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Controlling diurnal flower-opening time by manipulating the jasmonate pathway accelerates development of *indica–japonica* hybrid rice breeding

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Summary

Inter-subspecific *indica–japonica* hybrid rice (*Oryza sativa*) has the potential for increased yields over traditional *indica* intra-subspecies hybrid rice, but limited yield of F_1 hybrid seed production (FHSP) hinders the development of *indica–japonica* hybrid rice breeding. Diurnal flower-opening time (DFOT) divergence between *indica* and *japonica* rice has been a major contributing factor to this issue, but few DFOT genes have been cloned. Here, we found that manipulating the expression of jasmonate (JA) pathway genes can effectively modulate DFOT to improve the yield of FHSP in rice. Treating *japonica* cultivar Zhonghua 11 (ZH11) with methyl jasmonate (MeJA) substantially advanced DFOT. Furthermore, overexpressing the JA biosynthesis gene *OPDA REDUCTASE 7* (*OsOPR7*) and knocking out the JA inactivation gene *CHILLING TOLERANCE 1* (*OsHAN1*) in ZH11 advanced DFOT by 1- and 2-h respectively; and knockout of the JA signal suppressor genes *JASMONATE ZIM-DOMAIN PROTEIN 7* (*OsJAZ7*) and *OsJAZ9* resulted in 50-min and 1.5-h earlier DFOT respectively. The yields of FHSP using *japonica* male-sterile lines GAZS with manipulated JA pathway genes were significantly higher than that of GAZS wildtype. Transcriptome analysis, cytological observations, measurements of elastic modulus and determination of cell wall components indicated that the JA pathway could affect the loosening of the lodicule cell walls by regulating their composition through controlling sugar metabolism, which in turn influences DFOT. This research has vital implications for breeding *japonica* rice cultivars with early DFOT to facilitate *indica–japonica* hybrid rice breeding.

Introduction

Hybrid rice (*Oryza sativa* L.) breeding has dramatically increased rice yields, but yields of traditional *indica* intra-subspecies hybrid rice have plateaued, suggesting that maximum yield potential may have been reached in this breeding system. In contrast, inter-subspecific *indica–japonica* hybrid rice exhibits strong heterosis and the potential to increase yields by up to 30% compared with *indica* intra-subspecies hybrid rice (Qian *et al.*, 2016). However, the asynchrony in diurnal flower-opening times (DFOTs, the time of day when the flower opens) between *indica* and *japonica* varieties challenges the breeding of inter-subspecific *indica–japonica* hybrids. This asynchrony reduces the yield of F_1 hybrid seed production (FHSP), increasing the seed price for farmers and is the main factor restricting *indica–japonica* hybrid breeding (Liu *et al.*, 2017; Zhang *et al.*, 2016). Rice pollen is highly susceptible to temperature fluctuations, and high temperature reduces pollen viability during pollination. The *indica* rice varieties, growing in tropical and subtropical regions of low latitude with warmer climatic conditions, tend to bloom in the morning, avoiding peak diurnal temperatures and thus enhancing seed setting rates. Conversely, *japonica* rice varieties, growing in high latitudes, tend to bloom closer to noon, mitigating the risk of low temperatures during flower opening (Bheemanahalli *et al.*, 2017; Hirabayashi *et al.*, 2015; Jagadish, 2020; Li *et al.*, 2023). Therefore, the variation in DFOTs between *indica* and *japonica* rice varieties likely represents an adaptation to their respective environmental conditions, shaped by long-term evolution and artificial selection processes.

The pair of lodicules situated at the base of the floret in grasses function in flower opening by rapidly taking in water and expanding (Ciaffi *et al.*, 2011; Wang *et al.*, 2022; Yoshida, 2012). The precise timing of rice flower opening is determined by a delicate balance between osmotic pressure within the lodicule cells and the loosening of cell walls, which is influenced by genetic, environmental factors and plant hormones (Jagadish *et al.*, 2016; Kazuhiro *et al.*, 2010; Wang *et al.*, 2023). We recently identified *DIURNAL FLOWER OPENING TIME 1*

(*DFOT1*), which is specifically expressed in rice lodicules. DFOT1 directly interacts with members of the pectin methylesterase family and increases their enzymatic activity, modulating pectin methylesterification levels in the cell walls of lodicules and consequently impacting DFOT (Wang *et al*., 2022). Moreover, Xu *et al*. (2022) discovered that DFOT1 (named EMF1 in their study) also interacts with endo-1,4-β-glucanase (GLN2) to influence pectin and cellulose biosynthesis in lodicule cell walls, further impacting DFOT.

Jasmonates (JAs) are the primary phytohormones involved in DFOT in rice (Gou *et al*., 2022; Li *et al*., 2018; Liu *et al*., 2017; Wang *et al*., 2016). JAs constitute a category of fatty acid derivatives, including jasmonic acid and its various forms, such as jasmonoyl-L-isoleucine (JA-Ile), methyl jasmonate (MeJA) and 12-hydroxy jasmonoyl sulfate (12-HSO_4-JA). Jasmonic acid biosynthesis starts with the release of polyunsaturated fatty acids from chloroplast/plastid membranes by phospholipases (Howe *et al*., 2018). These fatty acids then undergo a series of conversions, with 12-oxo-phytodienoic acids (OPDAs) being produced through the catalytic activity of 13-lipoxygenase (LOX), 13-allene oxide synthase (AOS), and 13-allene oxide cyclase (AOC) in chloroplasts. Subsequently, OPDAs are transported to peroxisomes, where they undergo reduction by OPDA reductase (OPR) and multiple β-oxidation steps to generate JAs (Han, 2017; Hou *et al*., 2016; Ruan *et al*., 2019). Finally, JAs are transported to the cytoplasm and undergo further metabolism to generate derivatives. For example, JASMONATE RESISTANT 1 (JAR1), in collaboration with JA amido synthetase, catalyses the formation of the biologically active JA-Ile (Chini *et al*., 2007; Miersch *et al*., 2008; Thines *et al*., 2007). *CHILLING TOLERANCE 1* (*OsHAN1*), named for 'han' in Chinese, which means 'chilling', encodes a monooxygenase belonging to the cytochrome P450 superfamily, facilitating the conversion of active JA-Ile into inactive 12-hydroxy-JA-Ile (Mao *et al*., 2019). The CORONATINE INSENSITIVE 1 (COI1)-JASMONATE ZIM-DOMAIN (JAZ) protein complex plays a crucial role as the co-receptor for JA-Ile in the JA signalling pathway. COI1, a subunit of the E3 ubiquitin ligase SCF complex, detects the JA-Ile signal, binds to JAZ proteins and subsequently promotes JAZ protein ubiquitination and degradation (Sheard *et al*., 2010). JAZ proteins serve as inhibitory factors within the JA signalling pathway by binding to downstream transcription factors such as MYCs/MYBs to repress their activity (Katsir *et al*., 2008; Sheard *et al*., 2010; Thines *et al*., 2007).

In this study, we revealed that exogenous MeJA accelerates DFOT in rice, and the proportion of opened florets increases with MeJA concentration. Moreover, we generated rice lines with different DFOTs by manipulating the expression of key genes responsible for JA biosynthesis, deactivation and signal transduction. The seed setting rates of FHSP between *indica* cultivar Nanguizhan and *japonica* male sterile line GAZS with overexpressing *OsOPR7* (GS-*OPR7*-OE) and with knockout of *OsHAN1* (GS-*HAN1*-CAS) were 1.85 and 2.86 times higher than that of GAZS respectively. Further transcriptomic analysis suggested that JAs regulate DFOT by affecting genes related to sugar metabolism. Cytological observations, measurements of elastic modulus and determination of cell wall components indicated that JA pathway genes may mainly regulate DFOT by influencing the metabolism of the cell wall polysaccharides of lodicule cells, thereby affecting the rigidity of the lodicule cell wall. We, therefore, demonstrate that the JA signalling pathway is involved in regulating DFOT and reveal promising genes that could be manipulated to facilitate inter-subspecific *indica–japonica* hybrid rice breeding.

Results

Exogenous MeJA induces early flower opening in rice

The *japonica* rice cultivar Zhonghua 11 (ZH11) typically begins to bloom around 11:00 am in September in Guangzhou, China (with an average temperature of approximately 28 °C). We sprayed ZH11 plants with different concentrations of MeJA (0, 5, 10, 20, 30 or 50 mmol/L) at 7:00 am (Figure 1). These treatments substantially accelerated the blooming time of flowers, consistent with results reported from previous studies (Li *et al*., 2018; Liu *et al*., 2017; Wang *et al*., 2016). Furthermore, ZH11 commenced blooming approximately 0.5 h after MeJA treatment, and within 1 h, about half of the total number of florets for the day had opened. The number of flowering florets per panicle was positively associated with the concentration of MeJA, being highest at 30 and 50 mmol/L MeJA. These results indicate that exogenous MeJA accelerates the DFOT and peak flower opening of rice, increasing the number of open flowers.

Key genes in the JA pathway are associated with DFOT

To investigate whether JA pathway genes control DFOT in rice, we examined the expression patterns of key genes related to JA biosynthesis (*OsOPR7*/*Os08g0459600*/*LOC_Os08g35740*) and deactivation (*OsHAN1*/*Os11g0483000*/*LOC_Os11g29290*), as well as JAZ family genes that act as negative regulators of the JA pathway. Reverse transcription quantitative PCR (RT-qPCR) revealed that *OsOPR7* is expressed at low levels in roots and inflorescences, and in lodicules before flower opening. In contrast, its expression levels are higher in stems and leaves, and in lodicules during flower opening. Compared with its expression in lodicules at 5:00–6:00 pm the day before flower opening, *OsOPR7* was up-regulated in lodicules at 7:00–10:00 am on the day of flower opening, and its expression level increased substantially in lodicules at 11:00–11:59 am during flower opening (Figure 2a). The expression of *OsHAN1* was relatively low in roots, stems, leaves and inflorescences, and in lodicules before flower opening. *OsHAN1* expression in lodicules was nearly undetectable at 5:00–6:00 pm on the day before flower opening, was higher between 7:00 and 10:00 am on the day of flower opening and peaked during flower opening (Figure 2b). The substantial increase in *OsOPR7* and *OsHAN1* expression during flower opening suggests that these genes may be involved in this process.

The *JAZ* gene family in rice consists of 15 members (Figure 2c). We determined the expression levels of all 15 *JAZ* genes in rice lodicules at 9:00–10:00 am on the day of flower opening and selected those with relatively high expression levels for further analysis: *OsJAZ1* (Os04g0653000/LOC_Os04g55920), *OsJAZ4* (Os09g0401300/LOC_Os09g23660), *OsJAZ6* (Os03g0402800/LOC_Os03g28940), *OsJAZ7* (Os07g0615200/LOC_Os07g42370), *OsJAZ9* (Os03g0180800/LOC_Os03g08310) and *OsJAZ11* (Os03g0180900/LOC_Os03g08320) (Figure 2d). We analysed the expression patterns of these six *OsJAZ* genes in different tissues using RT-qPCR. *OsJAZ1* had the lowest expression level in stems and the highest in inflorescences, with similar expression levels in lodicules at all-time points (Figure 2e). *OsJAZ4* displayed the lowest expression in stems and the highest in inflorescences; its expression increased gradually in lodicules before flower opening and decreased gradually during flower opening (Figure 2f). *OsJAZ6*

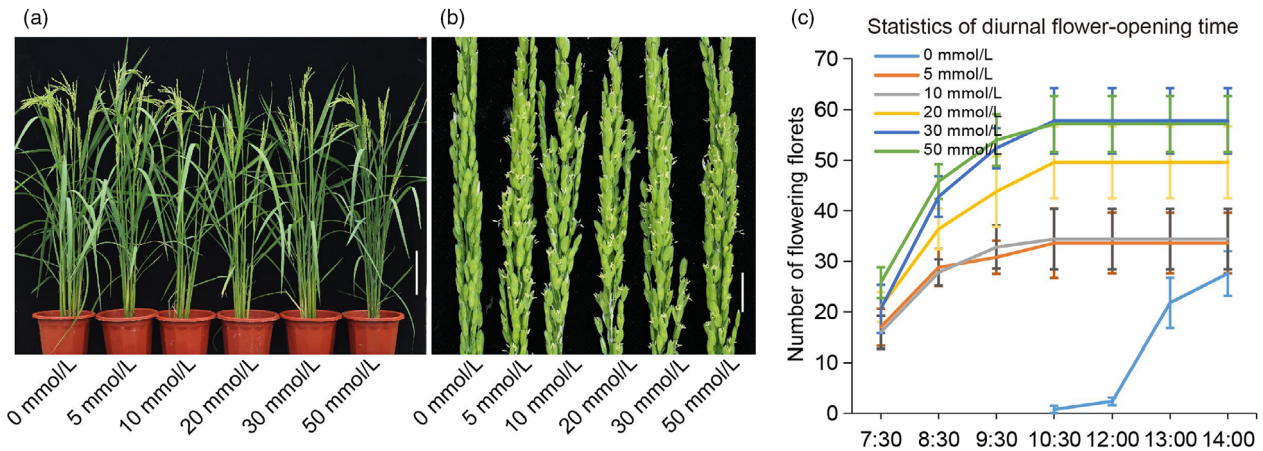

Figure 1 Exogenous MeJA can advance the DFOT of rice. (a) Rice plants sprayed with different concentrations of MeJA. Scale: 20 cm. (b) Rice inflorescence after 1 h spraying of different concentrations of MeJA. Scale: 2 cm. (c) The DFOT statistics of rice sprayed with different concentrations of MeJA, with the *y*-axis representing the total average number of florets per panicle that have blossomed and the *x*-axis representing the time. The data represent mean ± SD, $n = 5$.

and *OsJAZ7* exhibited similar expression patterns, with relatively low expression in roots, stems and inflorescences, and relatively high expression in leaves. Their expression was low in lodicules at 5:00–6:00 pm and gradually increased from 7:00 am to 11:59 am on the day of flower opening, peaking during active flower opening (11:00–11:59 am; Figure 2g,h). *OsJAZ9* had the relatively low expression in inflorescences, relatively higher expression in roots, and showed similar expression patterns to *OsJAZ6* and *OsJAZ7* in lodicules at different time points during flower opening (Figure 2i). *OsJAZ11* displayed the lowest expression in roots and the highest in inflorescences; expression in lodicules was relatively higher at 5:00–6:00 pm on the day before flower opening, with lower expression on the day of flower opening (Figure 2j). With the exception of *OsJAZ1*, the expression levels of *JAZ* genes changed in lodicules as flower opening approached, suggesting a potential association between *OsJAZ* genes and DFOT in rice.

We also detected the expression levels of JA-related genes *OsOPR7*, *OsHAN1*, *OsJAZ7* and *OsJAZ9* in a *japonica* cultivar Kongyu 131 (KY131) and an *indica* cultivar II32. The results showed the expression patterns of *OsOPR7*, *OsHAN1*, *OsJAZ7* and *OsJAZ9* in lodicules of the two varieties at various time points align with those of ZH11. Specifically, they exhibited lower expression levels before flowering, which increased during flowering (Figure S1).

Overexpression of JA biosynthesis gene *OsOPR7* increases JA levels and advances DFOT in rice

To study the role of *OsOPR7*, a key gene involved in JA biosynthesis, in the regulation of DFOT in rice, we developed overexpression lines of *OsOPR7* (*OsOPR7*-OE13 and *OsOPR7*-OE20) in the *japonica* rice ZH11 for subsequent study (Figure S2A). The DFOTs of the two *OsOPR7*-OE lines were 1 h earlier than that of the wild-type ZH11 in June and September in Guangzhou (Figure 3a–c). To analyse how *OsOPR7* mediates rice flower opening, we observed dynamic changes in lodicules at different time points of flower opening in September. Lodicules of both ZH11 and *OsOPR7*-OE lines increased in size before flower opening and wilted after flower opening. The lodicule sizes of the *OsOPR7*-OE and ZH11 plants were slightly different at 9:00 am on the day of flower opening, whereas *OsOPR7*-OE lodicules rapidly enlarged before flower opening at 10:00 am (Figures 3d and S2B).

OsOPR7 (OPR3) is a key enzyme in the biosynthetic pathway of JAs, and OPDA is an intermediate product of JA synthesis. OPDA is transported from chloroplasts to peroxisomes, where it undergoes catalysis by OsOPR7 and multiple β-oxidations to produce JAs (Tani *et al.*, 2008). To understand whether *OsOPR7* regulates DFOT through the JA pathway, we measured the contents of endogenous OPDA, JA and JA-Ile. At 3 h before flowering of *OsOPR7*-OE plants (7:00 am), the contents of OPDA, JA and JA-Ile in ZH11 and *OsOPR7*-OE is low, but as the DFOT approaches, the contents of OPDA, JA and JA-Ile in lodicules gradually increases (Figures 3e–g and S3). One hour before flower opening (9:00 am), JA and JA-Ile contents in lodicules were approximately 1.2 and 1.5 times higher in *OsOPR7*-OE plants than those in ZH11 respectively (Figure 3e–g). The contents of OPDA, JA and JA-Ile in the lodicules of *OsOPR7*-OE plants at flowering (10:30 am) increased explosively, which were 4.4 times, 32.4 times and 29.9 times higher than those of ZH11 respectively (Figure S3). This implies that overexpressing *OsOPR7* promotes JA biosynthesis and early flower opening in rice.

Knockout the JA deactivation gene *OsHAN1* enhances JAs levels and advances DFOT

To investigate the role of the JA deactivation gene *OsHAN1* in DFOT modulation in rice, we obtained two overexpression lines (*OsHAN1*-OE1 and *OsHAN1*-OE2) and two knockout lines (*OsHAN1*-CAS4 and *OsHAN1*-CAS15) of *OsHAN1* (Figure S4A, B). The DFOTs of *OsHAN1*-OE1 and *OsHAN1*-OE2 plants showed no substantial differences from that of the wild-type ZH11 (Figure S4C,D), whereas flowers of *OsHAN1*-CAS4 and *OsHAN1*-CAS15 plants opened approximately 2 h earlier than those in wild-type ZH11 in September in Guangzhou (Figure 4a–c). The lodicules of the *OsHAN1*-CAS plants were larger than those of ZH11 at 8:00 am on the day of flower opening, and this difference was most apparent during *OsHAN1*-CAS plants flower opening (Figures 4d and S4E). The early expansion of lodicules in *OsHAN1*-CAS4 and *OsHAN1*-CAS15 plants led to early flower opening. These results indicated that the enlargement of lodicules

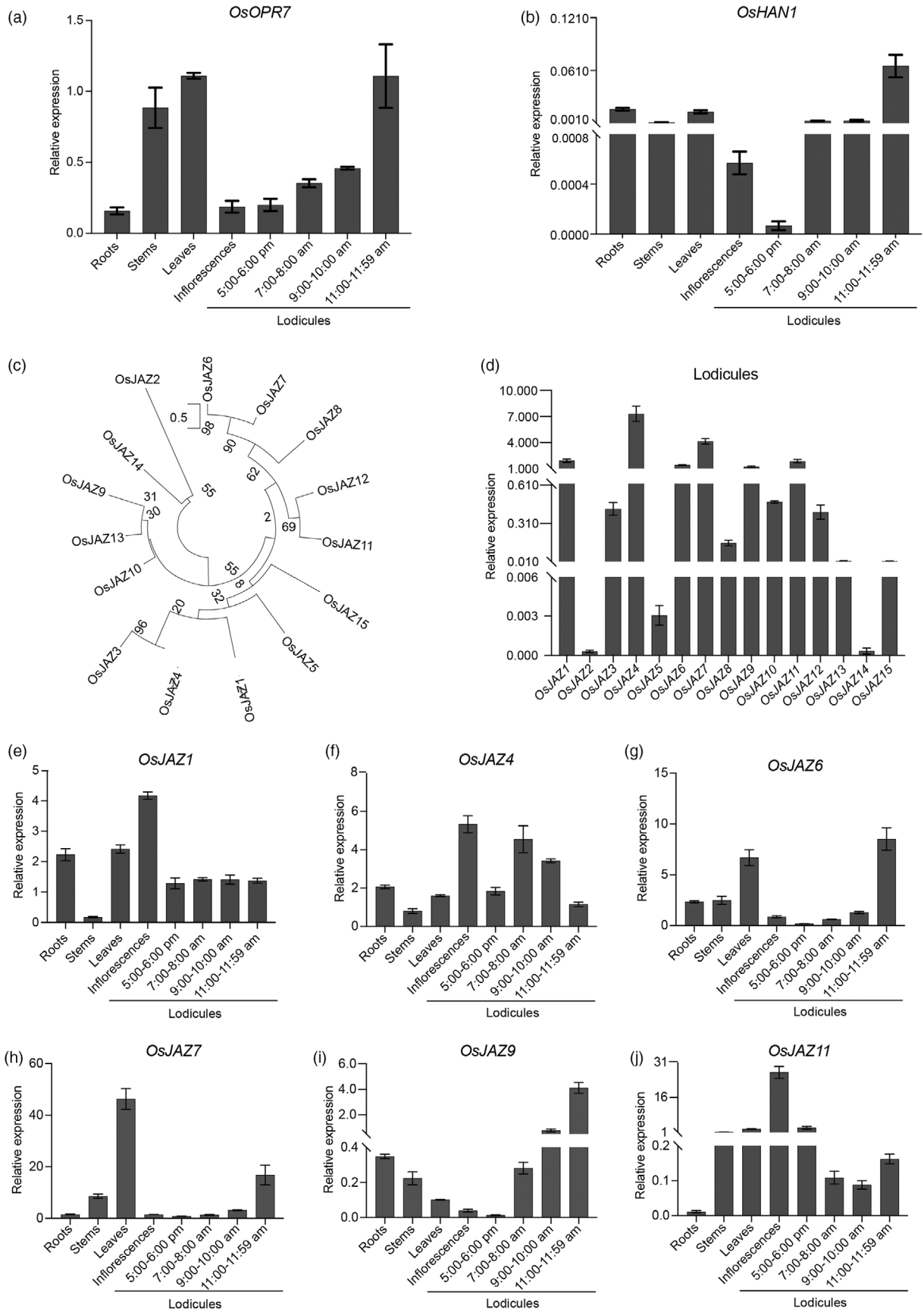

Figure 2 The expression characteristics of key genes in the JA pathway and the phylogenetic tree of rice JAZ protein family. (a, b) The relative expression levels of *OsOPR7* and *OsHAN1* in roots, stems, leaves, inflorescences and lodicules of *japonica* rice ZH11 from 5:00 pm on the day before flower-opening to 11:59 am on the day of flower-opening were analysed by RT-qPCR, and *Actin* was used as a control. (c) The phylogenetic tree of rice JAZ protein family was constructed by sequence alignment using MEGA 7.0 software, and the bootstrap repeats of the adjacent phylogenetic tree were 1000 times. (d) The relative expression levels of 15 genes of *JAZ* family in ZH11 lodicules during 9:00–10:00 am were analysed by RT-qPCR, and *Actin* was used as a control. (e–j) The relative expression levels of *OsJAZ1*, *OsJAZ4*, *OsJAZ6*, *OsJAZ7*, *OsJAZ9* and *OsJAZ11* in roots, stems, leaves, inflorescences and lodicules of ZH11 from 5:00 pm on the day before flower-opening to 11:59 am on the day of flower-opening were analysed by RT-qPCR, and *Actin* was used as a control.

is the primary force for pushing the glumes, and the early enlargement of lodicules in *OsHAN1*-CAS plants causes early flower opening.

OsHAN1 belongs to the cytochrome P450 superfamily and is responsible for the metabolism of the active forms of JA-Ile into the inactive form 12OH-JA-Ile, resulting in the inactivation of JAs. To investigate the changes in JA levels associated with early flower opening in *OsHAN1*-CAS plants, we measured the levels of endogenous OPDA, JA and JA-Ile in lodicules of both *OsHAN1*-CAS and ZH11 plants. Lodicules of both ZH11 and *OsHAN1*-CAS showed very low levels of OPDA, JA and JA-Ile contents at 2 h before flowering (7:00 am). However, as the time of flowering approaches, the levels of OPDA, JA and JA-Ile in lodicules gradually increased (Figures 4e–g and S3). At around 8:30 am on the day of flower opening, the contents of OPDA, JA and JA-Ile in the lodicules of *OsHAN1*-CAS plants were approximately 1.4, 5.6 and 4.3 times higher than those in wild-type ZH11 respectively (Figure 4e–g). During flowering (10:30 am), the levels of OPDA, JA and JA-Ile in *OsHAN1*-CAS increased dramatically, with amounts reaching 10.4, 62.6 and 38.6 times higher than those in ZH11 respectively (Figure S3). These findings suggest that *OsHAN1* knockout leads to an increase in active JA levels, which advances flower opening in *OsHAN1*-CAS plants.

Knockout of *JAZ* genes involved in JA signalling advances DFOT

JAZs are negative regulators in the JA signalling pathway, binding to transcription factors such as MYC and MYB to inhibit JA signal transduction (Katsir *et al*., 2008; Sheard *et al*., 2010; Thines *et al*., 2007). To investigate the role of JAZs in regulating DFOT in rice, we selected five genes, *OsJAZ1*, *OsJAZ4*, *OsJAZ7*, *OsJAZ9* and *OsJAZ11*, with relatively high expression levels and dissimilar expression patterns in lodicules for further study. These genes were knocked out individually in ZH11 (Figure S5). In September in Guangzhou, the *OsJAZ7* knockout lines showed a 50-min earlier DFOT compared to ZH11, whereas flowers of the *OsJAZ9* knockout lines opened approximately 1.5 h earlier (Figure 5). However, knockout lines of *OsJAZ1*, *OsJAZ4* and *OsJAZ11* did not exhibit substantial advancement in DFOT compared to ZH11 (data not shown).

To analyse how OsJAZ7 and OsJAZ9 regulate rice flower opening, we observed the dynamic changes in lodicules of *OsJAZ7* and *OsJAZ9* knockout plants at different time points. The lodicules of *OsJAZ7* knockout plants were similar in size compared with those of ZH11 at 8:00 am on the day of flower opening but enlarged considerably during flower opening (Figures 5d and S6A). In contrast, lodicule changes in *OsJAZ9*-CAS plants resembled those in *OsHAN1*-CAS plants, showing an earlier enlargement than in ZH11 at 8:00 am on the day of flower opening, with a more obvious difference during flower opening (Figures 5h and S6B). These results suggest that lodicule enlargement is the main driving force for flower opening in rice, and that early lodicule enlargement in *OsJAZ7*-CAS and *OsJAZ9*-CAS plants leads to earlier flower opening.

Deciphering the influence of JA signalling pathway genes on rice DFOT through transcriptome and cell wall polysaccharide metabolism analysis

To gain further insights into the mechanisms by which key genes in the JA signalling pathway affect rice DFOT, we conducted transcriptome analysis using the lodicules of *OsJAZ9*-CAS plants, which showed the significantly advanced DFOT, and those of wild-type ZH11. We identified 1319 differentially expressed genes (DEGs) between *OsJAZ9*-CAS and ZH11, including 1087 up-regulated genes and 232 down-regulated genes (Figure S7A; Table S1). This suggests that OsJAZ9 is a suppressor, which is consistent with the previous studies (Sheard *et al*., 2010; Thines *et al*., 2007). Gene ontology (GO) analysis identified seven out of the most enriched 20 GO terms of molecular function were related to various glycolytic enzyme activities and transport activities (Figure S7B).

Lodicule swelling is primarily influenced by the rising of cytoplasmic osmotic pressure and the loosening of cell wall (Gou *et al*., 2022). Sugar metabolism and transport both play important roles in osmotic pressure and cell wall loosening (Wang *et al*., 2023). Therefore, 11 up-regulated genes encoding sugar metabolism-related enzymes and two genes encoding sugar transport proteins were selected for validation by RT-qPCR. Among these genes, the sugar metabolism-related enzymes encoded by the selected genes include *Os01g0813800* (*beta-glucosidase 3*, *Os7BGlu3*), *Os01g0930800* (*Os1bglu5*), *Os07g0539100*, *Os08g0224500*, *Os06g0367100* and *Os01g0220100* (*endo-β-1,4-glycanase*, *OsCel9A*), are all predicted to be cellulases. These enzymes hydrolyse cellulose during cell wall development, releasing soluble oligosaccharides and monosaccharides (Chai *et al*., 2009; Opassiri *et al*., 2003; Wang and Li, 2021; Yoshida *et al*., 2006). *Os07g0529700* (*xyloglucan endotransglucosylase/hydrolase 1*, *XTH1*), *Os06g0696400* (*OsXTH11*) and *Os01g0134800* encode hemicellulases involved in the metabolic process of xyloglucan or xylan (Hara *et al*., 2014; Ishida and Yokoyama, 2022; Wang *et al*., 2014). *Os06g0713800* is predicted to encode an α-amylase, which is essential for hydrolysing starch and increasing soluble sugar content (Pujadas and Palau, 2001). *Os03g0401300* (*RSUS1*) encodes a sucrose synthase that participates in sucrose metabolism (Hirose *et al*., 2008). The genes involved in sugar transport include *Os07g0559700* (*monosaccharide transporter gene*, *OsMST6*), encoding a monosaccharide transporter, and *Os09g0416200*, encoding a glucose transporter (Deng and Yan, 2016; Wang *et al*., 2008). These two transporters contain transmembrane domains and facilitate the transport of sugars from the extracellular environment into cells. Our RT-qPCR results showed substantially higher expression levels of the above-mentioned

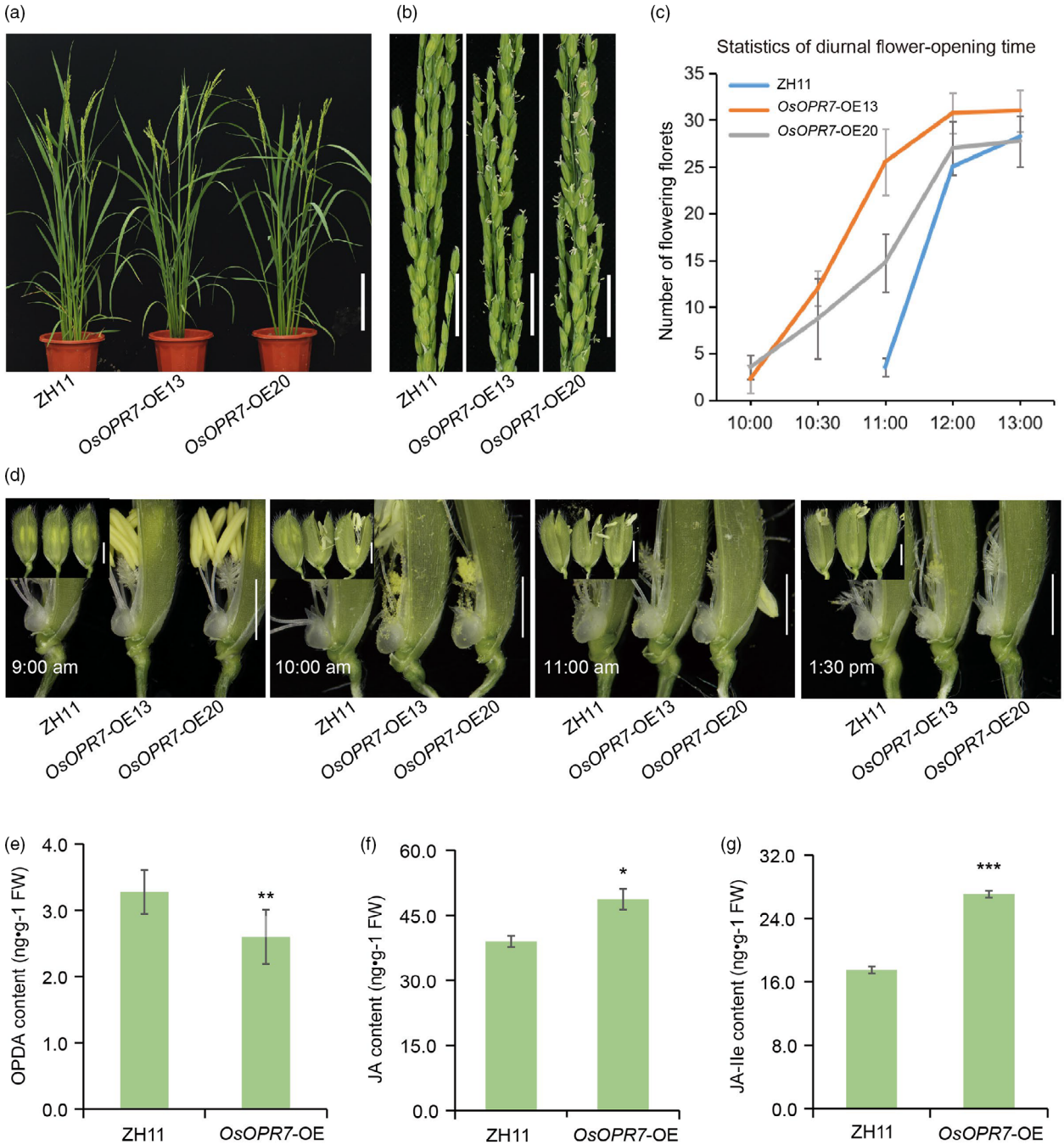

Figure 3 Overexpression of JA biosynthetic pathway gene *OsOPR7* advanced the DFOT of rice. (a) The phenotypes of ZH11, *OsOPR7*-OE13 and *OsOPR7*-OE20. *OsOPR7*-OE started to bloom at 10:00 am, while ZH11 started to bloom at 11:00 am. Scale: 20 cm. (b) The inflorescences of ZH11, *OsOPR7*-OE13 and *OsOPR7*-OE20 at 10:40 am. Scale: 2 cm. (c) The DFOT statistics of ZH11, *OsOPR7*-OE13 and *OsOPR7*-OE20. The *y*-axis represents the total number of florets per panicle that have bloomed, and the *x*-axis represents the time. The data represent mean ± SDs, $n = 5$. (d) The changes in glume opening and lodicule size of ZH11, *OsOPR7*-OE13 and *OsOPR7*-OE20 from 9:00 am to 1:30 pm on the day of flower-opening. Scale: 2 mm. (e–g) The contents of OPDA, JA and JA-Ile in the lodicules of ZH11 and *OsOPR7*-OE at 9:30 am. The data are the mean ± SDs, $n = 4$; *$0.01 \leq P < 0.05$; **$0.001 \leq P < 0.01$; ***$P < 0.001$ according to Student's *t*-test. FW stands for fresh weight.

sugar metabolism and transport-related genes in the lodicules of *OsJAZ9*-CAS plants compared to wild-type ZH11 plants (Figure S7C). This suggests that JA pathway genes may influence DFOT of rice via the sugar metabolism pathway and sugar transport.

In an effort to delve deeper into how JA pathway genes influence DFOT by modulating sugar metabolism, we measured soluble sugar content in the lodicules. Our results revealed that, in comparison to the ZH11, the lodicules of *OsOPR7*-OE, *OsHAN1*-CAS and *OsJAZ9*-CAS did not experience an increase in soluble sugar content. On the contrary, there was a slight decrease, which could be attributed to the larger size of the pre-flowering lodicules in the early DFOT transgenic lines and their relatively

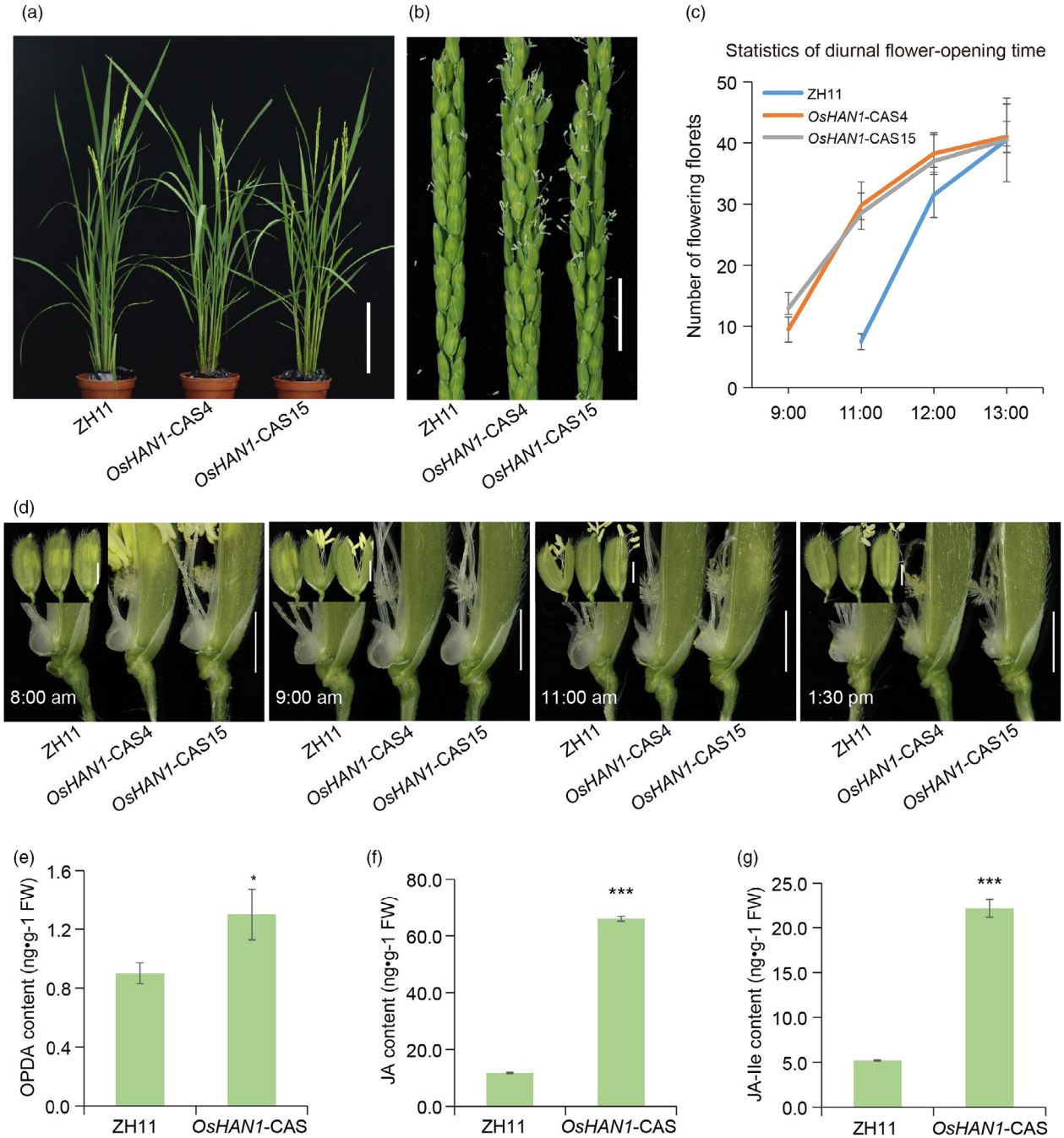

Figure 4 Knockout of the JA-inactivation regulator *OsHAN1* can increase the level of JA-Ile and advance the DFOT in rice. (a) The phenotypes of ZH11, *OsHAN1*-CAS4 and *OsHAN1*-CAS15 plants. *OsHAN1*-CAS started to bloom at 9:00 am, while ZH11 started to bloom at 11:00 am. Scale: 20 cm. (b) The inflorescences of ZH11, *OsHAN1*-CAS4 and *OsHAN1*-CAS15 at about 10:30 am. Scale: 2 cm. (c) The DFOT statistics of ZH11, *OsHAN1*-CAS4 and *OsHAN1*-CAS15. The *y*-axis represents the total number of florets per panicle that have bloomed, and the *x*-axis represents the time. The data represent mean ± SDs, $n = 5$. (d) The changes in glume opening and lodicule size of ZH11, *OsHAN1*-CAS4 and *OsHAN1*-CAS15 plants from 9:00 am to 1:30 pm on the day of flower-opening. Scale: 2 mm. (e–g) The contents of OPDA, JA and JA-Ile in the lodicules of ZH11 and *OsHAN1*-CAS of 8:30 am. The data are the mean ± SDs, $n = 4$; *$0.01 \leq P < 0.05$; ***$P < 0.001$ according to Student's *t*-test. FW Stands for fresh weight.

higher water content when compared to the ZH11 (Figure 6a). Cellulose content analysis showed that the cellulose content in lodicules of *OsOPR7*-OE, *OsHAN1*-CAS and *OsJAZ9*-CAS significantly decreased compared with ZH11 (Figure 6b). The immunofluorescence assay conducted with hemicellulose antibody LM15 revealed that the fluorescence intensity of lodicule cell walls of *OsOPR7*-OE, *OsHAN1*-CAS and *OsJAZ9*-CAS was weaker in comparison to ZH11. This suggests that the hemicellulose content in the lodicule cell walls of *OsOPR7*-OE, *OsHAN1*-CAS and *OsJAZ9*-CAS was lower than in ZH11 (Figure 6c–j). To verify the impact of the decrease of cellulose and hemicellulose on the cell wall of lodicule, we conducted ultra-thin sections to analyse

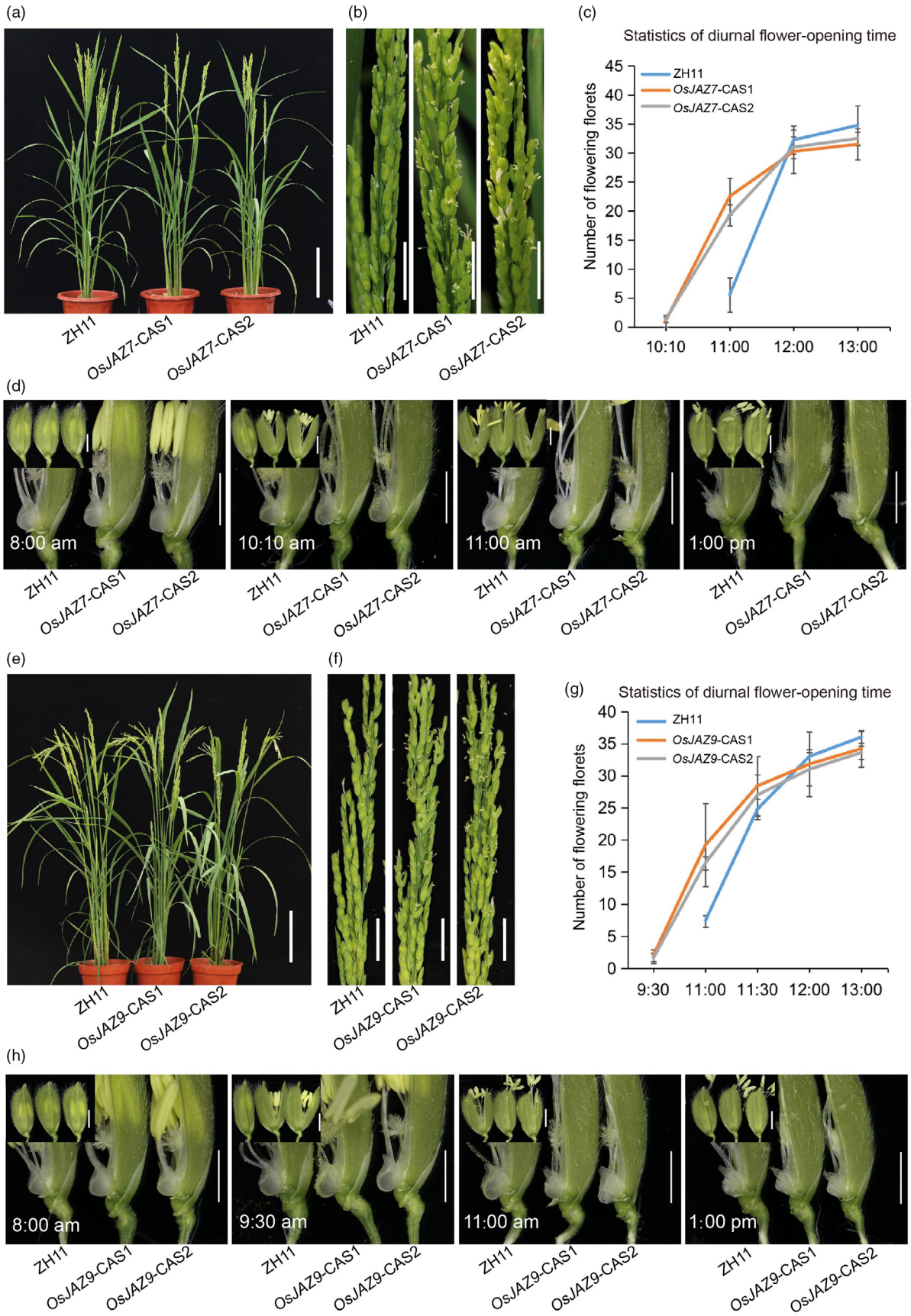

Figure 5 Knockout of the negative regulator *JAZ*s of the JA signalling pathway can advance the DFOT in rice. (a) The phenotypes of ZH11, *OsJAZ7*-CAS1 and *OsJAZ7*-CAS2 plants. *OsJAZ7*-CAS started to bloom at 10:10 am, while ZH11 started to bloom at 11:00 am. Scale: 20 cm. (b) The inflorescence of ZH11, *OsJAZ7*-CAS1 and *OsJAZ7*-CAS2 at around 10:40 am. Scale: 2 cm. (c) The DFOT statistics of ZH11, *OsJAZ7*-CAS1 and *OsJAZ7*-CAS2 plants. The *y*-axis represents the total number of florets per panicle that have bloomed, and the *x*-axis represents the time. The data represent mean ± SDs, $n = 5$. (d) The changes in glume opening and lodicule size of ZH11, *OsJAZ7*-CAS1 and *OsJAZ7*-CAS2 from 8:00 am to 1:00 pm on the day of flower-opening. Scale: 2 mm. (e) The phenotypes of ZH11, *OsJAZ9*-CAS1 and *OsJAZ9*-CAS2 plants. *OsJAZ9*-CAS started to bloom at 9:30 am, while ZH11 started to bloom at 11:00 am. Scale: 20 cm. (f) The rice inflorescence of ZH11, *OsJAZ9*-CAS1 and *OsJAZ9*-CAS2 at about 10:40 am. Scale: 2 cm. (g) The DFOT statistics of ZH11, *OsJAZ9*-CAS1 and *OsJAZ9*-CAS2. The *y*-axis represents the total number of florets per panicle that have bloomed, and the *x*-axis represents the time. The data represent mean ± SDs, $n = 5$. (h) The changes in glume opening and lodicule size of ZH11, *OsJAZ9*-CAS1 and *OsJAZ9*-CAS2 plants from 8:00 am to 1:00 pm on the day of flower-opening. Scale: 2 mm.

the cell wall thickness of lodicule. The results showed that the cell wall thickness of the lodicule of *OsHAN1*-CAS and *OsJAZ9*-CAS significantly decreased compared to ZH11 (Figure 6k,l). We then measured the elastic modulus of the lodicule cell walls of ZH11, *OsOPR7*-OE, *OsHAN1*-CAS and *OsJAZ9*-CAS, and the results showed that the elastic modulus of the lodicule cell walls of *OsOPR7*-OE, *OsHAN1*-CAS and *OsJAZ9*-CAS decreased, indicating the loosening of the cell wall of these transgenic plants (Figure 6m). The above results suggest that the JA pathway-related genes *OsOPR7*, *OsHAN1* and *OsJAZ9* regulate the cellulose and hemicellulose content of the cell walls of lodicule, affecting the rigidity of the cell walls and regulating DFOT in rice.

Application of JA synthesis and deactivation genes in FHSP

Currently, *indica–japonica* hybridization is primarily achieved through the use of *japonica* sterile lines and *indica* restorer lines. To further explore the potential of modulating JA synthesis and signal transduction pathway genes to improve hybrid seed production, we first assessed the agronomic traits of *OsOPR7*-OE, *OsHAN1*-CAS, *OsJAZ7*-CAS and *OsJAZ9*-CAS (Figure S8). Apart from a decrease in *OsHAN1*-CAS, there were no notable variances in the seed setting rate among the transgenic lines in comparison to ZH11. The 100-grain weight results indicated a significant but minor decrease in *OsOPR7*-OE, *OsJAZ7*-CAS and *OsJAZ9*-CAS in comparison to ZH11, with no significant change observed in *OsHAN1-CAS*. Tiller number assessment revealed no significant differences between ZH11 and the transgenic plants. The plant height was slightly decreased in the transgenic lines *OsHAN1*-CAS and *OsJAZ7*-CAS compared to ZH11, whereas *OsOPR7*-OE and *OsJAZ9*-CAS showed no significant differences. The *OsOPR7*-OE and *OsJAZ9*-CAS exhibited an increase in the number of grains per panicle compared to ZH11. In contrast, there was no significant difference observed in the *OsJAZ7*-CAS, whereas the *OsHAN1*-CAS lines showed a decrease in this regard. In general, the transgenic lines of *OsHAN1*-CAS, which flowered earliest, displayed a slightly lower yield compared to ZH11, whereas the yields of *OsOPR7*-OE and *OsJAZ9*-CAS were enhanced. Considering that knockout of *OsHAN1*, *OsJAZ7* and *OsJAZ9*, respectively, resulted in recessive traits that do not affect the agronomic characteristics of heterozygotes, we believe that the use of these genes in male sterile lines would not influence the agronomic traits of F_1 hybrid rice.

So we overexpressed the JA synthesis gene *OsOPR7* and knocked out the JA deactivation gene *OsHAN1* in the *japonica* thermo-sensitive genic male sterile (TGMS) line GAZS, respectively, which we developed previously (Zhou *et al*., 2016). As a result, we obtained GS-*OPR7*-OE1, GS-*OPR7*-OE2, GS-*HAN1*-CAS1 and GS-*HAN1*-CAS2 lines (Figure S9A,B). In trials conducted in September in Guangzhou, the DFOT of GS-*OPR7*-OE1 and GS-*OPR7*-OE2 was advanced from 11:30 to approximately 10:30, resulting in an advancement of approximately 1 h (Figures 7a,b and S9C). Furthermore, the knockout lines GS-*HAN1*-CAS1 and GS-*HAN1*-CAS2 flowered even earlier, from 11:30 to 10:00, resulting in a 1.5-h earlier DFOT compared with GAZS (Figures 7c,d and S9D). These findings indicate the potential for generating *japonica* male sterile lines with diverse DFOTs by modulating the expression of JA pathway genes.

In order to investigate whether DFOT affects the yields of FHSP, we conducted a trial test on the yields of *indica–japonica* hybrid rice seed production. The results demonstrated that the seed setting rate of the *japonica* TGMS line GAZS crossed with the *indica* variety Nanguizhan was 10.7%, and the yield per plant was 1.62 g. After modifying the DFOT of GAZS by overexpressing *OsOPR7* (*OPR7*-OE), the seed setting rate of GS-*OPR7*-OE crossed with Nanguizhan was 19.8% and the yield per plant was 2.69 g. The seed setting rate of GS-*HAN1*-CAS crossed with Nanguizhan was 30.6%, and the yield per plant was 4.50 g (Figures 7e–g and S10). These findings show that advancing the DFOT in *japonica* male sterile lines can significantly enhance in the yield of FHSP and reduce the cost of seed production.

Discussion

Genetic manipulation of DFOT reduces hybrid breeding costs through the JA pathway

Breeding of inter-subspecific *indica–japonica* hybrid rice holds promise for higher yields compared to traditional *indica* intra-subspecies hybrid rice. However, limited yield of F_1 hybrid seed hinders the development of *indica–japonica* hybrid rice breeding. The yields of FHSP from *indica* intra-subspecies crosses reached 300–400 catties per mu, whereas the average yield of F_1 hybrid seed from inter-subspecific *indica–japonica* crosses is only slightly over 100 catties per mu. This greatly increases the price of hybrid seed and discourages farmers from choosing *indica–japonica* hybrid rice varieties (Wang *et al*., 2023). Addressing the challenge of 'mismatched DFOT' between *indica* and *japonica* rice varieties and enhancing the yield of F_1 hybrid seed are urgent tasks for plant biologists and rice breeders (Zhang *et al*., 2016). Currently methods to improve the F_1 seed production of *indica–japonica* hybrid involve application of synthetic plant growth regulators, trace elements and employment of agronomic practices. These methods aim to increase the successful cross-pollination rate between parents. However, the exogenous plant growth regulators and trace elements can affect both *indica* and *japonica* rice, albeit with different effects. Therefore, these methods to improve the yield of hybrid seed production are limited. Furthermore, implementing agronomic practices is time-

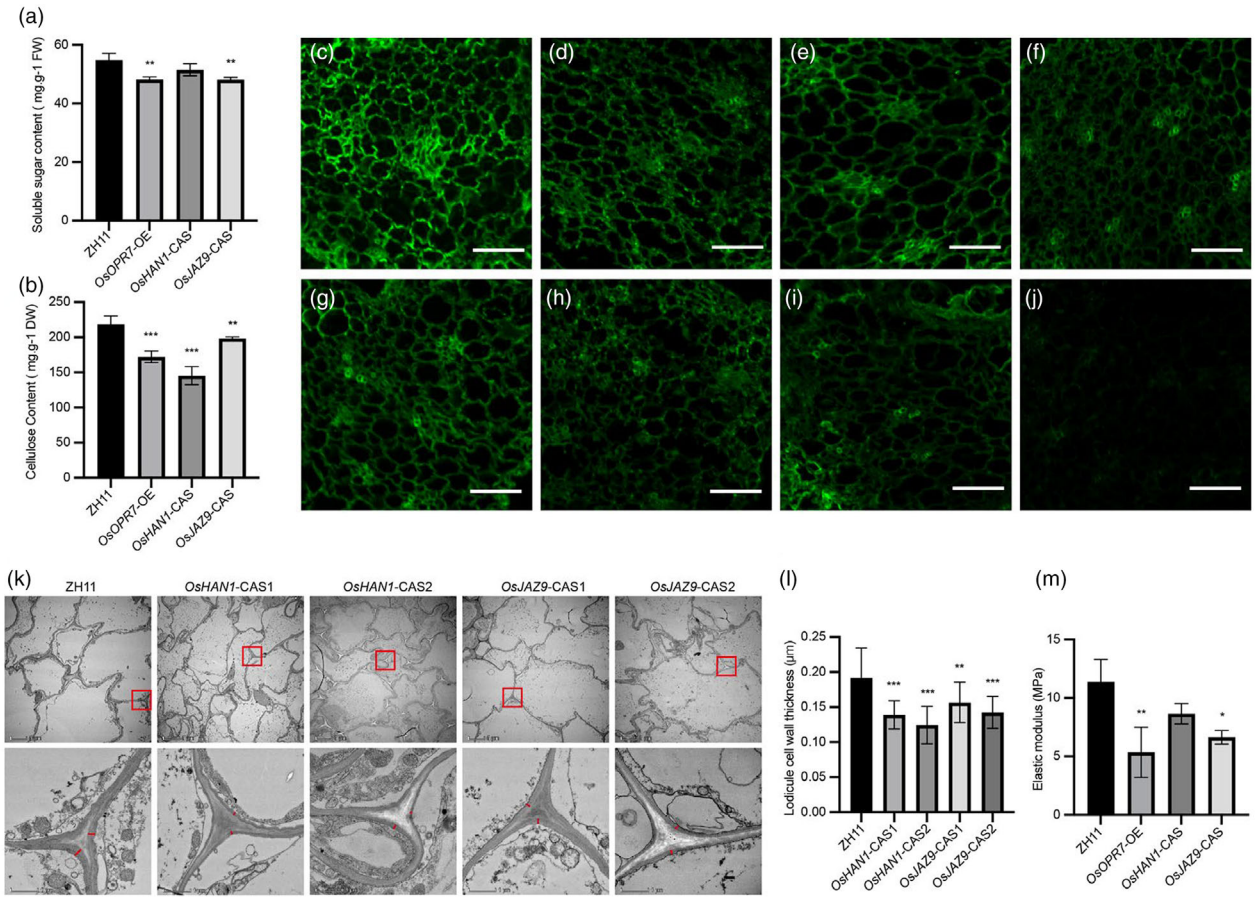

Figure 6 JA signalling pathway genes affect DFOT possibly through cell wall polysaccharide metabolism. (a) Soluble sugar contents in lodicules of *OsOPR7*-OE, *OsHAN1*-CAS and *OsJAZ9*-CAS plants at 8:30 am. The data are the mean ± SDs, $n = 4$; $^{**}0.001 \leq P < 0.01$ according to Student's *t*-test. FW stands for fresh weight. (b) Lodicules cellulose contents in *OsOPR7*-OE, *OsHAN1*-CAS and *OsJAZ9*-CAS plants at 8:30 am. The data are the mean ± SDs, $n = 5$; $^{**}0.001 \leq P < 0.01$; $^{***}P < 0.001$ according to Student's *t*-test. DW stands for dry weight. (c-j) Immunofluorescence assay of the lodicules of ZH11 (c), *OsOPR7*-OE (d, e), *OsHAN1*-CAS (f, g) and *OsJAZ9*-CAS (h, i) plants (primary antibody LM15). (j) Negative control. Rabbit anti-rat secondary antibody was added, but no primary antibody was added. Scale: 50 μm. (k) The cell structures of ZH11, *OsHAN1*-CAS and *OsJAZ9*-CAS lodicules were observed by transmission electron microscopy. Scales were 5 and 1 μm for top panel and bottom panel respectively. (l) The cell wall thickness of ZH11, *OsHAN1*-CAS and *OsJAZ9*-CAS lodicules calculated by ImageJ software. The data are the mean ± SDs, $n = 23$; $^{**}0.001 \leq P < 0.01$; $^{***}P < 0.001$ according to Student's *t*-test. (m) Elastic modulus of *OsOPR7*-OE, *OsHAN1*-CAS and *OsJAZ9*-CAS detected by atomic force microscopy. The data are the mean ± SDs, $n = 3$; $^{*}0.01 \leq p < 0.05$; $^{**}0.001 \leq p < 0.01$ a ccording to Student's *t*-test.

consuming and requires significant labour, posing challenges in practical seed production. Ultimately, these methods do not adequately address the issue of asynchronous DFOTs between *indica* and *japonica* parental lines (Wang *et al*., 2023; Zhang *et al*., 2016). Therefore, cloning DFOT genes and developing *japonica* rice varieties with earlier DFOT through genetic improvement is the most effective and cost-effective solution to address the mismatched DFOT issue.

In this study, we determined that manipulating genes in the JA pathway modulates DFOT in rice. Increasing the expression of *OsOPR7*, a gene in the JA biosynthesis pathway, elevated JA levels in lodicules and advanced DFOT by approximately 1 h (Figure 3). Knockout of *OsHAN1*, a gene responsible for JA deactivation, increased the levels of active JAs in lodicules and substantially advanced DFOT by approximately 2 h (Figure 4). Knockout of *OsJAZ7* or *OsJAZ9*, negative regulators in the JA signal transduction pathway, also accelerated DFOT by about 50 min and 1.5 h respectively (Figure 5). These findings highlight the potential of manipulating key genes in the JA pathway to advance flower opening in *japonica* rice.

In inter-subspecific *indica–japonica* hybrid breeding, *indica* restorer lines typically flower between 10:30 am and 12:30 pm, with peak flower opening around 11:00 am, whereas *japonica* sterile lines usually flower after 12:00 pm; each floret generally opens for about 1 h (Zhang *et al*., 2016). Modifying the JA pathway in parental lines could produce plants with various DFOTs for hybrid breeding, allowing breeders to synchronize DFOT using different parental lines. In this study, we modified the *japonica* TGMS line GAZS, obtaining the sterile lines GS-*OPR7*-OE and GS-*HAN1*-CAS, which flowered approximately 1 and 1.5 h earlier respectively (Figure 7b,d). F_1 seed production trials with *indica–japonica* hybrid rice showed that the seed-setting rate significantly increased to 19.8% and 30.6% when the transgenic lines GS-*OPR7*-OE and GS-*HAN1*-CAS were crossed with the *indica* rice Nanguizhan compared to the original GAZS rate of 10.7% (Figure 7e). The yields of FHSP using GS-*OPR7*-OE and

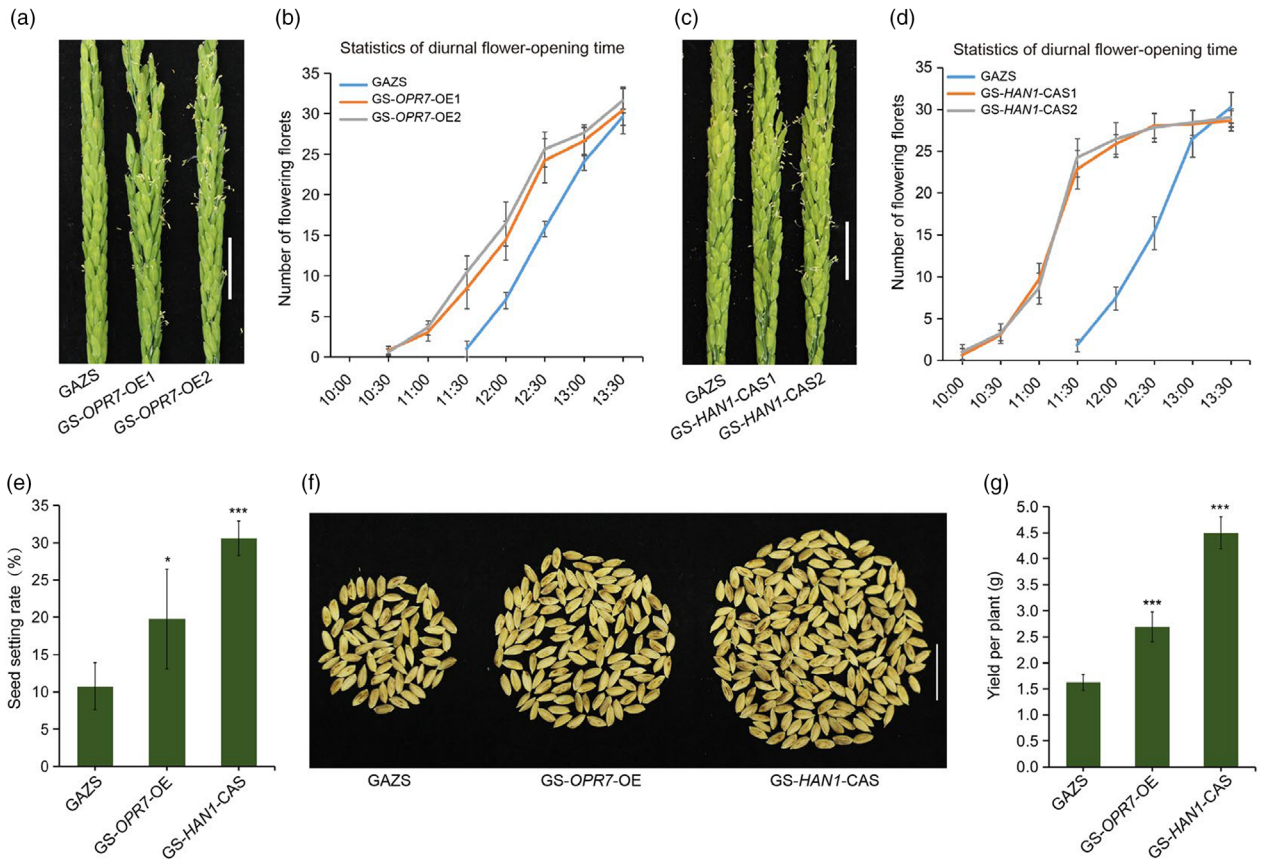

Figure 7 Application of *OsOPR7* overexpression and *OsHAN1* knockout in FHSP. (a) The inflorescence of GAZS, GS-*OPR7*-OE1 and GS-*OPR7*-OE2 at around 11:20 am. Scale: 2 cm. (b) The DFOT statistics of GAZS, GS-*OPR7*-OE1 and GS-*OPR7*-OE2 plants. The *y*-axis represents the total number of florets per panicle that have bloomed, and the *x*-axis represents the time. The data represent mean ± SDs, $n = 5$. (c) The inflorescence of GAZS, GS-*HAN1*-CAS1 and GS-*HAN1*-CAS2 at around 11:10 am. Scale: 2 cm. (d) The DFOT statistics of GAZS, GS-*HAN1*-CAS1 and GS-*HAN1*-CAS2 plants. The *y*-axis represents the total number of florets per panicle that have bloomed, and the *x*-axis represents the time. The data represent mean ± SDs, $n = 5$. (e) Statistics of F_1 hybridization rate between *japonica* male sterile lines GAZS, GS-*OPR7*-OE and GS-*HAN1*-CAS and *indica* cultivar Nanguizhan. The data are the mean ± SDs, $n = 5$; *$0.01 \leq P < 0.05$; ***$P < 0.001$ according to Student's *t*-test. (f) The seeds obtained by crossing five panicles of *japonica* male sterile lines GAZS, GS-*OPR7*-OE, and GS-*HAN1*-CAS with *indica* rice Nanguizhan. Scale: 2 cm. (g) Statistics of F_1 hybridization yield per plant between *japonica* male sterile lines GAZS, GS-*OPR7*-OE, and GS-*HAN1*-CAS and *indica* cultivar Nanguizhan. The data are the mean ± SDs, $n = 9$; ***$P < 0.001$ according to Student's *t*-test.

GS-*HAN1*-CAS as the female parents were 1.85 and 2.86 times greater, respectively, than those using GAZS. These results suggest that by modifying genes studied in the present study to advance the DFOT of *japonica* male sterile lines can significantly enhance the yield of FHSP and reduce the cost of seed production. In addition, JA pathway genes could also be modified to promote rice flower opening in the morning to avoid high mid-day temperatures. Furthermore, enhancing JA signalling also improves plant resistance to biotic and abiotic stresses (Ghorbel *et al*., 2021; Raza *et al*., 2021). Consequently, the manipulation of JA pathway genes holds immense significance for breeding stress-resistant rice varieties with early DFOT. Our findings provide a potential strategy for reducing hybrid breeding costs and advancing *indica–japonica* hybrid rice breeding.

JA controls DFOT through loosening of the lodicule cell walls in rice

DFOT is a complex trait influenced by multiple factors, and research in this field has been relatively limited (Zheng *et al*., 2020). Previous studies have identified certain quantitative trait loci on different chromosomes (1, 2, 3, 4, 5, 7, 8, 10, 12) that control DFOT in rice, but the functional genes have not been successfully cloned through map-based cloning (Hirabayashi *et al*., 2015; Ma *et al*., 2011; Thanh *et al*., 2010; Wan *et al*., 2013; Wang *et al*., 2023). Using reverse genetics, we previously discovered *DFOT1*/*EMF1* as a functional gene modulating DFOT in rice. *DFOT1*/*EMF1* encodes a protein of unknown function, and specifically expressed in lodicules (Wang *et al*., 2022; Xu *et al*., 2022). Loss of DFOT1 function results in the advancement of DFOT by approximately 2.5 h in summer in Guangzhou.

JAs have emerged as critical players in the flower-opening process of rice; for example, exogenous MeJA treatment induces flower opening in rice (Zeng and Zhou, 1999). In the rice CMS line Zhenshan 97A (ZS97A), a decrease in JA content in lodicules is related to delayed and dispersed DFOTs (Liu *et al*., 2017). These studies suggest a role for JAs in rice flower opening.

In this study, we observed that exogenous MeJA applied at various concentrations induced rice flower opening after

30 min (Figure 1). Furthermore, the proportion of blooming florets was dependent on MeJA concentration, with the optimal concentration for getting the maximum proportion of blooming florets being 30 mmol/L. Subsequently, we found that the expression level of key genes in the JA biosynthesis and signalling pathways changed during the process of rice flower opening (Figure 2). These results imply a role for JA in DFOT in rice.

Previous studies have revealed that the *OPEN GLUME1* mutant (*og1*) of *OsOPR7* displays dispersed flower opening. Down-regulation of sugar transport-related genes, such as *OsSWEET*, in the *og1* mutant affects the sugar content in lodicules, resulting in delayed closure of the glume after flower opening (Li *et al*., 2018). Here, overexpressing *OsOPR7* led to an increase in endogenous levels of JA, resulting in larger lodicules during flower opening, thereby advancing DFOT in rice (Figure 3). Knockout of *OsHAN1*, encoding a JA-inactivating enzyme, increased the level of active JA-Ile, resulting in larger lodicules before flower opening and a substantial advancement of DFOT (Figure 4). Individual knockouts of *OsJAZ7* and *OsJAZ9* resulted in an early DFOT phenotype (Figure 5). Similar to *OsHAN1*, knockout of *OsJAZ7* or *OsJAZ9* led to larger lodicules before flower opening.

We found that 3 h before flowering of *OsOPR7*-OE plants (7:00 am), the levels of OPDA, JA and JA-Ile in the lodicules of the ZH11, *OsOPR7*-OE and *OsHAN1*-CAS plants were all very low (Figure S3). As the DFOT approaching, the contents of OPDA, JA and JA-Ile gradually increased in the rice lodicules. In the lodicules of *OsOPR7*-OE plants, displaying a DFOT approximately 1 h earlier than wild-type ZH11, the JA and JA-Ile contents in lodicules were approximately 1.2 and 1.5 times higher than those in ZH11 plants about 1 h before flowering (9:00 am) respectively (Figure 3f,g). In *OsHAN1*-CAS lines, displaying a DFOT approximately 2 h earlier than wild-type ZH11, the JA and JA-Ile contents in lodicules were approximately 5.6 and 4.3 times higher than those in ZH11 about 0.5 h before flowering (8:30 am) respectively (Figure 4f,g). The *OsOPR7*-OE lines undergoing flowering at 10:30 am exhibited increased levels of OPDA, JA and JA-Ile that were 4.4, 32.4 and 29.9 times higher, respectively, than those in ZH11. The OPDA, JA and JA-Ile levels of lodicules of *OsHAN1*-CAS undergoing flowering showed 10.4, 62.6 and 38.6 times higher than those in ZH11 respectively (Figure S3). In summary, these results suggest that the levels of JA and JA-Ile in lodicules are positively associated with the DFOT. Additionally, on the day of flowering, the JA content progressively increases as the DFOT approaches.

DEG analysis of lodicule transcriptomes in the *OsJAZ9*-CAS and ZH11 plants indicated an up-regulation of genes associated with sugar metabolism, such as genes encoding cellulase and hemicellulase (Figure S7). Subsequent investigations showed that the cellulose and hemicellulose contents in the cell walls of the lodicules from the early-flowering lines *OsOPR7*-OE, *OsHAN1*-CAS and *OsJAZ9*-CAS were reduced compared to the ZH11. Moreover, ultra-thin sectioning observation revealed decreased cell wall thickness in lodicules of *OsHAN1*-CAS and *OsJAZ9*-CAS. Atomic force microscopy measurements also indicated a reduction in the elastic modulus of the lodicule cell walls for *OsOPR7*-OE, *OsHAN1*-CAS and *OsJAZ9*-CAS lines (Figure 6). These results suggest that the JA pathway-related genes *OsOPR7*, *OsHAN1* and *OsJAZ9* mainly influence the composition and thus the stiffness of lodicule cell walls by regulating sugar metabolism genes, thereby controlling the DFOT in rice.

Materials and methods

The construction of the expression vectors and genetic transformation

According to the previous description (Zhou *et al*., 2012), the coding region of *OsOPR7* was cloned into the pOX vector to construct the overexpression vector. According to the previous description (Ma *et al*., 2015; Zhou *et al*., 2016), two target site sequences of *OsHAN1*, *OsJAZ1*, *OsJAZ4*, *OsJAZ7*, *OsJAZ9* and *OsJAZ11* genes were, respectively, cloned into pYLgRNA, and then the expression cassette containing two target sites was amplified from pYLgRNA and connected to pYLCRISPR/Cas9 to construct the knockout vector. The above vectors were transformed into rice by *Agrobacterium*-mediated genetic transformation. All primers are shown in Table S2.

Plant materials and planting

The homozygous knockout mutants *OsHAN1*-CAS, *OsJAZ1*-CAS, *OsJAZ4*-CAS, *OsJAZ7*-CAS, *OsJAZ9*-CAS and *OsJAZ11*-CAS were obtained by CRISPR/cas9 technology in *japonica* rice (*Oryza sativa* L. ssp. *geng/japonica*) variety ZH11; the homozygous knockout mutants GS-*HAN1*-CAS were obtained by CRISPR/cas9 technology in *japonica* rice sterile line GAZS. *OsOPR7* was expressed in ZH11 and GAZS under the control of the ubiquitin promoter to obtain overexpressed lines *OsOPR7*-OE13, *OsOPR7*-OE20, GS-*OPR7*-OE1 and GS-*OPR7*-OE2. All materials were planted in the experimental farm of South China Agricultural University in Guangzhou, or Lingshui, Hainan.

Total RNA extraction and RT-qPCR analyses

RNA was extracted from different tissues of rice using Trizol reagent (Invitrogen). HiScript III 1st Strand cDNA Synthesis Kit (Vazyme, Nanjing) or the PrimeScript II 1st Strand cDNA Synthesis Kit (Takara, Kyoto) were used for reverse transcription to obtain cDNA. RT-qPCR was performed using 2× RealStar Green Fast Mixture Kit (Genstar, Beijing) according to the corresponding experimental steps. Each experiment was repeated three times, *Actin* was used as the control for normalizing cDNA amounts, and data were processed according to BioRad's 'Fluorescent Quantitative PCR Application Guide' by calculating the relative expression levels of target genes through $2^{-\Delta Ct}$ value. For RNA extraction, the plant materials of the *japonica* rice Kongyu131 (KY131) and *indica* rice II32 RNA were taken from Lingshui, Hainan in March, and plant materials for other rice lines were taken from summer in Guangzhou.

Statistics of the number of florets opened

Diurnal flower-opening time (DFOT) is an important agronomic trait of rice. The concept of DFOT can be divided into two categories: the time from floret opening to floret closing, and the time or duration of floret flowering in the field population within a day. The latter can be further divided into the beginning of flower opening, the peak time of flower opening (when 50% of the florets have opened), and the end time of flowering (Gou *et al*., 2022; Ma *et al*., 2011; Zhang *et al*., 2016). In this paper, we use DFOT to represent the beginning of flower opening. Counting statistics of DFOT for knockout plants and overexpressed plants with wild-type plants started from 2 to 5 days after rice heading. The inflorescences with similar growth rates were selected on the day before flowering, and five inflorescences for each rice line was labelled and marked. From the

beginning of flower-opening, the number of opening florets was counted every 30 min until all florets were closed. The statistical results were averaged from the data of the five inflorescences.

Observation of dynamic changes in lodicules

Florets were selected at different times of the day, and then peeled off the lemma and palea to expose a pair of lodicules, and the dissected florets were observed and photographed under a dissecting microscope (Olympus SZX10). Subsequently, a pair of lodicules on the florets were peeled off and photographed with a dissecting microscope. The surface area of the lodicules was counted by ImageJ software, as shown in Figure S11.

Hormone treatment

One day before the treatment, inflorescences with similar growth status were selected in the field and labelled. At 7:00 am, different concentrations of MeJA (Yuanye, Shanghai) solution were sprayed on the marked inflorescence until all rice inflorescences were wet, and then the number of floret opening was counted every 30 min for each inflorescence until all florets were closed. The average value of five inflorescences was taken for each time point. The rice plants treated with MeJA solution for 1 h were pulled out from the field and then planted in pots to take pictures of the plants.

Hormone content measurement

The fresh lodicules of ZH11, *OsOPR7*-OE and *OsHAN1*-CAS plants at different time points were quickly frozen in liquid nitrogen. The contents of JA, JA-Ile and OPDA were measured according to the method described by Cai *et al*. (2016). The extraction and detection part of the experiment was completed by Wuhan Greensword Creation Technology Co., Ltd.

Transcriptome analysis

About 0.1 g of fresh lodicules were collected 0.5–1 h before flower-opening of *OsJAZ9*-CAS plants and then quickly frozen in liquid nitrogen (in March in Lingshui, Hainan). The collected lodicules were thoroughly ground in liquid nitrogen and the fine powder was transferred into a 2-mL Eppendorf tube with Trizol reagent and quickly mixed, and then stored at −80 °C before use. The RNA extraction, library construction, sequencing and transcriptome analysis of the samples was conducted by GENEWIZ Suzhou Co., Ltd. According to the transcriptome results, differential gene expression analysis was performed using DESeq2 software. Genes with $P < 0.05$ and log2-fold change ≥1 or log2-fold change ≤−1 were classified as DEGs and GO enrichment analysis was performed with DEGs and the RT-qPCR method was used to verify the expression of the selected DEGs.

Plant soluble sugar detection

Plant soluble sugar detection is based on the principle of anthrone colorimetry by using soluble sugar detection kit (Sangon, Shanghai). The experimental procedure was carried out according to the kit instructions. The lodicules at 8:30 am on the day of flowering were collected into Eppendorf tubes and then 1 mL of ddH_2O was added. For breaking the lodicules, the tubes were incubated in water bath at 100 °C for 10 min, and then cooled at room temperature followed by centrifuging at 8000 ***g*** for 10 min. After centrifugation, the supernatant was collected into a new tube and the solution volume was adjusted with ddH_2O for measurement. Different concentrations of anhydrous glucose were used as standard curve quantification and ddH_2O as blank control. For soluble sugar measurement, 0.2 mL of the extracted sample was transferred into a new tube followed by adding 0.2 mL of ddH_2O, 0.1 mL of working solution, and 1 mL of concentrated sulphuric acid, then close the tube lid and mix well. Put tubes in a 95 °C water bath for 10 min, and then cool to room temperature. Transfer 0.2 mL of the solution to a 96-well plate, and then measure the absorbance value at 620 nm by using a spectrophotometer (Biotek, USA).

Cellulose content detection

The lodicules were dried to constant weight, according to the experimental steps of the cellulose content detection kit (Sangon, Shanghai). The cellulose was decomposed into β-D-glucose by heating under acidic conditions, and reacted with anthrone reagent to produce colour under strong acidic conditions. The absorbance value was measured at 620 nm to determine the cellulose content.

Ultra-thin sections

The experimental procedure was based on the previous study (Peng *et al*., 2023; Wang *et al*., 2022). Briefly, soak the lodicules in 4% paraformaldehyde fixative at 8:00 am on the day of flowering. After fixation with 1% the osmium tetroxide solution, the alcohol dehydration, the acetone transition, resin infiltration and embedding were conducted. Ultra-thin sections with a thickness of 100 nm were prepared with a diamond blade. The cut slices were placed in a staining box, stained with uranyl acetate at room temperature for 15–30 min, rinsed with ddH_2O and stained with lead citrate for 5–30 min. Then rinse the slices with ddH_2O to clean, dry and the slices were observed by using transmission electron microscopy after drying.

Immunofluorescence

The experimental steps for paraffin embedding and immunofluorescence of rice lodicules refer to the previous study (Chen *et al*., 2023; Wang *et al*., 2022). The primary antibody was LM15 (PlantProbes) with the antigen of Xyloglucan, and the secondary antibody was FITC Rabbit Anti-Rat (BOSTER, Wuhan).

Measurement of elastic modulus

Elastic modulus of the lodicule was measured according to the previous experimental method (Wang *et al*., 2022). Briefly, fresh samples were attached to a glass slide with transparent nail polish, and several drops of sterile water were dripped at room temperature to prevent the lodicule from losing water and shrinking. The elastic modulus of the surface of the lodicule was detected using a BioScope Resolve atomic force microscope with a SCANASYST-FLUID probe in the liquid QNM mode. The peak force frequency is 2 kHz, and the peak force setting value is 3 nN. The topological image size is $5 \times 5\ \mu m^2$ with the resolution of 256×256 pixels, and the scanning rate is 0.2 Hz. The experiment was repeated three times, and the data were analysed using NanoScope analysis software version 3.0.

Indica–japonica F_1 hybrid seeds production test

In the crossing experiment, the *japonica* TGMS line GAZS and its transgenic lines GS-*OPR7*-OE and GS-*HAN1*-CAS were used as maternal parents, and the *indica* rice variety Nanguizhan as the paternal parent. The ratio of sterile lines to restorer lines was 6 : 2, with six rows of sterile lines in the middle and one row of restorer lines on both sides. Each combination was planted with 48 rice plants, and three replicates were conducted for each

combination. Pollinations were performed once a day at 11:30 am during the flowering period, with the process repeated over four consecutive days. Flowering observations indicated that at 9:40 am, Nanguizhan began to open its florets, with most prepared to close by 11:30 am. At this time, GAZS had just started to flower.

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Conflict of interest

The authors declare they have no conflict of interest.

Author contributions

H.Z. conceived the project, and H.Z., M.W. and X.Z. wrote the manuscript. H.Z., Z.L. and J.Z. designed experiments and guided students. M.W. and X.Z. performed most of the experiments and analysed the data. Z.H., M.C., P.X., S.L., Y.Z., Y.G., J.H., Y.L., H.C. and X.W. performed parts of experiments. Z.L., J.Z., C.Z., L.Z., S.N. and J.D. modified the manuscript.

Data availability statement

The data that supports the findings of this study are available in the supplementary material of this article.

References

Bheemanahalli, R., Sathishraj, R., Manoharan, M., Sumanth, H.N., Muthurajan, R., Ishimaru, S.T. and Krishna, J.S.V. (2017) Is early morning flowering an effective trait to minimize heat stress damage during flowering in rice? *Field Crop Res.* **203**, 238–242.

Cai, W.J., Ye, T.J., Wang, Q., Cai, B.D. and Feng, Y.Q. (2016) A rapid approach to investigate spatiotemporal distribution of phytohormones in rice. *Plant Methods*, **12**, 47.

Chai, G., Fu, X., Bai, Z., Huang, J. and Liu, S. (2009) Research progress on plant endo-β-1,4-glucanase genes. *J. Agric. Sci. Technol.* **11**, 17–22.

Chen, H., Zhang, S., Li, R., Peng, G., Chen, W., Rautengarten, C., Liu, M. *et al*. (2023) BOTRYOID POLLEN 1 regulates ROS-triggered PCD and pollen wall development by controlling UDP-sugar homeostasis in rice. *Plant Cell*, **35**, 3522–3543.

Chini, A., Fonseca, S., Fernández, G., Adie, B., Chico, J.M., Lorenzo, O., García-Casado, G. *et al*. (2007) The JAZ family of repressors is the missing link in jasmonate signalling. *Nature*, **448**, 666–671.

Ciaffi, M., Paolacci, A.R., Tanzarella, O.A. and Porceddu, E. (2011) Molecular aspects of flower development in grasses. *Sex. Plant Reprod.* **24**, 247–282.

Deng, D. and Yan, N. (2016) GLUT, SGLT, and SWEET: Structural and mechanistic investigations of the glucose transporters. *Protein Sci.* **25**, 546–558.

Ghorbel, M., Brini, F., Sharma, A. and Landi, M. (2021) Role of jasmonic acid in plants: the molecular point of view. *Plant Cell Rep.* **40**, 1471–1494.

Gou, Y., Zhu, X., Wang, H. and Shen, R. (2022) Regulation mechanism and breeding application of rice floret-opening-time. *J. South China Agric. Univ.* **43**, 48–59.

Han, G.Z. (2017) Evolution of jasmonate biosynthesis and signalling mechanisms. *J. Exp. Bot.* **68**, 1323–1331.

Hara, Y., Yokoyama, R., Osakabe, K., Toki, S. and Nishitani, K. (2014) Function of xyloglucan endotransglucosylase/hydrolases in rice. *Ann. Bot.* **114**, 1309–1318.

Hirabayashi, H., Sasaki, K., Kambe, T., Gannaban, R.B., Miras, M.A., Mendioro, M.S., Simon, E.V. *et al*. (2015) *qEMF3*, a novel QTL for the early morning flowering trait from wild rice, *Oryza officinalis*, to mitigate heat stress damage at flowering in rice, *O. sativa*. *J. Exp. Bot.* **66**, 1227–1236.

Hirose, T., Scofield, G.N. and Terao, T. (2008) An expression analysis profile for the entire sucrose synthase gene family in rice. *Plant Sci.* **174**, 534–543.

Hou, Q., Ufer, G. and Bartels, D. (2016) Lipid signalling in plant responses to abiotic stress. *Plant Cell Environ.* **39**, 1029–1048.

Howe, G.A., Major, I.T. and Koo, A.J. (2018) Modularity in jasmonate signaling for multistress resilience. *Annu. Rev. Plant Biol.* **69**, 387–415.

Ishida, K. and Yokoyama, R. (2022) Reconsidering the function of the xyloglucan endotransglucosylase/hydrolase family. *J. Plant Res.* **135**, 1145–12156.

Jagadish, S.V.K. (2020) Heat stress during flowering in cereals-effects and adaptation strategies. *New Phytol.* **226**, 1567–1572.

Jagadish, S.V.K., Bahuguna, R.N., Djanaguiraman, M., Gamuyao, R., Prasad, P.V. and Craufurd, P.Q. (2016) Implications of high temperature and elevated CO_2 on flowering time in plants. *Front. Plant Sci.* **7**, 913.

Katsir, L., Schilmiller, A.L., Staswick, P.E., He, S.Y. and Howe, G.A. (2008) COI1 is a critical component of a receptor for jasmonate and the bacterial virulence factor coronatine. *Proc. Natl. Acad. Sci.* **105**, 7100–7105.

Kazuhiro, K., Tsutomu, M., Mayumi, Y. and Toshihiro, H. (2010) Effects of temperature, solar radiation, and vapor-pressure deficit on flower opening time in rice. *Plant Prod. Sci.* **13**, 21–28.

Li, X., Wang, Y., Duan, E., Qi, Q., Zhou, K., Lin, Q., Wang, D. *et al*. (2018) OPEN GLUME1: a key enzyme reducing the precursor of JA, participates in carbohydrate transport of lodicules during anthesis in rice. *Plant Cell Rep.* **37**, 329–346.

Li, J.Y., Yang, C., Xu, J., Lu, H.P. and Liu, J.X. (2023) The hot science in rice research: how rice plants cope with heat stress. *Plant Cell Environ.* **46**, 1087–1103.

Liu, L., Zou, Z., Qian, K., Xia, C., He, Y., Zeng, H., Zhou, X. *et al*. (2017) Jasmonic acid deficiency leads to scattered floret opening time in cytoplasmic male sterile rice Zhenshan 97A. *J. Exp. Bot.* **68**, 4613–4625.

Ma, Z., Zhan, Z., Cheng, X., Gao, J., He, G., Liu, D., Xu, H. *et al*. (2011) Flowering time in filial generations of cross between *indica* and *japonica* rice and its response to external environment. *Hybrid Rice*, **26**, 70–76.

Ma, X., Zhang, Q., Zhu, Q., Liu, W., Chen, Y., Qiu, R., Wang, B. *et al*. (2015) A robust CRISPR/Cas9 system for convenient, high-efficiency multiplex genome editing in monocot and dicot plants. *Mol. Plant*, **8**, 1274–1284.

Mao, D., Xin, Y., Tan, Y., Hu, X., Bai, J., Liu, Z.Y., Yu, Y. *et al*. (2019) Natural variation in the *HAN1* gene confers chilling tolerance in rice and allowed adaptation to a temperate climate. *Proc. Natl. Acad. Sci.* **116**, 3494–3501.

Miersch, O., Neumerkel, J., Dippe, M., Stenzel, I. and Wasternack, C. (2008) Hydroxylated jasmonates are commonly occurring metabolites of jasmonic acid and contribute to a partial switch-off in jasmonate signaling. *New Phytol.* **177**, 114–127.

Opassiri, R., Cairns, J.R.K., Akiyama, T., Wara-Aswapati, O., Svasti, J. and Esen, A. (2003) Characterization of a rice beta-glucosidase highly expressed in flower and germinating shoot. *Plant Sci.* **165**, 627–638.

Peng, G., Liu, M., Zhu, L., Luo, W., Wang, Q., Wang, M., Chen, H. *et al*. (2023) The E3 ubiquitin ligase CSIT1 regulates critical sterility-inducing temperature by ribosome-associated quality control to safeguard two-line hybrid breeding in rice. *Mol. Plant*, **16**, 1695–1709.

Pujadas, G. and Palau, J. (2001) Evolution of α-amylases: architectural features and key residues in the stabilization of the (β/α)8 scaffold. *Mol. Biol. Evol.* **18**, 38–54.

Qian, Q., Guo, L., Smith, S. and Li, J. (2016) Breeding high-yield superior quality hybrid super rice by rational design. *Natl. Sci. Rev.* **3**, 283–294.

Raza, A., Charagh, S., Zahid, Z., Mubarik, M.S., Javed, R., Siddiqui, M.H. and Hasanuzzaman, M. (2021) Jasmonic acid: a key frontier in conferring abiotic stress tolerance in plants. *Plant Cell Rep.* **40**, 1513–1541.

Ruan, J., Zhou, Y., Zhou, M., Yan, J., Khurshid, M., Weng, W., Cheng, J. *et al*. (2019) Jasmonic acid signaling pathway in plants. *Int. J. Mol. Sci.* **20**, 2479.

Sheard, L.B., Tan, X., Mao, H., Withers, J., Ben-Nissan, G., Hinds, T.R., Kobayashi, Y. *et al*. (2010) Jasmonate perception by inositol-phosphate-potentiated COI1-JAZ co-receptor. *Nature*, **468**, 400–405.

Tani, T., Sobajima, H., Okada, K., Chujo, T., Arimura, S.-I., Tsutsumi, N., Nishimura, M. *et al*. (2008) Identification of the *OsOPR7* gene encoding 12-oxophytodienoate reductase involved in the biosynthesis of jasmonic acid in rice. *Planta*, **227**, 517–526.

Thanh, P.T., Phan, P.D., Ishikawa, R. and Ishii, T. (2010) QTL analysis for flowering time using backcross population between *Oryza sativa* Nipponbare and *O*. rufipogon. *Genes Genet. Syst.* **85**, 273–279.

Thines, B., Katsir, L., Melotto, M., Niu, Y., Mandaokar, A., Liu, G., Nomura, K. *et al*. (2007) JAZ repressor proteins are targets of the SCF^{COI1} complex during jasmonate signalling. *Nature*, **448**, 661–665.

Wan, G., Feng, Y., Zhang, F., Xu, Q., Wang, Y., Yuan, X., Yu, H. *et al*. (2013) Mapping QTLs for flowering time in rice. *Acta Agric. Nucl. Sin.* **27**, 562–567.

Wang, C. and Li, J. (2021) Research progress of plant *β*-glucosidase. *Biot. Resour.* **43**, 101–109.

Wang, Y., Xiao, Y., Zhang, Y., Chai, C., Wei, G., Wei, X., Xu, H. *et al*. (2008) Molecular cloning, functional characterization and expression analysis of a novel monosaccharide transporter gene *OsMST6* from rice (*Oryza sativa* L.). *Planta*, **228**, 525–535.

Wang, X., Wang, M., Ji, Z., Chen, Z., Liu, W., Han, Y. and Yang, C. (2014) Functional characterization of the glycoside hydrolase encoding gene *OsBE1* during chloroplast development in *Oryza sativa*. *Acta Agron. Sin.* **40**, 2090–2097.

Wang, Y., Wang, D., Gu, C., Wu, X. and Dai, G. (2016) Application effects of methyljasmonate (MeJA) in different *japonica* inter-subspecific hybrid rice seed production. *China Rice*, **22**, 78–80.

Wang, M., Zhu, X., Peng, G., Liu, M., Zhang, S., Chen, M., Liao, S. *et al*. (2022) Methylesterification of cell wall pectin controls the diurnal flower opening times in rice. *Mol. Plant*, **15**, 956–972.

Wang, M., Chen, M., Huang, Z., Zhou, H. and Liu, Z. (2023) Advances on the study of diurnal flower-opening times of rice. *Int. J. Mol. Sci.* **24**, 10654.

Xu, P., Wu, T., Ali, A., Zhang, H., Liao, Y., Chen, X., Tian, Y. *et al*. (2022) EARLY MORNING FLOWERING 1 (EMF1) regulates the floret opening time by mediating lodicule cell wall formation in rice. *Plant Biotechnol. J.* **20**, 1441–1443.

Yoshida, H. (2012) Is the lodicule a petal: molecular evidence? *Plant Sci.* **184**, 121–128.

Yoshida, K., Imaizumi, N., Kaneko, S., Kawagoe, Y., Tagiri, A., Tanaka, H., Nishitani, K. *et al*. (2006) Carbohydrate-binding module of a rice endo-beta-1,4-glycanase, *OsCel9A*, expressed in auxin-induced lateral root primordia, is post-translationally truncated. *Plant Cell Physiol.* **47**, 1555–1571.

Zeng, X.C. and Zhou, X. (1999) Methyl jasmonate induces the opening of spikelets in rice. *Acta. Bot. Sin.* **41**, 560–562.

Zhang, M., Dai, D., Li, X., Zhang, H. and Ma, L. (2016) Advances on the study of flowering time trait in hybrid rice. *Acta Agric. Nucl. Sin.* **30**, 267–274.

Zheng, W., Ma, Z., Zhao, M., Xiao, M., Zhao, J., Wang, C., Gao, H. *et al*. (2020) Research and development strategies for hybrid japonica rice. *Rice*, **13**, 36.

Zhou, H., Liu, Q., Li, J., Jiang, D., Zhou, L., Wu, P., Lu, S. *et al*. (2012) Photoperiod- and thermo-sensitive genic male sterility in rice are caused by a point mutation in a novel noncoding RNA that produces a small RNA. *Cell Res.* **22**, 649–660.

Zhou, H., He, M., Li, J., Chen, L., Huang, Z., Zheng, S., Zhu, L. *et al*. (2016) Development of commercial thermo-sensitive genic male sterile rice accelerates hybrid rice breeding using the CRISPR/Cas9-mediated *TMS5* editing system. *Sci. Rep.* **6**, 37395.

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1 The relative expression levels of *OsOPR7*, *OsHAN1*, *OsJAZ7* and *OsJAZ9* in lodicules of *japonica* rice KY131 and *indica* rice II32 at different time points analysed by RT-qPCR.

Figure S2 The expression levels of *OsOPR7* and lodicule surface area in the *OsOPR7* overexpression plants.

Figure S3 OPDA, JA and JA-Ile contents in lodicules of ZH11, *OsOPR7*-OE and *OsHAN1*-CAS plants.

Figure S4 The expression levels of *OsHAN1* and DFOT phenotypes of the *OsHAN1*-OE plants, and the sequences of *OsHAN1* and lodicule surface area in the *OsHAN1*-CAS.

Figure S5 The nucleotide sequences of different rice *JAZ* genes in *JAZ* mutants.

Figure S6 Statistic analysis of the lodicule surface area in ZH11, *OsJAZ7*-CAS and *OsJAZ9*-CAS plants.

Figure S7 Gene expression analysis of *OsJAZ9*-CAS lodicules.

Figure S8 The agronomic traits of ZH11, *OsOPR7*-OE, *OsHAN1*-CAS, *OsJAZ7*-CAS and *OsJAZ9*-CAS plants.

Figure S9 Overexpression of *OsOPR7* and knockout of *OsHAN1* in a *japonica* rice sterile line GAZS.

Figure S10 The panicles of *japonica* sterile lines GAZS, GS-*OPR7*-OE and GS-*HAN1*-CAS hybridized with *indica* cultivar Nanguizhan.

Figure S11 The schematic diagram of the surface area of the lodicule calculated by ImageJ software.

Table S1 Analysis of differentially expressed genes screened by transcriptome.

Table S2 Primers used in this study.

