Rice Genomics and Genetics 2024, Vol.15, No.1, 28-35
http://cropscipublisher.com/index.php/rgg

Scientific Review Open Access

Genetic Regulation of Diurnal Flowering Time Divergence in Rice: The Role of the OsMYB8 and OsJAR1 Module

Zhong Jianli, Xuanjun Fang
Hainan Institute of Tropical Agricultural Resources, Sanya, 572024, China
Corresponding author email: zhongjianli8888@gmail.com
Rice Genomics and Genetics, 2024, Vol.15, No.1 doi: 10.5376/rgg.2024.15.0004
Received: 01 Dec., 2023
Accepted: 03 Jan., 2024
Published: 15 Jan., 2024

Preferred citation for this article:
Zhong J.L., and Fang X.J., 2024, Genetic regulation of diurnal flowering time divergence in rice: the role of the OsMYB8 and OsJAR1 module, Rice Genomics and Genetics, 15(1): 28-35 (doi: 10.5376/rgg.2024.15.0004)

On March 13, 2024, a joint research achievement by the State Key Laboratory for Conservation and Utilization of Subtropical Agro-Bioresources, School of Life Sciences, South China Agricultural University, the Guangdong Provincial Key Laboratory of New Technology in Rice Breeding, Rice Research Institute of Guangdong Academy of Agricultural Sciences, and the Life Sciences Technology Center of China National Seed Group Co., Ltd. was published in Nature Communications. The paper, titled "Natural variation in OsMYB8 confers diurnal floret opening time divergence between indica and japonica subspecies," had Yajun Gou and Yueqing Heng as co-first authors, with Rongxin Shen and Haiyang Wang as co-corresponding authors. The study was funded by the National Natural Science Foundation of China Innovation Group Project and the Hainan Yazhou Bay Seed Laboratory Commander Project, among others. It identified the OsMYB8 gene as a key factor regulating the divergence in diurnal flowering time in rice. The interaction between OsMYB8 and OsJAR1 significantly affects the flowering time of indica and japonica rice. Transferring the indica allele of OsMYB8 into japonica rice effectively advances flowering time, providing a new strategy for indica-japonica hybrid breeding.

1 Experimental Data Analysis

This research meticulously recorded the behavior of indica and japonica rice varieties flowering at different times of the day. RNA sequencing (RNA-seq) analysis revealed that OsMYB8 expression levels increased before flowering, and this upregulation directly promoted the expression of the OsJAR1 gene. The interaction between OsMYB8 and OsJAR1 further influenced the expression of genes regulating cell permeability and cell wall remodeling, collectively facilitating floret opening.

Figure 1 showed the divergence in Diurnal Flowering Opening Time (DFOT) between the two rice subspecies. Figure 1a compared the DFOT of indica (n=28) and japonica (n=12) rice varieties in Guangzhou in October 2019, showing that indica varieties generally flowered earlier than japonica. Figures 1b and 1c compared the floret opening of the indica rice TFB and the japonica ZH11 at 10:30 AM and 12:00 PM, showing significant differences, with TFB opening earlier. Figures 1d and 1e recorded the DFOT changes of TFB and ZH11 in June and October 2020, showing the stability of their DFOT across different seasons. Figure 1f displayed the changes in pistil morphology of TFB and ZH11 at different times of the day using stereomicroscope photos, while figure 1g explained how the pistil was considered an ellipsoid for volume measurement. Figure 1h quantitatively compared the pistil volumes of ZH11 and TFB at different times, using the ellipsoid volume formula for calculations. Finally, figure 1i analyzed the water content of 100 pistils of ZH11 and TFB at different times, providing experimental data for subsequent exploration of the association between DFOT and water content.

Therefore, these results highlighted the significant divergence in DFOT between the two rice subspecies, which is of great significance for rice breeding and understanding the impact of different biological clocks on plant flowering.

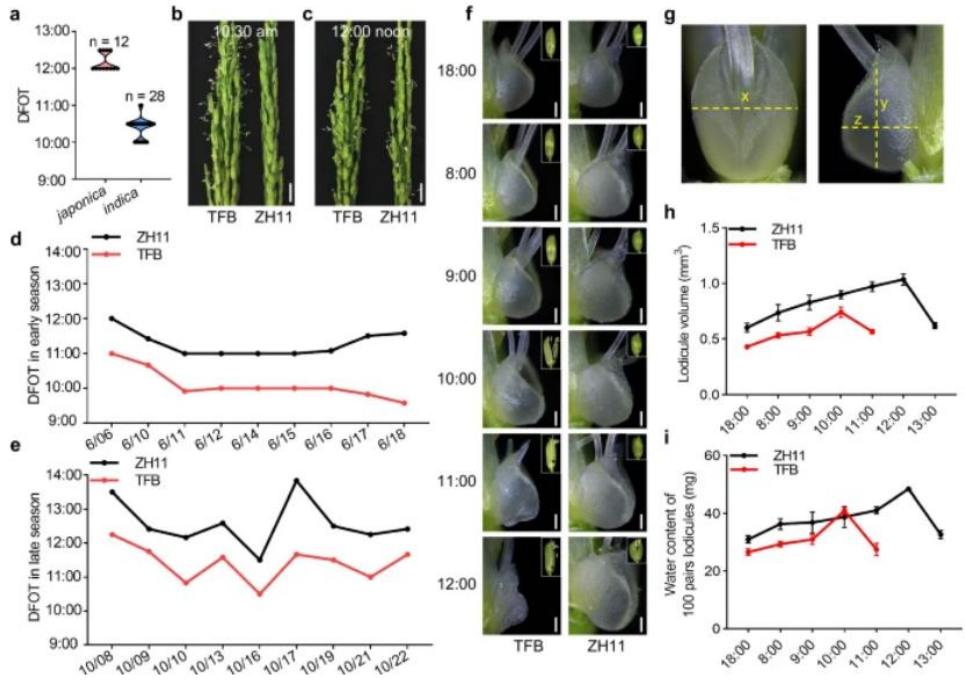

Figure 1 DFOT divergence in rice subspecies

Figure 2 through comparative transcriptomic analysis of lodicules between indica (TFB) and japonica (ZH11), revealed the gene expression differences at various time points. Figure 2a showed a Principal Component Analysis (PCA) highlighting the differences in the transcriptome datasets of TFB and ZH11 lodicules at different times. The time points T18 and Z18 represent 18:00 the day before floret opening, T9 and Z9 represent 9:00 (corresponding to 1 h and 3 h before opening for TFB and ZH11, respectively), Z11 represents 11:00 (1 h before ZH11 opening), and TF and ZF represent the peak of floret opening (approximately 10:00 for TFB and 12:00 for ZH11). The Venn diagram in figure 2b showed the number of upregulated differentially expressed genes (DEGs) in the comparison of Cluster18 genes with T9 versus Z9. Figure 2c displayed a Gene Ontology (GO) enrichment analysis of 251 intersecting genes, with Molecular Function (MF) and Biological Process (BP) indicated in different colors representing levels of P-value, with "transcription factor activity" highlighted in red. Figure 2d showed the expression analysis of 11 transcription factors selected through GO analysis, with the heatmap numbers representing average FPKM values. Figure 2e presented the RT-qPCR analysis of OsMYB8 expression levels in the lodicules of TFB and ZH11 at 9:00 AM in June and October 2021, with values representing mean ± standard error (SEM), n=3 biological replicates, and the significance at each time point evaluated by two-sided Student's t-test, providing P-values.

Therefore, indicating significant differences in the expression regulation of lodicules between indica and japonica rice, especially in the regulation of flowering time, where the OsMYB8 gene showed differential expression, possibly contributing to the DFOT differences between the two subspecies.

Figure 3 displayed the use of the CRISPR/Cas9 gene editing technique to create OsMYB8 mutants and analyzed their effects on the diurnal floret opening time (DFOT) in different backgrounds (ZH11 and TFB). Figure 3a showed the successful introduction of targeted mutations in the OsMYB8 gene using CRISPR/Cas9 technology. Figures 3b, e, j compared the inflorescences of ZH11 and Osmyb8ZH mutants (b), TFB and Osmyb8TF mutants (e), and ZH11 and OsMYB8TF/ZH11 transgenic lines (j) at different times. Figures 3c, f, k recorded the number of florets opened at different times in June, while figures 3d, g, l showed the number of florets opened on different days after flowering, both showing clear differences. Figure 3h presented the vector structure used to construct the OsMYB8TF/ZH11 transgenic plants. Figure 3i analyzed the expression levels of the OsMYB8 gene in the lodicules of OsMYB8TF/ZH11 transgenic lines by RT-qPCR, with ZH11 as a negative control, showing a

significant increase in OsMYB8 expression in the transgenic lines, determined by two-sided Student's t-test for significance.

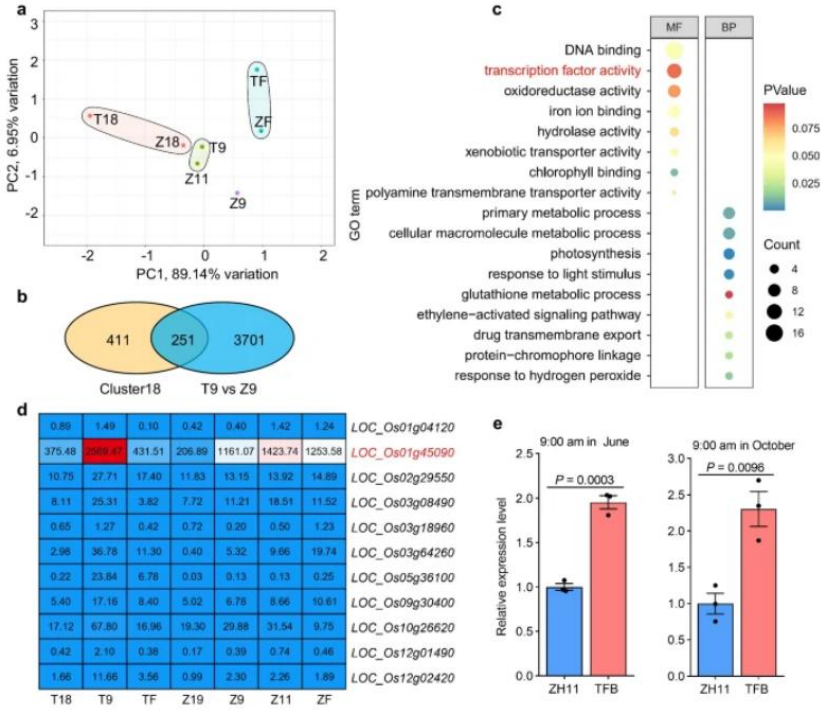

Figure 2 Identification of OsMYB8 by comparative transcriptomic analysis of lodicules between indica and japonica

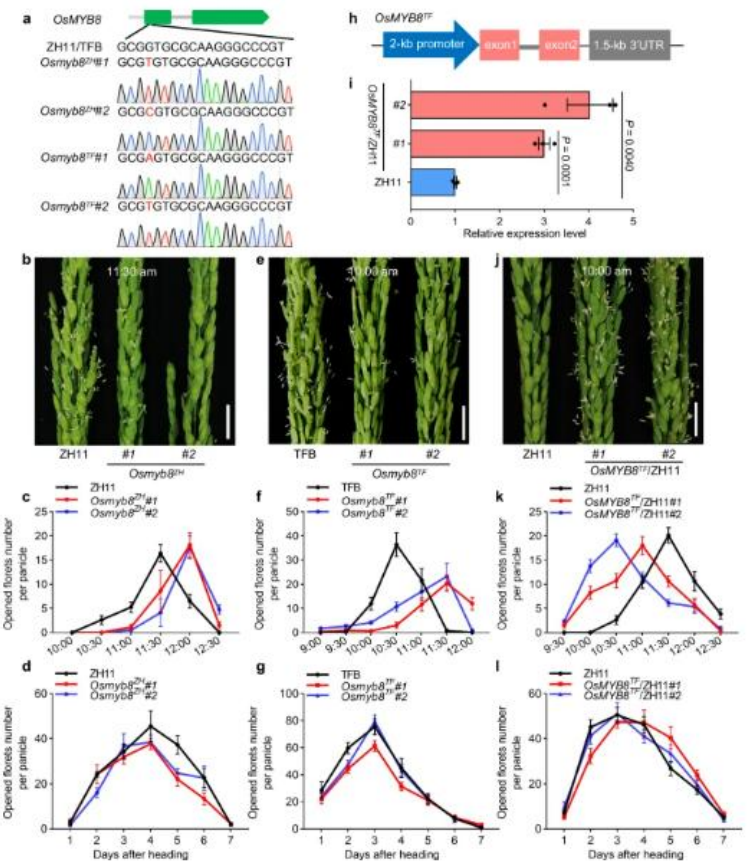

Figure 3 OsMYB8 positively regulates rice DFOT

Thus, emphasizing the role of the OsMYB8 gene in positively regulating rice DFOT and indicating its impact on the number of florets opened during inflorescence, thereby affecting the reproductive efficiency of rice. This way, researchers can better understand and manipulate key developmental stages in the rice growth process.

Figure 4 presented the process of identifying the genome-wide direct targets of the OsMYB8 protein and its functional analysis. a) The nuclear localization of OsMYB8-GFP in rice protoplasts was visualized by co-localization with D53-mCherry nuclear marker. b) Yeast two-hybrid experiments showed that full-length and truncated OsMYB8 proteins fused with the DNA-binding domain (BD) exhibited transcriptional activation activity. c) The core binding motif "TTHGGY" of OsMYB8 protein was identified using MEME-ChIP tool. d) Venn diagram compared the genes bound by DAP-seq technique with differentially expressed genes (DEGs) identified by RNA-seq in Osmyb8 mutants. e) GO (Gene Ontology) enrichment analysis of 345 overlapping genes, with Molecular Function (MF) and Biological Process (BP) represented in different colors indicating levels of P-value, and the size of circles representing the number of genes in each functional category. f) ChIP-qPCR analysis showed that OsMYB8 could bind in vivo to the promoter region of OsJAR1, with the red line indicating the position of the "TTHGGY" motif in the OsJAR1 promoter region. g) Electrophoretic mobility shift assay (EMSA) demonstrated that GST-OsMYB8 recombinant protein could directly bind to the OsJAR1 promoter region containing the "TTHGGY" motif. h) Transient dual-luciferase (LUC) reporter gene assay indicated that OsMYB8 could induce the transcription of OsJAR1 promoter in rice protoplasts, with the LUC/REN ratio representing the relative activity of the OsJAR1 promoter.

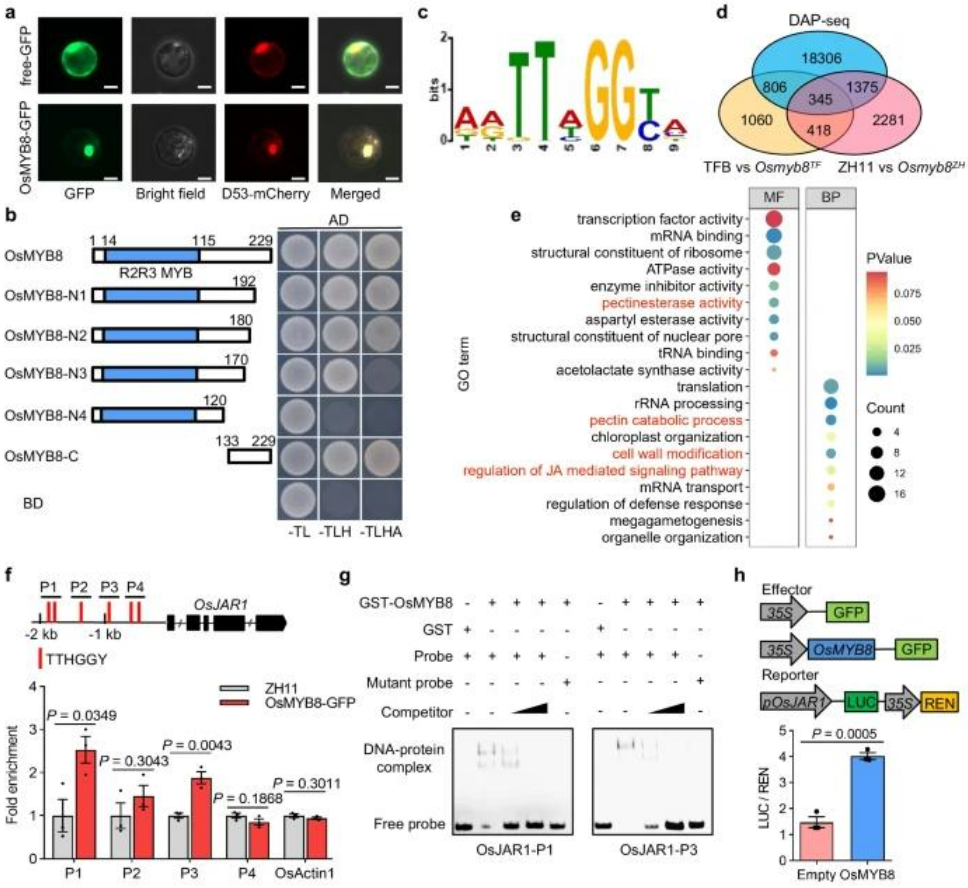

Figure 4 Identification of the genome-wide direct targets of OsMYB8

Therefore, the results further confirmed the key role of OsMYB8 in regulating rice diurnal floret opening time (DFOT), especially through its direct action on downstream target genes like OsJAR1, thereby affecting the JA-mediated signaling pathway and related biological processes. Moreover, these findings also revealed the important role of transcription factors in plant growth and development and response to environmental changes.

Figure 5 detailed the role of the OsJAR1 gene in regulating the diurnal floret opening time (DFOT) in rice and its relationship with the content of jasmonoyl-L-isoleucine (JA-Ile). a) Using CRISPR/Cas9 gene editing technology, Osjar1 mutants were created in the ZH11 background, with the mutation sites marked in red. b) Showed a comparison of the panicles of ZH11 and Osjar1 mutants at 11:30 AM in June 2022 in Guangzhou, with a scale of 1 cm. c) Recorded the number of florets opened per panicle at different times of the day for ZH11 and Osjar1 mutants, with the mean values presented as standard error (SEM) (n=10 panicles). d) and e) Measured the JA-Ile content in the lodicules of ZH11, Osjar1 mutants, and Osmyb8ZH mutants at 10:00 AM and 9:00 AM, respectively, with values presented as standard error (SEM) (n=3 biological replicates). f) Schematic diagram showing the vector structure used to construct OsJAR1com materials, where pOsMYB8TF represents the promoter amplified from TFB. g) Showed the relative expression levels of OsJAR1 in the lodicules of ZH11, OsJAR1com, and Osmyb8ZH, with values presented as standard error (SEM) (n=3 biological replicates). h) and j) Showed a comparison of the panicles of ZH11, OsJAR1com, and Osmyb8ZH as well as ZH11, OsMYB8TF/ZH11, OsMYB8TF/Osjar1, and Osjar1 at 11:30 AM and 12:00 PM in June and October 2022 in Guangzhou, respectively, with a scale of 1 cm. i) and k) Recorded the number of florets opened at different times of the day for ZH11, OsJAR1com, and Osmyb8ZH as well as ZH11, OsMYB8TF/ZH11, OsMYB8TF/Osjar1, and Osjar1, with mean values presented as standard error (SEM) (n=10 panicles).

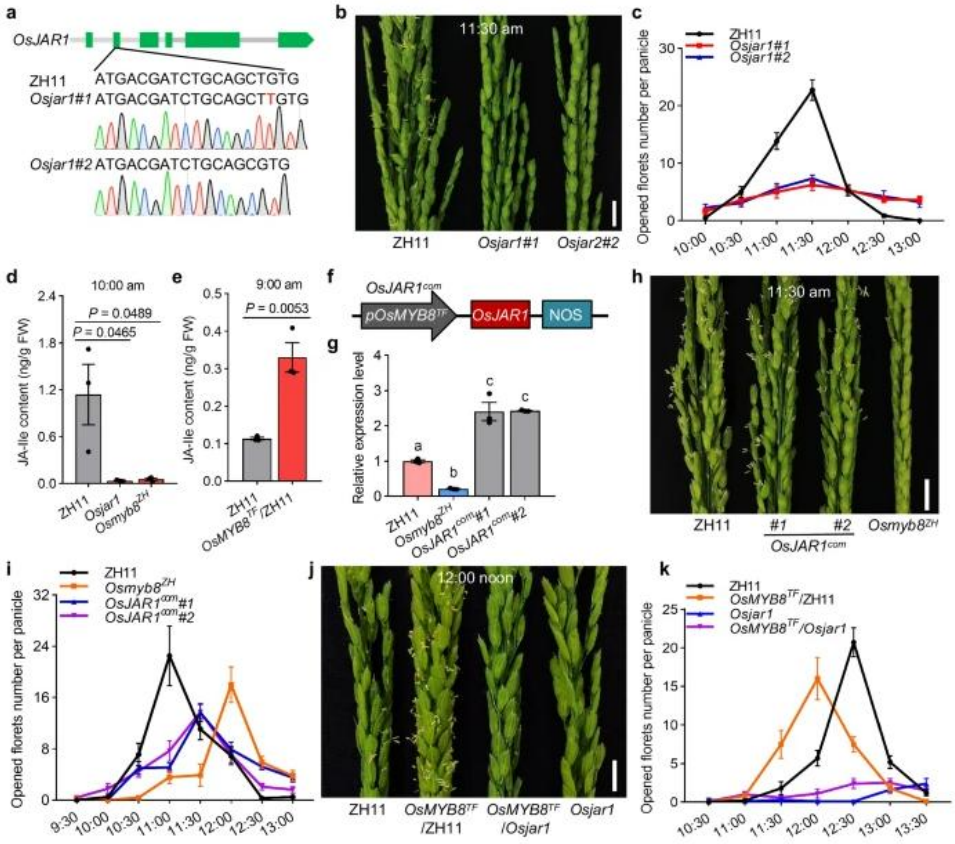

Figure 5 OsJAR1 influences JA-Ile content in lodicule to regulate rice DFOT

Therefore, the results indicate that OsJAR1 not only regulates rice DFOT by affecting the content of JA-Ile but also involves other complex biological processes. The data provided in the figures highlight the important application of gene editing technology in functional gene research and the potential for regulating flowering time in rice breeding.

Figure 6 revealed how natural variation in the OsMYB8 promoter causes differences in the diurnal floret opening time (DFOT) between indica and japonica rice. a) Single nucleotide polymorphism analysis of the OsMYB8 promoter was conducted in 3513 rice germplasm resources, showing the variation in the 2 kb promoter region. b)

Displayed the distribution frequency of three OsMYB8 haplotypes in different rice germplasms across Asia, with haplotype 1 (Hap1) being the most common. c) Analyzed the nucleotide diversity (π value) around the 100 kb genomic region surrounding the OsMYB8 gene, comparing the differences among indica (Ind), temperate japonica (TeJ), and wild rice (Ruf). d) FST values reflected the level of genetic differentiation in the 100kb region around OsMYB8 among the above three types. e) Recorded the DFOT of rice germplasm containing Hap1 and Hap2 in May 2022 in Guangzhou, finding that germplasm with Hap1 generally flowered earlier than those with Hap2. f) Measured the relative expression levels of OsMYB8 in the lodicules of rice germplasm containing Hap1 and Hap2. g) Measured the JA-Ile content in the lodicules of TFB and ZH11 at 9:00 AM. h) Transient dual-luciferase reporter gene assay showed the transcriptional activity of pOsMYB8Hap1 and pOsMYB8Hap2 in rice protoplasts. i) Measured the relative expression levels of OsMYB8 in the lodicules of ZH11, OsMYB8TF/Osmyb8ZH transgenic lines, and Osmyb8ZH mutants. j) Showed a comparison of the panicles of ZH11, OsMYB8TF/Osmyb8ZH transgenic lines, and Osmyb8ZH mutants at 11:30 AM in October 2022 in Guangzhou. k) Recorded the number of florets opened at different times of the day for ZH11, OsMYB8TF/Osmyb8ZH transgenic lines, and Osmyb8ZH mutants.

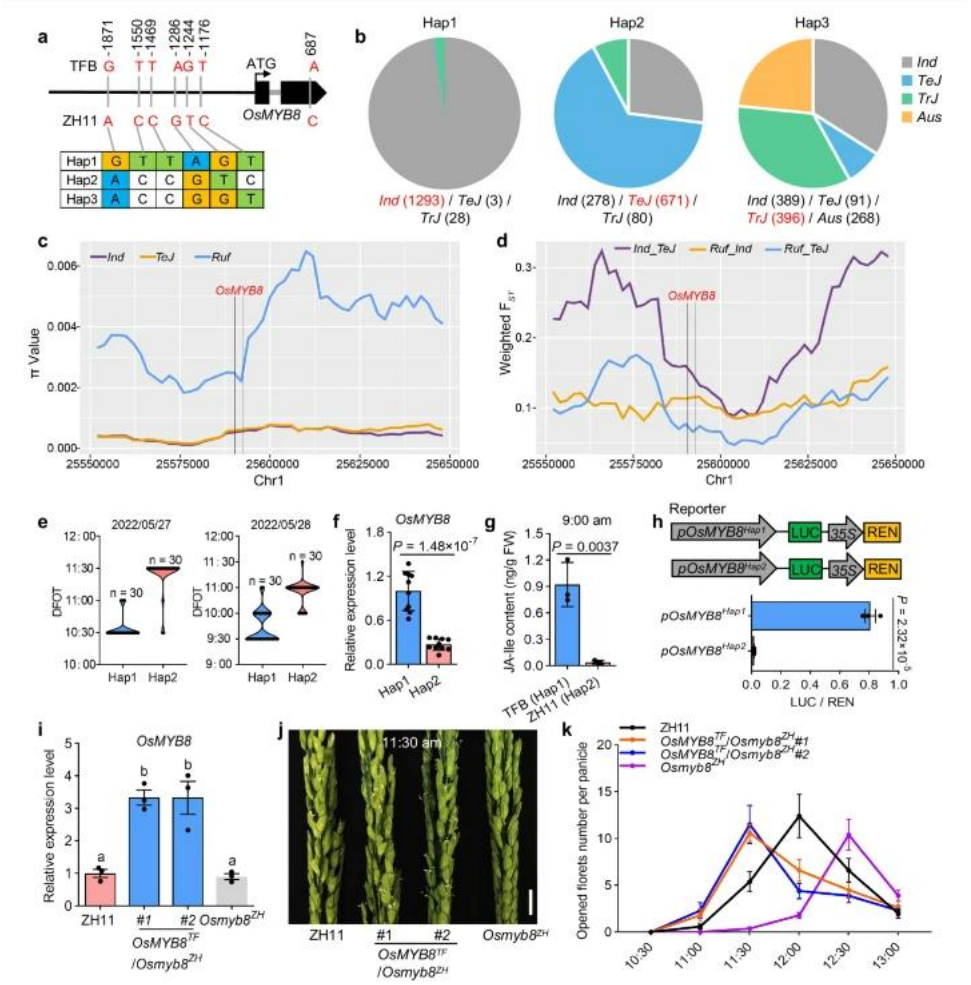

Figure 6 Natural variation in OsMYB8 promoter confers DFOT divergence in japonica and indica

Therefore, the results highlight the role of natural variation in the genetic regulation of rice DFOT, especially how promoter polymorphisms affect the expression of OsMYB8 and subsequent plant biological responses. These findings provide important genetic information for understanding the timing control mechanisms in rice growth and development, as well as for rice breeding.

Figure 7 showed the research results on how the indica allele of the OsMYB8 gene promotes the diurnal floret opening time (DFOT) in japonica rice. a) and d) Compared the panicles of ZH11 with NIL^TFB and XS134 with

CSSL9311 at 12:00 PM in Guangzhou, with a scale bar representing 1 cm. b) and e) Measured the number of florets opened per panicle at different times of the day for ZH11 with NIL^TFB and XS134 with CSSL9311, with values represented as mean ± standard error (SEM) (n=10 panicles). c) and f) Measured the relative expression levels of OsMYB8 and OsJAR1 in the lodicules of ZH11 with NIL^TFB and XS134 with CSSL9311, with values represented as mean ± standard error (SEM) (n=3 biological replicates). Significance was evaluated by two-sided Student's t-test and P-values were marked. g) Presented a model depicting the OsMYB8 and OsJAR1 module regulating the DFOT difference between indica and japonica rice. Natural variation in the OsMYB8 promoter sequence leads to higher expression levels of OsMYB8 in indica, resulting in greater accumulation of JA-Ile and, consequently, an earlier DFOT compared to japonica.

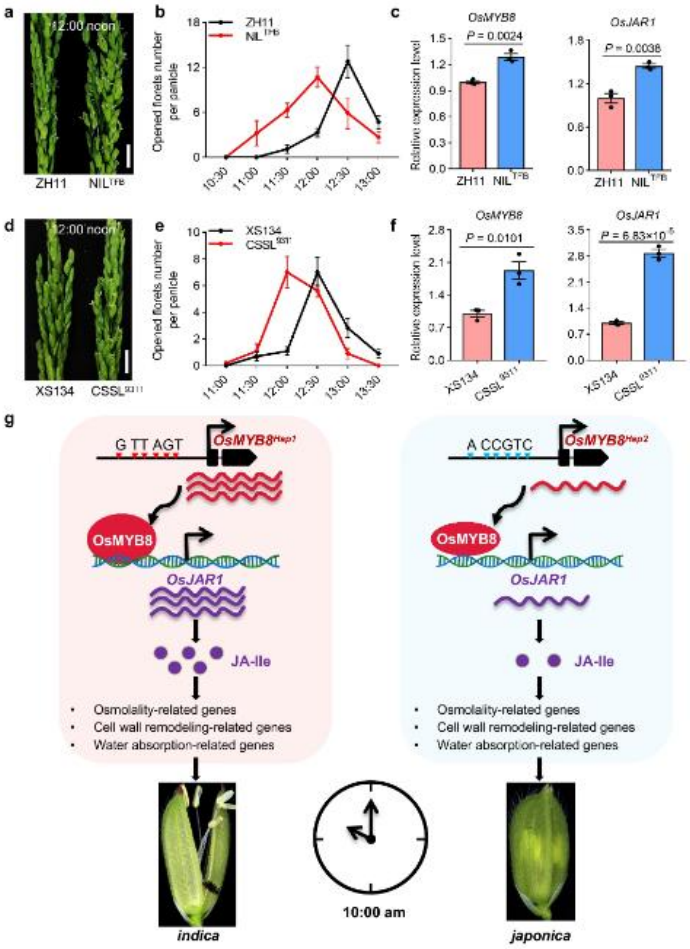

Figure 7 The indica allele of OsMYB8 promotes japonica DFOT

Through these results, figure 7 clearly illustrates how differences in gene expression affect the genetic variation in rice flowering time. These findings are not only crucial for understanding the plant biological clock and flowering mechanisms but also offer potential pathways for improving crop production traits through gene editing.

2 Analysis of Research Findings

The research results demonstrate that by comprehensively analyzing the interaction between the OsMYB8 and OsJAR1 gene modules, the roles in regulating rice cell permeability and cell wall remodeling were elucidated, revealing how these mechanisms are key in causing flowering time differences between indica and japonica rice. The study further shows that transferring the indica allele of OsMYB8 into japonica rice can significantly advance the flowering time of japonica rice, potentially offering an effective approach to improving the production of hybrid rice varieties. This finding not only deepens our understanding of plant flowering biology but also provides new molecular tools for rice breeding practice.

3 Evaluation of the Research

The study offers new insights into the genetic regulation of rice flowering time and provides a feasible strategy for improving the breeding efficiency of hybrid rice through genetic manipulation. This work not only advances our understanding of plant flowering time regulation but also offers new tools for the improvement of crops like rice.

4 Conclusions

By thoroughly analyzing the interaction between OsMYB8 and OsJAR1, the molecular mechanism regulating rice flowering time was revealed. The application of gene editing technology successfully adjusted the flowering time of rice. This discovery holds significant practical application value for enhancing the production efficiency of hybrid rice varieties.

5 Acknowledgement

Thanks to Nature magazine for providing the valuable opportunity of open access papers to a broad audience. This open access policy has allowed me to download, read, comment on, and disseminate this excellent research paper for free. Through such mechanisms, Nature magazine not only promotes the widespread dissemination of scientific knowledge but also provides valuable learning resources for researchers, students, and all individuals interested in science around the world. This spirit of openness and sharing is invaluable for advancing scientific progress and promoting public understanding and interest in scientific research.

6 Access the Full Text

Gou Y.J., Heng Y.Q., Ding W.Y. Xu C.H., Tang Q.S., Li Y.J., Fang Y.D., Zhou DG., Zhu X.Y., Zhang M.Y., Ye R.J., Wang H.Y., and Shen R.X., 2024, Natural variation in OsMYB8 confers diurnal floret opening time divergence between indica and japonica subspecies, Nat Commun, 15, 2262