

RESEARCH ARTICLE

Genetic analysis of the rice jasmonate receptors reveals specialized functions for *OsCOI2*

Hieu Trang Nguyen¹ [✉], Mohamad Cheaib¹, Marie Fournel^{1,2}, Maelle Rios¹, Pascal Gantet¹, Laurent Laplaze¹, Soazig Guyomarc'h¹, Michael Riemann³ , Thierry Heitz², Anne-Sophie Petitot¹, Antony Champion¹ ^{*}

1 DIADE, IRD, Univ Montpellier, Montpellier, France, **2** IBMP, CNRS, Univ Strasbourg, Strasbourg, France, **3** Karlsruhe Institute of Technology, Botanical Institute, Karlsruhe, Germany

✉ Current address: Department of Plant Biotechnology and Bioinformatics, VIB Center for Plant Systems Biology, Ghent University, Ghent, Belgium

* antony.champion@ird.fr


 OPEN ACCESS

Citation: Nguyen HT, Cheaib M, Fournel M, Rios M, Gantet P, Laplaze L, et al. (2023) Genetic analysis of the rice jasmonate receptors reveals specialized functions for *OsCOI2*. PLoS ONE 18(9): e0291385. <https://doi.org/10.1371/journal.pone.0291385>

Editor: Keith R. Davis, SativaGen, UNITED STATES

Received: January 5, 2023

Accepted: August 25, 2023

Published: September 8, 2023

Copyright: © 2023 Nguyen et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the paper and its [Supporting information files](#).

Funding: This work was supported by the Consultative Group for International Agricultural Research Program on rice-agrifood systems (CRP-RICE, 2017–2022). HTN is funded by a PhD fellowship from the French Embassy in Vietnam. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Abstract

COI1-mediated perception of jasmonate is critical for plant development and responses to environmental stresses. Monocots such as rice have two groups of *COI* genes due to gene duplication: *OsCOI1a* and *OsCOI1b* that are functionally equivalent to the dicotyledons *COI1* and *OsCOI2* whose function remains unclear. In order to assess the function of *OsCOI2* and its functional redundancy with *COI1* genes, we developed a series of rice mutants in the 3 genes *OsCOI1a*, *OsCOI1b* and *OsCOI2* by CRISPR Cas9-mediated editing and characterized their phenotype and responses to jasmonate. Characterization of *OsCOI2* uncovered its important roles in root, leaf and flower development. In particular, we show that crown root growth inhibition by jasmonate relies on *OsCOI2* and not on *OsCOI1a* nor on *OsCOI1b*, revealing a major function for the non-canonical *OsCOI2* in jasmonate-dependent control of rice root growth. Collectively, these results point to a specialized function of *OsCOI2* in the regulation of plant development in rice and indicate that sub-functionalisation of jasmonate receptors has occurred in the monocot phylum.

Introduction

Jasmonoyl-isoleucine (JA-Ile), the bioactive form of jasmonic acid (JA), regulates many physiological and developmental processes in plants such as flower and root development [1, 2]. Plants from monocot and eudicot lineages accumulate JA-Ile in response to stresses, such as injury or insect attack [3, 4]. Jasmonate perception and transcriptional regulation of JA-responsive genes relies on a conserved core protein complex, the main components of which are the F-box protein CORONATINE INSENSITIVE1 (COI1) protein, the JASMONATE ZIM DOMAIN (JAZ) transcriptional repressors and transcription factors (TFs) [5–9]. In the absence of JA-Ile, target gene expression is repressed due to TF and JAZ protein interaction within the TOPLESS (TPL)-NOVEL INTERACTORS OF JAZ (NINJA) repressor complex [10]. Upon JA-Ile accumulation, JAZ proteins are recruited by COI1, ubiquitinated and

Competing interests: The authors have declared that no competing interests exist.

rapidly degraded in a proteasome-dependent manner, thus freeing TFs and leading to the de-repression of JA-responsive genes and subsequent physiological responses.

In eudicot species such as Arabidopsis and tomato, JA-Ile is perceived by a single receptor encoded by *COI1*, that interacts with members of the JAZ family [5–7]. In contrast, monocots such as rice, maize and wheat have two groups of *COI* genes due to gene duplication [4, 11, 12]. In rice, there are three *COI* genes, named *OsCOI1a*, *OsCOI1b*, and *OsCOI2*. *OsCOI1a* and *OsCOI1b* are orthologous to *AtCOI1* as shown by complementation of the loss-of-function *coi1-1* mutant in Arabidopsis [13]. Several studies in rice demonstrated the involvement of *OsCOI1a* and *OsCOI1b* in plant defence. Transgenic rice plants with a reduced expression of both *OsCOI1a* and *OsCOI1b* genes display a phenotype similar to gibberellic acid over-accumulating plants, including elongated internodes which suggests that jasmonate signaling promotes defence response over growth [14]. In a second study, silencing of *OsCOI1a* and *OsCOI1b* gene expression led to an increased susceptibility to the rice leaf-folder insect [15] and to the rice stripe virus, the resistance to which is known to be conferred by JA signalling elements [16].

In contrast, very little is known about *OsCOI2* function in rice. *OsCOI2* fails to rescue the fertility and defence response defect of *coi1-1* Arabidopsis plants, nor does it interact with any AtJAZ co-receptor [13], suggesting that *OsCOI2* may have as-yet unidentified function(s) distinct from that of *OsCOI1*. Similarly, in maize (*Zea mays*), three closely related *ZmCOI1* genes could complement the *coi1-1* mutation in Arabidopsis, but *ZmCOI2* did not [11]. Interestingly, a substitution in *OsCOI2* of one amino acid predicted to be involved in JA-Ile interaction allowed it to interact with AtJAZs and to rescue *coi1-1* deficiency [13]. Monocot and dicot lineages split about 140 to 150 million years ago [17]. Phylogenetic analysis revealed that *OsCOI2* shares higher similarity to *COI2* proteins from other monocots than to *COI1* proteins from monocots and eudicots, suggesting that the monocot-specific *COI1-COI2* duplication and divergence occurred early in the monocot phylum [11]. This high similarity in the sequence of *COI2* proteins from monocot species, and the failure of *OsCOI2* and *ZmCOI2* to complement the Arabidopsis *coi1-1* mutant raised fundamental questions about the conservation of function of *COI2* and whether it was involved in jasmonate signalling or not. Here genetic analysis allowed us to address in parallel the functions of *OsCOI1a/b* and *OsCOI2*, and to uncover the unique roles of *OsCOI2* in jasmonate-dependent developmental processes.

Materials and methods

Edited rice lines

To generate the JA perception mutants *oscoi1a/b* and *oscoi2*, the CRISPR-Cas9 system was used on the background cultivar Kitaake (*Oryza sativa* L. ssp. japonica) as described in [18]. Briefly, the CRISPR Guides tool on Benchling platform (<https://benchling.com/crispr>) was used to design the gRNAs. One gRNA was designed for each gene, *OsCOI1a* and *OsCOI1b*, to obtain the *oscoi1a/b* double mutants and two different pairs of gRNAs were designed to obtain the *oscoi2* mutants. The sequences of the gRNAs were first inserted into the pUC57-sgRNA vector, then transferred by LR recombination into the binary vector pOS-Cas9 [19]. The pOS-Cas9-gRNAs vectors were introduced in the *Agrobacterium tumefaciens* strain EHA105 by electroporation. Rice embryogenic calli were transformed via *Agrobacterium*-mediated transformation protocols. The regenerated plants were checked for transformation, by PCR using T-DNA specific primers (HPT and Cas9, S1 Table in S1 File), and for mutation, by PCR using primers flanking the targeted sequences (S1 Table in S1 File) and sequencing (Eurofins). Edited plants were transferred to a greenhouse for multiplication. Edited lines were counter-

selected from T1 generation to obtain T3 homozygous lines without T-DNA insertion, except for *oscoi2-1* for which the limited number of seeds prevented such selection.

Plant phenotyping

Seeds were sterilized in 70% ethanol for 1 minute, then in 40% commercial bleach for 30 minutes, and were rinsed 6 times in sterilized water. Disinfected seeds were then germinated on half-strength Murashige & Skoog ($\frac{1}{2}$ MS) medium including Gamborg B5 vitamins (Duchefa Biochemie BV, Haarlem, the Netherlands) supplied with 0.7% plant agar (Duchefa) for 6 days. Plantlets of uniform size were transferred into soil and grown in a greenhouse under a 16h-day/8h-night photoperiod, at 28°C/24°C with a 75% relative humidity. Tiller and internode lengths were measured at the mature stage on the 3 highest tillers from 18 T3 homozygous lines and wild type (WT) plants. Fertility rate was measured on the same tillers as the ratio between the number of fertile spikelets and the total number of spikelets. Leaf necrotic lesions and adventitious roots were observed under an Axiozoom microscope (Zeiss). Spikelet and anther morphology were observed under a stereo microscope (Nikon SMZ1500).

Hormone treatments

Seeds were sterilized and sown as described above. Two days after germination, seedlings with both coleoptile and primary root were transferred into glass tubes containing 20 ml of $\frac{1}{2}$ MS including Gamborg B5 vitamins supplied with 0.2% phytigel (Sigma) and grown in a growth chamber at 26°C under a 12h-day/12h-night photoperiod with a 70% relative humidity. For phenotyping, 5 μ M JA (Sigma), 0.5 μ M COR (Sigma) both dissolved in DMSO, or DMSO only (control plants) were added to the media just before pouring. Eight days after transplanting, length of the crown roots was measured for each plant. For each line, 20–24 plants were used except for the *oscoi2-1* line for which very few seeds were available. Percentages of root growth inhibition were determined by randomly comparing the length values of the treated plants to those of control plants.

For gene expression analyses, plants were transferred 4 days after transplantation from the tubes into liquid media containing either 5 μ M JA or DMSO for control plants. Crown root tips (around 1 cm) from 20–24 plants were collected 6 hours after and immediately frozen in liquid nitrogen. Five independent biological samples were collected and analysed.

Gene expression analyses

Total RNAs were extracted from rice root samples using the RNeasy Plant mini kit (Qiagen, France), with addition of an on-column DNase I digestion. First-strand cDNAs were synthesized from 1 μ g of total RNA in 20 μ l final volume using an oligo-dT(18)-MN primer (Eurogentec, France) and the Omniscript RT kit (Qiagen). Specific primers were designed from *O. sativa* Nipponbare sequences using Primer3Plus (<https://primer3plus.com/>) (S1 Table in S1 File) and checked for specificity on *O. sativa* Kitaake sequences. Quantitative-PCR assays were performed on cDNAs samples (diluted 1/50e) in an Mx30005P thermal cycler (Stratagene, USA) using the Brilliant III Ultra-fast SYBR[®] Green QPCR Master mix with low ROX (Agilent, Santa Clara, CA, USA). Amplifications were performed in duplicate from the five biological samples. The *EXP* gene (LOC_Os06g11070) was used as a reference gene to normalize data. Relative gene expression levels were calculated using the $2^{-\Delta\Delta C_t}$ method.

Statistical analyses

Experimental data were analysed using GraphPad Prism (version 9.3.0). Values were considered statistically significant when $p \leq 0.05$.

Results

OsCOI2* regulates plant development and its function is not redundant with that of *OsCOI1a* and *OsCOI1b

To investigate *COI2* functions in monocots, CRISPR-Cas9 technology was used to generate *oscoi2* mutant alleles in rice. Two CRISPR-Cas9 constructs targeting the first (target #1 and #2), and the second exon (target #3 and #4) of *OsCOI2*, respectively, were used to generate edited lines in the rice cultivar Kitaake (Fig 1a). Three homozygous mutant lines, named *oscoi2-1*, *oscoi2-2* and *oscoi2-3*, were isolated and further characterized. The alleles *oscoi2-1* and *oscoi2-2* include short nucleotide insertions and deletions in the first and second exon leading to a translational frame shift and premature stop codon truncating 467 amino acids and 372 amino acids in the predicted proteins, respectively. The third allele *oscoi2-3* displays fragments deleted in exon 2 eliminating 18 amino acids in the LRR domain, which can affect the protein stability (Fig 1b) [20]. In order to compare their phenotype with that of *oscoi2* mutants and assess the putative functional redundancy between *OsCOI2* and *OsCOI1*, we also generated two double *oscoi1a oscoi1b* mutant lines (thereafter named *oscoi1a-1/b-1* and *oscoi1a-1/b-2*) by CRISPR-Cas9 (S1 Fig in S1 File).

Phenotypically, the *oscoi1a-1/b-1* and *oscoi1a-1/b-2* double mutant lines exhibited increased plant height in comparison with *oscoi2* single mutant and WT plants (Fig 1c, S2 and S3 Figs in S1 File). In addition, internode length was increased in the *oscoi1a-1/b-1* and *oscoi1a-1/b-2* compared to WT and *oscoi2* mutant lines (Fig 1d, S2 Fig in S1 File). These *oscoi1a/b* phenotypes are similar to those reported for the rice *coi1-RNAi* lines in which both *OsCOI1a* and *OsCOI1b* expression is reduced [14]. Thus, we used these new *oscoi1a-1/b-1* and *oscoi2* knock-out lines to compare *OsCOI1a/b* and *OsCOI2*-mediated regulation of plant development and JA signalling in rice.

The most prominent phenotype observed in *Arabidopsis coi1-1* and tomato *jail-1* JA receptor mutants is sterility [5, 21], but earlier studies reported only a weak reduction in fertility in *oscoi1-RNAi* and *oscoi1b-T-DNA* rice mutant lines [14, 22]. Consistent with these reports, seed-setting in our *oscoi1a/b* double mutant lines was close to WT level (Fig 1e–1g). In contrast, all three of our independent *oscoi2* mutant alleles conferred reduced seed setting (Fig 1e and 1h). None of the *oscoi1a/b*, nor *oscoi2* mutant lines displayed any abnormality in floret architecture (S4 Fig in S1 File); however, most of the *oscoi2* anthers did not dehisce (Fig 1f–1h and S4 Fig in S1 File). Hence, *OsCOI2*, rather than *OsCOI1a* or *OsCOI1b*, plays an important role during anther dehiscence stage in rice reproductive development. Importantly, even though *OsCOI2* does not restore fertility in the *Arabidopsis coi1-1* mutant background [13], our results indicated that *OsCOI2* is an essential factor for rice plant fertility, probably via jasmonate-dependent regulation of flower or seed development.

During the vegetative to reproductive transition, *oscoi2* mutant lines exhibited three distinct phenotypes not observed in WT or in *oscoi1a/b* plants. First, all three *oscoi2* mutant alleles showed spontaneous lesions on flag leaves and the last leaf mimicking disease symptoms, despite plants not being infected (Fig 1i). Second, when the main *oscoi2* panicle started to flower, above-ground adventitious roots emerged from node 1 to node 3, which resembles adventitious rooting induced by flooding in deep-water rice [23]. Third, 25 to 35% of the plants showed leaf rolling followed by tiller senescence and eventually plant death without

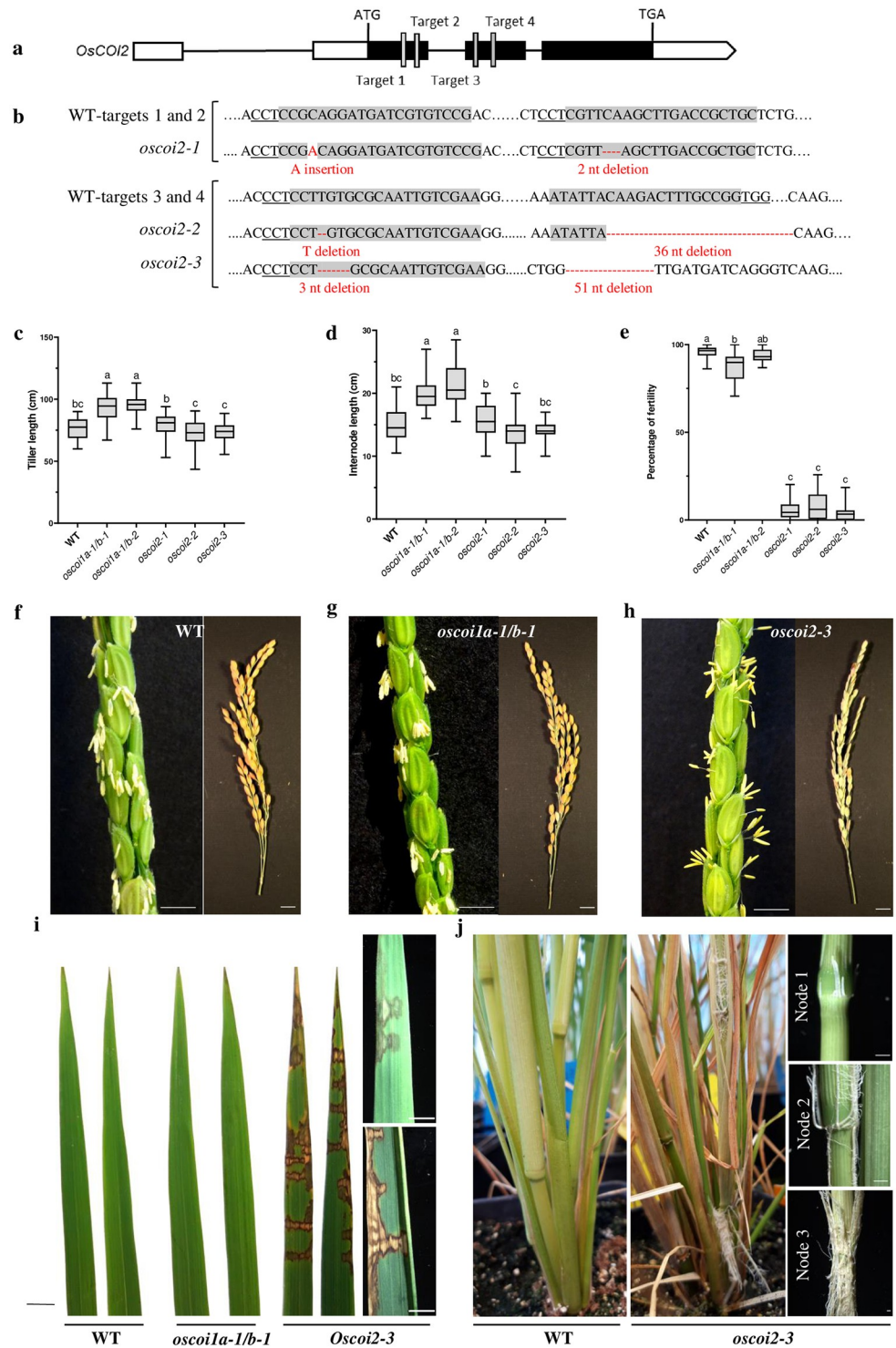


Fig 1. *OsCOI2* regulates vegetative and reproductive developmental programmes in rice distinct from *OsCOI1a/b*. **a**, *OsCOI2* gene structure and CRISPR-Cas9 target sites. **b**, insertion and deletion sites of three allelic mutations (*oscoi2-1*, *oscoi2-2* and *oscoi2-3*) generated by two different pairs of gRNA. **c**, quantification of tiller length and **d**, quantification of internode length. Letters indicate significant differences between lines (45 < n < 54, One way-ANOVA with Tukey's multiple comparisons test, $p < 0.05$). **e**, Percentage of fertile spikelet. Letters indicate significant differences between lines (43 < n < 53, Kruskal-Wallis multiple comparisons test, $p < 0.05$). In the boxplots, whiskers denote minimum/maximum values, the box defines the interquartile range and the centre line represents the median. (c, d, e). **f-h** images of WT (f), *oscoi1a-1/b-1* (g) and *oscoi2-3* (h) panicles. **i**, flag leaves. **j**, nodes. (f-j) Scale bar = 1 cm.

<https://doi.org/10.1371/journal.pone.0291385.g001>

imposing any water stress (S5 Fig in [S1 File](#)). Collectively, these data reveal that *OsCOI2* regulates plant development and that its function is not redundant with that of *OsCOI1a* and *OsCOI1b* jasmonate receptors.

***OsCOI2* is required for JA perception in rice crown roots**

Next we tested the hypothesis that *OsCOI2* participates in JA signalling in rice. Inhibition of seedling root growth by jasmonate or jasmonate analogue treatments has been extensively used as a bioassay to identify jasmonate response mutants in plants [5, 21, 24]. Under our growth conditions, JA treatment at 5 μ M inhibited significantly WT crown root growth (S6 Fig in [S1 File](#)). Interestingly, at this concentration, JA also inhibited root growth of *oscoi1a/b* double mutant plants to the same extent as in the WT ([Fig 2a and 2b](#)). In contrast, crown root growth was significantly less sensitive to exogenous JA treatment in *oscoi2-1*, *oscoi2-2*, and *oscoi2-3* genetic backgrounds ([Fig 2a and 2b](#) and S7 Fig in [S1 File](#)). Similarly, coronatine (COR), a bacterial mimic of JA-Ile, strongly inhibited root growth of WT and *oscoi1a/b* lines but had a significantly weaker effect on root growth of *oscoi2* mutant alleles ([Fig 2a and 2b](#)).

To explore the molecular mechanisms associated with inhibition of crown root growth by jasmonate, we focused on crown root tips, where cell proliferation and elongation occur. All three JA receptor genes *OsCOI1a*, *OsCOI1b* and *OsCOI2* were expressed in crown root tips ([Fig 2c](#)). Expression of the *OsCOI1a* and *OsCOI1b* was similar in WT and *oscoi2* mutant crown root tips thus indicating that no genetic compensation occurred (S8 Fig in [S1 File](#)). Similarly, *OsCOI2* expression was not different in WT and *oscoi1a-1/b-1* plants.

Induction of jasmonate responsive genes related to JA biosynthesis (*OsAOC*) and signalling (*OsJAZ5*, *OsJAZ8* and *OsMYC2*) by exogenous JA application was compromised in *oscoi2-2* crown roots relative to WT, but not in the *oscoi1a-1/b-1* mutant background ([Fig 2d](#), top panels). Consistent with the crown root growth phenotype, expression of the cell elongation-related genes *OsEXPB7*, *OsEXP13* and *OsXTH12* were significantly less repressed by JA treatment in *oscoi2-2* crown roots than in WT and in *oscoi1a-1/b-1* mutant background ([Fig 2d](#)). Conversely, expression of cell cycle or meristem marker genes (*OsCYCB1*, *OsCDKB1*, *OsCDKB2*, *OsPLT1* and *OsPLT6*) was not affected by JA treatment [25]. Furthermore, genes associated with defence responses (*OsPR5*, *OsPR10* and *OsWRKY71*) were significantly less induced by JA treatment in *oscoi2-2* than in WT and *oscoi1a-1/b-1*. Hence, expression profiling revealed that *OsCOI2*, and not *OsCOI1a/b*, is required for JA-dependent signalling in rice crown root.

Discussion

Here, we report that a monocot-specific member of the COI family *OsCOI2* mediates the regulation of a range of jasmonate-dependent vegetative and reproductive developmental processes. Significantly, the loss of *OsCOI2* function (and not that of *OsCOI1a* and *OsCOI1b* genes) was shown to strongly alter male fertility in rice, a phenotype that is *AtCOI1*-dependent in Arabidopsis. In rice, jasmonate is not only necessary for stamen fertility, but also required for floret development [26, 27]. Loss-of-function mutations or misexpression in key genes in the jasmonate biosynthetic or signalling pathways cause floral morphological alterations such as longer sterile lemmas and extra glume-like organs [24, 26, 27]. We found that *oscoi2* anthers did not dehisce properly, a phenotype also observed in the rice *osjar1* mutant impaired in JA-Ile biosynthesis [28]. Furthermore, previous reports showed that *OsCOI2* does not restore fertility and JA signal transduction in the Arabidopsis *coi1* mutant, unless the *OsCOI2* His-391 was substituted with Tyr-391 [13]. Recently, two reports also described that *oscoi2* mutant anthers do not dehisce properly [29, 30]. In addition, Wang et al., 2023 showed that the pollen

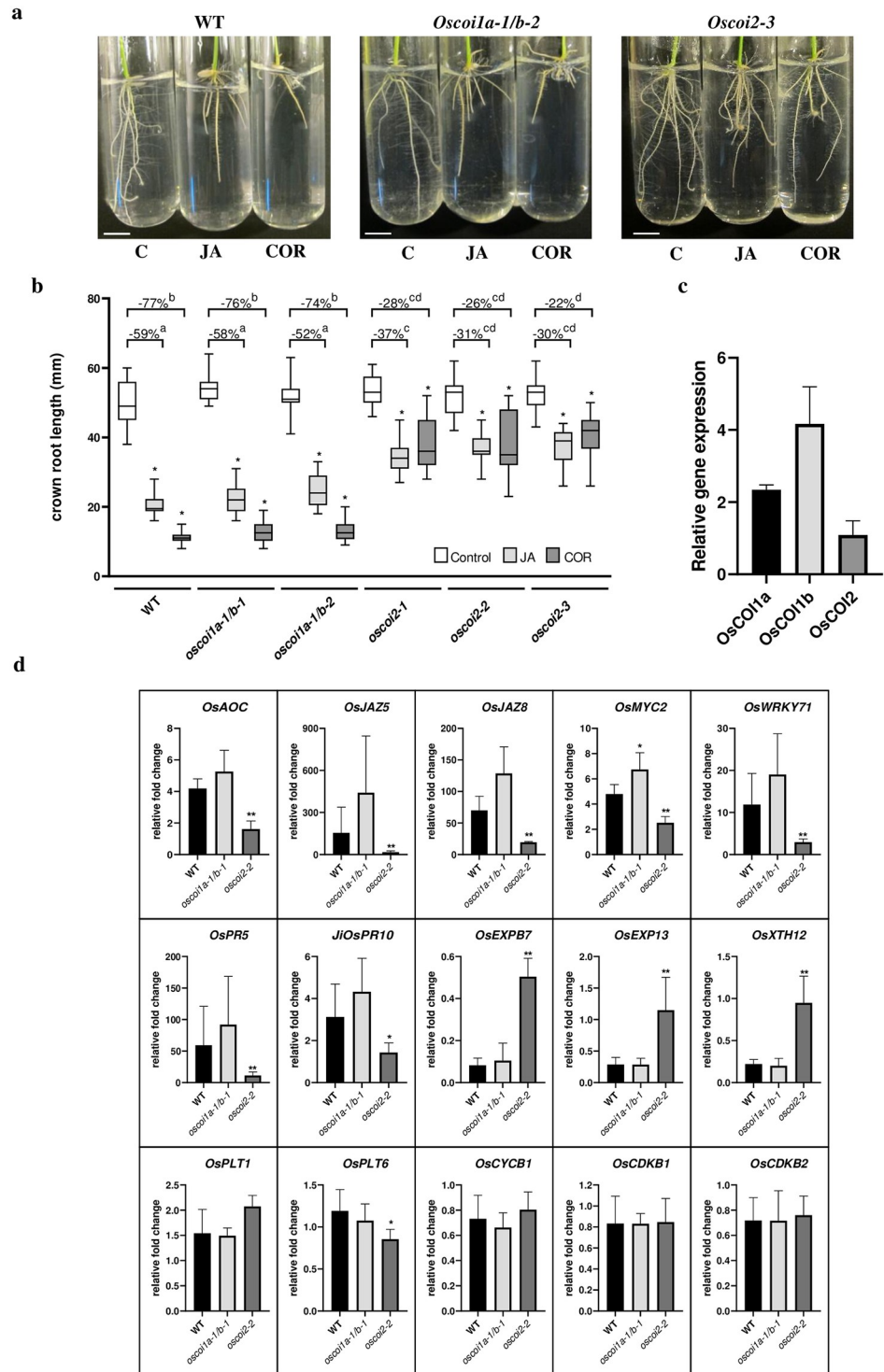


Fig 2. *OsCOI2* regulates jasmonate response in crown roots. **a**, Phenotype of the (WT) and two allelic mutations (*oscoi1a-1/b-2* and *oscoi2-3*) of roots in response to jasmonic acid (JA, 5 μ M), coronatine (COR, 0.5 μ M) and mock control (C, DMSO). Scale bars = 1 cm. **b**, effect of JA and COR compared to the mock treatment (DMSO) on crown root length in WT *oscoi1a/b* (*oscoi1a-1/b-1* and *oscoi1a-1/b-2*) and *OsCOI2* (*oscoi2-1*, *oscoi2-2* and *oscoi2-3*) mutant lines. In the boxplots, whiskers denote minimum/maximum values, the box defines the interquartile range and the centre line represents the median. Asterisks indicate significant differences between treated and control plants ($20 < n < 24$ except for *oscoi2-1* where $7 < n < 21$, One way-ANOVA with Bonferroni's multiple comparisons test, $p < 0.05$). The percentages of crown root growth inhibition by JA and COR treatments are indicated above the

respective plots from the WT, *oscoi1a-1/b-1*, *oscoi1a-1/b-2*, *oscoi2-1*, *oscoi2-2* and *oscoi2-3* mutants. Letters indicate significant differences between lines and treatments ($21 < n < 24$, One way-ANOVA with Tuckey's multiple comparisons test, $p < 0.05$). c. expression of *OsCOI1a*, *OsCOI1b* and *OsCOI2* genes in crown root tips from wild-type plants. Data are presented as the means \pm SD from five biological replicates. d. regulatory effects of JA on the transcription of jasmonate responsive genes, cell elongation and division and defence genes. Data are presented as means \pm SD from five biological replicates. Asterisks indicate significant differences between the WT and the double *oscoi1a-1/b-1* mutant or the *oscoi2-2* mutant (Mann-Whitney tests, * $p < 0.05$, ** $p < 0.01$).

<https://doi.org/10.1371/journal.pone.0291385.g002>

germination rate of *oscoi2* mutants was significantly lower than that of WT plants. Collectively, these data point to a specialized function of COI2 in the regulation of plant reproductive development in the Monocot phylum.

COI1 was shown to be the receptor of the jasmonate signal in Arabidopsis and in rice. We investigated the possible role of *OsCOI2* in jasmonate signalling using the JA-mediated repression of crown root growth. Surprisingly, our data suggested that inhibition of crown root growth by JA relies primarily on *OsCOI2*. Although the roots of *oscoi2* mutant plants showed better resistant to JA than that of *oscoi1a* and *oscoi1b* double mutant, its growth is still inhibited to some extent suggesting the minor contribution of *OsCOI1a* and *OsCOI1b*. Our observation is also in line with the report in Inagaki et al., (2023) and Wang et al., (2023) [29, 30], although the sensitivity to JA treatment varies to some extent between studies probably due to the differences in genetic background, alleles chosen, type of roots, and/or treatments. Consistently, expression profiling revealed that *OsCOI2* is specifically required for JA-dependent signalling in crown roots. This suggests that sub-functionalisation of JA receptors has occurred in the monocot lineage.

Besides its role in crown root growth inhibition, jasmonate is a potential regulator of adventitious root initiation. For example, JA treatment inhibited adventitious root formation in Arabidopsis hypocotyl [31]. Accordingly, *atcoi1-1* mutant produced more adventitious root compared to the WT indicating a negative role of JA in this developmental process [31]. Here, our *oscoi2* mutants also produced adventitious root on the above-ground nodes, a specific phenotype that has not been reported by Inagaki et al., (2023) and Wang et al., (2023) [29, 30]. In rice, adventitious root emergence is inducible by flooding and is tightly regulated by various hormones such as ethylene and auxin [32, 33]. Constitutive formation of adventitious roots from *oscoi2* nodes suggests that this non-canonical jasmonate receptor could be a central regulator of shoot-borne root development in rice.

Specific functions of *OsCOI2* could derive from (i) its spatial expression pattern being different from *OsCOI1a/b*, and/or (ii) distinct protein-protein interactions involving a subset of OsJAZs, and/or (iii) the perception of distinct jasmonate forms. For instance, the liverwort *Marchantia polymorpha* MpCOI1 receptor was shown to interact with distinct hormone forms that the eudicot Arabidopsis COI1 receptor did not bind [34]. Specifically, instead of perceiving JA-Ile, MpCOI1 binds the JA precursor dn-OPDA as an ancestral ligand to initiate jasmonate signalling [34]. In rice, despite of JA-Ile functions being established, OPDA- and distinct jasmonic acid-amino acid conjugates have been shown to be involved in defence response and could be additional ligands of *OsCOI* proteins [35, 36]. *OsCOI2* was shown to bind with different jasmonate derivatives and to interact with OsJAZ proteins with some selectivity compared to *OsCOI1a* and *OsCOI1b* JA receptors [29, 30]. This could explain the specificity of *OsCOI*s function.

In summary, we report that COI jasmonate receptor sub-functionalisation has occurred in rice, consistent with the hypothesis that different bioactive forms of jasmonate could modulate distinct responses to developmental cues and environmental stresses via *OsCOI1a/b* and *OsCOI2* perception. Our collection of *oscoi* mutant alleles offers a unique genetic resource for future dissection of the function of jasmonate receptor proteins in monocots.

Supporting information

S1 File.
(PDF)

Acknowledgments

We thank B.K. Pandey and M. Bennett for advice while drafting the manuscript. We thank E. Guillon and P. Serin for technical assistance with the IRD glasshouse work.

Author Contributions

Conceptualization: Antony Champion.

Formal analysis: Hieu Trang Nguyen, Mohamad Cheaib, Anne-Sophie Petitot.

Funding acquisition: Antony Champion.

Investigation: Hieu Trang Nguyen.

Methodology: Mohamad Cheaib, Marie Fournel, Maelle Rios, Anne-Sophie Petitot.

Resources: Marie Fournel, Maelle Rios.

Supervision: Anne-Sophie Petitot, Antony Champion.

Writing – original draft: Anne-Sophie Petitot, Antony Champion.

Writing – review & editing: Hieu Trang Nguyen, Mohamad Cheaib, Pascal Gantet, Laurent Laplace, Soazig Guyomarc'h, Michael Riemann, Thierry Heitz.

References

1. Yuan Z, Zhang D. Roles of jasmonate signalling in plant inflorescence and flower development. *Curr Opin Plant Biol.* 2015; 27: 44–51. <https://doi.org/10.1016/j.pbi.2015.05.024> PMID: 26125498
2. Lakehal A, Ranjan A, Bellini C. Multiple roles of jasmonates in shaping rhizotaxis: emerging integrators. *Methods Mol Biol.* 2020; 2085: 3–22. https://doi.org/10.1007/978-1-0716-0142-6_1 PMID: 31734913
3. Lyons R, Manners JM, Kazan K. Jasmonate biosynthesis and signaling in monocots: a comparative overview. *Plant Cell Rep.* 2013; 32: 815–827. <https://doi.org/10.1007/s00299-013-1400-y> PMID: 23455708
4. Schluttenhofer C. Origin and evolution of jasmonate signaling. *Plant Sci.* 2020; 298: 110542. <https://doi.org/10.1016/j.plantsci.2020.110542> PMID: 32771155
5. Xie DX, Feys BF, James S, Nieto-Rostro M, Turner JG. *COI1*: an *Arabidopsis* gene required for jasmonate-regulated defense and fertility. *Science.* 1998; 280: 1091–1094.
6. Chini A, Fonseca S, Fernández G, Adie B, Chico JM, Lorenzo O, et al. The JAZ family of repressors is the missing link in jasmonate signalling. *Nature* 2007; 448: 666–671. <https://doi.org/10.1038/nature06006> PMID: 17637675
7. Thines B, Katsir L, Melotto M, Niu Y, Mandaokar A, Liu G, et al. JAZ repressor proteins are targets of the SCF(COI1) complex during jasmonate signalling. *Nature.* 2007; 448: 661–665. <https://doi.org/10.1038/nature05960> PMID: 17637677
8. Yan Y, Stolz S, Chételat A, Reymond P, Pagni M, Dubugnon L, et al. A downstream mediator in the growth repression limb of the jasmonate pathway. *Plant Cell.* 2007; 19: 2470–2483. <https://doi.org/10.1105/tpc.107.050708> PMID: 17675405
9. Sheard LB, Tan X, Mao H, Withers J, Ben-Nissan G, Hinds TR, et al. Jasmonate perception by inositol-phosphate-potentiated COI1-JAZ co-receptor. *Nature.* 2010; 468: 400–405. <https://doi.org/10.1038/nature09430> PMID: 20927106
10. Pauwels L, Barbero GF, Geerinck J, Tilleman S, Grunewald W, Pérez AC, et al. NINJA connects the co-repressor TOPLESS to jasmonate signalling. *Nature.* 2010; 464: 788–791. <https://doi.org/10.1038/nature08854> PMID: 20360743

11. An L, Ahmad RM, Ren H, Qin J, Yan Y. Jasmonate signal receptor gene family *ZmCOIs* restore male fertility and defense response of *Arabidopsis* mutant *coi1-1*. *J Plant Growth Regul.* 2019; 38: 479–493.
12. Qi X, Guo S, Wang D, Zhong Y, Chen M, Chen C, et al. *ZmCOI2a* and *ZmCOI2b* redundantly regulate anther dehiscence and gametophytic male fertility in maize. *Plant J.* 2022; 10: 849–862.
13. Lee HY, Seo JS, Cho JH, Jung H, Kim JK, Lee JS, et al. *Oryza sativa* COI homologues restore jasmonate signal transduction in *Arabidopsis coi1-1* mutants. *PLoS One.* 2013; 8: e52802.
14. Yang DL, Yao J, Mei CS, Tong XH, Zeng LJ, Li Q, et al. Plant hormone jasmonate prioritizes defense over growth by interfering with gibberellin signaling cascade. *Proc Natl Acad Sci USA.* 2012; 109: 1192–1200.
15. Ye M, Song Y, Long J, Wang R, Baerson SR, Pan Z, et al. Priming of jasmonate-mediated antiherbivore defense responses in rice by silicon. *Proc Natl Acad Sci USA.* 2013; 110: 3631–3639. <https://doi.org/10.1073/pnas.1305848110> PMID: 24003150
16. Yang Z, Huang Y, Yang J, Yao S, Zhao K, Wang D, et al. Jasmonate signaling enhances RNA silencing and antiviral defense in rice. *Cell Host Microbe.* 2020; 28: 89–103. <https://doi.org/10.1016/j.chom.2020.05.001> PMID: 32504578
17. Chaw SM, Chang CC, Chen HL, Li WH. Dating the monocot-dicot divergence and the origin of core eudicots using whole chloroplast genomes. *J Mol Evol.* 2004; 58: 424–441. <https://doi.org/10.1007/s00239-003-2564-9> PMID: 15114421
18. Nguyen TH, Mai HTT, Moukouanga D, Lebrun M, Bellafiore S, Champion A. CRISPR/Cas9-Mediated Gene Editing of the Jasmonate Biosynthesis *OsAOC* Gene in Rice. *Methods Mol Biol.* 2020; 2085: 199–209.
19. Miao J, Guo D, Zhang J, Huang Q, Qin G, Zhang X, et al. Targeted mutagenesis in rice using CRISPR-Cas system. *Cell Res.* 2013; 23: 1233–1236. <https://doi.org/10.1038/cr.2013.123> PMID: 23999856
20. Yan J, Zhang C, Gu M, Bai Z, Zhang W, Qi T, et al. The *Arabidopsis* CORONATINE INSENSITIVE1 protein is a jasmonate receptor. *Plant Cell.* 2009; 21: 2220–2236.
21. Li L, Zhao Y, McCaig BC, Wingerd BA, Wang J, Whalon ME, et al. The tomato homolog of CORONATINE-INSENSITIVE1 is required for the maternal control of seed maturation, jasmonate-signaled defense responses, and glandular trichome development. *Plant Cell.* 2004; 16: 126–143. <https://doi.org/10.1105/tpc.017954> PMID: 14688297
22. Lee SH, Sakuraba Y, Lee T, Kim KW, An G, Lee HY, et al. Mutation of *Oryza sativa* CORONATINE INSENSITIVE 1b (*OsCOI1b*) delays leaf senescence. *J Integr Plant Biol.* 2015; 57: 562–576.
23. Lorbiecke R, Sauter M. Adventitious root growth and cell-cycle induction in deepwater rice. *Plant Physiol.* 1999; 119: 21–30. <https://doi.org/10.1104/pp.119.1.21> PMID: 9880342
24. Cao L, Tian J, Liu Y, Chen X, Li S, Persson S, et al. Ectopic expression of *OsJAZ6*, which interacts with *OsJAZ1*, alters JA signaling and spikelet development in rice. *Plant J.* 2021; 108: 1083–1096.
25. Zou X, Liu L, Hu Z, Wang X, Zhu Y, Zhang J, et al. Salt-induced inhibition of rice seminal root growth is mediated by ethylene-jasmonate interaction. *J Exp Bot.* 2021; 72: 5656–5672. <https://doi.org/10.1093/jxb/erab206> PMID: 33999128
26. Cai Q, Yuan Z, Chen M, Yin C, Luo Z, Zhao X, et al. Jasmonic acid regulates spikelet development in rice. *Nat Commun.* 2014; 5: 3476–3489. <https://doi.org/10.1038/ncomms4476> PMID: 24647160
27. Nguyen TH, Thi Mai To H, Lebrun M, Bellafiore S, Champion A. Jasmonates—the master regulator of rice development, adaptation and defense. *Plants.* 2019; 8: 339. <https://doi.org/10.3390/plants8090339> PMID: 31505882
28. Xiao Y, Chen Y, Charnikhova T, Mulder PP, Heijmans J, Hoogenboom A, et al. *OsJAR1* is required for JA-regulated floret opening and anther dehiscence in rice. *Plant Mol Biol.* 2014; 86: 19–33.
29. Inagaki H, Hayashi K, Takaoka Y, Ito H, Fukumoto Y, Yajima-Nakagawa A, et al. Genome Editing Reveals Both the Crucial Role of *OsCOI2* in Jasmonate Signaling and the Functional Diversity of COI1 Homologs in Rice. *Plant Cell Physiol.* 2023; 64: 405–421. <https://doi.org/10.1093/pcp/pcac166> PMID: 36472361
30. Wang X, Chen Y, Liu S, Fu W, Zhuang Y, Xu J, et al. Functional dissection of rice jasmonate receptors involved in development and defense. *New Phytol.* 2023; 238: 2144–2158. <https://doi.org/10.1111/nph.18860> PMID: 36869435
31. Gutierrez L, Mongelard G, Floková K, Pacurar DI, Novák O, Staswick P, et al. Auxin controls *Arabidopsis* adventitious root initiation by regulating jasmonic acid homeostasis. *Plant Cell.* 2012; 24:2515–2527. <https://doi.org/10.1105/tpc.112.099119> PMID: 22730403
32. Mergemann H, Sauter M. Ethylene induces epidermal cell death at the site of adventitious root emergence in rice. *Plant Physiol.* 2000; 124: 609–614. <https://doi.org/10.1104/pp.124.2.609> PMID: 11027711

33. Lin C, Sauter M. Polar auxin transport determines adventitious root emergence and growth in rice. *Front Plant Sci.* 2019; 10:444. <https://doi.org/10.3389/fpls.2019.00444> PMID: 31024605
34. Monte I, Ishida S, Zamarreño AM, Hamberg M, Franco-Zorrilla JM, García-Casado G, et al. Ligand-receptor co-evolution shaped the jasmonate pathway in land plants. *Nature Chem Biol.* 2018; 14: 480–488. <https://doi.org/10.1038/s41589-018-0033-4> PMID: 29632411
35. Shinya T, Miyamoto K, Uchida K, Hojo Y, Yumoto E, Okada K, et al. Chitooligosaccharide elicitor and oxylipins synergistically elevate phytoalexin production in rice. *Plant Mol Biol.* 2021; 109: 595–609. <https://doi.org/10.1007/s11103-021-01217-w> PMID: 34822009
36. Fu W, Jin G, Jiménez-Alemán GH, Wang X, Song J, Li S, et al. The jasmonic acid-amino acid conjugates JA-Val and JA-Leu are involved in rice resistance to herbivores. *Plant Cell Environ.* 2022; 45: 262–272. <https://doi.org/10.1111/pce.14202> PMID: 34661303