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

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14

15 **Abstract:**

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

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

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45 INTRODUCTION

46 Rice is a mainstay for global food security. The two-third population of the world is dependent
47 on rice as a staple food crop. Soil nutrient deficiency is one of the primary limitations of rice
48 production. Notably, Potassium (K) is one of the most important and abundant macronutrients
49 in plants, which can comprise up to 10% of the plant's total dry weight (Leigh & Wyn Jones,
50 1984). Its physiological functions fall into two categories; the first requires a high and relatively
51 stable concentration of K^+ to regulate the osmotic potential of the cell and activation of many
52 enzymes involved in respiration and photosynthesis (K^+ act as a cofactor for many enzymes).
53 The second category is based on the high mobility of K^+ ions resulting in osmotic changes,
54 which regulate the stomatal movement (Sawhney & Zelitch, 1969; Terry & Ulrich, 1973) and
55 phloem transport (Mengel and Viro, 1974). K^+ is also involved in key cellular processes such
56 as energy production, cell expansion and balancing the counter flux of charges across the
57 membranes (Tester and Blatt, 1989; Wu et al., 1991; Elumalai et al., 2002). Hence, its
58 deficiency affects fitness and overall growth as K deficient plants are more susceptible to salt
59 (Kaya et al., 2006), drought (Egilla et al., 2001; Wang et al., 2013), chilling (Kant and Kafkafi,
60 2002) and biotic stresses (Hardter, 2002; Sarwar, 2012).

61 Although K is among the most abundant minerals in the soil; its availability to plants is limited
62 because most of the soil K (about 98%) is in bound forms where its release into the soil solution
63 is far slower than the rate of acquisition by roots (Sparks, 1987). Notably, a very little fraction
64 of soil K is present in the soil solution or exchangeable form whose availability depends on
65 multiple factors like soil pH (Rich and Black, 1964; Rich, 1964), presence of other monovalent
66 cations like Na^+ and NH_4^+ (Qi and Spalding, 2004) and the type of soil particles (Sparks, 1987).
67 K deficiency results in both root (Singh et al., 2015) and shoot (Shankar et al., 2013) growth
68 inhibition. In roots, it strongly impairs primary root growth (Gruber et al., 2013); a similar
69 phenotype is also observed on exogenous Jasmonic Acid (JA) application (Staswick et al.,
70 1992; Cai et al., 2014). Further, a few transcriptome studies have shown many JA signaling
71 genes upregulated under K deficiency in rice (Ma et al., 2012; Shankar et al., 2013; Takehisa et
72 al., 2013). Interestingly, a substantial part of K-responsive transcriptome either was absent or
73 replaced in the Arabidopsis JA receptor, *coil-16* mutant (Armengaud et al., 2004) which
74 indicates active roles of JA signaling in K deficiency responses. Moreover, a significant
75 number of genes were also found commonly upregulated by exogenous JA treatment and K
76 deficiency in rice (Kobayashi et al., 2016), which further indicates crosstalk between JA
77 signaling and K deficiency response.

78 JA and its derivatives commonly called Jasmonates (JAs) are a group of oxylipin-derived
79 phytohormones, which are synthesized in response to a large number of biotic and abiotic
80 stresses in plants (Feys et al., 1994; Heitz et al., 2016; Kazan, 2015; Riemann et al., 2015).
81 Understanding of JA functions is most advanced in Arabidopsis, but the overall architecture
82 has been found conserved in all examined land plants. JA signaling has been the focus of
83 intensive research in rice and regulates many traits of agronomic importance (Reimann et al.,
84 2015; Nguyen et al., 2019). In absence of JA, JA-regulated genes are maintained silent by so-
85 called JASMONATE ZIM (JAZ) protein repressors that bind to and block the action of bHLH
86 transcription factors MYC2/3/4, with the involvement of additional repressors. Upon stress of
87 developmental cues, the bioactive conjugate jasmonoyl-isoleucine JA-Ile is formed and
88 triggers the assembly of the co-receptor JAZ-CO11 (Sheard et al., 2010). CO11
89 (CORONATINE INSENSITIVE 1), is the F-box component of the co-receptor, that will be
90 recruited into the ubiquitin E3 ligase complex SCF^{CO11} that targets JAZ protein for 26S
91 proteasome-mediated degradation (Wasternack and Hause, 2013). JAZ destabilisation de-
92 represses a myriad of transcription factors allows the transcription of numerous JA responsive
93 genes depending on organ or stimulus (Wasternack and Hause, 2013). JAZs form a small
94 family of 15 members in rice (Ye et al., 2009), and play roles in floral development, salt, and
95 biotic stress tolerance (Fu et al., 2017). However, their functions in nutritional, particularly K
96 deficiency response, remain elusive. Here, we examined the functions of a particular JAZ
97 isoform, OsJAZ9, because *OsJAZ9* was the most downregulated *JAZ* gene during K deficiency
98 among all the isoforms (Singh et al., 2015). Further, OsJAZ9 also influenced the rice response
99 to NaCl stress which also involves K homeostasis (Wu et al., 2015). We show that JA-Ile levels
100 are enhanced under K deficiency in rice and this response is further elevated in rice on *OsJAZ9*
101 overexpression. Also, we demonstrate that overexpression of *OsJAZ9* enhanced JA signaling,
102 and this was correlated with improved K deficiency tolerance.

103 **Materials and Methods**

104 **Plant growth conditions**

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

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

116 **qRT-PCR analysis**

117 Total RNA was extracted from seedlings of the desired age using Trizol reagent and cDNA
118 synthesis was performed as described (Mehra et al., 2017). Gene-specific primers were
119 designed from CDS (retrieved from Rice Gene Annotation Project) using PRIMER EXPRESS
120 version 3.0 (PE Applied Biosystems TM, USA) with default parameters. SYBR® Green
121 Master Mix was used to quantify the DNA product in Applied Biosystems 7500 Fast Real-
122 Time PCR. For the Relative gene expression analysis, we used the ΔΔCt method taking
123 *Ubiquitin5* (Os01g0328400) or *Actin1* (Os03g0718100) as endogenous control (Mehra et al.,
124 2016).

125 **Measurement of K and Na**

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

131 **Generation of transgenic lines**

132 Full-length cDNA (AK070649) of *OsJAZ9* was amplified using gene-specific primers
133 (Supplemental Table S1) and cloned under *ZmUbi1* promoter in gateway-compatible
134 overexpression vector as described (Pandey et al., 2017). For raising RNAi transgenics of
135 *OsJAZ9*, 416 bp region of *OsJAZ9* cDNA was amplified from the cDNA region encompassing
136 3'UTR and cloned in *pANIC8b* vector (Mann et al., 2012) by Gateway Technology. To
137 generate *p35S:OsJAZ9_GUS* and *p35S:OsJAZ9ΔC_GUS* lines, *OsJAZ9* coding region and
138 *OsJAZ9ΔC* (deleted 65 amino acids including jas motif from the C-terminal of OsJAZ9)
139 cDNAs were amplified using gene-specific primers, and cloned into *pCAMBIA1301*.

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

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

146 **Analysis of GUS Activity**

147 Newly emerged roots were selected for histochemical staining, i.e. GUS activity. Samples were
148 harvested at indicated time points and immersed in GUS buffer (1 mM 5-bromo-4-chloro-3-
149 indolyl-β-glucuronide sodium salt in 50 mM sodium phosphate, pH 7.0, ten mM Na-EDTA,
150 0.5 mM ferricyanide, 0.5 mM ferrocyanide, and 0.1% Triton X-100), and were incubated at
151 37°C for overnight. Chlorophyll was cleared from plant tissues by immersing them in 70%
152 ethanol, overnight. For detection of *OsJAZ9* degradation in-vivo, roots from transgenic lines
153 containing *p35S:OsJAZ9_GUS* and *p35S:OsJAZ9ΔC_GUS* plants were treated with MeJA
154 (100 μM) with or without the proteasome inhibitor MG132 (100 μM) for 1h. Histochemical
155 GUS staining of roots was performed to visualize the abundance/degradation of OsJAZ9 and
156 OsJAZ9ΔC. GUS signal was observed under a stereo-zoom microscope. Fifteen-days-old
157 seedlings of *p35S:OsJAZ9_GUS* and *p35S:OsJAZ9ΔC_GUS* transgenic lines were subjected
158 to K deficiency (4.08 μM external K⁺) and root samples were harvested on indicated time points
159 and immersed in GUS buffer. *pOsJAZ9:GUS* transgenic lines were subjected to K deficiency
160 (0 μM external K⁺) and root samples were harvested on indicated time points and immersed in
161 GUS buffer.

162 **Subcellular localization of OsJAZ9**

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

169 **MeJA mediated root growth inhibition assay**

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

176 **Exogenous MeJA treatment to analyze the impact on K deficiency**

177 To investigate the impact of exogenous application of MeJA on K deficiency, uniformly
178 germinated WT rice seedlings were transferred to hydroponic (Yoshida) media with 4.08 μM
179 K_2SO_4 , 8.16 μM K_2SO_4 and 510 μM K_2SO_4 for 5 days and then equal number of seedlings were
180 transferred to 0 μM , 0.1 μM , 0.01 μM and 0.001 μM MeJA with pre-existing K_2SO_4
181 concentrations for 10 days. These seedlings were analyzed for root length, shoot length and
182 lateral root length. After this analysis, the same seedlings were transferred to a hot air oven at
183 60°C for three days, and dry biomass was observed.

184 **Transcriptome Analysis**

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

198

199 **JA quantification**

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

212 RESULTS

213 K deficiency promotes JA signaling in rice

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

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

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

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

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

253 **Overexpression of *OsJAZ9* enhances K deficiency tolerance in rice**

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

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

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

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

284 Overexpression of several *JAZ* repressor encoding genes under the constitutive promoter has
285 resulted in partial JA insensitivity in Arabidopsis and rice (Thines et al., 2007; Yamada et al.,
286 2012). To understand the JA sensitivity level of the *JAZ9* transgenics, we analyzed root growth
287 inhibition of *OsJAZ9* OE and RNAi lines upon MeJA treatment. Consistent with previous
288 studies, we also observed a partial JA insensitive phenotype in *OsJAZ9* OE lines (Fig. 4). We
289 found ~36% root growth inhibition in RNAi line, whereas only 11% reduction in
290 overexpressing lines (OE10) as compared to WT (30% root length reduction) upon exogenous
291 MeJA treatment. This experiment also suggests that *OsJAZ9* OE may have higher level of
292 bioactive JA.

293 To uncover the global transcriptional response of *OsJAZ9* overexpression under K deficiency,
294 we performed RNA-seq analysis of WT and *OsJAZ9* OE (OE10) seedlings raised under normal
295 and K deficient conditions for 15 days. The comparative +K vs. -K analysis revealed a total of
296 5173 and 5505 genes were differentially expressed ($p < 0.05$, $q < 0.05$) in WT and *OsJAZ9* OE
297 line, respectively, during K deficiency. Out of these 2433 and 2670, genes were upregulated,
298 and 2740 and 2835 genes were downregulated, respectively, in WT and *OsJAZ9* OE line (Fig.
299 5 A-B).

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

306 We found up-regulation of *OsAOS2*, *OsLOX5*, *OsLOX7*, *OsLOX8*, *OsACX2*, *OsOPR5*, and
307 *OsJAO1* which play key roles in JA biosynthesis (or homeostasis) and higher expression of
308 key JA signaling marker genes like *OsVSP2* in *OsJAZ9* OE plants than WT plants subjected to
309 K deficiency (Table S3). This higher transcript abundance of JA biosynthesis, signaling and
310 marker genes confirmed the elevated JA response in *OsJAZ9* OE plants during K deficiency as
311 compared to the WT. Further analysis revealed that out of 13 known K transporters, 7 were
312 having higher transcript abundance in OE line as compared to WT under K deficiency, while
313 6 transporters were downregulated (Table S4). Among the lesser expressed ones, *OsHKT27*
314 encodes a K efflux transporter, *OsHKT2;2* and *OsHKT1;5* were described as more efficient in
315 Na transport than K (Kadar et al., 2006) while *OsK1.1* (*OsAKT1*) functions at the millimolar
316 range of external K (Ahmad et al., 2016). We found *OsHAK10*, *OsHAK24*, *OsK3.1*, *OsK5.2*,
317 *OsHKT1;1*, *OsHKT1;4*, *OsSOS1* and *OsGORK* genes having higher abundance in OE line as
318 compared to WT in K deficient conditions and thus supports the case for higher K uptake in
319 *OsJAZ9* overexpressing plants (Table S4).

320 ***OsJAZ9* modulates root system architecture**

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

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

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

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

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

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

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

380 **DISCUSSION**

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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502 **AUTHORS CONTRIBUTIONS**

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

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656

657

658 **FIGURE LEGENDS**

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

665

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

676

677 **Figure 3.** Enhanced K acquisition in K-starved *OsJAZ9* OE seedlings upon K resupply. (A)
678 Morphological data (root dry weight, shoot dry weight and number of roots per plant) of 10-
679 days-old normally grown seedlings (normal/S1), (B) followed by 15-days of K starvation (K
680 stress/S2) and (C) subsequent recovery with 10 μM K₂SO₄ for 5 days (Recovery/S3). K stress
681 represents the ratio of dry root, or shoot biomass K stressed plants with respect to normal
682 condition and recovery represents the ratio of recovered plants with respect to normal condition
683 (n=<15). (D) Na⁺/K⁺ and (E) Total K content per plant of WT and *OsJAZ9* transgenics during
684 S1, S2 and S3 treatments. K content experiment was performed with two replicates. Asterisks
685 indicate significant changes in transgenics compared to WT at respective time points ($p \leq 0.05$,
686 .01 and .001 for *, ** and *** calculated from Student's *t*-test). Scale =10 cm.

687

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

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

697

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

703

704 **Figure 6. *OsJAZ9* overexpression influences root system architecture.** (A) Representative
705 root images of 12-days-old WT and *OsJAZ9* transgenics showing lateral root lengths during K
706 deficient (upper panel) and K sufficient conditions (lower panel). (B) Average seminal root
707 length and (C) Average lateral root length of WT and *OsJAZ9* transgenics during K deficient
708 (upper row) and K sufficient (lower row) conditions. Four independent plants were used for
709 the analysis. Scale bar represents 1 cm while error bar represents SE among the replicates. (p
710 ≤ 0.05 , Student's *t*-test).

711

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

717

718 **Supporting Information**

719 **Figure S1.** Effect of K deficiency on rice growth.

720 **Figure S2.** Potassium deficiency induces JA signaling in rice.

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

722 **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.

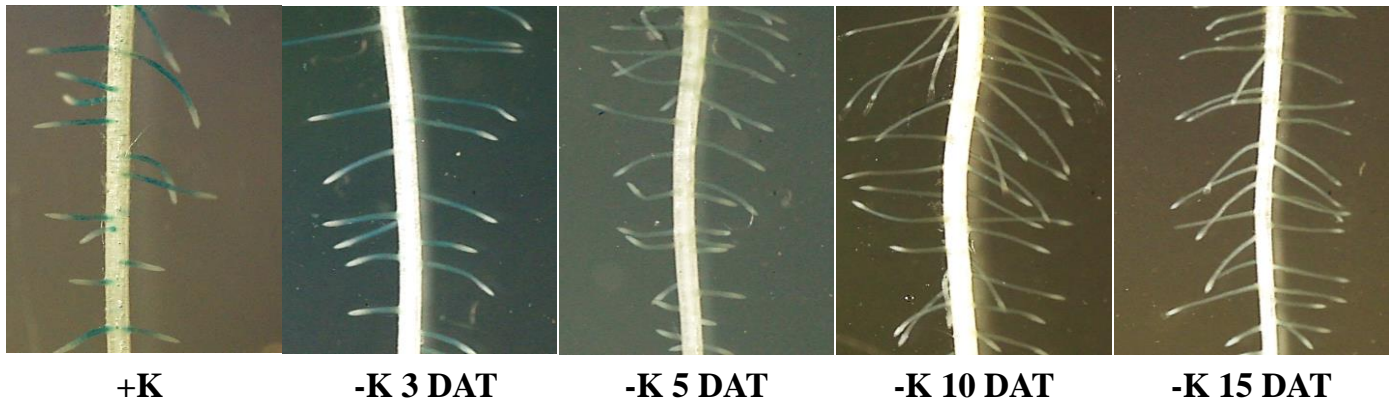


Figure 1. Promoter of *OsJAZ9* is K deficiency responsive. Gus expression in lateral roots on nodal roots of *pOsJAZ9:GUS* transgenic rice plants. Expression was mainly appeared in laterals which was monitored at different time points under K deficiency. *pOsJAZ9:GUS* expression faded away as the K deficiency 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).

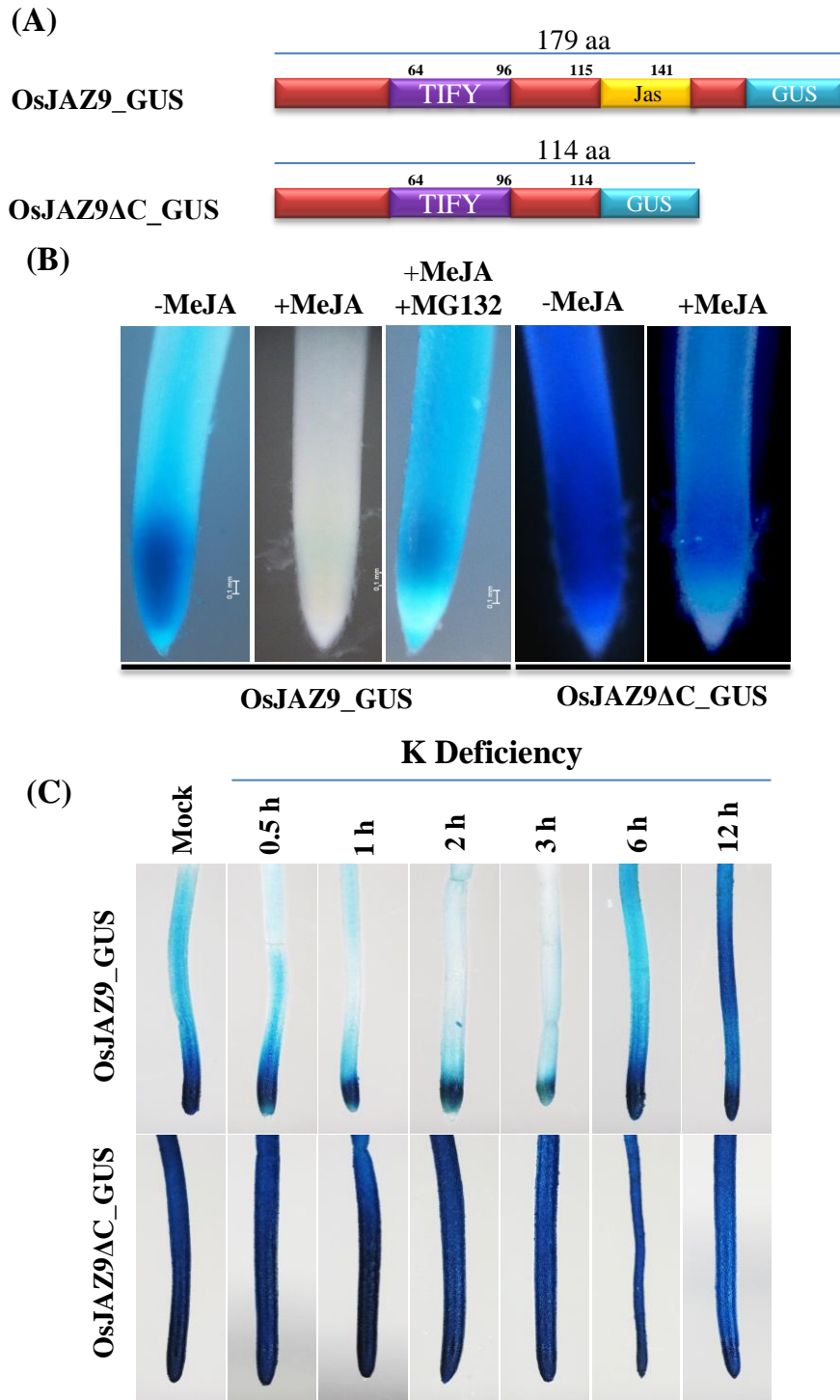


Figure 2. Potassium deficiency causes a rapid increase in JA signaling in rice. (A) Schematic representation of *OsJAZ9_GUS* and *OsJAZ9 Δ C_GUS* (Jas domain deleted) translational reporter constructs. (B) JA dependent proteasomal degradation of *OsJAZ9* through Jas domain. Root tips of plants overexpressing *OsJAZ9_GUS* and *OsJAZ9 Δ C_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 *OsJAZ9 Δ C_GUS* were transferred to K deficient media for indicated time points (0.5 to 12 h). Stability of *OsJAZ9_GUS* and *OsJAZ9 Δ C_GUS* proteins in response to K deficiency were analyzed by histochemical GUS staining of roots. The experiment was performed in three independent replicates.

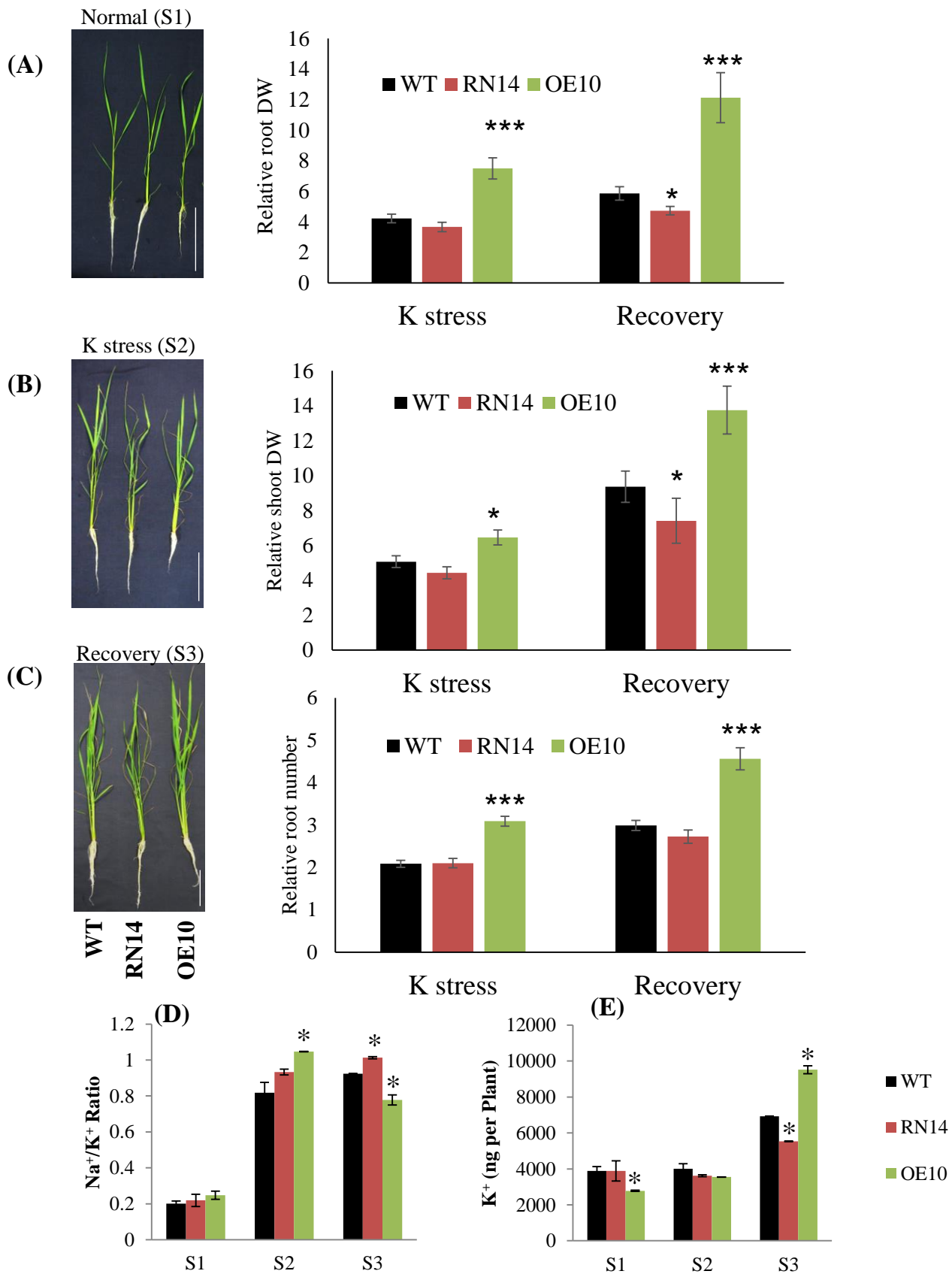


Figure 3. Enhanced K acquisition in K-starved *OsJAZ9* OE seedlings upon K resupply. (A) Morphological data (root dry weight, shoot dry weight and number of roots per plant) of 10-days-old normally grown seedlings (normal/S1), (B) followed by 15-days of K starvation (K stress/S2) and (C) subsequent recovery 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.

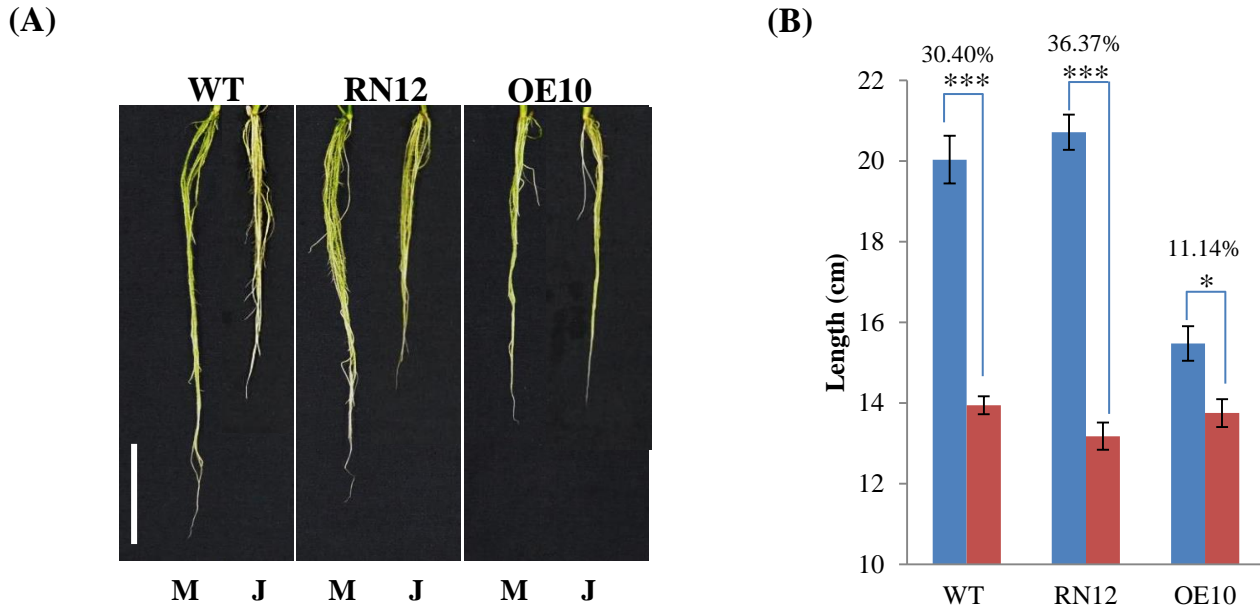


Figure 4. JA-induced root inhibition in WT and *OsJAZ9* transgenics. (A) Representative images of roots of 20-days-old WT, RN12 and OE10 seedlings supplemented with DMSO (Mock; M) or 10 μ M MeJA dissolved in DMSO (JA treated; J). Scale = 5 cm. (B) Average root lengths of WT, *OsJAZ9* RNAi and OE plants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

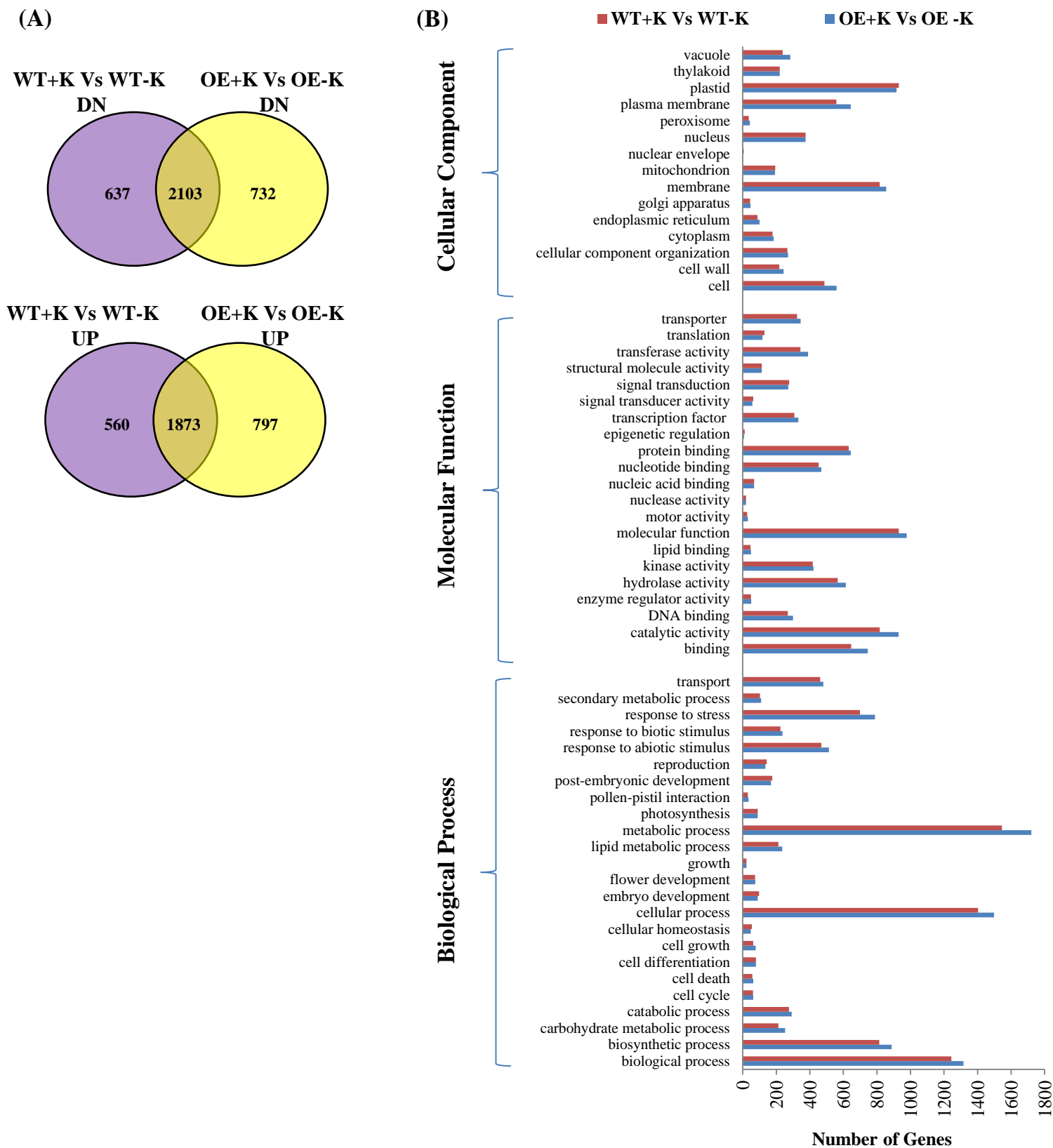


Figure 5. Overexpression of *OsJAZ9* enhances K deficiency response in rice. (A) Venn diagrams showing differentially regulated genes on K deficiency in WT and *OsJAZ9* OE lines after 15 days of deficiency. (B) Overview of transcriptome data showing category wise distribution of differentially genes 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.

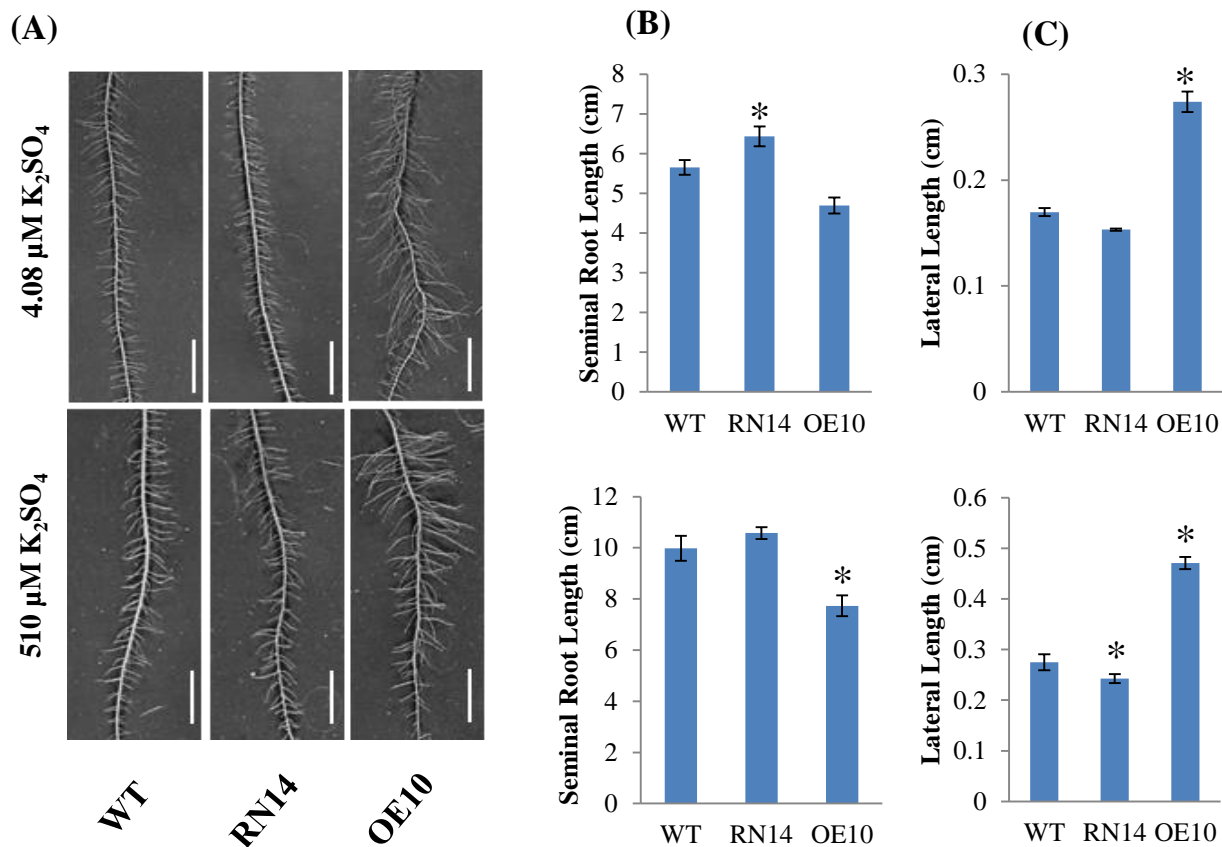


Figure 6. *OsJAZ9* overexpression influences root system architecture. (A) Representative root images of 12-days-old WT and *OsJAZ9* transgenics showing lateral root lengths during K deficient (upper panel) and K sufficient conditions (lower panel). (B) Average seminal root length and (C) Average lateral 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).

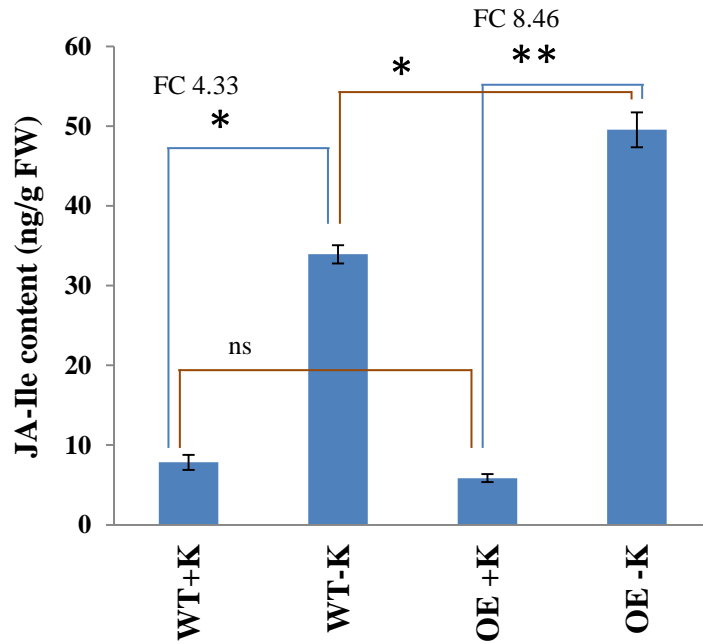


Figure 7. *OsJAZ9* OE lines accumulate higher JA-Ile than WT under K deficiency. JA-Ile content in WT and *OsJAZ9* OE (OE10) lines during normal (+K) and 15 days of K deficiency (-K) conditions. Bars represent the average change in JA-Ile content among at least four replicates. Error bar represents SE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

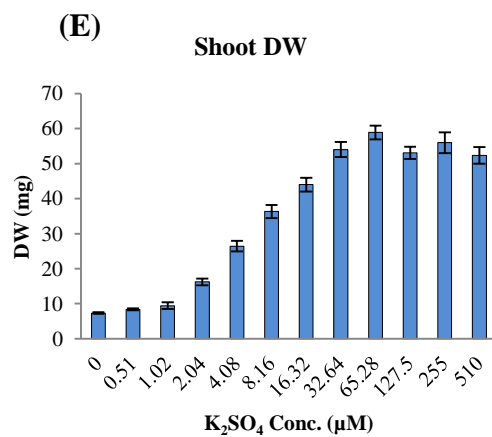
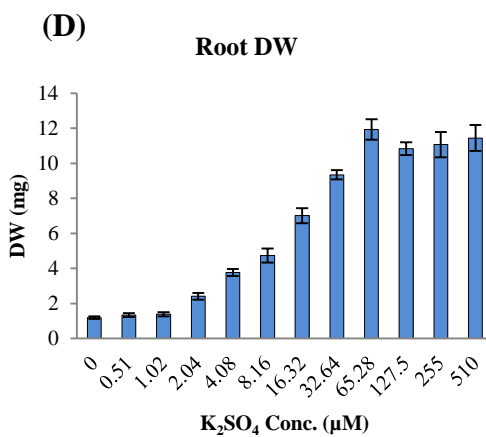
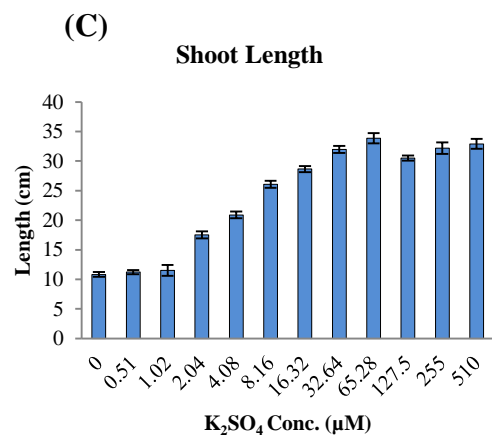
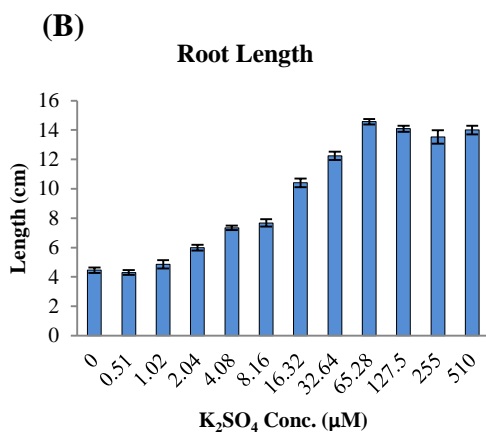
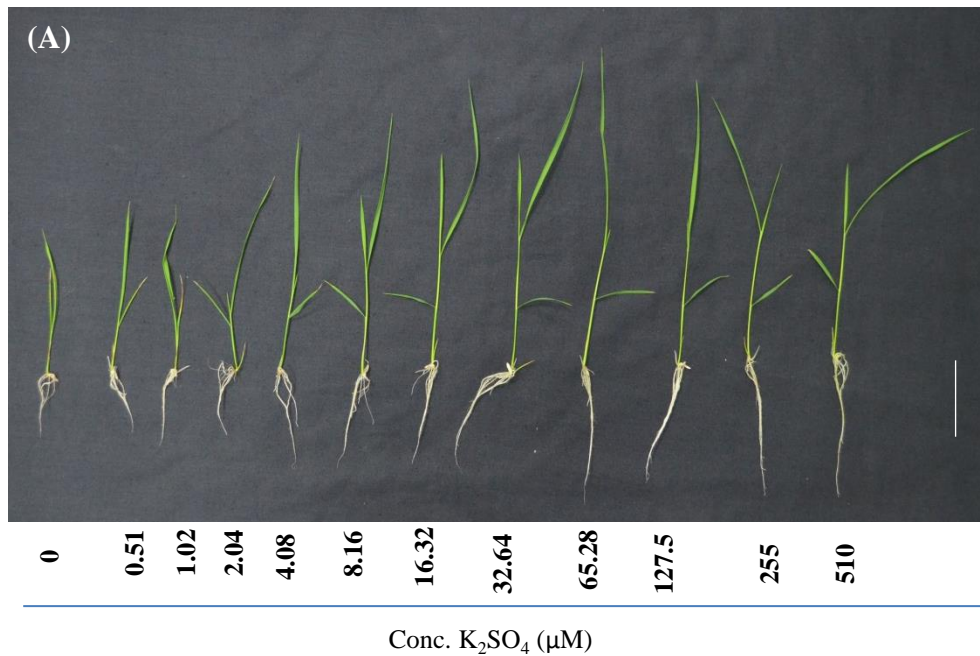


Figure S1. Effect of K deficiency on rice growth. (A) Representative images showing effect of different concentrations of K on plant growth. Uniformly germinated seedlings were transferred to Yoshida media containing different concentrations of K_2SO_4 (as indicated on x-axis). (B) Root length, (C) Shoot 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.

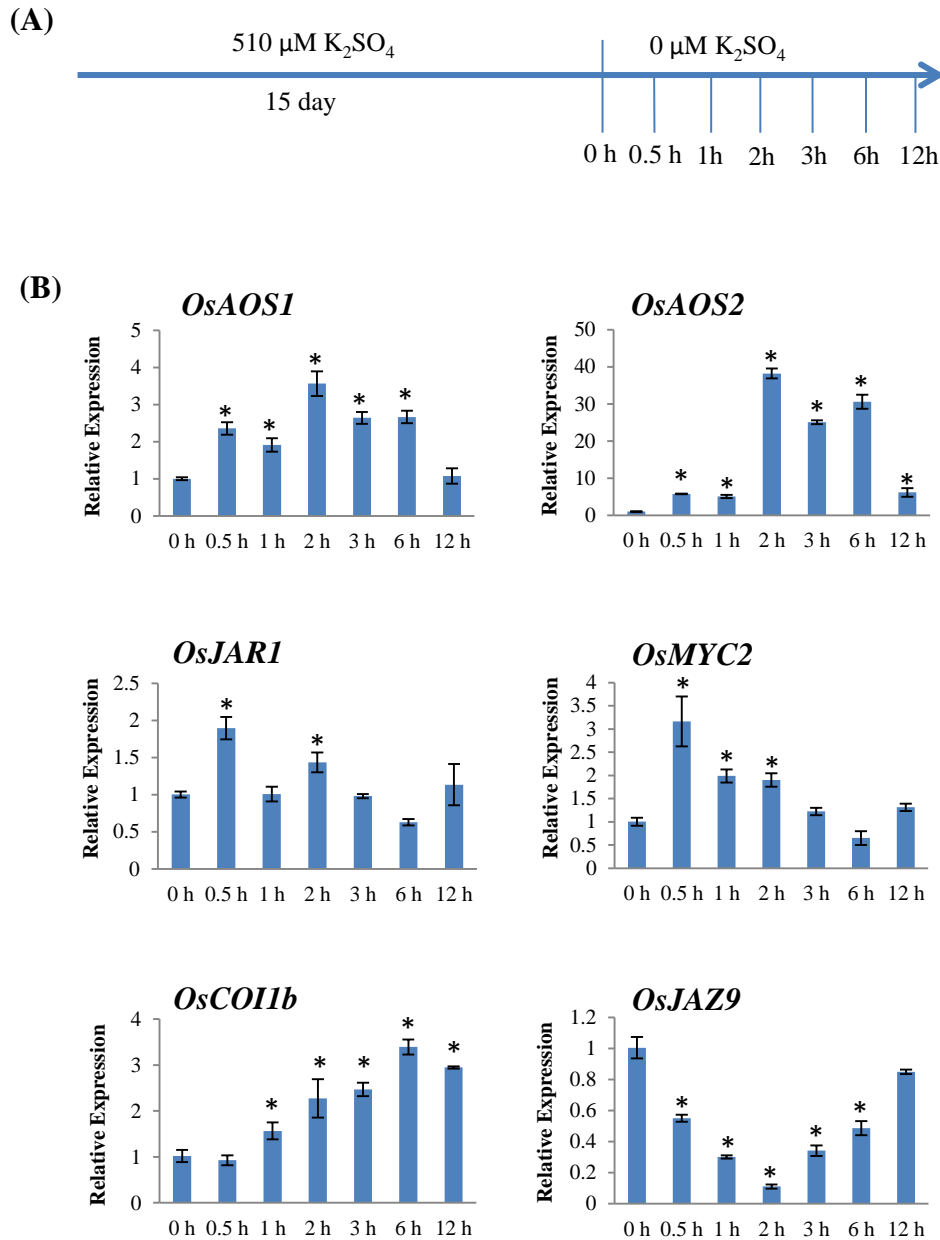


Figure S2. Potassium deficiency induces JA signaling in rice. (A) Schematic representation of experimental setup used for expression analysis of JA biosynthetic and signaling genes. Plants were raised under normal conditions ($510 \mu\text{M K}_2\text{SO}_4$) for 15 days before being subjected to different durations (0.5 to 12 h) of K deficiency treatment ($0 \mu\text{M K}_2\text{SO}_4$). (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).

(A)

Plant-mPLOC: Predicting subcellular localization of plant proteins including those with multiple sites

[Read Me](#) | [Data](#) | [Citation](#) |

Your input sequence (179aa) is:

```
>OsJAZ9
MASTDPMTRRFVACGVLSSQYVKANSSQPSTAAPVAQGVSGLMAAAAAAAAAPVVQEPGC
EVDGGGQQFTIFYAGKVVVIDRCTPAMAAELMRFASAAQGGGGAPEAPPALVDMPIARKA
SLKRFLAKRKATPASARSSYVVRAAAAEEEQPPAKKAKAAVERREDWLAGSLGHMHSR
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----- Plant-mPLOC Computation Result -----

Query protein	Predicted location(s)
OsJAZ9	Nucleus.

(B)

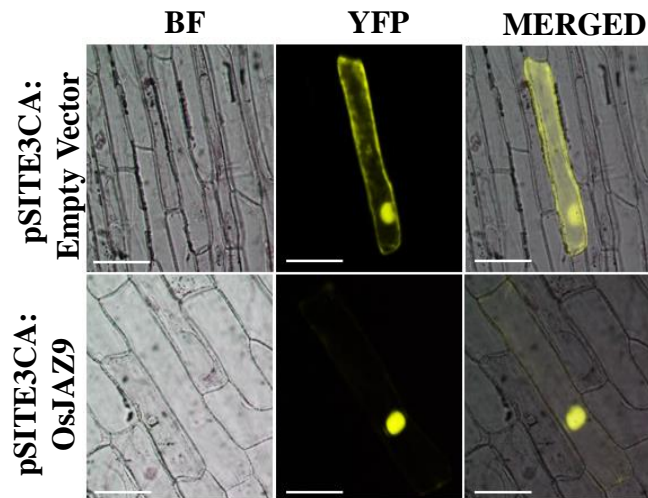


Figure S3. Nuclear localization of YFP-OsJAZ9. (A) *In-silico* prediction of OsJAZ9 localization using databases (Plant-mPLOC (<http://www.csbio.sjtu.edu.cn/cgi-bin/PlantmPLOC.cgi>)). (B) Subcellular localization of YFP-OsJAZ9 in onion epidermal cells. Merged images showing superimposition of YFP and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

(A)

```
OsJAZ9      MASTDPMTRRFVAVACGVL SQYVKANSSQPSTAAPVAQGV SGLMAAAAAAAAAAPVVQEPGC
OsJAZ9ΔC   MASTDPMTRRFVAVACGVL SQYVKANSSQPSTAAPVAQGV SGLMAAAAAAAAAAPVVQEPGC

OsJAZ9      EVDGGGQQFTI FYAGKVVVIDRCTPAMAAELMRFASAAQGGGGAPEAPPALVDMPIARKA
OsJAZ9ΔC   EVDGGGQQFTI FYAGKVVVIDRCTPAMAAELMRFASAAQGGGGAPEAPPALVDM-----

OsJAZ9      SLKRFLAKRKATPASARSSYV VRAAAAAEEEQPPAKKAKAAVERREDWLALGSLGHMHSR
OsJAZ9ΔC   -----
```

(B)

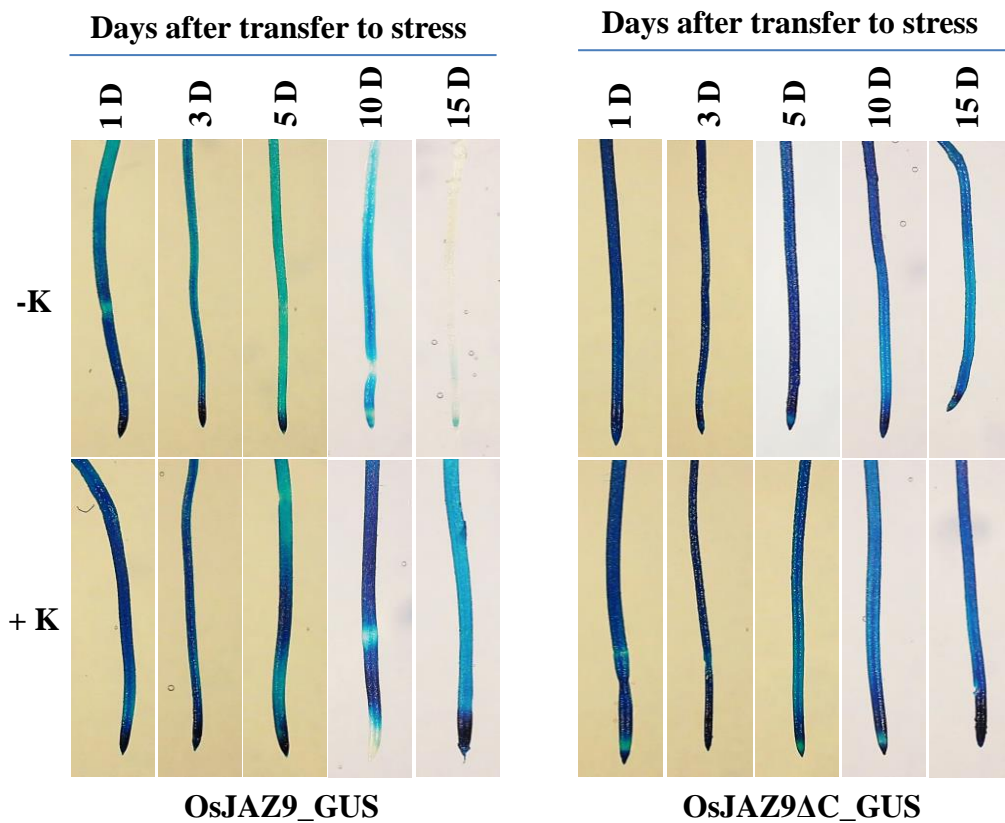


Figure S4. Effect of prolonged K deficiency on OsJAZ9 stability. (A) Protein sequence alignment of OsJAZ9 and OsJAZ9ΔC. Red text highlights TIFY domain while the yellow text highlights Jas motif. (B) OsJAZ9_GUS degradation on prolonged K deficiency. 15-days-old rice seedlings expressing *OsJAZ9_GUS* 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

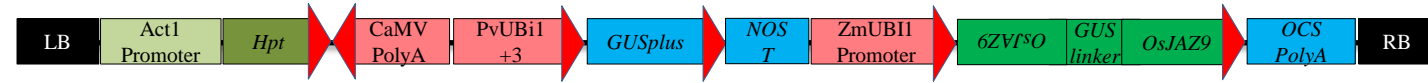
p35S:OsJAZ9_GUS



p35S:OsJAZ9ΔC_GUS



Vector constructs for *OsJAZ9* silencing



Vector constructs for *pOsJAZ9:GUS* reporter



(B)

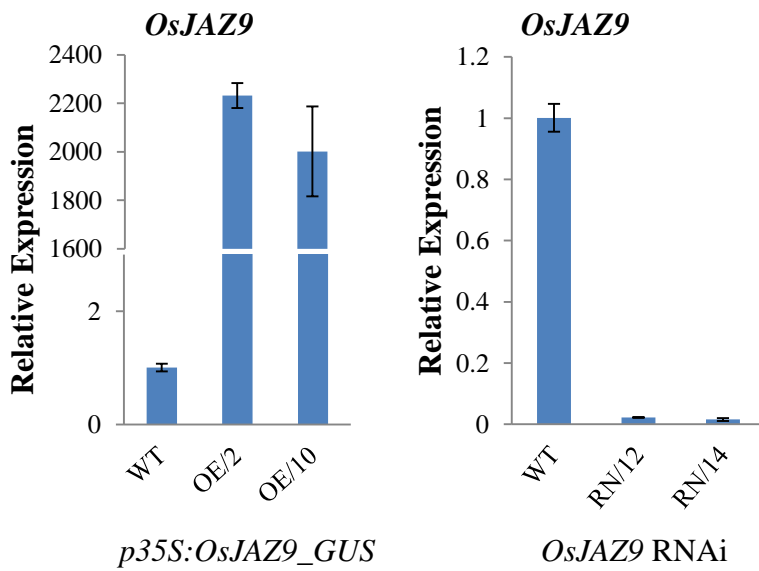


Figure S5. Raising and screening of *OsJAZ9* expressing rice transgenics. (A) Schematic of *OsJAZ9* overexpression and silencing constructs. (B) Relative expression of *OsJAZ9* in overexpression (*p35S:OsJAZ9_GUS*) and RNAi lines. Gene expression analysis was done with qRT-PCR with at least three independent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

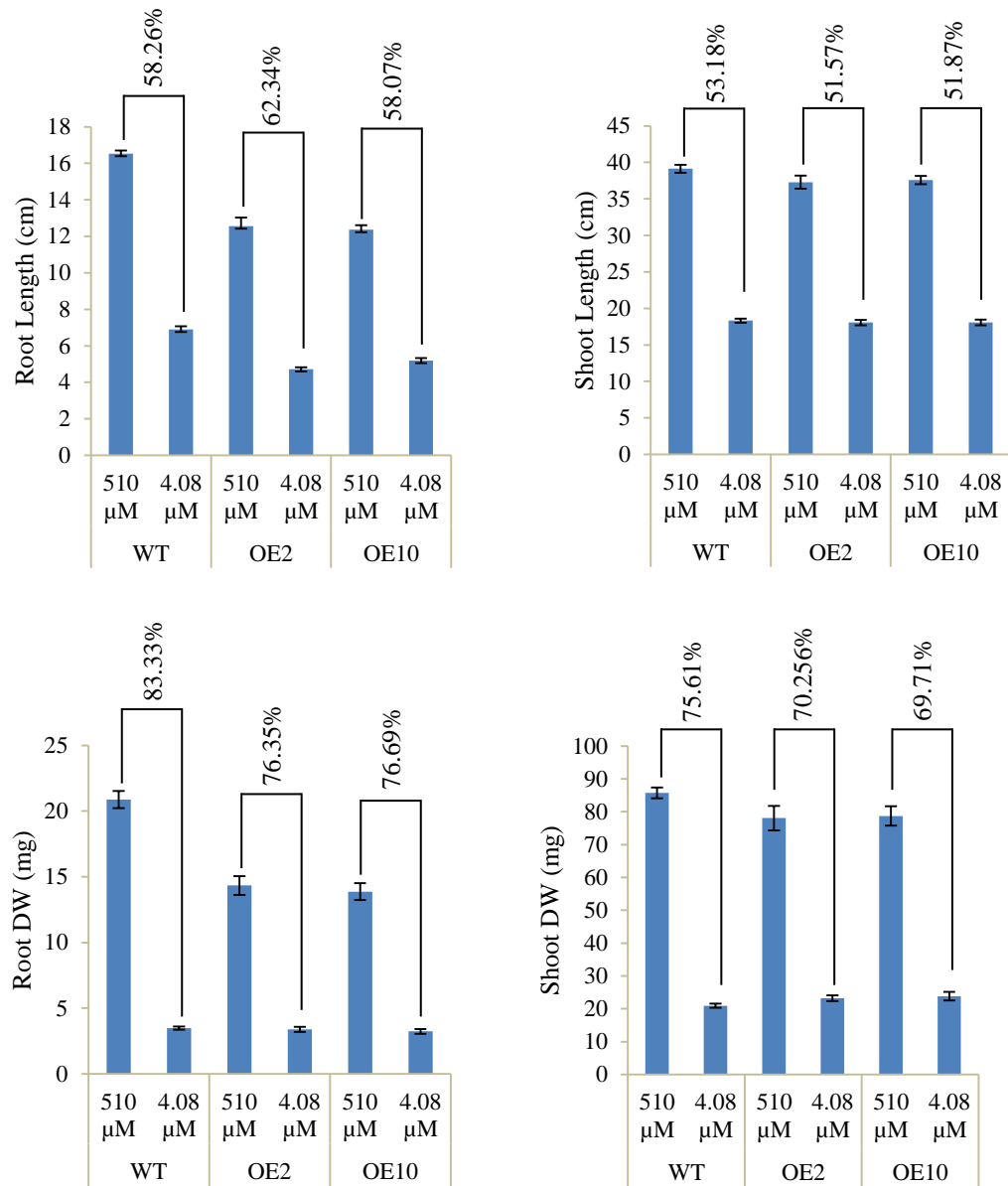


Figure S6. Effect of *OsJAZ9* overexpression on growth parameters of rice during K sufficient and deficient conditions. Growth parameters (length and dry biomass of root and shoot) of WT and *OsJAZ9* OE (OE2 and OE10) lines under K deficient and sufficient conditions. Each bar represents average of 12 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.

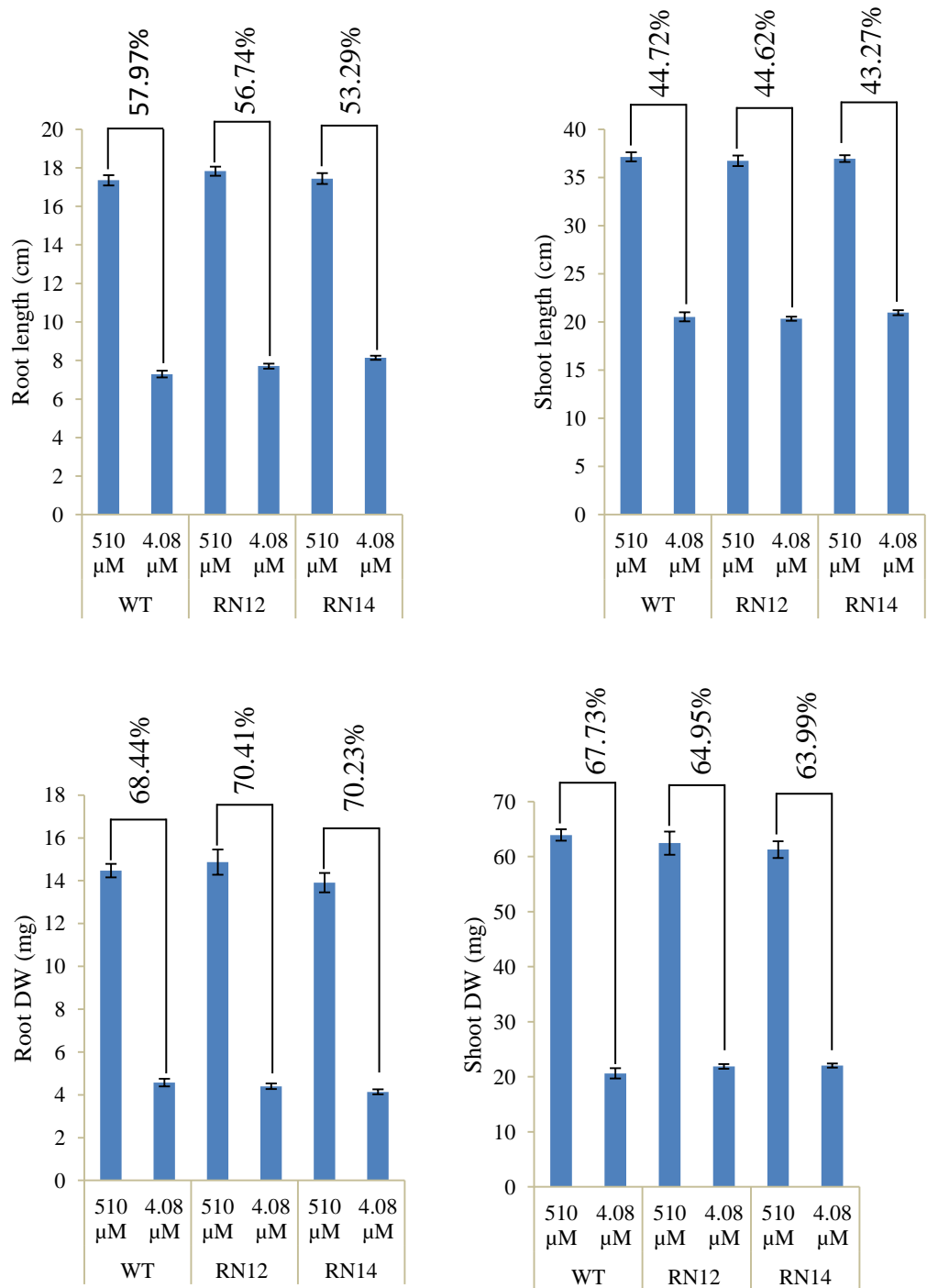


Figure S7. Effect of *OsJAZ9* knockdown on growth parameters of rice during K sufficient and deficient conditions. Growth parameters (length and dry biomass of root and shoot) of WT and *OsJAZ9* RNAi (RN12 and RN14) lines under K deficient and sufficient conditions. Each bar represents average of 12 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.

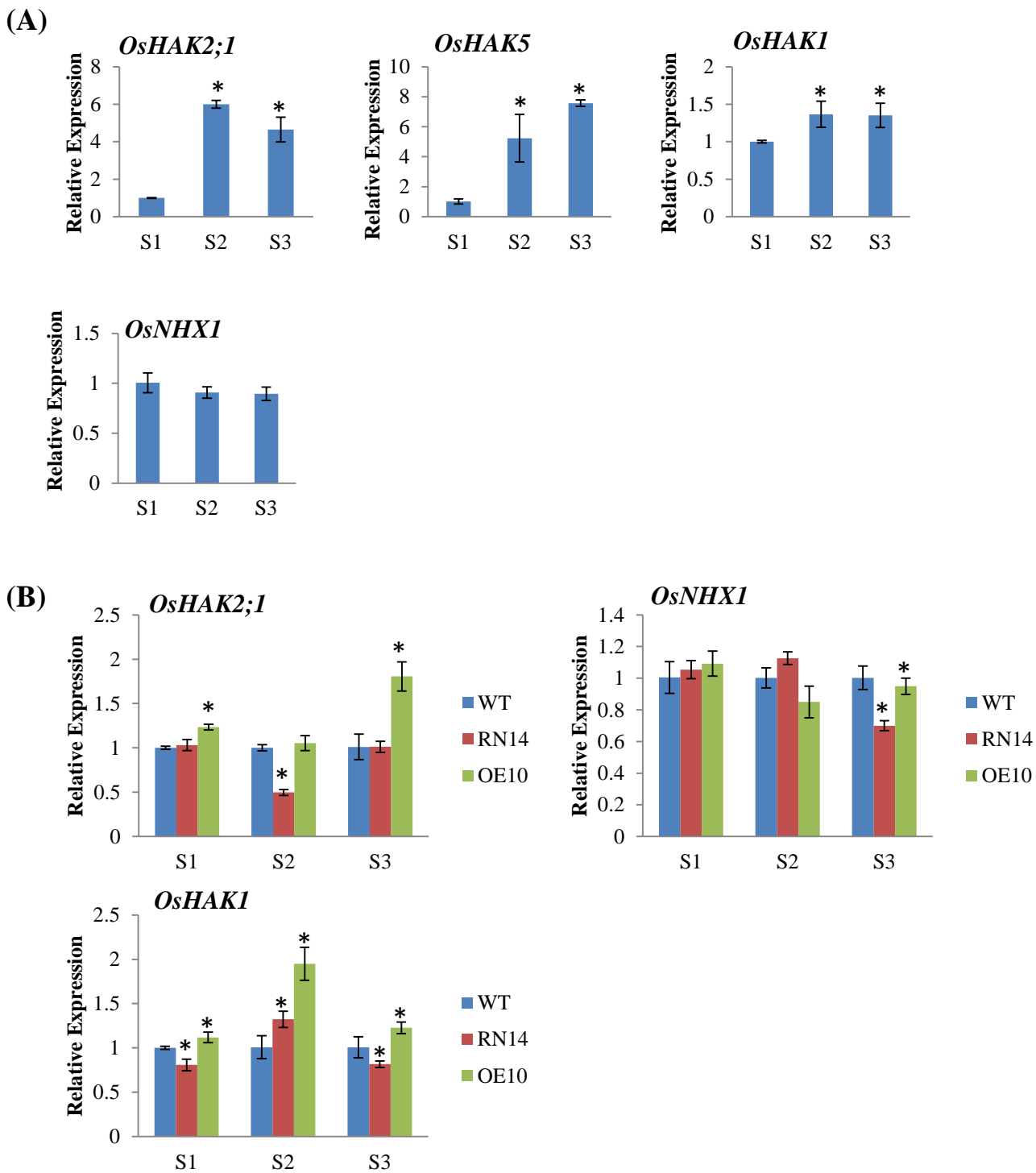


Figure S8. Overexpression of *OsJAZ9* enhances expression of K^+ transporters. (A) K^+ transporter encoding genes in WT under S2 (K deficiency, 15 days) and S3 (K recovery, 12 hrs) treatments with respect to S1 (K sufficient). **(B)** Relative expression levels of *OsHAK2;1*, *OsNHX1* and *OsHAK1* in *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).

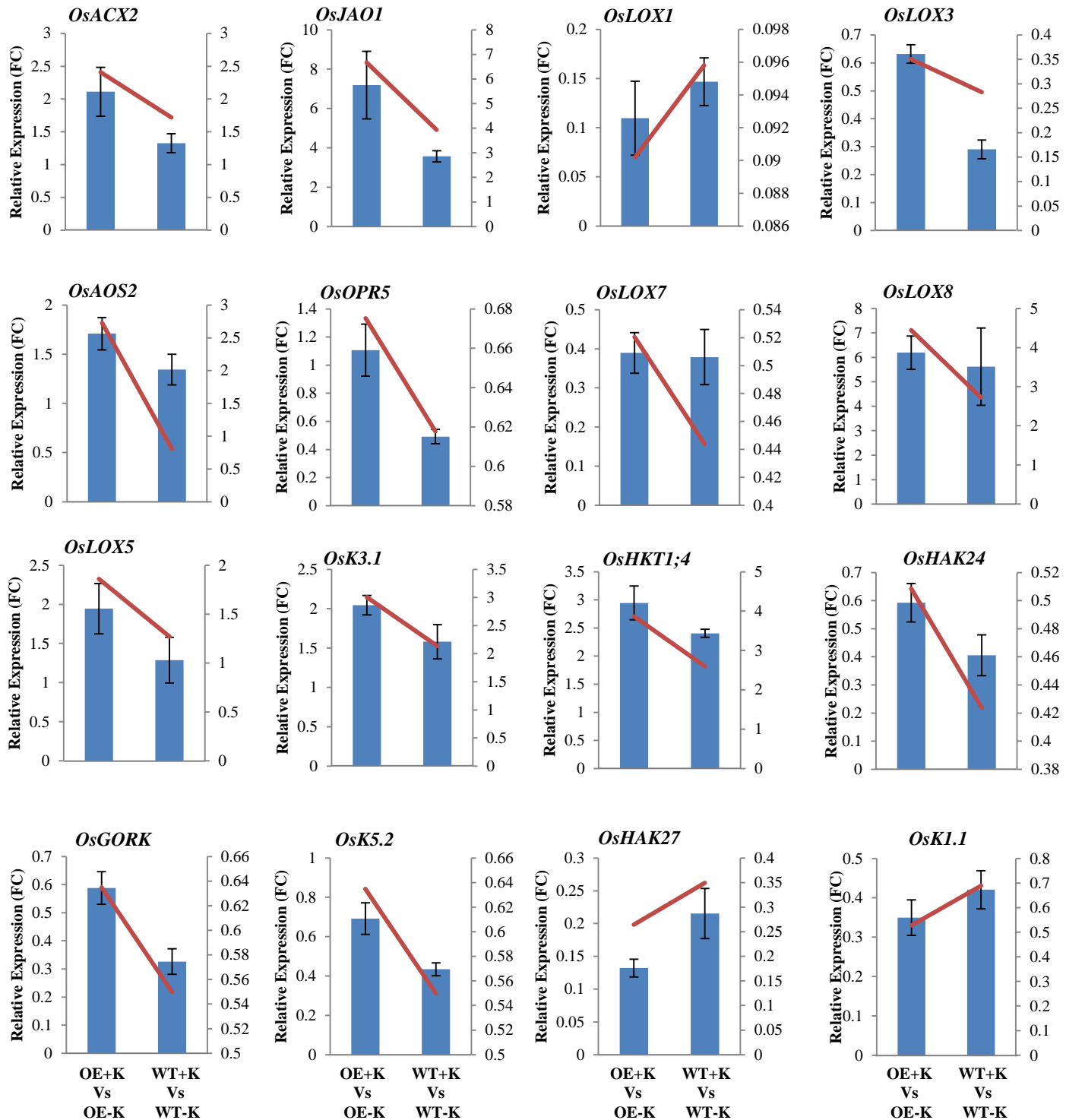


Figure S9. Validation of RNA-seq data with qRT-PCR. Relative expression levels (fold changes; FCs) of DEGs (shown in table S2 and S3) were analysed in WT and *OsJAZ9* OE line during K deficient conditions w.r.t their own controls. All expression levels were normalized with respect to endogenous control, *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.

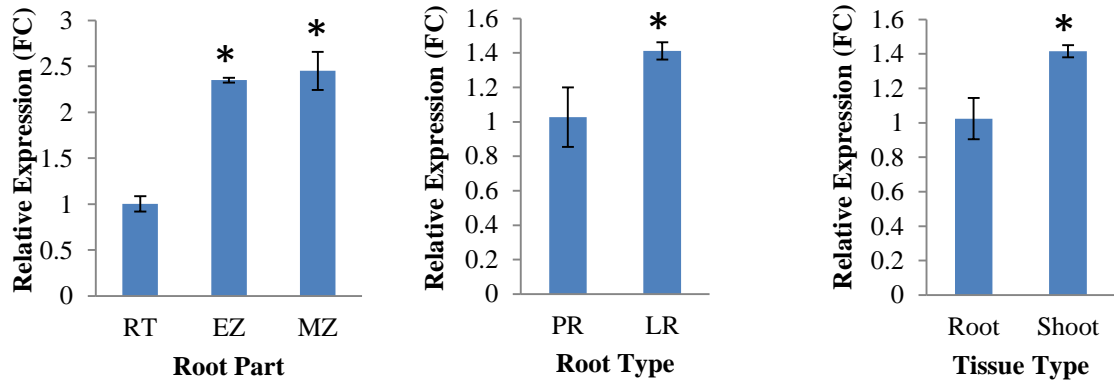


Figure S10. Tissue-specific expression of *OsJAZ9*. Expression of *OsJAZ9* shown by qRT-PCR analysis in different parts of root (Root Tip, RT; Elongation Zone, EZ; Maturation Zone, MZ), different root types (Primary Root, PR; Lateral Root, LR) and different tissues (Root and shoot). Each bar shows average 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, $p \leq 0.05$.

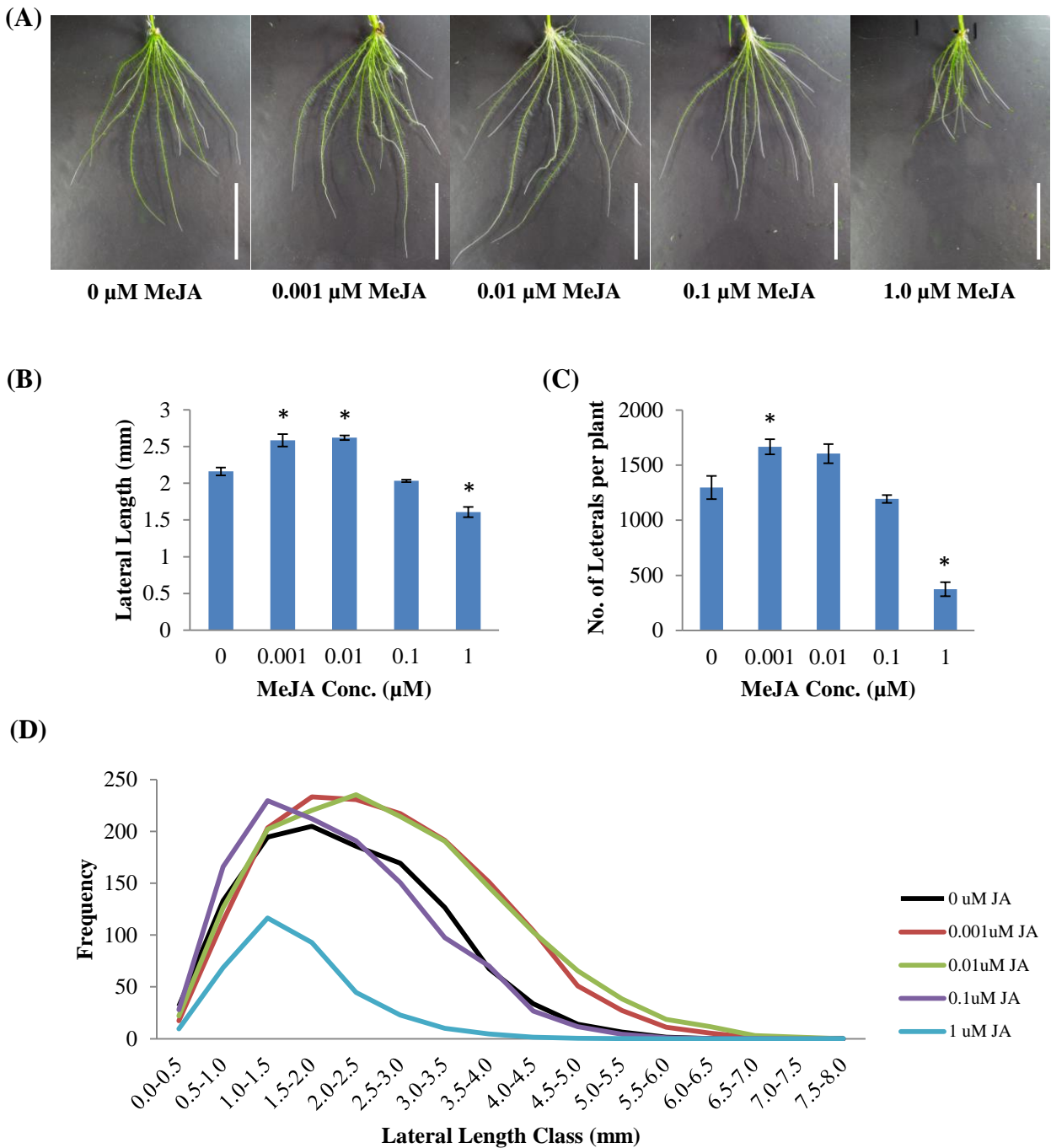


Figure S11. Exogenous application of MeJA modulates root system architecture under K sufficient conditions. (A) Representative image of 15-days-old WT plant roots grown with $510 \mu\text{M}$ K_2SO_4 and different concentrations of exogenous MeJA (0, 0.001, 0.01, 0.1 and $1 \mu\text{M}$). (B) Average 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.

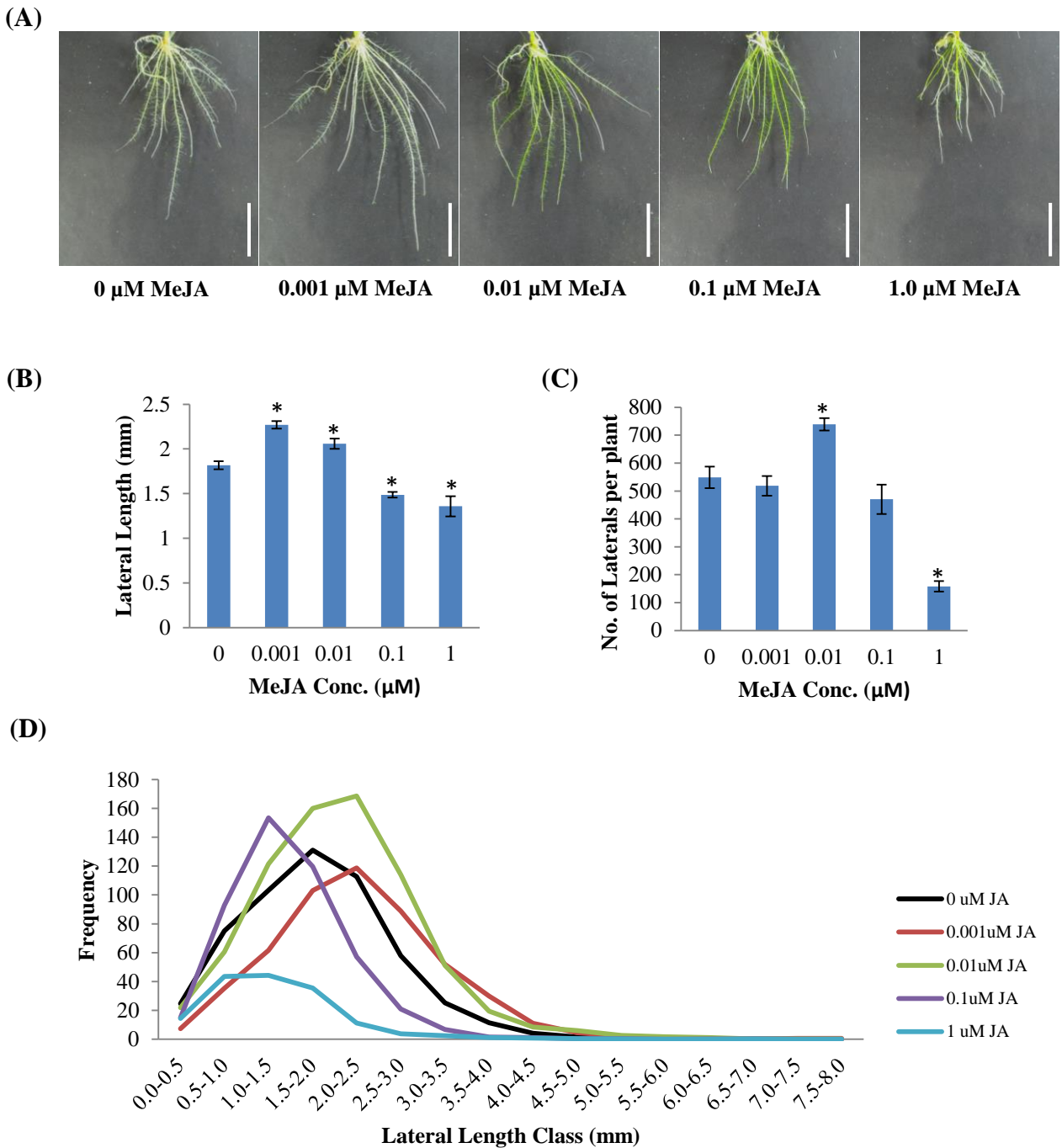


Figure S12. Exogenous application of MeJA modulates root system architecture under K deficient conditions. (A) Representative image of 15-days-old WT plant roots grown with 4.08 μM K_2SO_4 and different concentrations of exogenous MeJA (0, 0.001, 0.01, 0.1 and 1 μM). (B) Average lateral length, (C) Average 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)

pZmUbi:OsJAZ9



(B)

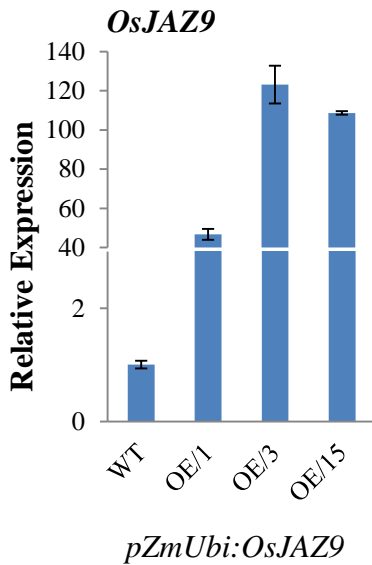


Figure S13. Raising and screening of *OsJAZ9* OE rice transgenic lines with *ZmUbi1* promoter. (A) Schematic of construct used for *OsJAZ9* overexpression. **(B)** Relative expression of *OsJAZ9* in overexpression lines. Gene expression analysis was done with qRT-PCR with at least three independent replicates.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

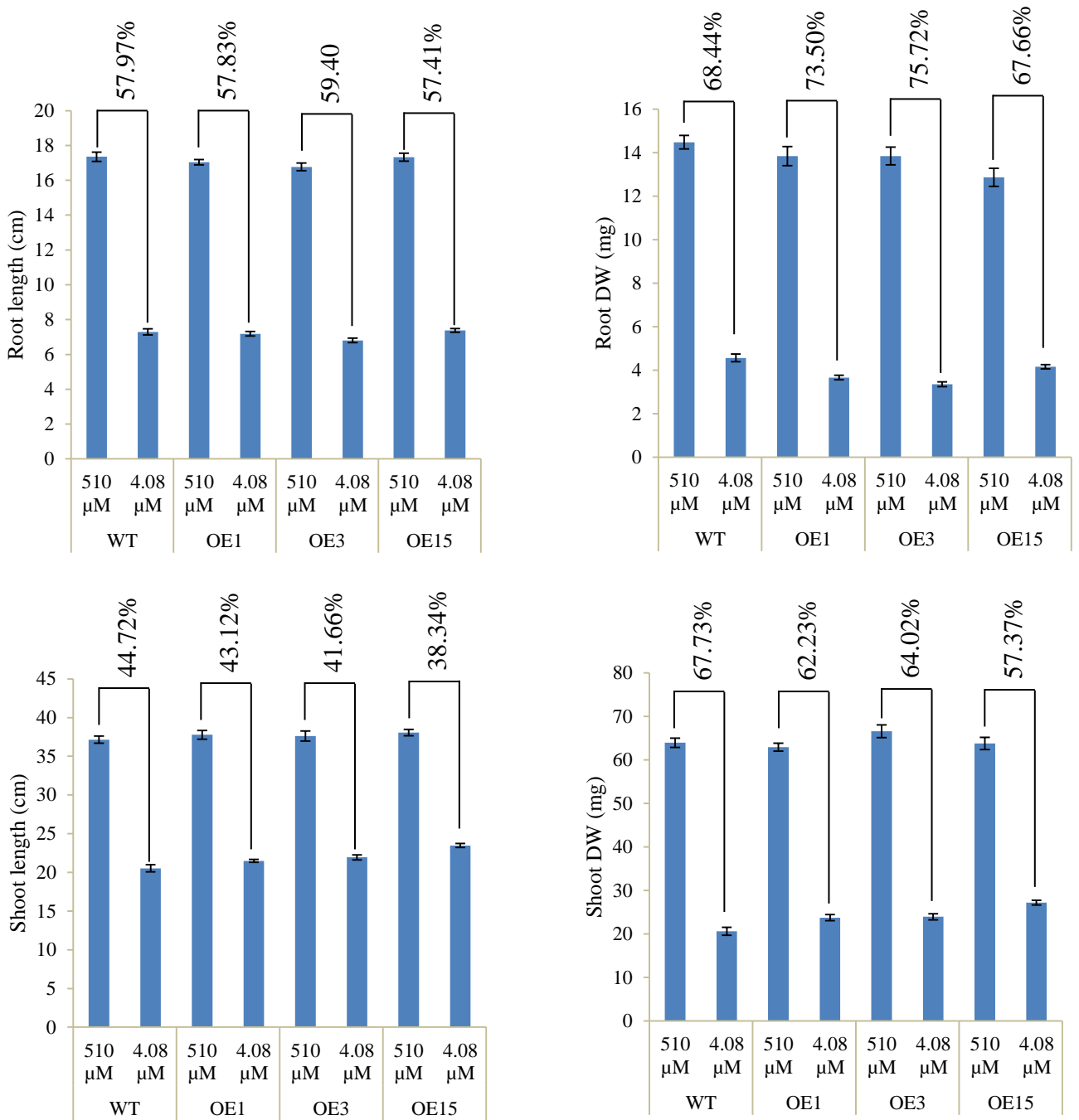


Figure S14. *OsJAZ9* is involved in K deficiency tolerance in rice. Growth parameters (length and dry biomass of root and shoot) of WT and *OsJAZ9* OE (OE1, OE3 and OE15) lines under K deficient and sufficient conditions. Each bar represents average of 12 seedlings with standard error while the values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

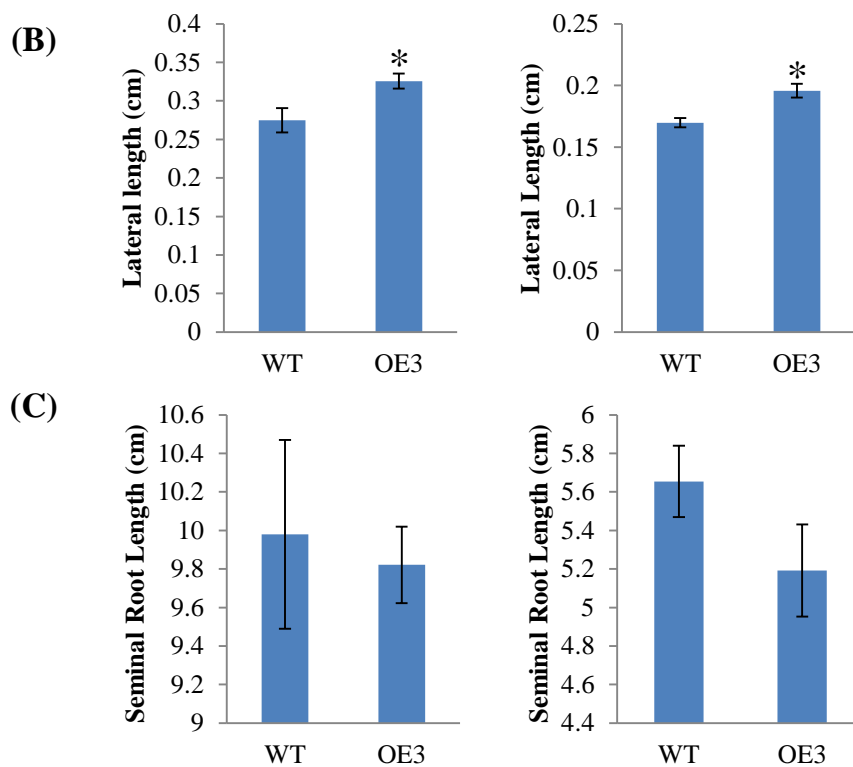
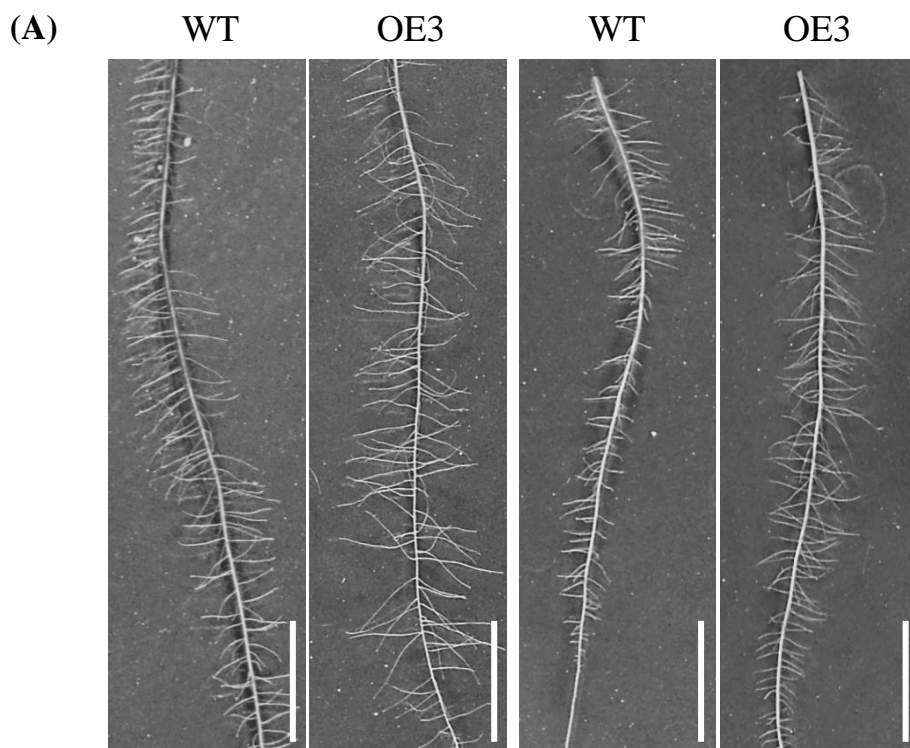


Figure S15. *OsJAZ9* overexpression influences root system architecture. (A) Representative root images of 12-days-old WT and *OsJAZ9* OE plants showing lateral root lengths during K sufficient (left panel) and K deficient conditions (right panel). (B) Average lateral root length and (C) Average seminal 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).

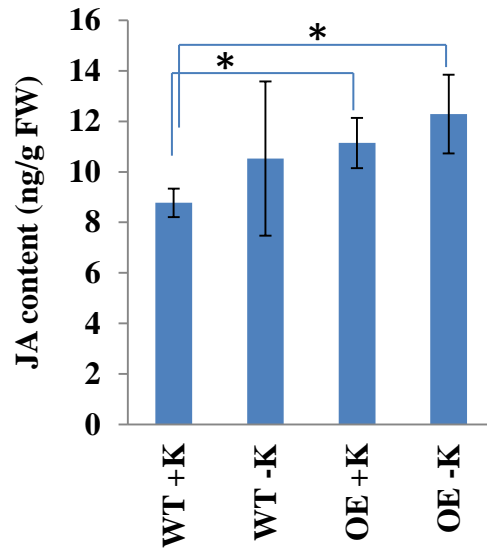


Figure S16. JA content in WT and *OsJAZ9* OE (OE/10) plants during normal (+K) and 15 days of K deficiency (-K) conditions. Bars represent the average change in JA content among at least four replicates. Error bar represents SE of all the replicates, asterisk marks the significant change with Student's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

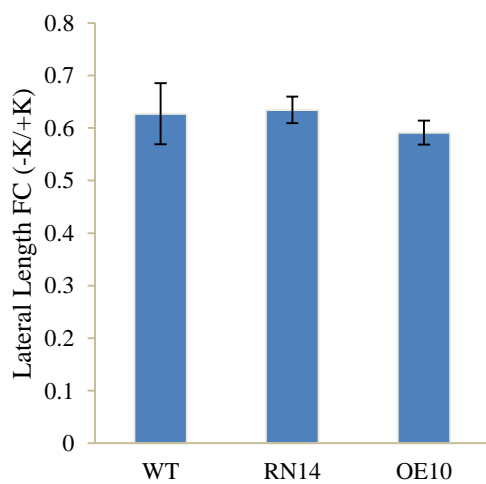


Figure X. *OsJAZ9* overexpression influences root system architecture. Average fold change in lateral root length of plants (WT, *OsJAZ9* RNAi and overexpression) grown under $-K/+K$ (K deficient/K sufficient) conditions. Four independent plants were used for the analysis. Error bar represents SE among 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	ACCGGCCATTTAGTTCTGT	CAGGTTCCGGTGCTTTCTCTTTC
qOsHKT1;4	TCCAGACCATCTCTGCACAGAT	GTTTGCCAGAGTCGCTCCAT
qOsJAO1	CCCGACGCCTTCATCGT	GTACATCGAGTTGCTCAAGATCTCA
qOsK5.2	CGTGGCCTACCGTGATCCT	GCGATGGAGGTGGGATTG
qOsLOX1	CATGCCGTCCAAGATGCA	GGAGTGCAGACAGGATGT
qOsLOX3	GCTGGTCTGAATCCCATGGT	TCAAGAGTACTTTTTGGTGGGAATT
qOsLOX5	GGGCCTCACGGTGCAA	TCGTGGTGGTCTAGGATGAAAA
qOsLOX7	CCGATATCTATGCCATTGGA	CCATCCCCCTCTTGATGAGA
qOsLOX8	GCAGCTCAGCGAGATGCA	CGTTGATCCGCATCGTGTAC
qOsOPR5	TGATGCGCCATTGAACAAGT	TCCAACAATTGGGTCTTGAGTGT
qOsK3.1	GGCATCCTCCGGCTATGG	TGATGTCCTTCTCCAGCCTTGT
qOsK1.1	TTGCGTTTGAATCGTACAGC	CCTTTACGACACCAGCCATT
qOsJAZ9	TCGACGGATCCCATGACC	GGCCTTGACGTACTGGCTG
OsJAZ9_RNAi	CGTACAGTACAGATCGTTCGCAAT	GGACAACATAAGCACAAAGCACAAA
qOsCOI1b	AGGATGCCGTGAACTGGAAT	TCCAGGGCCCCATTTGT
qOsMYC2	CTAGCGAGGAAACCCAATCG	CCATCCATCCATCCTAACAC
qOSAOS1	CGAGCTCTTCCTCCGATACG	GTCAGAAGGTGGCCTTCTTGAG
qOsAOS2	GGAGGAAGCTGCTGCAATAC	GGAGGTTGAAGCTTTGGTGA
OsJAR1	GGTTTGTGAACCCATCAAACAGC	CAAAATCCATAGGCAGTACTG
qOsHAK1	GTTGATGATGCTGATGTTGGAAG	CCAACACTTTCAGCTGAAAC
qOsHKT2;1	CACAGTCTCCTCGTTTGCAGAA	GCAAGAATCTGGCCGATGAA
qOsHAK5	CATTGTGGACTATTTTGAAAGAA	GGAGAACTACAGAAAAGCCAATC
qOsNHX1	TCCAGCCTCCGGATGCT	ATCAGCGCGTCGTCGAA
Primers used for cloning		
OsJAZ9_OE	CACCATCAGCGACGTACAGTACA	CGCCGCGATAACTAGGGTAAC
OsJAZ9_RNAi	CACCGACACATGCACTC	CAGAACAGTCGAAGGAATTTTA
OsJAZ9_GUS	TGCTTAAGATCTAATGGCGTCGACGGAT	TGCTTAAGATCTACGCGGAGTGCATGTG
OsJAZ9Δ_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	
	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
JA Biosynthesis genes	Os01g27230	<i>OPR10</i>	1.6846	1.8214	0.0005	0.0049	0.0002	0.0022	12-oxophytodienoate reductase, putative, expressed
	Os01g61610	<i>JAOI</i>	6.6732	3.9358	0.0002	0.0022	0.0001	0.0007	flavonol synthase/flavanone 3-hydroxylase, putative, expressed
JA Signaling Genes	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	
	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
JAZ Repressor	Os10g25230	<i>JAZ13</i>	0.4824	0.3100	0	0.3	0.02	0	ZIM domain containing protein, putative, expressed