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RESEARCH REPORT

The OsJAZ1 degron modulates jasmonate signaling sensitivity during rice development

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ABSTRACT

Jasmonates (JAs) are crucial to the coordination of plant stress responses and development. JA signaling depends on JASMONATE-ZIM DOMAIN (JAZ) proteins that are destroyed by the SCF^{COI1}-mediated 26S proteasome when the JAZ co-receptor COI1 binds active JA or the JA-mimicking phytotoxin coronatine (COR). JAZ degradation releases JAZ-interacting transcription factors that can execute stress and growth responses. The JAZ proteins typically contain Jas motifs that undergo conformational changes during JA signal transduction and that are important for the JAZ-COI1 interaction and JAZ protein degradation. However, how alterations in the Jas motif and, in particular, the JAZ degron part of the motif, influence protein stability and plant development have not been well explored. To clarify this issue, we performed bioassays and genetic experiments to uncover the function of the OsJAZ1 degron in rice JA signaling. We found that substitution or deletion of core segments of the degron altered the OsJAZ1-OsCOI1b interaction in a COR-dependent manner. We show that these altered interactions function as a regulator for JA signaling during flower and root development. Our study therefore expands our understanding of how the JAZ degron functions, and provides the means to change the sensitivity and specificity of JA signaling in rice.

KEY WORDS: Jasmonates, OsJAZ1, Degron, Sensitivity, Flower, *Oryza sativa*

INTRODUCTION

Jasmonates (JAs) are oxylipin phytohormones that are crucial to the coordination of plant stress responses and development. How JA specifically regulates different environmental and/or developmental cues, and manages to modulate multiple signaling outputs in specific cell types or organs, is however not well understood. Studies in the model eudicot plant *Arabidopsis* have revealed that the central JA signaling pathway is composed of CORONATINE INSENSITIVE1 (COI1)-JASMONATE-ZIM DOMAIN (JAZ) co-receptors, JAZ-interacting transcription factors (TFs) and other regulatory modules that balance JA signaling input and output responses (Browse, 2009; Howe et al., 2018; Wasternack and Hause, 2013). This signaling cascade appears to be conserved in land plants (Chini et al., 2016; Han, 2017; Howe et al., 2018; Monte et al., 2018). Upon JA binding, the JAZ proteins are destroyed by SKP1-CUL1-F-box protein $(SCF)^{COI1}$-mediated 26S proteasome, which releases JAZ-interacting TFs, such as MYC and MYB family members, and other TFs, to drive gene expression and thus change growth responses (Chico et al., 2008; Howe et al., 2018; Pauwels and Goossens, 2011; Wager and Browse, 2012). Hence, changes to JAZ protein stability and/or protein variants affect plant development (Cai et al., 2014; Chini et al., 2007; Chung et al., 2010; Chung and Howe, 2009; Gimenez-Ibanez et al., 2017; Hori et al., 2014; Hu et al., 2016; Huang et al., 2017; Oh et al., 2013; Song et al., 2011; Thines et al., 2007; Thireault et al., 2015; Yang et al., 2012; Yu et al., 2016; Zhai et al., 2015).

In the *Arabidopsis* genome, there are 12 JAZ genes belonging to the family of TIFY proteins, which are characterized by two conserved motifs (Chini et al., 2016; Howe et al., 2018; Pauwels and Goossens, 2011): a TIF[F/Y]XG motif [also called the zinc-finger protein expressed in the inflorescence meristem (ZIM) motif] in the central portion of the protein (Pauwels et al., 2010; Thireault et al., 2015); and a JA-associated (Jas) motif that is particularly important for controlling SCF^{COI1}-dependent stability, TF interactions and nuclear localization of the JAZ proteins (Chini et al., 2007; Howe, 2018; Melotto et al., 2008; Sheard et al., 2010; Thines et al., 2007; Withers et al., 2012; Yan et al., 2007). Some JAZ proteins also contain cryptic MYC-interaction domain/N-terminal (CMID/NT) motifs that are involved in interactions with other proteins (Goossens et al., 2015; Hou et al., 2010; Moreno et al., 2013; Zhai et al., 2015; Zhang et al., 2017), and ethylene-responsive element binding factor-associated amphiphilic repression (EAR) motifs that repress TF activity (Shyu et al., 2012).

Bioassays and structural studies in *Arabidopsis* have revealed that the Jas motif undergoes pronounced conformational changes during JA signal transduction. In the absence of JA, the Jas motif is in an α-helical state, which blocks interactions between MYC TFs and the transcriptional Mediator complex subunit MED25 (Cerrudo et al., 2012; Chen et al., 2012; Zhang et al., 2015). As a consequence, the expression of JA responsive genes is blocked. In the presence of active JA [(3R,7S)-jasmonoyl-L-isoleucine; JA-Ile], the N-terminal part of the Jas domain, also named the JAZ degron, undergoes conformational changes to form a bipartite structure which consists of a loop and an amphipathic α-helix. The loop structure interacts with JA-Ile, whereas the α-helix mediates docking of the JAZ protein to COI1, which leads to ubiquitylation of JAZ by the 26S proteasome (Sheard et al., 2010). Consequently, the JAZ degradation allows MYC2 to interact with MED25, which activates the expression of MYC2 target genes (An et al., 2017).

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Changes to the JAZ degron sequence impact JA signal transduction (Chini et al., 2007; Melotto et al., 2008; Sheard et al., 2010; Shyu et al., 2012; Thines et al., 2007; Zhang et al., 2015). In most cases this leads to abolished JA signaling, but different splice variants or divergences in the Jas motif, for example those of *Arabidopsis* JAZ10, JAZ2, JAZ7, JAZ8 and JAZ13, might also stabilize their repressor activity (Chung et al., 2010; Chung and Howe, 2009; Melotto et al., 2008; Moreno et al., 2013; Shyu et al., 2012; Thireault et al., 2015; Yan et al., 2014).

Rice contains conserved JA biosynthesis and signaling pathways (Cai et al., 2014; Yuan and Zhang, 2015). In the *osjaz1*/*extra glumes* (*eg*) *2-1D* dominant mutant, a substitution of Ala^5 (A^5) by Gly^5 (G^5) was identified in the OsJAZ1 degron (DLPIA^{5}RR changed to DLPIG^{5}RR), which blocked interaction between EG2-1D and OsCOI1b and, in turn, repressed E class MADS-box TF genes, such as *OsMADS1*, which resulted in the delayed transition of spikelet meristem to floral meristem (Cai et al., 2014). In addition, overexpression of mutated OsJAZ proteins (mJAZ with R^6R^7 or R^6K^7 substitution by A^6A^7) also affected spikelet meristem identity and determinacy (Hori et al., 2014), which indicates that OsJAZ degrons play similar roles to those of *Arabidopsis* JAZ degrons in shaping OsJAZ-OsCOI1b co-receptor interactions to implement JA-steered responses. But, the abnormal spikelet development was more severe in the overexpression of mJAZ lines than that seen in the *osjaz1*/*eg2-1D* mutant (Hori et al., 2014), which suggests that the mJAZ degron (XXPXAAA; X is any amino acid) might be more stable or insensitive to OsCOI1-dependent protein degradation than that of the EG2-1D degron (DLPIGRR). To clarify this finding, we investigated how changes to the OsJAZ degron in OsJAZ1 affected its ability to interact with COI and MYC proteins, and how these changes impacted rice development.

RESULTS AND DISCUSSION

To dissect the biochemical activity of the OsJAZ degron, we first compared sequences among the OsJAZ family proteins (Fig. S1). Similar to that of *Arabidopsis*, rice OsJAZ proteins contain several semi-conserved motifs, including CMID/NT, ZIM, Jas and EAR motifs (Fig. 1A and Fig. S1). Among the 15 OsJAZ proteins (Ye et al., 2009), 13 contained conserved or semi-conserved Jas motifs (Fig. 1A), composed of 27 amino acid segments that comprised the canonical JAZ degron (X_2PXARR/KX), a core sequence ($SLX_2FX_2KRX_2R$) and a C-terminal motif (X_5PY) (Fig. 1B). A conserved Jas intron sequence between the core sequence and the C-terminal X5PY motif was present in many of the OsJAZ proteins in proximity to the Jas motif (Fig. 1A), which typically is conserved across different plant species (Chung et al., 2010). The Jas motifs were present in all OsJAZ proteins, but some contained divergent Jas motifs, which might confer plasticity (Shyu et al., 2012) or specificity for their interactions with the different OsCOI isoforms (Lee et al., 2013).

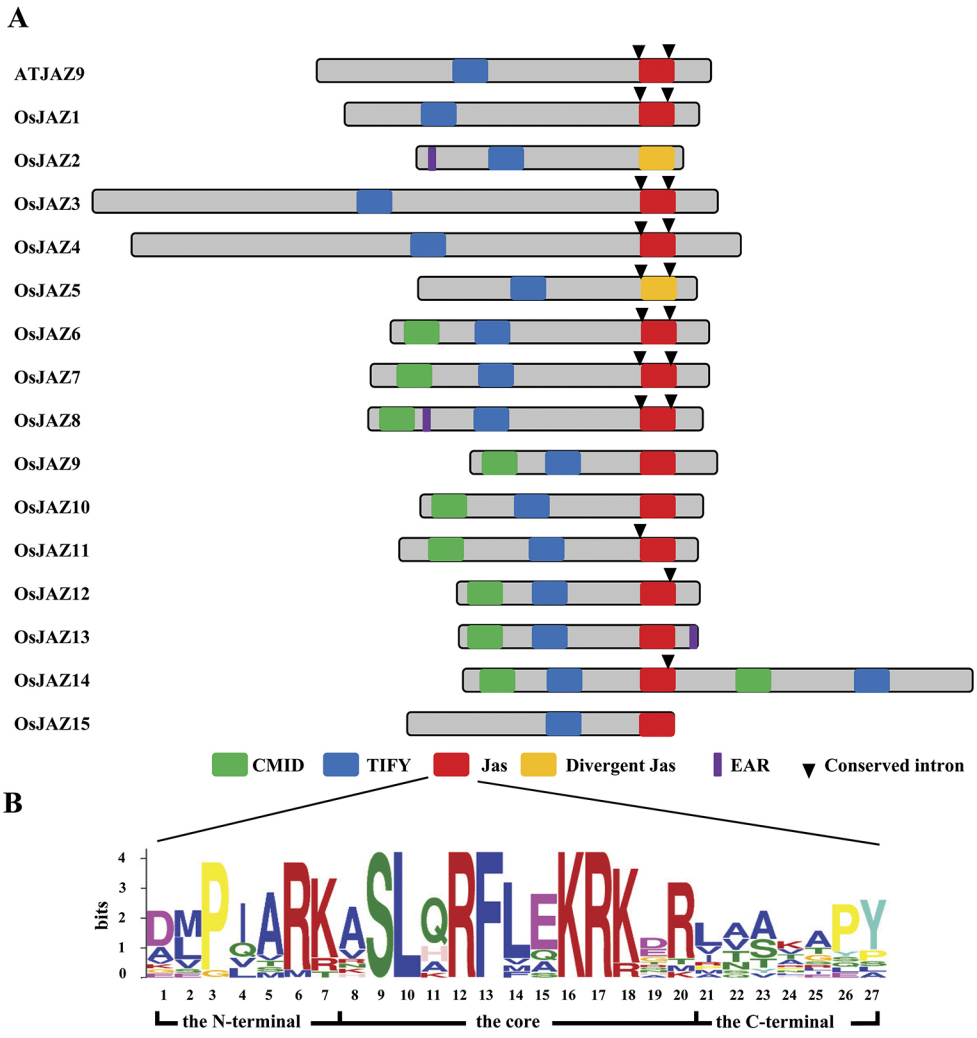

Fig. 1. Motif analysis of the rice OsJAZ protein family. (A) Diagrams show conserved motifs in the OsJAZ protein family. The positions of conserved functional domains are depicted by colored boxes. Arrowheads indicate conserved intron positions that flank the Jas motif in the DNA sequence. (B) Residue composition of consensus sequence in the Jas motif. Amino acids are color coded according to their properties, and the size indicates frequency.

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To explore how changes in the Jas motif affect the interactions between OsJAZ1, OsCOI1b and OsMYC2, we made series of amino acid deletions or substitutions in the Jas motif of OsJAZ1 and tested how this impacted the interactions in yeast two-hybrid assays (Fig. 2A). As expected, the interaction between OsJAZ1 and OsCOI1b was dependent on coronatine (COR; a structural mimic of JA-Ile). Deletion of the degron sequence (OsJAZ1$^{jasD1\text{-}8}$) abolished the interaction between OsJAZ1 and OsCOI1b, but did not affect the ability of it to interact with OsMYC2 (Fig. 2A). Furthermore, deletions of A^5, R^6, $A^5R^6R^7$ and $I^4A^5R^6$ in OsJAZ1 impaired its interactions with OsCOI1b, similar to when the R^6R^7 were substituted by A^6A^7 in OsJAZ1 (Fig. 2A). These data corroborate that the OsJAZ1 degron has a conserved role in the COR-dependent OsJAZ1-OsCOI1b interaction. Nevertheless, deletion of the first four amino acids in the degron (OsJAZ1$^{jasD1\text{-}4}$), or deletion of I^4A^5 and A^5R^6 did not affect its ability to interact with OsCOI1b and OsMYC2 in yeast (Fig. 2A). These results suggest that certain regions of JAZ1 might not be needed for the interactions to take place and highlights issues surrounding why these residues have been maintained in JAZ1. Perhaps these additional amino acids of the degrons provide some form of flexibility, plasticity or even specificity for JAZ interactions with different COI isoforms (Lee et al., 2013). It is also possible that some of the deletions could confer conformational changes to OsJAZ1. Protein structure studies revealed that the degron could change its conformation from an α-helix to a loop when active JA was presented (Zhang et al., 2015). This indicates that the interactions between OsJAZ1 and OsCOI1b might be tolerant to certain changes, as the deletions of $D^1L^2P^3I^4$, I^4A^5 and A^5R^6 in OsJAZ1 can still maintain interactions with OsCOI1b.

Apart from the degron length and conformation, research in *Arabidopsis* found that the interaction between AtJAZs and AtCOI1 depends on different COR concentrations (Melotto et al., 2008), e.g. different COR concentrations were needed for interactions to occur between AtCOI1 and alternatively spliced AtJAZ10 and AtJAZ2 isoforms (Chung et al., 2010; Chung and Howe, 2009). Indeed, the interaction strength differs between OsCOI1b and OsJAZ1^{jas} compared with several other OsJAZ1 mutations (Fig. 2). For example, OsCOI1b could interact with both EG2-1D and OsJAZ1^{jasDA5} under 50 µM and 100 µM COR, respectively (Fig. 2B). To further explore how COR concentrations affected

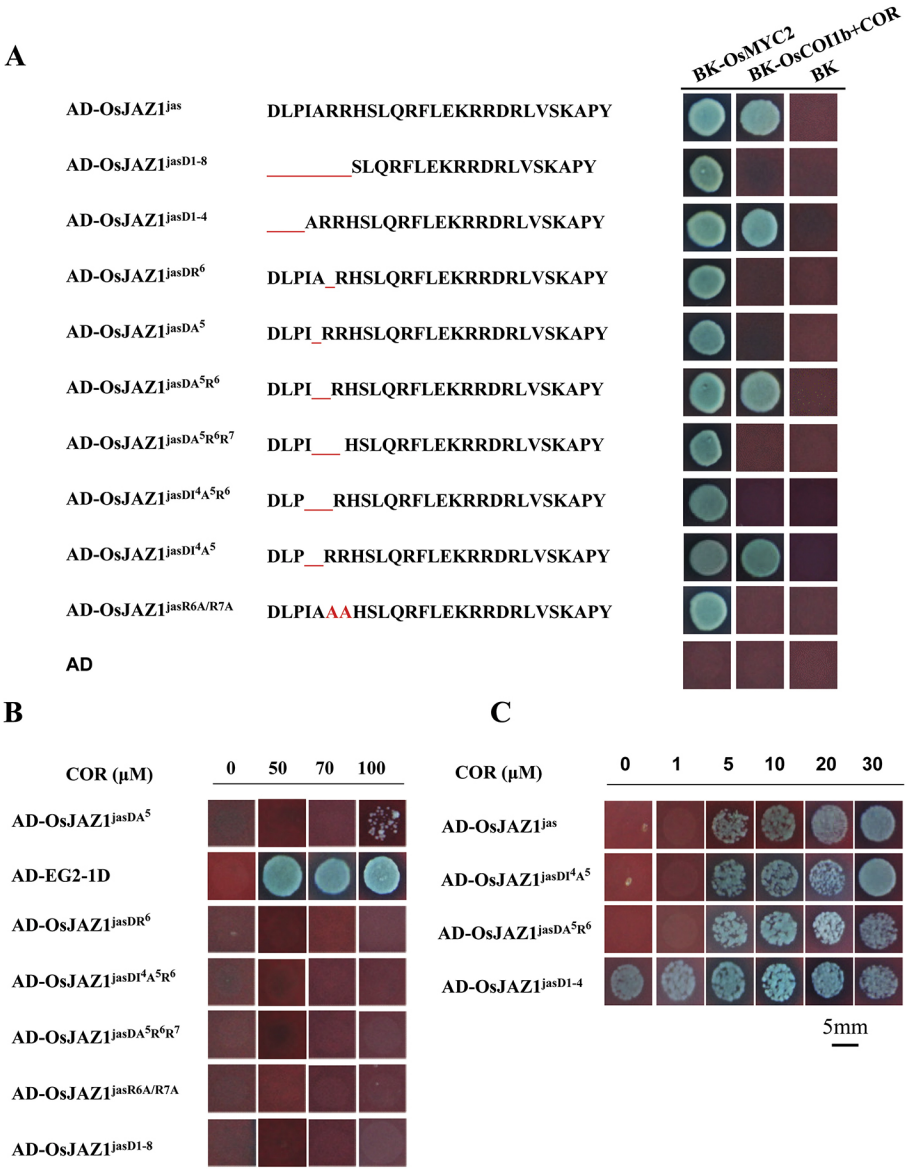

Fig. 2. COR-dependent OsJAZ1-COI1b interactions are determined by the degron domain. (A) The amino acid sequences of wild type (OsJAZ1^{jas}) and different OsJAZ1 variants. Deletion or substitution residues are underlined or in red, respectively. Yeast two-hybrid assays were applied to assess interactions between OsJAZ1 variants and OsCOI1b or OsMYC2. To detect the OsCOI1b-OsJAZ1 interactions, transformed yeast cells were grown on SD-Trp/-Leu/-His/-Ade/X-α-Gal medium that contained 30 µM COR. To detect the OsMYC2-OsJAZ1 variant interactions, transformed yeast cells were grown on SD-Trp/-Leu/-His/-Ade/X-α-Gal medium. AD, pGADT7; BK, pGBKT7. (B,C) Dose-dependent interactions were detected between OsCOI1b and different OsJAZ1 variants in the presence of different concentrations of COR.

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the interactions between OsCOI1b and different mutations of OsJAZ1, we tested the mutated OsJAZ1 versions that interacted with OsCOI1b under intermediate (30 µM) COR concentrations (Fig. 2A), i.e. OsJAZ1^{DI4A5}, OsJAZ1^{jasDA5R6} and OsJAZ1$^{jasD1\text{-}4}$, against OsCOI1b under lower (0 to 30 µM) COR concentrations. Although the interactions between OsCOI1b and OsJAZ1^{jasDI4A5}/OsJAZ1^{jasDA5R6} were similar to OsJAZ1^{jas}, the interaction between OsCOI1b and OsJAZ1$^{jasD1\text{-}4}$ was COR independent (Fig. 2C). In summary, these results suggested that degron sequence variants do play important roles in conferring COR sensitivity to JAZ-COI1b interactions, similar to the interaction between TIR1 and Aux/IAA (Calderón Villalobos et al., 2012). By contrast, we found that truncated Jas motifs after the core sequence did not affect the interactions between OsJAZ1, OsCOI1b and OsMYC2. Nevertheless, R^{12}, L^{14} and R^{17} substitution in OsJAZ1 affected the interaction between OsJAZ1 and OsMYC2, and F^{13} to A^{13} substitution in the core sequence abolished the interaction between OsJAZ1 and OsMYC2 or OsCOI1b (Fig. S2). These interaction relationships between OsJAZ1 and OsMYC2 are inconsistent with structural estimates of AtJAZ9 (Zhang et al., 2015), which suggests that interaction between OsJAZ variants and OsMYC2 might be sequence specific. However, reminiscent of studies of AtJAZ1 (Sheard et al., 2010), our data provide important evidence for how the core sequence of OsJAZ1 impacts interactions between OsJAZ1, OsCOI1b and OsMYC2.

Although bioassay and protein structure studies of JAZ3/9 in *Arabidopsis* indicate that the Jas degron sequence is pivotal for JA signaling, few studies have investigated how the sequence contributes to plant growth and development (e.g. Cai et al., 2014). We therefore applied a CRISPR-Cas9 system to edit the OsJAZ1 degron sequence (Xie et al., 2015), and established deletion and substitution lines within the degron (Fig. 3A). We

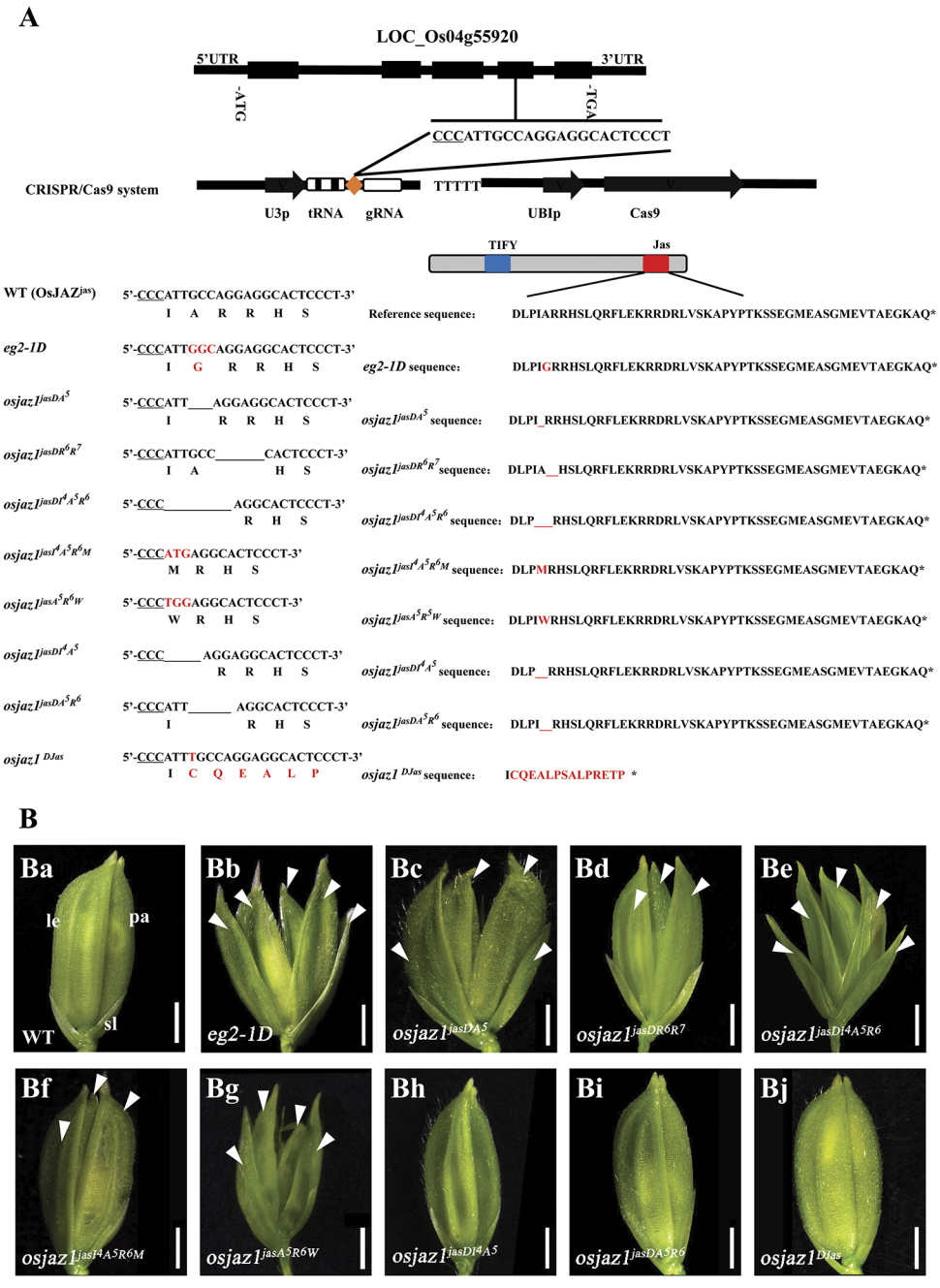

Fig. 3. CRISPR-Cas9 targeted editing of the degron domain leads to specific changes in spikelet development. (A) CRISPR/Cas9 editing of *OsJAZ1* in rice. Construct illustration of CRISPR/Cas9 systems for targeted editing of the *OsJAZ1* gene in rice. Deletion or substitution residues are underlined or in red, respectively. The sequence of lines *osjaz1*^{Djas} has a nucleotide insertion in target sequence. The sequences of lines *osjaz1*^{jasDA5}, *osjaz1*^{jasDA5R6}, *osjaz1*^{jasDI4A5}, *osjaz1*^{jasDR6R7} and *osjaz1*^{jasDI4A5R6} have a 3 nt, 6 nt, 6 nt, 6 nt or 9 nt deletion, respectively, in the target sequence. The sequences of lines *osjaz1*^{jasI4A5R6M} and *osjaz1*^{jasA5R6W} have three and two nucleotides replaced by ATG and TGG, respectively. (B) Floral organ phenotypes of different *OsJAZ1* CRISPR/Cas9 lines. Arrowheads indicate lemma-like structure. le, lemma; pa, palea; sl, sterile lemma. Scale bars: 1 mm.

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analyzed these lines for developmental phenotypes that deviated from the wild type. Consistent with our yeast data, the single A^5 deletion lines, as well as those lines with R^6R^7 and $I^4A^5R^6$ deletions, and substitutions of $I^4A^5R^6$ with M and A^5R^6 with W, were dominant and grew abnormal spikelets (Fig. 3A,B). These mutant phenotypes mimic those of *osjaz1*/*eg2-1D*, a dominant negative *osjaz1* mutant, which have extra lemma-like organs outside the lemma and palea, and reverted the lodicule to glume-like organs in the spikelets (Fig. 3A,B). These data confirmed that certain degron mutants impaired JA signaling during rice reproductive development. By contrast, the I^4A^5 and A^5R^6 deletion lines grew wild type-like spikelets (Fig. 3Bi and Bj), indicating that I^4A^5 and A^5R^6 deletions did not affect JA response to a degree that affected reproductive development in our growth conditions. The Jas domain deletion mutant (resulting in a truncated OsJAZ1) produced spikelets similar to wild type (Fig. 3Ba and Bj), which indicates functional redundancies among OsJAZ proteins, similar to that of AtJAZ proteins (Campos et al., 2016). These data are consistent with the Y2H data, and corroborate that degron modifications affect JA signal transduction. Indeed, the variants that abolished OsJAZ1-OsCOI1b interactions affected rice flower development by delaying the transition from spikelet meristem to floral meristem, mimicking phenotypes of *eg2-1D* (Cai et al., 2014).

Apart from floral development, JAs also affect root elongation (Cai et al., 2014; Wasternack and Hause, 2013), a process in which it is substantially easier to measure the sensitivity of JA signaling by applying methyl (Me)JA treatment. To assess whether the degron mutations affected JA signaling, we therefore observed root growth patterns when plants were grown on media supplemented with 0, 5, 10 and 15 µM MeJA. The root length of *eg1* (*Arabidopsis DAD1* homolog; a JA biosynthesis mutant) was longer than that of wild-type roots under mock conditions (Fig. 4 and Fig. S3) (Cai et al., 2014). Root growth of all lines was inhibited by MeJA, but the roots of *osjaz1*^{jasDA5} plants were significantly longer than those of wild-type, *eg2-1D* and *osjaz1*^{jasDA5R6} plants under different MeJA concentrations (Fig. 4). As expected, *eg1* and *osjaz1*^{jasDA5R6} roots were of a similar length to wild-type roots under 15 µM of MeJA (Fig. 4 and Fig. S3). These data indicate that the *osjaz1*^{jasDA5} and *eg2-1D* lines are less sensitive to changes in JA signaling than wild-type, *eg1* and *osjaz1*^{jasDA5R6} lines, and further corroborate that the degron sequence variation modulates the sensitivity of JA signaling.

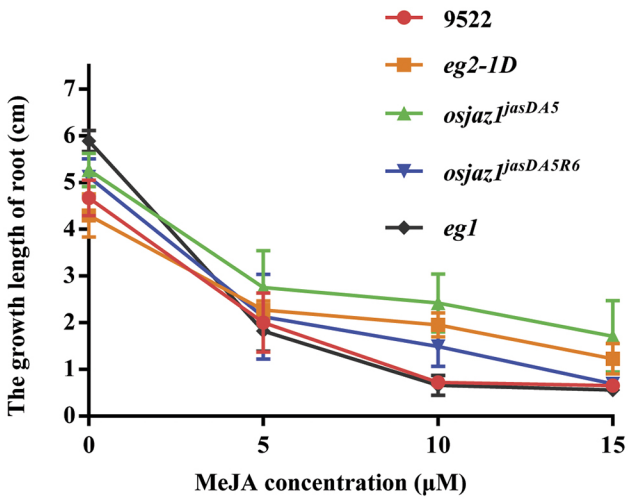

Fig. 4. OsJAZ1 degron sequence variation affects root growth by modulating the sensitivity of JA signaling. All seedlings were germinated and grown on 1/2 MS medium or on medium supplemented with 0, 5, 10 or 15 µM MeJA. The root lengths were calculated from ten independent plants. Data are mean±s.d. Note that root growth of *osjaz1*^{jasDA5} and *eg2-1D* plants was less sensitive to MeJA treatment, compared with that of *osjaz1*^{jasDA5R6}, *eg1* and wild-type 9522.

Altogether, our experiments provide new understanding on the function of the OsJAZ1 degron in rice JA signaling. We show that sequence variation in the degron of OsJAZ1 affects OsJAZ-OsCOI1b interaction, and possibly influences conformational changes of the OsJAZ1 protein that are needed for efficient signaling (Sheard et al., 2010; Zhang et al., 2015). Furthermore, this study extends our understanding of how the degron variation of OsJAZ1 contributes to complexity, specificity and sensitivity in the JA response.

MATERIALS AND METHODS

Yeast two-hybrid assay

For the yeast two-hybrid assay, full-length cDNAs of *OsCOI1b*, *OsMYC2*, *OsJAZ1* and site-directed mutagenesis of *OsJAZ1* were amplified and cloned into pGBKT7 and pGADT7 vectors, respectively (Clontech, primers are listed in Table S1). Site-directed mutagenesis of the Jas domain was performed using the Polymerase Incomplete Primer Extension method (Klock and Lesley, 2008). Mutations for all plasmid constructs were confirmed by Sanger sequencing [Generay Biotech (Shanghai) Co.]. Then, the experiments were carried out following the Matchmaker Gold Yeast Two-Hybrid System manufacturer's instructions (Clontech). Detection of protein-protein interactions between OsCOI1b and OsJAZ1 variants were performed in SD/-Trp/-Leu/-His/-Ade/X-α-Gal agar plates which contained 30 µM COR. To prepare 30 mM COR stock solution, 1 mg COR (Sigma-Aldrich, C8115) was dissolved in 1% methanol, which was further diluted to the final concentration with distilled water. Detection of protein-protein interactions between OsMYC2 and OsJAZ1 variants were performed in SD/-Trp/-Leu/-His/-Ade/X-α-Gal agar plates which contained 30 mM of 3-amino-1,2,4-triazole.

CRISPR/Cas9 construction and rice transformation

For targeted editing of OsJAZ1 degron, the sgRNA (ATTGCCAGGAGGCACTCCCT) in the fourth exon of the *OsJAZ1* gene was selected for CRISPR/Cas9 construction (Xie et al., 2015). Primers are listed in Table S1. *Agrobacterium*-mediated stable transformation in rice was performed as reported previously (Hiei and Komari, 2008).

Plant growth conditions and generation of transgenic plants

The variety 9522 (*Oryza sativa* L. ssp. *japonica*) and transgenic plants *osjaz1*^{jasDA5}, *osjaz1*^{jasDR6R7}, *osjaz1*^{jasDR6R7}, *osjaz1*^{jasI4A5R6M}, *osjaz1*^{jasA5R6W}, *osjaz1*^{jasDI4A5}, *osjaz1*^{jasDA5R6} and *osjaz1*^{DJas} were planted in the paddy fields of Shanghai Jiao Tong University (30°N, 121°E). The seeds of T_0 heterozygous plants and T_1 plants, segregated by self-pollination, were collected and genotyped for analysis. Plants were also grown in Conviron chambers (GR48), in which the parameters were set at 28°C, 14.0 h for long-day conditions and 10.5 h for short-day conditions. The morphology of 150-200 spikelets from panicles of five independent plants or transgenic lines was observed, and plants were photographed using a Leica S8APO stereomicroscope. For the root-sensitivity assay, plants were germinated and treated with 0, 5, 10 and 15 µM MeJA for 5 days. The length of root was then calculated from ten independent plants. To prepare MeJA stock solution, 573 µl MeJA (Sigma-Aldrich, 392707) was first dissolved in 95% ethanol to 5 ml, and was further diluted with distilled water to 20 mM and saved at 4°C. Data were analyzed using GraphPad Prism 7 software.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: Z.Y.; Methodology: J.T., Lichun Cao, X.C., Z.Y.; Validation: J.T.; Formal analysis: J.T., Lichun Cao, X.C., M.C., Z.Y.; Investigation: X.C., M.C., Liming Cao; Resources: Lichun Cao, P.Z., Liming Cao, D.Z.; Data curation: J.T.; Writing - original draft: J.T., Z.Y.; Writing - review & editing: S.P., Z.Y.; Supervision: S.P., D.Z., Z.Y.; Project administration: Z.Y.; Funding acquisition: Z.Y.

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Supplementary information

Supplementary information available online at
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