715L: ACGTCCGCAATGTGTTATTAA 2715R: AACGCTGATCAATTCCACAG

Figure S1

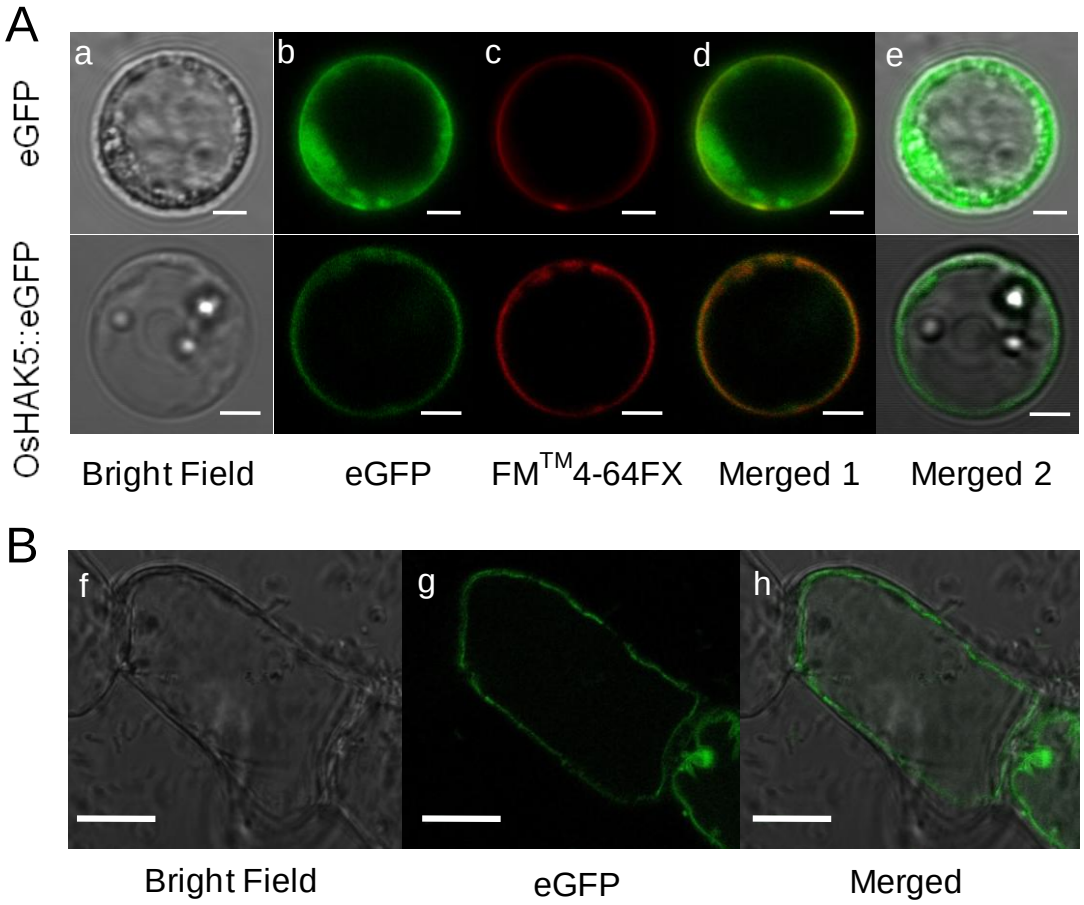


Figure S1: Localization of OsHAK5 in the cell plasma membrane.

A, An expression of eGFP and OsHAK5:eGFP fusion protein in rice protoplasts. Top row: images ofa control protoplast expressing eGFP. Bottom row: images of a protoplast expressing OsHAK5:GFP fusion protein. (a) GFP (green) fluorescence images (excitation: 490 nm, emission: 525 nm). (b) Bright-field images.(c) FM4-64FX dye (red) images (FM4-64FX is a membrane-selective fluorescent vital dye; excitation: 543 nm, emission: 660 nm). (d) Superposition of the GFP and FM4-64FX fluorescence images. (e) Superposition of the GFP fluorescence and bright-field images. Bars = 5 µm.

B, An expression of *OsHAK5*:GFP fusion protein in BY-2 cells.(f) GFP images, (g) Bright-field images, (h) Overlap of GFP fluorescence and bright-field images. Tobacco (*Nicotianatabacum* L.) cv. Bright Yellow 2 (BY2) suspension-cultured cells were maintained in a modified liquid Linsmaier and Skoog (LS) medium (pH 5.8) (Nagata et al., 1981). The cells were cultivated in

this medium at 27°C in the dark on an orbital shaker at 125 rpm, according to Nakayama et al. (2000).

Nagata T, Okada K, Takebe I, Matsui C (1981) Delivery of tobacco mosaic-virous RNA into plant-protoplasts mediated by reverse-phase evaporation vesicles (Liposome). Mol Gen Genet **184**: 161-165.

Nakayama H, Yoshida K, Ono H, Murooka Y, Shinmyo A (2000) Ectoine, the compatible solute of Halomonaselongata, confers hyperosmotic tolerance in cultured tobacco cells. Plant Physiol**122**: 1239-1247.

Figure S2

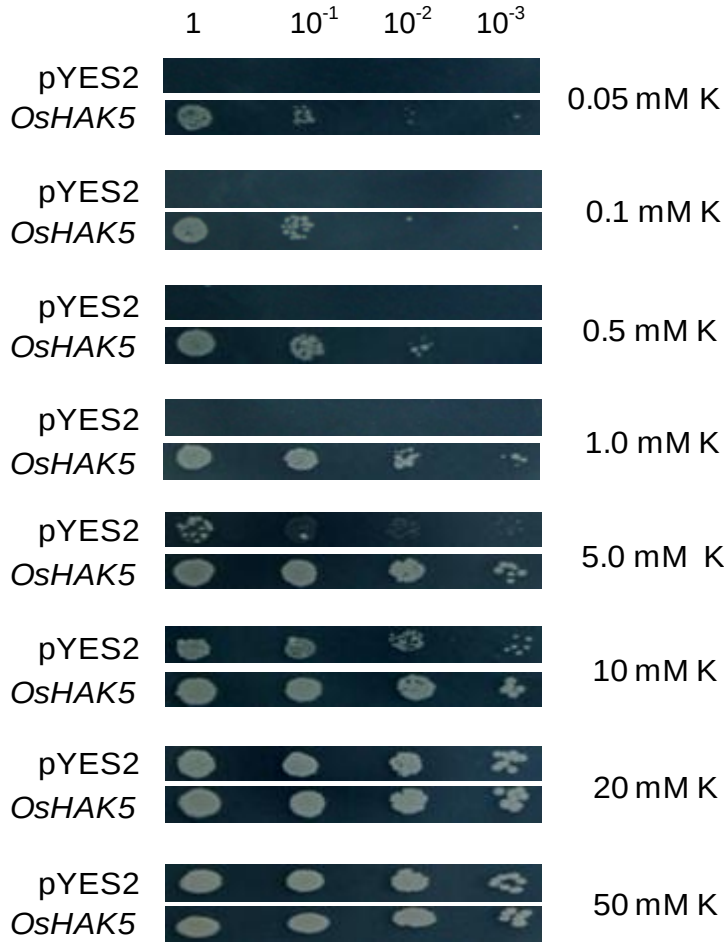
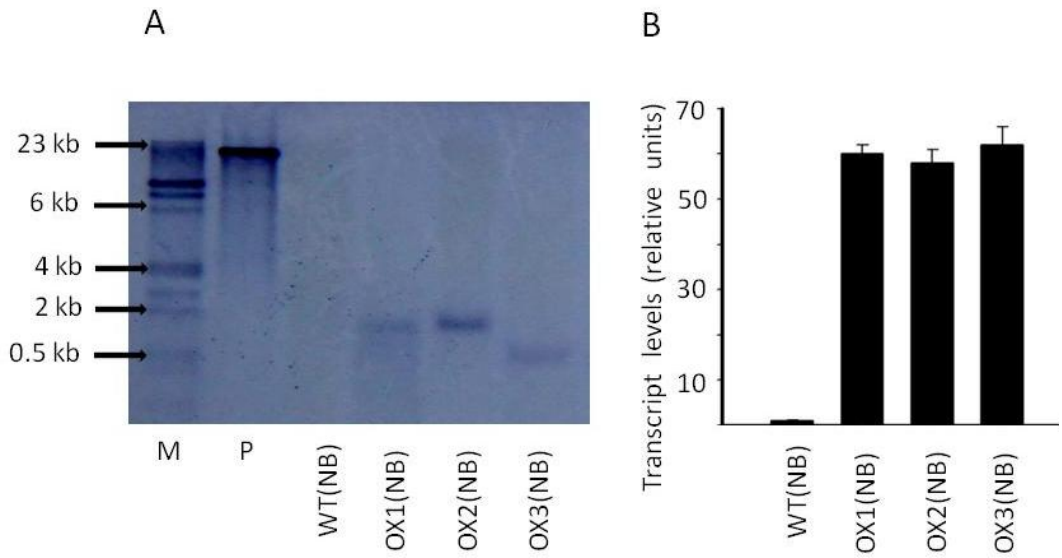


Figure S2: Functional complementation test of OsHAK5 in yeast for absorbing K from culture medium containing different levels of K.

The coding sequence of *OsHAK5* was clonedin pYES2 (Invitrogen) under control of the inducible Gal1 promoter. The expression vectors were transformed into the R5421 strain, an K uptake-deficient strain of Saccharomyces cerevisiae [ura3-52his3△200 leu2 △1 trp1 △1ade2 trk1 △::HIS3 trk2 △::HIS3] (kindly provided by Prof. Gaber from Northwest university in USA). The transformants were selected on Glc-containing SC-agar plates without uracil, supplemented with 100 mM K. Phosphoric acid (AP) medium was used for subsequent growth assays which were performed as described previously (Horie*et al.*, 2011). The complementation tests were conducted on solidmedia, and the plates were incubated at 30 ^{o}C for 6 d. The numbers at the top indicate yeast culture dilutions.

Figure S3



FigureS3: Molecular identification of *OsHAK5-overexpressing* transgenic rice lines in the background of the Nipponbare cultivar.

A, Southern blot analysis of the transgene copy number in T2 transgenic rice plants (OX1,OX2 and OX3)and WT plant. Genomic DNA was digested with two restriction enzymes *HindIII* and *EcoRI*, the Hygromycin gene was used as probe. The DNA was separated on 1%agarose gel. M: marker; P: pTK303-ubi as a positive control. **B**, Real-time quantitative RT-PCR analysis of endogenous *OsHAK5* gene levels in the leaves of WT, OX1, OX2 and OX3 plants using total RNA isolated from the leaves of two weeks-old seedlings.

Figure S4

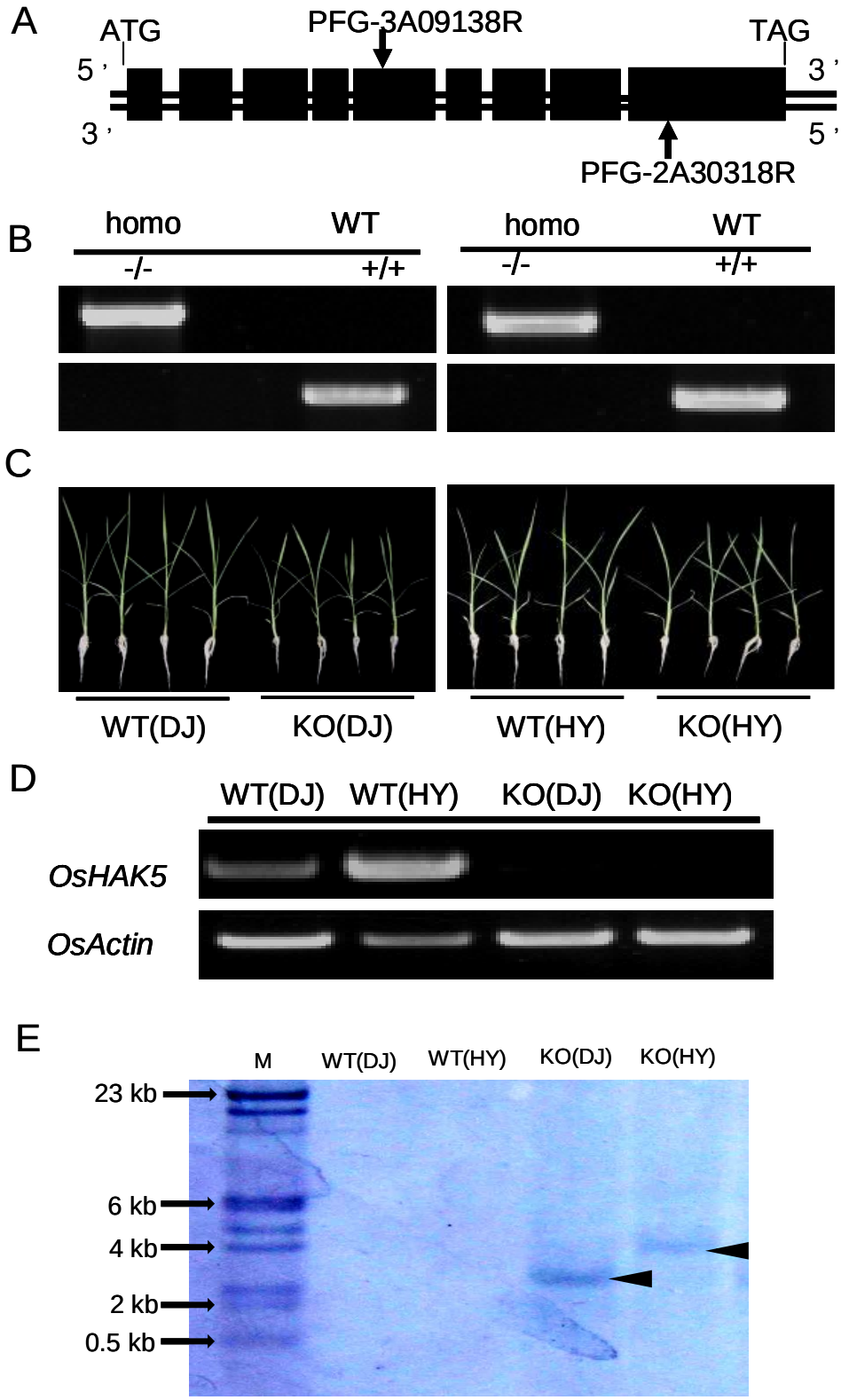


Figure S4: Homozygous T-DNA insertion mutants of *OsHAK5* gene in rice: phenotype and isolation. A, The position of two T-DNA insertions, identified by sequencing the regions flanking theright border of the T-DNA inserts in the

PCR products. **B**, Identification of plants homozygous (homo) for each of the T-DNA insertsusing two rounds of RT-PCR according to the detailed procedures for Japonica subspecies described in (http://signal.salk.edu/cgi-bin/RiceGE). All the primers are listed in the Supplemental Table S4. **C**, phenotype of 10-day-old seedlings of WTs and two homozygous T-DNA insertion (*oshak5* knockout mutant) lines grown in 0.3 mM K solution (KO(DJ) and KO(HY)). **D**, Expression levels of *OsHAK5* in leaves of WTs and *oshak5* mutants. RT-PCR was performed on total extracted RNA using the primers listed in Table S4. **E**, Southern blot analysis of WTs and the two T-DNA insertion lines. Genomic DNA from whole leaves was digested with two restriction enzymes *HindIII* and *EcoRI*, and separated by agarose gel of 1%. Arrow heads: indicated one copy insertion in the genomic DNA. M: marker; WTs: wild types of the Dongjin and Hwayoung cultivars. WT(DJ): wild type of the Dongjin cultivar, KO(DJ): *OsHAK5* knockout mutant line of the Dongjin cultivar. WT(HY): wild type of Hwayoung cultivar, KO(HY): *OsHAK5* knockout mutant line of the Hwayoung cultivar.

Figure S5

Figure S5: Effect of *OsHAK5* knockout on rice growth at conditions of low K supply. Phenotypes of WT and KO lines grown in IRRI solution containing 0.3 mM K for two weeks. Details of the treatment were described in Fig.4 legend. Bar is 5 cm.

Figure S6

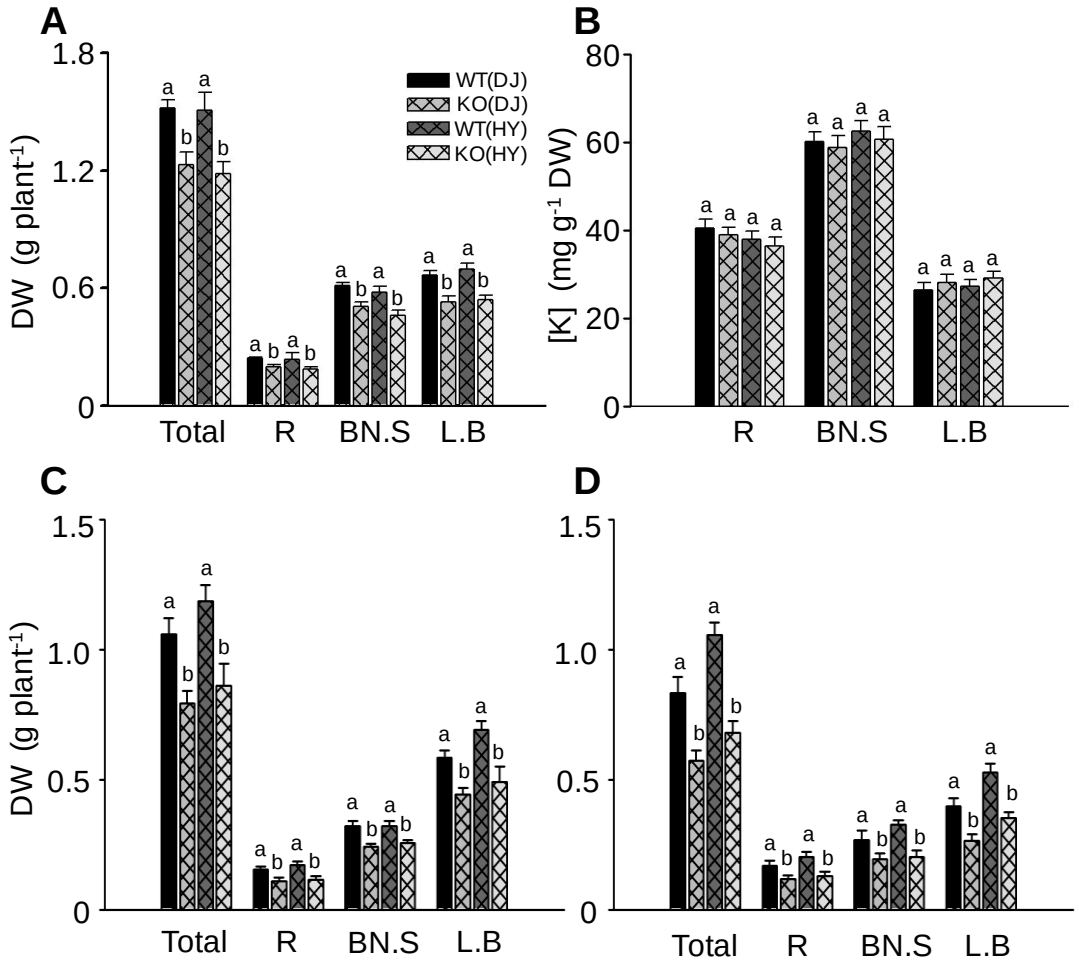


Figure S6: Effect of continuous supply of high K on plant growth and K accumulation of the *OsHAK5* knockout transgenic rice. A and **B**: Ten-day-old seedlings were grown continuously in IRRI solution containing 1 mM K for two weeks, and then supplied with 5 mM K for two more weeks. **C** and **D**: Independent experiment for growth comparison of WT and the *oshak5* mutants which were grown continuously in IRRI solution containing 1 mM K for one week, and then supplied with 10 mM K **(C)** and 20 mM K **(D)** for two more weeks. WT(DJ): wild type of the Dongjin cultivar, KO(DJ): *OsHAK5* knockout mutant line of the Dongjin cultivar. WT(HY): wild type of the Hwayoung cultivar, KO(HY): *OsHAK5* knockout mutant line of the Hwayoung cultivar, error bars: se (n=5 plants), DW: Dry Weight. Significant differences from WT in each group are indicated by different letters (P<0.05,one way ANOVA). R: root, BN.S: basal node + sheath, L.B: leaf blade.

Figure S7

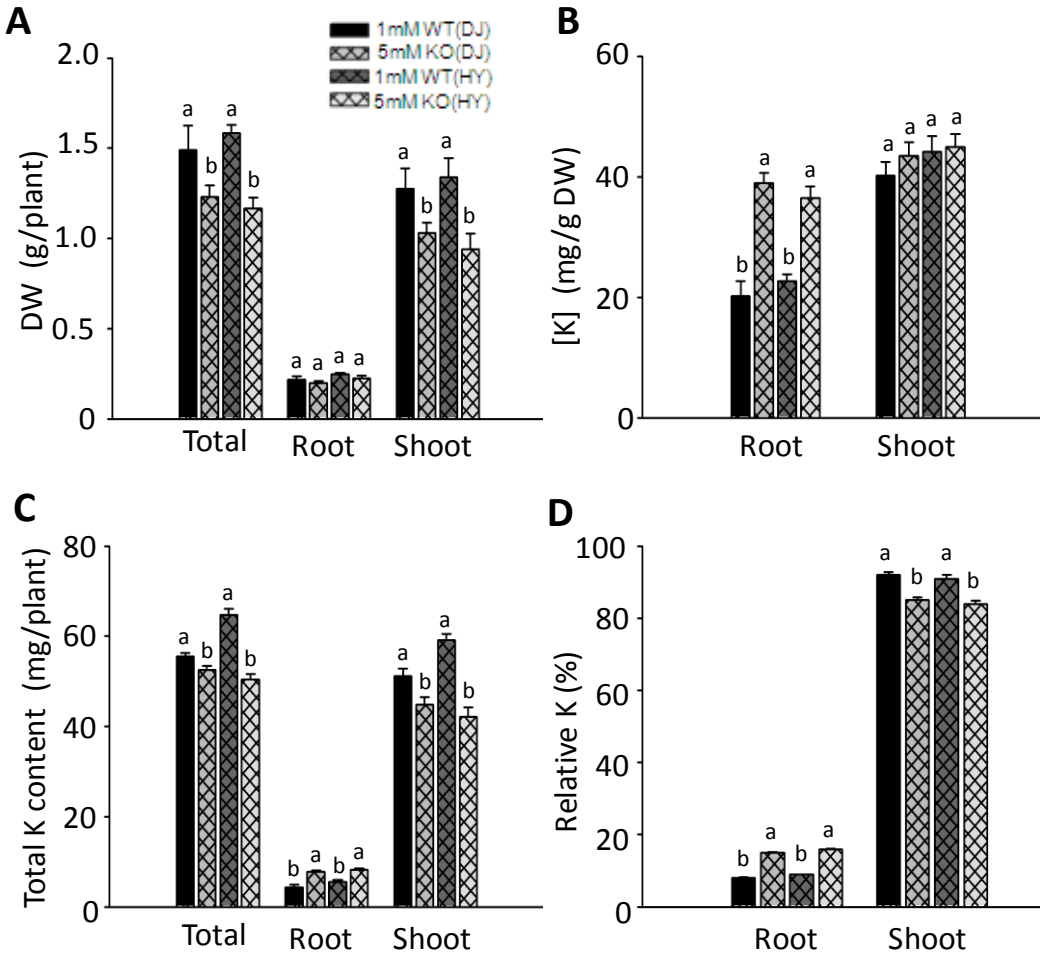


Figure S7: Effect of *OsHAK5* on K partitioning betweenroot and shoot of the rice plants with similar root size and total K contents. WT plants were grown in 1 mM K and KO plants in 5 mM K solution. **A** and **B:** Dry biomass and K concentration of WT and KO of DJ and HYc ultivars; WT data (plants in 1 mM K) are from Figs. 7 B&D and KO data (plants in 5 mM K) are from Figs.S6A&B, re-plotted here with the biomass of basal node and sheath and leaf blade merged into shoot biomass. **C**, Total K content per plant. **D**, Relative amount of total K distributed between roots and shoots of WT and KO plants.

Figure S8

Figure S8: Effect of *OsHAK5* knockout on plant growth in the presence of 100 mM NaCl. Phenotypes of WT and KO lines grown in IRRI solution containing 1 mM K and 100 mM NaCl. Details of the treatment were described in Fig.10 legend. Bar is 5 cm.