Transcriptome analysis of the impact of exogenous methyl jasmonate on the opening of sorghum florets

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Abstract

Background
Methyl Jasmonate (MeJA) could promote the opening of sorghum florets, but the molecular mechanism remains unclear.

Objective
We aimed to investigate the molecular mechanism of exogenous MeJA in promoting the opening of sorghum florets.

Methods
Hybrid sorghum Aikang-8 was selected as the test material in this study. Sorghum plants of uniform growth with approximately 20%-25% florets open were selected and treated with 0, 0.5 and 2.0 mmol/L of MeJA. Totally there were 27 samples with lodicules removed were obtained at different time points and used for the transcriptome analysis using the BGI-SEQ_500RS platform.

Results
The results showed the sorghum florets opened earlier than the control after the treatment with exogenous MeJA, and the promotive effect increased along with the increase of exogenous MeJA concentration. The number of differentially expressed genes (DEGs) in plasma cells increased with the increase of MeJA concentration, whether up- or down-regulated, after the exogenous MeJA treatment. Besides, the number of metabolic pathways was also positively correlated with the concentration of MeJA. GO and KEGG analysis suggested the DEGs were mainly enriched in starch and sucrose metabolism-related pathways (i.e., LOC8063704, LOC8083539 and LOC8056206), plant hormone signal transduction pathways (i.e., LOC8084842, LOC8072010, and LOC8057408), energy metabolic pathway (i.e., LOC8076139) and the α-linolenic acid metabolic pathway (i.e., LOC8055636, LOC8057399, LOC8063048 and LOC110430730). Functional analysis of target genes...
showed that two genes named LOC-1 (LOC8063704) and LOC-2 (LOC8076139) could induce the earlier flowering of *Arabidopsis thaliana*.

**Conclusion**

The results of this study suggest that exogenous MeJA treatments could induce the up- or down-regulation of genes related to starch and sucrose metabolism, -linolenic acid metabolism and plant hormone signal transduction pathways in the plasma cells of sorghum florets, thereby promoting the opening of sorghum florets.

**Introduction**

In northern regions of China, sorghum was widely planted as a major food crop as well as other important resources for forage, food and brewing industries. So the research of floret opening became increasingly important in sorghum for the growing demand of people. Different species and varieties, and even the flowering time of spikelet at different positions on the same spike in one day are also different, which are affected by the characteristics of varieties and external environment factors [1]. At present, there are many documents on the regulation of spikelets opening influenced by external environment factors, resembling light, temperature, CO₂ and mechanical stimulation, but the research about the spikelets opening regulated by endogenous signal molecules were still unknown [2–4].

Floret opening in grass plants is mainly caused by the swelling of a pair of lodicules at the base of the floret after water absorption [5]. In this process, the lodicules first swell and push chaff outward and palet inward, leading to the separation of chaff and palet, then, florets open [6]. The normal growth of plants are always regulated by a series of plant hormones, such as Ethylene (ETH), Auxin (IAA), Cytokinin (CTK), Gibberellic acids (GAs), Abscisic Acid (ABA), Jasmonic Acids (Jas), Brassinosteroids (BRs), etc. To date, at least five kinds of plant endogenous hormones can influence the open of plant flowers, including ETH, Jas, IAA, GAs and ABA [7,8]. As a typical C₄ plant, sorghum is one of the model plant for the research of comparative genomics of cereal crops [9,10], as well as one of important food crops and energy crops with the greatest potential in China [11]. As it is known to all, the opening peak of sorghum is mainly at night, which is quite different with rice and some other crops. Documents indicated that the opening of floret in excised spikelets in *Sorghum bicolor* L. Moench and *Sorghum Sudanesis* (Piper) Stapf was significantly stimulated by immersing into 2 mM methyl jasmonate (MeJA) solution [12]. So it is quite necessary to research the effect of exogenous methyl jasmonate on the opening of florets in sorghum.

RNA-seq is an very effective tool for studying the regulatory mechanism in plants, such as wheat [13], rice [14], cotton [15]. To date, several transcriptomics studies had been completed on sorghum using RNA-seq method to monitor gene expression or to identify genome-wide SNPs that potentially enhance genetic analysis and the application of molecular markers in sorghum in response to osmotic and abscisic acid and some other biological processes [10,16]. In addition to physiologic or agronomic approaches, RNA-seq could offer new opportunities for dissecting quantitative traits, paving the way to marker-assisted selection (MAS) breeding [17]. In this study, the BGISEQ_500RS sequencing platform was used to compare the transcriptome changes of lodicules of sorghum treated with 0, 0.5 and 2.0 mmol/L MeJA. We focused on the genes and pathways which were closely related to the regulation of floret
opening to further elucidate the impact of exogenous jasmonate acids on the floret opening of grass plants.

**Materials and methods**

**Materials**

In the study, sorghum (*S. bicolor* L. Moench) cultivar Aikang-8 was purchased from Shandong Ruiyou Agricultural Science and Technology Development Co., Ltd. in China and used to conduct the experiment. Aikang-8 seeds were planted in the training base of the Jiangxi Agricultural Engineering College On May 1st and May 10th for two times. Each time Aikang-8 was planted for 40 m² with normal cultivation managements. The MeJA (analytical reagent) was purchased from BOMEI (China). It was diluted into 10.0 mmol/L mother liquor with 1.0% ethanol, followed by further dilutions with 1.0% ethanol to 0.5 and 2.0 mmol/L. 1.0% alcohol was used for the blank control. *Arabidopsis thaliana* of wild-type was planted in small pots with a uniformly mixed mixture of peat, vermiculite, perlite by the ratio of 2:7:1 in growth chamber, respectively. The humidity was set at 60% and the temperature was set at 20–22˚C. The photoperiod was 24h and the illumination intensity was set 80–200 μmol·M⁻²·S⁻¹. Agrobacterium-mediated genetic transformation method was used to infect the inflorescence of *Arabidopsis thaliana* for 2–3 times. Arabidopsis plants were moved to dark environment for 16-24h after the infection. Arabidopsis seeds were harvested and used for the screening of transgenic plants.

**MeJA treatments and sampling**

In total, 10 robust and uniform sorghum plants with similar floret opening states were selected and treated with 20 mL of 0, 0.5 and 2.0 mmol/L MeJA, and the plants were then covered with green plastic film. Treatments were conducted at 18:00 PM. The duration from the beginning of treatment to the opening of a large quantity of florets, the number of sorghum plants with a large quantity of florets opened and the numbers of opened florets were recorded. The experiment was repeated three times.

After the treatments, nine samples were collected: samples treated with 2.0 mmol/L MeJA at 19:00 (1h) and 20:30 (2.5h) were labeled as SorgHM_1 and SorgHM_2, respectively; samples treated with 0.5 mmol/L MeJA at 19:00 (1h), 20:30 (2.5h) and 22:30 (4.5h) were labeled as SorgLM_1, SorgLM_2 and SorgLM_3, respectively; and samples treated with 0 mmol/L MeJA at 19:00 (1h), 20:30 (2.5h), 22:30 (2.5h) and 0:30 (6.5h) were labeled as SorgCK_1, SorgCK_2, SorgCK_3 and SorgCK_4, respectively. Each sample was repeated three times. Therefore, there were 27 samples were obtained with lodicules removed, followed by the storage in cold-proof RNase-free tubes at -80˚C. The weight of each sample was greater than 0.13 g.

**Total RNA extraction, library construction and transcriptome sequencing of sorghum lodicules**

Total RNAs of lodicules were extracted using pBIOZOL method, and RNA concentrations were measured using Nanodrop 2000, followed by RNA integrity test using 1.0% agarose gels. Agilent 2100 Bioanalyzer (Agilent RNA 6000 Nano Kit) was used to detect the concentrations, RIN values, 28S/18S values and fragment sizes of the total RNAs. The results indicated that 27 samples were qualified for library construction. The same amount of RNA from each of the three replicates at each time point were collected and uniformly mixed into nine samples for the sequencing and cDNA library construction. Nine libraries were named libSorgHM_1, libSorgHM_2, libSorgLM_1, libSorgLM_2, libSorgLM_3, libSorgCK_1, libSorgCK_2,
libSorgCK_3 and libSorgCK_4, respectively. Library was constructed as follow procedures: Magnetic beads with oligo (dT)s were used to enrich for mRNAs having polyA tails. A DNA probe was hybridized with the rRNA, and RNaseH was used to selectively digest DNA/RNA hybrids. Then, the DNA probe was digested using DNasel. Target RNA was obtained through purification, and interruption buffer was used to obtain RNA fragments. This was followed by reverse transcription using a random N6 primer. Double-stranded DNA was obtained through the synthesis of double-stranded cDNA. The 5'-end of the synthesized double-stranded DNA was phosphorylated, with an "A" tail at its 3' end. A connector with a "T" bulge at its 3' end was ligated as well. The ligated product was PCR amplified using specific primers. The PCR product was heated and denatured into single strands. The obtained single-stranded DNA was circularized using a bridge primer to obtain a single-stranded circular DNA library for further sequencing. Library construction and sequencing were performed by BGI, China.

Data quality control, filtration and comparison
The filtration of raw reads was performed using SOAPnuke (v1.5.2) [18] by BGI, including the removal of joint reads (joint contamination), the removal of reads with unknown bases at a proportion greater than 10% and the removal of low quality reads (reads with mass values of less than 15 are low quality reads, that accounted for more than 50% of the total bases). The clean reads after filtration (clean reads) were saved in FASTQ format [19]. Hierarchical indexing for spliced alignment of transcripts (HISAT, v2.0.4) [20] was used to blast the clean reads to a reference genome (sorghum genome) and reference gene sequences (sorghum reference genome database information was from https://www.ncbi.nlm.nih.gov/genome/?term=Sorghum%20bicolor%20) [21]. The Mapping rate was calculated using bowtie2 (v2.2.5), and the relative abundances of transcripts were quantified by the RSEM package [22,23]. The average comparison rate of each sample reached 95.39%, and the uniform comparison rate among samples indicated that the data were comparable. Only the clean reads which were successfully mapped on the reference genome were used for subsequent bioinformatics analysis.

Screening and functional annotation of differentially expressed genes (DEGs)
Clean reads were mapped to the reference sequence using Bowtie2 [24], and gene expression levels were calculated using RNA-seq Expectation–Maximization [25]. The method previously published by Audic and co-workers [26] was used to screen DEGs with the false discovery rates \( \leq 0.01 \) and \( |\log_2 \text{ratio}| \geq 1 \). The up- and down- regulation modes of the corresponding samples were described and analyzed. The DEG sequences were mapped to the Kyoto Encyclopedia of Genes and Genomes (KEGG) database using BLAST (parameter: -p blastx-e1e-5-m8) [27]. An enrichment analysis was performed for potential metabolic pathways containing the DEGs. The significant enrichment for a DEG was defined as Q-value \( \leq 0.05 \). The GO framework for model biological systems included three main categories: biological process, cellular component and molecular function [28] and GO terms with a FDR-corrected \( p \)-value \( < 0.05 \) were considered as significant enrichments.

Verification of DEGs by quantitative real-time fluorescence PCR (qRT-PCR)
The extracted RNAs were used as templates for reverse transcription with a RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific) and several DEGS were randomly selected. The expression levels of genes were verified using SYBR \textsuperscript{®} Select Master Mix (2X) reagents
(ABI Inc.) and qRT-PCR. GAPDH (AK064960) was selected and used as the reference gene. The relative expression analysis of selected genes was calculated using the $2^{-\Delta\Delta Ct}$ method with triplicate for each sample [29,30].

**Construction of plant overexpression vector**

Two target genes LOC-1 and LOC-2 were cloned with cDNA by PCR method using 50 μl reaction system, containing 10×PCR Buffer 5 μl, MgCl$_2$ (25 mM) 3 μl, dNTP mixture (2.5 mM) 4 μl, forward primer (10 μM) 1 μl, reverse primer (10 μM) 1 μl, Taq (5U/μl) 0.5μl, cDNA 2μl, ddH$_2$O 3.5μl. After the purification, PCR products were linked to pMD19-T Vector and transformed into DH5α according to the optimized procedure. At the same time, vector pCAMBIA1300 and pBI121 were digested with EcoRI and HindIII simultaneously. Klenow fragment of pCAMBIA1300 and small fragment of PBI121 were recycled and linked to construct an integrated expression vector pCAMBIA1300-BI containing 35S promoter, GUS gene and terminator. Then the GUS gene was replaced with two target gene LOC-1 and LOC-2, which were added CaMV35s promoter and Nos terminator at 5’ and 3’ end, respectively. The constructed plasmid was transformed into Arabidopsis thaliana to verify its functions [31].

**Results**

**MeJA can significantly stimulate the opening of sorghum florets**

After the treatment at 18:00 PM, the florets did not open until 19:00 after treatment with the three MeJA concentrations. At 20:30 (2.5h), a large number of sorghum florets started to open under the treatment of 2.0 mmol/L MeJA, while other sorghum florets didn’t open in abundance. At 22:30 (4.5h), a large number of sorghum florets started to open under the treatment of 0.5 mmol/L MeJA while the control sorghum florets (0 mmol/L MeJA) still didn’t open in abundance. At 0:30 (6.5h), a large number of control sorghum florets started to open (Fig 1). Besides, the numbers of opening florets after the treatments were also recorded in Fig 2, showing higher concentration of MeJA can obviously induce the opening of sorghum florets in advance. In order to investigate the regulation mechanism in this process, RNA-sequencing work was conducted at each time point.

**Sequencing quality analysis**

As shown in Table 1, 27 samples were obtained and sequenced. Finally an average of 24.11 Mb raw read and an average of 24.03 Mb clean reads were obtained. The average total size was 1.2 Gb. Bases with mass values of greater than 20 accounted for 97.70% of the total bases [Clean...](https://doi.org/10.1371/journal.pone.0248962.g001)

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**Fig 1. The opening state of sorghum florets after exogenous methyljasmonate treatments.** a-0h after the treatment of 0.0 mmol/L, b-6.5h after the treatment of 0.0 mmol/L, c-0h after the treatment of 0.5 mmol/L, d-4.5h after the treatment of 0.5 mmol/L, e-0h after the treatment of 2.0 mmol/L, f-2.5h after the treatment of 2.0 mmol/L.

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Reads Q20 (%). Bases with mass values of greater than 30 accounted for 90.04% of the total bases. Filtered reads (Clean Reads Q30 (%)) accounted for 99.68% of the total bases. Clean reads which were successfully mapped to the referenced genome (Total Mapping Ratio) accounted for 95.39% of the total reads. Clean reads which were successfully mapped to unique sites on the referenced genome (Unique Mapping Ratio) accounted for 87.74%. In addition, a total of 29,123 genes were detected. All results showed that the data of sequencing quality was qualified for further analysis.

Analysis of differentially expressed genes (DEGs)

As shown in Fig 3, the number of differentially expressed gene (DEGs) in sorghum florets increased along with treatment duration. For example, the number of DEGs in groups SorgCK_1-VS-SorgCK_3 and SorgLM_1-VS-SorgLM_3 were higher than those in groups SorgCK_1-VS-SorgCK_2 and SorgLM_1-VS-SorgLM_2, respectively. When the sorghum florets opened, DEGs were the most compared with the control, more than those in sorghum florets unopened. For example, the up- or down-regulated genes in SorgCK_1-VS-SorgCK_4 and SorgLM_1-VS-SorgLM_3 were 3,371 and 5,388, respectively. The number of DEGs increased along with the MeJA concentration within the same treatment time. For example, the number of DEGs in group SorgCK_1-VS-SorgHM_1 and SorgCK_2-VS-SorgHM_2 were higher than those in group SorgCK_1-VS-SorgLM_1 and SorgCK_2-VS-SorgLM_2, respectively. All results indicated so many DEGs were involved in the biological regulation of floret opening in sorghum. Besides, it was found that several genes may have similar expression patterns at different time points among different samples based on their expression quantity. So these genes could be clustered into one gene cluster which was correlated with floret opening based on their similar expression patterns. In our study, totally 9 gene clusters were discovered (Fig 4), suggesting that these genes followed certain expression rules at the opening stage of sorghum florets.

Verification of DEGs using qRT-PCR

To further verify the reliability of the transcriptome results, 17 genes with significantly different expression levels were selected (Table 2) for fluorescence quantitative RT-PCR to verify their expressions. Although the relative expression values determined by qRT-PCR did not exactly match the value from the transcriptional sequencing analysis, the variation trends of
gene expression were the same at the given time points (Fig 5), which indicated the results of the RNA-seq analysis of gene expression were credible. Interestingly, 10 genes were obtained in several significant pathways by blast analysis in NCBI database. Among of which, LOC8075740, LOC8056099, LOC8064482, LOC8058060, LOC110437037 and LOC8069151 were involved in starch and sucrose metabolism, and LOC8084355 was involved in hormone signal transduction pathways and LOC110429834 was involved in the α-linoleic acid metabolic pathway and LOC8072504 was involved in the regulation of plant circadian rhythm.

### Functional pathway analysis of DEGs

Based on the results of DEGs detection, biological pathway classification and enrichment analysis was performed in KEGG. Total of 29,135 unigenes were used to blast in the KEGG database.

### Table 1. Read quality levels of 27 samples and comparisons with reference genomes after genomic filtration.

| Sample          | Total Raw Reads(Mb) | Total Clean Reads(Mb) | Total Clean Bases(Gb) | Clean Reads Q20(%) | Clean Reads Q30(%) | Clean Reads Ratio(%) | Total Mapping Ratio(%) | Uniquely Mapping Ratio(%) |
|-----------------|---------------------|-----------------------|-----------------------|-------------------|-------------------|----------------------|------------------------|--------------------------|
| SorgCK_1_1      | 24.14               | 24.06                 | 1.20                  | 98.06             | 91.23             | 99.67                | 95.50                  | 87.57                    |
| SorgCK_1_2      | 24.14               | 24.05                 | 1.20                  | 97.86             | 90.53             | 99.66                | 95.62                  | 87.87                    |
| SorgCK_1_3      | 24.05               | 23.37                 | 1.19                  | 96.55             | 87.18             | 99.68                | 94.94                  | 5.85                     |
| SorgCK_2_1      | 24.14               | 24.06                 | 1.20                  | 97.76             | 90.03             | 99.69                | 95.46                  | 87.80                    |
| SorgCK_2_2      | 24.14               | 24.06                 | 1.20                  | 98.01             | 91.08             | 99.69                | 95.32                  | 87.72                    |
| SorgCK_2_3      | 24.14               | 24.06                 | 1.20                  | 98.00             | 90.98             | 99.68                | 95.63                  | 87.94                    |
| SorgCK_3_1      | 24.14               | 24.06                 | 1.20                  | 97.89             | 90.72             | 99.68                | 95.78                  | 88.09                    |
| SorgCK_3_2      | 24.14               | 24.06                 | 1.20                  | 98.00             | 91.17             | 99.67                | 93.92                  | 86.48                    |
| SorgCK_3_3      | 24.06               | 23.99                 | 1.20                  | 97.49             | 89.13             | 99.70                | 95.30                  | 87.60                    |
| SorgCK_4_1      | 24.05               | 23.96                 | 1.20                  | 98.10             | 91.36             | 99.66                | 94.69                  | 87.63                    |
| SorgCK_4_2      | 24.06               | 23.98                 | 1.20                  | 97.93             | 90.78             | 99.70                | 95.33                  | 88.10                    |
| SorgCK_4_3      | 24.05               | 23.97                 | 1.20                  | 97.98             | 90.86             | 99.68                | 95.31                  | 87.98                    |
| SorgHM_1_1      | 24.14               | 24.05                 | 1.20                  | 97.88             | 90.65             | 99.67                | 95.50                  | 87.69                    |
| SorgHM_1_2      | 24.14               | 24.06                 | 1.20                  | 98.02             | 91.19             | 99.68                | 95.27                  | 87.50                    |
| SorgHM_1_3      | 24.14               | 24.06                 | 1.20                  | 97.78             | 90.15             | 99.69                | 95.55                  | 87.69                    |
| SorgHM_2_1      | 24.08               | 24.01                 | 1.20                  | 96.65             | 86.38             | 99.71                | 95.49                  | 88.06                    |
| SorgHM_2_2      | 24.14               | 24.06                 | 1.20                  | 96.98             | 87.65             | 99.7                | 95.37                  | 87.79                    |
| SorgHM_2_3      | 24.14               | 24.07                 | 1.20                  | 96.12             | 85.05             | 99.72                | 95.72                  | 88.03                    |
| SorgLM_1_1      | 24.14               | 24.06                 | 1.20                  | 98.08             | 91.27             | 99.68                | 95.36                  | 87.70                    |
| SorgLM_1_2      | 24.14               | 24.06                 | 1.20                  | 97.78             | 90.33             | 99.68                | 95.09                  | 87.11                    |
| SorgLM_1_3      | 24.14               | 24.06                 | 1.20                  | 97.86             | 90.64             | 99.68                | 95.81                  | 87.96                    |
| SorgLM_2_1      | 24.14               | 24.06                 | 1.20                  | 97.9              | 90.68             | 99.68                | 95.80                  | 88.11                    |
| SorgLM_2_2      | 24.14               | 24.06                 | 1.20                  | 97.97             | 91.03             | 99.67                | 95.34                  | 87.96                    |
| SorgLM_2_3      | 24.14               | 24.06                 | 1.20                  | 97.74             | 90.09             | 99.67                | 95.76                  | 87.92                    |
| SorgLM_3_1      | 24.04               | 23.96                 | 1.20                  | 97.93             | 90.77             | 99.66                | 95.24                  | 87.96                    |
| SorgLM_3_2      | 24.14               | 24.06                 | 1.20                  | 97.75             | 90.08             | 99.68                | 95.71                  | 88.61                    |
| SorgLM_3_3      | 24.14               | 24.06                 | 1.20                  | 97.70             | 90.01             | 99.68                | 95.79                  | 88.38                    |
| Average         | 24.11               | 24.03                 | 1.20                  | 97.70             | 90.04             | 99.68                | 95.39                  | 87.74                    |

Sample: Sample name; Total Raw Reads (Mb): Number of reads before filtration; Total Clean Reads (Mb): Number of reads after filtration; Total Clean bases (Gb): Total number of bases after filtration; Clean Reads Q20 (%): Proportion of filtered reads with mass values greater than 20 out of the total number of bases; Clean reads Q30 (%): Proportion of filtered reads with mass values greater than 30 out of the total number of bases; Clean Reads Ratio (%): Proportion of filtered reads ratio; Total Clean Reads: Filtered reads; Total Mapping Ratio: Ratio of clean reads mapped to the reference genome; Unique Mapping Ratio: Ratio of clean reads uniquely mapped to specific sites on the reference genome.

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Fig 3. Number of differentially expressed genes (DEGs). X-axis represents different VS groups, and down-regulated and up-regulated DEGS were calculated. Y-axis represents the number of DEGS in each VS groups. SorgCK_1, SorgCK_2, SorgCK_3 and SorgCK_4 represented samples treated with 0 mmol/L MeJA at 19:00 (1h), 20:30 (2.5h), 22:30 (2.5h) and 0:30 (6.5h), respectively. SorgHM_1 and SorgHM_2 represented samples treated with 2.0 mmol/L MeJA at 19:00 (1h) and 20:30 (2.5h), respectively. SorgLM_1, SorgLM_2 and SorgLM_3 represented samples treated with 0.5 mmol/L MeJA at 19:00 (1h), 20:30 (2.5h) and 22:30 (4.5h), respectively.

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Fig 4. Nine gene clusters were discovered among different samples. Many genes could be classed into one cluster according to their similar expression patterns at different time points. X-axis represents different time points. Y-axis represents the expression value after homogenization.

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database, and finally 20,803 (70.96%) unigenes were annotated (Fig 6). In the 17 control groups, group SorgCK_1-VS-SorgCK_2 enriched the least metabolic pathways while group SorgCK_2-VS-SorgHM_2 enriched the most metabolic pathways (134). In the group SorgCK_2-VS-SorgHM_2, 4,198 DEGs were totally annotated. The number of metabolic pathways and annotated genes increased with prolonged treatment duration under the same treatment of exogenous MeJA in sorghum. For example, the number of enriched metabolic pathways and annotated DEGs in group SorgCK_2-VS-SorgHM_2 were significantly greater than that in group SorgCK_1-VS-SorgHM_1. Besides, the number of enriched metabolic pathways and annotated DEGs in group SorgCK_3-VS-SorgLM_3 were obviously greater than that in group SorgCK_2-VS-SorgLM_2 and SorgCK_1-VS-SorgLM_1.

Table 2. Genes and primers for DEG expression verification using qRT-PCR.

| NO | Gene ID | Forward primer | Reverse primer |
|----|---------|----------------|----------------|
| 1  | LOC8063704 | GGCTACGCCCATATCTCCGACG | AACGTAGAGATCCGTCAGC |
| 2  | LOC8083539 | GTGCTGTGCTTACTTGGCC | TGCCCTCGGAAGACCTGCAC |
| 3  | LOC8056206 | GGTACCGCATACATCTCCAGG | CTGGTGCTCCCTGCTTTAGT |
| 4  | LOC8060604 | AGGCAAGCTAGCCAATGGGGA | CATCTCTGGAAACCAGCTTGT |
| 5  | LOC8084355 | GTGACCAAGCGGTACCCTCTC | TCCCTGCTCCCTGCTTTAGT |
| 6  | LOC110437037 | TTTCTGGAGGAAATGACTTT | TGGGAGGAGACGTCATAGAA |
| 7  | LOC8069151 | TGCTCTGATTGGGAGAATG | CTACCAGTATCCAGAACAGAAT |
| 8  | LOC8082558 | GTGCTAGCCAGGACGTCAGC | ACAACCGGCGGATACAG |
| 9  | LOC8058060 | GGGAGATTCAGAAGTCTAGG | CTGACAGCTCTGCTTACT |
| 10 | LOC8070048 | CCTAACAGATAGCTAATGGGT | TGGTGCTCTAATGATCTCCTG |
| 11 | LOC8075740 | AGTGCGGCAACCTACCTCCTCAT | CAGGGGAGACGTCATAG |
| 12 | LOC8072504 | ACTCTCTGTTAGCTTCTACCTTGC | AGATTCAGTCAGGCACAAG |
| 13 | LOC8064482 | GTCCTCCTGCTGCTCCTGTC | AACCCCTGTGGCTCCCTTGAT |
| 14 | LOC110429834 | TTTACCTCTGCTCTCAGCCCACCAC | CAGCATCTCTCAGTCTTCAG |
| 15 | LOC8056099 | ATTTGAGGACAGGGACGACT | GAGCCACAAACACCATAC |
| 16 | LOC8083790 | TAGCGAAAGCTAGGCAAGGTCATTCAAGG | GTAATGCCATCTGATCTCCTCCTAAAA |
| 17 | LOC8155361 | CCACTTTAAGATGCTACCTGAGGAA | ACCGTGGGACTCCAGAG |

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Fig 5. Comparison of the DEGs between qRT-PCR and RNA-seq. qRT-PCR was an effective method to verify gene expression changes originated from RNA-seq analysis. Although their relative expression values were not the same, the variation trends were consistent. Genes above the X-axis were up-regulated and the below genes were down-regulated.

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At the same time point, the number of enriched metabolic pathways and DEGs increased along with the increase of MeJA concentration. For example, the number of enriched metabolic pathways and DEGs in SorgCK_1-VS-SorgHM_1 was greater than that in group SorgCK_1-VS-SorgLM_1. And the number of enriched metabolic pathways and DEGs in group SorgCK_2-VS-SorgHM_2 was greater than that in group SorgCK_2-VS-SorgLM_2.

In order to dig out the pathways which were most closely associated with spikelets opening, screening was performed with the following rules: 1) total number of DEGs > 3,000; 2) number of enriched metabolic pathways > 100; and 3) Q value < 0.05. Finally four comparison groups met the criteria, including SorgCK_3-VS-SorgLM_3, SorgCK_2-VS-SorgHM_2, SorgLM_1-VS-SorgLM_3 and SorgLM_3-VS-SorgLM_3. Totally nine metabolic pathways were enriched, including alpha-Linolenic acid metabolism, glycerolipid metabolism, glycerophospholipid metabolism, plant hormone signal transduction, anthocyanin biosynthesis, phenylpropanoid biosynthesis, starch and sucrose metabolism, limonene and pinene degradation, and circadian rhythm—plant (Table 3).

Analysis of DEGs in JA related pathway

JA and isoleucine (Ile) could form a conjugate (JAalle) when catalyzed by jasmonic acid-amido synthetase (JAR1), which induces the binding of the Jas motif in the jasmonate ZIM domain and COI1. The JAZ motif was then degraded, which was followed by the dissociation of the NINJA-TPL and transcription factor MYC2, resulting in the transcription of JA signaling-related genes [32–34]. On the basis of the transcriptome analysis, 19 DEGs were discovered at the same time point from four control groups (excluding SorgCK_1-VS-SorgLM_1) in JA signal transduction pathways (Fig 7), including two JAR1-related genes (LOC8084842 and LOC8065295) that catalyze the binding of JA and Ile. The expression level of LOC8084842 significantly increased after the treatment with 2.0 mmol/L MeJA for 2 hr, and the score reached to 7.6. In addition, 10 genes, including OsJAZ13 (LOC8067868, LOC8067866 and LOC8067865), OsJAZ10 (LOC8083641, LOC8062786 and LOC8072010), OsJAZ8 (LOC8062740), OsJAZ2 (LOC8079184), OsJAZ9 (LOC8059788) and OsJAZ6 (LOC8077075),
were found to be correlated with the JAZ protease activity. These genes could promote the degradation of JAZ, and significant differences were observed after the treatments of 2.0 mmol/L MeJA for 2 hr and 0.5 mmol/L for 4.5 hr compared with the controls. The difference score for LOC8072010 was 6.4. Seven genes, including LOC8069641, LOC8057408, LOC110436805, LOC8055721, LOC110433436, LOC8059158 and LOC8060491, were found to be correlated with bHLH zip transcription factors. These genes promoted MYC2 transcription initiation and the difference score reached 2.0 compared with the controls when treated with 2.0 mmol/L MeJA for 2 hr and 0.5 mmol/L MeJA for 4.5 hr. All results indicated that exogenous MeJA could induce the differential expression of these important genes, including JAR1, JAZM and MYC2. This demonstrated the important regulatory function of exogenous MeJA in sorghum floret opening at the molecular level.

The JAs are a class of fatty acid derivatives which play important regulatory roles in plant growth and development, mechanical damage and the induction of defense-related gene expression. JAs are mainly biosynthesized through the α-linolenic acid metabolic pathway. Lecithin on the cell membrane is catalyzed to release the precursor linolenic acid for the synthesis of JA, which is then oxidized to 13S-hydroperoxylinolenic acid through catalysis by lipoxygenase (LOX, EC 1.13.11.12). 13S-Hydroperoxylinolenic acid subsequently forms 12S,13S-epoxylinolenic acid, followed by cyclization to form 12-oxo-phyto-dienoicacid, which is then subject to be catalyzed to form MeJA or other complexes/metabolites [35]. As shown in S1 Fig, except for the group SorgCK_1-VS-SorgLM_1, 22 DEGs in the five control groups were involved in the JA biosynthetic pathway, including one gene related to the activity of

![Fig 7. Detailed information for the JA signal transduction pathway. JAR1: Jasmonate amino acid-binding enzyme, COI1: Coronatoxin insensitive protein 1; JAZ: Jasmonate ZIM domain protein, MYC2: MYC2 transcription factor, ORCA2/3: AP2 transcription factor DNA-binding protein.](https://doi.org/10.1371/journal.pone.0248962.g007)
lecithin lipid A1 (EC: 3.1.1.32) (LOC8085653) and four genes related to the activity of lecithin A2 (EC: 3.1.1.4) (LOC8070964, LOC8057399, LOC8079812 and LOC8077453). The differential expression score of LOC8085653 in SorgCK_2-VS-SorgHM_2 was 5.4, which was the most remarkable differential expression among the five groups. Besides, three genes related with lipoxygenase activity were also found, including LOC8082015, LOC8055177 and LOC8065835. All these genes were up-regulated in the α-linolenic acid metabolic pathway, positively regulating the biosynthesis of JA.

**Two genes named LOC-1 and LOC-2 played an important role in the opening process of sorghum florets**

In addition, another pathway named starch and sucrose metabolic pathway was found so important in the process of sorghum floret opening. As is well-known, starch and sucrose metabolism is a complex process of sugar metabolism. Starch synthesis starts from the degradation of sucrose by sucrose synthase (EC: 2.4.1.13) into uridine diphosphate glucose (UDPG) and fructose, followed by a series of biochemical reactions to form starch [36]. AGPase is the first enzyme in the process of plant starch biosynthesis, as well as being the rate-limiting enzyme of the whole process. The analysis in the study showed that LOC110437037, LOC8069151, LOC8064663 and LOC8060513 were related to the AGPase activity and five genes LOC8066439, LOC8081295, LOC8066807, LOC8068976 and LOC8071331 were related to GSS and GBE activity levels (S2 Fig). Expression analysis showed that these genes were downregulated at varying degrees. On the other hand, two enzymes named AMY and BMY (LOC8063704, LOC8083539, LOC8056206 and LOC8072062) were significantly upregulated to promote the degradation of starch. Two target genes named LOC-1, playing an important role in the starch and sucrose metabolic pathway, and LOC-2, playing an important role in energy metabolic pathway, were selected and cloned, then transformed into wild-type Arabidopsis thaliana (S1 Table and Fig 8a–8d). The results indicated the transgenic plants started to flower on the 30th day, while the wild-type A. thaliana delayed to flower by 7 days. The statistical results of the opening spikelets showed the wild-type A. thaliana does not begin to produce its first flower until the transgenic A. thaliana has reached 30 or more flowers, indicating

![Fig 8. Functional identification of two targets LOC-1 and LOC-2. a, Gene cloning of target LOC-1. Lines 1–8 were the PCR results of LOC-1. b, Gene cloning of target LOC-2. Lines 1and 3 were the PCR results of LOC-2. c, PCR identification of positive plants of LOC-1. Lines 1–13 represent 13 positive plants of Arabidopsis thaliana by PCR. d, PCR identification of positive plants of LOC-2. e, Comparison of flowering time in Arabidopsis thaliana.](https://doi.org/10.1371/journal.pone.0248962.g008)
overexpression of two genes could induce the earlier flowering of transgenic plants (Fig 8e). In summary, sorghum floret opening was consistent with the reduction of starch biosynthesis in cells accompanied by an accelerated metabolism. Therefore, the starch level decreased in lodicules cells, degrading into other sugars to reduce the osmotic pressure in the cells, which was correlated with the earlier opening of florets in sorghum.

Discussion

Sorghum is a short-day tropical species with the characters of substantial photoperiod sensitivity and delayed flowering in long days [37]. Sorghum genotypes show a wide-range of photoperiod sensitivity and historic genetic studies uncovered six flowering-time loci, namely \( M_{a1}, M_{a2}, M_{a3}, M_{a4}, M_{a5} \) and \( M_{a6} \) [38,39], but less literatures about the molecular mechanism of sorghum florets opening regulated by exogenous MeJA. Literatures reported that MeJA or JA treatments could significantly induce rice floret open [12]. Documents also reported that qRT-PCR method could be used to study the dynamic expression levels of JA signal-related genes during rice floret opening and showed that the expression levels of JA biosynthetic-related genes \( OsDAD1, OSAOS1, OSAOC \) and \( OSOPR7 \), signal transduction related genes \( OsJAR1 \) and \( OsCOI1b \), and 13 genes of the OsJAZ family, except \( OsJAZ5 \) and \( OsJAZ15 \), in lodicules cells were significantly up-regulated compared with the expressions at 18 h before floret open [39]. This further demonstrated the involvement of endogenous JA in the regulation of floret-opening in rice.

In this study, RNA-seq method was used to explore transcriptome changes in lodicule cells during sorghum floret opening after exogenous MeJA treatments. 0.5 and 2.0 mmol/L MeJA were applied to treat sorghum florets and the results showed the number of DEGs annotated and functionally metabolic pathways increased along with the increase of MeJA concentration. Functional enrichment analysis of DEGS showed plant hormone signal transduction and starch and sucrose metabolism were closely related with the open of sorghum florets. Thus, the results in this study showed MeJA could induce various physiological metabolisms in lodicules of sorghum florets, thereby promoting the floret open in advance. The results of this study are consistent with the previous reports, further confirming the regulatory mechanism we proposed in gramineal crops.

JA biosynthesis begins with the release of the precursor \( \alpha \)-linolenic acid from lecithin on the cell membrane, followed by a cascade of reactions catalyzed by multiple enzymes, including \( DAD1, LOX, AOS, AOC \) and \( OPR3 \). As shown in S1 Fig, differentially expressed genes (DEGs) identified under the treatment of 0.5 mmol/L MeJA at three time points were 2, 19 and 30 compared with the control, respectively. Differentially expressed genes (DEGs) identified under the treatment of 2.0 mmol/L MeJA were 10 and 37 at two time points compared with the control, respectively. This result showed that 2.0 mmol/L MeJA could induce more DEGs than 0.5 mmol/L MeJA at the same time point. Taking \( \log_2\text{ratio} = 3.0 \) as the criterion, significant expression differences were observed under the high concentration of 2.0 mmol/L treatment, including \( DAD1 \) activity related genes \( LOC8057399 \) (\( \log_2\text{ratio} = 3.5 \)), \( LOC8085653 \) (\( \log_2\text{ratio} = 5.4 \)) and \( LOC8055636 \) (\( \log_2\text{ratio} = 4.2 \)), \( AOS \) activity related genes \( LOC8056861 \) (\( \log_2\text{ratio} = 3.1 \)), 12-oxo-dienoic acid reductase related genes \( LOC8070772 \) (\( \log_2\text{ratio} = 3.9 \)) and \( LOC8070773 \) (\( \log_2\text{ratio} = 3.3 \)), \( OPC-8:0 \) coenzyme A ligase related gene \( LOC8063048 \) (\( \log_2\text{ratio} = 5.0 \)). Interestingly, 19 genes including \( LOC8057399, LOC8082061, LOC80770776 \) and \( LOC8068239 \) and etc., were significantly upregulated under the treatment of 2.0 mmol/L, while no expressions were found under the treatment of 0.5 mmol/L. These 19 genes were actively involved in the biosynthesis of JA, and high expression levels of these genes could further increase the content of JA, which was favorable to the open of sorghum florets.
The JA signal transduction pathway starts with the JAZ complex, followed by enzyme catalysis by JAR1, JAZ and MYC2 to induce JA signals. As shown in Fig 7, it was also found that several genes, including LOC8062786, LOC8083641, LOC8059386, LOC8056732, LOC110436805, LOC110429687, LOC8055721, LOC8055143, LOC8068250 and LOC110430094 were only differentially expressed under the 2.0 mmol/L MeJA treatment and these genes were barely expressed under the 0.5 mmol/L MeJA treatment, which suggested that 2.0 mmol/L MeJA treatment induced the expression of these genes to promote the open of sorghum florets by JA signal transduction.

In this study, four genes correlated with AGPase, which is a rate-limiting enzyme in starch synthesis and metabolism, were all downregulated, while four genes correlated with AMY and AMS were significantly upregulated to promote the degradation of starch. This result suggested that exogenous MeJA induced the up-regulation of starch synthesis related genes and the down-regulation of starch degradation related genes, thereby promoting the degradation of starch in lodicules cells into sucrose, maltose or other sugars, which caused the decrease of the osmotic potentials in lodicule cells. This further allowed lodicule cells to absorb water and

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**Exogenous MeJA**

![Diagram of Exogenous MeJA](https://doi.org/10.1371/journal.pone.0248962.g009)

**Expression of DEGs in pathways below**

starch and sucrose metabolism-related pathways (i.e., LOC-1),
energy metabolic pathway (i.e., LOC-2),
JA related pathway (i.e., LOC8084842),
the α-linolenic acid metabolic pathway (i.e., LOC8055636),
plant hormone signal transduction pathways (i.e., LOC8084842),
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**Early flowering**

Fig 9. A finally proposed model involved in the florets opening mechanism.

https://doi.org/10.1371/journal.pone.0248962.g009
swell, resulting in floret open. Previous research reported that the altered expression patterns of genes involved in glycolysis/gluconeogenesis pathway could cause early flowering in rice [40,41]. In addition, two important genes named LOC-1 and LOC-2 were cloned and transformed into Arabidopsis thaliana, overexpression of these two genes resulted the earlier flowering in transgenic Arabidopsis plants. This was not reported before.

In conclusion, floret opening is initiated by water absorption and lodicule swelling that pushes the chaff out. This process requires the loosening of lodicule cell walls and the reduction of the solute potential in lodicule cells. The results of this study showed that exogenous MeJA could promote the degradation of starch and the reduction of the solute potential in sorghum lodicule cells at the molecular level (Fig 9), but the detailed molecular mechanism that promotes starch degradation and its possible impact on the loosening of lodicule cell walls still requires further investigation.

Supporting information
S1 Fig. Pathway analysis of linolenic acid metabolism. (TIF)
S2 Fig. Pathway analysis of starch and sucrose metabolism. (TIF)
S1 Table. Full information of two targets LOC-1 and LOC-2. (XLSX)

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References
1. Okechukwu MF, Chikezie U, Charles U (2006) Isothermic sorption kinetics and intraparticulate diffusivity of dyes on modified and unmodified nano sorghum. Abstracts of Papers of the American Chemical Society 231.
2. Zeng XC, Zhou X, Zhang W, Murofushi N, Kitahara T, et al. (1999) Opening of rice floret in rapid response to methyl jasmonate. Journal of Plant Growth Regulation 18: 153–158. https://doi.org/10.1007/pl00007063 PMID: 10688703
3. Song P, Xia K, Wu CW, Bao DP, Chen LL, et al. (2001) Differential response of floret opening in male-sterile and male-fertile rices to methyl jasmonate. Acta Botanica Sinica 43: 480–485.
4. Wang YG, Funnell KA, Eason JR, Morgan ER, Woolley DJ (2016) Osmotic regulation via carbohydrate metabolism drives petal expansion and floret opening in gentian 'Showtime Spotlight'. Scientia Horticulturae 211: 19–25.
5. van Doorn WG, Van Meeteren U (2003) Flower opening and closure: a review. Journal of Experimental Botany 54: 1801–1812. https://doi.org/10.1093/jxb/erg213 PMID: 12869518
6. Chen J, Xu Y, Fei K, Wang R, He J, et al. (2020) Physiological mechanism underlying the effect of high temperature during anthesis on spikelet-opening of photo-thermo-sensitive genic male sterile rice lines. Sci Rep 10: 2210. https://doi.org/10.1038/s41598-020-59183-0 PMID: 32042005

7. Santner A, Calderon-Villalobos LJ, Estelle M (2009) Plant hormones are versatile chemical regulators of plant growth. Nat Chem Biol 5: 301–307. https://doi.org/10.1038/nchembio.165 PMID: 19377456

8. Wang YH, Irving HR (2011) Developing a model of plant hormone interactions. Plant Signal Behav 6: 494–500. https://doi.org/10.4161/psb.6.4.14558 PMID: 21406974

9. Niu H, Ping JA, Wang YB, Lv X, Li HM, et al. (2020) Population genomic and genome-wide association analysis of lignin content in a global collection of 206 forage sorghum accessions. Molecular Breeding 40.

10. Fracasso A, Trindade LM, Amaducci S (2016) Drought stress tolerance strategies revealed by RNA-Seq in two sorghum genotypes with contrasting WUE. BMC Plant Biol 16: 115. https://doi.org/10.1186/s12870-016-0800-x PMID: 27208977

11. Yang B, Chen Y, Zhang C (2019) Characterization of the complete chloroplast genome of the Chinese sorghum, Sorghum bicolor from China. Mitochondrial DNA Part B-Resources 4: 1421–1423.

12. Gao XQ, Zeng XC, Xia K, Yoshihara T, Zhou X (2004) Interactive effects of methyl jasmonate and salicylic acid on floret opening in spikelets of Sorghum. Plant Growth Regulation 43: 269–273.

13. Okada M, Yoshida K, Nishijima R, Michikawa A, Motoi Y, et al. (2018) RNA-seq analysis reveals considerable genetic diversity and provides genetic markers saturating all chromosomes in the diploid wild wheat relative Aegilops umbellulata. BMC Plant Biol 18: 271. https://doi.org/10.1186/s12870-018-1498-8 PMID: 30409135

14. Kong W, Zhang C, Qiang Y, Zhong H, Zhao G, et al. (2020) Integrated RNA-Seq Analysis and Meta-QTLs Mapping Provide Insights into Cold Stress Response in Rice Seedling Roots. International Journal of Molecular Sciences 21. https://doi.org/10.3390/ijms21134615 PMID: 32610550

15. Zhu G, Li W, Zhang F, Guo W (2018) RNA-seq analysis reveals alternative splicing under salt stress in cotton, Gossypium davidsonii. BMC Genomics 19: 73. https://doi.org/10.1186/s12864-018-4449-8 PMID: 29361913

16. Yazawa T, Kawahigashi H, Matsumoto T, Mizuno H (2013) Simultaneous transcriptome analysis of Sorghum and Bipolaris sorghicola by using RNA-seq in combination with de novo transcriptome assembly. Plos One 8: e62460. https://doi.org/10.1371/journal.pone.0062460 PMID: 23638091

17. Tuberosa R, Salvi S (2006) Genomics-based approaches to improve drought tolerance of crops. Trends Plant Sci 11: 405–412. https://doi.org/10.1016/j.tplants.2006.06.003 PMID: 16843036

18. Chen Y, Shi C, Huang Z, Zhang Y, Li S, et al. (2018) SOAPnuke: a MapReduce acceleration-supported software for integrated quality control and preprocessing of high-throughput sequencing data. Gigascience 7: 1–6. https://doi.org/10.1093/gigascience/gix120 PMID: 29220494

19. Cock PJA, Fields CJ, Goto N, Heuer ML, Rice PM (2010) The Sanger FASTQ file format for sequences with quality scores, and the Solexa/Illumina FASTQ variants. Nucleic Acids Res 38: 1767–1771. https://doi.org/10.1093/nar/gkq1137 PMID: 20015970

20. Kim D, Landmead B, Salzberg SL (2015) HISAT: a fast spliced aligner with low memory requirements. Nature Methods 12: 357–U121. https://doi.org/10.1038/nmeth.3317 PMID: 25751142

21. Paterson AH, Bowers JE, Bruggmann R, Dubchak I, Grimwood J, et al. (2009) The Sorghum bicolor genome and the diversification of grasses. Nature 457: 551–556. https://doi.org/10.1038/nature07723 PMID: 19189423

22. Langmead B, Salzberg SL (2012) Fast gapped-read alignment with Bowtie 2. Nat Methods 9: 357–359. https://doi.org/10.1038/nmeth.1923 PMID: 22386286

23. Li B, Dewey CN (2011) RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. BMC Bioinformatics 12: 323. https://doi.org/10.1186/1471-2105-12-323 PMID: 21816040

24. Langmead B, Salzberg SL (2012) Fast gapped-read alignment with Bowtie 2. Nature Methods 9: 357–U354. https://doi.org/10.1038/nmeth.1923 PMID: 22386286

25. Li B, Dewey CN (2011) RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. Bmc Bioinformatics 12.

26. Audic S, Claverie JM (1997) The significance of digital gene expression profiles. Genome Res 7: 986–995. https://doi.org/10.1101/gr.7.10.986 PMID: 9331369

27. Kanehisa M, Araki M, Goto S, Hattori M, Hirakawa M, et al. (2008) KEGG for linking genomes to life and the environment. Nucleic Acids Res 36: D480–484. https://doi.org/10.1093/nkjm882 PMID: 18077471

28. He B, Tao X, Gu Y, Wei C, Cheng X, et al. (2015) Transcriptomic Analysis and the Expression of Disease-Resistant Genes in Oryza meyeriana under Native Condition. PLoS One 10: e0144518. https://doi.org/10.1371/journal.pone.0144518 PMID: 26640944
29. Cheng XJ, He B, Chen L, Xiao SQ, Fu J, et al. (2016) Transcriptome analysis confers a complex disease resistance network in wild rice Oryza meyeriana against Xanthomonas oryzae pv. oryzae. Sci Rep 6: 38215. https://doi.org/10.1038/srep38215 PMID: 27905546

30. Tang W, Tu Y, Cheng X, Zhang L, Meng H, et al. (2019) Genome-wide identification and expression profile of the MADS-box gene family in Erigeron brevius. PLoS One 14: e0226599. https://doi.org/10.1371/journal.pone.0226599 PMID: 31860684

31. Han M, Lu X, Yu J, Chen X, Wang X, et al. (2019) Transcriptome Analysis Reveals Cotton (Gossypium hirsutum) Genes That Are Differentially Expressed in Cadmium Stress Tolerance. Int J Mol Sci 20. https://doi.org/10.3390/ijms20061479 PMID: 30909634

32. Katsir L, Chung HS, Koo AJK, Howe GA (2008) Jasmonate signaling: a conserved mechanism of hormone sensing. Current Opinion in Plant Biology 11: 428–435. https://doi.org/10.1016/j.yplb.2008.05.004 PMID: 18583180

33. Pauwels L, Barbero GF, Geerinck J, Tilleman S, Grunewald W, et al. (2010) NINJA connects the corepressor TOPLESS to jasmonate signalling. Nature 464: 788–U169. https://doi.org/10.1038/nature08854 PMID: 20360743

34. Suza WP, Staswick PE (2008) The role of JAR1 in Jasmonoyl-L-isoleucine production during Arabidopsis wound response. Planta 227: 1221–1232. https://doi.org/10.1007/s00425-008-0694-4 PMID: 18247047

35. Creelman RA, Mullet JE (1997) Biosynthesis and action of jasmonates in plants. Annual Review of Plant Physiology and Plant Molecular Biology 48: 355–381. https://doi.org/10.1146/annurev.arplant.48.1.355 PMID: 15012267

36. Kaplan F, Sung DY, Guy CL (2006) Roles of beta-amylase and starch breakdown during temperatures stress. Physiologia Plantarum 126: 120–128.

37. Murphy RL, Klein RR, Morishige DT, Brady JA, Rooney WL, et al. (2011) Coincident light and clock regulation of pseudoresponse regulator protein 37 (PRR37) controls photoperiodic flowering in sorghum. Proc Natl Acad Sci U S A 108: 16469–16474. https://doi.org/10.1073/pnas.1106212108 PMID: 21930910

38. Craufurd PQ, Mahalakshmi V, Bidinger FR, Mukuru SZ, Chantarette J, et al. (1999) Adaptation of sorghum: characterisation of genotypic flowering responses to temperature and photoperiod. Theoretical and Applied Genetics 99: 900–911.

39. Rooney WL, Aydin S (1999) Genetic control of a photoperiod-sensitive response in Sorghum bicolor (L.) Moench. Crop Science 39: 397–400.

40. Ohto M, Onai K, Furukawa Y, Aoki E, Araki T, et al. (2001) Effects of sugar on vegetative development and floral transition in Arabidopsis. Plant Physiol 127: 252–261. https://doi.org/10.1104/pp.127.1.252 PMID: 11553753

41. Mukherjee R, Gayen S, Chakraborty A, Bhattacharyya J, Maiti MK, et al. (2012) Double-Stranded RNA-Mediated Downregulation of pdhк Gene Expression to Shorten Maturation Time of a Late Maturing Native indica Rice Cultivar, Badshahbhog. Crop Science 52: 1743–1753.