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***OsJAZ9* overexpression modulates jasmonic acid biosynthesis and potassium deficiency responses in rice**

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Abstract:

Potassium (K) represents up to 10% of the plant's total dry biomass, and its deficiency makes plants highly susceptible to both abiotic and biotic stresses. K shortage results in the inhibition of root and shoots growth, but the underlying mechanism of this response is unclear. Our RNA-Seq and qPCR analysis suggested leading roles for JA pathway genes under K deficiency in rice. Notably, K deficiency and JA application produced similar phenotypic and transcriptional responses. Here, we integrated molecular, physiological and morphological studies to analyze the role of *OsJAZ9* in JA homeostasis and K deficiency responses. We raised *OsJAZ9* over-expression, knockdown, transcriptional reporter, translational reporter and C-terminal deleted translational reporter lines in rice to establish the role of JA signaling in K ion homeostasis. JA profiling revealed significantly increased JA-Ile levels in *OsJAZ9* OE lines under K deficiency. Furthermore, we established that *OsJAZ9* overexpression and knockdown result in K deficiency tolerance and sensitivity, respectively, by modulating various K transporters and root system architecture. Our data provide evidence on the crucial roles of *OsJAZ9* for improving K deficiency tolerance in rice by altering JA levels and JA responses.

Keywords: Abiotic stress, Potassium deficiency, JAZ repressor, Root system architecture, Lateral roots

INTRODUCTION

Rice is a mainstay for global food security. The two-third population of the world is dependent on rice as a staple food crop. Soil nutrient deficiency is one of the primary limitations of rice production. Notably, Potassium (K) is one of the most important and abundant macronutrients in plants, which can comprise up to 10% of the plant's total dry weight (Leigh & Wyn Jones, 1984). Its physiological functions fall into two categories; the first requires a high and relatively stable concentration of K^+ to regulate the osmotic potential of the cell and activation of many enzymes involved in respiration and photosynthesis (K^+ act as a cofactor for many enzymes). The second category is based on the high mobility of K^+ ions resulting in osmotic changes, which regulate the stomatal movement (Sawhney & Zelitch, 1969; Terry & Ulrich, 1973) and phloem transport (Mengel and Viro, 1974). K^+ is also involved in key cellular processes such as energy production, cell expansion and balancing the counter flux of charges across the membranes (Tester and Blatt, 1989; Wu et al., 1991; Elumalai et al., 2002). Hence, its deficiency affects fitness and overall growth as K deficient plants are more susceptible to salt (Kaya et al., 2006), drought (Egilla et al., 2001; Wang et al., 2013), chilling (Kant and Kafkafi, 2002) and biotic stresses (Hardter, 2002; Sarwar, 2012).

Although K is among the most abundant minerals in the soil; its availability to plants is limited because most of the soil K (about 98%) is in bound forms where its release into the soil solution is far slower than the rate of acquisition by roots (Sparks, 1987). Notably, a very little fraction of soil K is present in the soil solution or exchangeable form whose availability depends on multiple factors like soil pH (Rich and Black, 1964; Rich, 1964), presence of other monovalent cations like Na^+ and NH_4^+ (Qi and Spalding, 2004) and the type of soil particles (Sparks, 1987). K deficiency results in both root (Singh et al., 2015) and shoot (Shankar et al., 2013) growth inhibition. In roots, it strongly impairs primary root growth (Gruber et al., 2013); a similar phenotype is also observed on exogenous Jasmonic Acid (JA) application (Staswick et al., 1992; Cai et al., 2014). Further, a few transcriptome studies have shown many JA signaling genes upregulated under K deficiency in rice (Ma et al., 2012; Shankar et al., 2013; Takehisa et al., 2013). Interestingly, a substantial part of K-responsive transcriptome either was absent or replaced in the Arabidopsis JA receptor, *coil-16* mutant (Armengaud et al., 2004) which indicates active roles of JA signaling in K deficiency responses. Moreover, a significant number of genes were also found commonly upregulated by exogenous JA treatment and K deficiency in rice (Kobayashi et al., 2016), which further indicates crosstalk between JA signaling and K deficiency response.

JA and its derivatives commonly called Jasmonates (JAs) are a group of oxylipin-derived phytohormones, which are synthesized in response to a large number of biotic and abiotic stresses in plants (Feys et al., 1994; Heitz et al., 2016; Kazan, 2015; Riemann et al., 2015). Understanding of JA functions is most advanced in Arabidopsis, but the overall architecture has been found conserved in all examined land plants. JA signaling has been the focus of intensive research in rice and regulates many traits of agronomic importance (Reimann et al., 2015; Nguyen et al., 2019). In absence of JA, JA-regulated genes are maintained silent by so-called JASMONATE ZIM (JAZ) protein repressors that bind to and block the action of bHLH transcription factors MYC2/3/4, with the involvement of additional repressors. Upon stress or developmental cues, the bioactive conjugate jasmonoyl-isoleucine JA-Ile is formed and triggers the assembly of the co-receptor JAZ-CO11 (Sheard et al., 2010). CO11 (CORONATINE INSENSITIVE 1), is the F-box component of the co-receptor, that will be recruited into the ubiquitin E3 ligase complex SCF^{CO11} that targets JAZ protein for 26S proteasome-mediated degradation (Wasternack and Hause, 2013). JAZ destabilisation derepresses a myriad of transcription factors allowing the transcription of numerous JA responsive genes depending on organ or stimulus (Wasternack and Hause, 2013). JAZs form a small family of 15 members in rice (Ye et al., 2009), and play roles in floral development, salt, and biotic stress tolerance (Fu et al., 2017). However, their functions in nutritional, particularly K deficiency response, remain elusive. Here, we examined the functions of a particular JAZ isoform, OsJAZ9, because *OsJAZ9* was the most downregulated *JAZ* gene during K deficiency among all the isoforms (Singh et al., 2015). Further, OsJAZ9 also influenced the rice response to NaCl stress which also involves K homeostasis (Wu et al., 2015). We show that JA-Ile levels are enhanced under K deficiency in rice and this response is further elevated in rice on *OsJAZ9* overexpression. Also, we demonstrate that overexpression of *OsJAZ9* enhanced JA signaling, and this was correlated with improved K deficiency tolerance.

Materials and Methods

Plant growth conditions

Rice seeds var. PB1 (Indica) were surface-sterilized with 0.1% HgCl₂ for 10 min and then washed with sterile water five times. After that, seeds were germinated on autoclaved wet tissue paper for two days in the dark, followed by two days in the light. Equally germinated seeds were then transferred to full strength liquid Yoshida media (pH 5.0-5.5) with NH₄NO₃ (1.40 mM), NaH₂PO₄ (0.32 mM), K₂SO₄ (0.51 mM), CaCl₂·2H₂O (1 mM), MgSO₄·7H₂O (1.7 mM),

H₃BO₃ (19 µM), ZnSO₄.7H₂O (0.15 µM), CuSO₄.5H₂O (0.15 µM), (NH₄)₆Mo₄O₂.4H₂O (0.015 µM), Citric Acid (70.75 µM), Na-Fe-EDTA (60 µM) and MnCl₂.4H₂O (9.46 µM). The concentration of K₂SO₄ was adjusted as required to make-up the desired concentration of K. All the experiments were carried out in a controlled growth chamber maintained at 16 h photoperiod, 30°C day and 28°C night temperature, 280-300 µM photons/m²/sec photon density and ~70% relative humidity.

qRT-PCR analysis

Total RNA was extracted from seedlings of the desired age using Trizol reagent and cDNA synthesis was performed as described (Mehra et al., 2017). Gene-specific primers were designed from CDS (retrieved from Rice Gene Annotation Project) using PRIMER EXPRESS version 3.0 (PE Applied Biosystems TM, USA) with default parameters. SYBR® Green Master Mix was used to quantify the DNA product in Applied Biosystems 7500 Fast Real-Time PCR. For the Relative gene expression analysis, we used the $\Delta\Delta C_t$ method taking *Ubiquitin5* (Os01g0328400) or *Actin1* (Os03g0718100) as endogenous control (Mehra et al., 2016).

Measurement of K and Na

Root and shoot tissues were harvested separately and repeatedly washed with milliQ water. Dried 50 mg tissue was ground and digested in di-acid mixture (20 ml) containing HNO₃ and HClO₄ acid (9:4 ratio) using a hot plate digestion system. The diluted cooled digest was filtered and stored in polypropylene bottles with a final volume of 50 ml. Analysis of Na and K content was performed using a flame photometer (Systronics Flame Photometer 128µC).

Generation of transgenic lines

Full-length cDNA (AK070649) of *OsJAZ9* was amplified using gene-specific primers (Supplemental Table S1) and cloned under *ZmUbi1* promoter in gateway-compatible overexpression vector as described (Pandey et al., 2017). For raising RNAi transgenics of *OsJAZ9*, 416 bp region of *OsJAZ9* cDNA was amplified from the cDNA region encompassing 3'UTR and cloned in *pANIC8b* vector (Mann et al., 2012) by Gateway Technology. To generate *p35S:OsJAZ9_GUS* and *p35S:OsJAZ9ΔC_GUS* lines, *OsJAZ9* coding region and *OsJAZ9ΔC* (deleted 65 amino acids including jas motif from the C-terminal of OsJAZ9) cDNAs were amplified using gene-specific primers, and cloned into *pCAMBIA1301*.

Overexpression, RNAi, *p35S:OsJAZ9_GUS* and *p35S:OsJAZ9ΔC_GUS* transgenic rice lines were raised as described (Mehra et al., 2017).

For generating *OsJAZ9* promoter lines (*pOsJAZ9:GUS*), 1495 kb upstream region of the gene CDS (*OsJAZ9*) was cloned in *pMDC163* driving GUS reporter gene. Regenerated plants were selected on hygromycin (50 μg/ml) and screened by qRT-PCR and GUS histochemical staining. All the experiments were performed in T3 homozygous transgenic lines.

Analysis of GUS Activity

Newly emerged roots were selected for histochemical staining, i.e. GUS activity. Samples were harvested at indicated time points and immersed in GUS buffer (1 mM 5-bromo-4-chloro-3-indolyl-β-glucuronide sodium salt in 50 mM sodium phosphate, pH 7.0, ten mM Na-EDTA, 0.5 mM ferricyanide, 0.5 mM ferrocyanide, and 0.1% Triton X-100), and were incubated at 37°C for overnight. Chlorophyll was cleared from plant tissues by immersing them in 70% ethanol, overnight. For detection of *OsJAZ9* degradation in-vivo, roots from transgenic lines containing *p35S:OsJAZ9_GUS* and *p35S:OsJAZ9ΔC_GUS* plants were treated with MeJA (100 μM) with or without the proteasome inhibitor MG132 (100 μM) for 1h. Histochemical GUS staining of roots was performed to visualize the abundance/degradation of OsJAZ9 and OsJAZ9ΔC. GUS signal was observed under a stereo-zoom microscope. Fifteen-days-old seedlings of *p35S:OsJAZ9_GUS* and *p35S:OsJAZ9ΔC_GUS* transgenic lines were subjected to K deficiency (4.08 μM external K⁺) and root samples were harvested on indicated time points and immersed in GUS buffer. *pOsJAZ9:GUS* transgenic lines were subjected to K deficiency (0 μM external K⁺) and root samples were harvested on indicated time points and immersed in GUS buffer.

Subcellular localization of OsJAZ9

In-silico prediction of OsJAZ9 was analyzed with the help of Plant-PLoc database (<http://www.csbio.sjtu.edu.cn/cgi-bin/PlantmPLoc.cgi>). For in-plants validation, CDS of *OsJAZ9* was cloned into pSITE3CA vector to produce YFP:OsJAZ9 fusion protein. Subcellular localization was analyzed in onion epidermal cells using the particle bombardment method (Singh et al., 2015). YFP fluorescence was visualized under a fluorescence microscope (Nikon Eclipse 80i).

MeJA mediated root growth inhibition assay

Uniformly, germinated seedlings were transferred to Yoshida liquid media and allowed to grow for 10 days under normal conditions. After 10 days of growth, 10 μ M MeJA dissolved in DMSO was added to one set of plants (including WT, RN12, and OE10) while another set was supplied with DMSO alone (mock). pH was maintained at 5.4 and the media was replenished after five days. Root growth inhibition was analyzed after 10 days of MeJA treatment by calculating the percentage change in root growth between DMSO and MeJA treated plants.

Exogenous MeJA treatment to analyze the impact on K deficiency

To investigate the impact of exogenous application of MeJA on K deficiency, uniformly germinated WT rice seedlings were transferred to hydroponic (Yoshida) media with 4.08 μ M K_2SO_4 , 8.16 μ M K_2SO_4 and 510 μ M K_2SO_4 for 5 days and then equal number of seedlings were transferred to 0 μ M, 0.1 μ M, 0.01 μ M and 0.001 μ M MeJA with pre-existing K_2SO_4 concentrations for 10 days. These seedlings were analyzed for root length, shoot length and lateral root length. After this analysis, the same seedlings were transferred to a hot air oven at 60°C for three days, and dry biomass was observed.

Transcriptome Analysis

Uniformly germinated WT and *OsJAZ9* overexpressing line OE (OE10) seedlings were transferred to 4.08 μ M and 510 μ M concentrations of K_2SO_4 for 15 days along with other nutrients as per Yoshida liquid media. After that, total RNA was isolated with Qiagen RNeasy Mini Kit from the whole seedling according to the manufacturer's protocol. RNA integrity was analyzed using Bioanalyzer (2100 Agilent technologies), and samples with RIN value ≥ 7.5 proceeded for library preparation. Transcriptome sequencing was done using the Illumina HiSeq 2500/4000 platform with two independent biological replicates for each WT and *OsJAZ9* OE10. Adapter sequences and low-quality bases were trimmed using AdapterRemoval v2 (version 2.2.0). Further, rRNA sequences were removed by aligning reads with silva database. Finally, processed reads were aligned with the MSU7 genome (http://rice.plantbiology.msu.edu/pub/data/Eukaryotic_Projects/o_sativa/annotation_dbs/pseudomolecules/version_7.0/all.dir/) using the STAR program (Version 2.5.3a). Differentially expressed genes were identified as described (Bandyopadhyay et al., 2017).

JA quantification

For JA and JA-Ile quantification, uniformly germinated WT and *OsJAZ9* overexpression (OE10) seedlings were transferred to 4.08 μ M and 510 μ M concentrations of K_2SO_4 in Yoshida liquid media for 15 days. Whole seedlings were harvested, freeze-dried and stored at -80°C until used. For JAs and JA-Ile estimation, 250mg of total seedlings was homogenised and extraction was done with 1.5 ml methanol containing 60 ng of 9,10-d2-9,10-dihydrojasmonic acid and 15 ng of JA-[13C6]Ile conjugate as internal standards. The homogenate was vortexed for 30 min using vortex followed by spin at 14000 rpm for 20 min at 4°C. A second extraction was again done with 0.5 ml methanol and the supernatant was pooled. The combined supernatant was evaporated in a SpeedVac and re-dissolved in 0.5 ml methanol. JA and JA-Ile concentrations were quantified in at least four replicates, as described in Vadassery et al. (2012). JAs and JA-Ile were quantified on Exion LC coupled to Triple Quad 6500+ (Sciex). JAs and JA-Ile were quantified relative to the signal of their corresponding internal standard.

RESULTS

K deficiency promotes JA signaling in rice

Our low potassium dose-response experiment showed drastic root and shoot growth inhibition of rice seedlings (Fig. S1). To underpin the underlying genes responsible for root and shoot growth inhibition, we performed RNA-seq analysis and compared the gene expression in WT plants grown under K sufficient and deficient conditions. Interestingly, we found induction of several JA biosynthetic genes like *OsLOX8*, *OsACX2*, *OsOPR10*, *OsJAO1* etc. (Table S2). To validate this observation, we performed temporal expression profiling of selected JA-associated genes under K deficiency using qRT-PCR. This analysis confirmed the induced expression of *OsCOI1b* and *OsMYC2* on K deficiency suggesting elevated JA signalling. Moreover, we also found increased expression of *OsAOS1*, *OsAOS2* and *OsJAR1* suggesting increased JA biosynthesis under K deficiency (Fig. S2). These results indicate a higher JA level and concomitantly induced JA signalling during the initial phase of K deficiency in rice.

In our previous study, we had reported highest downregulation of *OsJAZ9* especially at early time points under low K conditions (Singh et al., 2015). To extrapolate this result, we performed an early time point expression analysis of *OsJAZ9* under K deficiency and found down-regulation of *OsJAZ9* transcripts at initial time point (Fig. S2A). Our *pOsJAZ9:GUS* transcriptional reporter lines also showed transcriptional down-regulation of *OsJAZ9* in a temporal fashion (Fig. 1). On the basis of these results, we hypothesized that K deficiency

provokes increased JA signalling and biosynthesis, and *OsJAZ9* may be involved here. Moreover, we also found *OsJAZ9* as a nuclear-localized JAZ protein (Fig. S3).

JA signalling alters both RNA and protein levels of JAZ repressors. Therefore, to study the effect of *OsJAZ9* on K deficiency, we raised dual purpose overexpression lines of *OsJAZ9* (*CaMV35S:OsJAZ9_GUS*). In addition to overexpression of *OsJAZ9*, these lines also serve the function of a translational reporter. Expectedly, MeJA treatment promoted the degradation of complete JAZ9-GUS signal however, GUS signal was intact in the roots of the *OsJAZ9*-GUS reporter without C-terminal Jas domain implying that *OsJAZ9* is degraded in the presence of JA through Jas domain (Fig. 2B). Further, MeJA supplemented with MG-132 (proteasome inhibitor) was unable to degrade *OsJAZ9_GUS* signal, which confirms that JAs degrade *OsJAZ9* via proteasomal pathway (Fig. 2B).

Therefore, we utilized these two lines to investigate JA response during K deficiency considering *OsJAZ9_GUS* stability as a proxy for JA signalling response. Interestingly, initial exposure of K deficiency (up to 3h) showed a rapid decrease in *OsJAZ9_GUS* signals. However, after 6h, *OsJAZ9_GUS* signal was stabilized indicating early induction of JA signaling under K deficiency (Fig. 2C). However, on prolonged K deficiency exposure, *OsJAZ9_GUS* signal remains stabilized until 12 h and starts decreasing after that. Moreover, after 15 days of K deficiency, *OsJAZ9_GUS* signal again disappeared completely (Fig. S4B). This revealed a kind of rhythmic stability of *OsJAZ9* levels during the early phase of K deficiency. Expectedly, *OsJAZ9 Δ C_GUS* line showed consistent GUS signal throughout the duration of K deficiency (Fig. 2C and S4B). These results confirmed that JA levels are indeed enhanced during K deficiency.

Overexpression of *OsJAZ9* enhances K deficiency tolerance in rice

To characterize the functional roles of *OsJAZ9* under K deficiency, we analysed homozygous lines of *OsJAZ9* overexpression (OE2 and 10) and RNAi (RN12 and 14). The overexpression lines had significant upregulation while RNAi lines showed around 98% reduction in the *OsJAZ9* expression as compared to WT (Fig. S5). We next tested the growth performance of WT and transgenic lines grown in +K (control) and -K (K deficient) media for fifteen days. Phenotypic analysis revealed that K deficiency inflicted lesser plant growth inhibition in *OsJAZ9* overexpressing lines as compared to WT while RNAi lines showed no significant difference in the shoot biomass (Fig. S6; S7).

Furthermore, we performed another experiment including *OsJAZ9* OE, RNAi and WT seedlings in K sufficient media for 10 days followed by K deficiency for the next 15 days and thereafter recovery in K sufficient media for next 5 days. *OsJAZ9* OE plants were performing significantly better than WT and RNAi lines by accumulating more root and shoot dry biomass under both K deficiency and K recovery conditions. (Fig. 3). Even, the root numbers (seminal and crown root) in *OsJAZ9* OE plants were also significantly higher than WT and RNAi lines under K deficiency and recovery conditions (Fig. 3). Interestingly, we also observed a higher Na^+/K^+ ratio in OE plants than both WT and RNAi lines during K deficient conditions (Fig. 3). Further, during recovery conditions this Na^+/K^+ in OE plants was found significantly lower than both WT and RNAi lines. Moreover, we observed higher K content in OE plants during recovery conditions which remained non-significant during K deficient conditions. Since, during K deficient conditions there was no external K available for uptake and OE plants absorb Na^+ which is the structural mimic of K^+ which resulted into higher Na^+/K^+ in OE lines.

In order to investigate better K uptake in *OsJAZ9* overexpression plants, we analysed the expression pattern of known K^+ transporters under K sufficient, deficient and recovery conditions. Expression profiling (including *OsHKT2;1*, *OsHAK5* and *OsHAK1*) showed induced expression of these K^+ transporting genes under K deficient and recovery conditions (Fig. S8A). Moreover, we also found higher expression of *OsHKT2;1* and *OsHAK1* in *OsJAZ9* OE lines, especially after 12 h of K recovery (Fig. S8B). The higher expression of K transporters well supports the better K uptake in *OsJAZ9* OE plants during K recovery conditions.

Transcriptome profiling revealed enhanced JA biosynthesis in *OsJAZ9* OE lines in rice

Overexpression of several *JAZ* repressor encoding genes under the constitutive promoter has resulted in partial JA insensitivity in Arabidopsis and rice (Thines et al., 2007; Yamada et al., 2012). To understand the JA sensitivity level of the *JAZ9* transgenics, we analyzed root growth inhibition of *OsJAZ9* OE and RNAi lines upon MeJA treatment. Consistent with previous studies, we also observed a partial JA insensitive phenotype in *OsJAZ9* OE lines (Fig. 4). We found ~36% root growth inhibition in RNAi line, whereas only 11% reduction in overexpressing lines (OE10) as compared to WT (30% root length reduction) upon exogenous MeJA treatment. This experiment also suggests that *OsJAZ9* OE may have higher level of bioactive JA.

To uncover the global transcriptional response of *OsJAZ9* overexpression under K deficiency, we performed RNA-seq analysis of WT and *OsJAZ9* OE (OE10) seedlings raised under normal and K deficient conditions for 15 days. The comparative +K vs. -K analysis revealed a total of 5173 and 5505 genes were differentially expressed ($p < 0.05$, $q < 0.05$) in WT and *OsJAZ9* OE line, respectively, during K deficiency. Out of these 2433 and 2670, genes were upregulated, and 2740 and 2835 genes were downregulated, respectively, in WT and *OsJAZ9* OE line (Fig. 5 A-B).

We further analyzed the expression of all known JA biosynthesis and signaling genes in our transcriptome data. Out of 17 JA-biosynthesis related genes detected in the transcriptome, seven were showing higher expression in OE plants as compared to WT during K deficiency considering their respective controls while four genes were downregulated (Table S3). We also validated our RNA-seq results by qRT-PCR analysis and most of the selected genes showed very similar expression pattern (Fig. S9).

We found up-regulation of *OsAOS2*, *OsLOX5*, *OsLOX7*, *OsLOX8*, *OsACX2*, *OsOPR5*, and *OsJAO1* which play key roles in JA biosynthesis (or homeostasis) and higher expression of key JA signaling marker genes like *OsVSP2* in *OsJAZ9* OE plants than WT plants subjected to K deficiency (Table S3). This higher transcript abundance of JA biosynthesis, signaling and marker genes confirmed the elevated JA response in *OsJAZ9* OE plants during K deficiency as compared to the WT. Further analysis revealed that out of 13 known K transporters, 7 were having higher transcript abundance in OE line as compared to WT under K deficiency, while 6 transporters were downregulated (Table S4). Among the lesser expressed ones, *OsHKT27* encodes a K efflux transporter, *OsHKT2;2* and *OsHKT1;5* were described as more efficient in Na transport than K (Kadar et al., 2006) while *OsK1.1* (*OsAKT1*) functions at the millimolar range of external K (Ahmad et al., 2016). We found *OsHAK10*, *OsHAK24*, *OsK3.1*, *OsK5.2*, *OsHKT1;1*, *OsHKT1;4*, *OsSOS1* and *OsGORK* genes having higher abundance in OE line as compared to WT in K deficient conditions and thus supports the case for higher K uptake in *OsJAZ9* overexpressing plants (Table S4).

***OsJAZ9* modulates root system architecture**

As overexpression of *OsJAZ9* resulted in higher K levels in transgenic plants, we analyzed root traits that could contribute to K uptake in addition to the K transporter activity. Interestingly, *OsJAZ9* OE plants showed shorter seminal roots while RNAi plants have longer seminal roots both in K deficient and sufficient conditions (Fig 6). However, *OsJAZ9* OE lines had longer

lateral roots in both low and optimal K conditions (Fig. 6C). In WT background, we also found higher expression of *OsJAZ9* in elongation and maturation zones as compared to the root tip of seminal roots. On comparison, qRT-PCR analysis showed higher *OsJAZ9* expression in lateral roots than primary root suggesting a role for *OsJAZ9* in regulating lateral root growth (Fig. S10).

Previously, low doses of exogenous JA were reported to enhance the lateral root length in *Arabidopsis* and rice, while at higher doses, JA inhibits lateral elongation (Raya-Gonzalez et al., 2012; Hsu and Kao, 2011). We speculated that this slow growth of seminal roots along with enhanced lateral elongation could be either due to higher levels of intrinsic JA or enhanced JA signaling in *OsJAZ9* OE lines. To test these possibilities, we analyzed the effect of exogenous MeJA application on RSA (Root System Architecture) of WT plants grown in control (510 μ M K_2SO_4) and K deficient (4.08 μ M K_2SO_4) conditions for 15 days under varying concentrations of MeJA. Interestingly, we observed increased lateral root length at lower concentrations of MeJA (0.001 and 0.01 μ M) while the higher level of MeJA (1 μ M) inhibited the lateral root elongation under both control and K deficient conditions (Fig. S11; S12). In agreement with Raya-Gonzalez et al. (2012) and Hsu and Kao (2011), we also observed increased lateral root numbers at lower concentrations of exogenous MeJA (Fig. S11C; S12C). Again, longer laterals roots were overrepresented in lower MeJA treatment as compared to no MeJA or higher MeJA concentrations (Fig. S11D; S12D). These observations also validated the hypothesis that *OsJAZ9* OE plants are experiencing higher JA level which is also reflected in our transcriptome data.

As we found *OsJAZ9* overexpression transgenics modulate RSA, we were interested to understand tissue specific expression of *OsJAZ9* in various organ of roots. For this, we generated stable transcriptional reporter lines of *OsJAZ9* with GUS (*pOsJAZ9:GUS*). GUS staining of roots of *pOsJAZ9:GUS* reporter line showed expression of *OsJAZ9* only confined in lateral roots (Fig. 1). Remarkably, *OsJAZ9* promoter:GUS signal was faded away when the reporter lines were transferred to a low K regime and completely vanished after 15 days of low K treatment in lateral roots (Fig. 1). This lateral root-specific expression pattern confirms the role of *OsJAZ9* as evidenced in regulating lateral root elongation. qRT-PCR analysis further confirmed the higher expression of *OsJAZ9* in lateral roots (LR) than the rest of the root (primary root, PR). This validated the qRT-PCR result of *OsJAZ9* expression in other root organs.

As overexpression lines of *OsJAZ9* contained a large GUS tag, we suspected that our findings may be influenced by under/over-estimation of *OsJAZ9* functions in K deficiency. To exclude any such possibility or observation bias, we also raised overexpression lines of ‘untagged *OsJAZ9*’ under *ZmUbi1* promoter. We screened these transgenic lines and reanalysed their phenotypes under K sufficient and deficient conditions (Fig. S13). Notably, the new overexpression lines also behaved similar to the studied translational reporter lines under K deficiency (Fig. S14; S15). This reconfirmed our conclusions about roles of *OsJAZ9* under K deficiency.

Overexpression of *OsJAZ9* enhances in-vivo JA-Ile levels under K deficiency

The observed phenotypes of *OsJAZ9* OE lines so far suggest invariably heightened JA response which might be contributed by either increased intrinsic JA levels or the increased JA signaling in *OsJAZ9* OE lines as compared to the WT. To test whether there was indeed higher JA content in *OsJAZ9* OE plants or these plants are only experiencing elevated JA signaling; we analyzed the levels of JA and JA-Ile levels in WT and *OsJAZ9* overexpression (OE10) seedlings raised under K deficient and sufficient conditions as compared to their respective controls. We found slightly higher JA levels in OE plants as compared to WT plants under both K sufficient and K deficient conditions (Fig. S16). To our surprise, we observed no change in JA-Ile content in OE plants as compared to WT under K sufficient conditions. However, JA-Ile content was significantly increased in OE plants as compared to WT plants in response to K deficiency. The induction was four fold in WT plants while it was around 8 fold in OE plants under K deficiency (Fig. 7). Based on this evidence, we conclude that overexpression of *OsJAZ9* results in enhanced bioactive JA levels during K deficiency, which is also reflected in the transcriptome data.

DISCUSSION

Potassium is one of the essential minerals for plant growth. Despite its high concentration in soil, it remains mostly unavailable for root uptake and utilization. Therefore, its deficiency in soil remains one of the major constraints for crop productivity. As K^+ being a structural mimic of Na^+ , its deficiency also results in high Na^+ uptake by the plant and causes salinity stress. Therefore, K availability in the soil also influences the level of salt stress experienced by the plant. In contrast to Phosphorus (P) deficiency responses, where enzymes like purple acid phosphatase can elevate the P availability in soil (Mehra et al., 2017), there is no such enzyme known in rice to hydrolyze locked-up K in the soil. Therefore, strategies targeting exploiting

of K transporters and reshaping of root architecture are more suited for enhancing K-uptake-efficiency in rice.

While JAs are well-known for controlling plant defence against fungal and insect attacks (Peng et al., 2012; Wasternack and Hause, 2013), emerging evidences have established their role in diverse plant processes like flower development, reproduction, and response to abiotic and biotic stresses (Cai et al., 2014; Kazan, 2015; Nguyen et al., 2019). JAs are also known for inhibiting root growth, and recently intricate details of this response were revealed (Wasternack and Hause, 2013; Wasternack and Song, 2017). Root growth inhibition during K deficiency mimics the root growth inhibition phenotype observed on exogenous JA treatment in various plants (Staswick et al., 1992; Gruber et al., 2013; Cai et al., 2014). Recently, Li et al. (2017) and Troufflard et al. (2010) have found enhanced JA levels during K deficiency in Arabidopsis, rice, and wheat. JAZ repressors are pivotal to JA signaling, and their genetic manipulation can lead to many altered plant processes (Wasternack and Song, 2017). We have reported previously that *OsJAZ* genes encoding JA signaling repressors are transcriptionally responsive to K deficiency (Singh et al., 2015). However, it was not known if *JAZs* can also regulate K deficiency responses. Here, we found that *OsJAZ9* expression and stability form a rhythmic/wavy pattern indicating a faster early response and a slower late response towards K deficiency (Fig. 1, S2 and S4). Similar wavy patterns were also observed for *JAZ* genes expression in Arabidopsis upon exogenous JA treatment (Hickman et al., 2018). These observations strengthen our hypothesis that K deficiency response involves enhanced JA response.

Here using comprehensive transgenics, physiological and morphological approaches, we showed that *OsJAZ9* is a crucial component of JA signaling for K deficiency responses. *OsJAZ9* OE lines performed better in terms of root and shoot growth under K deficiency, while RNAi lines performed poorly. We also noticed that *OsJAZ9* overexpression plants are showing both better K-uptake-efficiency (higher K levels in *OsJAZ9* OE plants under recovery as compared to WT) and K-use-efficiency (*OsJAZ9* OE plants were able to produce more biomass during K deficient conditions). In addition, higher Na^+/K^+ found in OE lines during K deficient conditions was reflecting increased Na^+ uptake while K^+ contents were less affected. This could be explained well as Na^+ and K^+ are the structural mimics of each other and Na^+ can be taken up more when K^+ is not available to the plants, which can result in a higher Na^+/K^+ ratio under extremely K deficient conditions (0 μM K). This becomes clearer when the K starved plants were recovered with 10 μM K_2SO_4 and Na^+/K^+ goes down in OE lines, but it remains high in

RNAi lines. This decrease of Na^+/K^+ ratio was more due to the efficient K^+ uptake rather Na^+ uptake by OE lines as indicated by the K^+ content per plant. These results suggested that *OsJAZ9* has improved K^+ over Na^+ uptake during low K^+ availability. The elevated K^+ uptake of *OsJAZ9* OE lines was well supported by the higher transcript abundance of K transporters, *OsHAK1* and *OsHKT2;1*.

Interestingly, we did not find *OsNHX1* induction under K deficiency while its transcripts were induced during NaCl excess stress (Wu et al., 2015). Thus its non-responsive nature towards K deficiency shows that *OsNHX1* is very specific for its exclusive expression in NaCl stress. This expression behaviour demarcates the thin boundary between salt and K deficiency stress. A higher magnitude of transcriptome changes in *OsJAZ9* OE lines further also showed greater responsiveness towards K deficiency than WT. Particularly, enhanced expression of JA biosynthesis and marker genes like *OsAOS2*, *OsLOX5/7/8*, *OsJAO1* and *OsVSP2*, respectively, confirms the higher JA levels in *OsJAZ9* OE plants during K deficiency.

The enhanced JA response on overexpression of a repressor is surprising; however, similar observations on activated JA signaling were also made on *JAZ7* overexpression in Arabidopsis (Thatcher et al., 2016). Therefore, the intricate contribution of *OsJAZ9* with positively and negatively interacting partners of the JA signaling machinery needs to be elucidated. How the overexpression of a JAZ repressor (*OsJAZ9*) influence the increased JA biosynthesis is not clear. Does *JAZ9* also activate some component of JA biosynthesis machinery would answer this question. If overexpression of *OsJAZ9* suppresses the downstream JA signalling by suppressing the *OsMYC2* then why JA response is elevated in *OsJAZ9* overexpressing transgenic seems counterintuitive. In fact we observed some JA signalling genes being upregulated in *OsJAZ9* OE plants. This implies that JA biosynthesis machinery is directly or indirectly influenced by *OsJAZ9*. Further pulldown assay of *OsJAZ9* interactome will reveal several aspects of its action.

In addition to enhanced K transporters activity, longer laterals might have also contributed to improved K uptake in *OsJAZ9* OE plants. It appears that seminal root length was inhibited in OE lines, but an increase in laterals length might have increased root surface area for better K acquisition. This also suggest that plant have different level of JA sensitivity for lateral elongation and seminal elongation. It would be fascinating to reveal how same level of JA act differently to augment lateral elongation and seminal growth suppression. We cannot rule out the possibility of having different signal amplification (JA response) in lateral and seminal

roots. Our transcriptional reporter lines show that *OsJAZ9* is preferentially expressed in elongating lateral roots than seminal root tips. Therefore, relatively (seminal vs lateral) a small dose of external JA application might have distinct effect on laterals than seminal roots. Previously, exogenous MeJA has resulted in the enhanced lateral root length and density in *Arabidopsis* (Raya-González et al., 2012; Hsu and Kao, 2011). Our observations of increased lateral root elongation by low concentrations of MeJA, confirm the growth-promoting nature of JA at a low level, and parallel the longer lateral phenotype of *OsJAZ9* OE lines. However, it may hold true only at lower levels of JA as higher exogenous JA is growth inhibitory irrespective of the stress.

At morphological levels, JA treatment is known to inhibit the primary root growth in plants (Staswick et al., 1992; Cai et al., 2014). Consistent with these studies, we also found *OsJAZ9* OE plants having shorter roots than WT, mimicking the canonical JA overproduced or enhanced JA signaling response. This phenotype is very appealing as most of the other *JAZ* repressors overexpressed transgenics in *Arabidopsis* (*JAZ1*, *JAZ2*, *JAZ3*, *JAZ5*, *JAZ7*, and *JAZ9*) and rice (*JAZ1*) reported earlier, either promoted growth or showed no change (Chini et al., 2007; Hakata et al., 2017; Yang et al., 2012). Like other *JAZs*, root growth inhibition assay revealed reduced JA sensitive behavior of *OsJAZ9* OE lines while knockdown lines were JA hypersensitive.

The induction of JA biosynthetic genes and the phenotypes mimicking the JA treatment made us analyze the JA and JA-Ile content in transgenic lines. The slightly enhanced JA and highly induced JA-Ile accumulation in *OsJAZ9* transgenic lines explain higher JA response in OE lines during K deficiency. Mostly, overexpression of *Arabidopsis JAZs* inhibits JA induced growth suppression while the loss of function mutants of *AtJAZs* exhibited hypersensitivity to JA (reviewed in Wasternack and Song, 2017). In agreement with Wu et al. (2015), we also found JA hypersensitive response in *OsJAZ9* knockdown lines. However, elevated JA-Ile levels and signaling in *OsJAZ9* OE lines are very intriguing.

We suspect here a negative feedback regulation of JA homeostasis wherein constitutive overexpression of a *JAZ* repressor hyperactivated JA signaling (Chini et al., 2007; Thatcher et al., 2016). *JAZs* activities are tightly controlled at tissue levels. By constitutively overexpressing a *JAZ* repressor, we may have disturbed that fine-tuned JA homeostasis resulting in enhanced JA levels which in-turn lead to enhanced JA response in *OsJAZ9* overexpression plants as also reported earlier in *Arabidopsis* (Thatcher et al., 2016).

Noticeably, JA levels were also enhanced in knockdown lines of *AtJAZ1* while it remained unchanged in the knockdown lines of *NaJAZh* (Li et al., 2017; Oh et al., 2012).

A closer look at the transcriptome data revealed the suppression of all *JAZ* repressors under both normal and low K conditions in *OsJAZ9* overexpression plants (Table S5). This makes OsMYC2 free from the clutch of *JAZ* repressors, which leads to enhanced JA response. This elevated JA response can shape the overall phenotype and its effect on root growth. The suppression of other *JAZs* can underscore the effect of *OsJAZ9* overexpression. How does low K level invokes JA biosynthesis will pave the way for a broader understanding of low K perception and its close association with JA machinery. However, our multifaceted evidence supports the role of *OsJAZ9* as an important element regulating JA-biosynthesis and response and thus influencing root system architecture and K homeostasis.

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AUTHORS CONTRIBUTIONS

A.P.S., B.K.P., and P.M. conducted experiments and analyzed data. J.G. designed the project, supervised experiments. A.P.S., B.K.P., P.M., T.H., and J.G. wrote the manuscript.

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656

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FIGURE LEGENDS

Figure 1. Promoter of *OsJAZ9* is K deficiency responsive. GUS expression in lateral roots on nodal roots of *pOsJAZ9:GUS* transgenic rice plants. Expression was mainly appeared in laterals which was monitored at different time points under K deficiency. *pOsJAZ9:GUS* expression faded away as the K deficiency is prolonged from 3 days to 15 days. Uniformly germinated fifteen-days-old seedlings from +K media were transferred to -K media in a hydroponic system and GUS signal was analysed. (DAT, Day after K deficiency treatment).

Figure 2. Potassium deficiency causes a rapid increase in JA signaling in rice. (A) Schematic representation of *OsJAZ9_GUS* and *OsJAZ9ΔC_GUS* (Jas domain deleted) translational reporter constructs. **(B)** JA dependent proteasomal degradation of OsJAZ9 through Jas domain. Root tips of plants overexpressing *OsJAZ9_GUS* and *OsJAZ9ΔC_GUS* were analysed for GUS activity after 3h of treatment with 100 μM Me-JA alone or combined with 100 μM proteasome inhibitor, MG132. **(C)** 15-days-old rice seedlings expressing *OsJAZ9_GUS* and *OsJAZ9ΔC_GUS* were transferred to K deficient media for indicated time points (0.5 to 12 h). Stability of OsJAZ9_GUS and OsJAZ9ΔC_GUS proteins in response to K deficiency were analyzed by histochemical GUS staining of roots. The experiment was performed in three independent replicates.

Figure 3. Enhanced K acquisition in K-starved *OsJAZ9* OE seedlings upon K resupply. **(A)** Morphological data (root dry weight, shoot dry weight and number of roots per plant) of 10-days-old normally grown seedlings (normal/S1), **(B)** followed by 15-days of K starvation (K stress/S2) and **(C)** subsequent recovery with 10 μM K₂SO₄ for 5 days (Recovery/S3). K stress represents the ratio of dry root, or shoot biomass K stressed plants with respect to normal condition and recovery represents the ratio of recovered plants with respect to normal condition (n=<15). **(D)** Na⁺/K⁺ and **(E)** Total K content per plant of WT and *OsJAZ9* transgenics during S1, S2 and S3 treatments. K content experiment was performed with two replicates. Asterisks indicate significant changes in transgenics compared to WT at respective time points ($p \leq 0.05$, .01 and .001 for *, ** and *** calculated from Student's *t*-test). Scale =10 cm.

Figure 4. JA-induced root inhibition in WT and *OsJAZ9* transgenics. (A) Representative images of roots of 20-days-old WT, RN12 and OE10 seedlings supplemented with DMSO (Mock; M) or 10 μM MeJA dissolved in DMSO (JA treated; J). Scale = 5 cm. **(B)** Average root

lengths of WT, *OsJAZ9* RNAi and OE plants under DMSO (blue bars) and MeJA (Red bars) treatments. Each bar represents average of 9 seedlings with standard error. Values over the bars represent JA mediated percentage inhibition in the root length as compared to DMSO treated plants. Significant differences between DMSO vs MeJA treatment were evaluated by Student's *t*-test. Asterisks; *, **, *** and **** indicate *p* values, $\leq 10^{-3}$, 10^{-4} , 10^{-8} and 10^{-10} , respectively.

Figure 5. Overexpression of *OsJAZ9* enhances K deficiency response in rice. (A) Venn diagrams showing differentially regulated genes on K deficiency in WT and *OsJAZ9* OE lines after 15 days of deficiency. (B) Overview of transcriptome data showing category wise distribution of differentially genes in WT and *OsJAZ9* OE lines during K deficiency. Error bar represents SE of all the replicates. ($p \leq 0.05$, Student's *t*-test). FC=Fold Change.

Figure 6. *OsJAZ9* overexpression influences root system architecture. (A) Representative root images of 12-days-old WT and *OsJAZ9* transgenics showing lateral root lengths during K deficient (upper panel) and K sufficient conditions (lower panel). (B) Average seminal root length and (C) Average lateral root length of WT and *OsJAZ9* transgenics during K deficient (upper row) and K sufficient (lower row) conditions. Four independent plants were used for the analysis. Scale bar represents 1 cm while error bar represents SE among the replicates. ($p \leq 0.05$, Student's *t*-test).

Figure 7. *OsJAZ9* OE lines accumulate higher JA-Ile than WT under K deficiency. JA-Ile content in WT and *OsJAZ9* OE (OE10) lines during normal (+K) and 15 days of K deficiency (-K) conditions. Bars represent the average change in JA-Ile content among at least four replicates. Error bar represents SE of all the replicates. ($p \leq 0.05$, Student's *t*-test). FC=Fold Change.

Supporting Information

Figure S1. Effect of K deficiency on rice growth.

Figure S2. Potassium deficiency induces JA signaling in rice.

Figure S3. Nuclear localization of YFP-*OsJAZ9*.

Figure S4. Effect of prolonged K deficiency on *OsJAZ9* stability.

723 **Figure S5.** Raising and screening of *OsJAZ9* expressing rice transgenics.

724 **Figure S6.** Effect of *OsJAZ9* overexpression on growth parameters of rice during K sufficient
725 and deficient conditions.

726 **Figure S7.** Effect of *OsJAZ9* knockdown on growth parameters of rice during K sufficient and
727 deficient conditions.

728 **Figure S8.** Overexpression of *OsJAZ9* enhances expression of K⁺ transporters.

729 **Figure S9.** Validation of RNA-seq data with qRT-PCR.

730 **Figure S10.** Tissue-specific expression of *OsJAZ9*.

731 **Figure S11.** Exogenous application of MeJA modulates root system architecture under K
732 sufficient conditions.

733 **Figure S12.** Exogenous application of MeJA modulates root system architecture under K
734 deficient conditions.

735 **Figure S13.** Raising and screening of *OsJAZ9* OE rice transgenic lines with ZmUbi1 promoter.

736 **Figure S14.** *OsJAZ9* is involved in K deficiency tolerance in rice.

737 **Figure S15.** *OsJAZ9* overexpression influences root system architecture.

738 **Figure S16.** JA content in WT and *OsJAZ9* OE (OE10) plants during normal (+K) and 15 days
739 of K deficiency (-K) conditions.

740 **Supporting Table S1.** List of primers used for qRT-PCR and gene cloning.

741 **Supporting Table S2.** Effect of K deficiency on expression of JA associated genes.

742 **Supporting Table S3.** Effect of *OsJAZ9* expression on expression of JA associated genes
743 under K deficiency.

744 **Supporting Table S4.** Effect of *OsJAZ9* expression on K transporter expression under K
745 deficiency.

746 **Supporting Table S5.** Effect of *OsJAZ9* expression on expression of *OsJAZ* genes.

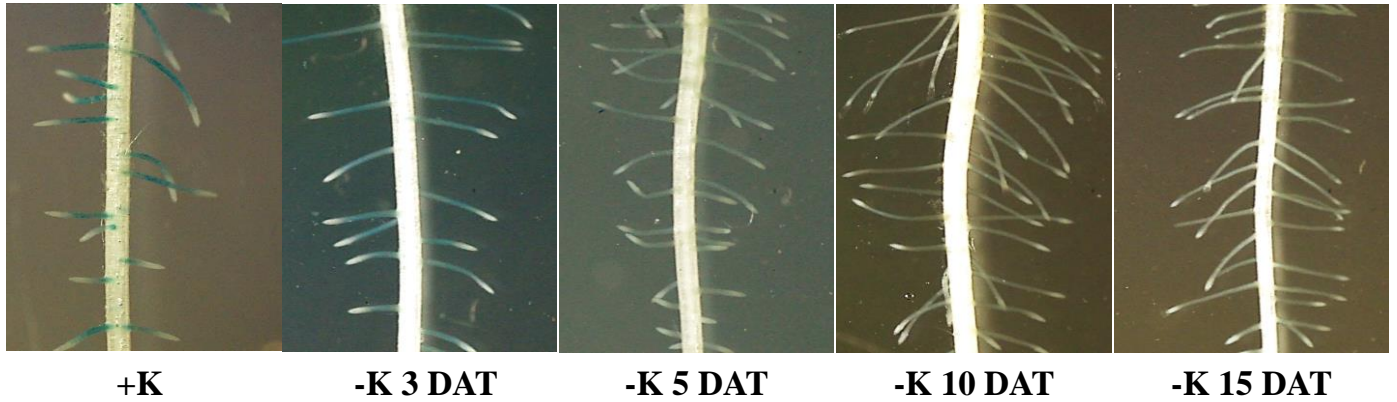


Figure 1. Promoter of *OsJAZ9* is K deficiency responsive. Gus expression in lateral roots on nodal roots of *pOsJAZ9:GUS* transgenic rice plants. Expression was mainly appeared in laterals which was monitored at different time points under K deficiency. *pOsJAZ9:GUS* expression faded away as the K deficiency is prolonged from 3 days to 15 days. Uniformly germinated fifteen-days-old seedlings from +K media were transferred to –K media in a hydroponic system and gus signal was analysed. (DAT, Day after K deficiency treatment).

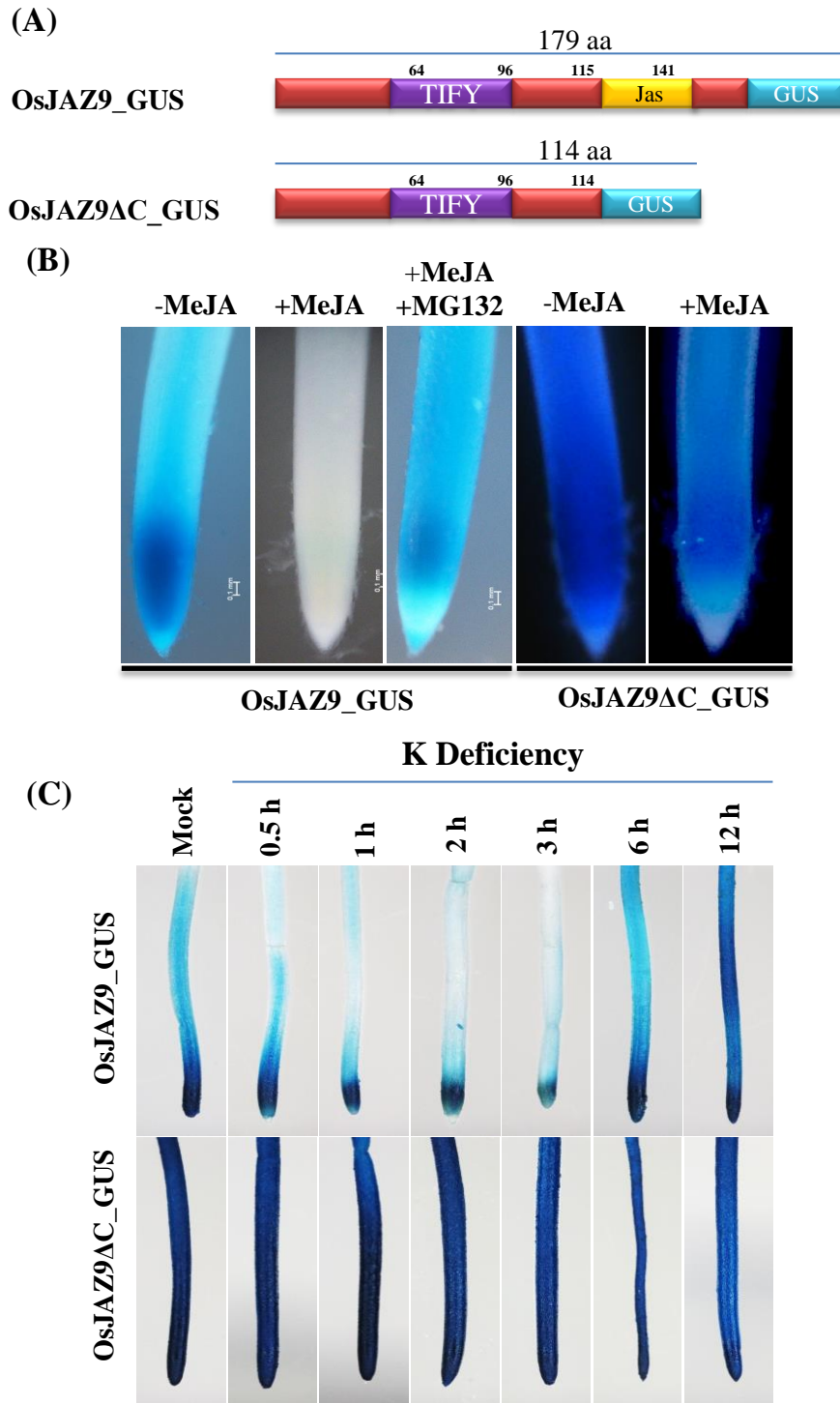


Figure 2. Potassium deficiency causes a rapid increase in JA signaling in rice. (A) Schematic representation of *OsJAZ9_GUS* and *OsJAZ9ΔC_GUS* (Jas domain deleted) translational reporter constructs. (B) JA dependent proteasomal degradation of OsJAZ9 through Jas domain. Root tips of plants overexpressing *OsJAZ9_GUS* and *OsJAZ9ΔC_GUS* were analysed for GUS activity after 3h of treatment with 100 μ M MeJA alone or combined with 100 μ M proteasome inhibitor, MG132. (C) 15-days-old rice seedlings expressing *OsJAZ9_GUS* and *OsJAZ9ΔC_GUS* were transferred to K deficient media for indicated time points (0.5 to 12 h). Stability of OsJAZ9_GUS and OsJAZ9ΔC_GUS proteins in response to K deficiency were analyzed by histochemical GUS staining of roots. The experiment was performed in three independent replicates.

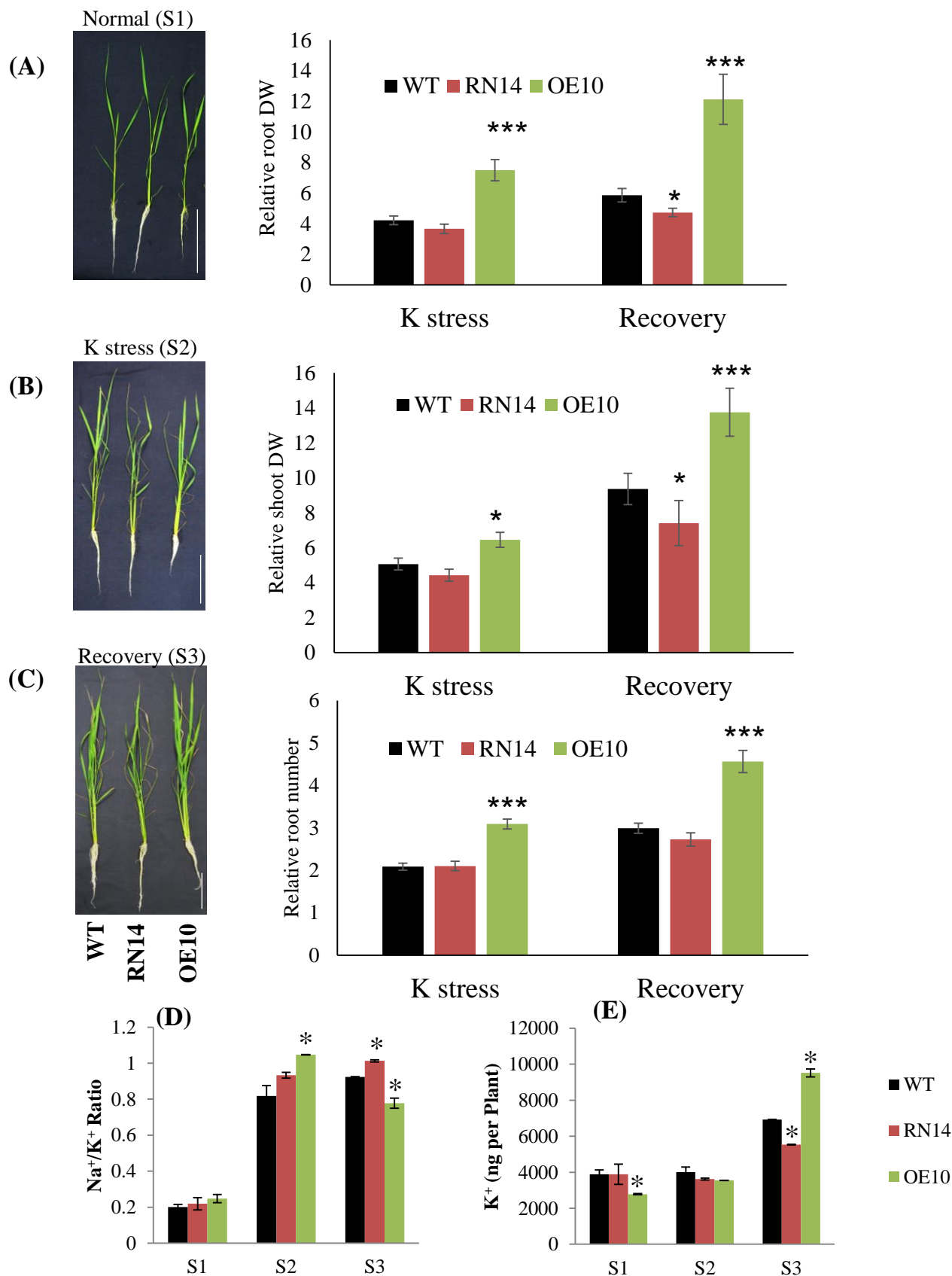
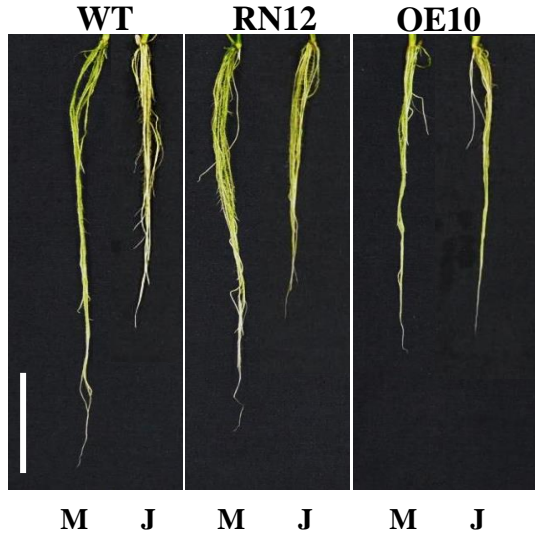


Figure 3. Enhanced K acquisition in K-starved *OsJAZ9* OE seedlings upon K resupply. (A) Morphological data (root dry weight, shoot dry weight and number of roots per plant) of 10-days-old normally grown seedlings (normal/S1), (B) followed by 15-days of K starvation (K stress/S2) and (C) subsequent recovery with 10 μ M K_2SO_4 for 5 days (Recovery/S3). K stress represent the ratio of dry root or shoot biomass K stressed plants with respect to normal condition and recovery represent the ratio of recovered plants with respect to normal condition ($n < 15$). (D) Na^+/K^+ and (E) Total K content per plant of WT and *OsJAZ9* transgenics during S1, S2 and S3 treatments. K content experiment was performed with two replicates. Asterisks indicate significant changes in transgenics compared to WT at respective time points ($p \leq 0.05$, .01 and .001 for *, ** and *** calculated from Student's *t*-test). Scale = 10 cm.

(A)



(B)

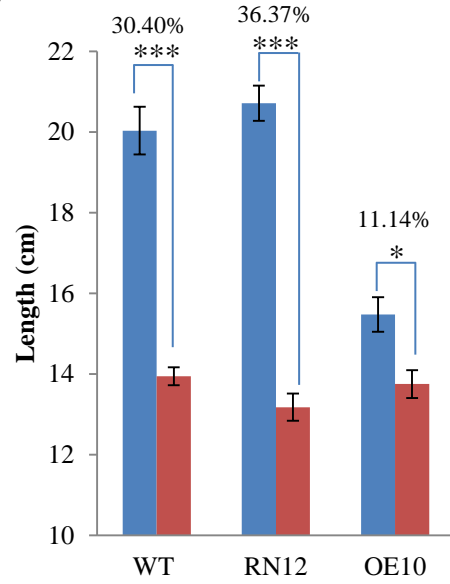


Figure 4. JA-induced root inhibition in WT and *OsJAZ9* transgenics. (A) Representative images of roots of 20-days-old WT, RN12 and OE10 seedlings supplemented with DMSO (Mock; M) or 10 μ M MeJA dissolved in DMSO (JA treated; J). Scale = 5 cm. (B) Average root lengths of WT, *OsJAZ9* RNAi and OE plants under DMSO (blue bars) and MeJA (Red bars) treatments. Each bar represents average of 9 seedlings with standard error. Values over the bars represent JA mediated percentage inhibition in the root length as compared to DMSO treated plants. Significant differences between DMSO vs MeJA treatment were evaluated by Student's *t*-test. Asterisks; *, **, *** and **** indicate *p* values, $\leq 10^{-3}$, 10^{-4} , 10^{-8} and 10^{-10} , respectively.

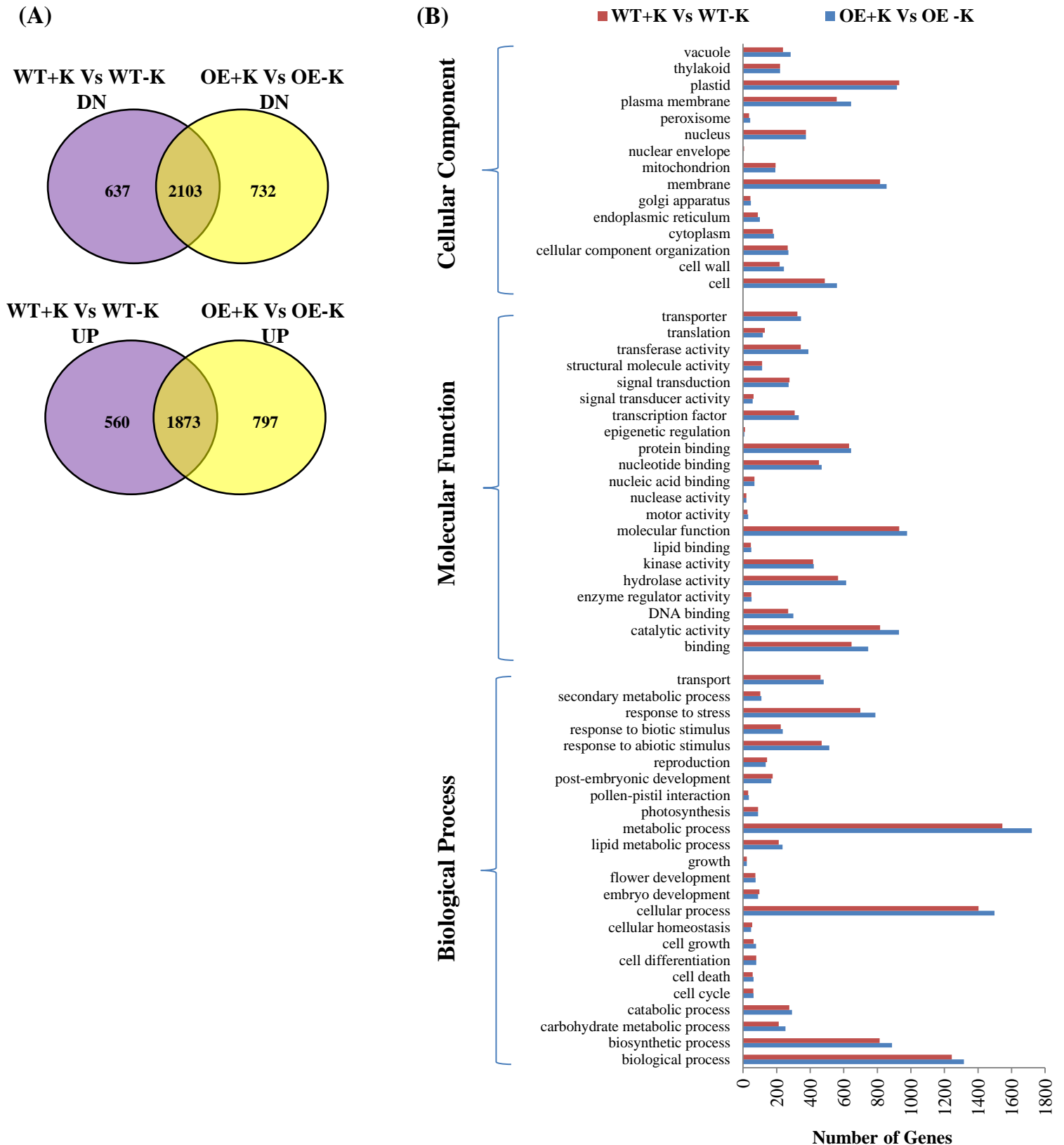


Figure 5. Overexpression of *OsJAZ9* enhances K deficiency response in rice. (A) Venn diagrams showing differentially regulated genes on K deficiency in WT and *OsJAZ9* OE lines after 15 days of deficiency. (B) Overview of transcriptome data showing category wise distribution of differentially genes in WT and *OsJAZ9* OE lines during K deficiency. Error bar represents SE of all the replicates. ($p \leq 0.05$, Student's *t*-test). FC=Fold Change.

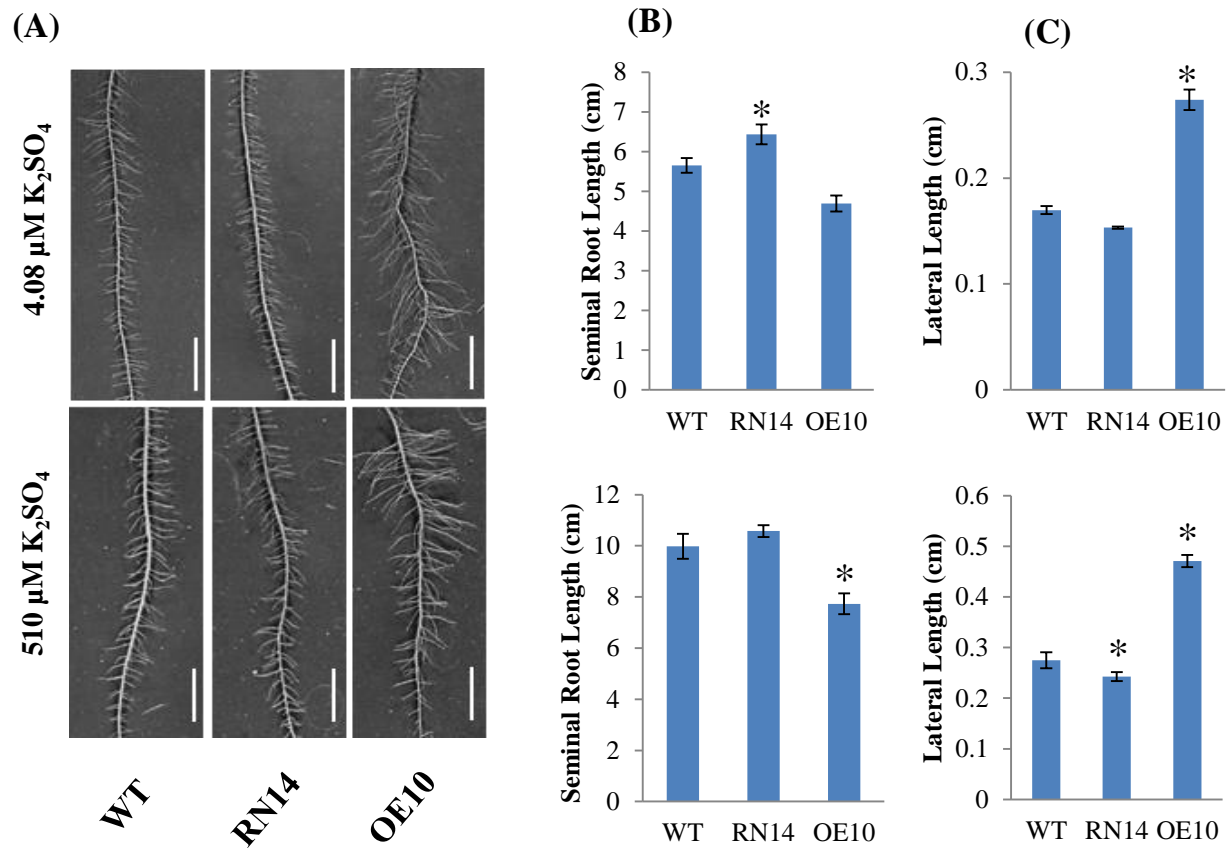


Figure 6. *OsJAZ9* overexpression influences root system architecture. (A) Representative root images of 12-days-old WT and *OsJAZ9* transgenics showing lateral root lengths during K deficient (upper panel) and K sufficient conditions (lower panel). (B) Average seminal root length and (C) Average lateral root length of WT and *OsJAZ9* transgenics during K deficient (upper row) and K sufficient (lower row) conditions. Four independent plants were used for the analysis. Scale bar represents 1 cm while error bar represents SE among the replicates. ($p \leq 0.05$, Student's *t*-test).

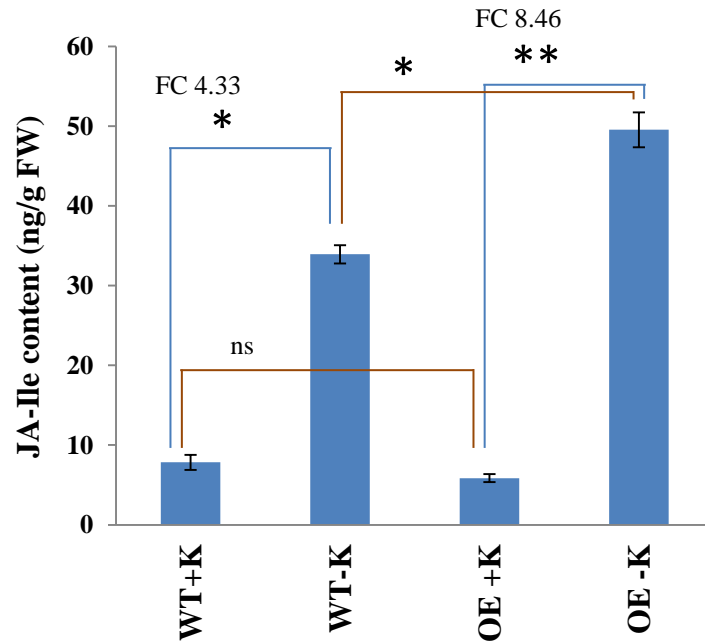


Figure 7. *OsJAZ9* OE lines accumulate higher JA-Ile than WT under K deficiency. JA-Ile content in WT and *OsJAZ9* OE (OE10) lines during normal (+K) and 15 days of K deficiency (-K) conditions. Bars represent the average change in JA-Ile content among at least four replicates. Error bar represents SE of all the replicates. ($p \leq 0.05$, Student's *t*-test). FC=Fold Change.

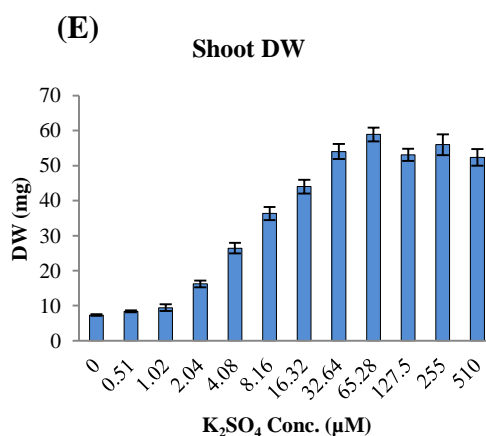
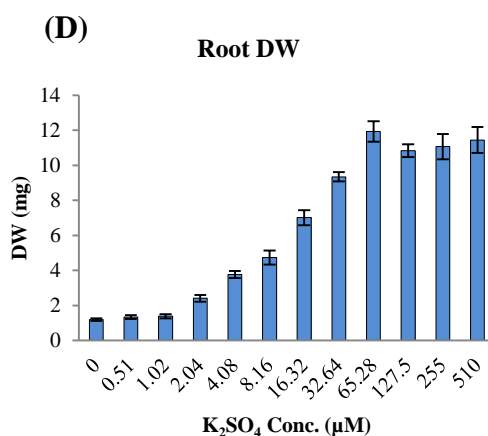
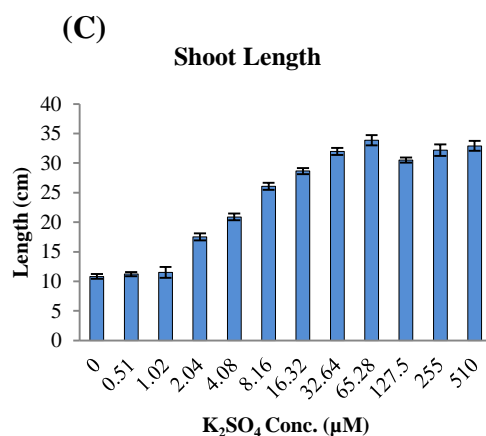
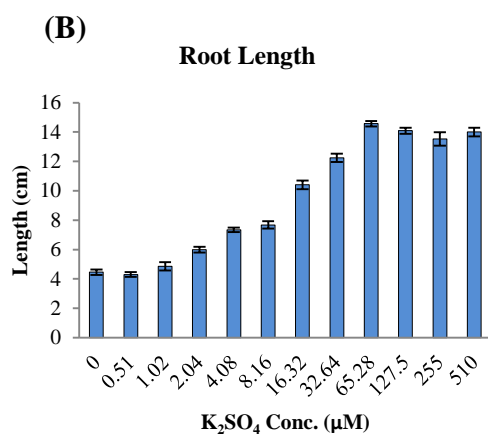
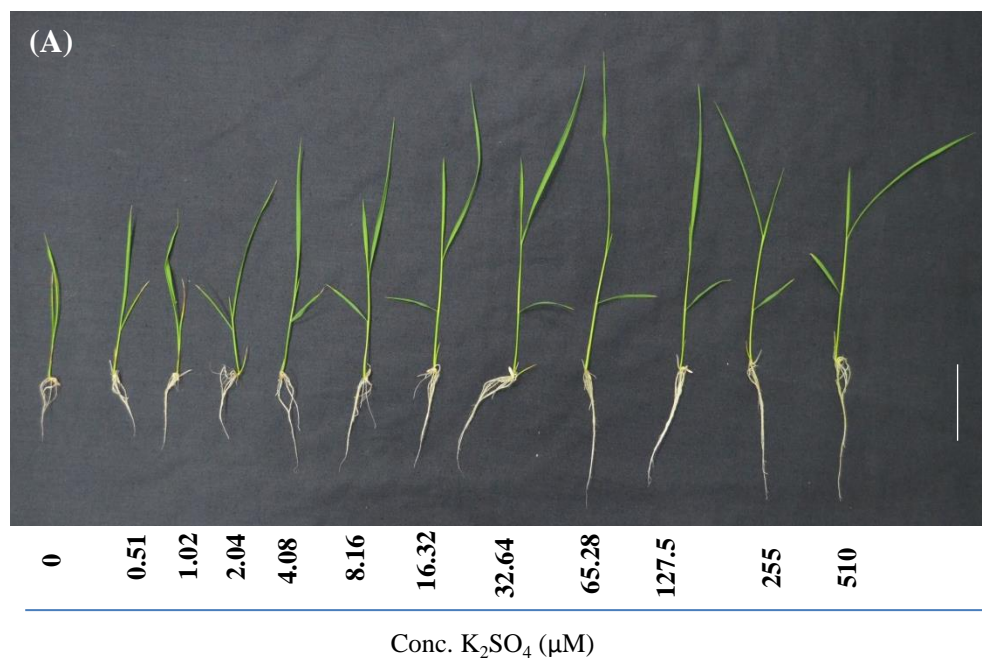


Figure S1. Effect of K deficiency on rice growth. (A) Representative images showing effect of different concentrations of K on plant growth. Uniformly germinated seedlings were transferred to Yoshida media containing different concentrations of K_2SO_4 (as indicated on x-axis). (B) Root length, (C) Shoot length, (D) Root dry biomass and (E) Shoot dry biomass of rice seedlings after 15 days of growth under varied K supply. Each bar represents average of 10 seedlings with standard error. Scale = 5 cm.

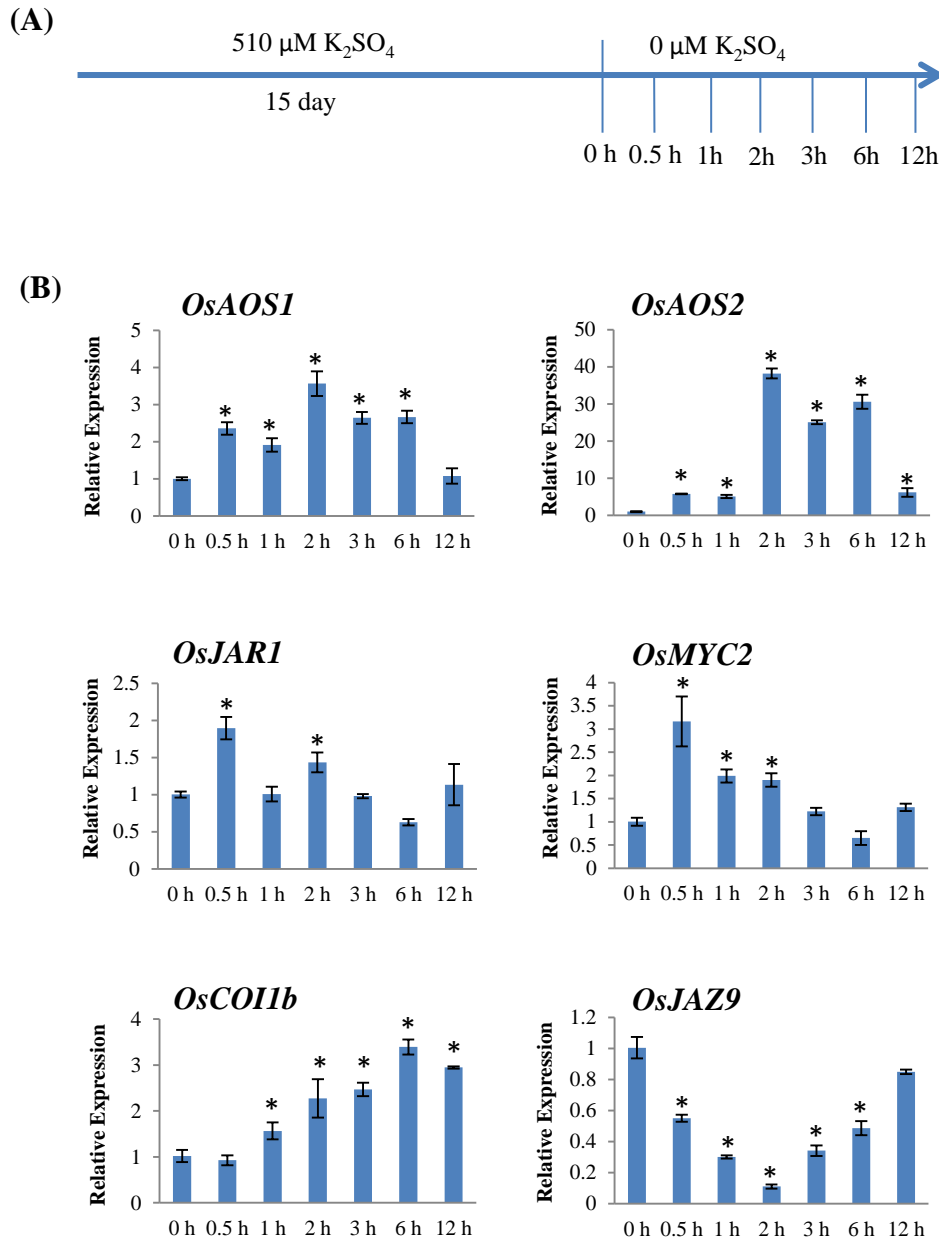


Figure S2. Potassium deficiency induces JA signaling in rice. (A) Schematic representation of experimental setup used for expression analysis of JA biosynthetic and signaling genes. Plants were raised under normal conditions (510 μM K_2SO_4) for 15 days before being subjected to different durations (0.5 to 12 h) of K deficiency treatment (0 μM K_2SO_4). (B) Short-term K deficiency responses of JA biosynthetic and signaling genes. Relative expression levels at different time points of K deficiency were analysed with respect to 0 h. Error bar indicates standard error derived from three independent replicates. Asterisk indicates significant changes at different time points compared to 0 h treatment ($p \leq 0.05$, Student's t -test).

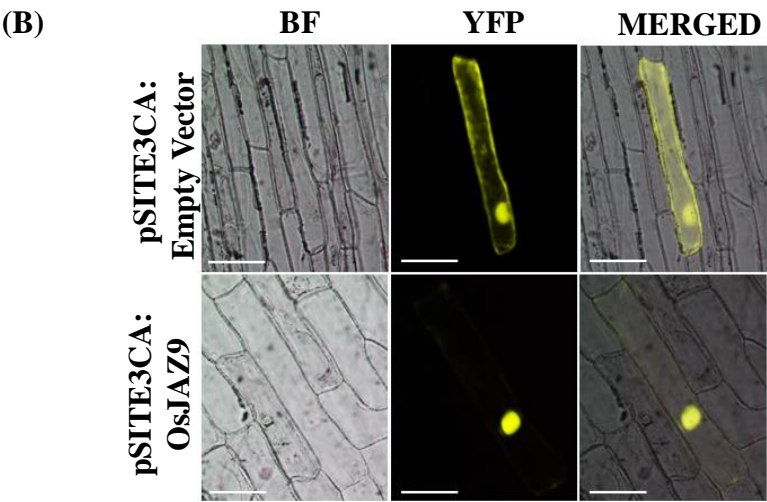
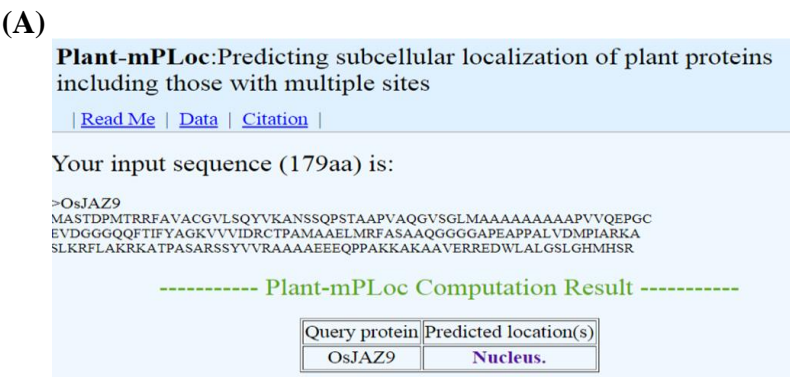


Figure S3. Nuclear localization of YFP-OsJAZ9. (A) *In-silico* prediction of OsJAZ9 localization using databases (Plant-mPLOC (<http://www.csbio.sjtu.edu.cn/cgi-bin/PlantmPLOC.cgi>)). (B) Subcellular localization of YFP-OsJAZ9 in onion epidermal cells. Merged images showing superimposition of YFP and Bright field (BF) images.

(A)

OsJAZ9	MASTDPMTRRFAVACGVL	SQYVKANSSQPSTAAPVAQGV	SGLMAAAAAAAAAAPVVQEPGC
OsJAZ9ΔC	MASTDPMTRRFAVACGVL	SQYVKANSSQPSTAAPVAQGV	SGLMAAAAAAAAAAPVVQEPGC
OsJAZ9	EVD	GGGQQFTIFYAGKVVIDRCTPAMAAELMRFASAAQGGGGAPEAPPALVDM	PIARKA
OsJAZ9ΔC	EVD	GGGQQFTIFYAGKVVIDRCTPAMAAELMRFASAAQGGGGAPEAPPALVDM	-----
OsJAZ9	SLKRFLAKRKATPASARSSYV	VRAAAAAEEEQPPAKKAKAAVERREDWLALGSLGHMHSR	
OsJAZ9ΔC	-----	-----	-----

(B)

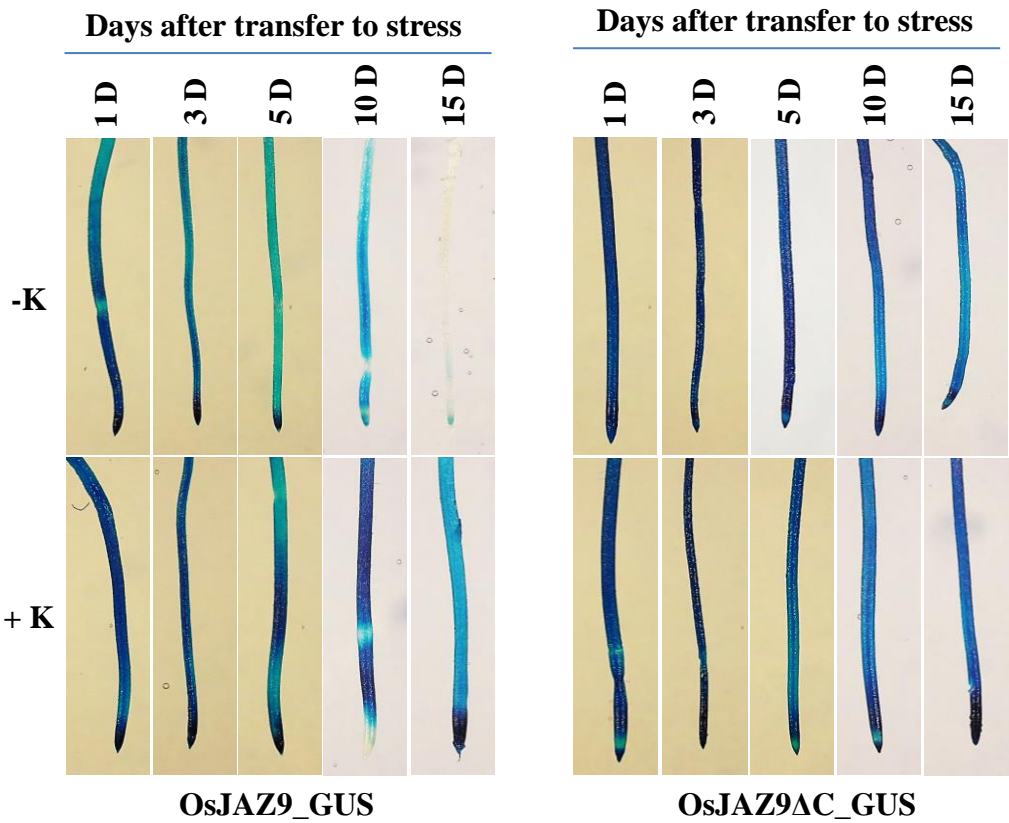
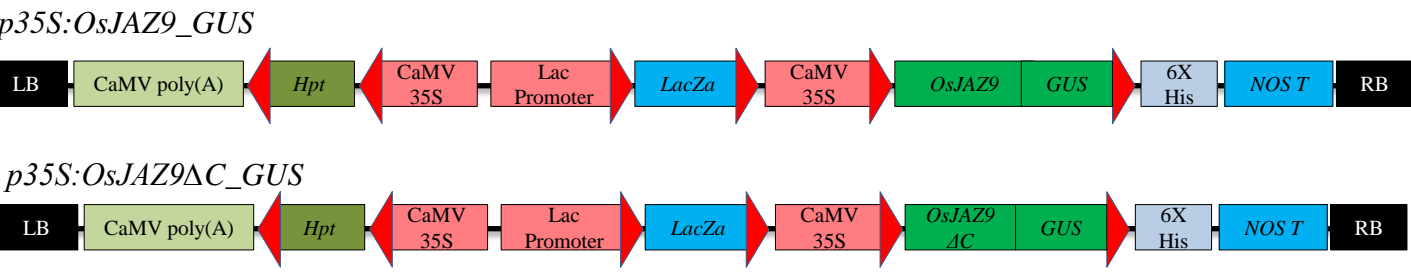
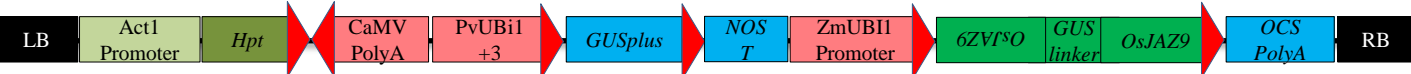


Figure S4. Effect of prolonged K deficiency on OsJAZ9 stability. (A) Protein sequence alignment of OsJAZ9 and OsJAZ9ΔC. Red text highlights TIFY domain while the yellow text highlights Jas motif. (B) OsJAZ9_GUS degradation on prolonged K deficiency. 15-days-old rice seedlings expressing *OsJAZ9_GUS* and *OsJAZ9ΔC_GUS* were transferred to K deficient media for indicated time points (1 day to 15 days). Stability of OsJAZ9_GUS and OsJAZ9ΔC_GUS proteins in response to K deficiency was analysed by histochemical GUS staining of root sections. Experiment was performed in three independent replicates.

(A)
Vector constructs for *OsJAZ9* overexpression



Vector constructs for *OsJAZ9* silencing



Vector constructs for *pOsJAZ9:GUS* reporter



(B)

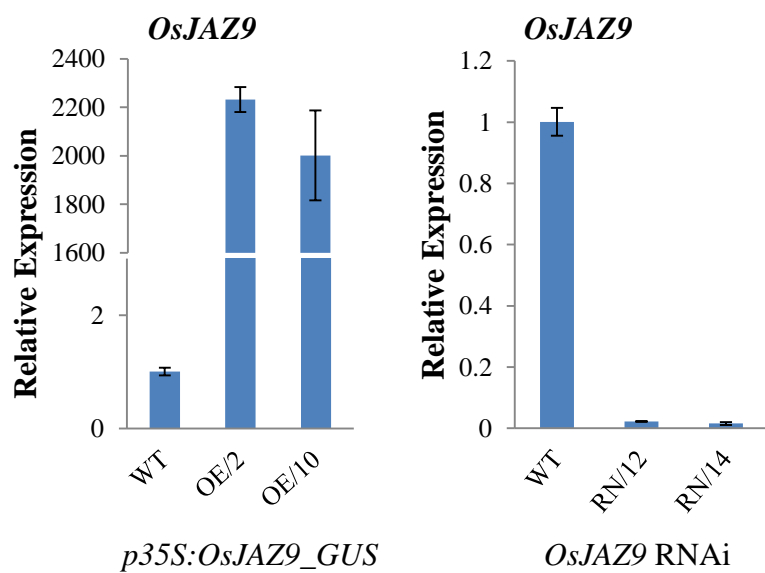


Figure S5. Raising and screening of *OsJAZ9* expressing rice transgenics. (A) Schematic of *OsJAZ9* overexpression and silencing constructs. (B) Relative expression of *OsJAZ9* in overexpression (*p35S:OsJAZ9_GUS*) and RNAi lines. Gene expression analysis was done with qRT-PCR with at least three independent replicates. Asterisk indicates significant change compared to WT ($p \leq 0.05$, Student's *t*-test).

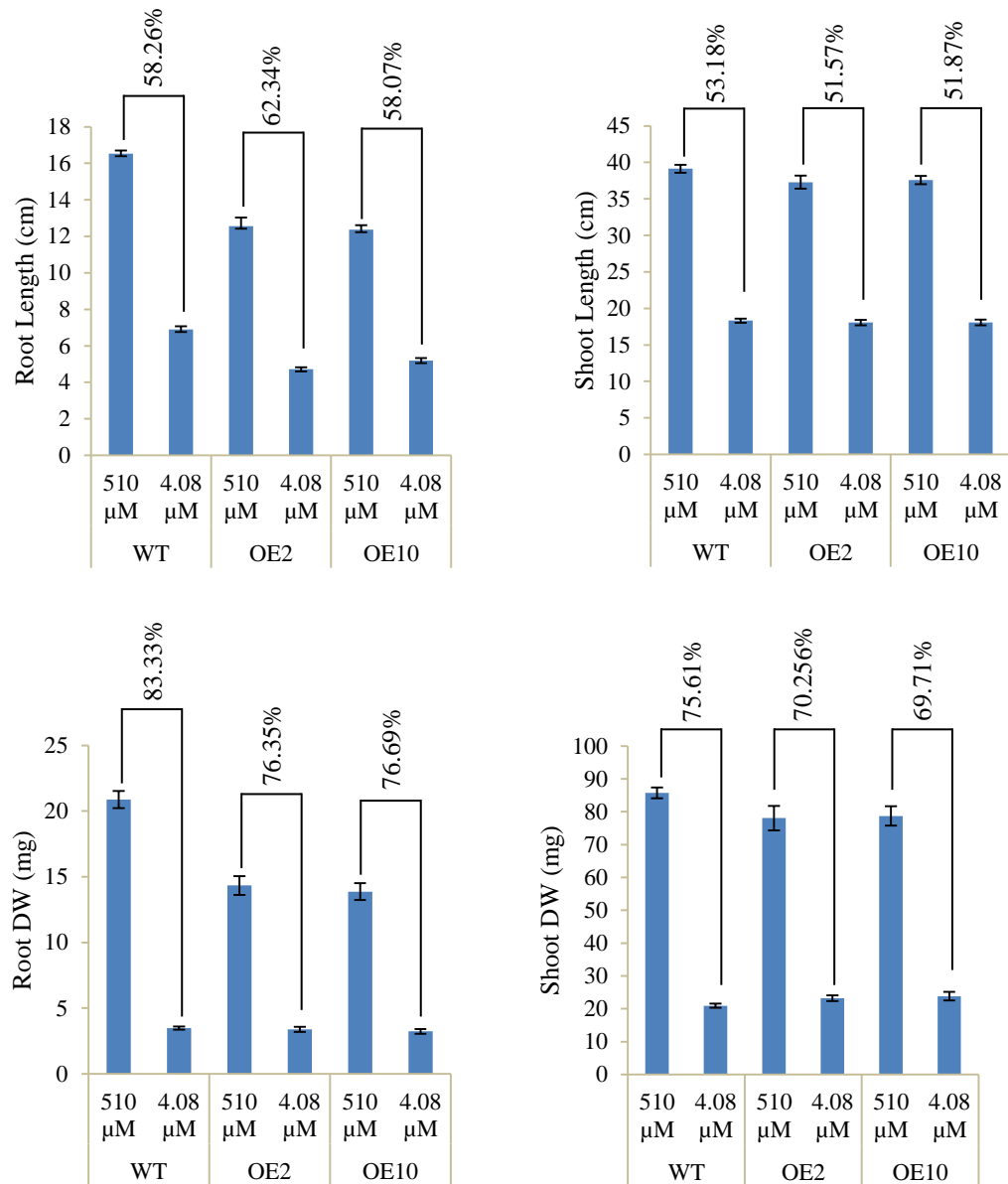


Figure S6. Effect of *OsJAZ9* overexpression on growth parameters of rice during K sufficient and deficient conditions. Growth parameters (length and dry biomass of root and shoot) of WT and *OsJAZ9* OE (OE2 and OE10) lines under K deficient and sufficient conditions. Each bar represents average of 12 seedlings with standard error while the values above the bars represents percentage change in that specific line due to K deficiency. Values of the top of the bars are showing per cent reduction due to K deficiency.

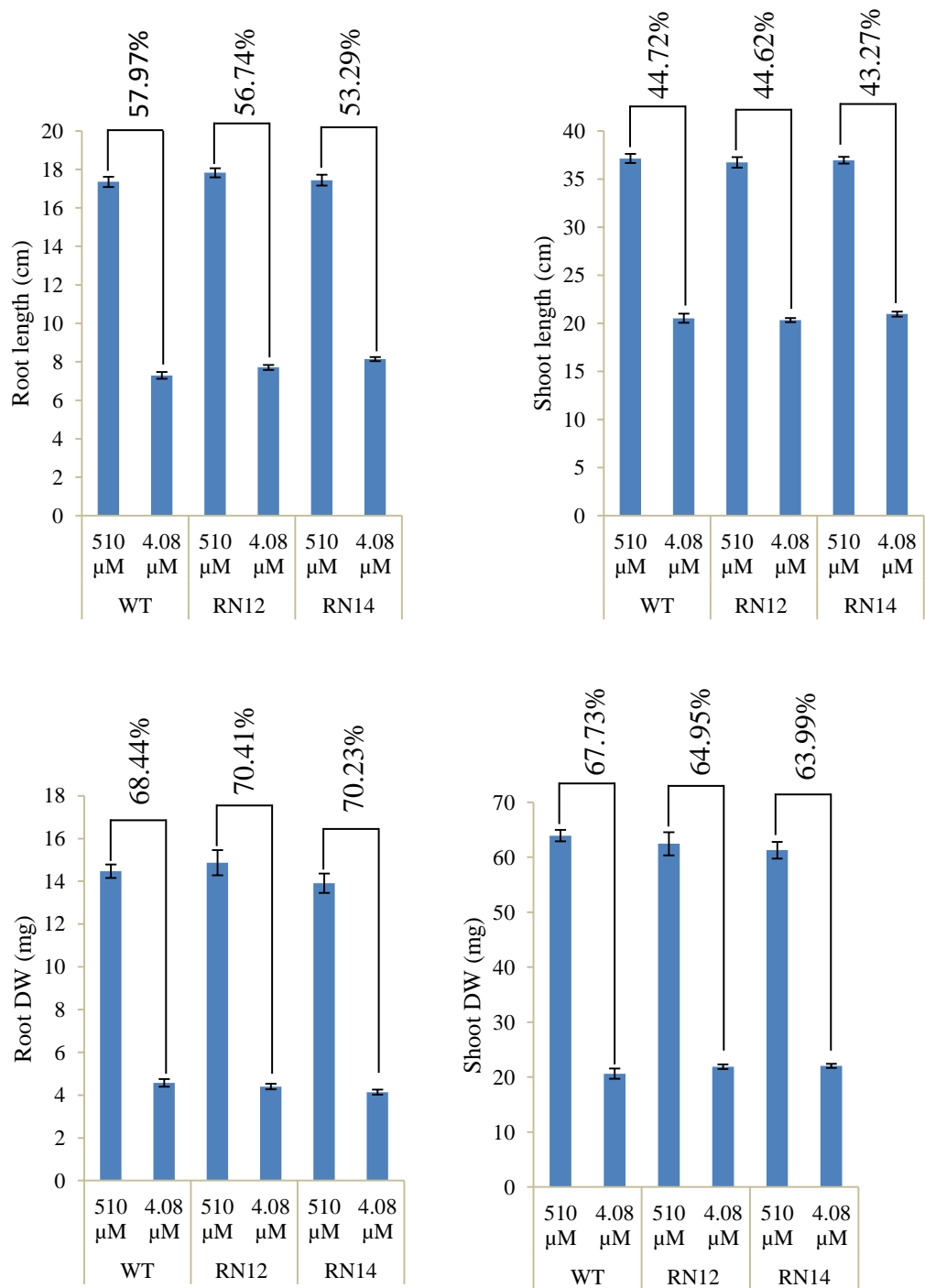


Figure S7. Effect of *OsJAZ9* knockdown on growth parameters of rice during K sufficient and deficient conditions. Growth parameters (length and dry biomass of root and shoot) of WT and *OsJAZ9* RNAi (RN12 and RN14) lines under K deficient and sufficient conditions. Each bar represents average of 12 seedlings with standard error while the values above the bars represents percentage change in that specific line due to K deficiency. Values of the top of the bars are showing per cent reduction due to K deficiency.

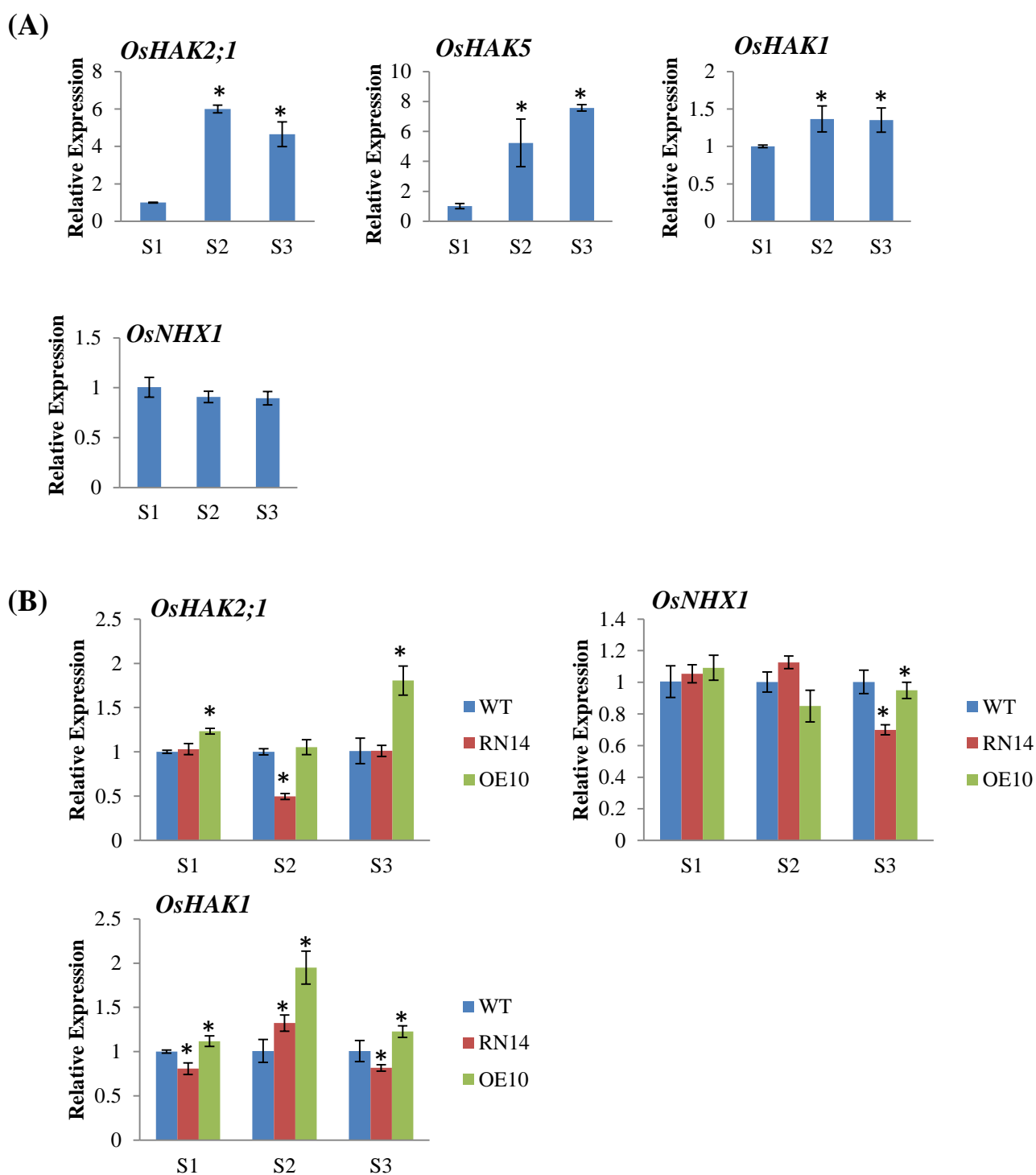


Figure S8. Overexpression of *OsJAZ9* enhances expression of K^+ transporters. (A) K^+ transporter encoding genes in WT under S2 (K deficiency, 15 days) and S3 (K recovery, 12 hrs) treatments with respect to S1 (K sufficient). **(B)** Relative expression levels of *OsHAK2;1*, *OsNHX1* and *OsHAK1* in *OsJAZ9* transgenics with respect to WT during S1, S2 and S3 treatments. Bars represent average of three biological replicates with standard error. Asterisk indicates significant change compared to WT of respective time point ($p \leq 0.05$, Student's t -test).

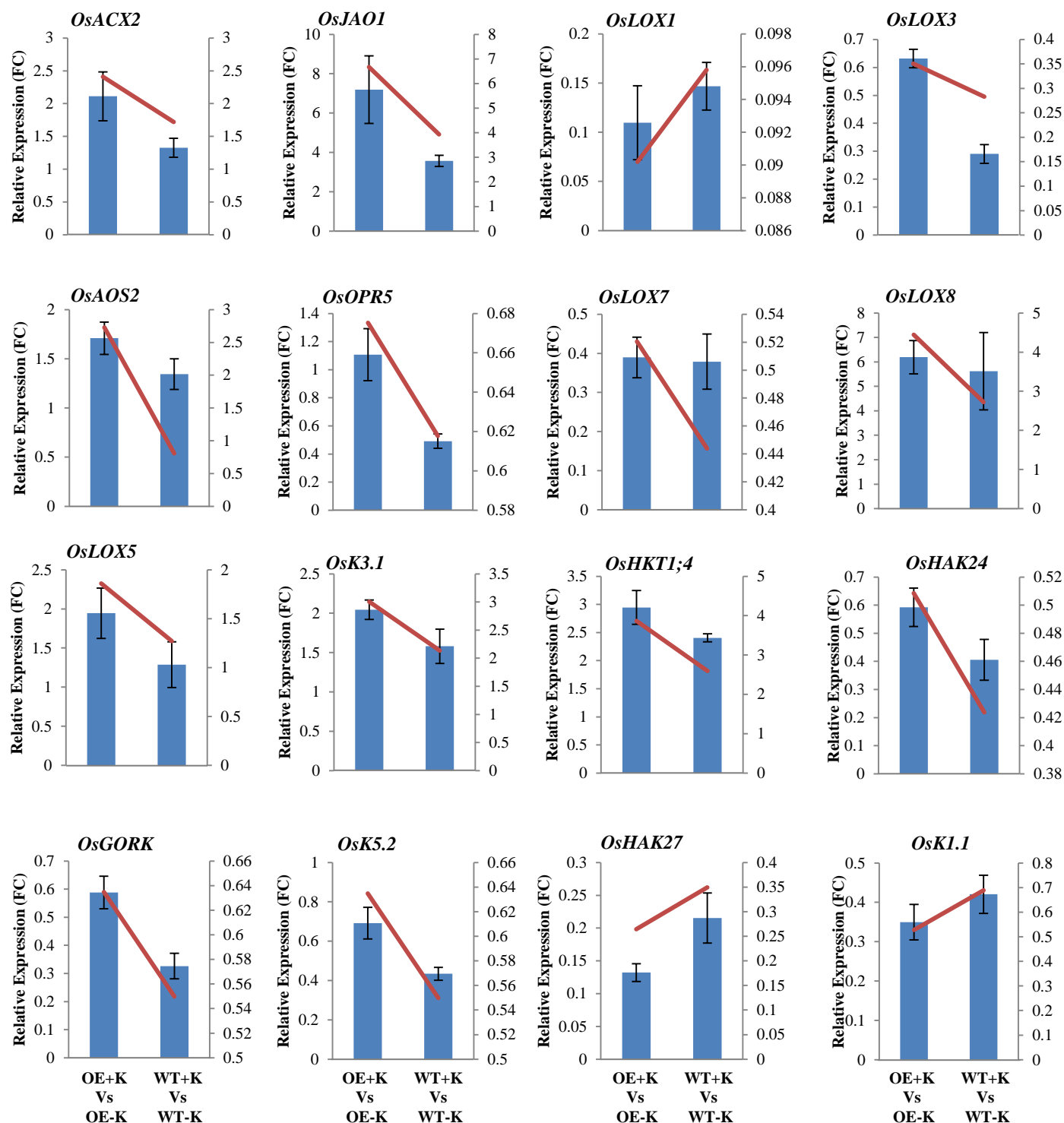


Figure S9. Validation of RNA-seq data with qRT-PCR. Relative expression levels (fold changes; FCs) of DEGs (shown in table S2 and S3) were analysed in WT and *OsJAZ9* OE line during K deficient conditions w.r.t their own controls. All expression levels were normalized with respect to endogenous control, *OsAct1* and FCs were determined by $2^{-\Delta\Delta CT}$ method. Bar Graphs shows the expression obtained using qRT-PCR (right scale) and line graphs (left scale) shows the expression pattern obtained in RNA-seq data.

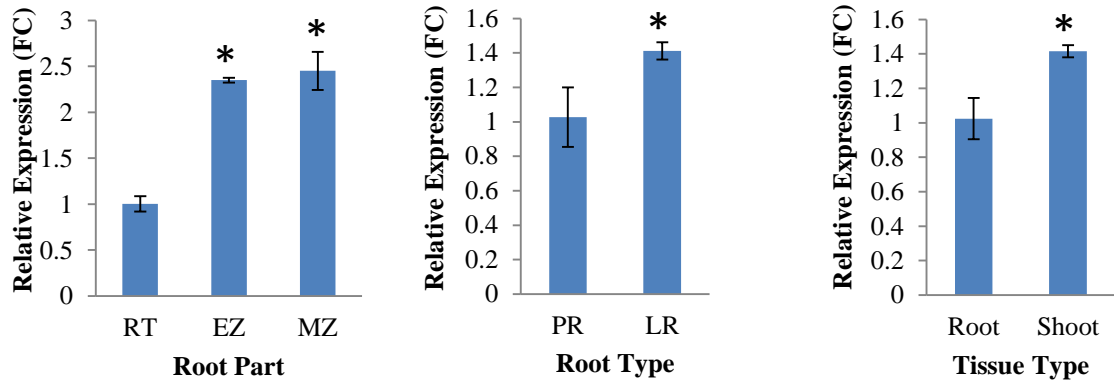


Figure S10. Tissue-specific expression of *OsJAZ9*. Expression of *OsJAZ9* shown by qRT-PCR analysis in different parts of root (Root Tip, RT; Elongation Zone, EZ; Maturation Zone, MZ), different root types (Primary Root, PR; Lateral Root, LR) and different tissues (Root and shoot). Each bar shows average fold change normalized to RT, PR and Root in respective graphs. $n \geq 3$, error bar represents standard error, asterisk marks the significant change with Student's *t*-test, $p \leq 0.05$.

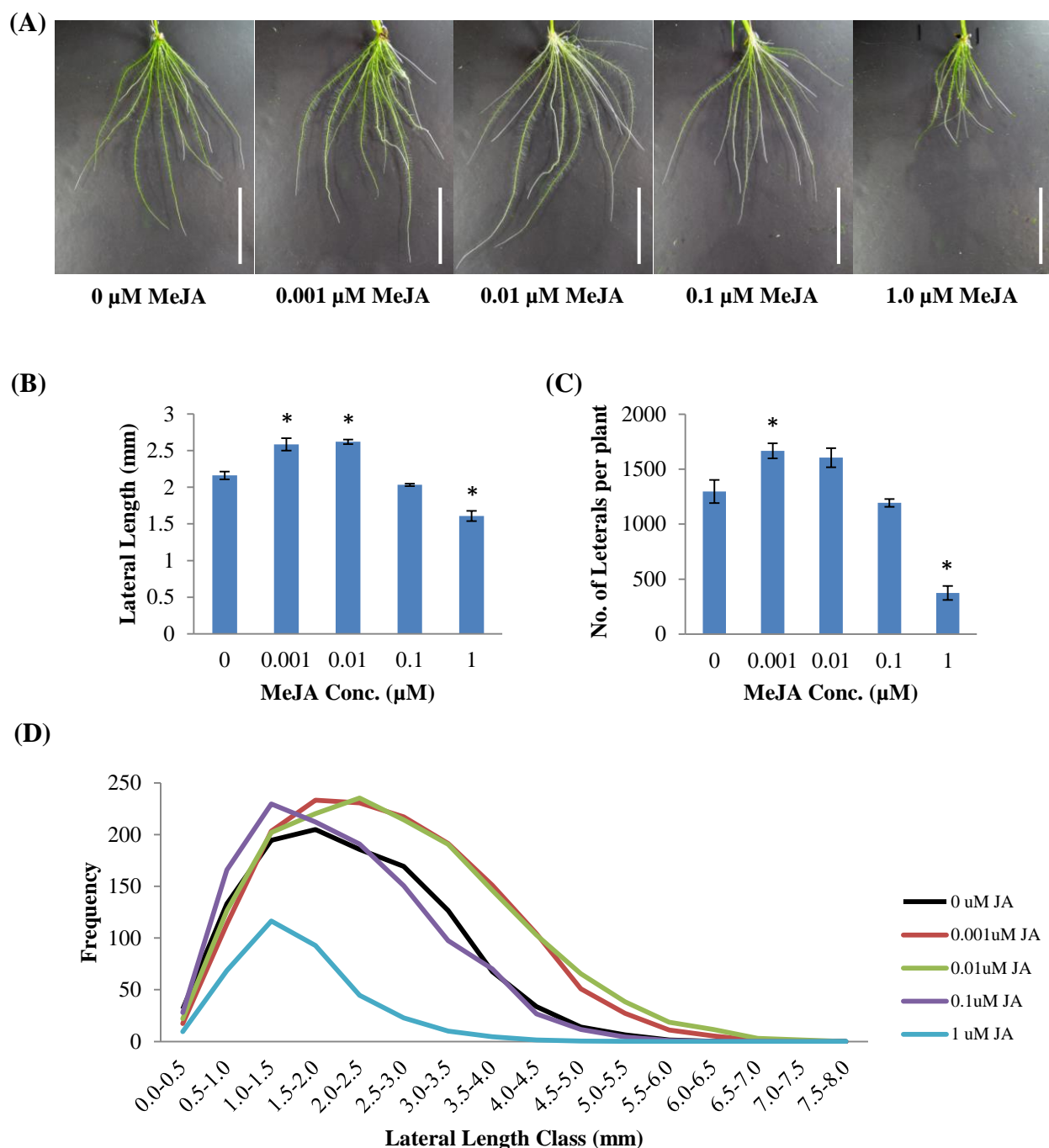


Figure S11. Exogenous application of MeJA modulates root system architecture under K sufficient conditions. (A) Representative image of 15-days-old WT plant roots grown with 510 μM K_2SO_4 and different concentrations of exogenous MeJA (0, 0.001, 0.01, 0.1 and 1 μM). (B) Average lateral length, (C) Average number of laterals per plant and (D) Frequency distribution curve showing abundance of different classes of laterals during the MeJA treatment for 15 days. Bar represents average of 4 seedlings (taking average of all the laterals in one plant as one replicate) with standard error. Frequency distribution graph shows the average distribution of the classes in 3 plants. Asterisks indicate significant changes in transgenics compared to WT at respective time points ($p \leq 0.05$, Student's *t*-test). Scale = 5 cm.

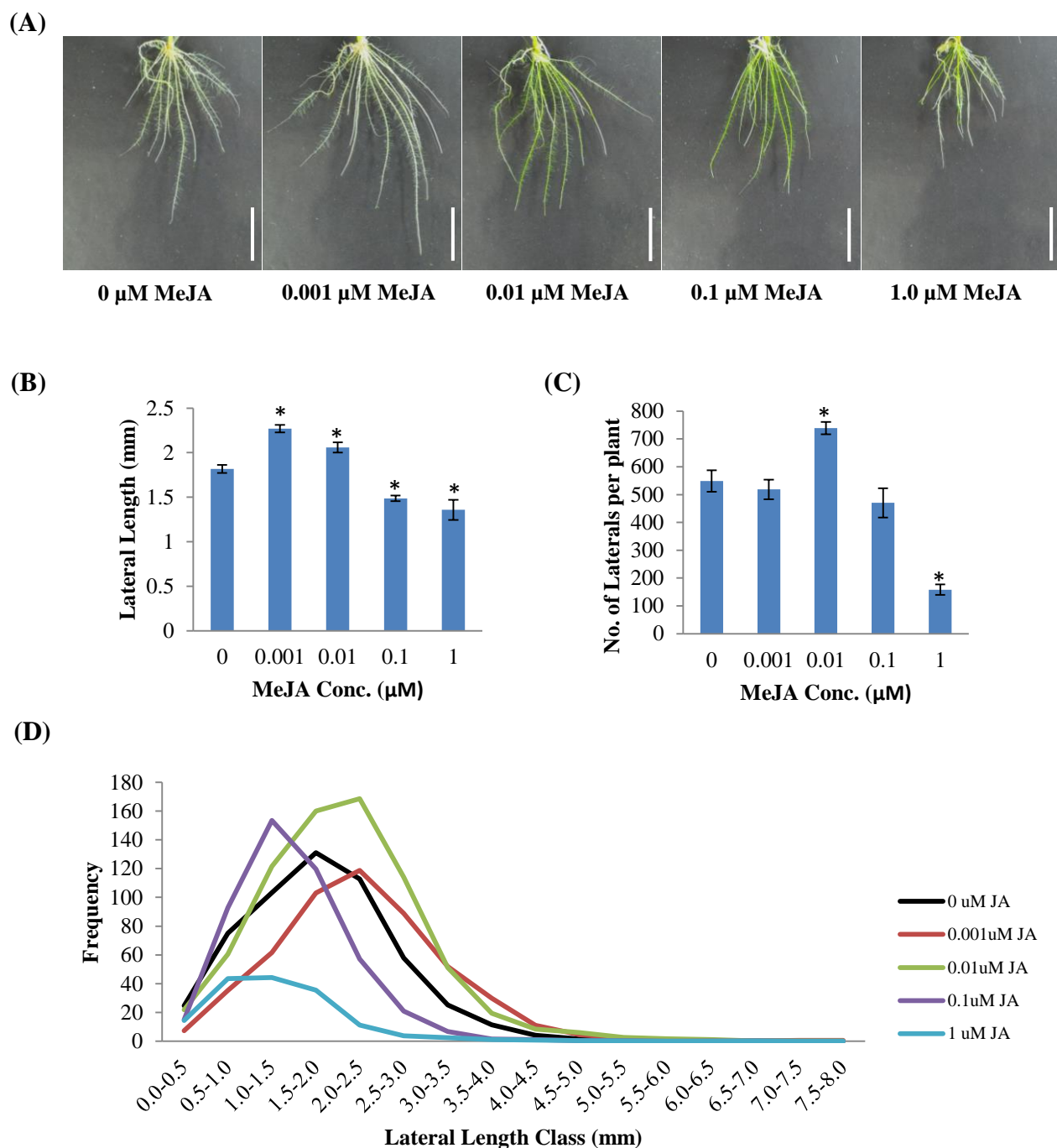
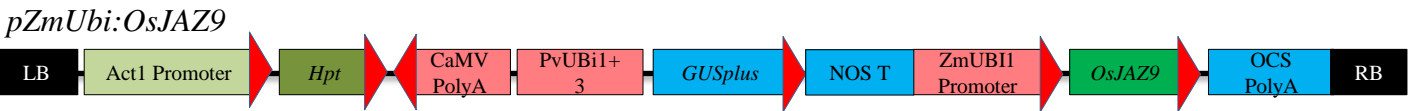


Figure S12. Exogenous application of MeJA modulates root system architecture under K deficient conditions. (A) Representative image of 15-days-old WT plant roots grown with 4.08 μM K_2SO_4 and different concentrations of exogenous MeJA (0, 0.001, 0.01, 0.1 and 1 μM). (B) Average lateral length, (C) Average number of laterals per plant and (D) Frequency distribution curve showing abundance of different classes of laterals during the MeJA treatment for 15 days. Bar represents average of 4 seedlings (taking average of all the laterals in one plant as one replicate) with standard error. Frequency distribution graph shows the average distribution of the classes in 4 plants. Asterisks indicate significant changes in transgenics compared to WT at respective time points ($p \leq 0.05$, Student's t -test). Scale = 2 cm.

(A)



(B)

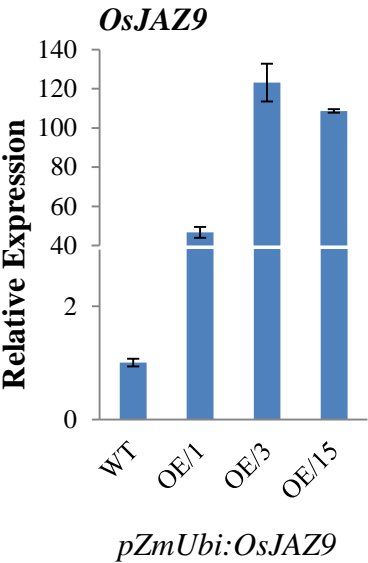


Figure S13. Raising and screening of *OsJAZ9* OE rice transgenic lines with *ZmUbi1* promoter. (A) Schematic of construct used for *OsJAZ9* overexpression. **(B)** Relative expression of *OsJAZ9* in overexpression lines. Gene expression analysis was done with qRT-PCR with at least three independent replicates. Asterisk indicates significant change compared to WT ($p \leq 0.05$, Student's *t*-test).

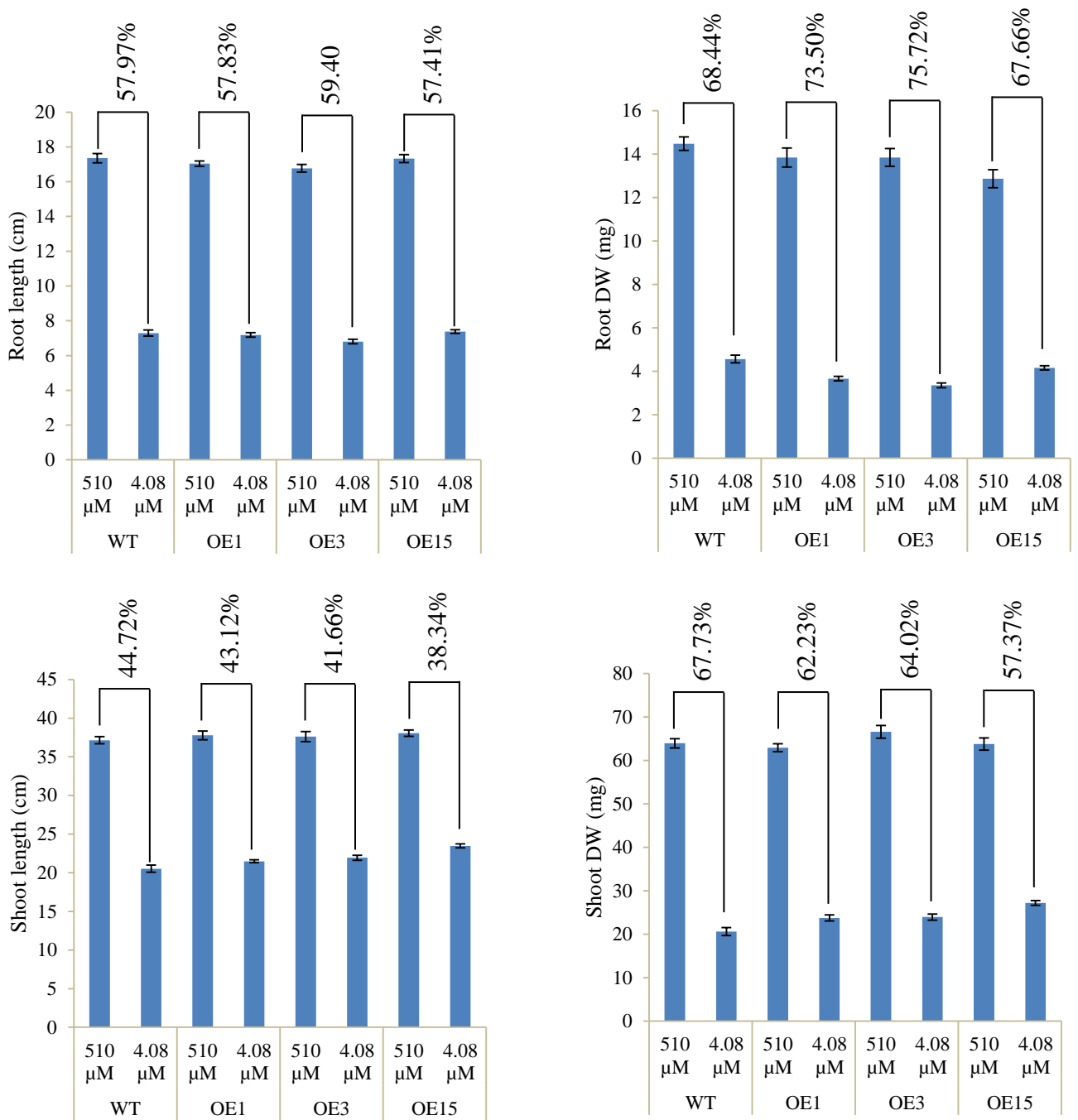


Figure S14. *OsJAZ9* is involved in K deficiency tolerance in rice. Growth parameters (length and dry biomass of root and shoot) of WT and *OsJAZ9* OE (OE1, OE3 and OE15) lines under K deficient and sufficient conditions. Each bar represents average of 12 seedlings with standard error while the values above the bars represents percentage change in that specific line due to K deficiency.

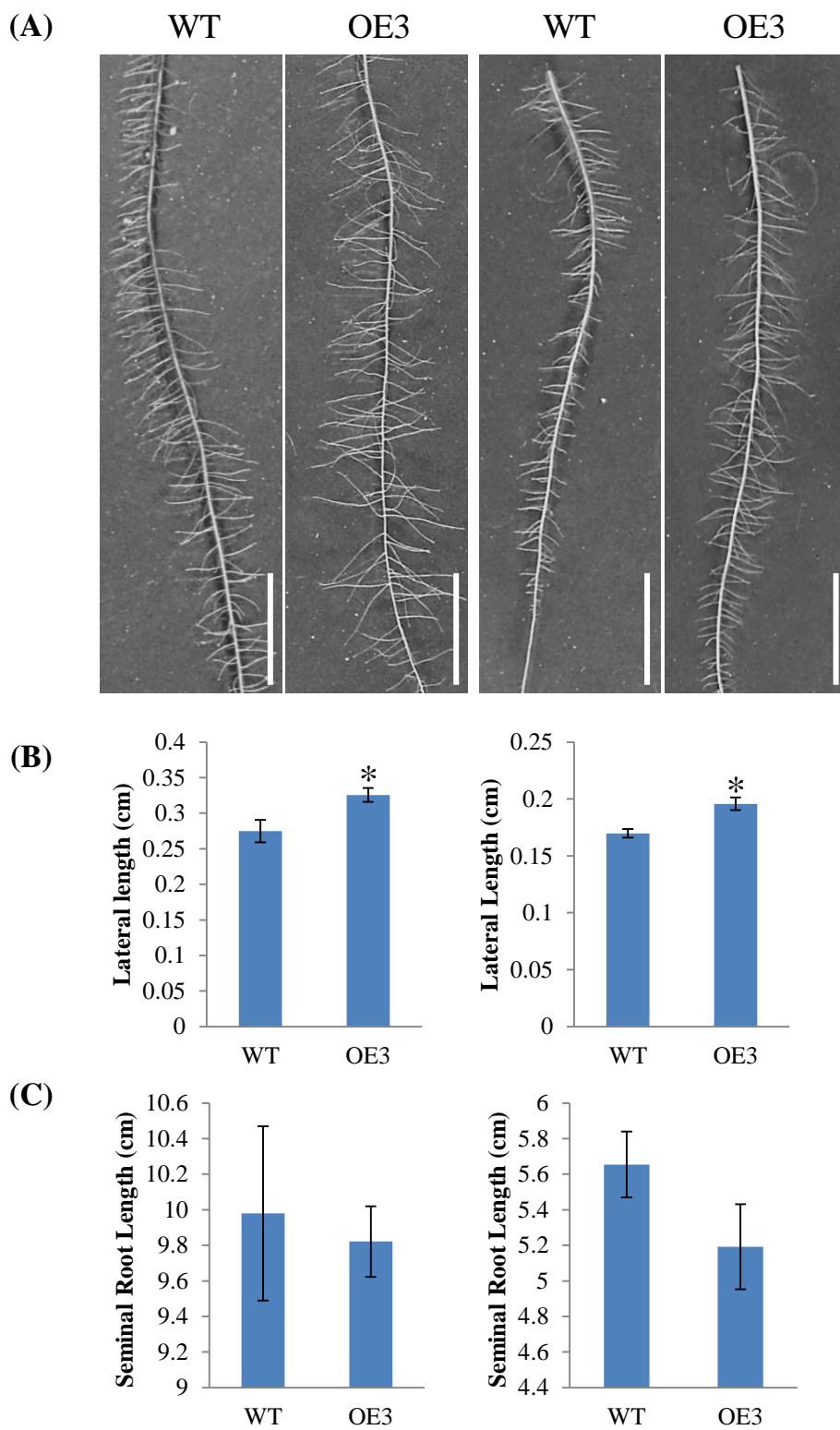


Figure S15. *OsJAZ9* overexpression influences root system architecture. (A) Representative root images of 12-days-old WT and *OsJAZ9* OE plants showing lateral root lengths during K sufficient (left panel) and K deficient conditions (right panel). (B) Average lateral root length and (C) Average seminal root length of WT and *OsJAZ9* OE plants during K deficient and K sufficient conditions. Four independent plants were used for the analysis. Scale bar represents 1 cm while error bar represents SE among the replicates. ($p \leq 0.05$, Student's *t*-test).

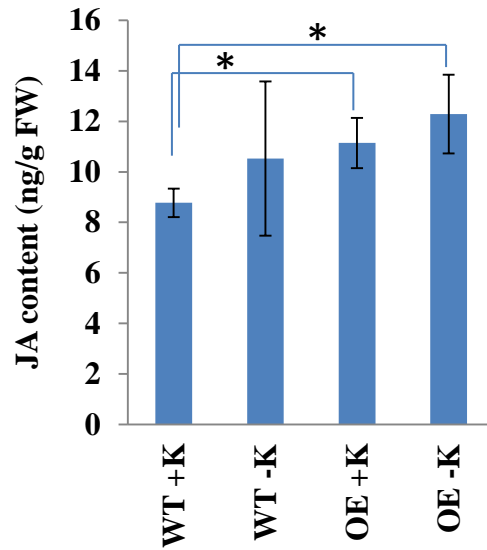


Figure S16. JA content in WT and *OsJAZ9* OE (OE/10) plants during normal (+K) and 15 days of K deficiency (-K) conditions. Bars represent the average change in JA content among at least four replicates. Error bar represents SE of all the replicates, asterisk marks the significant change with Student's *t*-test, $p \leq 0.05$.

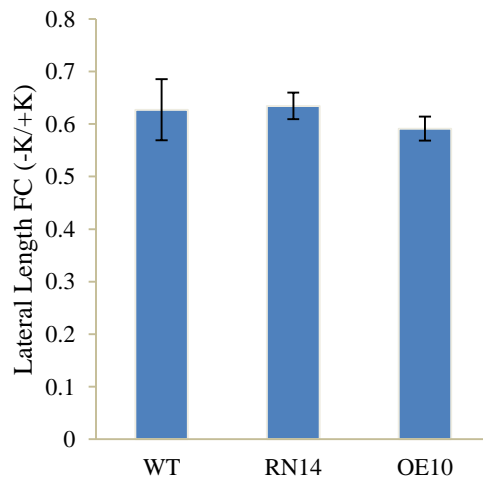


Figure X. *OsJAZ9* overexpression influences root system architecture. Average fold change in lateral root length of plants (WT, *OsJAZ9* RNAi and overexpression) grown under $-K/+K$ (K deficient/K sufficient) conditions. Four independent plants were used for the analysis. Error bar represents SE among the replicates.

Table S1 List of primers used for qRT-PCR and gene cloning

Primer used for qRT-PCR		
Primer ID	Forward Primer	Reverse Primer
qOsUBQ5	ACCACTTCGACCGCCACTACT	ACGCCTAAGCCTGCTGGTT
OsActin1	CAGCCACACTGTCCCCATCTA	AGCAAGGTCGAGACGAAGGA
qOsACX2	CATGTGGTGGCCATGGGTAT	TCATGATCATTGCGCAAAGC
qOsGORK	CGTGGCCTACCGTGATCCT	GCGATGGAGGTGGGATTG
qOsHAK24	CAAGGGAGGCAGGCATGT	CGCTTTTCGCCTTCATATGG
qOsHAK27	ACCGGCCATTTCAGTTCTGT	CAGGTTTCGGTGCTTTCTCTTTC
qOsHKT1;4	TCCAGACCATCTCTGCACAGAT	GTTTGCCAGAGTCGCTCCAT
qOsJAO1	CCCGACGCCTTCATCGT	GTACATCGAGTTGCTCAAGATCTCA
qOsK5.2	CGTGGCCTACCGTGATCCT	GCGATGGAGGTGGGATTG
qOsLOX1	CATGCCGTCCAAGATGCA	GGAGTGCGACGACAGGATGT
qOsLOX3	GCTGGTCTGAATCCCATGGT	TCAAGAGTACTTTTTGGTGGGAATT
qOsLOX5	GGGCCTCACGGTGCAA	TCGTGGTGGTCTAGGATGAAAA
qOsLOX7	CCGATATCTATGCCCATTGGA	CCATCCCCCTCTTGATGAGA
qOsLOX8	GCAGCTCAGCGAGATGCA	CGTTGATCCGCATCGTGTAC
qOsOPR5	TGATGCGCCATTGAACAAGT	TCCAACAATTGGGTCTTGAGTGT
qOsK3.1	GGCATCCTCCGGCTATGG	TGATGTCCTTCTCCAGCCTTGT
qOsK1.1	TTGCGTTTGAATCGTACAGC	CCTTTACGACACCAGCCATT
qOsJAZ9	TCGACGGATCCCATGACC	GGCCTTGACGTACTGGCTG
OsJAZ9_RNAi	CGTACAGTACAGATCGTTCGCAAT	GGACAATAAGCACAAAGCACAAA
qOsCOI1b	AGGATGCCGTGAACTGGAAT	TCCAGGGCCCCATTTGT
qOsMYC2	CTAGCGAGGAAACCCAATCG	CCATCCATCCATCCTAACAC
qOSAOS1	CGAGCTCTTCCTCCGATACG	GTCAGAAGGTGGCCTTCTTGAG
qOsAOS2	GGAGGAAGCTGCTGCAATAC	GGAGGTTGAAGCTTTGGTGA
OsJAR1	GGTTTGTGAACCCATCAAACAGC	CAAAATCCATAGGCAGTACTG
qOsHAK1	GTTGATGATGCTGATGTTGGAAG	CCAACACTTTCAGCTGAAAC
qOsHKT2;1	CACAGTCTCCTCGTTTGCGAA	GCAAGAATCTGGCCGATGAA
qOsHAK5	CATTGTGGACTATTTTGAAAGAA	GGAGAACTACAGAAAAGCCAATC
qOsNHX1	TCCAGCCTCCGGATGCT	ATCAGCGCGTCGTCGAA
Primers used for cloning		
OsJAZ9_OE	CACCATCAGCGACGTACAGTACA	CGCCGCGATAACTAGGGTAAC
OsJAZ9_RNAi	CACCGACACATGCACTC	CAGAACAGTCGAAGGAATTTTA
OsJAZ9_GUS	TGCTTAAGATCTAATGGCGTCGACGGAT	TGCTTAAGATCTACGCGGAGTGCATGTG
OsJAZ9ΔC_GUS	TGCTTAAGATCTAATGGCGTCGACGGAT	TGCTTAAGATCTACCATGTCCACCAGCGC
pOsJAZ9_Gus	CACCACTGCGCACCATAGTTC	TGCCGTCTTCTAGCACGGAC

Table S2. Effect of K deficiency on expression of JA associated genes.

Gene Category	MSU ID	Gene Name	WT+K Vs WT-K	P Value	Q Value	GOSlime Annotation
	Os02g12680	<i>AOS3</i>	2.5484	0.0017	0.0130	cytochrome P450, putative, expressed
	Os08g39850	<i>OsLOX8</i>	2.7217	0.0001	0.0007	lipoxygenase, chloroplast precursor, putative, expressed
	Os11g39220	<i>OsACX2</i>	1.7181	0.0001	0.0012	acyl-coenzyme A oxidase, putative, expressed
	Os01g27230	<i>OPR10</i>	1.8214	0.0002	0.0022	12-oxophytodienoate reductase, putative, expressed
	Os01g61610	<i>JAO1</i>	3.9358	0.0001	0.0007	flavonol synthase/flavanone 3-hydroxylase, putative, expressed
	Os05g10210	<i>VSP2</i>	1.6605	0.0012	0.0101	HAD superfamily phosphatase, putative, expressed

Table S3. Effect of *OsJAZ9* expression on expression of JA associated genes under K deficiency.

Gene Category	MSU ID	Gene Name	Fold Change		OE+K Vs OE -K		WT+K Vs WT-K		GOSlime Annotation
			OE+K Vs OE -K	WT+K Vs WT-K	P value	Q Value	P Value	Q Value	
JA Biosynthesis genes	Os03g12500	<i>AOS2</i>	2.7263	NS	0.0001	0.0007	0.3161	0.6305	cytochrome P450, putative, expressed
	Os02g12680	<i>AOS3</i>	5.4734	2.5484	0.0001	0.0007	0.0017	0.0130	cytochrome P450, putative, expressed
	Os02g12690	<i>AOS4</i>	0.1591	0.3320	0.0001	0.0007	0.0001	0.0012	cytochrome P450, putative, expressed
	Os03g32314	<i>AOC</i>	0.6586	0.6601	0.0026	0.0186	0.0026	0.0189	allene oxide cyclase 4, chloroplast precursor, putative, expressed
	Os02g43700	<i>DAD1</i>	0.4736	0.6386	0.0002	0.0017	0.0059	0.0367	lipoxygenase
	Os02g10120	<i>OsLOX1</i>	0.0902	0.0958	0.0001	0.0007	0.0001	0.0007	lipoxygenase, putative, expressed
	Os12g37260	<i>LOX2</i>	0.4736	NS	0.0061	0.0376	0.1148	0.3374	lipoxygenase 2.1, chloroplast precursor, putative, expressed
	Os03g49260	<i>LOX3</i>	0.3508	0.2829	0.0001	0.0007	0.0001	0.0007	lipoxygenase, putative, expressed
	Os03g49380	<i>OsLOX5</i>	1.8601	NS	0.0001	0.0007	0.1102	0.3279	lipoxygenase, putative, expressed
	Os05g23880	<i>OsLOX7</i>	0.5205	0.4439	0.0001	0.0007	0.0001	0.0007	lipoxygenase, putative, expressed
	Os08g39850	<i>OsLOX8</i>	4.4474	2.7217	0.0001	0.0007	0.0001	0.0007	lipoxygenase, chloroplast precursor, putative, expressed
	Os03g52860	<i>OsLOXL-2</i>	0.2465	0.3115	0.0001	0.0007	0.0001	0.0007	lipoxygenase, putative, expressed
	Os11g39220	<i>OsACX2</i>	2.4093	1.7181	0.0001	0.0007	0.0001	0.0012	acyl-coenzyme A oxidase, putative, expressed
	Os06g20920	<i>JMT</i>	0.5246	0.4895	0.0058	0.0361	0.0015	0.0121	SAM dependent carboxyl methyltransferase, putative, expressed
	Os06g11210	<i>OPR5</i>	NS	0.6178	0.0183	0.0885	0.0034	0.0236	12-oxophytodienoate reductase, putative, expressed
	Os01g27230	<i>OPR10</i>	1.6846	1.8214	0.0005	0.0049	0.0002	0.0022	12-oxophytodienoate reductase, putative, expressed
JA Signaling Genes	Os01g61610	<i>JAOL1</i>	6.6732	3.9358	0.0002	0.0022	0.0001	0.0007	flavonol synthase/flavanone 3-hydroxylase, putative, expressed
	Os10g25290	<i>JAZ12</i>	2.7992	NS	0.0001	0.0007	0.0113	0.0612	ZIM domain containing protein, putative, expressed
JA Marker Genes	Os05g46480	<i>LEA3</i>	22.0807	NS	0.0001	0.0007	0.0001	0.0007	late embryogenesis abundant protein, group 3, putative, expressed
	Os05g10210	<i>VSP2</i>	4.1088	1.6605	0.0001	0.0007	0.0012	0.0101	HAD superfamily phosphatase, putative, expressed

Table S4. Effect of *OsJAZ9* expression on K transporter expression under K deficiency

Family	MSU ID	Gene Name	Fold Change		OE+K Vs OE -K		WT+K Vs WT-K		UP/DN
			OE+K Vs OE -K	WT+K Vs WT-K	P VALUE	Q VALUE	P VALUE	Q VALUE	
KUP/HAK/KT family	Os01g70490	<i>OsHAK5</i>	2.05340	3.00194	0.0001	0.0007	0.0001	0.0007	DN
	Os06g42030	<i>OsHAK10</i>	0.43768	0.37120	0.0001	0.0007	0.0001	0.0007	UP
	Os09g27580	<i>OsHAK17</i>	0.51064	0.60262	0.0001	0.0007	0.0007	0.0061	DN
	Os06g15910	<i>OsHAK24</i>	0.50844	0.42377	0.0002	0.0017	0.0001	0.0007	UP
	Os03g37830	<i>OsHAK27</i>	0.26458	0.34944	0.0001	0.0007	0.0001	0.0007	DN
AKT family	Os01g45990	<i>OsK1.1</i>	0.52729	0.68904	0.0001	0.0007	0.0080	0.0465	DN
	Os05g35410	<i>OsK3.1</i>	3.00689	2.13439	0.0001	0.0007	0.0001	0.0012	UP
	Os06g14030	<i>OsK5.2</i>	0.63491	0.54976	0.0051	0.0327	0.0002	0.0017	UP
Trk family	Os06g48810	<i>OsHKT2;2</i>	4.73023	5.67469	0.0001	0.0007	0.0001	0.0007	DN
	Os04g51830	<i>OsHKT1;4</i>	3.86436	2.59602	0.0012	0.0101	0.0087	0.0497	UP
	Os01g20160	<i>OsHKT1;5</i>	0.19389	0.25082	0.0037	0.0253	0.0067	0.0406	DN
Other K transporters	Os12g44360	<i>SOS1</i>	0.52463	0.47277	0.0001	0.0007	0.0001	0.0007	UP
	Os06g14030	<i>GORK</i>	0.63491	0.54976	0.0051	0.0327	0.0002	0.0017	UP

Table S5. Effect of *OsJAZ9* expression on expression of *OsJAZ* genes.

Category	MSU ID	Gene Name	Fold Change		WT+K Vs OE+K		WT-K Vs OE-K		GOSlim Annotation
			WT+K Vs OE+K	WT-K Vs OE-K	P value	Q value	P value	Q value	
JAZ Repressor	Os04g55920	<i>JAZ1</i>	0.8657	0.7747	0	0.59	0.06	0	zinc-finger protein, putative, expressed
	Os07g05830	<i>JAZ2</i>	0.0027	0.3799	1	1	1	1	zinc-finger protein, putative, expressed
	Os08g33160	<i>JAZ3</i>	0.9049	0.9561	0	0.78	0.75	1	ZIM motif family protein, expressed
	Os09g23660	<i>JAZ4</i>	0.8980	0.8640	1	0.83	0.4	1	ZIM motif family protein, expressed
	Os04g32480	<i>JAZ5</i>	0.6802	0.7559	0	0.6	0.35	1	zinc-finger protein, putative, expressed
	Os03g28940	<i>JAZ6</i>	0.5110	0.6006	0	0	0	0	ZIM domain containing protein, putative, expressed
	Os07g42370	<i>JAZ7</i>	0.6822	0.6394	0	0.03	0	0	zinc-finger protein, putative, expressed
	Os09g26780	<i>JAZ8</i>	0.3315	0.3787	0	0	0	0	zinc-finger protein, putative, expressed
	Os03g08310	<i>JAZ9</i>	204.2619	163.9600	0	0	0	0	
	Os03g08320	<i>JAZ10</i>	0.4277	0.6511	0	0	0.03	0	ZIM domain containing protein, putative, expressed
	Os10g25290	<i>JAZ12</i>	0.3498	0.6259	0	0	0.01	0	ZIM domain containing protein, putative, expressed
	Os10g25230	<i>JAZ13</i>	0.4824	0.3100	0	0.3	0.02	0	ZIM domain containing protein, putative, expressed