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Jasmonic acid regulates spikelet development in rice

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The spikelet is the basal unit of inflorescence in grasses, and its formation is crucial for reproductive success and cereal yield. Here, we report a previously unknown role of the plant hormone jasmonic acid (JA) in determining rice (*Oryza sativa*) spikelet morphogenesis. The *extra glume 1* (*eg1*) and *eg2* mutants exhibit altered spikelet morphology with changed floral organ identity and number, as well as defective floral meristem determinacy. We show that EG1 is a plastid-targeted lipase that participates in JA biosynthesis, and EG2/OsJAZ1 is a JA signalling repressor that interacts with a putative JA receptor, OsCOI1b, to trigger OsJAZ1's degradation during spikelet development. OsJAZ1 also interacts with OsMYC2, a transcription factor in the JA signalling pathway, and represses OsMYC2's role in activating *OsMADS1*, an E-class gene crucial to the spikelet development. This work discovers a key regulatory mechanism of grass spikelet development and suggests that the role of JA in reproduction has diversified during the flowering plant evolution.

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Rice (*Oryza sativa*) is a major staple food for half of the world population and a model plant to investigate evolutionary developmental biology in grasses, monocot plants that compose one of the largest families in angiosperms[1]. One unique feature of grasses is that each plant contains specialized spikelets, structural units of inflorescences that show great morphological diversity even within the grass family[2].

Compared with dicot plants, grasses evolved more types of specialized axillary meristems during reproduction. For instance, rice initiates branch meristem from the inflorescence meristems, produces spikelet meristem from inflorescence branches and converts spikelet meristem to floral meristem[3]. Each rice spikelet contains two pairs of leaf-like structures: rudimentary glumes and sterile lemmas, and a single fertile flower that consists of a lemma, a palea, two lodicules, six stamens and one pistil[4]. Rice spikelet morphogenesis is pivotal to the determination of inflorescence architecture and seed production.

In *Arabidopsis thaliana*, floral meristem arises from inflorescence meristem in response to multiple flowering signals, such as the phytohormone auxin[5]. Auxin-responsive transcription factor *MONOPTEROS* (*MP*) induces the expression of a floral meristem identity gene, *LEAFY* (*LFY*), which is also regulated by other flowering regulators including *FLOWERING LOCUS T* (*FT*), *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*), and *AGAMOUS-LIKE 24* (*AGL24*), resulting in the transition from inflorescence meristem to floral meristem[5,6]. *LFY* and *APETALA1* (*AP1*) induce each other's expression to initiate flower primordium emergence and morphogenesis[7,8]. Unlike their counterparts in *Arabidopsis*[9], rice *AP1*-like and *LFY*-like genes are involved in inflorescence meristem specification[10–12]. Instead, *OsMADS1*/*LEAFY HULL STERILE1* (*LHS1*), an E-class gene, functions similarly to *LFY* and *AP1* in determining floral meristem initiation and specification[13,14]. Loss-of-function of *OsMADS1* leads to reiterative formation of glumes or extra spikelet formation within spikelets, and defective organ identity[13,15]. In contrast to *Arabidopsis*, in which *APETALA2* (*AP2*) genes play a role in specifying floral organ identity[9], rice, maize and possibly other grasses use *AP2/ERF*-like genes to control spikelet meristem transition and deteminacy[16–19]. These findings suggest that grasses and dicots seem to use the same sets of genes in different aspects of meristem identity specification in reproduction.

Jasmonic acid (JA) and its derivatives are lipid-derived hormones that control plant defence response and developmental events, such as seed germination, root growth, tuber formation, tendril coiling, trichome initiation, reproduction and senescence[20–24]. Two *Arabidopsis* phospholipases, DONGLE (DGL) and DEFECTIVE IN ANTHER DEHISCENCE1 (DAD1), release α-linolenic acid (C18:3) from chloroplastic membrane lipids as the first step of JA biosynthesis[25,26]. Recent evidence showed that DGL and DAD1 are not essential for wound- or pathogen-induced JA biosynthesis and that other lipases may be involved in stress-induced JA production, suggesting that plants may use different lipases for jasmonate biosynthesis in various biological processes[27]. In the well-characterized *Arabidopsis* JA signalling pathway, JA is perceived by the receptor CORONATINE-INSENSITIVE1 (COI1), an F-box protein that activates the expression of JA-responsive genes by degrading the JAZ (jasmonate ZIM-domain) repressors[28–35]. Despite our understanding of JA biosynthetic and signalling pathways, relatively little is known about the precise functions of JA in plant (particularly crop) development.

In this study, we characterized, *extra glume 1* (*eg1*) and *eg2*, two rice mutants with aberrant spikelet morphology, and identified the *EG1* and *EG2* genes through fine-mapping. EG1 is a lipase in JA biosynthesis and EG2/OsJAZ1 is a repressor of the JA signalling pathway. We further showed that EG2/OsJAZ1 interacts with the putative JA receptor, OsCOI1b, and with the transcription factor OsMYC2, to repress OsMYC2's function in activating the E-class gene *OsMADS1* in spikelet development. This work reveals a unique role of JA in regulating rice spikelet development, which is to specify floral organ identity and floral meristem determinacy.

Results

***eg1-3* and *eg2-1D* exhibit abnormal spikelet morphology**. To understand the mechanism that controls rice spikelet development, we identified two spikelet morphological mutants with similar phenotypes, and named them *extra glumes* (*eg*) *1-3* and *eg2-1D*. Both mutants displayed defects in spikelet development, including extra glume-like structures between the sterile lemma and lemma and altered floral organ numbers and identities (Fig. 1a, Supplementary Fig. 1a–g and Supplementary Table 1). Genetic analyses suggested that *eg1-3* was caused by a single recessive mutation (wt:*eg1-3* = 78:27, $\chi^2 = 0.03$, $P > 0.05$, χ^2-test used), whereas *eg2-1D* was caused by a single dominant mutation (wt:*eg2-1D* = 30:87, $\chi^2 = 0.03$, $P > 0.05$, χ^2 test used).

To characterize the spikelet defects in *eg1-3* and *eg2-1D*, we performed detailed histological observations (Fig. 1b). The spikelets of *eg1-3* and *eg2-1D* contained palea that exhibited lemma-like characteristics, such as five vascular bundles, no mrps and opened flower hull (Fig. 1b and Supplementary Fig. 1c–g). In addition, both mutants displayed transformation of lodicules into glume–lodicule mosaic structures (Fig. 1a,b), and decreased number of stamens and pistil (Supplementary Table 1). To determine the genetic relationship between *EG1* and *EG2*, we constructed *eg1-3 eg2-1D* double mutant. The double mutant showed phenotypes similar to the two single mutants but to stronger degrees. It had lost floral organ identity, displaying more glume-like structures outside the lemma and palea, homeotic transformation of floral organs to glume-like structures and in some cases, extra spikelets with elongated pedicels at their centre (Fig. 1a,b and Supplementary Fig. 1h).

Further analysis using scanning electron microscopy revealed that *eg1-3* and *eg2-1D* had early developmental defects in spikelet. In wild-type spikelet, after the differentiation of a pair of sterile lemma primordium, the spikelet meristem was converted into floret meristem and produced one lemma and one palea primordium (Fig. 1c and Supplementary Fig. 2a). However, the spikelets of *eg1-3* and *eg2-1D* developed extra glume-like structures after the formation of sterile lemma primordium, which resulted in additional floral whorls (Fig. 1c,d and Supplementary Fig. 2b–h). Floral meristem determinacy was also markedly affected in *eg1-3* and *eg2-1D*, which had bulged meristem and ectopic floral organ primordium and ectopic glume-like structures (Fig. 1c and Supplementary Fig. 2b–h). Moreover, compared with the singe mutant, *eg1-3 eg2-1D* grew more ectopic floral primordium, glume-like structures or double floral meristem in one spikelet, suggesting defects in floral meristem indeterminacy (Fig. 1c and Supplementary Fig. 2i–l). Together, these results suggested that *EG1* and *EG2* may be functionally associated or even act in the same pathway that determines rice floral meristem determinacy and floral organ identity.

***EG1* encodes a lipase for JA biosynthesis**. The *EG1* gene was mapped to chromosome 1 between two In-Del markers, CQ1 and Os116 (Fig. 2a). Sequence analysis revealed that *eg1-3* contains a G-to-A substitution in the coding region of an annotated gene[36] (LOC_Os01g67430, http://www.gramene.org/), causing the change of Gly to Arg at the 216th amino acid (Fig. 2b). Besides

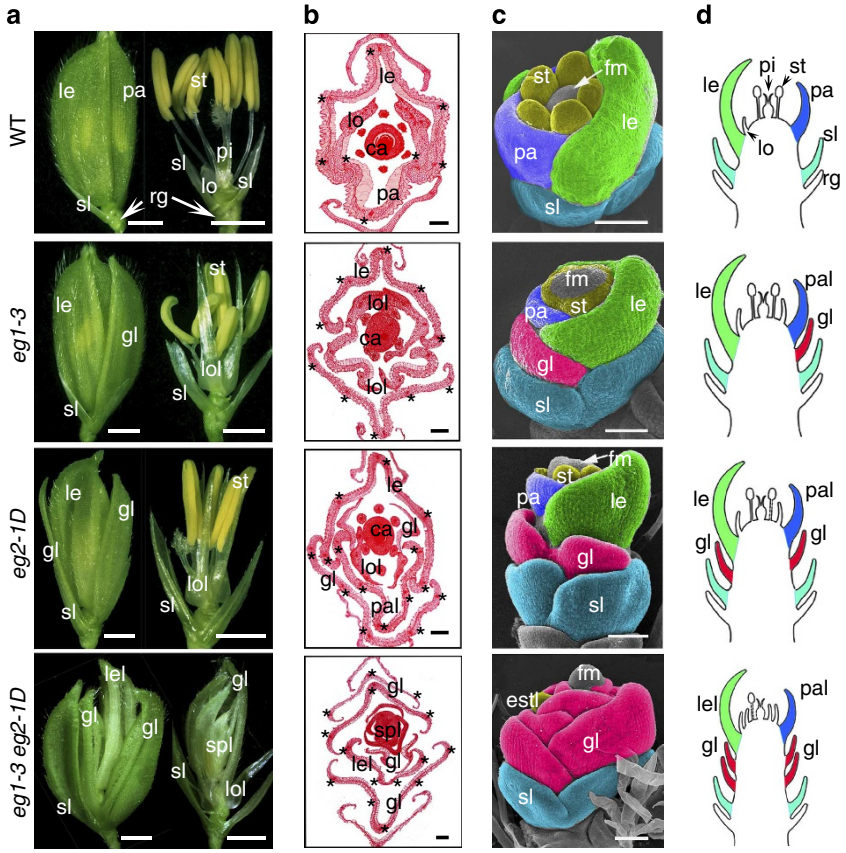

Figure 1 | Phenotypic analysis of spikelet development in mutants. (**a**) Rice spikelets. Scale bars, 1 mm. (**b**) Transverse paraffin sections of spikelets. Stars indicate vascular bundles. Scale bars, 100 μm. (**c**) Scanning electron microscopic pictures of floral organ primordium. Organ identities are indicated by pseudocolors: sterile lemma in navy blue, lemma in green, palea in blue, stamen in light yellow and ectopic glumes in red. Scale bars, 50 μm. (**d**) Diagrams of the spikelets, showing position and growth pattern of floral organs. estl, ectopic stamen-like; gl, glume-like; le, lemma; lel, lemma-like; lo, lodicule; lol, lodicule-like; pa, palea; pal, palea-like; pi, pistil; rg, rudimentary glume; sl, sterile lemma; spl, spikelet-like; st, stamen.

ubiquitous expression in seedlings, roots, shoot and leaves during vegetative growth, *EG1* also expressed at reproductive stage, in inflorescence meristem, spikelet meristem and floral organs (Supplementary Fig. 3), further supporting the role of *EG1* in spikelet development.

Genetic analysis demonstrated that *eg1-3* was allelic to *eg1-1* and *eg1-2* (Fig. 2c,d), two spikelet development mutants identified from a previous study, which showed that the stronger allele (*eg1-1*) grew ectopic floral organs at each organ whorl or in extra ectopic whorls, resulting in an indeterminate floral meristem[36]. This previous study identified EG1's role in rice spikelet development, yet the biochemical function of EG1 was not investigated. EG1 belongs to class I of the phospholipase A_1 family[36], in the same clade as the homologous *Arabidopsis* JA biosynthetic enzymes DGL and DAD1 (refs 25,26). To test whether EG1 is an enzyme in JA production, we overexpressed *EG1* cDNA in the *Arabidopsis dad1* mutant, and observed rescue of the male sterile and silique development phenotypes (Fig. 3a). Conversely, expression of $EG1_{pro}$:*DGL* in *eg1-3* also rescued the abnormal spikelet phenotypes (Fig. 3b). Thus, *EG1* is the rice ortholog of *DAD1* and *DGL*, functioning as a lipase in JA production during rice spikelet development.

Using the rice protoplasts and *Nicotiana benthamiana* leaf cells as protein expression systems, we observed localization of EG1-GFP (green fluorescent protein) in chloroplasts (Supplementary Fig. 4), where DAD1 and DGL function[25,26]. Measurement of endogenous JA levels showed that compared with the wild type, the total amount of free JA in *eg1-3* decreased 28, 32 and 54% in seedling, inflorescence and spikelets, respectively (Fig. 3c). In agreement with the previously reported function of JA in repressing root growth[24], root length of *eg1-3* was also increased (Supplementary Fig. 5). Exogenous application of MeJA rescued the abnormal spikelet (Fig. 3d,e) and longer root (Supplementary Fig. 5) phenotypes in the *eg1-3* mutant, further confirming that the spikelet development defect in *eg1-3* was caused by reduced content of JA resulted from the lack of a functional EG1 protein in JA biosynthesis.

EG2 is a repressor of JA response and interacts with OsCOI1b. The *EG2* gene was mapped to chromosome 4 between CQQ2 and CQQ3, two In-Del markers that define a 2.2 cM (centimorgan) genomic region (Fig. 4a). Sequence analysis identified a mutation in LOC_Os04g55920 (http://www.gramene.org/) in *eg2-1D* (Fig. 4b). We named the gene *OsJAZ1* because of its high sequence similarity with the *Arabidopsis* JAZ proteins. The mutation in *eg2-1D* was a C-to-G transition, resulting in a substitution of Ala by Gly in the fifth amino acid of the Jas domain (Fig. 4b), a motif known to be critical for JAZ's interaction with the JA receptor COI1 (refs 28–31).

To confirm that the mutated *OsJAZ1* is responsible for defective spikelet development in *eg2-1D*, a plasmid containing a 6.6-kb *OsJAZ1* genomic fragment amplified from *eg2-1D* ($OsJAZ1_{pro}$:*osjaz1-1D gDNA*) was introduced into the wild type and *eg1-3* (Fig. 4c–i). Transgenic lines exhibited abnormal spikelets similar to those in *eg2-1D* (Fig. 4c–e,i) and lines with

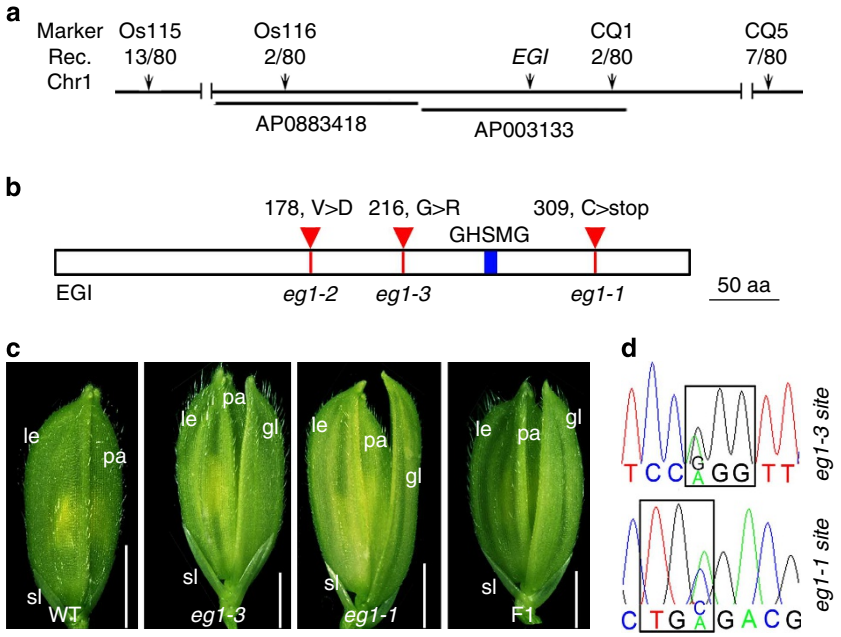

Figure 2 | Positional cloning of *EG1*. (**a**) The location of *EG1* on the BAC clone AP003133 on chromosome 1. (**b**) A schematic representation of the EG1 protein. The mutant alleles are indicated by red lines and triangles. The conserved lipase III domain (GMSMG) is indicated by a blue bar. (**c**) Spikelet morphology of the wild type, *eg1-3*, *eg1-1* and F_1 progeny from *eg1-3* x *eg1-1*. Scale bars, 1 mm. (**d**) Sequence analysis of the *EG1* gene in F_1 progeny, showing mutated nucleotides in the mutant alleles. gl, glume-like; le, lemma; pa, palea; sl, sterile lemma.

higher level expression of *osjaz1-1D* exhibited stronger phenotypes, that is, having multiple layers of glume-like structures in the spikelet (Fig. 4f,i). In addition, the *eg1-3 OsJAZ1*$_{pro}$*:osjaz1-1D gDNA* lines showed stronger phenotypes than *eg1-3*, mimicking the phenotype of *eg1-3 eg2-1D* double mutants (Fig. 4g–i). These results demonstrated that the abnormal spikelet development in *eg2-1D* was caused by the mutation in the Jas domain of *OsJAZ1*. *EG2* expressed ubiquitously in the plant, from roots, stems and leaves, to spikelets and seeds (Supplementary Fig. 6), overlapping with the expression pattern of *EG1*, further supporting the notion that these two proteins may function in the same pathway.

To investigate whether OsJAZ1 is involved in JA signalling, we treated the *eg2-1D*-mutant seedlings with MeJA. Unlike the wild type, the *eg2-1D* mutant was insensitive to jasmonate's inhibition of root growth (Supplementary Fig. 7), a phenotype similar to those reported for *Arabidopsis jai3-1* mutant that was defective in the *JAZ3* gene and transgenic lines overexpressing the *JAZ1* gene deleted for the Jas domain[28,32].

To further characterize the role of OsJAZ1 in JA response, we used yeast two-hybrid (Y2H) assays to test the interaction between OsJAZ1 and three rice COI1-like proteins: OsCOI1a, OsCOI1b and OsCOI1c[37]. OsJAZ1 and OsCOI1b interacted, and the interaction was dependent on JA-Ile's mimic, coronatine (COR), but not JA or MeJA (Fig. 5a). This JA-Ile-dependent interaction is similar to what was reported in *Arabidopsis* between JAZ proteins and COI1 (refs 28–32). No interaction was observed between OsJAZ1 and OsCOI1a or OsCOI1c (Fig. 5a). Furthermore, the Ala-to-Gly mutation in the Jas domain of OsJAZ1 in *eg2-1D* blocked osjaz1-1D-OsCOI1b interaction (Fig. 5b), suggesting that the Jas domain is required for the interaction between OsJAZ1 and OsCOI1b.

To test how OsJAZ1 affects spikelet development, we generated transgenic plants overexpressing OsJAZ1-GUS and osjaz1-1D-GUS fusion proteins, respectively. After 1-h treatment of 100 μM MeJA, GUS signals were greatly reduced in the *OsJAZ1-GUS* transgenic plants and the addition of 100 μM MG132, a specific inhibitor of the 26S proteasome, prevented JA-mediated elimination of the GUS signals (Fig. 5c and Supplementary Fig. 8). By contrast, GUS signals were stable after JA treatment in the *osjaz1-1D-GUS* transgenic lines (Fig. 5c and Supplementary Fig. 8), suggesting that the osjaz1-1D in *eg2-1D* rendered the protein resistant to JA-induced proteasome-mediated degradation. In addition, overexpression of *osjaz1-1D-GUS*, but not *OsJAZ1-GUS*, resulted in defective spikelet development in the wild type, similar to the phenotype of *eg1-3* and *eg2-1D* (Fig. 5d), suggesting that *OsJAZ1* is a key component of the JA signalling pathway during rice spikelet development and its regulation is similar to that of the *Arabidopsis* JAZ proteins.

JA pathway affects the expression of E-class genes. To uncover downstream components in the pathway by which JA regulates spikelet development in rice, we analysed the expression of possible target genes in the *eg1-3* and *eg2-1D* mutants. In rice, five E-class (*SEPALLATA*, *SEP*) genes (*OsMADS1, OsMADS5, OsMADS7, OsMADS8* and *OsMADS34*) play a key role in specifying inflorescence and spikelet development[38–40]. The *osmads1* mutant and *OsMADS1/5/7/8-RNAi* lines display spikelet defect similar to *eg1-3 eg2-1D* double mutant and some *osjaz1-1D* gain-of-function lines (Figs 1a and 4f)[15,38]. Thus, we analysed the expression of *OsMADS1*, *OsMADS5, OsMADS7, OsMADS8* and *OsMADS34* in the *eg1-3* and *eg2-1D* mutants by quantitative reverse transcriptase–PCR (qRT–PCR). The expression of *OsMADS1*, *OsMADS7* and *OsMADS8* was reduced in *eg1-3* and *eg2-1D* mutants at early stages of spikelet development (Fig. 6a), but no obvious changes were detected for *OsMADS5* and *OsMADS34* (Supplementary Fig. 9). In addition, *in situ* hybridization analysis of the *eg1-3* and *eg2-1D* mutants revealed that the expression signal of *OsMADS1* became much weaker in lemma and palea primordium and the expression of *OsMADS7* and *OsMADS8* was reduced in the floral meristem (Fig. 6b). Furthermore, expression analysis showed close correlation between higher expression level of *osjaz1-1D* and reduced expression level of *OsMADS1*, *OsMADS7* and *OsMADS8*

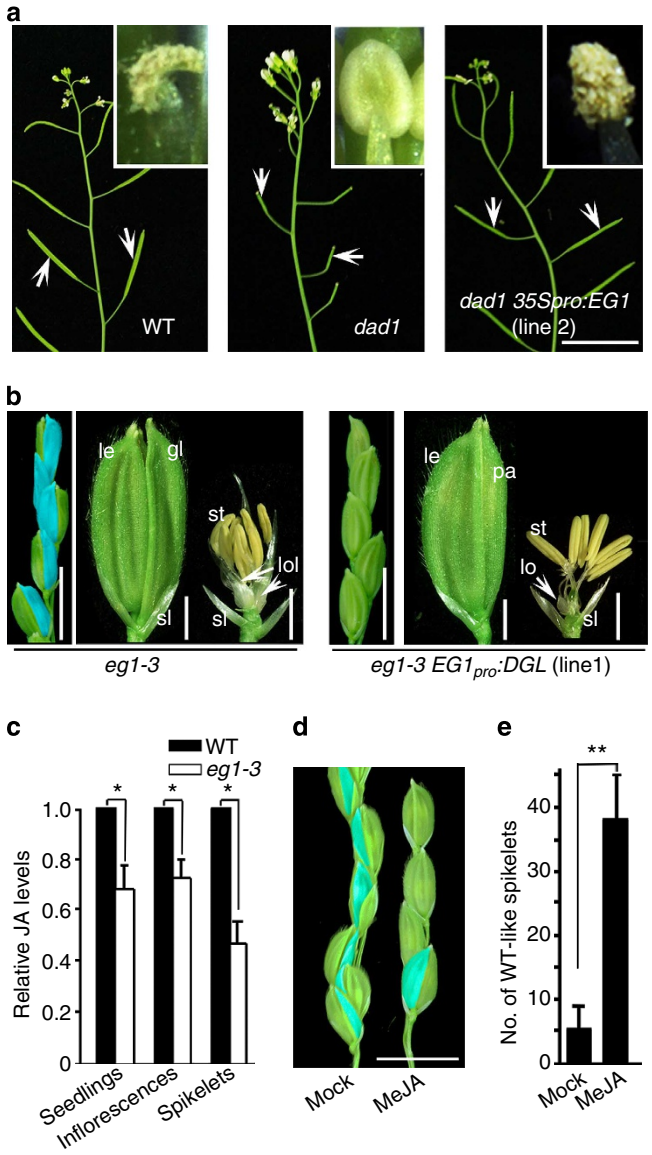

Figure 3 | EG1 is involved in JA production. (**a**) Overexpression of *EG1* in the *dad1* mutant rescued anther indehiscence (insets) and silique development (arrows) phenotypes. Scale bar, 1 cm. (**b**) The defects in *eg1-3* spikelets were rescued by expressing $EG1_{pro}$:*DGL*. Sky blue indicate extra glumes. Scale bars, 1 cm for the branches, 1 mm for spikelets. (**c**) Comparison of endogenous JA content in the wild type and *eg1-3*. The aerial part of 6-day-old seedlings, 2–4 mm long inflorescence and spikelets at late stage of Sp8 were used. Each data point is the average of three biological repeats, and error bars indicate s.d. Asterisks indicate significant difference between samples (Student's *t*-test *P*-values, $*P<0.05$; $**P<0.01$). (**d**) Exogenous application of MeJA rescued the abnormal spikelet development phenotype in *eg1-3*. Sky blue indicates extra glumes in *eg1-3* spikelets. Scale bar, 1 cm. (**e**) Quantification of wild type-like spikelets in the inflorescence after MeJA treatment in (**d**). Each data point is the average of 10 biological repeats, and error bars indicate s.d. Asterisks indicate significant difference between samples (Student's *t*-test *P*-values, $*P<0.05$; $**P<0.01$). gl, glume-like; le, lemma; lo, lodicule; lol, lodicule-like; pa, palea; sl, sterile lemma; st, stamen.

in $OsJAZ1_{pro}$:*osjaz1-1D gDNA* transgenic lines (Supplementary Fig. 10). These results suggest that JA regulates early spikelet development possibly by activating the expression of *OsMADS1*, *OsMADS7* and *OsMADS8*.

To check the genetic interaction between these *SEP*-like proteins and JA signalling pathway, we crossed *eg1-1* with the available mutant, *osmads1-z*[39]. Similar to the phenotype in *osmads1-z* and *OsMADS1/5/7/8-RNAi* lines, *eg1-1 osmads1-z* double mutant and *osmads1-z* mutant containing $OsJAZ1_{pro}$:*osjaz1-1D gDNA* showed elongated lemma and palea, and homeotic transformation of inner floral organs into glume-like structures (Fig. 6c,d). These data suggested that *OsMADS1* (and possibly *OsMADS7* and *OsMADS8*) is downstream from the action of EG1 and OsJAZ1 in the JA pathway.

OsMYC2 directly regulates *OsMADS1* expression. The *Arabidopsis* basic helix–loop–helix transcription factor MYC2 is a crucial activator of JA response genes[28,41,42]. To determine whether rice MYC2 homologues are involved in JA signalling in spikelet development, we cloned the only rice homologue of *Arabidopsis* MYC2, OsMYC2 (ref. 43), which was highly expressed in spikelet and floral organs (Supplementary Fig. 11), and tested its interaction with OsJAZ1 using Y2H assays. Both OsJAZ1 and osjaz1-1D interacted with OsMYC2, and the interaction required the Jas domain, as the deletion of this domain abolished the interaction (Fig. 7a,b). Bimolecular fluorescence complementation assays and pull-down assays also showed that OsMYC2 interacted with full-length OsJAZ1, and the mutation in the Jas domain in osjaz1-1D did not affect this interaction (Fig. 7c,d). These results demonstrate that OsMYC2 may function as a direct target of OsJAZ1 in spikelet development.

The basic region of the *Arabidopsis* MYC2 protein binds to the G-box motif (5′-CACGTG/CACATG-3′) in the promoters of MYC2 target genes[42]. There are five G-box motifs in the *OsMADS1* genomic region; G1 and G2 are in the promoter and G3, G4, and G5 are in the first intron (Fig. 8a). To determine whether *OsMADS1* is a target of OsMYC2 in transcription, we produced antibody against OsMYC2 (Methods) and performed chromatin immunoprecipitation (ChIP)-PCR assays. In wild-type spikelet, G1 and G2, but not G3, G4 or G5, can be amplified from immunoprecipitation pulled down by the anti-OsMYC2 antibody (Fig. 8b). Yeast one-hybrid assays and DNA-binding assays further revealed that OsMYC2 was able to bind to the G2 region but not G1 in the *OsMADS1* promoter (Fig. 8c,d). Furthermore, dual-luciferase (dual-LUC) assays showed that the expression level of luciferase driven by the *OsMADS1* promoter was transiently increased by OsMYC2, but this increase was abolished when OsJAZ1 was co-expressed (Fig. 8e), indicating that OsJAZ1 interacts with OsMYC2 and suppresses the activity of OsMYC2 in triggering the transcription of *OsMADS1*. These results suggested that OsMYC2 directly binds to the *OsMADS1* promoter, at least through G2, and thus it directly regulates the expression of *OsMADS1*.

Discussion

The grass spikelet displays diverse morphologies among taxa, and its structure is a key determinant of grain production, yet the developmental and genetic mechanisms underlying this great diversity remain largely unclear. In this work, we discovered a novel regulatory mechanism in rice spikelet development that is dependent on JA (Fig. 9).

Results from genetic complementation and JA measurements suggested that, like its orthologs in *Arabidopsis*, rice EG1 is an important enzyme in JA biosynthesis. However, *EG1*'s expression does not seem to be induced by wounding (Supplementary Fig. 12), which differs from its *Arabidopsis* conterparts[25,26]. Based on these data, we propose that EG1 is largely if not exclusively dedicated to JA production in rice development and not in

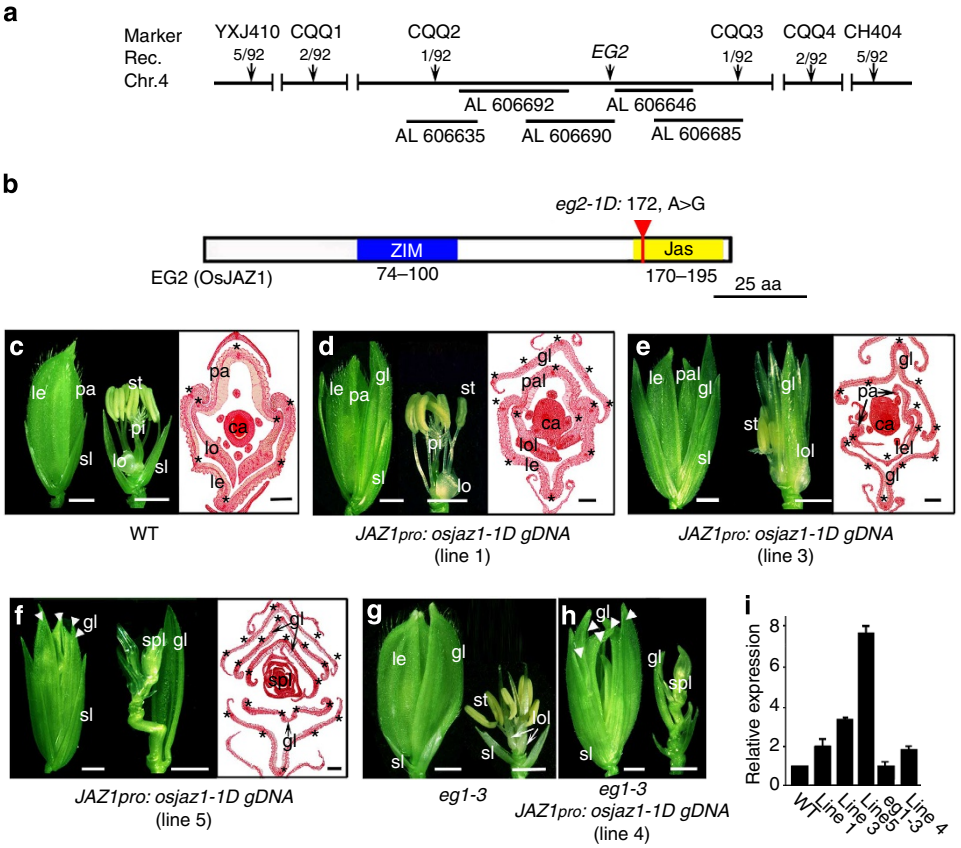

Figure 4 | Positional cloning of *EG2* (*OsJAZ1*). (**a**) The location of *EG2* (*OsJAZ1*) on chromosome 4. (**b**) A schematic representation of the EG2 protein. (**c**–**h**) Spikelets of transgenic lines expressing $OsJAZ1_{pro}$:*osjaz1-1D gDNA* in the wild-type (**d**–**f**) and *eg1-3* (**h**) background. Arrowheads indicate multiple layers of glumes. Transverse paraffin section of spikelets is shown at the right of each panel. Stars indicate vascular bundles. Scale bars, 1 mm in the spikelet images, 100 μm in the transverse paraffin sections. (**i**) Quantitative analysis of the expression of *OsJAZ1/osjaz1-1D* in spikelets in (**c**–**h**). Inflorescences were 3–4 mm, corresponding to stage Sp6 in the wild type. The expression level of *OsJAZ1* in the wild type was set as 1. Each data point represents the average of three biological repeats, and error bars indicate s.d. gl, glume-like; le, lemma; lo, lodicule; lol, lodicule-like; pa, palea; pal, palea-like; pi, pistil; spl, spikelet-like; st, stamen.

wounding response. The fact that the endogenous JA level in *eg1-3* mutant decreased ~50% compared with the wild type suggests that there might be unidentified genes encoding JA producing enzymes in rice spikelet development.

The role of JA in rice spikelet development is confirmed by the identification of the mutant of *EG2/OsJAZ1*, which encodes a protein homologous to the *Arabidopsis* JAZ proteins. Biochemical and genetic evidence in this study uncovered the role of JA in promoting the degradation of OsJAZ1 via the OsCO1b-mediated proteasome pathway. This mode of action for OsJAZ1 is similar to that of the *Arabidopsis* JAZ proteins[28–32]. No protein–protein interaction between OsJAZ1 and OsCOI1a or OsCOI1c was observed, possibly because that OsCOI1a and OsCOI1c interact with other JAZ proteins and regulate JA's action in other biological events. Rice genome contains 15 JAZ members and the expression of *OsJAZ1* and eight other JAZ genes was detected in rice spikelet at stage Sp4 (Supplementary Fig. 13), suggesting the possible redundant role of JAZ members in rice spikelet development, similar to the redundant role of *Arabidopsis* JAZ proteins[24].

Besides the spikelet defects, both *eg1-3* and *eg2-1D* exhibit phenotypes during vegetative development, such as longer roots and larger leaves in seedlings (Supplementary Fig. 14), confirming the previously reported role of JA in inhibiting vegetative organ growth[24]. However, *eg1-3* and *eg2-1D* showed no obvious changes in the height and leaf size of adult plants and inflorescence branch development, possibly due to functional redundancy of other JA biosynthetic and signalling genes.

Phenotypes of *eg1-3* and *eg2-1D* in spikelet morphology clearly differ from that of JA defective mutants reported in other species[21]. For example, *Arabidopsis* mutants impaired in JA biosynthesis and perception, such as *OPDA reductase 3* (*opr3*), *dad1*, *allene oxide synthase* (*aos*), *fatty acid desaturation* (*fad*) *3-2 fad7-2 fad8* and *coi1*, exhibit male sterility caused by insufficient filament elongation, non-viable pollen and delayed anther dehiscence[21]. In contrast to the male sterile phenotype of *Arabidopsis coi1* mutant[44], mutants of the tomato *COI1* homologue are female sterile with defective maternal control of seed maturation[45], indicating that JA signalling plays distinct roles in flower development in *Arabidopsis* and tomato. In maize (*Zea mays*), *Tasselseed1* (*TS1*) encodes a plastid-targeted lipoxygenase with predicted 13-lipoxygenase activity and participates in JA biosynthesis[46,47]. Two maize oxophyto-dienoate reductase genes, *OPR7* and *OPR8*, were shown to participate in JA production[48]. Both *ts1* and *opr7 opr8* mutants convert staminate to pistillate in tassel inflorescence, indicating that JA is required for male sex determination and suppression of female reproductive organ biogenesis in maize[46–48]. Based on these results from other plant species and our own data, we propose that the conserved JA biosynthetic and signalling pathways play an important role in distinct aspects of reproductive development in various plant species, targeting different downstream genes.

Control of the spikelet meristem transition and determinacy is a key event during grass inflorescence morphogenesis, and

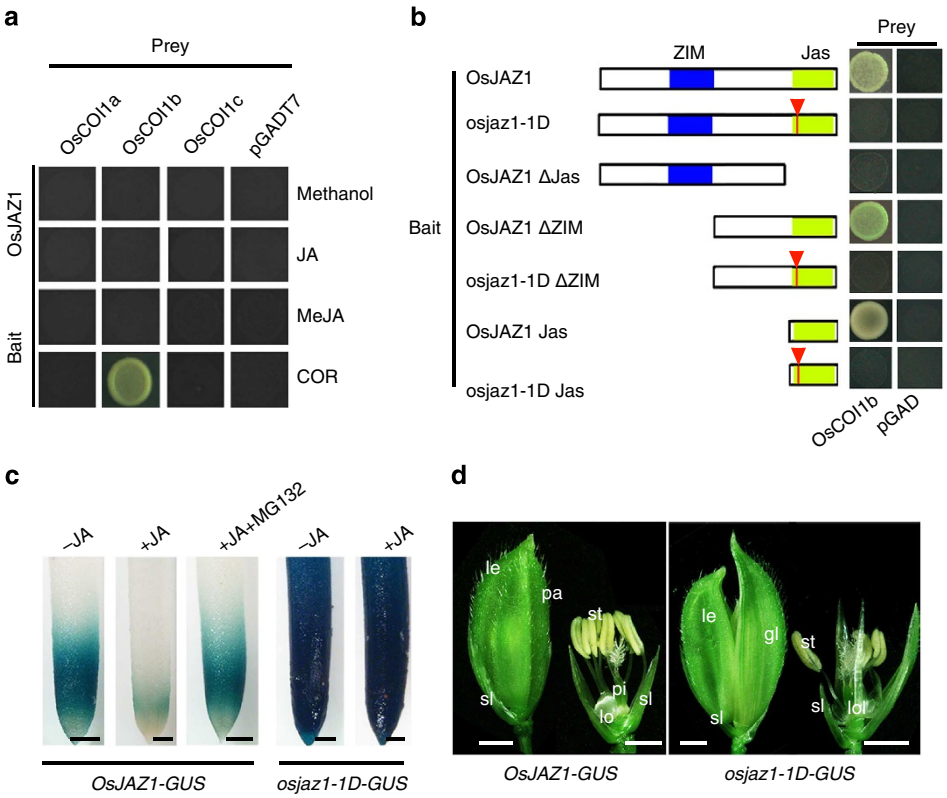

Figure 5 | *OsJAZ1* is involved in JA signalling and acts as a repressor of spikelet development. (**a**) Y2H assays to test interaction between OsJAZ1 and three rice COI1 homologues in the presence of JA derivatives. (**b**) Y2H analysis showing the coronatine (COR)-dependent OsJAZ1-OsCOI1b interaction. Red and red line triangle indicates the mutation in *osjaz1-1D*. (**c**) JA-dependent proteasome-mediated degradation of OsJAZ1-GUS and osjaz1-1D-GUS in roots. MG132, a proteasome inhibitor. Scale bars, 1 mm. (**d**) Spikelets of transgenic lines expressing $35S_{pro}$:*OsJAZ1-GUS* and $35S_{pro}$:*osjaz1-1D-GUS* in wild-type background. Scale bars, 1 mm. gl, glume-like; le, lemma; lo, lodicule; lol, lodicule-like; pa, palea; pi, pistil; sl, sterile lemma; st, stamen.

successful formation of floral organs and fertile flowers determines grain production. To date, several proteins have been identified to restrict the development of axillary meristems within the spikelet meristem and subsequently specify meristem identity. These proteins include the grass ethylene response factor transcription factors, such as maize BRANCHED SILKLESS 1 (BD1) and rice FRIZZY PANICLE (FZP), AP2 transcription factors such as maize INDETERMINATE SPIKEKET 1 (IDS1), SISTER of INDETERMIATE SPIKELET 1 (SID1), rice SUPERNUMERARY BRACT (SNB), OsIDS1 and MULTI-FLORET SPIKELET 1 (MFS1), and *Brachypodium distachyon* MORE SPIKELETS[49]. However, the roles of *AP2* gene products in the specification of spikelet meristem fate in monocot are distinct from that of their homologues in *Arabidopsis*, which is to specify floral organ identity[9]. Furthermore, LONG STERILE LEMMA (G1), a member of a plant-specific gene family that contains proteins with an uncharacterized ALOG (*Arabidopsis* LSH1 and Oryza G1) domain, specifies rice spikelet morphology by determining sterile lemma identity[50]. TONGARI-BOUSHI 1 (TOB1), a YABBY protein, controls proper lateral organ development and spikelet meristem maintenance in a non-cell-autonomous manner[51]. Whether the JA pathway interacts with these genes in regulating rice spikelet development remains to be elucidated.

In the past decade, investigations suggest that the classical ABCDE model generated from studies of dicots, including *Arabidopsis*, *Petunia hybrida* and *Antirrhinum majus,* is partially applicable to grasses such as rice and maize[52]. Grasses evolved diversified E-class (*SEPALLATA* [*SEP*]) genes[52]. Rice has at least five *SEP* members: *OsMADS1/LEAFY HULL STERILE1* (*LHS1*), *OsMADS5*, *OsMADS7*, *OsMADS8* and *OsMADS34* (ref. 52). The *lhs1/osmads1* mutant displays changed floral meristem identity[13]. Transgenic plants silenced for both *OsMADS7* and *OsMADS8* show late flowering, floral organ homeotic transformations and floral indeterminacy[38]. *OsMADS34/PANICLE PHYTOMER2* (*PAP2*) regulates the development of inflorescences and spikelets[39,40]. Emerging evidence suggests that ancient *AGAMOUS-LIKE6* (*AGL6*)-like genes conserved in gymnosperms and angiosperms, such as rice *OsMADS6/MOSAIC FLORAL ORGANS1* (*MFO1*)[53–55] and maize *zea agamous3* (*zag3*)[56], the close homologues of *SEP*-like genes, have *SEP*-like functions essential for flower development. In this study, we show a previously unreported JA pathway that regulates the expression of *OsMADS1*, *OsMADS7* and *OsMADS8* in rice spikelet development, linking the upstream JA pathway to the floral meristem identity genes (Fig. 9). In this pathway, OsMYC2, the only rice homologue of *Arabidopsis* MYC2 (ref. 43), binds to the G2 site in the promoter of *OsMADS1*. The phenotype of *eg1-3 eg2-1D* double mutant resembles that of *OsMADS1/5/7/8*-RNAi lines[38] and the *eg1-1 Osmads1-z* double mutant. That the *eg1-3* or *eg2-1D* single mutant displays more moderate phenotypes compared with that of *OsMADS1/5/7/8*-RNAi transgenic plants may be due to the redundant role of other JA pathway genes besides *EG1* and *EG2*, and/or the presence of other signalling pathway(s) that control the expression of *OsMADS1/7/8* during rice spikelet development. Whether JA signalling regulates other genes in spikelet development remains to be elucidated in the future.

In summary, we present a role of JA in regulating rice floral meristem determinacy and floral organ identity (Fig. 9). The mechanism discovered in this study may be applicable to many other grasses with similar spikelet structure, including species in

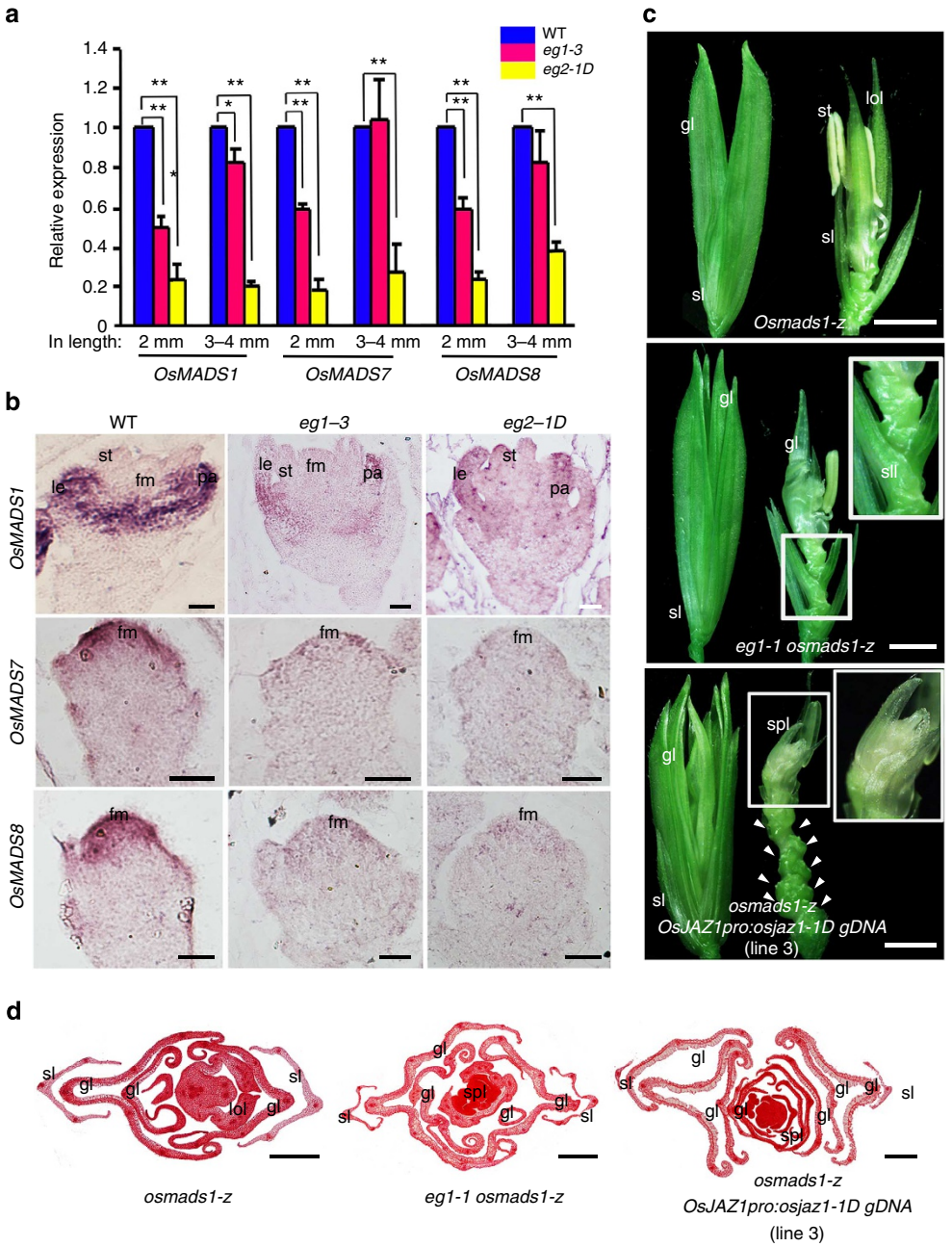

Figure 6 | JA signalling regulates the expression of three E-class genes in spikelets. (**a**) Quantitative analysis of *OsMADS1*, *OsMADS7* and *OsMADS8* transcripts in 2 mm (stage Sp4 in wild type) and 3–4 mm (stage Sp6 in wild type) inflorescences in the wild type, *eg1-3* and *eg2-1D*. *Actin* expression in each sample was used as a control. The expression level in the wild type was set as 1. Each data point represents the average of three biological repeats, and error bars indicate s.d. Asterisks indicate significant difference between samples (Student's -test *P*-values, $*P<0.05$; $**P<0.01$). (**b**) *In situ* analysis of the expression of three *OsMADS* genes in wild-type, *eg1-3* and *eg2-1D* spikelets. Scale bars, 25 μm. (**c**) Spikelet phenotypes of *osmads1-z*, *eg1-1 osmads1-z* and *osmads1-z OsJAZ1*$_{pro}$*:osjaz1-1D gDNA*. Insets are magnified images of the boxed regions to show spikelet-like structure formed in the centre of the spikelet. Arrowheads indicate the position of the extra whorls of glumes (which had been removed before images were taken) in the spikelet. Scale bars, 1 mm. (**d**) Transverse paraffin section of wild-type and mutants spikelets in (**c**). Scale bars, 100 μm. fm, floral meristem; gl, glume-like; in, inflorescences; le, lemma; lol, lodicule-like; pa, palea; sl, sterile lemma; sll, sterile lemma-like; spl, spikelet-like; st, stamen.

Oryza, *Sorghum* and *Panicum*, and provides basis to efforts aimed at improving crop yield.

Methods

Plant growth conditions and analyses of spikelet development. The strains *eg1-3*, *eg2-1D* and *osmads1-z* were in background of 9522 (wild type, *O. sativa* L. ssp. *japonica*), *eg1-1* was in *Zhefu 802* background (wild type, *O. sativa* L. ssp. *indica*). *eg1-3* and *eg2-1D* mutants were isolated by screening an ethyl-methane sulphonate-induced mutant library. *osmads1-z* was from our previous work[39]. *eg1-1* was kindly provided by Prof. Y.B. Xue[36]. The *dad1* mutant (Salk_025432, in Col-0 background) was obtained from the *Arabidopsis* Biological Resource Center (http://www.arabidopsis.org/). All rice plants were grown in the isolated paddy field in Shanghai Jiao Tong University in the summer.

The development stages of rice spikelet were defined by Ikeda *et al.*[57]. The histological, transverse section and scanning electron microscopic analyses were performed as described by Li. *et al.*[55].

Generation of transgenic lines. To generate transgenic *EG1*$_{pro}$*:GUS* (β-glucuronidase) lines, the 2-kb *EG1* promoter region was cloned into *pCAMBIA1301*

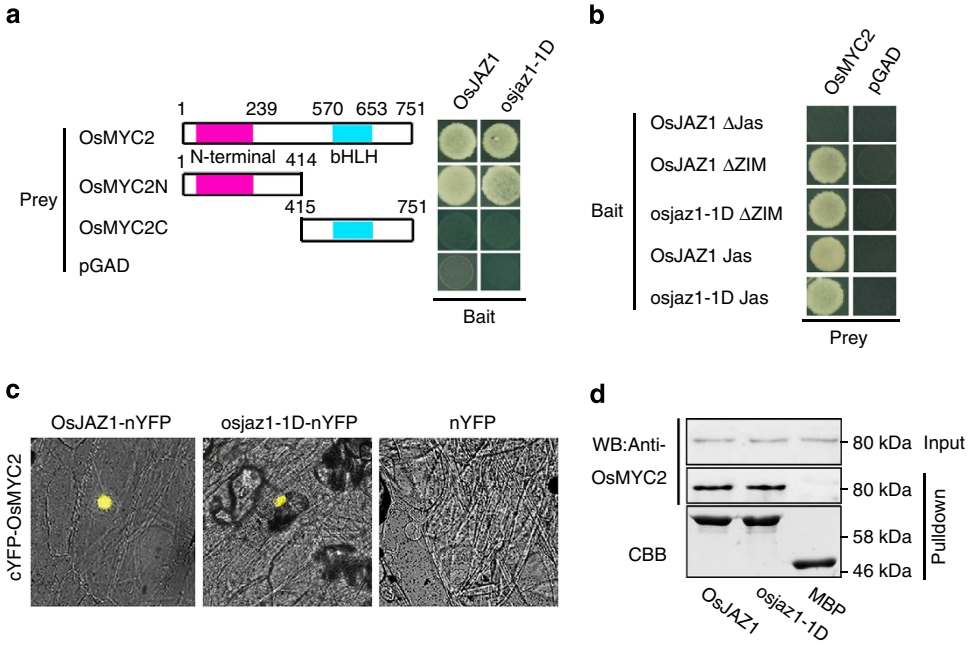

Figure 7 | Interaction of OsJAZ1/osjaz1-1D and OsMYC2 *in vitro* and *in vivo*. (**a**) Y2H analysis showing the requirement of the N-terminal conserved region of OsMYC2 for its interaction with OsJAZ1/osjaz1-1D. (**b**) Y2H analysis showing the requirement of the Jas domain in OsJAZ1/osjaz1-1D for its interaction with OsMYC2. (**c**) Bimolecular fluorescence complementation assays showing the interaction between OsMYC2 and OsJAZ1/osjaz1-1D. (**d**) *In vitro* pull-down assays showing the interaction between OsMYC2 and OsJAZ1/osjaz1-1D. The precipitated OsMYC2 was detected by anti-OsMYC2 antibody. Molecular weight of proteins, OsMYC2 = 81 kDa, OsJAZ1/osjaz1-1D = 65 kDa, MBP = 51 kDa. WB, western blot, CBB, Coomassie brilliant blue staining.

vector to fuse with GUS. To generate the $OsJAZ1_{pro}$:*osjaz1-1D gDNA* transgenic lines, a 6.6-kb genomic sequence containing the 3.0-kb promoter and 3.6-kb genomic sequence of the mutated *EG2* gene from the *eg2-1D* mutant was cloned into *pCAMBIA1301*. To generate $35S_{pro}$:*OsJAZ1-GUS* and $35S_{pro}$:*osjaz1-1D-GUS* lines, *OsJAZ1* and *osjaz1-1D* cDNAs were amplified from wild-type and *eg2-1D*-mutant background, respectively, and then cloned into p*CAMBIA1301*. To generate $35S_{pro}$:*EG1* lines, the cDNA of *EG1* was cloned into the *PHB* vector (kindly provided by Prof. H.Q.Yang). To generate $EG1_{pro}$:*DGL* lines, the 2-kb *EG1* promoter region and cDNA of *DGL* were cloned into p*CAMBIA1301*. See Supplementary Table 2 for all the primers used for making the constructs. All transgenic plants of rice and *Arabidopsis* were generated by Agrobacterium-mediated transformation and confirmed by PCR genotyping and qRT–PCR (Supplementary Fig. 15a–h).

Map-based cloning of *EG1* and *EG2*. To map *EG1*, crosses were made between *eg1-3* and Guang-lu-ai 4 (wild type, *O. sativa L. ssp. indica*). Plants with abnormal spikelet phenotypes were selected from the F_2 populations for mapping. For mapping of *eg2-1D*, crosses were made between *eg2-1D* and 9311 (wild type, *O. sativa L. ssp. indica*), and the wild type-like plants in the F_2 populations were used for mapping. In-Del molecular markers (Primers are described in Supplementary Table 2) were designed based on the sequence difference between *japonica* and *indica* genomes.

Rice protoplast transformation. Rice protoplast transformation was performed by using polyethylene glycol (PEG)-mediated transfections as described by Bart *et al.*[58]. Stem tissues of seedlings (14 days after germination) were cut into 0.5 mm pieces and incubated in enzyme solution (0.6 M mannitol, 10 mM MES, pH 5.7, 1.5% cellulase RS, 0.75% macerozyme, 0.1% BSA, 1 mM $CaCl_2$) for 4 h in the dark under shaking (40 r.p.m.). After the removal of the enzyme solution, tissues were incubated in W5 solution (154 mM NaCl, 125 mM $CaCl_2$, 5 mM KCl, 2 mM MES, pH 5.7) for 1 h under shaking (40 r.p.m.) to release protoplasts. After incubation, the incubated mixture was passed through a 35 μm nylon mesh filter, mixed with one volume of W5 solution and then centrifuged for 5 min at 1500 r.p.m. to pellet the protoplasts. Cells were re-suspended in Mmg solution (0.6 M mannitol, 15 mM $MgCl_2$, 4 mM MES, pH 5.7) for PEG-mediated transformation at 10^6 cells per ml. For transformation, 40% PEG (0.6 M mannitol, 100 mM $CaCl_2$, 40% v/v PEG 4000) and 10 μg plasmid were added to the protoplasts for 15 minutes at room temperature. Cells were washed 1 × with 10 volumes of W5 and then re-suspended in W5 solution at 28 °C in the dark overnight.

Protein subcellular localization analysis. $35S_{pro}$:*EG1-GFP* (full-length *EG1* cDNA fused with GFP) was generated in *pCAMBIA1301* (primers are described in Supplementary Table 2) and expressed in rice protoplasts using PEG-mediated transfections.

The localization of EG1-GFP was analysed in *Nicotiana benthamiana* (tobacco) leaves after agroinfiltration[59]. Briefly, overnight cultured *Agrobacteria* containing the plasmid were collected and re-suspended in Murashige and Skoog liquid medium that contained 10 μM MES (pH 5.6) and 200 μM Acetosyringone to $OD_{600} = 0.6$, and then incubated for 3 h at room temperature before being infiltrated into the young leaf of tobacco. Before images were taken, the plants were grown for about 48 h under weak light conditions.

Images were captured by a Confocal Laser Scanning Microscope equipped with an argon/krypton laser (Leica TCS SP5). GFP fluorescent and chlorophyll autofluorescent signals were imaged at the excitation wavelength of 488 nm, and emission wavelength of 505–530 nm and 650–798 nm, respectively.

Endogenous JA measurement and MeJA treatment. Endogenous JA was measured according to the method by Fu *et al.*[60]. Briefly, about 200 mg fresh weight of plant tissue from the wild type and *eg1-3* were homogenized, and endogenous JA was extracted for 24 h in methanol, with $^2H_5$-JA (CDN Isotopes) added as internal standard. JA levels were analysed by a LC/MS/MS system, which consisted of an Acquity Ultra Performance Liquid Chromatograph (Acquity UPLC; Waters) and a triple quadruple tandem mass spectrometer (Quattro Premier XE; Waters). Three biological replicates were performed for each group.

MeJA treatment of rice spikelet was performed 2 weeks before inflorescence initiation. MeJA (95%; Sigma-Aldrich) was dissolved in 0.05% aqueous Tween-20 to 50 μM, and then added into the soil medium in which *eg1-3* plants had been growing. For seedling treatment, 3-day-old seedlings of 9522, *eg1-3* and *eg2-1D* were transferred to 1/2 Murashige and Skoog medium containing 1 or 10 μM MeJA, and grew for 5 days before harvesting.

Gene expression analyses. For qRT–PCR analysis, total RNA was isolated with Plant Total RNA Mini Kit (TianGen), and first strand cDNA was synthesized from 1 μg of total RNA with M-MLV reverse transcriptase (PrimeScript RT reagent Kit, Takara). qRT–PCR was performed with the CFX384 real-time PCR detection system (Bio-Rad) using the SYBR Green mix (Bio-Rad) (Primers are described in Supplementary Table 2).

For RNA *in situ* hybridizations, digoxygenin-labeled antisense and sense probes of *EG1*, *OsMADS1*, *OsMADS7* and *OsMADS8* were transcribed *in vitro* using the *in vitro* transcription kit according to the user manual (ACCU-CHEK).

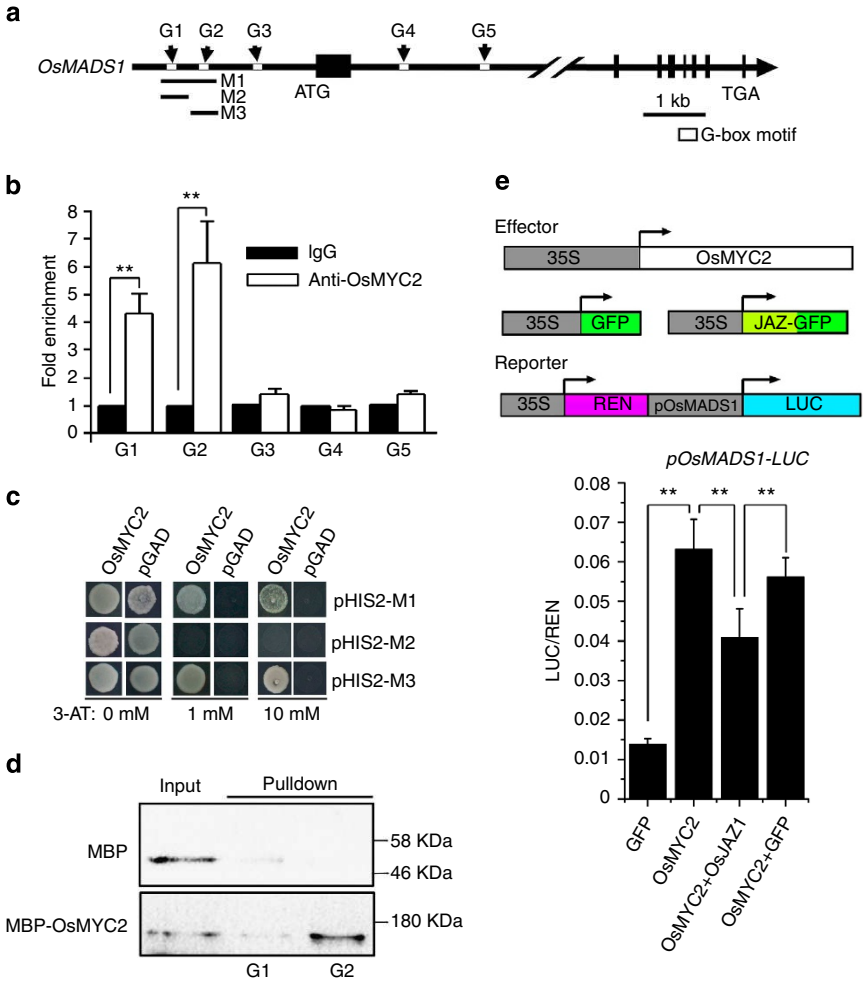

Figure 8 | OsMYC2 regulates the transcription of *OsMADS1*. (**a**) Diagram of the *OsMADS1* genomic region showing the five G-box motifs, G1 to G5. Black boxes are exons. (**b**) qChIP-PCR results showing that the promoter fragments G1 and G2 can be amplified from IP pulled down by the anti-OsMYC2 antibody. In the control, immunoglobulin G was set as 1. Each data point represents the average of three biological repeats, and error bars indicate s.d. Asterisks indicate significant difference between samples (Student's *t*-test *P*-values, $*P<0.05$; $**P<0.01$). (**c**) Yeast one-hybrid analysis showing interaction between OsMYC2 and the M2 region of *OsMADS1* promoter. M1, M2, M3 are shown in (**a**). 3-amino-1,2,4-triazole, 3-amino-1,2,4-triazole. (**d**) DNA-binding assays showing OsMYC2's binding to G2 in the promoter of *OsMADS1*. The precipitated OsMYC2 was detected by anti-OsMYC2 antibody. Molecular weight of proteins, MBP-OsMYC2 = 123 kDa, MBP = 51 kDa. (**e**) Transient dual-LUC reporter gene assays showing OsJAZ1's ability to inhibit OsMYC2's transcription activity on the *OsMADS1* promoter. Constructs used in the transient transactivation assays are shown in the upper panel. Five biological repeats were performed for each sample, error bars indicate s.d. Asterisks indicate significant difference between samples (Student's *t*-test *P*-values, $*P<0.05$; $**P<0.01$). There was no significant difference between OsMYC2 + GFP and OsMYC2 (Student's *t*-test *P*-values, $P>0.05$).

Floral primordia of young inflorescences were fixed using formalin/acetic acid/alcohol fixative solution at 4 °C overnight, dehydrated and embedded in Paraplast Plus paraffin (Sigma). *In situ* hybridization procedures used were from Li *et al.*[55]. Sequences of the probes used, that is, *EG1*, *OsMADS1*, *OsMADS7* and *OsMADS8*, were from previous reports[36,39,55]. Images were captured with a Leica DM750M fluorescence microscope.

Procedure for GUS staining of $EG1_{pro}$:*GUS* lines was modified from a previously published protocol[61]. Samples were incubated in GUS staining solution (50 mM $NaPO_4$ buffer, pH 7.0, 10 mg ml^{-1} X-Gluc and 0.02% (v/v) Triton X-100), at 37 °C in the dark. After staining, samples were washed with 70% ethanol at room temperature. The GUS staining images were taken by a Leica MZ8 dissecting microscope.

Detection of protein degradation *in vivo*. Transgenic $35S_{pro}$:*OsJAZ1-GUS* and $35S_{pro}$:*osjaz1-1D-GUS* plants were treated with MeJA (100 μM) with or without the proteasome inhibitor MG132 (100 μM) (MERCK) for 1 h. Histochemical GUS staining of roots was performed as described previously[61], and results were observed under a Leica MZ8 dissecting microscope. The quantity of the GUS-fusing protein was also detected by western blot using anti-β glucuronidase (GUS) antibody (Abcam, 1:2,000 dilution).

Yeast one/two-hybrid assays. For Y2H assays, the full-length or specific domains of *OsCOI1a*, *OsCOI1b*, *OsCOI1c*, *OsJAZ1*, *osjaz1-1D* and *OsMYC2* were amplified and cloned into the Y2H vectors p*GBKT7* and p*GADT7* (Clontech), respectively (primers are described in Supplementary Table 2). Y2H experiments were then carried out following the manual of Matchmaker Gold Yeast Two-Hybrid System (Clontech). Detection of protein–protein interactions between OsJAZ1 and OsCOI1a/OsCOI1b/OsCOI1c were performed in SD/-Trp/-Leu/-His/-Ade medium containing 30 μM JA, MeJA or Coronatine, all of which were dissolved in 1% methanol.

For yeast one-hybrid assays, specific fragments of the *OsMADS1* promoter region were amplified and cloned into the yeast one-hybrid (Y1H) vector p*HIS2* (Clontech) (Primers are described in Supplementary Table 2). Y1H experiments were then carried out following the transformation protocol in the Yeastmaker Yeast Transformation System 2 user manual (Clontech). Detection of protein–DNA interactions between OsMYC2 and *OsMADS1* promoter region was performed in SD/-Trp/-Leu/-His medium containing 0, 1 or 10 mM of 3-amino-1,2,4-triazole.

Bimolecular fluorescence complementation assays. The cDNAs of *OsMYC2*, *OsJAZ1*, *osjaz1-1D* and *OsMYC2* were individually cloned into p*SAT1-nEYFP-N1*

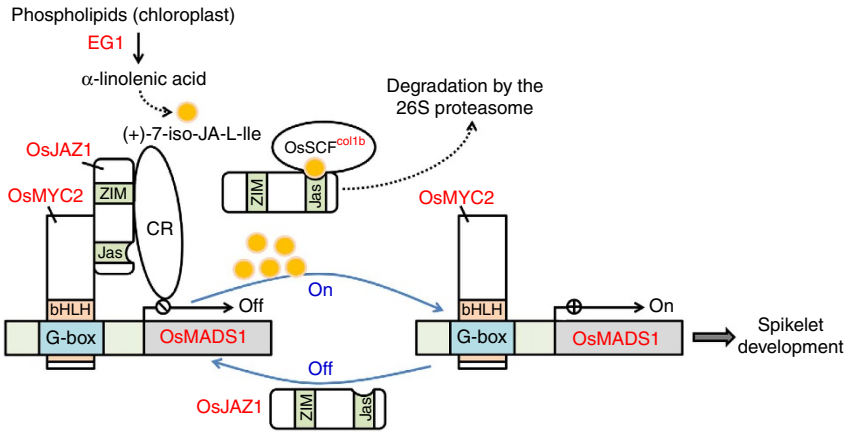

Figure 9 | A working model of the role of JA in regulating rice spikelet development. EG1-mediated JA biosynthesis is required for rice spikelet development. Upon perception of the JA signal, OsCOI1b recruits OsJAZ1 to the SCF^{COI1} complex for ubiquitination and degradation through the 26S proteasome. As a result, OsMYC2 is released from the OsJAZ1-OsMYC2 complex and proceeds with its function in activating the expression of downstream genes, such as the E-class gene *OsMADS1*. Proteins investigated in this study are in red. bHLH, basic-helix–loop–helix domain; CR: co-repressors.

and p*SAT1-cEYFP-C1-B* vectors that contained either amino- or carboxy-terminal EYFP fragments (Primers are described in Supplementary Table 2). The method for bimolecular fluorescence complementation assays using onion epidermal cells was modified from Lee *et al.*[62]. After removal of the dry outer layer, whole onion was sterilized by 2% NaOCl and washed with sterilized H_2O for five times. The onion was cut into 2 × 2 cm squares, and placed in a plate with Murashige and Skoog medium containing 40 g l^{-1} D-Mannitol for 4 h. For transformation, 5 μg of each plasmid DNA was coated with 1.0-μm gold particles (Bio-Rad) and delivered into onion epidermal cells using a PDS-100/He Particle Delivery System (Bio-Rad). Images were captured one night after transformation by a Confocal Laser Scanning Microscope equipped with an argon/krypton laser (Leica TCS SP5). YFP fluorescence was imaged at the excitation wavelength of 514 nm and emission wavelength of 525–546 nm.

OsMYC2 polyclonal antibody preparation. A 120-amino acid OsMYC2-specific peptide, which consisted of amino acids 16–84, 320–345 and 396–420 of the protein, was synthesized *in vitro* using optimized codons for *E. coli* genes (the sequence is described in Supplementary Table 3) and cloned into the *pMAL-c2X* vector (NEB) to fuse with MBP. The recombinant protein was expressed in *E. coli* DE3 (BL21) (Novagen) and purified using Affinity Resin (Clontech) to produce rabbit polyclonal antibodies (prepared by Abclonal of China). The antibody (1:1,000 dilution) was tested by immunoblot analysis using total proteins and nuclear proteins extracted from wild-type spikelet at late stage Sp8, which detected a single band of the expected size at 81 kD (Supplementary Fig. 15i).

***In vitro* pull-down assays.** For *in vitro* pull-down experiment, full-length cDNAs of *OsJAZ1* and *osjaz1-1D* were synthesized *in vitro* using optimized codons for *E. coli* genes (primers are described in Supplementary Table 2 and sequences are described in Supplementary Table 3) and cloned into the p*MAL-c2X* vector (NEB) to fuse with maltose binding protein (MBP). A quantity of 0.5 mg total proteins extracted from wild-type rice spikelet at stage Sp8 was incubated with 1 μg immobilized MBP, MBP-OsJAZ1 or MBP-osjaz1-1D at 4 °C for 1 h. Proteins retained on the beads were eluted and separated by SDS–PAGE, and stained with Coomassie Brilliant R250 (Fisher Scientific) or subjected to immunoblotting with the anti-OsMYC2 antibody (1:1,000 dilution) (Uncropped images of blots are shown in Supplementary Fig. 16).

ChIP assays. Chromatin immunoprecipitation (ChIP) assays involved the procedures described by Li. *et al.*[55]. Chromatin was isolated from rice spikelets at stage Sp4-Sp8 and sonicated, and the solubulized chromatin was immunoprecipitated by either anti-OsMYC2 antibody or rabbit immunoglobulin G at 4 °C overnight. After immunoprecipitation, DNA was collected by protein A/G Plus Agarose (Calbiochem). For each qRT–PCR reaction, the recovered DNA from immunoprecipitation was used as template. Three biological replicates were included. All primers used in the ChIP assays are listed in Supplementary Table 2.

DNA-binding assays. Protein–DNA-binding assays were performed as described by Bai *et al.*[63]. Full-length *OsMYC2* cDNA was synthesized *in vitro* using optimized codons for *E.coli* genes (Sequences are described in Supplementary Table 3) and cloned into the p*MAL-c2X* vector to fuse with MBP. MBP and MBP-OsMYC2 were expressed and affinity-purified from *E. coli.* (BL21) using amylose resin (NEB). The DNA probe fragments were amplified from genomic DNA of wild type by PCR using biotin-labeled primers (primers are described in Supplementary Table 2). Proteins were pulled down using streptavidin-agarose beads (Sigma) that were bound to the DNA probe fragments, and detected by western blot with anti-MBP antibody (EarthOx, 1:2,000 dilution) (uncropped images of blots are shown in Supplementary Fig. 16).

Protoplast transient reporter gene assays. To generate the effector and reporter constructs, *pGreenII-0000* vector containing the *35S* promoter (kindly provided by Hao Yu) and *pGreenII-0800-LUC* (kindly provided by Hongquan Yang) were used[64]. All primers used for generating constructs for transient transactivation assays are listed in Supplementary Table 2. The transient assays were performed in rice protoplasts using PEG-mediated transfections (See the section of Rice Protoplast Transformation), and LUC and REN activities were analysed using the Dual-Luciferase reporter kit according to the user manual (Promega). Absolute LUC/REN was measured in a Turner 20/20 luminometer (Promega).

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Author contributions

Z.Y., M.C. and Z.L. conducted the mutant screen, identified *eg1-3* and *eg2-1D* and performed preliminary genetic and phenotypes analyses. X.Z. performed genetic analyses of the *eg1-3*, *eg2-1D* and *eg1-1 osmads1-1*mutants. Q.C. mapped the *EG1* and *EG2* genes and conducted follow-up studies on the function of the EG1 and EG2 proteins. C.Y. performed *in situ* hybridization analysis. Z.Y., W.L. and D.Z. conceived the study, supervised the work and analysed the data. J.H. and X.Z. participated in the project discussions. D.Z., J.H., Q.C. and Z.Y. co-wrote the manuscript.

Additional information

Accession codes: The genomic sequence of *EG1*, *EG2* (*OsJAZ1*) and *OsMYC2* have been deposited in GRAMENE (http://www.gramene.org/) as LOC_Os01g67430, LOC_Os04g55920, and LOC_Os10g42430, respectively.

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