

ANALYSIS OF FLOWER BUD DIFFERENTIATION OF *JATROPHA NIGROVIENSRUGOSUS* AT DIFFERENT DEVELOPMENTAL STAGES

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Abstract. The female to male flower ratio of *Jatropha nigroviensrugosus*, a new cultivar, is higher than that of the common *J. curcas*. However, the sex determination mechanism of *J. curcas* remains elusive. In the present study, gene expression analysis of inflorescence buds, female and male flowers, and new leaf buds of *J. nigroviensrugosus* was performed, using second-generation sequencing. The results demonstrated that high expression of genes related to sucrose phosphate synthase, soluble starch synthase (EC:2.4.1.21) and granule-bound starch synthase promoted the formation and development of male flowers, and the genes CUP-SHAPED COTYLEDON 2 (*CUC2*) and *CUC3*, played pivotal roles in regulating the formation and development of female flowers. The initial development of female flower primordia required cytokinins (CTK) induction, while late developmental stages only required a low concentration of CTK. The characteristic genes for floral organ development were conservatively expressed in the four tested parts. In this investigation, we not only provide genetic information regarding the differential developmental processes between female and male flowers, but also deepen our understanding of *J. nigroviensrugosus*.

Keywords: *Jatropha*, new cultivar, differential expressed gene, transcription factor

Introduction

Jatropha curcas L., a plant of the *Jatropha* genus in the *Euphorbiaceae* family, is an important energy-bearing tree which is extensively distributed worldwide. *J. curcas* has a high, untapped potential to contribute towards sustainable production of food and bioenergy, rehabilitation of degraded land, and reduction of atmospheric carbon dioxide, its potential oil productivity is 1.9 t/ha, beginning the fourth year after planting (Montes et al., 2016; Dias et al., 2012). However, the low seed yield of has been a stumbling block in realizing its full potential as an ideal bioenergy crop, the small number of female flowers and a low female to male flower ratio (ranges from 1:20 to 1:108) are the main factors limiting its seed yield (Gangwar et al., 2018; Ashoke et al., 2005). Breeders are mainly concerned with shortening the young growth cycle of *J. curcas*; increasing the ratio of female to male flowers; increasing oil content, seed weight, seed volume and other traits. Seed weight had positive correlation with seed length, breadth, thickness and oil content (Kaushik et al., 2007). The shortening of the breeding cycle and the increasing the number of female flowers contribute to the number of seeds, and the traits such as seed size, density and volume are important indicators affecting yield. Changing the offspring traits through interspecific hybridization in *J. curcas*, or through the method of discovery, identification, promotion and application of high-yield new varieties is a necessary way to genetic improvement.

J. nigroviensrugosus CV Yang, a new variant of *J. curcas* discovered in 2005, its main features are the downward cotyledons and true leaves, bulged leaf tissues (wrinkled leaves). It has a larger number of female flowers and a higher female to male flower ratio, which means a higher seed yield, the experimental plots for many years of afforestation showed that the dry seed yield per hectare of five-year-old stands in *J. nigroviensrugosus* reached 3196.8 kg/ha, which was 6 times higher than *J. curcas* (349.5~559.5 kg/ha); the oil content of the seeds in *J. nigroviensrugosus* was 40~42%, which was higher than *J. curcas* (30 ~ 40%) (Yang et al., 2012, 2013, 2015). Comparison of the $\delta^{13}\text{C}$ values and various photosynthetic indices between *J. nigroviensrugosus* and *J. curcas*, with respect to different flowering stages, demonstrated that during the leaf bud stage, *J. nigroviensrugosus* transported a large amount of nutrients to the leaf buds for their differentiation to flower buds. Therefore, flower differentiation is likely determined during the transformation from leaf to flower buds, and the flowering numbers may be determined during the inflorescence stage (He et al., 2016).

Flower formation has continuously been a focus in Plant developmental biology. Studies regarding sex expression and floral morphology are essential for deep understanding of breeding and for the determination of the reproductive potential of plant genotypes (De Lourdes Adriano-Anaya et al., 2016). The transcriptome describes all RNA transcribed by a certain tissue or cell at a certain developmental stage or in a specific physiological state, reflecting the overall expression pattern and regulation of genes. The female or male favorable analysis of floral development in both monoecious and dioecious plants by transcriptome profiling or microarray analysis may help to better understand flower development and the gender regulatory network (Mao et al., 2017; Gao et al., 2013; Rocheta et al., 2014). Hence, we performed high-throughput RNA sequencing (RNA-Seq) and differential analysis on the inflorescence buds, leaf buds, and male and female flowers of *J. nigroviensrugosus*. The present study shed light on the molecular basis of sex determination of *J. nigroviensrugosus* and elucidated the expression patterns of flowering-related genes prior to and following inflorescence and leaf bud differentiation, providing directions for the study of the flowering mechanism of *J. nigroviensrugosus*.

Materials and methods

Materials

The tested plants were collected from Qiaoma Experimental Forest, Ceheng County, Guizhou Province, China. The place is a typical dry-hot valley climate which is situated 830 m above sea level, with an annual average temperature 19.2 °C, rainfall 1340.7 mm, sunshine duration 1514 h and annual frost-free period is 305 days. It is known as the “natural greenhouse”. The soil is yellow sandy loam developed from sand shales, slightly acidic. Three 8-year-old *J. nigroviensrugosus* (JCw) plants were selected for the present investigation. The following four tissues were collected from each of the three plants: inflorescence buds at the terminal vegetative shoots (JCw_p) (early-forming adaxial bulges with a diameter <0.3 cm); slightly red new leaf buds at the branch tips (JCw_l); female flowers not yet open (JCw_f) (slightly visible white ovules showing after vertical cut); and male flowers not yet open (JCw_m) (slightly visible yellow anthers showing after vertical cut). Immediately after sample collection, wrap it

in foil paper and put it into liquid nitrogen. Bring it back to the laboratory and transpose it in a -80 °C ultra-low temperature freezer.

RNA extraction and transcriptome sequencing

Total RNA was extracted from the above-mentioned tissue samples, using TRIzol (Invitrogen, USA). After passing the quality examination, the RNA samples were sent to Shanghai Meiji Biological Cooperation for sequencing from both ends with an Illumina HiSeq sequencing platform.

Sequencing

Following completion of the sequencing, the raw data were quality controlled and compared with the genome of *J. curcas* (<https://www.ncbi.nlm.nih.gov/genome/915>). The read counts were transformed into FPKM (expected number of fragments per kilobase of transcript sequence per million base pairs sequenced) values for gene expression analysis. The thresholds of $|\log_2(\text{fold change})| \geq 1$ and $q\text{-value} < 0.05$ were used to determine the differential expressed genes (DEGs) in the resulting reads. The related DEGs were subjected to Gene Ontology (GO, <http://www.geneontology.org/>) functional enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG, <http://www.genome.jp/kegg/>) pathway enrichment.

Results

Transcriptome sequencing and alignment

FastQC (<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>) was used to perform quality control on the raw data, demonstrating a Q20 above 97%, a Q30 above 93%, and an average QC content of 43.5%. HISAT2 (<https://ccb.jhu.edu/software/hisat2/index.shtml>) was used to align the sequenced transcriptome data of *J. nigroviensrugosus* with the genome of *J. curcas* (<https://www.ncbi.nlm.nih.gov/genome/915>), demonstrating alignment rates higher than 96% (Table 1).

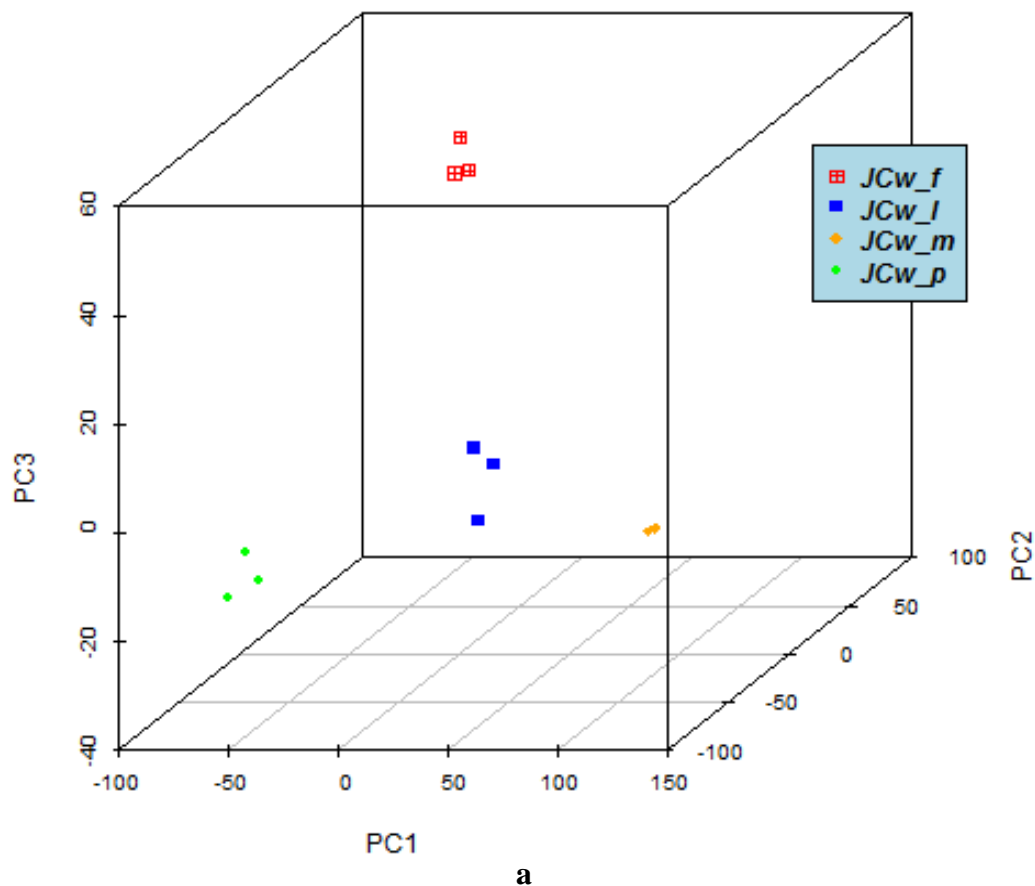
Table 1. Transcriptome sequence number of *J. nigroviensrugosus* and alignment rate against *J. curcas* genome

Sample	Total_seq_num	Aligned_seq_num	Alignment_rate	Uniq_aligned_num	Uniq_rate	Multi_aligned_num	Multi_rate
JCw_p1	61561080	59770212	97.09%	47954394	77.90%	11815818	19.19%
JCw_p2	68567902	66625809	97.17%	53415546	77.90%	13210263	19.27%
JCw_p3	59863830	58056039	96.98%	46249509	77.26%	11806530	19.72%
JCw_l1	65790812	63842133	97.04%	50803720	77.22%	13038413	19.82%
JCw_l2	57042156	55510903	97.32%	44681167	78.33%	10829736	18.99%
JCw_l3	54306178	52727027	97.09%	42234790	77.77%	10492237	19.32%
JCw_f1	63398574	61833649	97.53%	49485973	78.06%	12347676	19.48%
JCw_f2	51699528	50312809	97.32%	40301836	77.95%	10010973	19.36%
JCw_f3	65665646	63948655	97.39%	51294438	78.11%	12654217	19.27%
JCw_m1	61817828	60212667	97.40%	47821892	77.36%	12390775	20.04%
JCw_m2	55453444	54036050	97.44%	42977841	77.50%	11058209	19.94%
JCw_m3	59762748	58184393	97.36%	46342932	77.54%	11841461	19.81%

Form 40.30 to 53.41 million sequences were aligned to unique positions in the genome, the alignment rates is ranging from 77.22 to 78.33%, form 18.99 to 20.04% sequences is aligning to multiple positions in the genome. These indicating overall matched *J. nigroviensrugosus* sequences with the *J. curcas* genome. Consequently, the sequences may be analyzed as reference-based transcriptome. The small differences between the sequences indicate that the data were highly qualified to reveal differences in inflorescences prior to and subsequent to leaf bud differentiation.

PCA and gene expression analyses

The gene expression of four samples of *J. nigroviensrugosus*, based on the quantitative results of FPKM, was subjected to principal component analysis (PCA), using R (<https://www.r-project.org/>) which is an open source software. The results denote that the tip inflorescence buds (JCw_p) were closely related to the new leaf buds (JCw_l), with a PC1 value on the X axis of 44.4% and a PC2 value on the Y axis of 31.5%. Three-dimensional (3D) visualization shows significantly clustered samples with no outliers, suggesting good biological repeatability (Fig. 1a). According to the DEG screening thresholds, statistical analysis of all pairs of *J. nigroviensrugosus* samples, there were 2995 DEGs between JCw_m and JCw_f, with 1733 upregulated and 862 downregulated genes in JCw_m; 4238 DEGs between JCw_p and JCw_f, with 2138 upregulated and 2100 downregulated genes in JCw_p; 6153 DEGs between in JCw_p and JCw_m, with 3226 upregulated and 2927 downregulated genes in JCw_p. There were 74 genes specifically expressed in JCw_f or JCw_m (Fig. 1b).



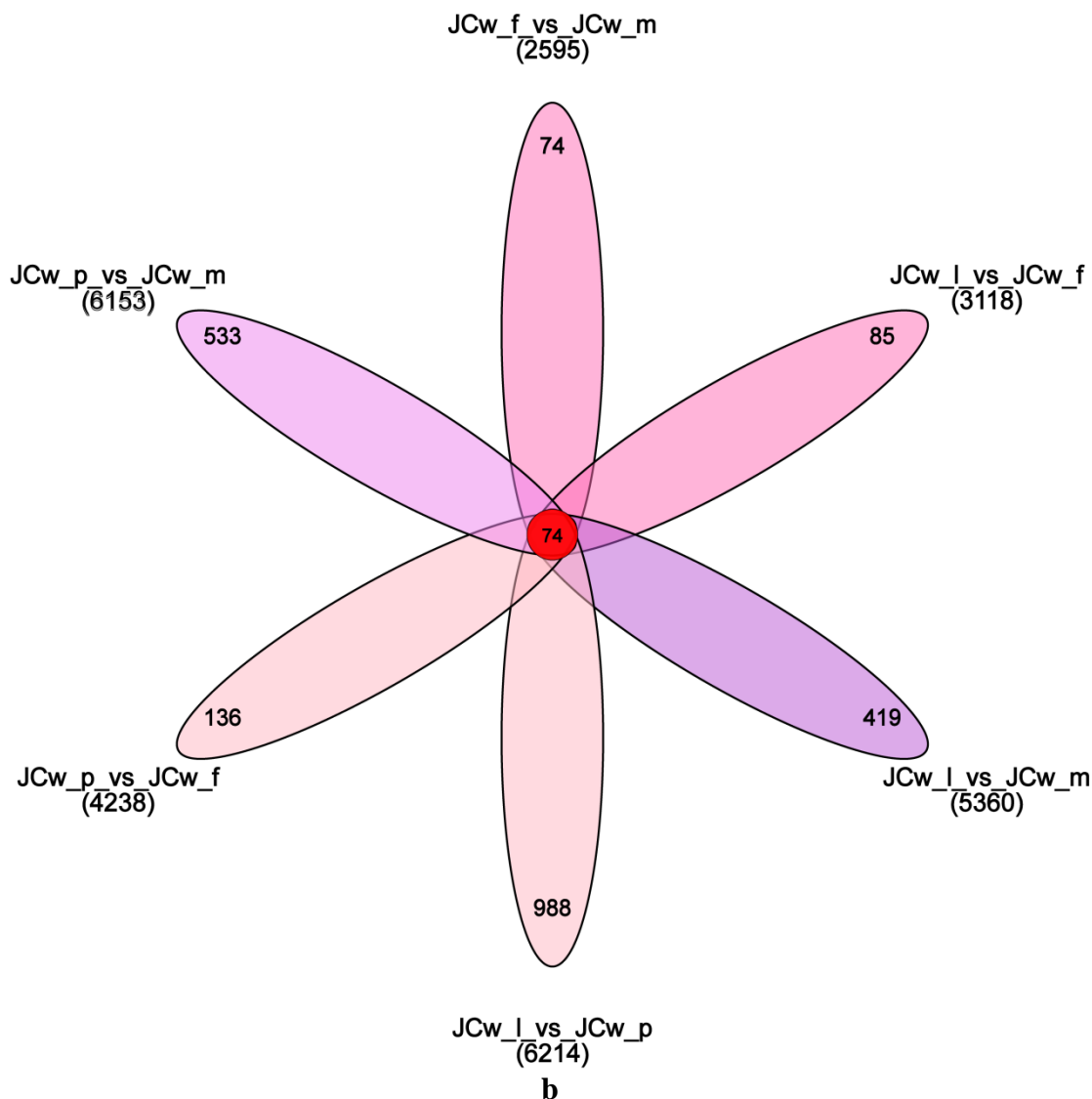


Figure 1. a. PCA of the different tissues of *J. nigroviensrugosus* with 3D visualization. **b.** Venn diagram of DEGs between pairs of *J. nigroviensrugosus* samples

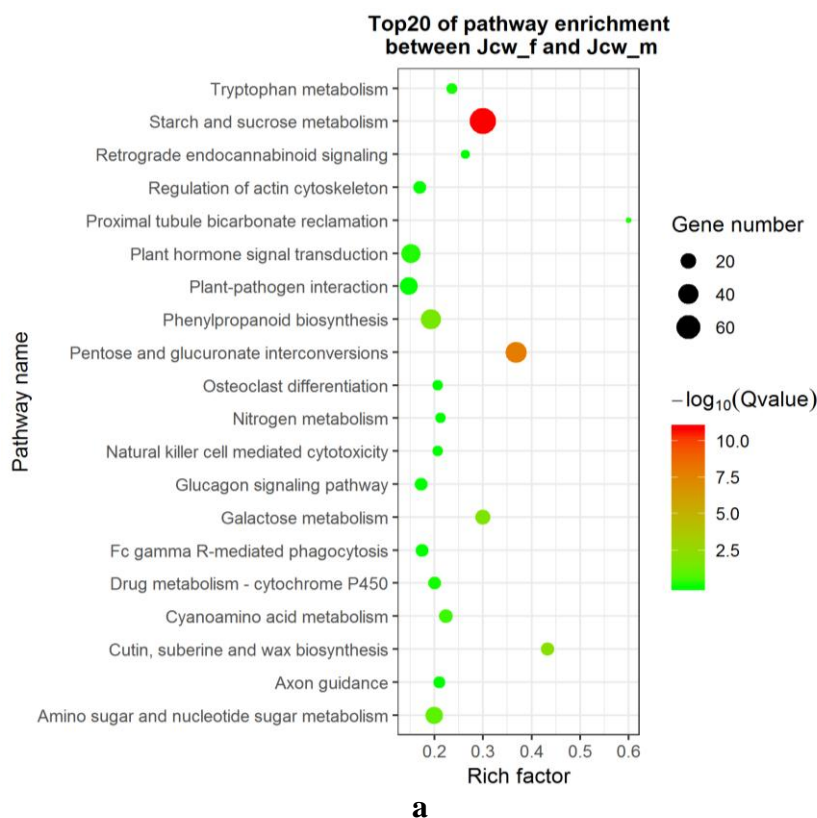
Functional enrichment of female and male flowers of *J. nigroviensrugosus*

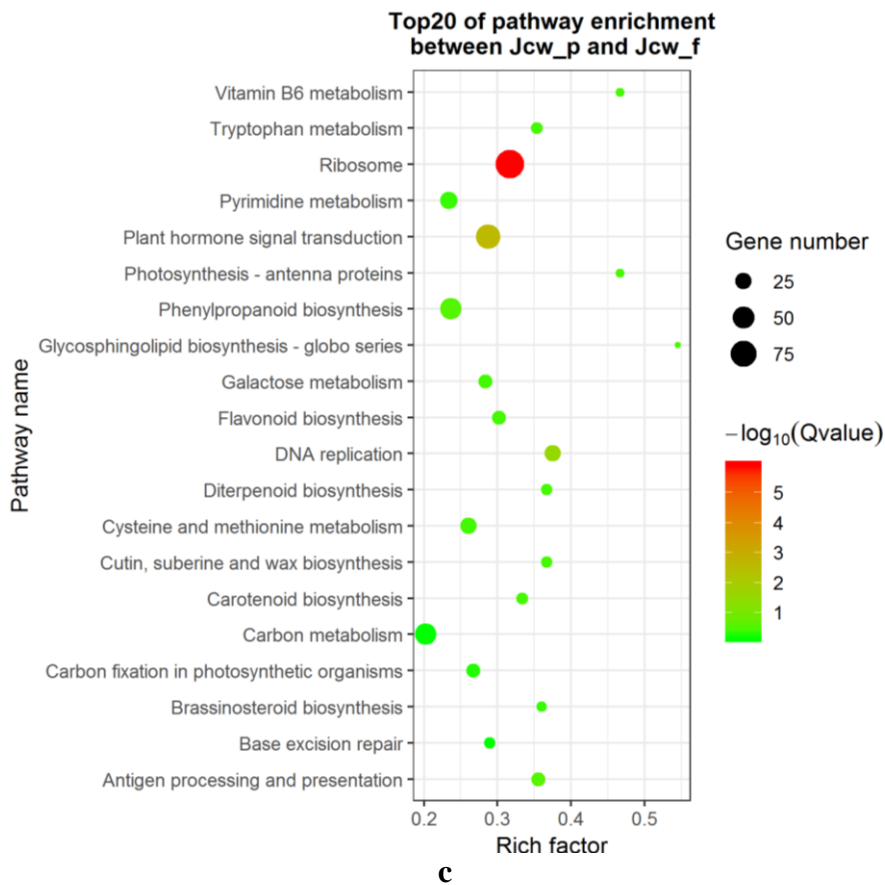
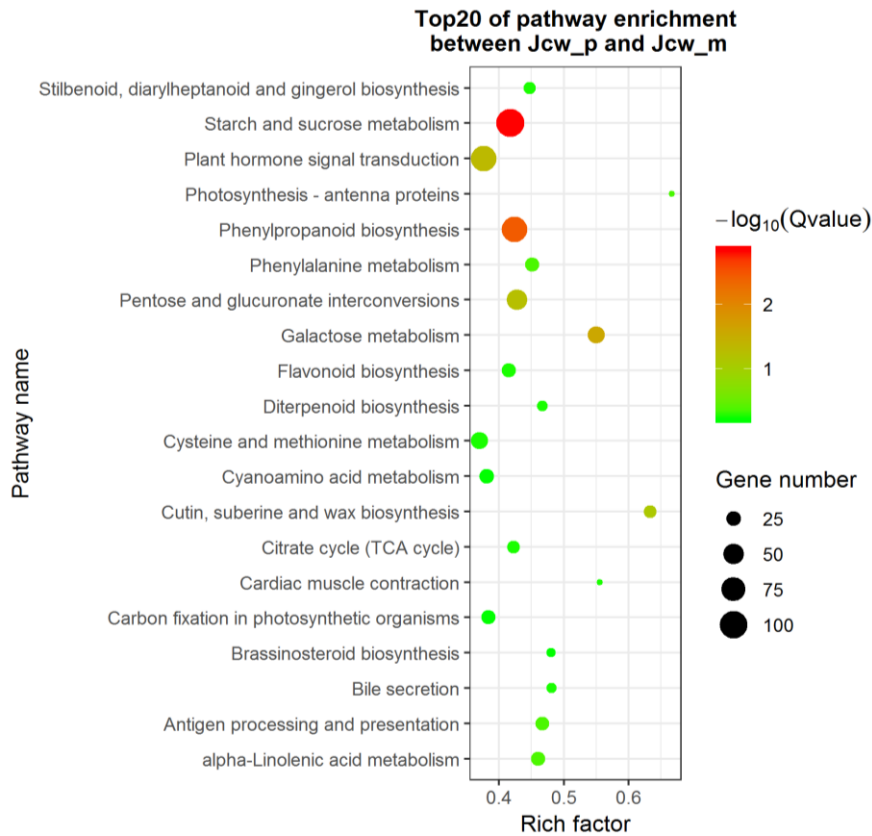
GO functional enrichment analysis was conducted on the DEGs between male and female flowers. With respect to the biological process, genes related to carbohydrate metabolism, including glucose, sucrose, polysaccharides and starch, were significantly enriched. With respect to the cell components, genes associated with inner and outer membranes, extracellular regions, transcription factor complexes and the cell skeleton were significantly enriched. In molecular function, genes coupled to glucosidase, hydrolase and oxidoreductase were significantly enriched. Pathway enrichment analysis of DEGs between male and female flowers (Fig. 2a) displays significantly enriched metabolic pathways such as starch and sucrose (ko00500), and pentose and glucuronic acid interconversion (ko00040). Moreover, there were significant differences in the plant hormone signal transduction pathway (ko04075), with a total of 16 hormone synthesis-related genes upregulated in female flowers and 18 upregulated in male

flowers. The abscisic acid (ABR) synthesis-related genes SnRK2 (gene 20150) and ABF (gene 13255 and gene 14512) were significantly upregulated in male flowers. The cytokinin (CTK) synthesis-related gene A-ARR (gene 13354) was significantly upregulated in female flowers, and B-ARR (gene 20147) was significantly upregulated in male flowers.

DEGs analysis between inflorescence and leaf buds, and female and male flowers

Between inflorescence buds and male flowers, the starch and sucrose metabolic pathway (ko00500) was significantly enriched (*Fig. 2b*). In male flowers, the expression of 84 genes related to fructokinase (EC: 2.7.1.4) (gene 5753, gene 8905, and gene 17326), starch synthase (EC: 2.4.1.21) (gene 4801), granule-bound starch synthase (EC: 2.4.1.242) (gene 13471) and sucrose-phosphate synthase (EC: 2.4.1.14) (gene 7836, gene 12091, and gene 14471) were significantly upregulated, and 21 were downregulated. However, there was no significant enrichment of the starch and sucrose metabolic pathway between inflorescence buds and female flowers. The pathway enrichment of the DEGs between inflorescence buds and female flowers reveals enriched metabolic processes such as ribosomes, plant hormone signaling and DNA replication (*Fig. 2c*). With respect to the phytohormone signaling pathway, the ethylene synthesis-related gene ERF1/2 (gene 18694) was upregulated in female flowers. The pathway enrichment of the DEGs between inflorescence and leaf buds displayed enriched metabolic processes such as ribosomes, photosynthesis, photosynthesis-antenna proteins, and plant hormone signaling (*Fig. 2d*). With respect to the photosynthetic pathway, with the exception of gamma (gene 7618) being upregulated in inflorescence buds, the remaining 48 DEGs involved in photosynthesis and 15 DEGs associated with photosynthesis-antenna proteins were all upregulated in leaf buds.





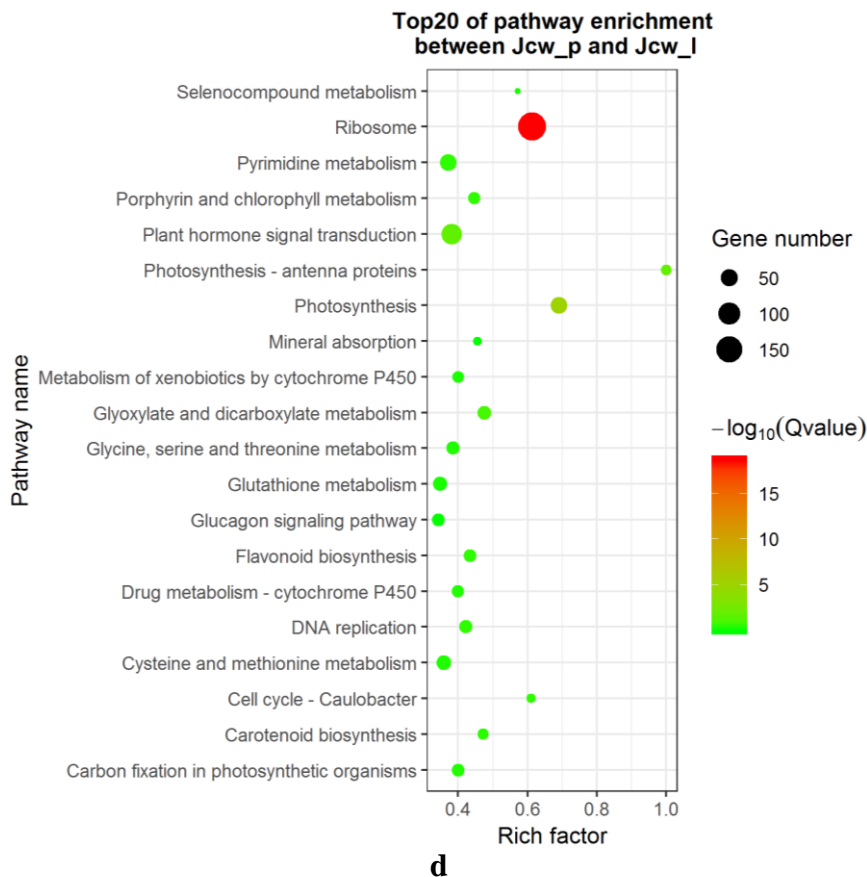


Figure 2. *a.* Bubble map for the top 20 enriched pathways in female and male flowers. *b.* Bubble map for the top 20 enriched pathways in inflorescence buds and male flowers. *c.* Bubble map for the top 20 enriched pathways in inflorescence buds and female flowers. *d.* Bubble map for the top 20 enriched pathways in inflorescence and leaf buds

Changes in MADS-box gene expression

Download the *J. curcas* proteome data (<http://www.ncbi.nlm.nih.gov/genome/915>) (Sato et al., 2010) and the MADS-box Hidden Markov Model (HMM) model file (PF00319, <http://pfam.sanger>), using the HMM file as a seed, run HMM search in the *J. curcas* proteome database (e-value < 1e-20). Construct a *J. curcas*-specific HMM model based on the screening results, then perform HMM search again (e-value < 0.01), non-repetitive MADS-box related protein ID are extracted and the corresponding gene ID sequence and length information are extracted according to genomic annotation file. Search through the Pfam database, NCBI conserved domain database (<https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi>) and SMART (<http://smart.embl-heidelberg.de/>) to confirm that all predicted genes contain the MADS-box domain. The MADS-box gene family is divided into two main lineages, type-I and type-II, that originated from a duplication of ancestor genes during the divergence of plants and animals, the major difference between type-I and type-II genes is that type-II genes possess the K-box domain (Alvarez-Buylla et al., 2000; Theissen et al., 1996). Contained the K-box domain was identified as the type-II MADS-box gene, the otherwise is identified as the type-I MADS-box gene. Heatmap clustering and multiple annotations are performed using the circlize package (Gu et al., 2014). Two

types of MADS-box-related genes were subjected to hierarchical cluster analysis and visualized as four clusters according to the number of tissue samples (Fig. 3). The results show that for *J. nigroviensrugosus*, the MADS-box-related genes could be divided into two expression patterns (clustered by sample), one expressed in female and male flowers, and the other in inflorescence and leaf buds. In addition, the gene highly expressed in all samples is the type-II MADS-box gene.

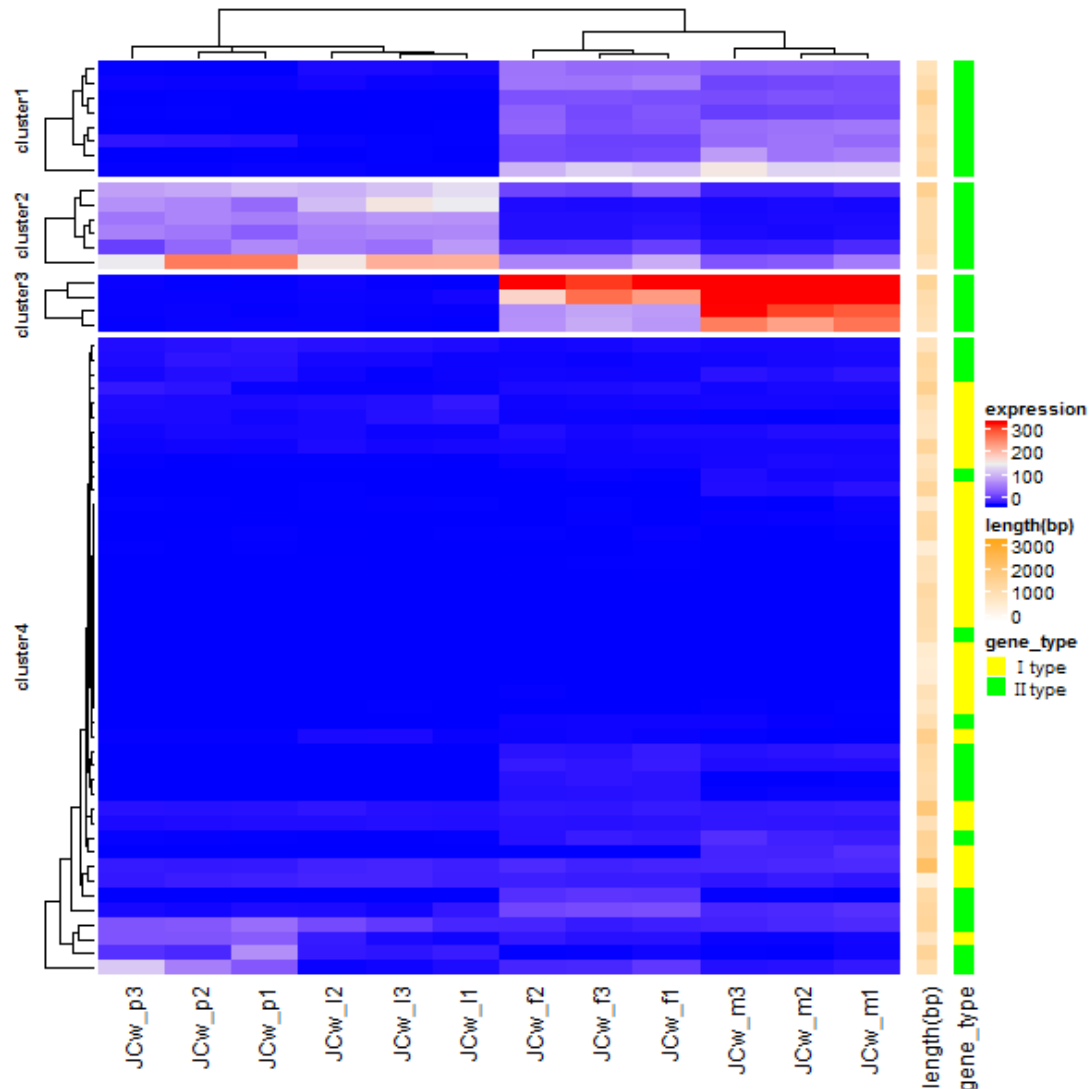


Figure 3. MADS-box gene expression profile of the four parts of *J. nigroviensrugosus*

Discussion

The low yield and low oil content of *J. curcas* seeds are the biggest problems encountered in the current planting of *J. curcas* trees; therefore, the identification of the most favorable genotypes is extremely important (Junqueira et al., 2016). As a new species, *J. nigroviensrugosus* has a higher female to male ratio and a higher seed yield than common *J. curcas*. Several papers have investigated flower induction of *J. curcas* in China and abroad, for instance, histological analysis of the differentiation of male and female flowers at different developmental stages (Xu et al., 2016), transcriptome

differences between pure female and bisexual inflorescences (Chen et al., 2017), and gene responses subsequent to hormone induction (Ghosh et al., 2010; Pan et al., 2016). However, there is a lack of information regarding the flowering mechanism of *J. nigroviensrugosus*, and whether the relevant measures applied to the common *J. curcas* can be applied to *J. nigroviensrugosus*. Studies of *J. nigroviensrugosus* prior to and subsequent to inflorescence differentiation are beneficial for expanding knowledge of the sex determination mechanism of this energy-rich plant.

A plant undergoes a major physiological change during the transition from vegetative growth to reproductive development, and this transition is a result of responses to various endogenous and exogenous signals (Srikanth and Schmid, 2011). The expression of a specific gene, including the tissue specificity and the difference in transcriptional level, may influence the sex determination of a flower (Ramos et al., 2017). The differentially-expressed genes in the comparison group of female and male flowers may be involved in the regulation of gender differentiation. During this period, carbohydrates may act as mobile signals to regulate flower morphogenesis (Turnbull, 2011; Corbesier et al., 2006). Sugar catabolism enzymes may increase the assimilation ability of the shoots, thereby accelerating shoot growth and increasing primordium numbers (Ito et al., 2002). Low concentrations of sucrose may promote flowering, whereas high concentrations may delay flowering (Ohto et al., 2001). Sucrose-mediated signaling is integrated into the photoperiod pathway, located downstream of the CONSTANS (CO) gene and upstream of the FLOWERING LOCUS T (FT) gene (Seo et al., 2011). Sucrose-phosphate synthase (SPS) (EC: 2.4.1.14) is considered to be a key enzyme in the control of sucrose synthesis (Sawitri et al., 2018). With respect to inflorescence buds and male flowers, the SPS-related genes (gene 7836, gene 12091 and gene 14471) were upregulated in male flowers. In addition, polygalacturonase (PG) was involved in the development of pollen (Ogawa et al., 2009), which was significantly upregulated in male flowers. The distribution of sugars and starch is known to be related to flower transformation. Studies conducted on various angiosperm species have shown a correlation between flower abortion and starch content (Reale et al., 2009). Starch is composed of linear and branched glucose (Glc) polymers (Zeeman et al., 2010). The soluble starch synthase (EC: 2.4.1.21) is primarily related to amylopectin catalysis, while granule-bound starch synthase (EC: 2.4.1.242) is primarily associated with the production of amylose, both of which were found to be highly expressed in the male flowers of *J. nigroviensrugosus*. This data indicates that high expression of sucrose and starch synthesis-related genes plays an important role in the development of male flowers in *J. nigroviensrugosus*.

Plant hormones play essential roles in gender determination (Aryal and Ming, 2014). Auxin plays a dominant role in floral organ initiation and organogenesis (Chandler, 2011), and CTK triggers the initiation of female flower primordium (Hui et al., 2017). Application of exogenous cytokinin (6-benzyladenine, BA) on inflorescence buds can significantly increase the number of female flowers, BA treatment may delay floral organ formation by inhibiting the transcription of the A, B and E classes of floral organ-identity genes, which allowing extra time for the plant to produce more primordia in inflorescence meristems (Chen et al., 2014). Type-B Arabidopsis response regulators (ARRs) are positive regulators of the CTX response (Mason et al., 2005), and type-A ARRs are thought to work as negative regulators of CTX to inhibit the activity of type-B ARRs (To and Kieber, 2008; To et al., 2004). B-ARR (gene 8030 and gene 17670) was significantly upregulated in inflorescence buds compared with leaf buds.

Comparing the male and female flowers, A-ARR (gene 13354) was significantly upregulated in female flowers, whereas B-ARR (gene 20147) was significantly upregulated in male flowers. These data suggest that the initial development of *J. nigroviensrugosus* female flower primordia requires the induction of CTK, but the late developmental stage only needs a low concentration of CTK. As a gaseous plant hormone, ethylene regulates the flowering time of plants through interactions with other plant hormones (Iqbal et al., 2017). In *J. curcas*, treatment with a low concentration of ethephon (25 ppm) is thought to increase the number of female flowers (Makwana and Robin, 2013), and increasing the ethephon concentration has been shown to decrease the number of male and female flowers per inflorescence as well as the total flower number (Costa et al., 2016). Compared with inflorescence buds and male flowers, the expression of ERF1/2 (gene 18694) was significantly increased in female flowers of *J. nigroviensrugosus*, highlighting the importance of ethylene in the regulation of female flower formation and development. In the ABA signaling pathway, the SnRK2 signal plays a crucial role in the sex determination of ferns and regulates the sex ratio of male to hermaphrodite in the reproductive cycle (McAdam et al., 2016). However, there is no related report regarding the impact of SnRK2 on the female to male flower ratio of angiosperms, especially *J. curcas*.

The differential expression of transcription factors (TFs), also known as trans-acting factors, play an essential role in the control of organ development (Latchman, 1997). Six signaling pathways have been identified to regulate the flowering of *A. thaliana*: photoperiod, vernalization, autonomous, gibberellin (GA), temperature-sensitive, and age-dependent control (Fornara et al., 2010). These signaling pathways are both independent and connected, and converge the flowering signals to several key integrators such as FT, TWIN SISTER OF FT (TSF) and SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1), activating the expression of meristem-specific genes such as LEAFY (LFY), APETALA1 (AP1) and FRUITFULL (FUL), eventually initiating plant flowering (Cho et al., 2017). Among these, the expression of SOC1 was high in leaf and inflorescence buds, but low in male and female flowers. Conversely, FT was highly expressed in male and female floral organs. The zinc finger transcription factor CONSTANS contains a B-box domain (Gangappa et al., 2014), which plays a central role in photoperiod sensing (Lee et al., 2017). The expression of CONSTANS-LIKE 7 (gene 24482) and CONSTANS-LIKE 3 (gene 12833) were upregulated in both male and female flowers, with respect to inflorescence buds.

NAC genes encode plant-specific transcription factors that are closely related to vegetative reproduction, and embryo and flower development (Olsen et al., 2005). CUP-SHAPED COTYLEDON 1 (CUC1), CUC2 and CUC3 have been shown to regulate organ segregation and meristem formation (Vroemen et al., 2003). In addition to controlling shoot meristem activity, CUC1 and CUC2 also play key roles in female organ development (Kamiuchi et al., 2014). Genetic interaction analyses using single, double and triple mutants of *cuc1-1D*, *cuc2-1D* (a CUC2 mutant similar to *cuc1-1D*), and *hws-1*, demonstrate that HWS (HAWAIIAN SKIRT), CUC1 and CUC2 act together to control floral organ number (González-Carranza et al., 2017). It is believed that CUC2 is associated with female flower opening of *J. curcas* (Gangwar et al., 2016). Through comparison of female and male flowers of *J. nigroviensrugosus*, we found nine NAC-related genes upregulated in female flowers (including homologous genes of CUC2 and CUC3), and 12 NAC-related genes upregulated in male flowers (including

NAC2 and NAC29). These data indicate that CUC2 and CUC3 make a contribution in the formation and development of *J. nigroviensrugosus* female flowers.

The MADS-box TFs are the primary components of the current well-known ABCDE model which are closely related to the origin and evolution of plant reproductive organs (Smaczniak et al., 2012; Theissen et al., 2000). The MADS-box TFs play a pivotal role in floristic differentiation (Theissen and Melzer, 2007). Ming et al. (2011) showed that the structural determinants of floral organs are randomly distributed in autosomes, therefore only the spatiotemporal patterns are expressed between sexes, but the expression are not sex-specific. With respect to the DEGs selected in the comparison group of female and male flowers in *J. nigroviensrugosus*, seven genes including ANR1, AGL14 and AGL7 were upregulated in female flowers, while 11 genes including AGL11, AGL62 and AGL20/SOC1 were upregulated in male flowers. The B- and C-class genes, the characteristic genes of flower organ development (Pařenicová et al., 2003), and the dependent SEP1, SEP2 and SEP3 genes, were all similarly expressed in both female and male floral organs of *J. nigroviensrugosus*, and the expression was not sex-specific. The MADS-box-related genes from the four different tissues of *J. nigroviensrugosus* were primarily divided into two expression patterns (Fig. 3). Cluster 3-related genes were expressed only in male and female flowers, while cluster 2-related genes were expressed in inflorescences and leaf buds, with a certain degree of conservativity. Two MADS-box-related genes in cluster 3 were only highly expressed in male flowers, spatiotemporally. In addition, the gene highly expressed in all samples belongs to the type-II MADS-box gene. Several cluster 4-related genes, including AGL62 and AGL80, were not expressed in any of the four tissues, suggestive of involvement in early endosperm development (Kang et al., 2008; Portereiko et al., 2006) and regulation of certain physiological activities.

Conclusion

As a new cultivar, transcriptome sequencing of inflorescence and leaf bud differentiation in *J. nigroviensrugosus* was beneficial to analyze the nutrient supply relationship and hormonal regulation of different plant parts. Genes related to sucrose phosphate synthase, soluble starch synthase and granule-bound starch synthase were highly expressed in male flowers of *J. nigroviensrugosus*, suggestive of an important role in the formation and development of male flowers. Initial development of female flower primordia required the induction of CTK, while late stage development only required a low concentration of CTK in coordination with other hormones. CUC2 and CUC3 were very important in the formation and development of female flowers of *J. nigroviensrugosus*. As the most important transcription factors, MADS-box-related genes were conserved throughout different parts of *J. nigroviensrugosus*. Moreover, a number of DEGs were screened during the differentiation of male and female flowers, this study provided an important basis for further research regarding the differentiation of flowers and the molecular mechanism of high-yield *J. nigroviensrugosus* breeding.

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