Rice MEDIATOR25, OsMED25, is an essential subunit for jasmonate-mediated root development and OsMYC2-mediated leaf senescence

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Abstract

The Mediator multiprotein complex acts as a universal adaptor between transcription factors (TFs) and RNA polymerase II. MEDIATOR25 (MED25) has an important role in jasmonic acid (JA) signaling in Arabidopsis. However, no research has been conducted on the role of MED25 in JA signaling in rice, which is one of the most important food crops globally and is a model plant for molecular studies in other monocotyledonous species. In the present study, we isolated the loss-of function mutant of MED25, *osmed25*, through the map-based cloning and phenotypic complementation analysis by the introduction of *OsMED25* and investigated the role of OsMED25 in JA signaling in rice. The *osmed25* mutants had longer primary (seminal) roots than those of the wild-type (WT) and exhibited JA-insensitive phenotypes. S-type lateral root densities in *osmed25* mutants were lower than those in the WT, whereas L-type lateral root densities in *osmed25* mutants were higher than those in the WT. Furthermore, the *osmed25* mutants retarded JA-regulated leaf senescence under dark-induced senescence. Mutated osmed25 protein could not interact with OsMYC2, which is a positive TF in JA signaling in rice. The expression of JA-responsive senescence-associated genes was not upregulated in response to JA in the *osmed25* mutants. The results suggest that OsMED25 participates in JA-mediated root development and OsMYC2-mediated leaf senescence in rice.

Key words

Jasmonate · Leaf senescence · OsMYC2· Rice· Root

Introduction

Transcriptional regulation of gene expression is one of the most important processes in plant growth. Transcription factors (TFs) act as key regulators that induce or suppress the expression of genes in response to environmental stresses in plants. The expression and activation of TFs are strictly regulated by plant hormones such as jasmonic acid (JA) [1]. Numerous studies have demonstrated that JA plays an important role in plant growth and biotic/abiotic responses in rice [2]. Our recent studies have also revealed that JA participates in the dark-induced senescence of leaves [3,4,5] and defense responses by regulating induction of plant volatiles in rice [6,7,8,9,10].

Numerous TF families have been reported to be involved in JA-mediated stress responses in plants [1]. A basic helix-loop-helix (bHLH)-type TF, OsMYC2, acts as a positive regulator of JA signaling in chlorophyll degradation and leaf senescence [4, 11], and is regulated through interactions with several rice JASMONATE ZIM (JAZ)-domain proteins [11]. JAZ proteins block TF activity in the absence of bioactive JAs by recruiting general corepressors, including TOPLESS (TPL) and NOVEL INTERACTOR OF JAZ (NINJA) [12]. OsNINJA1 and the OsNINJA1-interacting protein, OsSRO1a, act as negative regulators in the OsMYC2-mediated leaf senescence in rice [3,5]. These results above indicate that OsMYC2 is strictly regulated through the formation of complexes with various types of regulatory proteins in response to JA.

TFs, including OsMYC2, do not directly interact with the general transcription machinery, including RNA polymerase II, during transcription. TFs bind to specific cis-acting sequences presented in the promoter regions of target genes through their DNA-binding domains, and recruit the Mediator multiprotein complex, which bind TFs to the RNA Pol II. The Mediator complex contains numerous subunits, and approximately 30 and 50 subunits have been found in the Arabidopsis and rice genomes, respectively [13]. Some Mediator complex subunits have important roles in plant growth, including abiotic and biotic stress responses [14,15,16,17,18,19]. Such subunits are structurally divided into three modules: the head, middle, and tail modules, which are known as the core Mediator [20]. The head module interacts with RNA Pol II and is involved in

basal transcription. The middle module connects the head and tail modules, and facilitates flexibility with regard to conformational changes in the Mediator complex during transcription. The tail module interacts with the TFs [21].

The MEDIATOR25 (MED25) subunit was first isolated as a regulator involved in the flowering process [22]. Since then, it has been demonstrated to have important roles in the other plant growth processes regulated by plant hormones, including JA [23]. Arabidopsis *atmed25* mutants exhibited a JA-insensitive phenotype and increased susceptibility to a necrotrophic pathogen, *Alternaria brassicicola* [17,24,25]. In wheat, the suppression of the expression of *TsMED25* induced resistance to a biotrophic pathogen, *Blumeria graminis* f. sp. *tritici* [26]. It has also been demonstrated that AtMED25 interacts with several TFs involved in JA signaling, including AtMYC2, and AtMED25 is required for the regulation of AtMYC2-responsive genes [24], which suggests that MED25 is associated with the tail module of the Mediator complex.

In the present study, we isolated a novel mutant that has a longer primary (seminal) root than that of the wild-type (WT), and identify OsMED25 as the causative gene. We investigated the role of OsMED25 in JA signaling using *osmed25* mutant, which is a first step toward the understanding of the role of OsMED25 in JA signaling in rice.

Materials and methods

Plant materials, growth conditions, and morphological characterization

The N2-64 mutant was obtained by mutagenizing *Oryza sativa* cv. Nipponbare using *N*-methyl-*N*-nitrosourea (MNU). The seeds of wild-type (WT), N2-64 mutant, and F_2 plants derived from crosses between the mutants and *Oryza sativa* cv. Kasalath were grown in tap water without nutrient supplementation in a growth chamber at 28°C under continuous light. Transgenic plants were grown in Murashige and Skoog (MS) medium [27] containing 3% (w/v) sucrose and 0.3% Gelrite. For phenotypic characterization, seedlings of the N2-64 mutant and its WT were grown for 20 days under the aforementioned growth conditions, and plant height and root phenotypic traits were evaluated at the end of the growth experiments.

Map-based cloning, plasmid constructs, and plant transformation

To map the causative gene of the N2-64 mutant, we performed a linkage analysis using F_2 plants derived from a cross between the mutant and *O. sativa* cv. Kasalath. For the complementation of the mutation and expression analysis of the causative gene, *OsMED25*, the WT genomic sequence of 'Nipponbare' was amplified in the region extending from approximately −3 kbp to +12 kbp (considering the *OsMED25* translation site as +1 bp) and was cloned into the pHGW vector to generate the *ProOsMED25:OsMED25* construct. To generate the *ProOsMED25:NSL-3×Venus* construct, we first prepared the plasmid vector pENTR-ProOsMED25:NSL-3×Venus by linking the *OsMED25* promoter sequence with a fusion gene encoding the nuclear localization signal and tandem triplicate of the fluorescent protein Venus. The ProOsMED25:NSL-3×Venus fragment was then transferred from pENTR into pGWB1 [28] using a Gateway LR reaction. The generated fusion constructs were introduced into the EHA105 strain of *Agrobacterium tumefaciens* via electroporation.

Subsequently, the *ProOsMED25:OsMED25* and *ProOsMED25:NSL-3×Venus* constructs were transformed into the *osmed25* mutant and WT plants, respectively, via *Agrobacterium*-mediated

transformation, as described previously [29,30]. Transgenic plants were selected on MS medium containing 50 mg L^{-1} hygromycin at 30°C.

Jasmonic acid treatment

To examine the effects of JA rice plants were grown up to the four-leaf stage in a growth chamber in Kimura-B liquid medium [31] at 25°C (24 h light) and then incubated in the same medium supplemented with 100 μM JA (Sigma-Aldrich, St. Louis, MO, USA). Root length and chlorophyll contents were measured 5 days after the JA treatment. The chlorophyll contents were determined using the method of Arnon (1949) [32]. The lateral root densities on seminal roots were measured 10 days after treatment with 0.1 μM JA.

Dark-induced senescence (DIS)

Leaf blades detached from three-week-old WT and *osmed25* mutant rice plants were incubated on 3 mM MES buffer (pH 5.8) and maintained at 25 °C for 4 days in complete darkness, with the abaxial side upwards, as described by Uji et al. (2017) [4]. The chlorophyll contents were determined using the method of Arnon (1949) [32].

Yeast two-hybrid system

The MATCHMAKER yeast two-hybrid system [Clontech (Takara Bio), Shiga, Japan] was and the yeast strain AH109 were used, as previously described by Kashihara et al. (2020) [5]. The cDNAs of *OsMYC2* (*Os10g42430*), *OsbHLH034* (*Os02g49480*), *OsNINJA1* (*Os05g48500*), *OsSRO1a* (*Os10g42710*), and *OsJAZs* were ligated into the pGADT7 vector. The cDNAs of *OsMED25* (*Os09g0306700*) and mutated *osmed25* were ligated into the pGBKT7 vector. The *OsJAZ* gene sequences has been reported previously by Ye et al. (2009) [33].

Bimolecular fluorescence complementation (BiFC) assay

We used the Kusabira Green (mKG) system (MBL, Nagoya, Japan) for the bimolecular fluorescence complementation (BiFC) assay as previously described by Onohata and Gomi (2020) [34]. To visualize the nuclei, rice histone H4 (Os10g39410) was fused with DsRed as previously described by Onohata and Gomi (2020) [34]. The constructed vectors were expressed in onion epidermal cells using a particle bombardment system (PDS-1000/He; BioRad, Hercules, USA) as described by Kim et al. (2009) [35]. We used a KEYENCE BIOREVO BZ-9000 microscope (KEYENCE, Osaka, Japan) to observe mKG and DsRed fluorescence. The conditions were similar to those previously described by Onohata and Gomi (2020) [34].

Reverse transcription-quantitative PCR (RT-qPCR)

The fourth leaf blades were used for RT-qPCR analyses. Total RNA was extracted from rice leaf blades using TRIzol (Invitrogen, Carlsbad, CA, USA). RT-qPCR was performed using TB Green *Premix Ex Taq* (Takara, Shiga, Japan) in a Thermal Cycler Dice TP850 System (Takara) according to the manufacturer's instructions. Analysis of the obtained data was performed according to Gomi et al. (2010) [36]. The sequences of the gene-specific primers used in RT-qPCR are listed in Table S1.

Results

Phenotypic characterization of N2-64 mutant

To enhance our understanding of the molecular mechanisms underlying the regulation of root system development, we screened a rice mutant line, N2-64, which exhibited enhanced root growth (Fig. 1A, B). Although there was no significant difference on plant height between WT and the mutant seedlings (Fig. 1E), both the seminal root lengths and total lateral root numbers on seminal roots in the mutant were approximately 50 % longer and higher than those in the WT (Fig. 1A, B, F and G). Conversely, because of greater seminal root lengths in the mutant, total lateral root density in the seminal roots were not significantly different from those in the WT (Fig. 1H). Rice lateral roots are classified into two distinct morphological types including the S-type (thin in

159 diameter and short) and the L-type (thick in diameter and long) [37,38]. Consequently, we evaluated
160 them separately and found that compared to the WT, S-type lateral root density in the mutants was
161 lower, whereas L-type lateral root density was higher in the mutants than in the WT (Fig. 1I, J).
162
163 **Isolation of the causative gene of the N2-64 mutant and its expression patterns in roots**
164 To map the causative gene of the N2-64 mutant, an F_2 population was generated by crossing
165 the mutant (derived from Nipponbare, a *japonica* variety) with WT Kasalath (an *indica* variety).
166 Seedlings displayed a well-developed root system among the progeny segregated into a 3:1
167 WT:mutant ratio, indicating that the mutant phenotype is caused by a single recessive gene. We
168 employed a map-based cloning approach using the seedlings to isolate the causal gene, and identified
169 a locus on chromosome 9, located in an approximately 83-kb region between the molecular markers
170 RM23946 and RM23950 (Fig. 2A). Within the region, we detected a single nucleotide substitution
171 from guanine to adenine, which resulted in a single amino acid substitution from glycine to glutamic
172 acid, in the 10th exon of an open reading frame (ORF), Os09g0306700 (Fig. 2B). Subsequently, we
173 introduced the WT candidate gene under the control of its promoter into the N2-64 mutant. We
174 succeeded in producing more than 10 transgenic plants and found that all of them lacked a developed
175 root phenotype (Fig. 2D), whereas regenerated N2-64 mutant plants without the construct were
176 characterized by well-developed roots (Fig. 2C). Accordingly, we concluded that the root phenotype
177 of the N2-64 mutant is caused by a mutation in the Os09g0306700 ORF.
178 The causative gene encodes OsMED25, a member of the Mediator complex, which is
179 known to interact with the TFs and to recruit RNA polymerase II for the transcription of important
180 genes. Consequently, OsMED25 is a key regulator of several plant processes [39,40]. Hereafter, we
181 named the N2-64 mutant as *osmed25*. The mutation site is in the Activator Interacting Domain
182 (ACID) protein domain (Fig. 2B), which allows the gene to interact with the TFs, suggesting that the
183 *osmed25* mutant could not interact with the TFs, giving rise to the loss-of-function *osmed25* mutant.
184 We examined the expression patterns of *OsMED25* in WT roots using Venus fluorescence

controlled by the *OsMED25* promoter. We observed fluorescence in regions of the crown root tip (Fig. 2F). In addition, the fluorescence was observed in the lateral root tip and its stele region at the site of lateral root emergence (Fig. 2G, H). In contrast, we did not observed Venus fluorescence in the crown root tip of transformants carrying no *ProOsMED25:NLS-3×Venus* construct, which we used as the negative control (Fig. 2E).

It has been demonstrated that AtMED25 has an important role in the root growth including lateral root growth under the regulation of JA [23,41,42]. Consequently, to investigate whether the aberrant lateral root development in *osmed25* mutant is caused by defect in OsMED25-mediated JA signaling, we observed lateral root formation following JA treatment in WT. Both total and S-type lateral root densities were increased significantly by JA treatment (Fig. 3A, B, D). Conversely, significant reductions were observed in the case of L-type lateral root densities (Fig. 3C, D). In addition, we also observed similar tendency in the transgenic plants of N2-64 introduced the *OsMED25* under the control of its promoter (Supplementary Fig. 1). Such phenotypes in the WT were completely opposite those in the *osmed25* mutant.

JA-related phenotypes of *osmed25* mutant

We first investigated root growth inhibition in response to JA. Root growth was not significantly inhibited by JA treatment in *osmed25* mutants (Fig. 4A). Subsequently, we measured the chlorophyll contents following treatment with JA because JA promotes chlorophyll reduction in rice [43]. The levels of chlorophyll did not decrease significantly in *osmed25* mutants following treatment with JA, whereas those of the WT plants were decreased significantly (Fig. 4B). We have also demonstrated that OsMYC2-mediated JA signaling has an important role in the regulation of leaf senescence under the DIS conditions [3,4,5]. To investigate whether OsMED25 is involved in the leaf senescence, we compared the levels of chlorophyll in the WT plants and the *osmed25* mutants under DIS conditions. The detached leaf blades of the *osmed25* mutants had higher chlorophyll contents than those of the WT plants after four days under the DIS conditions (Fig. 4C,

211 D).

212

213 **Analysis of OsMED25-interacting proteins**

214 The mutation of *osmed25* was a single nucleotide substitution, guanine to adenine, which
215 resulted in a single amino acid substitution, glycine to glutamic acid, in the ACID domain of
216 OsMED25. Although the ACID region is required for the interactions with TFs such as AtMYC2 in
217 Arabidopsis [23], there is no data demonstrating that the ACID region in OsMED25 is required for
218 the interaction with rice TFs, including OsMYC2. Consequently, we investigated interaction between
219 OsMED25 and OsMYC2 in yeast and plant cells. We first confirmed interaction between intact
220 OsMED25 and OsMYC2 in both yeast and plant cells (Fig. 5A, B). However, mutated OsMED25
221 could not interact with OsMYC2 in both yeast and plant cells (Fig. 5A, B). We have previously
222 demonstrated that another bHLH-type TF, OsbHLH034, is involved in the JA signaling in rice [34].
223 OsMED25 did not interact with OsbHLH034 in yeast cells (Fig. 5A).

224 Subsequently, we investigated whether OsMED25 also interacts with OsJAZs and the other
225 OsMYC2-regulating proteins, OsNINJA1 and OsSRO1a [3,5]. OsMED25 strongly interacted with
226 OsJAZ4, OsJAZ9, OsJAZ11, OsJAZ12, and OsNINJA1, and weakly interacted with OsJAZ3,
227 OsJAZ7, OsJAZ8, and OsJAZ10 in yeast cells (Fig. 5A). We confirmed that OsMED25 interacted
228 with OsNINJA1 and OsJAZ9 in plant cells (Fig. 5B). All proteins tested could not interact with
229 mutated OsMED25 in yeast cells (Fig. 5A).

230

231 **Expression of JA-responsive *SAG*s in *osmed25* mutant**

232 OsMYC2-responsive senescence-associated genes (SAGs) have been identified in our
233 previous study [4,5]. In addition, an OsMYC2-independent JA-responsive SAG, OsNAP
234 (Os03g21060), which is a NAC-type TF involved in the leaf senescence, has been identified in rice
235 [4,44]. Therefore, we compared the expression of two OsMYC2-responsive *SAG*s, *OsSAG12*
236 (*Os04g13140*) and *similar to SAG* (*Os02g01220*), and the *OsNAP* in WT and *osmed25* mutants. The

levels of expression of all the *SAG*s in the *osmed25* mutants were significantly lower than those in the WT after JA treatment (Fig. 6).

Discussion

In Arabidopsis, *atmed25* mutants have longer primary roots and higher lateral root density than WT as well as *osmed25* rice mutants of rice [45]. However, *atmed25* mutants do not exhibit high JA-insensitivity in term of root growth in response to JA [25,45], whereas *osmed25* mutants exhibit a significantly JA-insensitive phenotype, including resistance to the JA-induced root growth inhibition, and inhibition of the JA- and dark-induced chlorophyll degradation. The results suggest that MED25-mediated JA signaling is slightly different between rice and Arabidopsis, which was supported by the results of responses to JA treatment based on the lateral root density; that is, JA signaling was reduced in Arabidopsis, while it was increased in rice [41,42,46].

As mentioned previously in the present paper, rice lateral roots are classified into S-type and L-type lateral roots [37,38]. Compared to the WT, S-type lateral root density was lower in the *osmed25* mutant, whereas L-type lateral root density was higher, indicating that OsMED25 induces S-type lateral root formation and suppresses L-type lateral root formation through the regulation of JA signaling. Recently, we have reported that high auxin concentrations are required for the formation of L-type lateral roots [47]. However, the detailed mechanisms via which auxins regulate of the formation of different types of lateral roots, and whether other hormones are also involved in the regulation mechanisms remain unknown. The findings in the present, for the first time, shed light into the mechanisms of regulation of lateral root formation via JA signaling in rice.

In the present study, we also revealed that OsMED25 interacts with OsMYC2. OsMYC2 interacts with all OsJAZ proteins, excluding OsJAZ14 [11]; however, OsMED25 selectively interacted with some OsJAZ proteins, suggesting that OsMED25 could be involved in the regulation of a part of OsMYC2/OsJAZs-mediating JA responses in rice. OsMYC2 acts as a positive regulator

of the JA-mediated leaf senescence by regulating the expression of JA-responsive *SAG*s in rice [4]. In the present study, we revealed that loss of function of OsMED25 significantly downregulated the expression of OsMYC2-responsive *SAG*s in response to JA, suggesting that OsMED25 is required for the expression of genes regulating by OsMYC2-mediated leaf senescence in rice. Furthermore, OsMED25 is involved in the regulation of an OsMYC2-independent JA-responsive SAG, suggesting that OsMED25 interacts with other uncharacterized TF(s) and is a key factor regulating leaf senescence via several JA signaling activities in rice. To the best of our knowledge, this is the first study to demonstrate the involvement of MED25 in JA-mediated leaf senescence in plants, particularly rice.

OsMED25 contains a von Willebrand factor type A domain, a non-conserved middle domain, an ACID, and a glutamine-rich domain (GD) as well as Arabidopsis AtMED25 [48]. In Arabidopsis, the C-terminal GD is necessary for AtMED25 interaction with the AtJAZs [49,50]. However, we demonstrated that single amino acid substitution, glycine to glutamic acid, in ACID of OsMED25 inhibited the formation of complexes with OsMYC2 and OsMYC2-regulating proteins such as OsJAZs and OsNINJA1. We have no experimental data to ascertain whether the mutation in the ACID domain causes a conformational change in the entire OsMED25 structure or the ACID domain only. Further studies, such as a three-dimensional analysis of the structures of OsMED25 and mutated osmed25, are required to clarify the nature of the interaction between OsMED25 and OsMYC2-regulating proteins in rice.

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Author Contributions

YI and KG designed the research project. GS, NL-A, KK, and YF performed the experiments. GS and NL-A wrote the manuscript. All of the authors reviewed and approved the manuscript.

Compliance with Ethical Standards

This article does not contain any studies with human participants or animals performed by any of the authors.

Conflicts of interest

The authors declare that they have no conflict of interest.

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Figure legends

Fig. 1. Phenotypic characterization of N2-64 mutant.

(A, B) Root phenotype of the wild-type (WT) (A) and N2-64 mutant (B) 20 days after germination. SR, seminal root; CR, crown root. Scale bars = 3 cm. (C, D) Close-up of the seminal roots and lateral roots in the WT (C) and N2-64 mutant (D). LR, lateral root. Scale bars = 1 cm. (E–J) Shoot and root traits measured 20 days after germination. Values represent means ± SE. * and ** indicate statistically significant differences at $P<0.05$ and $P<0.01$, respectively, in the means between genotypes as revealed by the Student's *t*-test.

Fig. 2. Map-based cloning of the causative gene locus of N2-64 mutant, the root phenotypic complementation, and expression analysis of the causative gene, *OsMED25*.

(A) High resolution linkage and physical map of the causative gene locus of the N2-64 mutant and the structure of the *OsMED25* on chromosome 9. The vertical bars represent molecular markers and the number of recombinant plants indicated above and below the linkage map, respectively. The black box underneath denotes the putative open reading frame for *OsMED25* (*Os09g0306700*). Black boxes and horizontal lines indicate the exon and intron, respectively. (B) The mutation site at the 10^{th} exon showing a single base substitution difference between WT and mutant. (C, D) Regenerated plants of N2-64 mutant with the vector (C) and *ProOsMED25:OsMED25* construct (D). Scale bars = 3 cm. (E–H) Localized Venus signal of *OsMED25:NLS-3×Venus* in crown root tip (F) and emerging lateral roots (G, H) from crown roots. (E) Expression pattern of negative control in crown root tip. Scale bars = 100 μm.

Fig. 3. Effects of Jasmonic acid (JA) treatments on WT roots.

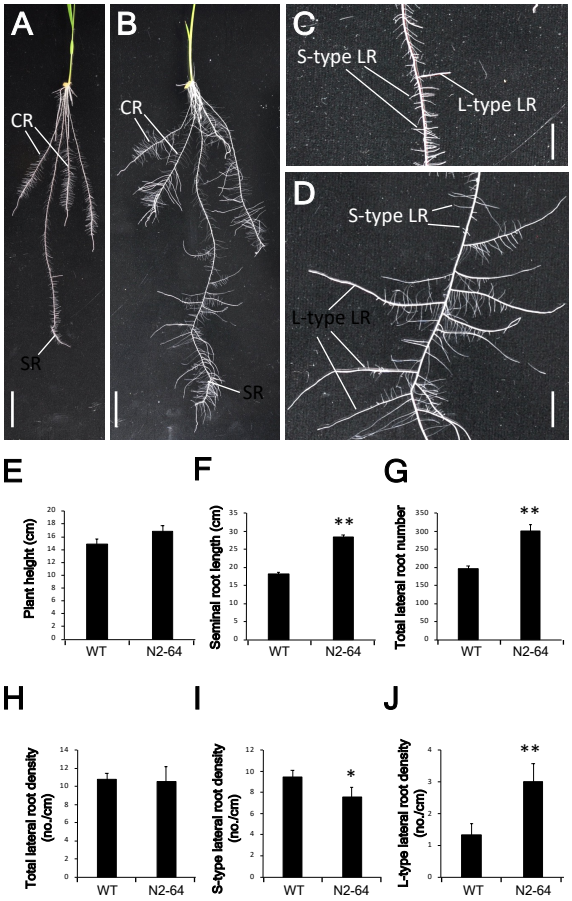
(A-C) Lateral root density of WT 10 days after germination grown in the absence or presence of 0.1 μM JA. Values represent means ± SE. * and ** indicate statistically significant differences at $P<0.05$ and $P<0.01$, respectively, in the means between genotypes, based on the Student's *t*-test. (D) Root

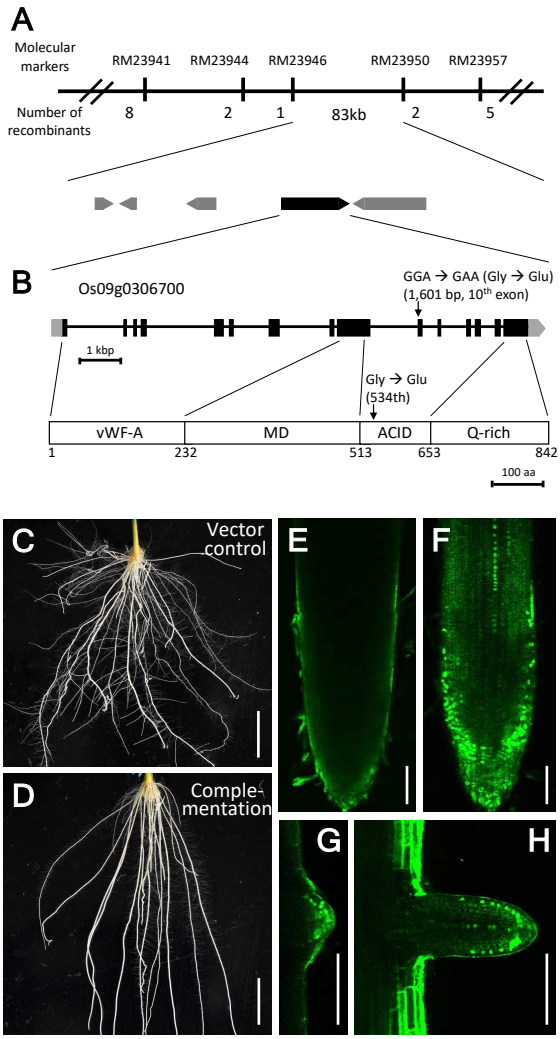
501 phenotypes of WT 10 days after germination in the absence (left) or presence (right) of 0.1 μM JA.
502 Arrowheads indicate L-type lateral roots. Scale bars = 1 cm.
503
504 **Fig. 4.** JA-related phenotypes of *osmed25* mutants. (A) Length of roots after 5 days of growth in the
505 absence or presence of 100 μM JA. Data were analyzed using the Tukey-Kramer test (n = 3 for both
506 WT Mock and WT JA; n = 7 for both *osmed25* Mock and *osmed25* JA). Values are means ± SE.
507 Means accompanied by different letters are significantly different at $P < 0.05$. (B) Total chlorophyll
508 contents in leaf blades after treatment with 100 μM JA for 5 days in WT and *osmed25* mutants. Data
509 were analyzed using the Tukey-Kramer test (n = 4 for both WT Mock and WT JA; n = 8 for both
510 *osmed25* Mock and *osmed25* JA). Values are means ± SE. Means accompanied by different letters
511 are significantly different at $P < 0.05$. (C) Photographs of leaf blades after incubation for 4 days
512 under dark-induced senescence (DIS) conditions in the WT and *osmed25* mutants. Scale bars = 10
513 mm. (D) Total chlorophyll contents in leaf blades after incubation for 4 days under DIS conditions in
514 the WT and *osmed25* mutants. Values are means ± SE of 6 replicates. Data were analyzed using the
515 Student's *t*-test. Asterisks indicate significant difference at $P < 0.05$.
516
517 **Fig. 5.** Interaction of OsMED25 with OsMYC2 and OsMYC2-regulating proteins. (A) Interaction of
518 OsMED25 with OsMYC2 and OsMYC2-regulating proteins in yeast cells. The yeast strain AH109
519 carrying each construct was dropped on synthetic dropout (SD) glucose medium lacking Leu and Trp
520 (-2) or on SD glucose medium lacking Ade, His, Leu, and Trp (-4). Images were obtained 3 days
521 after dropping. (B) Interaction of OsMED25 with OsMYC2 and OsMYC2-regulating proteins in
522 plant cells. From left to right, the images shown are: KG, fluorescence images of KG protein; DsRed,
523 fluorescence images of DsRed protein; Merge, overlap KG images and DsRed images; Bright,
524 light-microscopy images. Scale bars = 100 μm.
525
526 **Fig. 6.** Expression of the JA-responsive *SAG*s in *osmed25* mutants. RT-qPCR analysis of

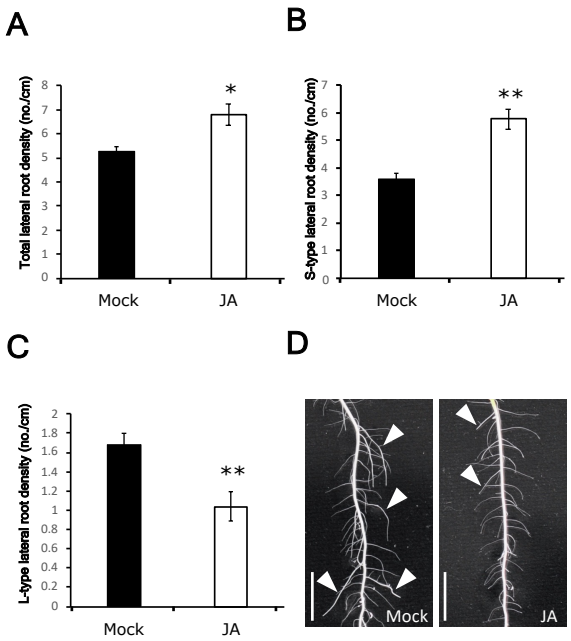
JA-responsive *SAG*s in the WT and *osmed25* mutants. Values are means ± SE. Data were analyzed using the Tukey-Kramer test (n = 4 for WT Mock, n = 5 for WT JA, *osmed25* Mock and *osmed25* JA). Means accompanied by different letters are significantly difference at $P < 0.05$.

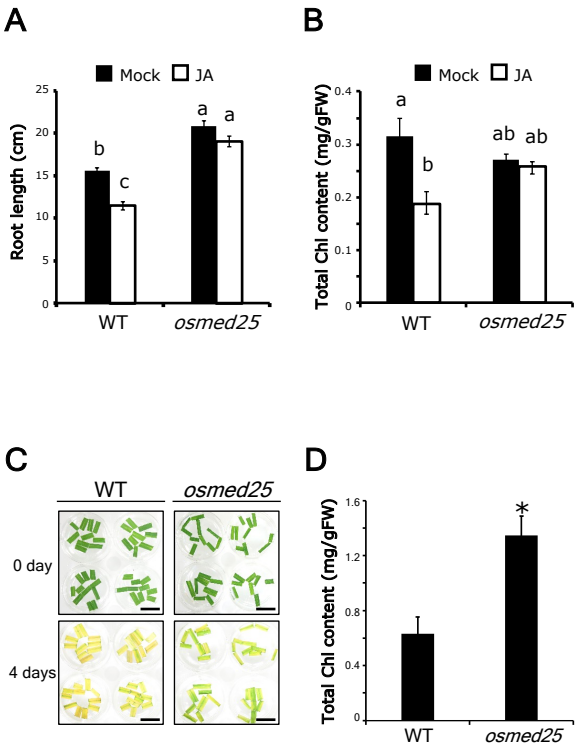
Supplementary Fig. 1. Effects of Jasmonic acid (JA) treatments on transgenic plant roots of N2-64 introduced the *OsMED25* under the control of its promoter.

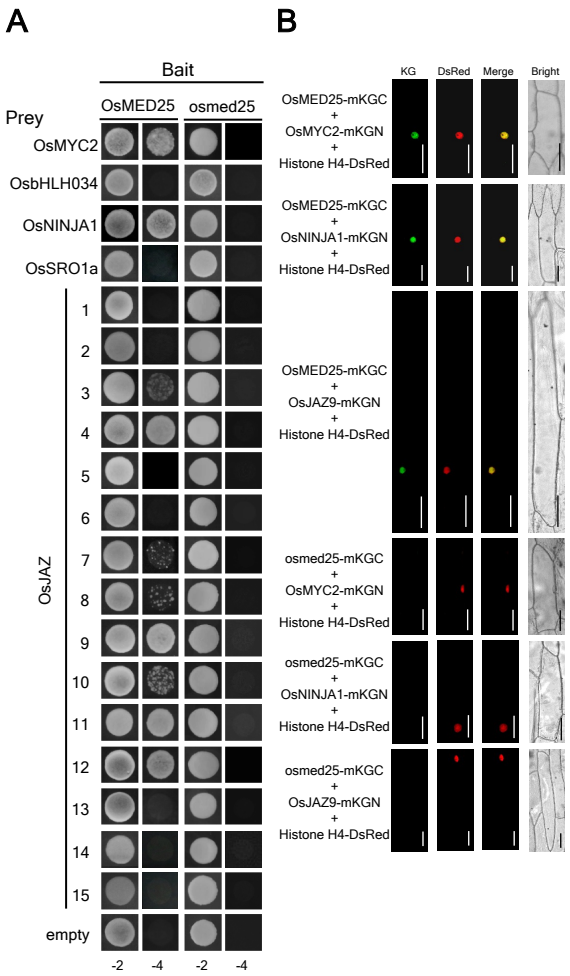
(A-C) Lateral root density of 10 days after germination grown in the absence or presence of 0.1 μM JA. Values represent means ± SE. * and ** indicate statistically significant differences at $P<0.05$ and $P<0.01$, respectively, in the means between genotypes, based on the Student's *t*-test.

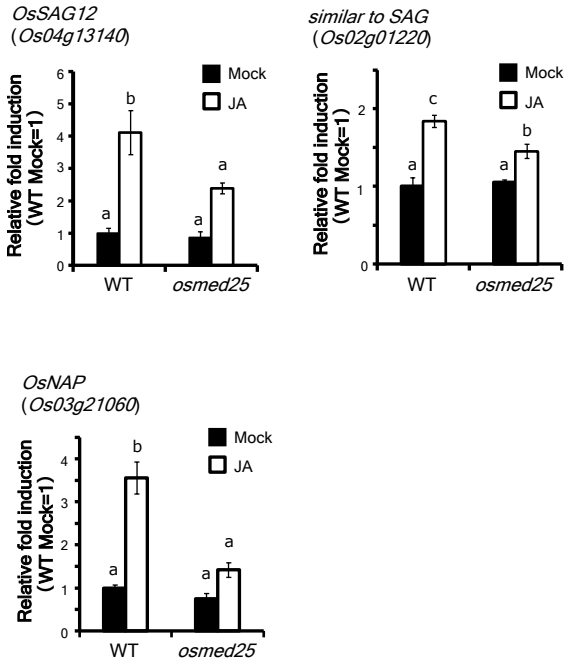

A
CR
SR
B
CR
SR
C
S-type LR
L-type LR
D
S-type LR
L-type LR
E
Plant height (cm)
WT
N2-64
F
Seminal root length (cm)
**
WT
N2-64
G
Total lateral root number
**
WT
N2-64
H
Total lateral root density (no./cm)
WT
N2-64
I
S-type lateral root density (no./cm)
*
WT
N2-64
J
L-type lateral root density (no./cm)
**
WT
N2-64


A
Molecular markers
RM23941
RM23944
RM23946
RM23950
RM23957
Number of recombinants
8
2
1
83kb
2
5
B
Os09g0306700
GGA → GAA (Gly → Glu)
(1,601 bp, 10th exon)
1 kbp
Gly → Glu
(534th)
vWF-A
MD
ACID
Q-rich
1
232
513
653
842
100 aa
C
Vector control
D
Comple-mentation
E
F
G
H


A
B
Total lateral root density (no./cm)
S-type lateral root density (no./cm)
*
**
Mock
JA
C
D
L-type lateral root density (no./cm)
**
Mock
JA


A
Mock
JA
Root length (cm)
25
20
15
10
5
0
b
c
a
a
WT
osmed25
B
Mock
JA
Total Chl content (mg/gFW)
0.4
0.3
0.2
0.1
0
a
b
ab
ab
WT
osmed25
C
WT
osmed25
0 day
4 days
D
Total Chl content (mg/gFW)
1.6
1.2
0.8
0.4
0
*
WT
osmed25


A
Bait
OsMED25
osmed25
Prey
OsMYC2
OsbHLH034
OsNINJA1
OsSRO1a
OsJAZ
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
empty
-2
-4
-2
-4
B
KG
DsRed
Merge
Bright
OsMED25-mKGC + OsMYC2-mKGN + Histone H4-DsRed
OsMED25-mKGC + OsNINJA1-mKGN + Histone H4-DsRed
OsMED25-mKGC + OsJAZ9-mKGN + Histone H4-DsRed
osmed25-mKGC + OsMYC2-mKGN + Histone H4-DsRed
osmed25-mKGC + OsNINJA1-mKGN + Histone H4-DsRed
osmed25-mKGC + OsJAZ9-mKGN + Histone H4-DsRed


OsSAG12
(Os04g13140)
Relative fold induction
(WT Mock=1)
Mock
JA
WT
osmed25
similar to SAG
(Os02g01220)
OsNAP
(Os03g21060)