INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
18F–018/2018/20–7–1523–1532
DOI: 10.17957/IJAB/15.0660
http://www.fspublishers.org

Full Length Article

Flowering Related Comparative Transcriptomics between *Jatropha curcas* and *Jatropha nigroviensrugosus*

Feng Xiao[1,2], Xiu Rong Wang[1], Yang Zhao[1,2*] and Hua He[1]
[1]*Foresty College of Guizhou University, Guiyang, 550225, China*
[2]*Institute for Forest Resources & Environment of Guizhou, Guiyang, 550225, China*
[*]For correspondence: zhy737@126.com

Abstract

The main factor limiting the yield is the low radio of female to male flowers. As a new variant, *J. nigroviensrugosus* cv. Yang has a larger female to male flower ratio than *Jatropha carcas* L. Leaf- and inflorescence buds and female and male flowers of *J. carcas* and its cultivar *J. nigroviensrugosus* were sequenced to carry out a comparative analysis among their transcriptome differences. Results demonstrated that the alignment rates for both of them with respect to the genome of *J. carcas* were above 96%. This indicates small sequence differences and closes genetic relationships among the two taxa. The morphological differences between the leaves of the two cultivars affect the plant's response to red/far-red light and blue light. From the gene expression trend, it was found that most of the genes that were up-regulated in both female and male flowers were associated with floral organ formation. There were significant differences in the gene expression in the inflorescence buds between the two cultivars, exhibiting a total of 1646 differential genes. Plant hormone signal transduction pathways were involved in the sexual differentiation and flower bud formation of *J. curcas*. While the genes DELLA, MYC2 and CYCD3 might be related to the stamen abortion and the female flower sex determination during the inflorescence bud stage of *J. nigroviensrugosus*. © 2018 Friends Science Publishers

Keywords: *Jatropha nigroviensrugosus*; Female to male flower ratio; Sex determination; Plant hormone signal transduction pathways

Introduction

Jatropha curcas L. (Fam.: Euphorbiaceae) is a deciduous shrub or small tree. The leaves are membranous or paper like, alternate, with no lateral lobes or sometimes with 3–5 weak lobes. The old branches are dark gray and the fresh branches are green containing milky watery juice inside. The inflorescence is cymose, usually as dichasia. It is monoecious with unisexual, auxiliary or terminal flowers (Wang *et al.*, 2012a). *J. carcas* is one of the most important energy-bearing species in the world (Fairless, 2007), it has high potential for development in promoting sustainable production of food and bioenergy, the restoration of degraded land and the reduction of atmospheric carbon dioxide (Montes and Melchinger, 2016). The ratio female-male flower is the most important character for *J. curcas* because it has a significant correlation to the yield (Wijaya *et al.*, 2009). But it has small number of inflorescences with fewer female flowers in *J. curcas* (Raju and Ezradanam, 2002). The female to male flower ratio ranges from 1:20 to 1:108 (Fresnedo-Ramírez, 2013). Currently its yield is low (Openshaw, 2000).

The biggest challenge for *J. curcas* breeding is to identify superior genotypes that present high seed yield and seed oil content with reduced toxicity levels (Junqueira *et al.*, 2016). Touse the full potential of *J. curcas* and to support further expansion and systematic selection, breeding and domestication are a prerequisite (Achten *et al.*, 2010). *J. nigroviensrugosus* cv. Yang is a newly discovered variant of *J. curcas* developed via selectively cross-breeding (Yang *et al.*, 2012; Yang, 2013). Its main features are the downward cotyledons and true leaves, bulged leaf tissues (wrinkled leaves), and the leaves are dark green. Compared with *J. curcas*, *J. nigroviensrugosus* has more female flowers, a larger female to male flower ratio. 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.*, 2015). During the leaf bud stage, a large amount of nutrient is transported to the flower buds for the differentiation and development of male flowers and the establishment of inflorescence morphology in *J. nigroviensrugosus* (He *et al.*, 2016). Till now there has been only a few research on the *J. nigroviensrugosus* which focused mainly on the growth phenology and field cultivation, the available reports

on the mechanism of flower blossom in *J. nigroviensrugosus* are very limited. During the flower bud differentiation process, the external environmental factors, the internal factors of plant itself, as well as the nutrient conditions all can affect the differentiation. The woodland, watershed and regional climates have significant effects on the flowering characteristics of the *J. curcas* (Liu *et al.*, 2010). The flowering season and number of flowering events, and male to female flower ratio in *Jatropha* is dependent upon soil fertility and available moisture (Kant and Wu, 2011). Even elevated CO_2 significantly influences reproductive characteristics of *Jatropha* and improve its fruit and seed yields (Kumar *et al.*, 2014). The nutritional status of *Jatropha* itself is also an essential factor affecting the number of flowers and the female to male flower ratio. It was found that high contents of Zn, Fe and a high K/Na ratio all contribute to the flower formation (especially the female flowers) of the *J. curcas* (Wang *et al.*, 2012b). So the same site conditions on the growth are crucial to the comparison of two *Jatropha* species.

The continuous development of high-throughput sequencing technologies has deepened the genomics and genetics of *J. curcas* in recent years. Sato *et al.* (2010) conducted a genome-wide sequencing of *Jatropha* species obtained 286 M assembly data. Genetic differences are the inherent factors that lead to differences in the female to male flower ratio. Understanding the transcriptome is essential for interpreting the functional elements of the genome and revealing the molecular constituents of cells and tissues, and also for understanding development and disease (Wang *et al*., 2009). Expression analysis at six floral developmental stages between high female to male flower ratio and a low ratio genotype in *J. curcas*, genes CUC2, TAA1, CKX1, PIN1, SUP and CRY2 play a significant role in increased transition towards female flowering by promoting abortion of male flower primordia (Gangwar *et al*., 2016). The search for differences in floral development between flowers is often used as a means of identification of sex differentiation regulators. Male floral initiation in *J. curcas* was associated with the flavonoid biosynthesis pathway while Cytokinin (CTK) signaling triggered the initiation of female floral primordium (Hui *et al*., 2017). Not only the uniqueness of flowers, but also different types of inflorescences. A comparative transcriptome analysis between gynoecious and monoecious inflorescences were performed, KNOTTED1-LIKE HOMEOBOX GENE 6 (KNAT6), MYC2, SHI-RELATED SEQUENCE 5 (SRS5), SHORT VEGETATIVE PHASE (SVP), TERMINAL FLOWER 1 (TFL1), and TASSELSEED2 (TS2), were considered as candidate regulators that may be involved in sex differentiation in *J. curcas* (Chen *et al*., 2017).The gender of the flower in *J. curcas* is also regulated by plant hormones. The application of plant growth regulators elevated the seed yield of *J. curcas* (Joshi *et al.*, 2011; Xu *et al.*, 2013; Chen *et al.*, 2014; Fröschle *et al.*, 2017). The increase in the number of female flowers after BA treatment was attributed to the induction of delayed floral organ formation and increased expression of CUC1 gene, leading to an increase in the number of flower primordium in inflorescence meristem (Pan *et al.*, 2014). However, differences between the phytohormone signal transduction pathways during flower sex differentiation between two *Jatropha* species are not yet clear.

Focus on breeding, identification and popularization of new varieties with high yield are necessary measures and ways of genetic improvement. In this study, the comparative transcriptomics was used to screen, enrich, and analyze the differential genes (DEGs) before and after flower bud differentiation as well as the newborn leaves of the two cultivars. Combined with the related phenotypic traits, physiological and biochemical differences factors related to the development of the female and male flower were investigated. The underlying mechanism for the female to male flower ratio was also explored. It is intended that the result of the present research would provide ideas and make available data sources for the reproductive regulation of *J. curcas*.

Materials and Methods

Eight years old three plants from each of *J. curcass* (JCn) and *J. nigroviensrugosu* cv. Yang (JCw) with comparative growth situation were selected for the present investigation. The plants were collected from Qiaoma Experimental Forst, Ceheng County, Guizhou Province, China. The place is situated 830 m above sea level, with an annual average temperature 19.2°C, rainfall 1340.7 mm and sunshine duration 1514 h. The soil is yellow sandy loam developed from sand shales, slightly acidic. The samples prepared and numbered for the present experiment have been provided in Table 1.

RNA Extraction and Library Construction

The total RNA content present in the tissue of plant samples were extracted using a Trizol kit (Invitrogen). Nandrop 2000 was used to determine the concentration and purity of the RNA. The RNA integrity was assessed by agarose gel electrophoresis while its integrity number (RIN) value was measured by Agilent 2100. After the quality of the total RNA sample met the requirements (with at least 1μg sample of concentration ≥50 ng/μL, OD260/280 = 1.8–2.2), the mRNA was enriched with Oligo (dT) magnetic beads. Further, the mRNA was added with fragmentation buffer and cut into 200 bp short fragments. Using mRNA as templates, cDNA was reverse transcribed using six-base random primers. The double-stranded cDNA samples were purified, end-repaired, added with poly (A) tails and then ligated to the sequencing adapters to create cDNA libraries. After the libraries passed quality test, they were sent to Shanghai Meiji Biological Cooperation for sequencing from both ends with an Illumina Hiseq sequencing platform.

Table 1: Description of test materials

Cultivars	Leaf	Inflorescence bud	Female flower	Male flower	Source
Jatropha curcass L. (JCn)	JCn_l1	JCn_p1	JCn_f1	JCn_m1	JCn plant #1
	JCn_l1	JCn_p1	JCn_f1	JCn_m1	JCn plant #1
	JCn_l3	JCn_p3	JCn_f3	JCn_m3	JCn plant #3
Jatropha nigroviensrugosus CV Yang (JCw)	JCw_l1	JCw_p1	JCw_f1	JCw_m1	JCw plant #1
	JCw_l2	JCw_p2	JCw_f2	JCw_m2	JCw plant #2
	JCw_l3	JCw_p3	JCw_f3	JCw_m3	JCw plant #3

Inflorescence bud: a terminal vegetative shoot at the sexual differentiation stage (with an early formed adaxial bulge of diameter less than 0.3 cm). Leaf: a slightly red new shoot bud at a branch tip. Female flower: a female flower that has not yet opened (with slightly visible white ovules shown after vertical cut). Male flower: a male flower that has not opened (with slightly visible yellow anthers shown after vertical cut)

Comparative Transcriptomic Data Analysis

At first, the raw data were quality filtered by SeqPrep (https://github.com/jstjohn/SeqPrep), removing the reads with adapters or with more than 10% N, as well as the poor quality sequences. Then the qualities of the clean reads thus obtained, were assessed with FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) (Cock *et al.*, 2009). Further HISAT2 (https://ccb.jhu.edu/software/hisat2/index.shtml) was used to align the sequenced transcriptomic data with the genome of *J. curcas* (https://www.ncbi.nlm.nih.gov/genome/915) (Kim *et al.*, 2015). The clean reads of each sample were mapped to the reference genome sequence and further assembled based on it. Compared with the reference transcripts some unannotated transcripts were obtained and defined as new transcripts. The resulting read counts were converted to FPKM (expected number of Fragments Per Kilobase of transcript sequence per millions base pairs sequenced) values to analyze the gene expression level. According to DEGseq (Anders and Huber, 2010), the thresholds of $|\log2(\text{Fold change})| \geq 1$ and q-value < 0.05 were used to determine the DEGs for the reads from the gene expression analysis. The related DEGs were subjected to GO (Gene Ontology, http://www.geneontology.org/) functional enrichment and KEGG (Kyoto encyclopedia of genes and genomes) pathway enrichment. GOA tools (https://github.com/tanghaibao/goatools) is used to determine significantly enriched GO terms from the four groups of DEGs based on Fisher's exact test (uncorrected $p< 0.05$).

Real-time Quantitative PCR Validation

Total RNA was extracted using a Trizol kit (Invitrogen). For each sample, an amount of 1 μg total RNA was used for cDNA synthesis using a Prime Script kit (Takara Biotechnology, China).Further qPCR was performed on a LightCycler ® 480 Instrument II (Roche) using a LightCycler ® FastStart DNA Master PLUS SYBR Green I Kit (Roche). The cycling procedure was 95°C for 3 min, followed by 40 cycles of 94°C for 10 s, 59°C for 10 s and 72°C for 40 s. There were three biological replicates for each sample. The $2^{-\Delta\Delta}$Ct method was used to calculate the relative expression level of DEGs (Schmittgen and Livak, 2008).

Results

Transcriptome Alignment and Quality Assessment

After using HISAT2 to align the sequenced transcriptomic data with the available genome of *J. curcas*, the statistical results of mapping demonstrated alignment rates of higher than 96% for both JCn and JCw samples. It indicated that the JCn and JCw shared the same genome with the available genome of *J. curcas*, thus the subsequent analysis could be based on the reference genome. The quality control of raw reads of all samples was performed. The rate of base position error was below 0.014%, the average value of Q20 was 97.83%, the average value of Q30 was 94.06% and the average content of GC was around 43.41%. The saturation, coverage and redundancy of the sequencing were analyzed (Fig. 1). All samples exhibited high levels of saturation. Most of the genes with moderate expression (the genes with FPKM values of >3.5) were almost saturated when the mapped reads were around 40% (the vertical axis value was close to 1). This indicated that the overall saturation quality was high and the sequencing could cover most genes. The genetic coverage result of the overall samples exhibited that the sequencing results was not biased and provided uniform results. In addition, none of the redundancy figures demonstrated any peaks, indicating normal contents.

Correlation between Samples and PCA Analysis

RSEM (http://www.biomedsearch.com/nih/RESM-accurate-transcript-qunantification-from/21816040.html) is a maximum likelihood estimation model of abundance based on the expectation–maximization (EM) algorithm. Using the reads from both ends, the gene expression levels were calculated (Li and Dewey, 2011). Further the correlation clustering heat maps between all pairs of samples were produced and the PCA analysis was performed (Fig. 2). The results showed that there were strong correlations between the tissue samples of JCn and JCw when they were from the same parts, no outliers were found, and the samples shared high similarities. Further, the inflorescence buds from JCn were significantly different from those of JCw. The female flowers and the new leaves were clustered into one group, exhibiting similar overall gene expression patterns and slight differences between the female flowers and the new leaves in the two cultivars.

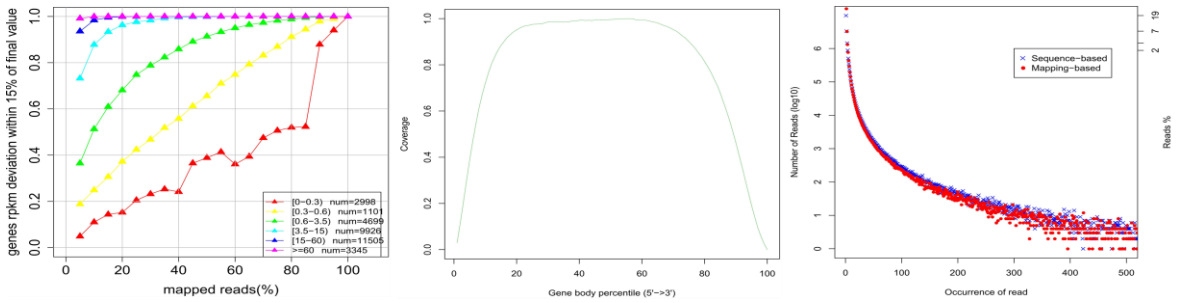

Fig. 1: Saturation, coverage and redundancy of the sequencing for some samples

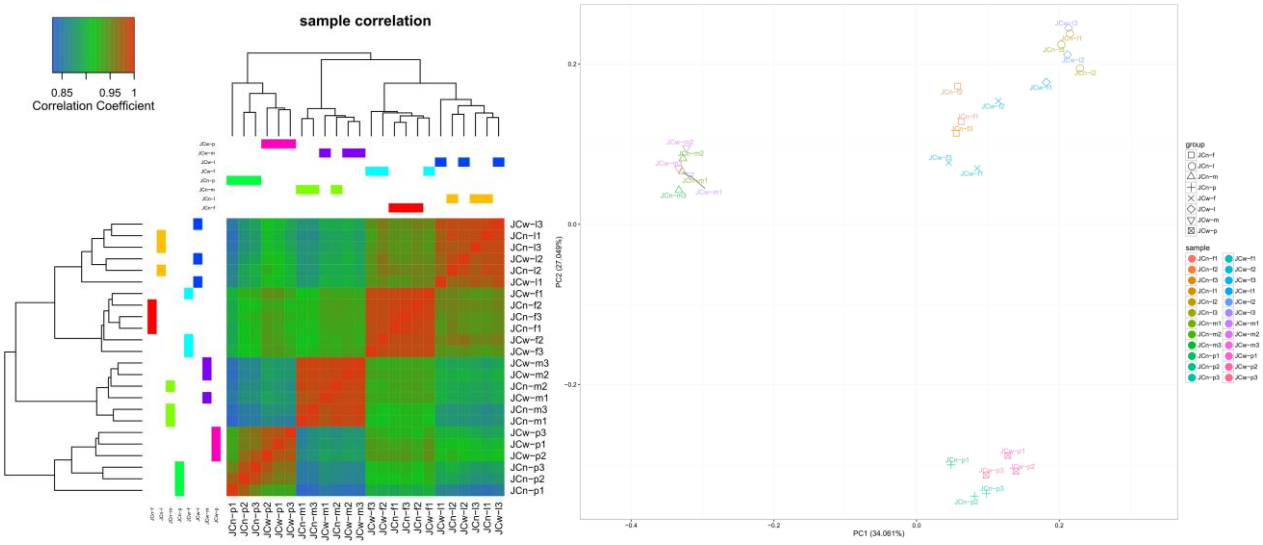

Fig. 2: Correlation clustering heat map of all pairs of samples. B: PCA analysis

Expression Analysis of Differential Genes

According to the differential gene screening thresholds, there were 1646 DEGs between sample JCn_p and JCw_p, with 1076 up-regulated genes and 570 down-regulated genes in JCw_p; 85 DEGs between JCn_m and JCw_m, with 31 up-regulated and 54 down-regulated genes in JCw_m; 231 DEGs between JCn_f and JCw_f, with 195 up-regulated and 36 down-regulated genes in JCw_f, and 75 DEGs between JCn_l and JCw_l, with 62 up-regulated and 13 down-regulated genes in JCw_l.

GO, KEGG Analysis of DEGs between the Two Cultivars

The enrichment analyses of DEGs regarding the samples from the same parts of the two cultivars were performed with a Venn diagram (Fig. 3A). Among them there were most specific DEGs between JCn_p and JCw_p, which was 1474. There were 10 DEGs standing out from the all pairs'. Further the DEGs were analyzed with h cluster and the trend line charts of the clusters were drawn (Fig. 3B). There were 1718 DEGs that were consistently expressed across all four samples from the two cultivars (Fig. 3B cluster1). There were nine DEGs (gene1736, gene3646, gene3969, gene16545, gene17100, gene17637, gene18164, gene20342, and gene20934) that were highly expressed in the male flowers from the two cultivars (Fig. 3B, cluster 6). And there were three DEGs (gene13819, gene18203, and gene24046) that were highly expressed in JCw samples (Fig. 3B cluster10). The trendline chart for the JCn regarding different parts (Fig. 3C cluster 2) showed that there were 171 DEGs that were highly expressed only in the male flowers. And this expression pattern in the male flower was generally consistent to the DEGs from different parts of JCn, which involved 183 genes (Fig. 3D cluster 2). While the DEGs that were only up-regulated in the female and male flowers demonstrated similar trends in both JCn (Fig. 3C cluster 7) and JCw (Fig. 3D cluster 6).

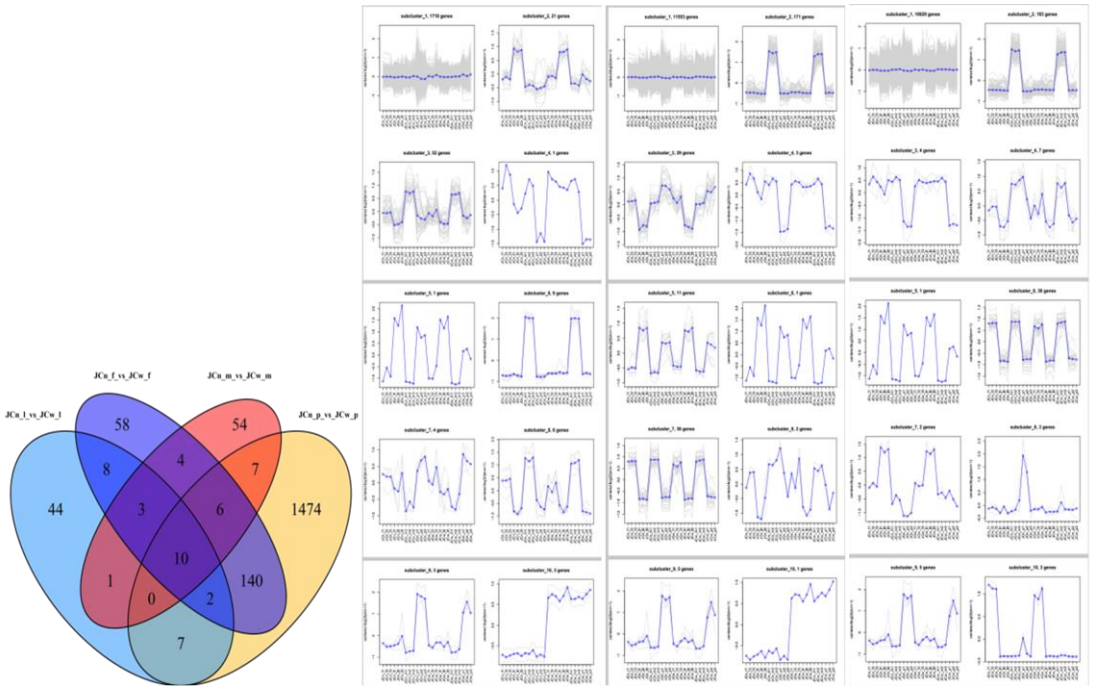

Fig. 3: Venn diagram of DEGs between the samples from the same parts of JCn and JCw; B: Trendline chart of the expression hcluster of DEGs from the same parts of the two cultivars (Cluster1-10); C: Trendline chart of the expression hcluster of DEGs from the different parts of JCn (Cluster1-10); D: Trendline chart of the expression hcluster of DEGs from the different parts of JCw (Cluster1-10)

New Leaves Related DEGs

The GO terms resulted from the GO enrichment analysis were visualized and color-coded based on their p-values. With respect to the comparison between JCn_l and JCw_l (Fig. 4), there were four genes involved in "response to stimulus" (GO: 0050896) and were up-regulated in JCw_l. Among them, three genes (gene2080, gene22291, and gene699) which were mapped to "response to red or far red light" (GO: 0009639) demonstrated significant differences.

Male and Female Flowers Related DEGs

As shown in Fig. 5A, the KEGG pathway enrichment of the DEGs from JCn_f and JCw_f demonstrated 18 enriched genes related to four metabolic pathways ($p<0.05$), which were plant hormone signal transduction, plant-pathogen interaction, pentose and glucuronate interconversions and galactose metabolism. Regarding the plant hormone signal transduction pathway, three GH3 genes (gene2529, gene7461, and gene14196), two SAUR genes (gene16489 and gene20105) and four TCH4 genes (gene10113, gene10112, gene10110, and gene10114) were all significantly up-regulated in JCw_f. With respect to the plant hormone signal transduction pathway, four CAM genes (gene749, gene22920, gene9629, and gene14095) were also significantly up-regulated in JCw_f. While the KEGG pathway enrichment of the DEGs from JCn_m and JCw_m exhibited 13 enriched genes related to eight metabolic pathways ($p<0.05$), mainly involving carotenoid biosynthesis, phenylalanine, tyrosine and tryptophan biosynthesis and alpha-linolenic acid metabolism pathways. The results are shown in Fig. 5B.

Inflorescence Buds Related DEGs

There were 276 genes enriched regarding JCn_p and JCw_p by KEGG pathway enrichment analysis (Fig. 6), involving 24 metabolic pathways including phenylpropanoid biosynthesis, pentose and glucuronate interconversions, cutin, suberine and wax biosynthesis, and plant hormone signal transduction ($p<0.05$).

With respect to the plant hormone signaling pathway (ko04075), there were 26 DEGs between JCn_p and JCw_p, including 21 up-regulated and 5 down-regulated genes in JCw_p. Regarding the gibberellin (GA) signaling pathway, the DELLA gene (gene13701) was significantly up-regulated in JCw_p. Regarding the jasmonic acid (jasmonate, JA) signaling pathway, the JAZ gene (gene6042) and MYC2 gene (gene11878) were significantly up-regulated in JCw_p. Regarding the brassinosteroid (BR) signaling pathway, five TCH4 genes (gene10110, gene10111, gene10112, gene10113, and gene10114) and one CYCD3 gene (gene38) were significantly up-regulated in JCw_p.

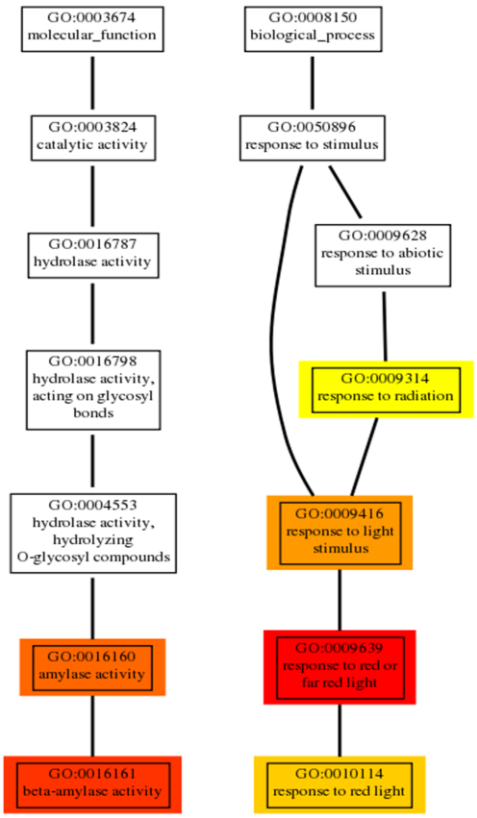

Fig. 4: Directed acyclic graph of the significant GO terms between the new leafs of the two cultivars

RT-qPCR Verification

To verify the acquired expression profile from RNA-Seq, five randomly selected DEGs were analyzed with real-time quantitative PCR (RT-qPCR). The five selected genes were CO, PG, DELLA, MYC2 and CYCD3, and the internal reference gene was actin (Zhang *et al.*, 2013). The specific primers were designed by Primer Premier 5.0 (Table 2). The comparison between the RT-qPCR results from the 24 samples from the two studied cultivars, the data were subjected to log-transformation and the transcriptome expression data showed that the expression pattern of the selected genes was consistent with the expression pattern from the transcriptome analysis (Fig. 7), confirming the reliability of the obtained transcriptome data in this study.

Discussion

In higher plants, flower bud differentiation process occurs via changes from the physiological and histological stage of the leaf bud to the flower bud and then develops into a flower embryo. Flower development can be divided into four stages. These are (1) phase transition from vegetative growth to reproductive growth, (2) formation of inflorescence meristems, (3) formation and identification of floral organs and (4) growth and maturation of floral organs (Okada and Shimura, 1994). Light not only provides energy for plants, but also could act as an environmental stimulus to regulate the growth and development of plants (Thomas *et al.*, 1997; Franklin and Whitelam, 2004). Phytochrome (PHY) and crytochrome (CRY) are two types of photoreceptors in plants. PHY regulate the flowering time by mediating the photoperiodic pathways (Yu *et al.*, 2010). EFL4 (EARLY FLOWERING 4), a gene regulated by PHY, was involved in the functions such as circadian clock and photoperiod sensing (Khanna *et al.*, 2003) and was up-regulated in JCn-1 (gene2080). As an E3 ubiquitin ligase, COP1 (CONSTITUTIVE PHOTOMORPHOGENIC 1) was involved in the ubiquitin-mediated protein degradation of many key photoreceptors. It was a downstream factor of the blue-light photoreceptor CRY, a negative regulator of photo controlled development and functioned in light signal transduction (Moon *et al.*, 2004). COP1 played a key role in stomatal opening and the stability of the negative regulation of constants (CO) (Liu *et al.*, 2008; Kang *et al.*, 2009; Chen *et al.*, 2012). COP1 (gene22422) was up-regulated and CO (gene4945) was down-regulated in JCw_l, the two of which demonstrated a negative correlation. The expression of CO was regulated by light, which provided the basis for the photoperiodic control of flowering (Suárez-López *et al.*, 2001). Red light reduced the accumulation of the CO protein and postponed the flowering (Valverde, 2011). In addition, elevated levels of PGR5 (PROTON GRADIENT REGULATION 5) cause a series of effects such as changes in electron transport, chloroplast defects, leaf development and decreases in chlorophyll content (Okegawa *et al.*, 2007), PRG5 (gene6855) was up-regulated in JCw_l. Therefore, it indicated that the morphological differences between the leaves of the two cultivars may affect the plant's response to red/far-red light and blue light, resulting in the difference in the photoperiodic pathways between *J. curcas* and *J. nigroviensrugosus* and the delayed flowering of *J. nigroviensrugosus*.

J. curcas is hermaphrodite, with male and female flowers in the same inflorescence. The male flowers are generated from unisexual flowers, most of which are born at the tips of the indeterminate inflorescences. However, the female flowers are generated from bisexual flowers, the majority of which are born at the tips of the cymose inflorescences. The stamen primordia cease growth during development, exhibiting as functionally unisexual flowers with degenerated stamen (Wu *et al.*, 2011). Among the DEGs who were only highly expressed in the male flowers (Fig. 3B Cluster 6), gene18164 and gene20342 belonged to polygalacturonase (PG) genes which were related to the physiological activities such as anther dehiscence and pollen maturation (González-Carranza *et al.*, 2007). And gene17100 and gene20934, as lipid-transfer protein (LTP) genes, were involved in the elongation of the pollen tube and the early stage

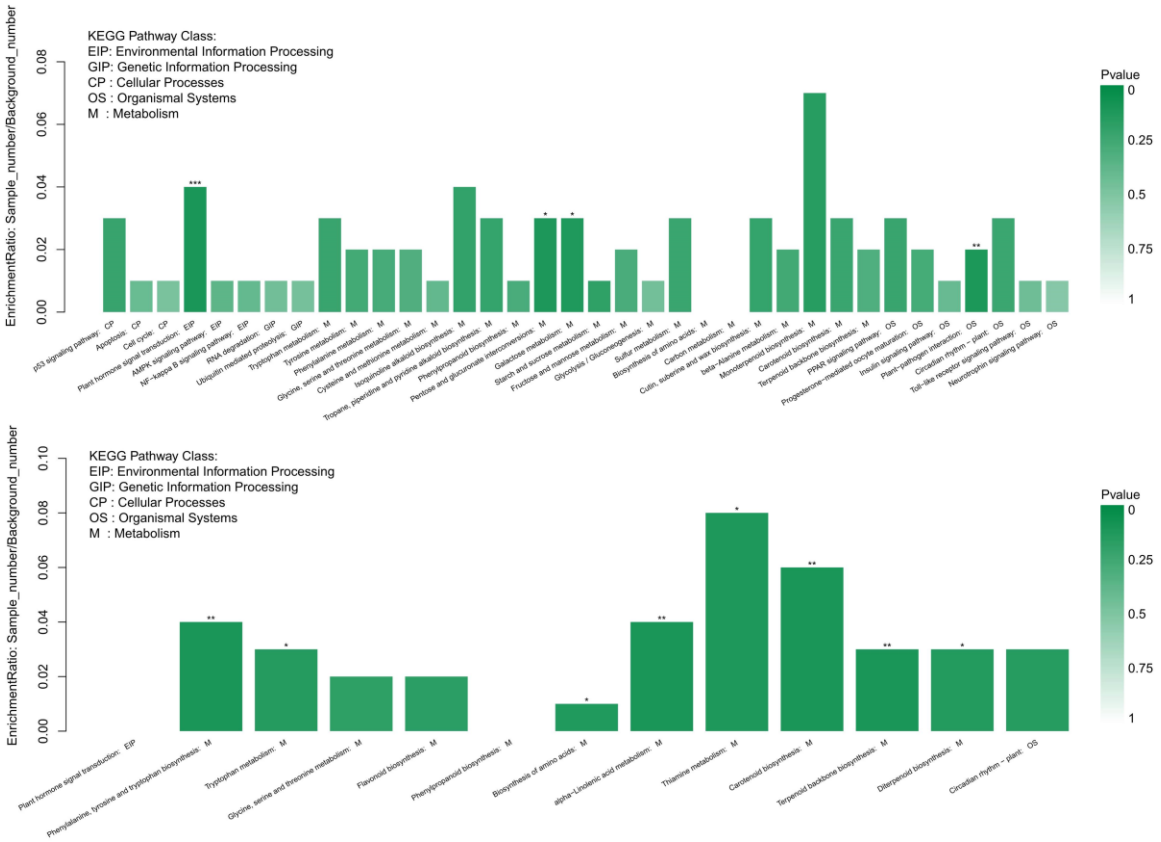

Fig. 5: KEGG pathway enrichment analysis of DEGs between the female flowers of the two cultivars; B: KEGG pathway enrichment analysis of DEGs between the male flowers of the two cultivars

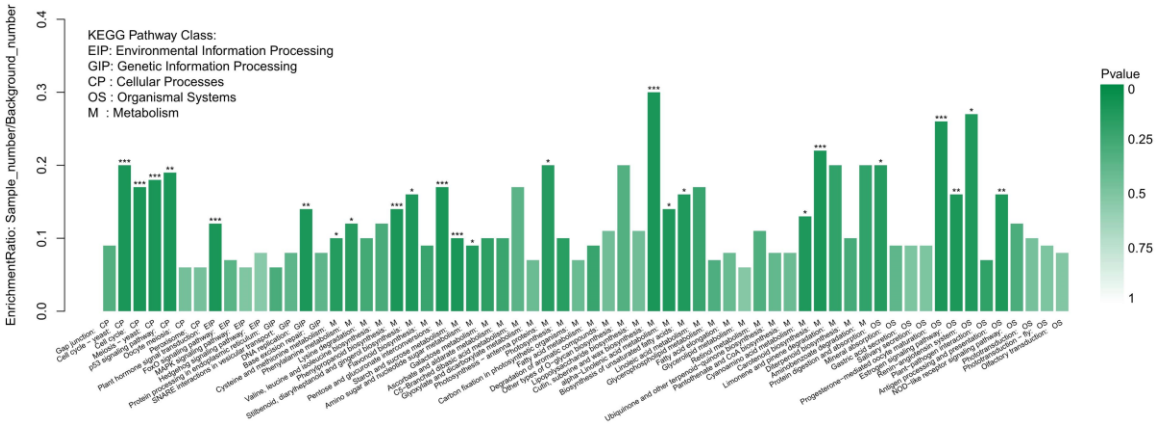

Fig. 6: KEGG pathway enrichment analysis of DEGs between the inflorescence buds of the two cultivars

development of pollen (Park *et al.*, 2000; Ariizumi *et al.*, 2002). While most of the genes with high expression in both the male and female floral organs (Fig. 3C Cluster 7 and Fig. 3D Cluster 6) were associated with MADS-box genes. For example, SEP1 (gene15032) and AGL6 (gene17327), as Category E genes, were involved in the floral organ formation and development (Pelaz *et al.*, 2000; Rijpkema *et al.*, 2009). In addition, it was found that three ncRNAs were highly expressed in all four parts of JCw which is significantly different from JCn (Fig. 3B Cluster 10). This may be related to the specific traits of *J. nigroviensrugosus*. Their related target genes and functions need to be further studied.

The low seed yield of *J. curcas* may be due to the excessive vegetative growth resulted from endogenous hormonal disposition during the competition on growth and

Table 2: The primers of the selected unigenes

Gene name	Primer name	Sequence(5' to 3')
Actin	Actin-F	CTCCTCTCAACCCCAAAGCCAA
	Actin-R	CACCAGAATCCAGCACGATACCA
CO	CO-F	TGGTCAAGGCCAAAACATGC
	CO-R	GACCTTTCACTCGCTGCCTT
PG	PG-F	CATGGTGCTAAGGCAGACGA
	PG-R	ACACACCCTTGCACCTCAAA
DELLA	DELLA-F	CCCGCCTAGAAACTCAGCAA
	DELLA-R	CTTCTAGCCGAGCTGATCCC
MYC2	MYC2-F	GCCTTCTTCTGGTGTGGTGA
	MYC2-R	ATTGCATCGCCAAGGAGTGA
CYCD3	CYCD3-F	ACACCACAAACCCTGACCAA
	CYCD3-R	ACGAGCAACAGAGAGGGAAG

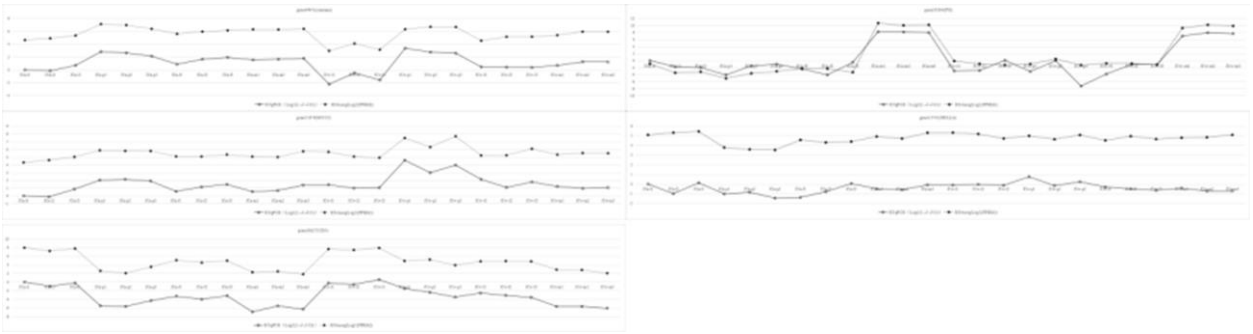

Fig. 7: Expression pattern of selected genes
"RNAseq(log_2(FPKM))" indicatesThe FPKM of the genes of the 24samples were calculated as the log2 value and are shown on the y-axis; "RTqPCR($log_2(2^{-\Delta\Delta}$Ct)" indicates the genes expression by RT-qPCR analysis were also calculated as the log2 value

development (Ghosh *et al.*, 2010). Plant hormones play an essential role in sex determination (Aryal and Ming, 2014). The formation of organ primordia and the differentiation of floral organs is thought to be mediated in part by the activities of different phytohormones (Wellmer *et al.*, 2013). There were also significant differences in plant hormonal signaling pathways in the inflorescence buds of the two cultivars. GAs are endogenous regulators of plant growth (Richards *et al.*, 2001). They regulated the leaf differentiation, light morphogenesis and pollen tube elongation (Fleet and Sun, 2005). By inhibiting DELLA, the constitutive repressor, they regulated the development of petal, stamen and anther (Cheng *et al.*, 2004). The expression of micro RNA159 (miR159) was negatively regulated by DELLA. The miR159 level might have interfered anther development and result in reduced flower fecundity (Achard *et al.*, 2004). The constitutive expression of bHLH48 and bHLH60 and two new DELLA interacting transcription factors have up-regulated the transcription of flowering locus T (FT) gene (Li *et al.*, 2017). The accumulation of DELLA protein could cause GA deficiency and result in male sterility, and JA deficiency might cause male sterility as well (Cheng *et al.*, 2009). In JCw_p, the expression of DELLA (gene13701) was up-regulated and this may be related to male sterile of *Jatropha*. Studies have shown that JA played an important role in plant reproduction, such as male sterility, sex determination and seed maturation (Yuan and Zhang, 2015). MYC2, as a transcriptional activator in JA signaling pathway, also played a significant role (Boter *et al.*, 2004). It was the main switch in plant hormonal signal transduction cascade (Kazan and Manners, 2013). It was also considered one of the dominant regulators in the sex determination of *Jatropha* (Chen *et al.*, 2017). JAS (gene6072) and MYC2 (gene11878) genes were both up-regulated in JCw. In the BR pathway, BR controlled both the pollen grain production and the filament elongation (Song *et al.*, 2013). CYCD3 plays a critical role in the switch from cell proliferation to the final stages of differentiation (Dewitte *et al*., 2003). The up-regulation of CYCD3 was thought to be correlated with an increase in inflorescence meristems (Pan *et al.*, 2014). Therefore, it is indicated that the plant hormones signal transduction pathways were widely involved in the sexual differentiation and the flower bud formation of the *Jatropha*, while DELLA, MYC2 and CYCD3 genes may be related to the stamen abortion and the sex determination of female flowers during the formation of the inflorescence buds in *J. nigroviensrugosus*.

Conclusion

There are significant differences in the leaf shape and the female to male flower ratio, however, the alignment rate of each of two *Jatropha* species with respect to the reference

genome is higher than 96%, exhibiting small sequence differences. The morphological differences between the leaf buds of the two cultivars affect the plant's response to red/far-red light and blue light, resulting in the difference in the photoperiodic pathways between *J. curcas* and *J. nigroviensrugosus* and the delayed flowering of *J. nigroviensrugosus*. The higher expression of genes DELLA, MYC2 and CYCD3 in inflorescence bud may be the reason that the higher female to male flower ratio in the *Jatropha nigroviensrugosus.*

Acknowledgements

The work was supported by National Natural Science Foundation of China-Study on flower bud differentiation and flowering mechanism of *Jatropha nigroviensrugosus* cv. Yang. (31360165).

References

Achard, P., A. Herr, D.C. Baulcombe and N.P. Harberd, 2004. Modulation of floral development by a gibberellin-regulated microRNA. *Development*, 131: 3357–3365

Achten, W.M., L.R. Nielsen, R. Aerts, A.G. Lengkeek, E.D. Kjær, A. Trabucco, J.K. Hansen, W.H. Maes, L. Graudal, F.K. Akinnifesi and B. Muys, 2010. Towards domestication of *Jatropha curcas*. *Biofuels*, 1: 91–107

Anders, S. and W. Huber, 2010. Differential expression analysis for sequence count data. *Genom. Biol*., 11: 106

Ariizumi, T., M. Amagai, D. Shibata, K. Hatakeyama, M. Watanabe and K. Toriyama, 2002. Comparative study of promoter activity of three anther-specific genes encoding lipid transfer protein, xyloglucan endotransglucosylase/hydrolase and polygalacturonase in transgenic *Arabidopsis thaliana*. *Plant Cell Rep*., 21: 90–96

Aryal, R. and R. Ming, 2014. Sex determination in flowering plants: papaya as a model system. *Plant Sci*., 217: 56–62

Boter, M., O. Ruíz-Rivero, A. Abdeen and S. Prat, 2004. Conserved MYC transcription factors play a key role in jasmonate signaling both in *tomato* and *Arabidopsis*. *Genes Dev.*, 18: 1577–1591

Chen, C., Y.G. Xiao, X. Li and M. Ni, 2012. Light-regulated stomatal aperture in *Arabidopsis*. *Mol. plant*, 5: 566–572

Chen, M.S., B.Z. Pan, Q. Fu, Y.B. Tao, J. Martínez-Herrera, L. Niu, J. Ni, Y.L. Dong, M.L. Zhao and Z.F. Xu, 2017. Comparative transcriptome analysis between gynoecious and monoecious plants identifies regulatory networks controlling sex determination in *Jatropha curcas*. *Front. Plant Sci.*, 7: 1953

Chen, M.S., B.Z. Pan, G.J. Wang, J. Ni, L. Niu and Z.F. Xu, 2014. Analysis of the transcriptional responses in inflorescence buds of *Jatropha curcas* exposed to cytokinin treatment. *BMC Plant Biol*., 14: 318

Cheng, H., S. Song, L. Xiao, H.M. Soo, Z. Cheng, D. Xie and J. Peng, 2009. Gibberellin acts through jasmonate to control the expression of MYB21, MYB24, and MYB57 to promote stamen filament growth in *Arabidopsis*. *PLoS Genet*., 5: e1000440

Cheng, H., L. Qin, S. Lee, X. Fu, D.E. Richards, D. Luo, N.P. Harberd and J. Peng, 2004. Gibberellin regulates *Arabidopsis* floral development via suppression of DELLA protein function. *Development*, 131: 1055–1064

Cock, P.J., C.J. Fields, N. Goto, M.L. Heuer and P.M. Rice, 2009. The Sanger FASTQ file format for sequences with quality scores, and the Solexa/Illumina FASTQ variants. *Nucl. Acids Res.*, 38: 1767–1771

Dewitte, W., C. Riou-Khamlichi, S. Scofield, J.S. Healy, A. Jacqmard, N.J. Kilby and J.A. Murray, 2003. Altered cell cycle distribution, hyperplasia, and inhibited differentiation in *Arabidopsis* caused by the D-type cyclin CYCD3. *Plant Cell*, 15: 79–92

Fairless, D., 2007. Biofuel: the little shrub that could-maybe. *Nat. News*, 449: 652–655

Fleet, C.M. and T.P. Sun, 2005. A DELLAcate balance: the role of gibberellin in plant morphogenesis. *Curr. Opin. Plant Biol*., 8: 77–85

Franklin, K.A. and G.C. Whitelam, 2004. Light signals, phytochromes and cross-talk with other environmental cues. *J. Exp. Bot*., 55: 271–276

Fresnedo-Ramírez, J., 2013. The floral biology of *Jatropha curcas* L.-a review. Trop. *Plant Biol*., 6: 1–15

Fröschle, M., H. Horn and O. Spring, 2017. Effects of the cytokinins 6-benzyladenine and forchlorfenuron on fruit-, seed-and yield parameters according to developmental stages of flowers of the biofuel plant *Jatropha curcas* L. (Euphorbiaceae). *Plant Growth Regul*., 81: 293–303

Gangwar, M., H. Sood and R.S. Chauhan, 2016. Genomics and relative expression analysis identifies key genes associated with high female to male flower ratio in *Jatropha curcas* L. *Mol. Biol. Rep*., 43: 305–322

Ghosh, A., J. Chikara, D.R. Chaudhary, A.R. Prakash, G. Boricha and A. Zala, 2010. Paclobutrazol arrests vegetative growth and unveils unexpressed yield potential of *Jatropha curcas*. *J. Plant Growth Regul*., 29: 307–315

González-Carranza, Z.H., K.A. Elliott and J.A. Roberts, 2007. Expression of polygalacturonases and evidence to support their role during cell separation processes in *Arabidopsis thaliana*. *J. Exp. Bot*., 58: 3719–3730.

He, H., X.R. Wang and Z.J. Hu, 2016. Nutritional transport and photosynthetic characteristics of Guizhou *Jatropha nigroviensrugosus* and ordinary *Jatropha curcas*. *J. West Chin. For. Sci*., 45: 79–83

Hui, W., Y. Yang, G. Wu, C. Peng, X. Chen and M.Z. Zayed, 2017. Transcriptome profile analysis reveals the regulation mechanism of floral sex differentiation in *Jatropha curcas* L. *Sci. Rep.*, 7: 16421

Joshi, G., A. Shukla and A. Shukla, 2011. Synergistic response of auxin and ethylene on physiology of *Jatropha curcas* L. *Braz. J. Plant Physiol*., 23: 66–77

Junqueira, V.S., L. de Azevedo Peixoto, B.G. Laviola, L.L. Bhering, S. Mendonça, T.D.S.A. Costa and R. Antoniassi, 2016. Bayesian Multi-Trait Analysis Reveals a Useful Tool to Increase Oil Concentration and to Decrease Toxicity in *Jatropha curcas* L. *PloS One*, 11: e0157038

Kang, C.Y., H.L. Lian, F.F. Wang, J.R. Huang and H.Q. Yang, 2009. Cryptochromes, phytochromes, and COP1 regulate light-controlled stomatal development in *Arabidopsis*. *Plant Cell*, 21: 2624–2641

Kant, P. and S. Wu, 2011. The extraordinary collapse of *Jatropha* as a global biofuel. *ACS Public.*, 2011: 7114–7115

Kazan, K. and J.M. Manners, 2013. MYC2: the master in action. *Mol. Plant*, 6: 686–703

Khanna, R., E.A. Kikis and P.H. Quail, 2003. EARLY FLOWERING 4 functions in phytochrome B-regulated seedling de-etiolation. *Plant Physiol*., 133: 1530–1538

Kim, D., B. Langmead and S.L. Salzberg, 2015. HISAT: a fast spliced aligner with low memory requirements. *Nat. Methods*, 12: 357–360

Kumar, S., B.S. Chaitanya, S. Ghatty and A.R. Reddy, 2014. Growth, reproductive phenology and yield responses of a potential biofuel plant, *Jatropha curcas* grown under projected 2050 levels of elevated CO2. *Physiol. Plant.*, 152: 501–519

Li, B. and C.N. Dewey, 2011. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC Bioinform.*, 12: 323

Li, Y., H. Wang, X. Li, G. Liang and D. Yu, 2017. Two DELLA-interacting proteins bHLH48 and bHLH60 regulate flowering under long-day conditions in *Arabidopsis thaliana*. *J. Exp. Bot.*, 68: 2757–2767

Liu, F., K. Li, X. Wang, C. Zhang, C. Luo, Y. Cui and H. Peng, 2010. Sex-differentiation and number of female flowers for polygamous inflorescences of *Jatropha curcas* in two drainage basins. *J. Zhejiang For. College*, 27: 684–690

Liu, L.J., Y.C. Zhang, Q.H. Li, Y. Sang, J. Mao, H.L. Lian, L. Wang and H.Q. Yang, 2008. COP1-mediated ubiquitination of CONSTANS is implicated in cryptochrome regulation of flowering in *Arabidopsis*. *Plant Cell*, 20: 292–306

Montes, J.M. and A.E. Melchinger, 2016. Domestication and Breeding of *Jatropha curcas* L. *Trends Plant Sci*., 21: 1045–1057

Moon, J., G. Parry and M. Estelle, 2004. The ubiquitin-proteasome pathway and plant development. *Plant Cell*, 16: 3181–3195

Okada, K. and Y. Shimura, 1994. Genetic analyses of signalling in flower development using *Arabidopsis*. *Plant Mol. Biol*., 26: 1357–1377

Okegawa, Y., T.A. Long, M. Iwano, S. Takayama, Y. Kobayashi, S.F. Covert and T. Shikanai, 2007. A balanced PGR5 level is required for chloroplast development and optimum operation of cyclic electron transport around photosystem I. *Plant Cell Physiol*., 48: 1462–1471

Openshaw, K., 2000. A review of *Jatropha curcas*: an oil plant of unfulfilled promise. *Biomass Bioenerg*., 19: 1–15

Pan, B.Z., M.S. Chen, J. Ni and Z.F. Xu, 2014. Transcriptome of the inflorescence meristems of the biofuel plant *Jatropha curcas* treated with cytokinin. *BMC Genom.*, 15: 974

Park, S.Y., G.Y. Jauh, J.C. Mollet, K.J. Eckard, E.A. Nothnagel, L.L. Walling and E.M. Lord, 2000. A lipid transfer–like protein is necessary for lily pollen tube adhesion to an in vitro stylar matrix. *Plant Cell*, 12: 151–163

Pelaz, S., G.S. Ditta, E. Baumann, E. Wisman and M.F. Yanofsky, 2000. B and C floral organ identity functions require SEPALLATA MADS-box genes. *Nature*, 405: 200–203

Raju, A.S. and V. Ezradanam, 2002. Pollination ecology and fruiting behaviour in a monoecious species, *Jatropha curcas* L. (Euphorbiaceae). *Curr. Sci*., 2002: 1395–1398

Richards, D.E., K.E. King, T. Ait-ali and N.P. Harberd, 2001. How gibberellin regulates plant growth and development: a molecular genetic analysis of gibberellin signaling. *Annu. Rev. Plant Biol*., 52: 67–88

Rijpkema, A.S., J. Zethof, T. Gerats and M. Vandenbussche, 2009. The petunia AGL6 gene has a SEPALLATA‐like function in floral patterning. *Plant J*., 60: 1–9

Sato, S., H. Hirakawa, S. Isobe, E. Fukai, A. Watanabe, M. Kato, K. kawashima, C. Minami, A. Muraki, N.N.C. Takahashi, S. Nakayama, S. Tabata, T. Aizu, A. Toyoda, T. Shin-i, Y. Minakuchi, Y. Kohara, A. Fujiyama, S. Tsuchimoto, S. Kajiyama, E. Makigano, N. Ohmido, N. Shibagaki, J.A. Cartagena, N. Wada, T. Kohinata, A. Atefeh, S. Yuasa, S. Matsunaga and K. Fukui, 2010. Sequence analysis of the genome of an oil-bearing tree, *Jatropha curcas* L. *DNA Res*., 18: 65–76

Schmittgen, T.D. and K.J. Livak, 2008. Analyzing real-time PCR data by the comparative CT method. *Nat. Protoc*., 3: 1101–1108

Song, S., T. Qi, H. Huang and D. Xie, 2013. Regulation of stamen development by coordinated actions of jasmonate, auxin, and gibberellin in *Arabidopsis*. *Mol. Plant*, 6: 1065–1073

Suárez-López, P., K. Wheatley, F. Robson, H. Onouchi, F. Valverde and G. Coupland, 2001. CONSTANS mediates between the circadian clock and the control of flowering in *Arabidopsis*. *Nature*, 410: 1116–1120

Thomas, T.H., P.D. Hare and J. Van Staden, 1997. Phytochrome and cytokinin responses. *Plant Growth Regul*., 23: 105–122

Valverde, F., 2011. CONSTANS and the evolutionary origin of photoperiodic timing of flowering. *J. Exp. Bot.*, 62: 2453–2463

Wang, X.R., G.J. Ding, L.Z. Liu and P. Li, 2012a. Flowering behavior and spatial-temporal distribution characteristics of *Jatropha curcas* in Luodian. *J. Zhejiang A&F Univ*., 29: 388–393

Wang, X.R., G.J. Ding, Y. Xie and L. Han, 2012b. Nutrition distributing in different organs of *Jatropha curcas*. *J. Central South Univ. For. Technol.*, 32: 26–31

Wang, Z., M. Gerstein and M. Snyder, 2009. RNA-Seq: a revolutionary tool for transcriptomics. *Nat. Rev. Genet.*, 10: 57

Wellmer, F., E. Graciet and J.L. Riechmann, 2013. Specification of floral organs in *Arabidopsis*. *J. Exp. Bot.*, 65: 1–9

Wijaya, A., M.U. Harun and H. Hawalid, 2009. Flower characteristics and the yield of Jatropha (*Jatropha curcas* L.) accessions. *HAYATI J. Biosci*., 16: 123–126

Wu, J., Y. Liu, L. Tang, F. Zhang and F. Chen, 2011. A study on structural features in early flower development of *Jatropha curcas* L. and the classification of its inflorescences. *Afr. J. Agric. Res.*, 6: 275–284

Xu, G., R. Luo and Y. Yao, 2013. Paclobutrazol improved the reproductive growth and the quality of seed oil of *Jatropha curcas*. *J. Plant Growth Regul*., 32: 875–883

Yang, C.Y., 2013. *Phenotypic Characteristics and Genetic Stability of New Varieties of Jatropha curcas*. Botany in Ecological Civilization: Present and Future - The Fifteenth Congress of Chinese Society of Botany and the 80th Academic Year Symposium - Session 4 Session: *Resour. Bot.*, 2013. (http://cpfd.cnki.com.cn/Article/CPFDTOTAL-ZGZO201310005036.htm)

Yang, C.Y., W. Lu, X. Wu, Z. Xu and Y. Long, 2015. Analysis on Components of Fatty Acids from New *Jatropha* Cultivars Seed Oils and Their Potential of Preparing Biodiesel. *Bot. Res.*, 4: 16–24

Yang, C.Y., Z. Fang, B. Li and Y.F. Long, 2012. Review and prospects of *Jatropha* biodiesel industry in China. *Renew. Sustain. Energ. Rev.*, 16: 2178–2190

Yu, X., H. Liu, J. Klejnot and C. Lin, 2010. The cryptochrome blue light receptors. *Arabidopsis Book*, 8: 0135

Yuan, Z. and D. Zhang, 2015. Roles of jasmonate signalling in plant inflorescence and flower development. *Curr. Opin. Plant Biol*., 27: 44–51

Zhang, L., L.L. He, Q.T. Fu and Z.F. Xu, 2013. Selection of reliable reference genes for gene expression studies in the biofuel plant *Jatropha curcas* using real-time quantitative PCR. *Int. J. Mol. Sci.*, 14: 24338–24354

(Received 26 January 2018; Accepted 05 march 2018)