

Molecular basis of differential sensitivity to MeJA in floret opening between indica and japonica rice

ZHIQIANG YAN*, RUYUE DENG, HUIHUI TANG, HONGWEI ZHANG, SUSONG ZHU*

Guizhou Provincial Academy of Agricultural Sciences, Guiyang, P.R. China

*Corresponding authors: 2414904364@qq.com; 13984033281@139.com

Zhiqiang Yan and Ruyue Deng contributed equally to this work.

Citation: Yan Z.Q., Deng R.Y., Tang H.H., Zhang H.W., Zhu S.S. (2024): Molecular basis of differential sensitivity to MeJA in floret opening between indica and japonica rice. Czech. J. Genet. Plant Breed., 60: 136–148.

Abstract: Methyl jasmonate (MeJA) accelerated floret opening in rice, crucial for addressing inconsistent floret opening in hybrid seed production of indica × japonica hybrids. However, indica and japonica rice exhibited notable differences in their sensitivity to MeJA's impact on floret opening, with indica responding faster. To clarify the molecular basis of MeJA-induced floret opening sensitivity differences between indica and japonica rice, after 1 h of MeJA spraying, transcriptomes and endogenous floret opening regulators were compared between indica and japonica rice, with histology differences observed. The results showed that MeJA quickly activated plant hormones in indica and japonica rice, but *JAZ* genes in JA signalling pathway upregulation were greater in indica rice. Alpha-linolenic acid, starch and sucrose metabolism pathways responded faster in indica rice. MeJA boosted endogenous JA synthesis more in indica rice, which had 5–6 middle-layer cells, more than japonica's 3–4 layers, resulting in thicker glumes. The metabolic pathways regulating floret opening, including plant hormone signal transduction, alpha-linolenic acid and starch and sucrose metabolism, along with endogenous JA and soluble sugars, could respond to exogenous MeJA. However, the more intense and rapid responses of these regulatory pathways and endogenous substances to MeJA in indica, resulted in the difference in sensitivity to MeJA between indica and japonica rice.

Keywords: floret opening time; indica and japonica subspecies; MeJA; RNA-seq

Hybrids between indica and japonica subspecies show a 30% higher yield than those within the same subspecies (Yan et al. 2015). However, variations in floret opening time both in indica and japonica subspecies restricted the seed production. Previous studies examined the response of floret opening time to environmental factors, such as region (Quan et al. 2016), temperature (Tao et al. 2008; Ma et al. 2011; Zhou et al. 2014; Zhang et al. 2019; Huang & Zeng 2021), humidity (Peng et al. 1994; Ma 1999), light (Kobayasi 2012), and CO₂ (Wang et al. 1993; Kobayasi et al. 2019; Huang & Zeng 2023). Several genes regulating floret opening time in rice have been iden-

tified. For instance, *Diurnal Flower Opening Time 1* (*DFOT1*), which is expressed in lodicule specifically, regulated floret opening time in rice by interacting with pectin methylesterase (PME) (Wang et al. 2022), *Oryza sativa Myeloblastosis Virus Binding Factor 8* (*OsMYB8*), a pivotal regulator of DFOT, induced transcription of *Oryza sativa Jasmonate Resistant 1* (*OsJAR1*) and ultimately enhancing rice floret opening (Gou et al. 2024). Mutations in *OsJAR1* (Xiao et al. 2014) and *Open Glume 1* (*OGI*) (Li et al. 2017), which is involved in jasmonic acid biosynthesis, resulted in dispersed floret opening in rice, the mutation in *Split-husk 1* (*SH1*), which involved in jasmonic

Supported by National Natural Science Foundation of China (No. 32260530) and Central Funds for National Guide to Guizhou Provincial Project (QKHZYD [2023]019 and QKHZYD [2023]014).

© The authors. This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International (CC BY-NC 4.0).

<https://doi.org/10.17221/43/2024-CJGPB>

acid biosynthesis, leading to failure of floret closure (Pan et al. 2019). *Oryza sativa* 9-cis-epoxycarotenoid dioxygenase 3 (*OsNCED3*) and *Oryza sativa* pyrabactin resistance-like abscisic acid receptor 1 (*OsPYL1*) controlled rice floret closure through the endogenous abscisic acid pathway (Zhai et al. 2023). Previous studies employed histological approach elucidated the multiple regulatory pathways responsible for floret opening and closure in rice, such plant hormone signal transduction, starch and sucrose metabolism, alpha-linolenic acid metabolism, jasmonate (JA) signal pathway and so on (Fu et al. 2016; Yan et al. 2022b). These findings provided important theoretical support for regulating floret opening and closure.

To address the issue of inconsistent floret opening time, two primary strategies can be employed: genetic enhancement of floret opening time through breeding and manipulation of floret opening time through cultivation or exogenous chemicals, however chemical regulation was straightforward and held immense potential for widespread use in hybrid seed production. Currently, multiple chemicals could control rice floret opening and closure: methyl jasmonate (MeJA), epi-brassinolide (epi-BR) and gibberellic acid (GA) promote floret opening (Zeng et al. 1999; Xia et al. 2019; Gou et al. 2022), ethephon (Eth), methyl salicylate (MeSA), indole acetic acid (IAA) and naphthaleneacetic acid (NAA) delayed floret opening (Zeng 2000; Yan et al. 2015; He & Zhang 2023); moreover, IAA, indole-3-butyric acid (IBA), NAA, 2,4-dichlorophenoxy acetic acid (2,4-D), and abscisic acid (ABA) facilitate floret closure (Huang et al. 2018).

Preliminary findings from our study showed MeJA promoted floret opening in both indica and japonica subspecies, however, sensitivity to MeJA is different between subspecies, with indica responding to lower concentrations and japonica to higher. In addition, spraying high MeJA could not synchronize floret opening time, and indica responded faster (Yan et al. 2014). Furthermore, by analysing the transcriptomes of multiple indica and japonica rice varieties, we pinpointed several metabolic pathways that played a pivotal role in floret opening. These pathways included plant hormone signal transduction, mitogen active protein kinase (MAPK) signaling pathway-plant, starch and sucrose metabolism, alpha-linolenic acid metabolism, plant-pathogen interaction, diterpenoid biosynthesis, glucuronate interconversions, and phenylpropanoid biosynthesis (Yan et al. 2022b). To elucidate the molecular basis of differential sensitivity to MeJA-induced floret opening time between indica and japonica

subspecies, and whether the floret opening regulatory pathways responds differently to methyl jasmonate in these two subspecies, thereby accounting for their floret opening differential sensitivity to MeJA, we conducted transcriptome analysis on spikelet treated with 4 mmol/L MeJA and validated our findings through RT-PCR and endogenous substance quantification. This study establishes a theoretical foundation for the applying of MeJA in hybrid seed production between indica and japonica subspecies.

MATERIAL AND METHODS

Trail materials and treatments. This study utilized the indica rice variety Hefengsimiao (Ind) and the japonica rice variety Jingdao104 (Jap), both sourced from the Guizhou Rice Research Institute, as materials. In 2023, the experimental materials were planted in Guiyang, located in southwest China, at an altitude of 1 100 m in a humid subtropical climate. Each variety occupied 6 rows of 10 plants, placed 30 × 16 cm apart. The phytohormone MeJA was purchased from Tokyo Chemical Industry Ltd., Japan, with purity > 95%. Spraying with 4 mmol/L MeJA serves as treatment, with an ethanol concentration of 2% and emulsifier TWEEN®80 concentration of 0.1%. A water solution containing the same concentration of ethanol and emulsifier serves as the control. One day before treatment, the opened florets must be removed. Then, the next morning (08:00 a.m.), spray the entire rice plant. Each treatment involved spraying three individual plants, with three replicates, at a rate of 20 mL per plant. The sample information is listed in Table S1 in Electronic Supplementary Material (ESM).

Measurements of heading date and floret opening time. The heading date was determined when 50% of the rice panicles heading were heading. Manual observation was performed to assess the timing of floret opening in rice, considering the first floret opening on the rice panicle as the initiation time, with 8 a.m. as the starting point (Yan et al. 2014).

RNA extraction and sequencing. Spikelets were sampled for transcriptome sequencing one hour following spraying, with three biological replicates for each sample. TRIzol reagent (Thermo Fischer) was used for RNA extract. The quantity and purity of the total RNA were thoroughly analysed using the Bioanalyzer 2100 and RNA 6000 Nano LabChip Kit (Agilent, USA, 5067–1511). To construct the sequencing library, only high-quality RNA samples with a RIN number exceeding 7.0 were utilized (Pandey

et al. 2022). The 2×150 bp paired-end sequencing (PE150) was performed using the Illumina NovaseqTM 6000 (LC-Bio Technology CO., Ltd., Hangzhou, China) following the manufacturer's recommendation. The reference genome used for the mapping was Ensembl_v54. A total of 12 cDNA libraries were constructed in the trial (Table S2 in ESM). The raw sequencing data were deposited in China National Center for Bioinformation (CNCB) under the Bio-Project accession number PRJCA024794.

Quantitative realtime PCR analysis. Primers were designed utilizing Beacon Designer 7.9, with GAPDH as the reference gene. Detailed primer information is listed in Table S3 in ESM. Real-time RT-PCR was conducted using the qTOWER 2.0/2.2 Quantitative Real-Time PCR Thermal Cyclers (Germany). The reaction conditions were as follows: step 1: 95 °C for 3 min, step 2: 95 °C for 10 s, step 3: 58 °C for 30 s + plate read, step 4: 39 cycles of 95 °C for 10 s, step 5: melt curve analysis (60–95 °C, + 1 °C/cycle, holding time 4 s). Relative expression was calculated using the $2^{-\Delta\Delta CT}$ method.

Determination of endogenous substances. To extract endogenous phytohormones, the spikelets were ground to dry powder in liquid nitrogen, and then isopropanol-water-hydrochloric acid (configuration ratio, 2 : 1 : 0.002) was added. Add 8 μ L (1 μ g/mL) internal standard solution and oscillated for 30 min at 4 °C. After adding dichloromethane, oscillate and centrifuge at 13 000 r/min using high-speed centrifuge (TGL-20bR, Anting Scientific Instrument, China) at 4 °C for 5 min to obtain the lower organic phase, then dry it with termovap sample concentrator (DC150-2, YouNing Instrument, CN) and redissolve methanol (0.1% formic acid). Centrifuge at 4 °C for 10 min (13 000 r/min), and then filter the supernatant through a 0.22 μ m filter membrane. Gradient standard solutions of SA, JA, MeJA, and hormones-gibberellic acid (GA3) were prepared using methanol (0.1% formic acid) at concentrations of 0.1, 0.2, 0.5, 2, 5, 20, 50 and 200 ng/mL, with the addition of an internal standard solution at a final concentration of 20 ng/mL. Endogenous JA, MeJA, salicylic acid (SA) and GA3 were determined by high-performance liquid Chromatography (Agilent, 1290) and tandem mass spectrometry (Applied Biosystems 6500 Quadrupole Trap). Chromatographic condition: chromatographic column is Agilent Poroshell 120 SB-C18 (2.7 μ m 2.1×150 mm); Column temperature: 40 °C; the mobile phases A and B are respectively 0.1% formic acid in methanol and 0.1% formic acid in wa-

ter. The injection volume is 2 μ L, and the flow rate is 0.2 mL/min. The elution gradient is set from 0 to 13.5 min. Mass spectrometer conditions: the electron spray ionization (ESI) source is adopted. with an atomization temperature of 400 °C, a curtain gas (CUR) pressure of 15 psi, an ionspray voltage (IS) of 4 500 V, a nebulizer gas pressure (Gas1) of 65 psi, and an auxiliary gas pressure (Gas2) of 70 psi. The monitoring mode is set to multiple reaction monitoring (MRM). The Analyst software is adopted to process mass spectrometry data. Alpha-amylase activity was performed using the α -amylase (a-AL) activity kit (Solarbio, 50T/24S, China). Measurement is conducted using a spectrophotometer (SpectraMax190, Molecular Devices, USA). Soluble sugar was determined using anthrone colorimetry (Wang 2006). The spikelet samples were ground into powder using liquid nitrogen. Then 0.3 g of the powder was mixed with 10 mL of distilled water, and after a 30-min boiling bath, the supernatant was collected. Another 10 mL of distilled water was added to the residue, followed by a 20-min boiling bath, cooling, and retrieval. The residue was then repeatedly washed until the volume was adjusted to 25 mL. Eventually, 1 mL of the sample was pipetted, topped up to 2 mL with distilled water, and anthrone-ethyl acetate reagent and concentrated sulfuric acid solution were added sequentially for measurement. The above experiments were conducted in three replications. The chromatograms and standard curves are shown in Figure S1–S9 in ESM.

Analysis of histology. Glumes were collected and fixed with FAA, then dehydrated and cleared under different concentrations of ethanol and xylene, stained with Safranin O and Fast green, and sectioned to 8 μ m thickness in paraffin using a microtome (Leica RM2016) and scanned under 3D HISTECH (Pannoramic Scan; Hungary), and measured with CaseViewer software.

Statistical analyses. The student's *t*-test was performed using SPSS 20.0. Data visualization was done using the GraphPad prism 9.

RESULTS

Investigations of floret opening time. The floret opening time of Ind was significantly earlier than Jap (Figure 1A). Spraying 4 mmol/L MeJA accelerated floret opening in both cultivars (Figure 1B, C), with Ind responding within an hour, while japonica rice requires one and a half to two hours. Furthermore, Ind exhibited higher sensitivity to MeJA than Jap in terms of floret opening time (Figure 1D, E).

<https://doi.org/10.17221/43/2024-CJGPB>

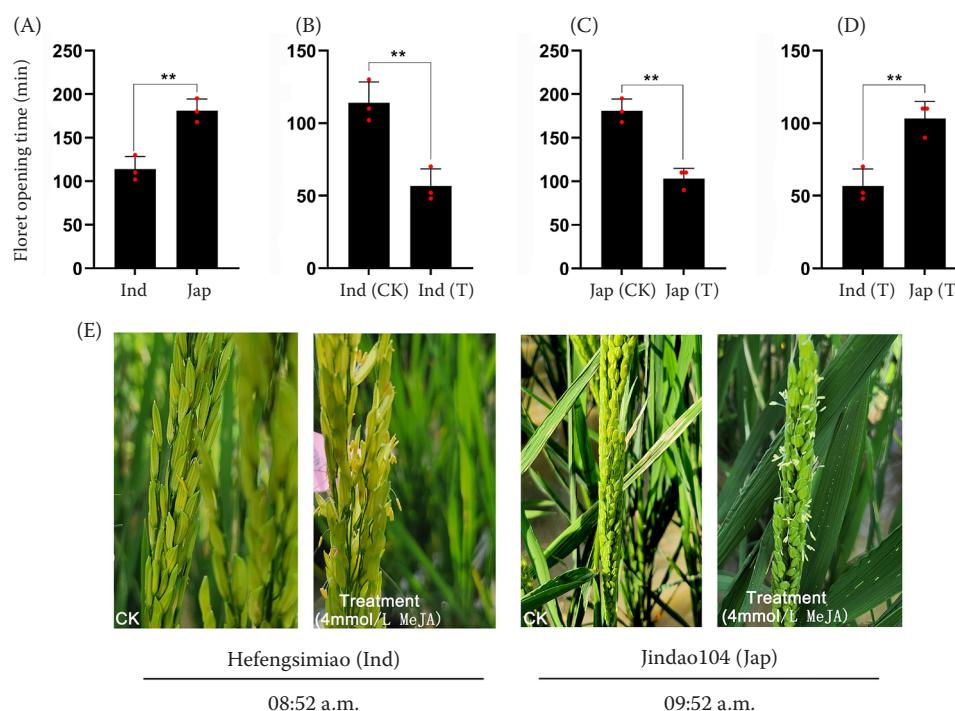


Figure 1. Investigations of floret opening time: comparing the floret opening time of Hefengsimiao (Ind) and Jindao104 (Jap) (A), comparison of floret opening time between treatment and control for Ind and Jap (B–C), comparison of floret opening time of treatment between Ind and Jap (D), field photographs of treatment and control groups for Ind and Jap (E) *, **significant and highly significant at the levels of 0.05 and 0.01

Significantly differentially expressed genes (DEGs) shared by indica and japonica rice 1 hour after MeJA treatment.

One hour after MeJA treatment, 1 186 significantly DEGs were shared by Ind and Jap, with 776 genes exhibiting concordant regulation and 410 genes exhibiting discordant regulation (Figure 2). The 776 genes aligned for regulation were selected for Kyoto Encyclopedia of Genes and Genomes (KEGG) annotation, and the results showed that plant hormone signal transduction, biosynthesis of secondary metabolites and ABC transporters were the significantly enriched pathways (Figure 3A), suggesting that these three pathways both in indica and japonica rice can respond to MeJA. Previous studies have shown that the plant hormone signal transduction, JA biosynthesis and signal transduction pathways were closely related to floret opening (Fu et al. 2016; Yan et al. 2022b). These finds revealed that the plant hormone signal transduction pathway comprised 17 genes, including 9 genes from the *JAZ* family (Table S4 in ESM), such as *OsJAZ12*, *OsJAZ10*, *OsJAZ8*, *OsJAZ6*, *OsJAZ11*, *OsJAZ1*, *OsJAZ13*, *OsJAZ9*, and *OsJAZ5*, which participated in jasmonic acid signal transduction (Ye et al. 2009; Yamada et al. 2012; Feng et al. 2020; Cao et al. 2021; Singh et al. 2021; Mehra et al. 2022). After 1 h

of MeJA treatment, the expression of these genes increased significantly, with Ind exhibiting significantly higher levels compared to Jap, except for *OsJAZ8* and

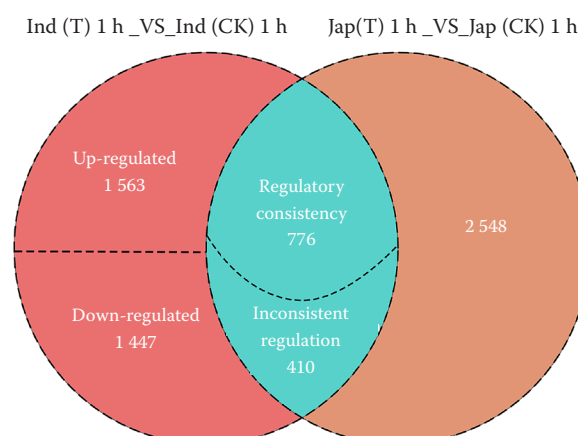


Figure 2. Venn diagram exhibiting the differentially expressed genes (DEGs) distribution in two comparison groups, the red circle represents the DEGs in the Hengfengsimiao (Ind (T) 1 h _VS_ Ind (CK) 1 h), the yellow circle represents the DEGs in the Jindao104 (Jap(T) 1 h _VS_ Jap (CK) 1 h), and the blue circle represents the DEGs shared by the two comparison groups

OsJAZ13 (Figure 3B). Consistent with the transcriptome, RT-PCR further corroborated that the expression of these genes, *OsJAZ12*, *OsJAZ10*, *OsJAZ6*, *OsJAZ11*, *OsJAZ1*, *OsJAZ9*, and *OsJAZ5*, showed a significant increment in indica rice compared to japonica rice one hour after spraying with MeJA (Figure 3C).

Significantly DEGs Specific to indica rice 1 hour after MeJA treatment. Following 1 h of MeJA treatment, 3 010 significantly differentially expressed genes were identified specific to indica rice, with 1 563 up-regulated and 1 447 down-regulated (Figure 2). Significantly up-regulated genes were significantly enriched in three pathways, biosynthesis of secondary metabolites, metabolic pathways and alpha-linolenic acid metabolism (Figure 4A). As alpha-linolenic acid initiated JA biosynthesis, our focus was on analysing its metabolism pathway, comprising 10 genes. The cloned ones include

OsAOC, *OsAOS1*, *OsAOS3*, and *OsAOS4*, which were the key genes in the JA biosynthesis pathway (Table S5 in ESM). Utilizing transcriptome analysis and RT-PCR validation, it was unequivocally confirmed that the expression of these four cloned genes was markedly upregulated 1 h following MeJA treatment (Figure 4B, C).

Significantly down-regulated genes were significantly enriched in five pathways, pentose and glucuronate interconversions, metabolic pathways, starch and sucrose metabolism, biosynthesis of secondary metabolites and cutin, suberine and wax biosynthesis (Figure 5A). Previous studies indicating the involvement of starch and sucrose metabolism in rice floret opening led us to analyse this pathway, comprising 16 genes, with 9 already cloned (Table S6 in ESM). The transcriptome and RT-PCR confirmed that the expression of these

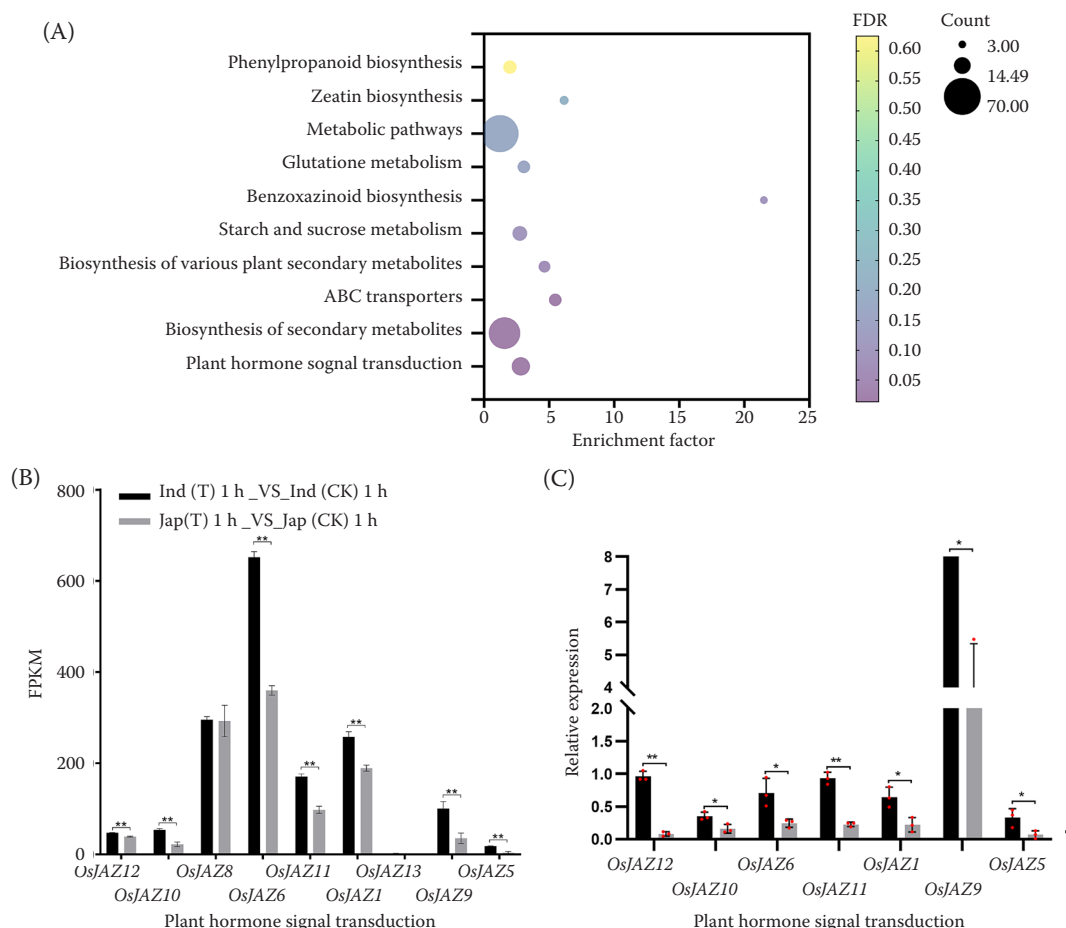


Figure 3. Analysis of shared and concordant regulated differentially expressed genes (DEGs) between two comparison groups: Hengfengsimiao (Ind (T) 1 h_VS_Ind (CK) 1 h) and Jindao104 (Jap (T) 1h_VS_Jap (CK) 1 h): bubble diagram for KEGG enrichment analysis (A), analysis of the expression of *JAZ* genes on plant hormone signal transduction between two comparison groups (B), RT-PCR validation for the results of the transcriptome (C)

*, **significant and highly significant at the levels of 0.05 and 0.01

<https://doi.org/10.17221/43/2024-CJGPB>

9 cloned genes were significantly down-regulated 1 h after MeJA treatment (Figure 5B, C).

Variation of endogenous hormones in indica and japonica rice 1 hour after MeJA treatment.

Endogenous JA's role in rice floret opening has been confirmed (Huang et al. 2015). Hence, we analysed changes in JA and MeJA content in both indica and japonica rice. Our results indicated a significant increase in endogenous JA and MeJA in both indica and

japonica rice 1 h after MeJA treatment (Figure 6A–D). Specifically, MeJA significantly elevated these levels more in indica rice than japonica rice (Figure 6E, F).

It has been demonstrated that salicylic acid (SA) and MeJA exhibit antagonistic effects on floret opening in rice (Zeng 2000). Consequently, we examined variations in endogenous SA in indica and japonica rice. The results indicated that the endogenous SA in indica and japonica rice increased significantly 1 h after MeJA

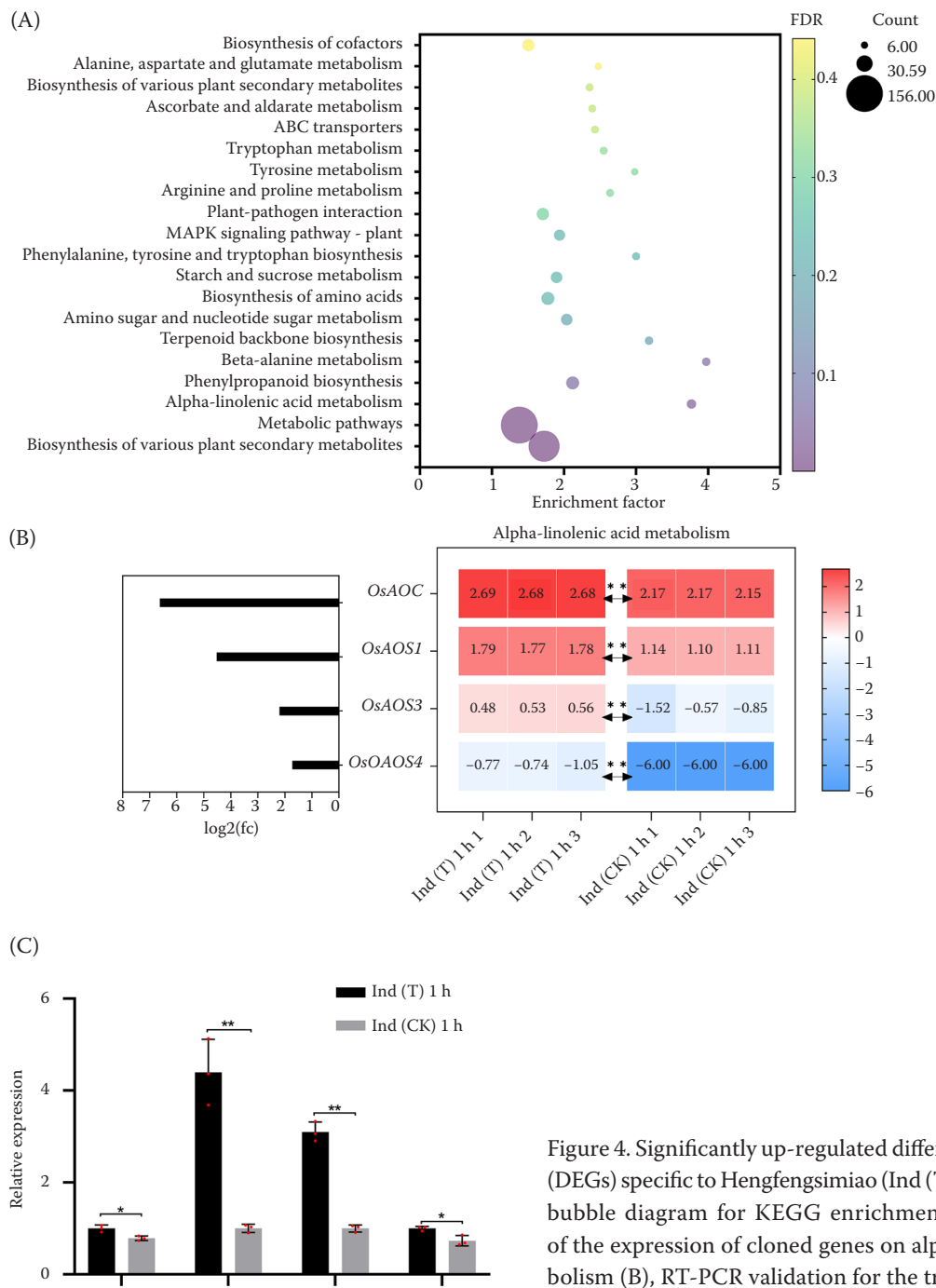


Figure 4. Significantly up-regulated differentially expressed genes (DEGs) specific to Hengfengsimiao (Ind (T) 1 h_VS_Ind (CK) 1 h): bubble diagram for KEGG enrichment analysis (A), analysis of the expression of cloned genes on alpha-linolenic acid metabolism (B), RT-PCR validation for the transcriptome (C)

*, **significant and highly significant at the levels of 0.05 and 0.01

spraying (Figure 6G, H), with a significant increment observed in indica compared to japonica rice (Figure 6I).

Additionally, we analysed the changes in endogenous GA3 levels resulting from the application

of gibberellins to promote floret opening in rice. The results showed that spraying MeJA significantly decreased the endogenous GA3 of indica rice (Figure 6J), with no significant decrease in japonica rice

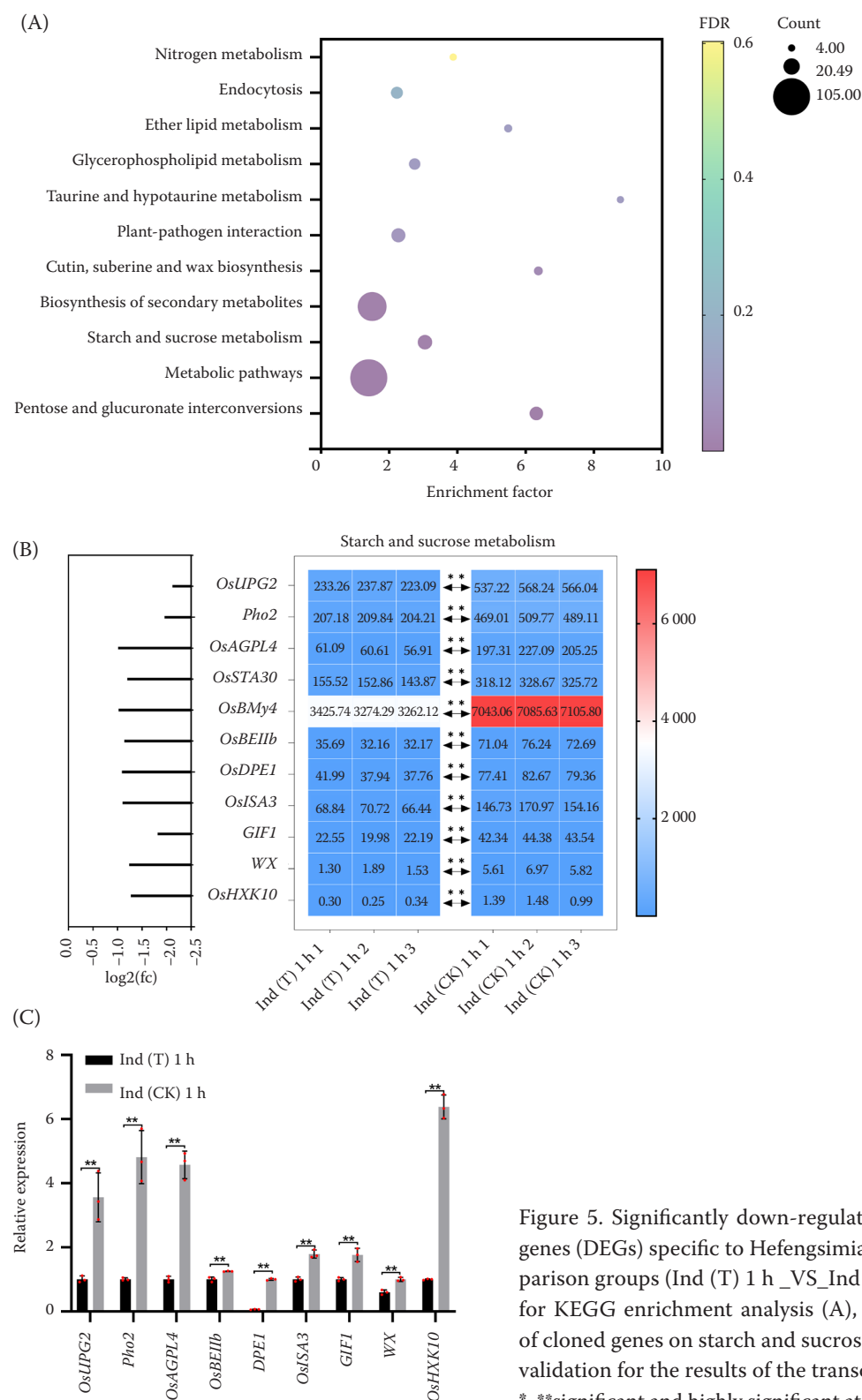


Figure 5. Significantly down-regulated differentially expressed genes (DEGs) specific to Hefengsimiao (Ind) between two comparison groups (Ind (T) 1 h_VS_Ind (CK) 1 h): bubble diagram for KEGG enrichment analysis (A), analysis of the expression of cloned genes on starch and sucrose metabolism (B), RT-PCR validation for the results of the transcriptome (C)

*, **significant and highly significant at the levels of 0.05 and 0.01

<https://doi.org/10.17221/43/2024-CJGPB>

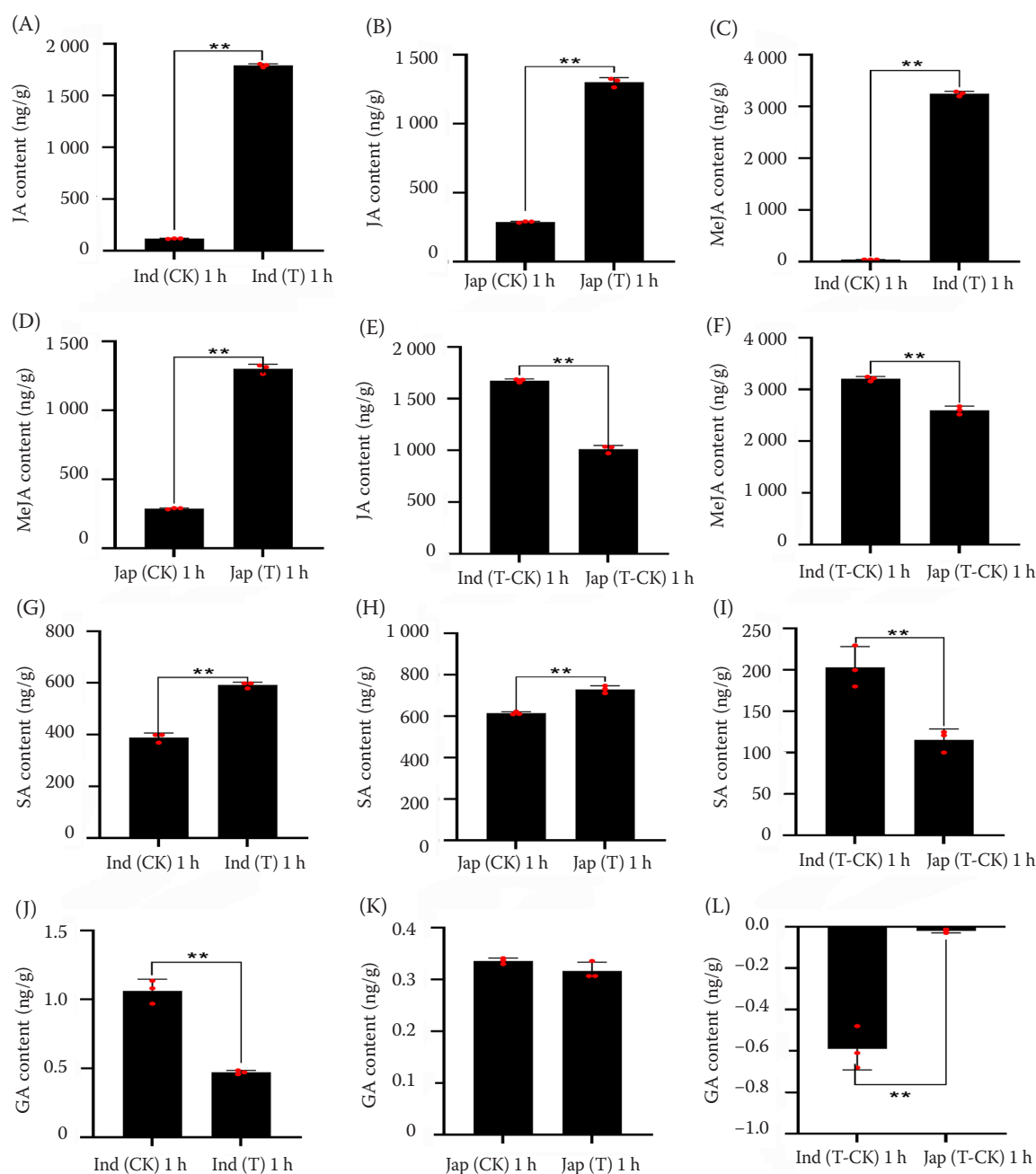


Figure 6. Endogenous hormones in Hengfengsimiao (Ind) and Jindao104 (Jap): endogenous jasmonate (JA) (A–C), endogenous methyl jasmonate (MeJA) (D–F), endogenous salicylic acid (SA) (G–I), endogenous hormones-gibberellic acid (GA3) (J–K), **, significant and highly significant at the levels of 0.05 and 0.01

(Figure 6K), and the reduction of endogenous GA3 in indica rice was significantly greater than that in japonica rice by MeJA (Figure 6L).

Osmotic adjustment substance in floret opening. Rice floret opening involves the turgor pressure movement induced by lodicule, where soluble sugars play a crucial role in regulating lodicule osmotic pressure (Zeng et al. 2004). Additionally, α -amylase can

decompose starch into soluble sugars. Therefore, we analysed the variations in endogenous α -amylase and soluble sugars in indica and japonica rice varieties. Following MeJA spraying, there was a notable surge in endogenous α -amylase content in both indica and japonica rice varieties after 1 h (Figure 7A, B). Notably, the increase was more pronounced in indica than in japonica rice (Figure 7C). The soluble sugar

content in both indica and japonica rice significantly increased after treatment (Figure 7D, E), however, the increase in indica rice was notably smaller compared to japonica rice (Figure 7F).

Cytological observations of the glumes of both indica and japonica rice. Morphological differences existed between indica and japonica rice glumes. It was presumed that indica glumes have fewer cell layers than japonica rice, allowing exogenous MeJA to diffuse and be absorbed more easily. Thus, glume transverse sections were observed. The results indicated that both indica and japonica rice glumes were composed of outer mastoid, and inner large parenchyma cells. Notably, indica rice exhibited greater number of middle-layer cells, ranging from 5 to 6 layers (Figure 8A), compared to 3 to 4 layers in japonica rice (Figure 8B), resulting in thicker glumes in indica rice (Figure 8C). Therefore, the variation in sensitivity to MeJA between the floret opening time of indica and japonica rice was not directly associated with glume thickness or the number of cell layers.

DISCUSSION

We have previously demonstrated variations in the sensitivity of floret opening time between subsp.

indica and subsp. *japonica* rice in response to MeJA. Notably, indica rice exhibited responsiveness to both low (40 μ mol/L) and high (4 mmol/L) concentrations of MeJA, whereas japonica rice responded solely to relatively high concentrations (400 μ mol/L and 4 mmol/L). 4 mmol/L MeJA could not synchronize the floret opening time between these two subspecies, with indica rice exhibiting a shorter response time than japonica. (Yan et al. 2014). Given the substantial influence of environmental factors on floret opening time, the previous experiments, conducted in Northeast China, exhibited geographical specificity in the conclusions. Nonetheless, the current study, executed in Southwest China, corroborated the previous findings, establishing the universality of the differential sensitivity to MeJA across different rice subspecies and geographical regions.

Our previous research established that several metabolic pathways, encompassing plant hormone signal transduction, starch and sucrose metabolism, and alpha-Linolenic acid metabolism, played crucial roles in the regulation of rice floret opening (Yan et al. 2022b). Specifically, genes such as *OsJAZ1-OsJAZ13* in jasmonic acid signal transduction, *OsAOC*, *OsAOS1*, *OsAOS2* in alpha-linolenic acid metabolism, and *OsHXX3* in the *OsHXX* family involved

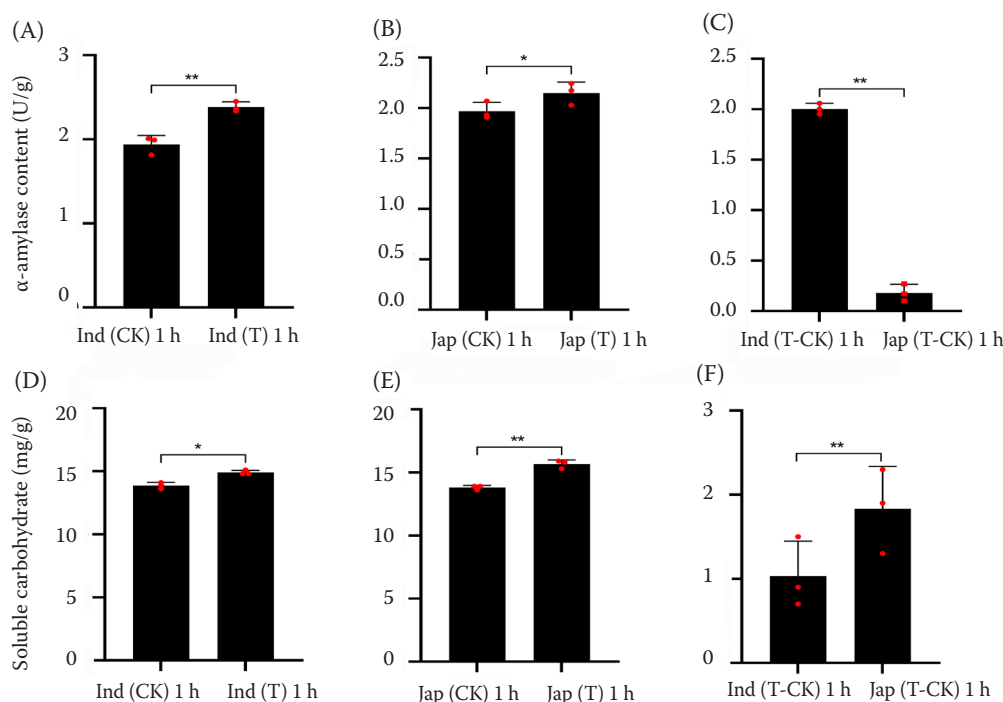


Figure 7. Alpha-amylase and soluble sugars in Hengfengsimiao (Ind) and Jindao104 (Jap): α -amylase (A–C), soluble sugars (D–F)

*, **significant and highly significant at the levels of 0.05 and 0.01

<https://doi.org/10.17221/43/2024-CJGPB>

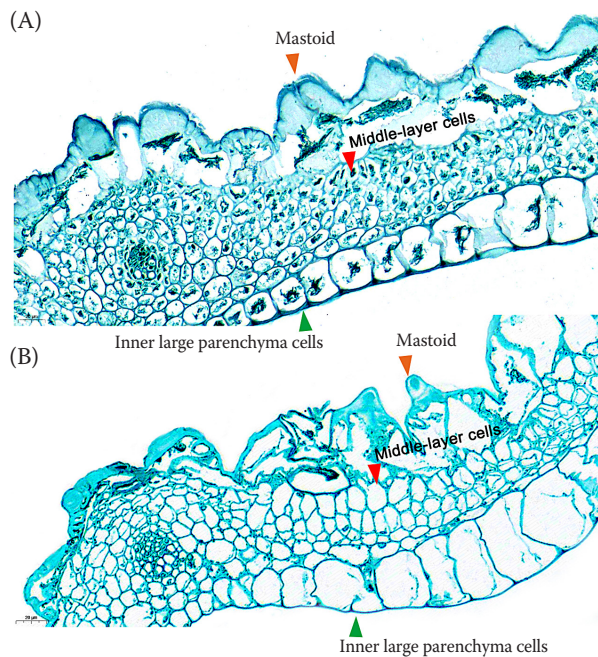


Figure 8. Glume transverse sections between Hengfengsimiao (Ind) and Jindao104 (Jap): transverse slice of Hengfengsimiao (Ind) (A), transverse slice of Jindao104 (Jap) (B), analysis of glume thickness (C)

The orange, red and green triangles represent mastoid, middle-layer cells and inner large parenchyma cells; *, **significant and highly significant at the levels of 0.05 and 0.01

in starch and sucrose metabolism, exhibiting marked up-regulation in expression levels close to floret opening (Huang et al. 2015; Fu et al. 2016; Yan et al. 2022b). The current study demonstrated that MeJA activated these three pathways, leading to significant increases in the expression of genes including *OsJAZ1*, *OsJAZ5*, *OsJAZ6*, *OsJAZ9*, *OsJAZ10*, *OsJAZ11*, *OsJAZ12* in jasmonic acid signal transduction, *OsAOC*, *OsAOS1* in alpha-linolenic acid metabolism, and *OsHKK10* in starch and sucrose metabolism. These findings indicated that MeJA promoted floret opening by modulating genes within the regulatory pathways governing floret opening.

Salicylic acid and MeJA had antagonistic effects on floret opening in *Poaceae* plants (Liu et al. 2001). The present study revealed that spraying MeJA significantly boosted endogenous SA in indica and japonica rice, indicating that MeJA did not promote rice floret opening by suppressing endogenous SA synthesis. Exogenous GA3 treatment efficiently accelerated rice floret opening and enhanced hybrid seed production (Gou et al. 2022), concurrently stimulating endogenous GA3 synthesis (Dong et al. 2016). However, our study revealed that spraying MeJA reduced the endogenous GA3, potentially attributed to the independent regulatory roles of MeJA and GA3 in floret opening. GA3 facilitated internode elongation through the activation of cell division and elongation. Conversely, decreased GA biosynthesis or weakened signal transduction resulted in shorter

internodes and reduced plant height (Nagai et al. 2020). Notably, spraying MeJA in indica-japonica rice significantly shortens the plant height (Yang et al. 2021; Yan et al. 2022a), indicating that MeJA may inhibit GA3 biosynthesis, decreasing rice plant height.

Soluble sugars were the primary osmotic agents affecting the osmotic potential of lodicule (Zeng et al. 2004). Suggesting floret opening time of indica subspecies was more sensitive to MeJA than japonica subspecies, the increase in α -amylase and soluble sugar content in indica rice was expected to surpass that in japonica rice. However, surprisingly japonica rice exhibiting a higher increase in soluble sugar content. A study indicated that during floret opening of rice, the soluble sugar in lodicules originated not only from starch hydrolysis but also potentially from the input of Spikelet axis (Wang et al. 1991). Therefore, we speculated that one hour after the MeJA treatment, the soluble sugar content of the spikelet in japonica was higher than that in indica rice, potentially due to the external input of the spikelet axis.

The study showed the variation in sensitivity to MeJA between the floret opening time of indica and japonica rice was not directly associated with glume thickness or the number of cell layers. However, research showed sterile rice lines' later floret opening was due to underdeveloped lodicule vascular tissue (Wang et al. 1992). Next, we will investigate the dif-

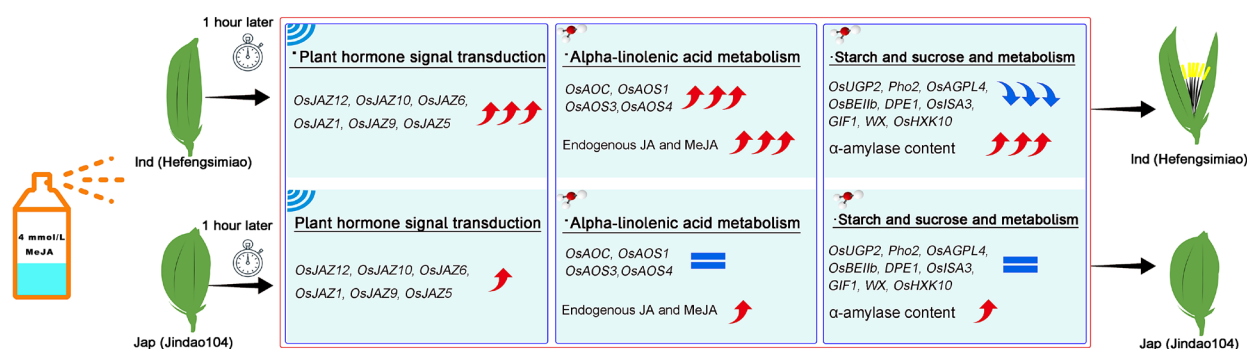


Figure 9. Molecular basis for indica-japonica difference in MeJA-mediated floret opening

The red and blue arrows represent upregulation and downregulation of expression, respectively, the number of arrows represents the intensity of increase or decrease, the blue double line represents no significant change in expression level

ference in the lodicule structure between indica and japonica rice, analyse its impact on floret opening, and assess its role in their varying sensitivity to MeJA.

CONCLUSION

The metabolic pathways regulating floret opening, including plant hormone signal transduction, alpha-linolenic acid and starch and sucrose metabolism, along with endogenous JA, MeJA and α-amylase, could respond to exogenous MeJA. However, given the more intense and rapid responses of these regulatory pathways and endogenous substances to MeJA in indica, resulting in the difference in sensitivity to MeJA between indica and japonica rice (Figure 9).

REFERENCES

- Cao L., Tian J., Liu Y., Chen X., Li S., Persson S., Lu D., Chen M., Luo Z., Zhang D. (2021): Ectopic expression of OsJAZ6, which interacts with OsJAZ1, alters JA signaling and spikelet development in rice. *The Plant Journal*, 108: 1083–1096.
- Dong C., Gu H., Ding C., Xu N., Zhang W. (2016): Effects of gibberellic acid on forage quality of rice (*Oryza sativa*) straw. *Acta Prataculturae Sinica*, 25: 94–102. (in Chinese)
- Feng X., Zhang L., Wei X., Zhou Y., Dai Y., Zhu Z. (2020): OsJAZ13 negatively regulates jasmonate signaling and activates hypersensitive cell death response in rice. *International Journal of Molecular Sciences*, 21: 4379.
- Fu y., Xiang M., Jiang H., He Y., Zeng X. (2016): Transcriptome profiling of lodicules before floret opening in *Oryza sativa* L. *Scientia Agricultura Sinica*, 49: 1017–1033. (in Chinese)
- Gou Y., Zhu X., Wang H., Shen R. (2022): Regulation mechanism and breeding application of rice floret-opening-time. *Journal of South China Agricultural University*, 43: 48–59. (in Chinese)
- Gou Y., Heng Y., Ding W., Xu C., Tan Q., Li Y., Fang Y., Li X., Zhou D., Zhu X., Zhang M., Ye R., Wang H., Shen R. (2024): Natural variation in OsMYB8 confers diurnal floret opening time divergence between indica and japonica subspecies. *Nature Communications*, 15: 2262.
- He Y., Zhang F. (2023): Study of regulating effect of auxin on floret opening in rice. *Acta Agronomica Sinica*, 49: 1690–1698. (in Chinese)
- Huang J., He Y., Zeng X., Xiang M., Fu Y. (2015): Changes of JA levels in foral organs and expression analysis of JA signaling genes in lodicules before floret opening in rice. *Scientia Agricultura Sinica*, 48: 1219–1227. (in Chinese)
- Huang Y., Zeng X. (2021): Effects of environmental factors and spikelet structure on closure of rice spikelets. *Jiangsu Agricultural Sciences*, 49: 94–100. (in Chinese)
- Huang Y., Zeng X. (2023): Influences of carbon dioxide concentration on spikelet closure in fertile and sterile rice (*Oryza sativa* L.). *Acta Agriculturae Universitatis Jiangxiensis*, 45: 373–383. (in Chinese)
- Huang Y., Zeng X., Cao H. (2018): Hormonal regulation of floret closure of rice (*Oryza sativa*). *PLoS ONE*, 13: e0198828.
- Kobayasi K. (2012): Effects of solar radiation on fertility and the flower opening time in rice under heat stress conditions. *Solar Radiation*, 245: 66.
- Kobayasi K., Sakai H., Tokida T., Nakamura H., Usui Y., Yoshimoto M., Hasegawa T. (2019): Effects of free-air CO₂ enrichment on flower opening time in rice. *Plant Production Science*, 22: 367–373.

<https://doi.org/10.17221/43/2024-CJGPB>

- Li X., Wang Y., Duan E., Qi Q., Zhou K., Lin Q., Wang D., Wang Y., Long W., Zhao Z. (2017): OPEN GLUME1: A key enzyme reducing the precursor of JA, participates in carbohydrate transport of lodicules during anthesis in rice. *Plant Cell Reports*, 37: 329–346.
- Liu S., Xia K., Zeng X., Zhou X. (2001): Inductive effect of methyl jasmonate (MeJA) on floret opening in wheat and its inhibition by salicylic acid (SA). *Acta Agronomica Sinica*, 27: 123–126. (in Chinese)
- Ma Q. (1999): Studies on ecological and physiological principle of blooming time overslip in hybrid rice seed production. *Journal of Hubei Agricultural College*, 19: 2–6. (in Chinese)
- Ma Z., Zhan Z., Cheng X., Gao J., He G., Liu D., Xu H., Xu Z. (2011): Flowering time in filial generations of cross between indica and japonica rice and its response to external environment. *Hybrid Rice*, 26: 70–76. (in Chinese)
- Mehra P., Pandey B.K., Verma L., Prusty A., Singh A.P., Sharma S., Malik N., Bennett M.J., Parida S.K., Giri J. (2022): *OsJAZ11* regulates spikelet and seed development in rice. *Plant Direct*, 6: e401.
- Nagai K., Mori Y., Ishikawa S., Furuta T., Gamuyao R., Niimi Y., Hobo T., Fukuda M., Kojima M., Takebayashi Y. (2020): Antagonistic regulation of the gibberellic acid response during stem growth in rice. *Nature*, 584: 109–114.
- Pan X., Liu W., Li Y., Xiong H., Sheng X., Duan Y., Yu Y., Zhao W., Wei X., Li X. (2019): Identification and genetic analysis of split husk mutant *sh1* in rice. *Chinese Journal of Rice Science*, 33: 323–330. (in Chinese)
- Pandey A., Khan M., Hamurcu M., Brestic M., Topal A., Gezgin S. (2022): Insight into the root transcriptome of a boron-tolerant *Triticum zhukovskyi* genotype grown under boron toxicity. *Agronomy*, 12: 2421.
- Peng J., Mo H., Hui D. (1994): The relationship between flowering time of rice and weather factor. *Chinese Journal of Rice Science*, 8: 75. (in Chinese)
- Quan D., Meng W., Jin C., Zhou G. (2016): The effect of flowering habits of CMS lines in Hainan ecological conditions. *Crops*, 32: 28–32. (in Chinese)
- Singh A.P., Mani B., Giri J. (2021): *OsJAZ9* is involved in water-deficit stress tolerance by regulating leaf width and stomatal density in rice. *Plant Physiology and Biochemistry*, 162: 161–170.
- Tao L., Tan H., Wang X., Cao L., Song J., Cheng S. (2008): Effects of high temperature stress on flowering and grain-setting characteristics for Guodao 6. *Acta Agronomica Sinica*, 34: 669–674. (in Chinese)
- Wang M., Zhu X., Peng G., Liu M., Zhang S., Chen M., Liao S., Wei X., Xu P., Tan X. (2022): Methylsterification of cell-wall pectin controls the diurnal flower-opening times in rice. *Molecular Plant*, 15: 956–972.
- Wang X. (2006): Principles and Techniques of Plant Physiological and Biochemical Experiments. Beijing, Higher Education Press.
- Wang Z., Gu Y., Gao Y. (1991): Studies on mechanism of the anthesis of rice III. Structure of the lodicule and changes of its contents during flowering. *Acta Agronomica Sinica*, 17: 96–101. (in Chinese)
- Wang Z., Gu Y., Gao Y. (1992): Studies on mechanism of the anthesis of rice IV. Structure of rice rachilla and its relations to spikelet opening and closing. *Acta Agronomica Sinica*, 18: 331–336.
- Wang Z., Gu Y., Gao Y. (1993): Application of CO₂ to induce rice flowering. *Plant Physiology Communications*, 29: 282–287. (in Chinese)
- Xia Y., Du Z., Yang Y., Gong Y., Yan Z., Xu H. (2019): Effects of Epi-Brassinolide treatments on floret opening time of indica and japonica rice. *Crops*, 35: 139–147. (in Chinese)
- Xiao Y., Chen Y., Charnikhova T., Mulder P.P.J., Heijmans J., Hoogenboom A., Agalou A., Michel C., Morel J.-B., Dreni L. (2014): *OsARI* is required for JA-regulated floret opening and anther dehiscence in rice. *Plant Molecular Biology*, 86: 19–33.
- Yamada S., Kano A., Tamaoki D., Miyamoto A., Shishido H., Miyoshi S., Taniguchi S., Akimitsu K., Gomi K. (2012): Involvement of *OsJAZ8* in jasmonate-induced resistance to bacterial blight in rice. *Plant and Cell Physiology*, 53: 2060–2072.
- Yan Z., Xu H., Ma Z., Gao D., Xu Z. (2014): Differential response of floret opening to exo-methyl jasmonate between subsp. indica and subsp. japonica in rice. *Scientia Agronomica Sinica*, 47: 2529–2540. (in Chinese)
- Yan Z., Xu H., Gong Y., Xia Y., Ma Z., Xu Z. (2015): Difference of floret opening time between subsp. indica and subsp. japonica rice in response to ethephon. *Journal of Shenyang Agricultural University*, 46: 641–647. (in Chinese)
- Yan Z., Deng R., Gong Y., Wang Z., Zhu S. (2022a): Effects of MeJA on agronomic and quality traits of indica and japonica rice. *Hybrid Rice*, 37: 107–114. (in Chinese)
- Yan Z., Deng R., Zhang H., Li J., Zhu S. (2022b): Transcriptome analysis of floret opening and closure both Indica and Japonica rice. *3 Biotech*, 12: 188.
- Yang Y., Xia Y., Yan Z., Wang L., Yan B., Wang J., Wang Y., Luan J., Xu H. (2021): Effect of spraying methyl jasmonate during heading date on floret opening time and plant type of japonica rice from different ecological areas. *Chinese Journal of Rice Science*, 35: 69–77. (in Chinese)
- Ye H., Du H., Tang N., Li X., Xiong L. (2009): Identification and expression profiling analysis of TIFY family genes involved in stress and phytohormone responses in rice. *Plant Molecular Biology*, 71: 291–305.

<https://doi.org/10.17221/43/2024-CJGPB>

- Zeng X. (2000): Induction effect of jasmonates on floret opening in *Oryza sativa* L., *Sorghum bicolor* (L.) Moench and *Dactylis glomerata* L. [PhD. Thesis] Nanjing Agricultural University.
- Zeng X.C., Zhou X., Zhang W., Murofushi N., Kitahara T., Kamuro Y. (1999): Opening of rice floret in rapid response to methyl jasmonate. *Journal of Plant Growth Regulation*, 18: 153.
- Zeng X., Zhou X., Wu X. (2004): Advances in study of opening mechanism in rice florets. *Scientia Agricultura Sinica*, 37: 188–195. (in Chinese)
- Zhai X., Kai W., Huang Y., Chen J., Zeng X. (2023): *OsNCED3* and *OsPYL1* promote the closure of rice florets by regulating sugar transporters through endogenous abscisic acid. *Journal of Integrative Agriculture*, doi: 10.1016/j.jia.2023.11.004.
- Zhang Q., Wang Y., Zhu D., Chen H., Xiang J., Zhang Y., Zhang Y. (2019): Effect of increasing night temperature on floret opening and grain setting of rice. *Chinese Journal of Agrometeorology*, 40: 180–185. (in Chinese)
- Zhou J., Zhang Y., Zhu D., Lin X., Xiang J., Chen H., Hu S. (2014): Influence of flowering characteristics on spikelet fertility under high temperature. *Chinese Journal of Rice Science*, 28: 297–303. (in Chinese)

Received: April 22, 2024

Accepted: June 11, 2024

Published online: June 27, 2024