Comparative transcriptomics reveals a reduction in carbon capture and flux between source and sink in cytokinin-treated inflorescences of *Jatropha curcas* L.

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Abstract

The low seed yield of *Jatropha curcas* has been a stumbling block in realizing its full potential as an ideal bioenergy crop. Low female to male flower ratio is considered as a major limiting factor responsible for low seed yield in *Jatropha*. An exogenous cytokinin application was performed on floral meristems to increase the seed yield. This resulted in an increase of total flowers count with a higher female to male flower ratio. However, the seed biomass did not increase in the same proportion. The possible reason for this was hypothesized to be the lack of increased photosynthesis efficiency at source tissues which could fulfil the increased demand of photosynthates and primary metabolites in maturing seeds. After cytokinin application, possible molecular mechanisms underlying carbon capture and flux affected between the source and sink in developing flowers, fruits and seeds were investigated. Comparative transcriptome analysis was performed on inflorescence meristems (treated with cytokinin) and control (untreated inflorescence meristems) at time intervals of 15 and 30 days, respectively. KEGG-based functional annotation identified various metabolic pathways associated with carbon capture and flux. Pathways such as photosynthesis, carbon fixation, carbohydrate metabolism and nitrogen metabolism were upregulated after 15 days of cytokinin treatment; however, those were downregulated after 30 days. Five genes *FBP*, *SBP*, *GS*, *GDH* and *AGPase* showed significant increase in transcript abundance after 15 days of treatment but showed a significant decrease after 30 days. These genes, after functional validation, can be suitable targets in designing a suitable genetic intervention strategy to increase overall seed yield in *Jatropha*.

Keywords *Jatropha curcas* · Transcriptome · Carbon flux · Starch · Yield

Abbreviations

BA Benzyl adenine
BLAST Basic local alignment search tool

CDS	Coding sequence
CKX	Cytokinin oxidase
C/N	Carbon/nitrogen
FPKM	Fragment per kilo base per million mapped reads
GO	Gene ontology
IPT	Isopentenyl transferase
KEGG	Kyoto encyclopedia of genes and genomes
KAAS	KEGG automatic annotation server
TR15	Inflorescence after 15 days of cytokinin treatment
TR30	Inflorescence after 30 days of cytokinin treatment
VS	Vegetative stage

Manali Gangwar and Archit Sood contributed equally to this work.

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Introduction

Today's rising demand for fuels has diverted the attention towards biofuels from bioenergy plants and algae. *Jatropha curcas* L. (Jatropha), a potential bioenergy plant with high seed oil content (45–50%), can be grown in extreme conditions such as in tropical savannah and monsoon climates, temperate and semi-arid climates (Maes et al. 2009). Jatropha, primarily originating from Central America, has been introduced into many tropical and subtropical countries in Asia and Africa (Akbar et al. 2009; Sood and Chauhan 2015).

One of the major concerns with its commercialization has been low seed yield, majorly depending on the number of female flowers per inflorescence and the number of branches per plant. About 300 flowers are formed at each inflorescence, of which only 10–12 female flowers further result in 8–10 ovoid fruits (Kochhar et al. 2008). Increase in flower number, sex alteration and subsequently fruit number has been achieved successively by exogenous application of growth hormones such as cytokinins and gibberellic acids, etc. (Pan and Xu 2011; Gayakwad et al. 2014). Different strategies such as comparative genomics and transcriptome profiling have also been used to capture genes associated with female flowering (Pan et al. 2014; Gangwar et al. 2016). Recently, comparative transcriptome analysis for sex differentiation stages has identified a plethora of genes differentiating the development pattern of staminate and instaminate flowers in *J. curcas* (Xu et al. 2016).

Cytokinins have proven to be the most promising growth regulator in increasing the total flower number and seeds. Benzyladenine (BA) and thidiazuron treatment resulted in a drastic increase in number of flowers and female flowers along with induced bisexual flowers in *J. curcas* (Pan and Xu 2011; Pan et al. 2016). However, the fruiting rate was relatively low, i.e. 2–3 folds compared to increased female flowers with up to 9–10 folds. Also, there was a reduction in 100 seed weight in fruits formed after cytokinin treatment (Pan and Xu 2011; Chen et al. 2014). This reduction in seed weight led to compromised seed yield even after the application of phytohormones such as cytokinins. Apart from Jatropha, reduction in seed yield after cytokinin application has also been reported in other plant species such as soybean, jojoba and lupin (Ma et al. 1998; Nagel et al. 2001; Prat et al. 2008). However, the reasons for reduction in seed yield have not been investigated. We hypothesize that the compromised seed yield could be a consequence of the inability of the photosynthetic source to fully support sink, i.e. seed development in spite of having significantly increased number of flowers. The reduction in final seed formation is also due to

abortion of floral buds in cytokinin-treated plants at later stages of development because all female flowers do not transit to formation of fruits due to higher flower abortion rate, which may be due to non-fulfillment of increased requirement of sink (Pan et al. 2016; Yashima et al. 2005). Limited supply of photo assimilates to increased flowers is due to increased competition among leaves, stems, nodules and reproductive organs which might be the possible reasons for flower abortion (Brun and Betts 1984; Antos and Wiebold 1984). Also, the negative correlation between fruiting rate and seed formation with the number of female and bisexual flowers per inflorescence on plants treated with cytokinins, may be a result of either the shortage of photosynthetic products or reduced source to sink strength (Pan and Xu 2011; Pan et al. 2016).

Apart from exogenous cytokinin treatment, increase in endogenous cytokinin level either by enhancing the cytokinin biosynthetic machinery or reducing the rate of cytokinin degradation has also been attempted in various plant species to increase the overall seed yield. Isopentenyl transferases (IPTs), the genes involved in cytokinin biosynthesis have been overexpressed to increase seed number, weight and thus overall yield in many plants (Peleg et al. 2011; Qin et al. 2011; Kant et al. 2015). Also, genes involved in cytokinin degradation, i.e. cytokinin oxidases (*CKXs*) have been downregulated to increase seed number and size which in turn enhanced seed yield in important food plants such as rice, wheat, maize, and cotton (Zalewski et al. 2010; Yeh et al. 2015; Zhao et al. 2015).

Various studies focusing on understanding molecular mechanisms associated with seed yield in *J. curcas* has been carried out in the recent past. Transcriptome profiling of different stages of stamen and carpel development identified pathways associated with sex differentiation. Genes associated with male and female flower development and involvement of various hormonal pathways at different stages of organ development have been deciphered (Xu et al. 2016). Differential transcriptional profiling of *J. curcas* inflorescence after cytokinin treatment at different intervals revealed the association of molecular cues for enhanced flower number and promotion of female flowering as well as induced bisexual flowers (Pan et al. 2014). Also, the effect of cytokinin application on phytohormones metabolism and signaling regulating the flower development has been depicted (Chen et al. 2014). However, molecular mechanisms underlying role of carbon flux towards increased fruit/seed biomass and overall yield, in response to exogenous phytohormone application, is not known in *J. curcas*. Also, no information exists on molecular signals associated with compromised seed yield following exogenous hormonal application in *J. curcas*.

The present study was, therefore, conducted to study the effect of cytokinins on physiological, biochemical and

molecular precursors affecting carbon flux from source to sink and establish a correlation with the floral biomass and final seed yield. Comparative transcriptome analysis of *Jatropha* inflorescence meristems treated with benzyladenine (BA) at different time intervals was performed, following reference-based genome mapping approach. The study provided insights on how cytokinin treatment affects carbon fixation, carbon availability and nitrogen metabolism, thereby altering C/N ratio, which might be affecting biomass production, thus fruit/seed yield in *J. curcas*. Also, molecular cues linked with vegetative to reproductive phase transition and that of promoting female flowering have been investigated. It was observed that various metabolism-associated processes such as photosynthesis, Calvin cycle, oxidative phosphorylation (energy metabolism), and carbohydrate and nitrogen metabolism were upregulated in response to cytokinin treatment after 15 days and then decreased after 30 days of cytokinin treatment.

Materials and methods

Plant material and benzyl adenine treatment

Seven years old, seed-propagated plants of *J. curcas* (accession IC561235) at the experimental farm of Himalayan Forest Research Institute at Sultanpur (altitude 1400 m asl), HP, India, were treated with cytokinin. The shoot apices with and without inflorescence treated with BA (160 mg/l) were collected. Shoot apical meristem tissues were frozen immediately in liquid nitrogen, and stored at -80°C until use. For cytokinin treatment, 18–20 inflorescences of five plants each were sprayed with BA (160 mg/l). Working solution (0.5 mM) of BA is prepared by adding Tween-20 (0.05% v/v). It was sprayed on each inflorescence meristem and the surrounding leaves by hand sprayer. Control inflorescence meristems (3–4) were sprayed with distilled water containing 0.05% (v/v) Tween-20. Spraying was done thrice at 2 days interval. Shoot apices with inflorescence were collected from CONTROL (untreated), TR15 (15 days of BA treatment) and TR30 (30 days of BA treatment). For identification of floral transition genes vegetative stage (VS), i.e. shoot apices (no inflorescence developed) which have not been primed to form inflorescence buds were collected (Fig. 1).

Isolation of total RNA

Total RNA was isolated from shoot apices of four stages (vegetative, control, TR15 and TR30), each in two biological replicates using RaFlex Total RNA isolation Kit (Genei) as per manufacturer's instruction. The quality and integrity of total RNA was checked on 1% denatured agarose gel. Total RNA was quantified using Qubit RNA BR kit (fluorometer).

Illumina NextSeq library preparation and sequencing

Transcriptomes were outsourced and generated at Xcelris Genomics, Ahmedabad, India (<http://www.xcelrisgenomics.com>) as per the following protocol: paired-end cDNA sequencing libraries were prepared in duplicates using Illumina TruSeq stranded Total RNA Library Preparation Kit as per the protocol described (Illumina). The fragmented RNA depleted of rRNA was used for first-strand and second-strand cDNA synthesis. The paired-end adaptor ligated fragments were used for PCR amplification. Qubit dsDNA HS kit and High Sensitivity Assay Kits were used for library quantification and validation, respectively.

Data generation

The raw reads generated on NextSeq of *Jatropha* tissue samples were filtered using trimmomatic (v 0.30) with quality value QV > 20. Parameters such as adapter trimming, sliding window, leading, trailing and minimum length (40 bp) were used for filtration. About 57,437 coding sequences (CDS) were present in reference genome of *J. curcas* (<ftp.kazusa.or.jp/pub/Jatropha>). The Illumina NextSeq transcriptome data for three *Jatropha* plant samples (CONTROL, TR15 and TR30) were mapped separately to the reference genome using default parameters of BWA version 0.7.5a (<http://bio-bwa.sourceforge.net/>) (Fig. 2).

Differential gene expression

Transcript abundance of CDS mapped to *Jatropha* genome was calculated in terms of FPKM (Fragment per kilobase per million mapped reads) with DESeq package. FPKM value was calculated for each gene with a combination of control and treated samples. Log-fold change [$\log_2(\text{FPKM}_{\text{Treated}}/\text{FPKM}_{\text{Control}})$] change was determined for commonly expressed genes reported between control and treated samples. Differentially expressed genes (DEG) were screened with p value < 0.05 and absolute $\log_2$ -fold ($\log_2$ -fold value > 0.5 for upregulation and $\log_2$ fold value < 0.5 downregulation). Genes were further categorized as upregulated genes and downregulated genes based on their transcript abundance in the treated sample compared to control sample.

Heat map analysis

Heat map of top 100 differentially expressed genes was constructed for hierarchical clustering by multiple experiment viewer (MEV v4.9). Two parameters, i.e. $\log_2$ ratio and normalized value of genes were considered. Pearson's uncentered correlation distance and the complete linkage

Fig. 1 *Jatropha curcas* tissues used for transcriptome analysis. **a** Vegetative stage (VS): shoot apex without floral buds. **b** CONTROL: shoot inflorescence meristem without BA application. **c** TR15: inflorescence meristem after 15 days of BA treatment, with increased flo-

ral buds (biomass). **d** TR30: inflorescence meristem after 30 days of BA treatment, with less number of floral buds (biomass) compared to TR15

method was used for normalized value of genes. Differentially expressed genes identified in vegetative vs control and control vs experimental conditions were analyzed by hierarchical clustering representing transcript abundance (Fig. 3).

Gene ontology (GO) analysis

GO assignments were used to classify the functions of predicted CDS. GO mapping provides the ontology of gene product properties. These properties are grouped into three main classes: biological process, cellular component and molecular function. BLAST2GO program was used with default parameters to execute GO annotation of the CDS

in all four tissues. Genes were further analyzed for mapping and annotation and finally mapping into main GO categories.

Pathway analysis

Functional annotation of CDS for CONTROL, TR15 and TR30 samples was done using KAAS. KAAS annotate pathways by BLAST comparisons against KEGG GENES database by BBH (Bi-directional best hit). KEGG Orthology database (<http://www.genome.jp/kegg/ko.html>) was applied for pathway mapping.

Fig. 2 Workflow depicting methodology followed for transcriptome generation and differential gene expression analysis

Gene expression validation through RT-qPCR

The expression of 31 selected genes was measured via RT-qPCR to validate the transcript abundance. First-strand cDNA synthesis was done using Verso cDNA synthesis kit (Thermo Scientific, USA) from total RNA (2 µg) as per manufacturer's instructions, in two biological replicates.

With gene-specific primers (Supplementary Table 1), RT-qPCR was performed on CFX96 system (Bio-Rad, USA) with iScript One-Step RT PCR kit (Bio-Rad, USA). The PCR protocol started with denaturation at 94 °C for 5 min followed by 40 cycles of denaturation at 94 °C for 20 s and then annealing at 50–55 °C for 30 s followed by one elongation step of 20 s at 72 °C. 26S rRNA and actin genes were

Fig. 3 Heat map representing top 100 differentially expressed genes. **a** Vegetative vs control, **b** control vs TR15 and TR30 (MultiRankSeq differentially expressed CDS based on DESeq 2 rank ≤ 200)

used as an internal reference for data normalization. All quantitative PCR experiments were performed in triplicates and were repeated two times.

Results and discussion

Transcriptome sequencing and data generation

The libraries were sequenced on NextSeq 500 platform of Illumina using 2x150 PE Chemistry. The high-quality reads (QV > 20) were used for the RNA-Seq analysis. A total of 11,608,257, 12,184,924, 11,811,964 and 10,592,622 high-quality reads were obtained in VS, CONTROL, TR15 and TR30, respectively (Table 1). High-quality reads were mapped on to the reference genome using mapping software BWA with optimized parameters. The transcriptome data generated have been uploaded at <http://14.139.240.55/NGS/download.php> for access to public and other users.

Differential gene expression analysis

The expression analysis of genes was carried out with R package DESeq and was calculated in terms of FPKM (Fragment per kilobase per million mapped reads). Upon annotation, it was observed that a total of 55,755 transcripts associated with multiple pathways were expressed in CONTROL, TR15 and TR30 based on FPKM values. 253, 510 and 444 transcripts were expressed uniquely in CONTROL, TR15 and TR30, respectively. 1350 and 1236 transcripts

were upregulated and downregulated, respectively, in TR15 compared to CONTROL. 3192 and 375 transcripts were upregulated and downregulated, respectively, in TR15 compared to TR30. In VS vs CONTROL tissues, about 413 and 419 transcripts were uniquely expressed, whereas 3486 and 191 transcripts were upregulated and downregulated, respectively, in CONTROL (Fig. 4).

Pathway analysis

KAAS (KEGG Automatic Annotation Server) was used for functional annotation in CONTROL, TR15 and TR30 tissues with BLAST comparisons against KEGG GENES database. KEGG orthology database was used for pathway mapping. Upon KEGG annotation, it was observed that different pathways from carbon fixation and flow to C/N ratio were upregulated by cytokinin treatment. This was revealed by the increased expression of the genes associated with these pathways in TR15 compared to CONTROL. However, genes associated with these pathways were downregulated after 30 days of cytokinin treatment (TR30) compared to CONTROL and TR15. Out of these, majority of genes were mapped to pathways such as photosynthesis, Calvin cycle, oxidative phosphorylation, carbohydrate metabolism, nitrogen metabolism and glycolysis which implied that these were the main pathways that were upregulated in TR15 and downregulated in TR30 compared to CONTROL.

Gene ontology (GO) mapping and CDS distribution

The GO analysis revealed that majority of assignments were in category ‘biological process’ (15,834 in CONTROL, 12,752 in TR15 and 15,584 in TR30) followed by ‘molecular function’ (12,073 in CONTROL, 11,945 in TR15 and 14,423 in TR30) and ‘cellular component’ (3592 in CONTROL, 2380 in TR15 and 3218 in TR30) categories in CONTROL, TR15 and TR30 (Fig. 5). In biological process category, metabolic process, cellular process, single-organism process, localization, biological regulation and response to stimulus were highly represented. Binding, catalytic activity, transporter activity, nucleic acid-binding transcription factor

Table 1 Statistics of transcriptome datasets

Sample	Number of reads	Number of high-quality reads
Vegetative	22,735,557	11,608,257
Control	22,707,846	12,184,924
TR15	22,988,570	11,811,964
TR30	20,562,981	10,592,622

Fig. 4 Distribution of genes expressed **a** vegetative stage (VS) vs CONTROL, **b** CONTROL vs TR15, **c** TR15 vs TR30

Fig. 5 GO classification and distribution of GO annotated genes in CONTROL, TR15 and TR30 tissues of *J. curcas*

Table 2 Effect of BA treatment on floral buds at different time intervals

Parameter	Control	TR15	TR30
Number of floral buds	150	1050	500
Number of female flowers	10	— ^a	55–60

^aNo differentiation between female and male flowers in TR15

and structural molecule activity were highly represented in molecular component category. Cell, membrane, organelle and macromolecular complex were the most represented groups in the cellular component category. Overall analysis revealed that the metabolic processes were significantly repressed in TR30 compared to TR15.

Effect of cytokinin treatment on biomass and yield

BA-treated inflorescence showed an increase in floral buds (biomass) up to 7–8 folds after 15 days of application, however, the floral buds (biomass) were reduced to 3–4 folds after 30 days of treatment (Table 2). This might be due to the increased abortion of flowers and also reduction in photoassimilates/photosynthates accumulation (Pan et al. 2014; Pan et al. 2016). No change was observed in number of leaves surrounding inflorescence following BA application at different intervals (TR15 and TR30) which might be attributed to the fact that the cytokinins are most

effective when applied at the early stage of development whereas the surrounding leaves were matured at the time of BA treatment (Hoffman et al. 2009). Further, yield parameters such as seed number, seed size and seed weight were recorded (after 80 days of treatment). For yield parameters, current observations are in accordance with previous reports in *Jatropha* suggesting cytokinin treatment could enhance the overall yield, however, there was a reduction in parameters such as seed size and seed weight but seed number was increased after BA application (Fig. 6; Table 3) (Chen et al. 2014). These results revealed that overall seed yield was compromised after application of cytokinin.

Transition from vegetative to reproductive phase

Transcriptome profiling of vegetative tissues with respect to control (shoot apices with floral buds) identified genes associated with the reproductive phase transition. On comparing and analyzing transcriptomes of VS and CONTROL, transcripts for genes *FPGS1*, *BBX19*, *MED13*, *EBS*, *REV*, *MED12*, *FCA*, *MSI4*, *SPA1*, *SPA2*, *MAF5*, *FPA*, *PIN1* were found exclusively in the CONTROL, which denoted their association with reproductive phase transition and flowering induction (Supplementary Table 2a). *BBX19*, a transcription factor which acts as a circadian clock regulates flowering by regulating the *FT* expression (Wang et al. 2014). Genes *MSI4*, *MED12*, *MED13*, *FPA* and *FCA* induce flowering by repressing *FLC* in *Arabidopsis* (Pazhouhandeh et al. 2011;

Fig. 6 Morphology of seeds collected from control and BA-treated inflorescence (scale bar—1.27 cm)

Gillmor et al. 2014; Schomburg et al. 2001; Macknight et al. 2002). *MSI4* controls the epigenetic regulation of flowering time in *Arabidopsis* by interacting with *CLF-PRC2* complex (Pazhouhandeh et al. 2011). *PIN1*, an auxin efflux carrier plays an important role in primordial development by defining the auxin polarity at different stages of flower development (Gangwar et al. 2016). *Arabidopsis SPA* proteins regulate photoperiodic flowering by interacting with a floral inducer *CONSTANS*, whereas *SPA1* is required for normal flower development (Laubinger et al. 2006). *MAF5* a paralog of *FLC* (flowering repressor) induces flowering by playing an opposite role to *FLC* in response to vernalization in *Arabidopsis* (Ratcliffe et al. 2003). Genes *AGL12*, *AGL17*,

AGL24, *SPL3*, *SPL5*, *SPL15*, *CRY1* and *CRY2* showed higher transcript abundance in CONTROL compared to VS, which inferred their involvement in transition towards flowering. Various reports regarding regulation of flowering transition by agamous-like proteins (*AGL12*, *AGL17*, *AGL24*) exist, especially *AGL12* which acts by up-regulating *SOC*, *FT*, and *LFY*; whereas, *AGL17* acts in an *FT*-independent pathway for flowering by regulating *LFY* and *AP1* (Tapia-Lopez et al. 2008; Han et al. 2008). *AGL24* controls flowering along with *SOC1* and *FUL* in *Arabidopsis* (Torti and Fornara 2012). Also, their expression was detected in vegetative tissues as they are involved in developmental shoot transition (Díaz-Riquelme et al. 2014). Squamosa promoter-binding protein-like transcription factors (*SPL3*, *SPL5* and *SPL15*) are actively involved in reproductive phase change (Schmid et al. 2003). Through this study, we identified two circadian rhythms genes, i.e. *CRY1* and *CRY2*, which are involved in transition towards flowering (Gangwar et al. 2016). Transcript of *AP2* gene found only in stages with floral buds (CONTROL, TR15 and TR30), as it is known to regulate the floral meristem and floral organ development. It has also been known to play an important role in determining seed size, seed weight, and the accumulation of seed oil and protein (Jofuku et al. 2005). In TR30, the transcript abundance of *AP2* was reduced, thus might be affecting the seed size and seed weight in *J. curcas*. Transcript abundance of *AP1* and *LEAFY* was increased in TR15 whereas reduced in TR30, which might be the possible cause of reduced floral mass and eventually the seed yield in TR30 compared to TR15 (Song et al. 2011).

Cytokinin application promotes female flowering

We have previously identified genes involved in female flower transition such as *SUP* and *CRY2* in *J. curcas* (Gangwar et al. 2016). In the current study, transcript abundance of *SUP* gene was upregulated in TR15. This indicated that cytokinin treatment increases the transition towards female flowering as *SUP* has been identified to promote female flower development by aborting stamens (Gangwar et al. 2016). Transcript level of *CK1I* and *PIN1* increased in TR15 compared to TR30, indicating the

Table 3 Effect of BA treatment on seed yield in *J. curcas*

Yield parameter	Control	BA treated
Seed size	Length: 1.8 ± 0.02 cm Diameter: 0.9 ± 0.012 cm	Length: 1.4 ± 0.03 cm Diameter: 0.7 ± 0.01 cm
Seed weight	0.78 ± 0.02 g	0.48 ± 0.01 g
100 seed weight	65–67 g	50–52 g
Seed weight per plant	65–70 g ^a	750–800 g ^a
Seed number per inflorescence	21–23	48–50

^aOnly 3–4 inflorescences were kept as control, whereas 18–20 inflorescences were treated with BA

enhanced development of female flowering. Also, *TyPAI* gene identified for male flowering was downregulated in TR15 compared to TR30. Validation through RT-qPCR showed that *SUP* and *PIN1* were upregulated (up to 10-folds) in TR15 compared to TR30 (Fig. 8a). *TCP18*, a *CYC* subfamily has been identified in *J. curcas* transcripts and its increased abundance in TR15 indicated that the stamen abortion rate might have increased as it causes the stamen repression (Song et al. 2009). Recently, Xu et al. (2016) also reported that genes like ATP-binding protein degenerate stamens in female flowers at later developmental stages. Our observations along with these studies implied that the cytokinin treatment enhanced female flower formation either by promoting the female flower development or by increasing the stamen abortion rate (Pan et al. 2014).

Effect of cytokinin application on endogenous cytokinin metabolism

Cytokinins play important role in the formation and maintenance of shoot apical meristem (SAM) (Werner et al. 2001). Comparative transcriptome analysis identified 11 transcripts of gene-encoding isopentenyltransferases homologs (*IPT1*, *IPT3*, *IPT5*, *IPT6*), cytokinin hydroxylase (*CYP735A2*), cytokinin oxidase/dehydrogenase homologs (*CKX1*, *CKX3*, *CKX5*, *CKX6*, *CKX7*) and ciszeatin O-glucosyltransferase (*CISZOG*) associated with cytokinin metabolism, out of which transcripts for *IPT3*, *IPT5*, *IPT6*, *CISZOG* and *CYP735A* genes were upregulated in TR15. *IPT* catalyzes the rate-limiting step of cytokinin biosynthesis and its upregulation in TR15 showed its association with increased floral buds (biomass) (Atkins et al. 2011). According to previous findings, *IPT2*, *IPT3*, *IPT9* and *CYP735A2* genes showed higher expression in floral buds indicating their involvement in floral bud formation and increased cytokinin accumulation augmenting the reproductive organ development (Gangwar et al. 2016; Ashikari et al. 2005). RT-qPCR analysis revealed that *IPT3* gene showed up to 9.5-folds increased expression in TR15 whereas *CKX5* showed up to 1.5-folds higher expression in TR15. No significant change was observed in transcript levels of cytokinin oxidases (*CKX1* and *CKX3*) in all three tissues. Increase in transcript level of *CKX5* and *CKX7* was observed in TR15 compared to CONTROL and TR30. This might be due to the fact that *CKX* activity was induced due to an elevated level of cytokinins in TR15 (Motyka et al. 2003; Blagoeva et al. 2004). These results showed a positive correlation between exogenous cytokinins and endogenous cytokinins levels, well associated with growth and development of plant (Blagoeva et al. 2004).

Effect of cytokinin application on other phytohormones

Comparative transcriptome analysis identified 33 transcripts of genes associated with phytohormones (such as ethylene, brassinosteroids, abscisic acids and jasmonic acids). Genes encoding EIN3-binding F-box protein, ethylene receptor and ethylene-insensitive protein 3 were associated with ethylene signaling, of which EIN3-binding F-box protein and ethylene-insensitive protein 3 (*EIN3*) were upregulated in TR15 compared to CONTROL. These are the positive regulators of ethylene signaling. However, *ETR* gene, a negative regulator of ethylene signaling was downregulated in TR15. These results showed that cytokinin may have a positive effect on ethylene signaling. It has been reported that ethylene is involved in floral organogenesis; thus, the increased expression of ethylene genes in TR15 might resulted in dramatic increase in floral buds where floral organs are formed. Genes encoding *SAUR* family protein, auxin-responsive protein *IAA*, auxin-responsive *GH3* gene family were identified to be associated with auxin signaling. *SAUR* protein, *IAA* auxin-response factors (*ARFs*) functions as transcriptional activators or repressors which regulate the expression of auxin-response genes by binding specifically to auxin-response elements (*AREs*) during the development of plant. Auxin-inducible genes *IAA* and *SAUR* were upregulated by BA treatment in TR15 and gene-encoding auxin-response factors were downregulated in TR15. Genes encoding BR-signaling kinases (*BSK*) were induced by BA application as they showed upregulation in TR15 and brassinosteroid resistant ½ (*BSR*), a transcriptional repressor was downregulated in TR15 compared to CONTROL. This indicates that cytokinin application might have a positive effect on BR signaling. Jasmonate ZIM domain-containing protein and jasmonic acid-amino synthetase associated with jasmonate metabolism and signaling were upregulated in TR15 showing a positive correlation between cytokinins and jasmonic acids. Absciscic acid receptor (*PYL* family) and *PP2C* were upregulated in TR15 whereas ABA-responsive element binding factor was downregulated in TR15 compared to CONTROL. *GID1*, a positive regulator of GA signaling was downregulated in TR15 (Supplementary Table 2b).

Effect of cytokinin application on photosynthesis

Plant feedstock is largely influenced by the rate of photosynthesis, carbon fixation and carbon to nitrogen ratio ultimately leading to the availability of carbon skeleton for the synthesis of various compounds involved in number of biological activities (Lawlor 2002). We studied the effect of exogenous cytokinin treatment at differential time intervals on pathways/genes contributing to photosynthesis, pentose phosphate pathway, citrate cycle, oxidative phosphorylation,

pyruvate metabolism, starch metabolism, nitrogen metabolism which might affect the overall feedstock yield in *J. curcas* (Fig. 7). Differential gene expression analysis was carried out for genes associated with various pathways in three tissues (CONTROL, TR15, TR30) of *J. curcas*.

Light reaction plays an important role in carbon fixation through photosynthesis. Photosynthesis is a tightly controlled process, in which light energy is captured and converted into ATP and NADPH. Out of 30 genes identified for enzymes coding for the photosynthetic pathway, genes coding for *LHCI*, *LHCII*, *PSI*, *PSII* and F-type H⁺-transporting ATPase were upregulated in TR15 whereas showed downregulation in TR30. This might be the consequence of less photosynthetic activity in TR30 compared to TR15 due to reduced floral biomass (Renau-Morata et al. 2012). In photosynthesis, photosystems (*PSI* and *PSII*) regulate the primary photochemistry of photosynthesis, transfer of energy and light absorption (Caffarri et al. 2014). Photosynthetic capacity mainly depends on photosynthetic pigments, cytokinin application induced the photosynthetic activity by inducing the photosynthesis genes and by increasing the transcript levels of *PSI* and *PSII* genes thus increasing the

photosynthetic pigment complexes in TR15, however, their activity was reduced in TR30 where the floral buds were reduced due to increased abortion rate which can be correlated with reduced photosynthetic activity. Current results are in accordance with findings of Liu et al. (2010), who reported the increased photosynthetic activity initially in transgenic rice with overexpression of genes associated with cytokinin biosynthesis but decreased later. These observations indicated that cytokinin application did induce the photosynthetic activity initially, however, its prolonged effect was not observed. Further, it could not keep pace with the increased number of floral buds thus source to sink ratio was weakened, causing the abortion/abscission of floral buds in TR30.

Genes encoding plastocyanin, ferredoxin-NADP⁺ reductase, F-type H⁺-transporting ATPase showed decreased transcript levels in TR30 compared to TR15 whereas no change was observed in TR15 as compared to control (Supplementary Table 3a). Apart from reduction in photosynthetic pigments, the reduction in photosynthetic rate might be due to the debilitating metabolic reactions such as RuBP synthesis, ATP synthesis, and electron transfer (Lawlor

Fig. 7 Representation of enzymes upregulated and downregulated in metabolic pathways associated with carbon flux in *J. curcas*. Enzymes are shown in blue and red colors, Blue color signifies upregulation in TR15 and downregulation in TR30 and Red color signifies downregulation in TR30 with no significant change in TR15 and CONTROL. *RBCL* ribulose-bisphosphate carboxylase large chain; *PGK* phosphoglycerate kinase; *GAPDH* glyceraldehyde 3-phosphate dehydrogenase; *ALDO* aldolase, class I; *FBP* fructose-

1,6-bisphosphatase I; *TK* transketolase; *SBP* sedoheptulose-bisphosphatase; *PRK* phosphoribulokinase; *RPIA* ribose 5-phosphate isomerase A; *GPI* glucose-6-phosphate isomerase; *PGM* phosphoglucomutase; *GLGC* glucose-1-phosphate adenyltransferase; *GLGA* starch synthase; *GLGB* 1,4-alpha-glucan branching enzyme; *AMYA* alpha-amylase; *AMYB* beta-amylase; *HK* hexokinase; *GPI* glucose-6-phosphate isomerase; *PFK* 6-phosphofructokinase 1; *PDH* pyruvate dehydrogenase

2002). As per our observations, the decreased transcript abundance of gene-encoding enzyme NADP⁺ reductase in TR30 is associated with reduced photosynthetic electron flow further limits ATPs required for carbon fixation (Kramer and Evans 2011; Sood and Chauhan 2017). Thus, from our findings, it is inferred that the reduced biomass in TR30 might be due to decrease in overall photosynthetic capacity or availability of photosynthates required for the proper development of increased number of flowers and subsequent formation of fruits and seeds (Wang et al. 1996).

Effect of cytokinin application on carbon fixation

ATP and NADPH generated through photosynthesis are utilized in carbon fixation through Calvin cycle. Among 18 genes identified for the Calvin cycle, 12 were differentially expressed in CONTROL, TR15 and TR30. Genes 6-phosphogluconolactonase, fructose-1,6-bisphosphatase I, ribulose-bisphosphate carboxylase small chain, glyceraldehyde-3-phosphate dehydrogenase exhibited higher transcript abundance in TR15 compared to CONTROL and TR30, whereas genes phosphoglucomutase, phosphoglycerate kinase, fructose-bisphosphate aldolase (*FBP*), ribose 5-phosphate isomerase A, ribulose-phosphate 3-epimerase and transaldolase, sedoheptulose-bisphosphatase (*SBP*) showed higher transcript abundance in TR15 compared to TR30 but same to that of CONTROL (Supplementary Table 3b). *FBP* and *SBP* are considered as the major control points in the Calvin cycle for CO₂ fixation in plants. Previous studies have reported that the overexpression of *SBP/FBP* in various plants resulted in increased photosynthetic CO₂ fixation and carbohydrate (starch and sucrose) accumulation (Lefebvre et al. 2005; Tamoi et al. 2006; Gong et al. 2015). It is implied that the decreased transcript abundance of *SBP* in TR30 could be the possible reason for reduction in floral biomass (Harrison et al. 2001). We hereby inferred that *SBP/FBP* could be targeted through genetic engineering approaches to enhance the carbon flux and thus biomass and further fruit or seed yield in *Jatropha*.

Apart from genes involved in Calvin cycle, other genes associated with carbon fixation are glutamate–glyoxylate aminotransferase, phosphoenolpyruvate carboxykinase, aspartate aminotransferase, malate dehydrogenase, pyruvate orthophosphate dikinase, acetyl-CoA carboxylase carboxyl transferase subunit alpha, ATP citrate (pro-*S*)-lyase, aconitate hydratase, methylenetetrahydrofolate reductase (*NADPH*), isocitrate dehydrogenase, acetyl-CoA carboxylase which were largely influenced by cytokinin treatment as these were overexpressed in TR15 compared to CONTROL and TR30. ATPs required for carbon fixation are also generated by oxidative phosphorylation from oxidation of *NADH* and *FADH2* from photosynthesis. Out of 69 genes identified for oxidative phosphorylation, 33 showed

differential transcript abundance in three tissues. Genes encoding enzymes inorganic pyrophosphatase, NADH dehydrogenase, NAD(P)H-quinone oxidoreductase subunit 5, H⁺-transporting ATPase, ubiquinol-cytochrome c reductase, cytochrome c oxidase subunit 6a showed higher transcript abundance in TR15 compared to CONTROL and TR30. Also, genes such as succinate dehydrogenase, V-type H⁺-transporting ATPase, F-type H⁺-transporting ATPase subunit g, F-type H⁺-transporting ATPase subunit beta showed lower transcript abundance in TR30 compared to TR15 (Supplementary Table 3c). Thus, our observations inferred that carbon fixation increased after 15 days but decreased after 30 days of BA treatment suggesting that reduced carbon availability might be affecting the floral biomass in TR30 and the seed yield. The current observations are in line with previous studies for plants such as *Arabidopsis*, tobacco and rice supporting the correlation of carbon fixation and biomass accumulation (Kurek et al. 2007; Zhu et al. 2007; Raines 2011).

Effect of cytokinin application on carbohydrate metabolism

Carbon fixed through Calvin cycle is further utilized for the synthesis of carbohydrates. In plants, starch and sucrose serve as the major carbohydrates storage points (Berg et al. 2002; Kumar et al. 2016). Based on KEGG pathway assignments, we identified 23 genes involved in starch and sucrose metabolism out of which 18 genes were downregulated in TR30 compared to TR15. Transcript abundance showed that genes encoding enzymes pectinesterase, sucrose synthase, trehalose 6-phosphate phosphatase, beta-glucosidase, beta-fructofuranosidase, polygalacturonase, endoglucanase, beta-D-xylosidase 4 and alpha-1, 4-galacturonosyltransferase were upregulated in TR15 compared to CONTROL and TR30. Genes encoding enzymes linked to starch and sucrose metabolism such as glucose-1-phosphate adenylyltransferase (*AGPase*), trehalose 6-phosphate synthase/phosphatase, 4-alpha-glucanotransferase, phosphoglucomutase (*PGM*), hexokinase, 1,4-alpha-glucan branching enzyme and sucrose phosphate synthase were downregulated in TR30 compared to TR15. Transcript abundance of genes encoding *PGM* and *AGPase* decreased in TR30 compared to TR15 suggesting a lower rate of starch formation in TR30. In starch biosynthetic pathway, *PGM* converts glucose-6-phosphate into glucose-1-phosphate and its reduced activity has been reported to reduce the rate of starch accumulation (Andriotis et al. 2012). Further, the rate of starch biosynthesis is regulated by *AGPase*, which catalyzes an irreversible reaction and is strongly regulated through allosteric (*3PGA* activator and orthophosphate as an inhibitor) and redox control (George et al. 2010). The activity of this enzyme probably determines the flux of carbon into starch as its reduced expression might

limit the carbon flux and starch synthesis (Vigeolas et al. 2004). Thus, it is implicit that the decreased floral biomass in TR30 is possibly due to the reduction of carbohydrate synthesis and supply to the florets which are required for the proper development of reproductive organs (Corbesier et al. 1998). Apart from floral biomass reduction in TR30, the reduction in seed size and weight might be due to less carbohydrate reserves and their translocation to the reproductive sink tissue further affecting seed yield at later stages of development. These observations suggest that cytokinin application initially enhanced the carbon flux, resulting in increased biomass. However, after a particular time, the required carbon flux could not meet the required demand, due to the reduced triose phosphate availability needed for carbohydrate synthesis (Walters et al. 2004).

During night hours, the carbon stored in the form of starch is mobilized by conversion to sucrose in the cytoplasm. Triose phosphate form fructose 1,6-bisphosphate which is then converted into fructose-6 phosphate by fructose-1,6-bisphosphatase (*FBP*). *FBP* regulates sucrose metabolism pathway which was supported by its upregulation in TR15 compared to CONTROL and TR30. Sucrose phosphate synthase (*SPS*) which forms sucrose phosphate by utilizing UDP-glucose and D-fructose-6-phosphate was downregulated in TR30 compared to TR15. (Supplementary Table 3d). *SPS* plays a crucial role in carbohydrate metabolism by regulating the partitioning of carbon between starch production and carbohydrate accumulation (Chen et al. 2005). Based on the above observations, it is imperative that there was a reduction in carbohydrate synthesis as well as flow to the sink organs (florets), thus, weakened sink strength causing the reduction in biomass in TR30 and further affected the seed yield (Koch 2004; Hikosaka and Sugiyama 2015).

Starch phosphorylase, which transfers glucose from non-reducing end of α -1,4-linked glucan to orthophosphate and generates glucose 1-phosphate, was found to be repressed in TR30. This glucose 1-phosphate enters glycolysis for sugar breakdown. The pyruvate formed at the end of glycolysis enters the citric acid cycle (TCA) providing precursors for carbon skeleton (Berg et al. 2002). TCA cycle plays a central role in generating ATP and providing carbon skeletons to many biosynthetic processes. Overall, the citric acid cycle has been affected after the treatment thus reducing the carbon flow, as the enzyme ATP citrate lyase has been downregulated (Daloso et al. 2015). The reduction in TCA activity might have affected the biomass after 30 days due to compromise in the efficient use of carbohydrates (reduced starch and sucrose level), which has already been reduced as stated above (Schroder et al. 2014). Overall, these observations suggest that the increased floral buds might produce more photoassimilates, i.e. carbon gain and carbon assimilation, however, premature exhaustion of carbohydrate reserves might trigger starvation leading to decreased

biomass, further affecting yield (Buchanan-Wollaston et al. 2005). Thus, it is inferred that the pathways contributing towards carbon accumulation were reduced and could not meet the increased demand due to high flowering intensity, which in turn affected the development of fruits further leading to reduction in seed weight and size, thereby overall compromising the yield.

Effect of cytokinin application on nitrogen assimilation and carbon to nitrogen (C/N) ratio

Nitrogen assimilation depends upon a regular co-ordination between nitrogen and carbon, due to the requirement of carbon in the form of 2-oxoglutarate and ATP (Lancien et al. 2000). Therefore, certain metabolites such as sucrose and amino acids play an important role in the regulation of enzymes involved in nitrogen assimilation. Nitrogen plays a significant role in the formation of compounds required for cellular activities. 9 genes associated with nitrogen metabolism were identified, of which six were differentially expressed in TR15. Transcript abundance of genes encoding enzymes glutamine synthetase, carbonic anhydrase, formamidase, nitrate reductase and nitrate/nitrite transporters was repressed in TR30 (Supplementary Table 3e) showing less rate of nitrogen metabolism. Transcript abundance of gene-encoding glutamine synthetase was (*GS*) decreased in TR30 compared to TR15 which indicates less nitrogen availability, as it is a key enzyme assimilating inorganic nitrogen (Lea et al. 1990). Glutamate dehydrogenase (*GDH*) activity was decreased from TR15 to TR30, thus reducing the release of carbon required under low C/N ratios as it acts as a shunt to the glutamate synthase cycle releasing carbon from amino compounds as keto-acids (Mifflin and Habash 2002). *GDH* also catalyzes the amination of 2-oxoglutarate in glutamate synthesis. This indicated that due to the repressed activity of this enzyme, carbon availability has been decreased causing reduced floral biomass in TR30 and further yield. Many reports deliberating an increase in biomass and yield with *GS* transformation in various plant species exist (Gallardo et al. 1999; Harrison et al. 2000; Czyzewicz and Below 1994). The reduction in biomass after 30 days of cytokinin treatment (TR30) might be due to reduced nitrogen availability, thus affecting the assimilate partitioning between vegetative and reproductive organs (Czyzewicz and Below 1994). Availability of nitrogen has the potential to increase total yield and genes encoding enzymes *GS* and *GDH* are hereby referred as potential targets to increase yield of *J. curcas* as they regulate the C/N ratio (Yong et al. 2010).

RT-qPCR-based experimental validation

Transcript abundance of genes from transcriptome data was validated for 31 genes involved in flowering (2),

Fig. 8 a RT-qPCR-based expression pattern of genes involved in female flowering and cytokinin metabolism. **b** RT-qPCR-based expression pattern of genes involved in photosynthesis, carbon fixation, starch and nitrogen metabolism and glycolysis. *LCI* light-harvesting complex I; *LCII* light-harvesting complex I; *PSI* photosystem I; *PSII* photosystem II; *PLST* plastocyanin; *FER* ferredoxin; *PGM* phosphoglucosyltransferase; *GLGC* glucose-1-phosphate adenylyltransferase; *FBP* fructose-1,6-bisphosphatase; *G6PE1* glucose-6-phos-

phate 1-epimerase; *HK* hexokinase; *PGK* phosphoglycerate kinase; *GAPDH* glyceraldehyde 3-phosphate dehydrogenase; *RBCL* ribulose-bisphosphate carboxylase small chain; *RPIA* ribose 5-phosphate isomerase A; *PGLS* 6-phosphogluconolactonase; *ACCA* acetyl-CoA carboxylase carboxyl transferase subunit alpha; *IDH* isocitrate dehydrogenase; *SBP* sedoheptulose-bisphosphatase; *ACLY* ATP citrate (pro-*S*)-lyase; *GDH* glutamate dehydrogenase; *GS* glutamine synthetase; *CA* carbonic anhydrase; *NR* nitrate reductase

cytokinin metabolism (5), photosynthesis (6), carbon fixation (9), starch metabolism (3) nitrogen metabolism (4) and glycolysis (2) in all three tissues by RT-qPCR. Genes *FBP*, *SBP*, *GS*, *GDH*, *RPIA*, *RBCL* and *GLGC* showed significant upregulation of up to fourfolds in TR15 compared to CONTROL and up to 0.03-folds of downregulation in TR30 compared to TR15. Flowering genes *SUP* and *PIN1* showed up to 10-folds increase in TR15 and up to 0.4-folds decrease in TR30. Genes involved in cytokinin metabolism showed up to 9.5-folds increase in TR15 compared to CONTROL and up to 0.1-folds decrease in TR30. Cytokinin oxidase *CKX1* did not show significant fold difference in all the three tissues, i.e. CONTROL,

TR15 and TR30; however, *CKX5* showed up to 1.5-folds increase in TR15 compared to CONTROL and up to 0.93-folds in TR30. Thus, a positive correlation between RT-qPCR analysis and RNA-Seq data was observed, i.e. higher expression of genes in TR15 compared to TR30 and CONTROL (Fig. 8b).

Availability of transcriptome data

The raw reads and annotated material are available at the URL: <http://14.139.240.55/NGS/download.php>.

Conclusion

Comparative transcriptomic analysis of control and cytokinin-treated shoot apical meristems at different time intervals shed light on physiological, biochemical and molecular cues affecting floral biomass and total seed yield in *J. curcas*. The present study will contribute not only to genetic engineering and breeding of *Jatropha* for better yield but also provided insights into the mechanisms associated with the effect of cytokinin application on carbon fixation, carbon availability and nitrogen metabolism which further affects biomass production and thus feedstock yield of *J. curcas*. Through this study, genes associated with carbon flux mechanisms have emerged as possible targets to increase the total floral biomass and seed yield in *Jatropha*.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

- Akbar E, Yaakob Z, Kamarudin S, Ismail M (2009) Characteristics and composition of *Jatropha curcas* oil seed from Malaysia and its potential as biodiesel feedstock. *Eur J Sci Res* 29:396–403
- Andriotis VM, Pike MJ, Schwarz SL, Rawsthorne S, Wang TL, Smith AM (2012) Altered starch turnover in the maternal plant has major effects on Arabidopsis fruit growth and seed composition. *Plant Physiol* 1160:1175–1186. <https://doi.org/10.1104/pp.112.205062>
- Antos M, Wiebold WJ (1984) Abscission, total soluble sugars, and starch profiles within a soybean canopy. *Agron J* 76:715–719. <http://doi.org/10.2134/agronj1984.00021962007600050002x>
- Ashikari M, Sakakibara H, Lin S, Yamamoto T, Takashi T, Nishimura A, Angeles ER, Qian Q, Kitano H, Matsuoka M (2005) Cytokinin oxidase regulates rice grain production. *Science* 309:741–774. <https://doi.org/10.1126/science.1113373>
- Atkins CA, Emery RJN, Smith PM (2011) Consequences of transforming narrow leaved lupin (*Lupinus angustifolius* [L.]) with an *ipt* gene under control of a flower-specific promoter. *Transgenic Res* 20:1321–1332. <https://doi.org/10.1007/s11248-011-9497-7>
- Berg JM, Tymoczko JL, Stryer L (2002) Biochemistry, 5th edn. W H Freeman, New York
- Blagoeva E, Dobrev PI, Malbeck J, Motyka V, Strnad M, Hanus J, Vankova R (2004) Cytokinin *N*-glucosylation inhibitors suppress deactivation of exogenous cytokinins in radish, but their effect on active endogenous cytokinins is counteracted by other regulatory mechanisms. *Physiol Plant* 121:215–222. <https://doi.org/10.1111/j.1365-3054.2004.00320.x>
- Brun WA, Betts KJ (1984) Source/sink relations of abscising and no abscising soybean flowers. *Plant Physiol* 75:187–191. <https://doi.org/10.1104/pp.75.1.187>
- Buchanan-Wollaston V, Page T, Harrison E, Breeze E, Lim PO, Nam HG et al (2005) Comparative transcriptome analysis reveals significant differences in gene expression and signalling pathways between developmental and dark/starvation-induced senescence in Arabidopsis. *Plant J* 42:567–585. <https://doi.org/10.1111/j.1365-3113.2005.02399.x>
- Caffarri S, Tibiletti T, Jennings RC, Santabarbara S (2014) A comparison between plant photosystem I and photosystem II architecture and functioning. *Curr Protein Pept Sci* 15:296–331. <http://doi.org/10.2174/1389203715666140327102218>
- Chen S, Hajirezaei M, Bornke F (2005) Differential expression of sucrose-phosphate synthase isoenzymes in tobacco reflects their functional specialization during dark-governed starch mobilization in source leaves. *Plant Physiol* 139:1163–1174. <https://doi.org/10.1104/pp.105.069468>
- Chen MS, Pan BZ, Wang GJ, Ni J, Niu L, Xu ZF (2014) Analysis of the transcriptional responses in inflorescence buds of *Jatropha curcas* exposed to cytokinin treatment. *BMC Plant Biol* 14:318. <https://doi.org/10.1186/s12870-014-0318-z>
- Corbesier L, Lejeune P, Bernier G (1998) The role of carbohydrates in the induction of flowering in *Arabidopsis thaliana*: comparison between the wild type and a starchless mutant. *Planta* 206:131–137. <https://doi.org/10.1007/s004250050383>
- Czyzewicz JR, Below FE (1994) Genotypic variation for nitrogen uptake by maize kernels grown in vitro. *Crop Sci* 34:1003–1008. <https://doi.org/10.2135/cropsci1994.0011183X003400040032x>
- Daloso DM, Müller K, Obata T, Florian A, Tohge T, Bottcher A, Riondet C et al (2015) Thioredoxin, a master regulator of the tricarboxylic acid cycle in plant mitochondria. *Proc Natl Acad Sci USA* 112:E1392–E1400. <https://doi.org/10.1073/pnas.1424840112>
- Díaz-Riquelme J, Martínez-Zapater JM, Carmona MJ (2014) Transcriptional analysis of tendrils and inflorescence development in grapevine (*Vitis vinifera* L.). *PLoS ONE* 9:e92339. <https://doi.org/10.1371/journal.pone.0092339>
- Gallardo F, Fu J, Canton FR, Garcia-Gutierrez A, Canovas FM, Kirby EG (1999) Expression of a conifer glutamine synthetase gene in transgenic poplar. *Planta* 210:19–26. <https://doi.org/10.1007/s004250050649>
- Gangwar M, Sood H, Chauhan RS (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. <https://doi.org/10.1007/s11033-016-3953-7>
- Gayakvad P, Jadeja DB, Bhalawe S (2014) Effect of foliar application of GA3, ethrel and copper sulphate on flowering behaviour and sex ratio of *Jatropha curcas* L. *J Appl Nat Sci* 6:286–289
- George GM, van der Merwe MJ, Nunes-Nesi A, Bauer R, Fernie AR, Kossmann J, Lloyd JR (2010) Virus-induced gene silencing of plastidial soluble inorganic pyrophosphatase impairs essential leaf anabolic pathways and reduces drought stress tolerance in *Nicotiana benthamiana*. *Plant Physiol* 154:55–66. <https://doi.org/10.1104/pp.110.157776>
- Gillmor CS, Silva-Ortega CO, Willmann MR, Buendía-Monreal M, Poethig RS (2014) The *Arabidopsis* Mediator CDK8 module genes *CCT (MED12)* and *GCT (MED13)* are global regulators of developmental phase transitions. *Development* 141:4580–4589. <https://doi.org/10.1242/dev.111229>
- Gong HY, Li Y, Fang G, Hu DH, Jin WB, Wang ZH et al (2015) Transgenic rice expressing *Ictb* and *FBP/Sbpase* derived from cyanobacteria exhibits enhanced photosynthesis and mesophyll conductance to CO₂. *PLoS ONE* 10:e0140928. <https://doi.org/10.1371/journal.pone.0140928>
- Han P, García-Ponce B, Fonseca-Salazar G, Alvarez-Buylla ER, Yu H (2008) *AGAMOUS-LIKE 17*, a novel flowering promoter, acts in a *FT*-independent photoperiod pathway. *Plant J* 55:253–265. <http://doi.org/10.1111/j.1365-3113.2008.03499.x>

- Harrison J, Brugière N, Phillipson B, Ferrario-Méry S, Becker T, Limami A, Hirel B (2000) Manipulating the pathway of ammonium assimilation through genetic engineering and breeding: consequences on plant physiology and development. *Plant Soil* 221:81–93. <https://doi.org/10.1023/A:1004715720043>
- Harrison EP, Olcer H, Lloyd JC, Long SP, Raines CA (2001) Small decreases in SBPase cause a linear decline in the apparent RuBP regeneration rate, but do not affect Rubisco carboxylation capacity. *J Exp Bot* 52:1779–1784. <https://doi.org/10.1093/jexbot/52.362.1779>
- Hikosaka S, Sugiyama N (2015) Effects of exogenous plant growth regulators on yield, fruit growth, and concentration of endogenous hormones in gynocious parthenocarpic cucumber (*Cucumis sativus* L.). *Hortic J* 84:342–349. <https://doi.org/10.2503/hortj.MI-051>
- Hoffman EW, Bellstedt DU, Jacobs G (2009) Exogenous cytokinin induces “Out of Season” flowering in *Protea* cv. Carnival. *J Am Soc Hortic Sci* 134:308–318
- Jofuku KD, Omidyar PK, Gee Z, Okamuro JK (2005) Control of seed mass and seed yield by the floral homeotic gene *APETALA2*. *Proc Natl Acad Sci USA* 102:3117–3122. <https://doi.org/10.1073/pnas.0409893102>
- Kant S, Burch D, Badenhorst P, Palanisamy R, Mason J, Spangenberg G (2015) Regulated expression of a cytokinin biosynthesis gene *IPT* delays leaf senescence and improves yield under rainfed and irrigated conditions in Canola (*Brassica napus* L.). *PLoS ONE* 10:e0116349. <https://doi.org/10.1371/journal.pone.0116349>
- Koch K (2004) Sucrose metabolism: regulatory mechanisms and pivotal roles in sugar sensing and plant development. *Curr Opin Plant Biol* 7:235–246. <https://doi.org/10.1016/j.pbi.2004.03.014>
- Kochhar S, Singh SP, Kochhar VK (2008) Effect of auxins and associated biochemical changes during clonal propagation of the biofuel plant—*Jatropha curcas*. *Biomass Bioenergy* 32:1136–1143. <https://doi.org/10.1016/j.biombioe.2008.02.014>
- Kramer DM, Evans JR (2011) The importance of energy balance in improving photosynthetic productivity. *Plant Physiol* 155:70–78. <https://doi.org/10.1104/pp.110.166652>
- Kumar V, Shitiz K, Chauhan RS, Sood H, Tandon C (2016) Tracking dynamics of enzyme activities and their gene expression in *Picrorhiza kurroa* with respect to picoside accumulation. *J Plant Biochem Biotechnol* 25:125–132. <https://doi.org/10.1007/s13562-015-0317-7>
- Kurek I, Chang TK, Bertain SM, Madrigal A, Liu L, Lassner MW, Zhu G (2007) Enhanced thermostability of *Arabidopsis* RuBisCO activase improves photosynthesis and growth rates under moderate heat stress. *Plant Cell* 19:3230–3241. <https://doi.org/10.1105/tpc.107.054171>
- Lancien M, Gadal P, Hodges M (2000) Enzyme redundancy and the importance of 2-oxoglutarate in higher plant ammonium assimilation. *Plant Physiol* 123:817–824. <https://doi.org/10.1104/pp.123.3.817>
- Laubinger S, Marchal V, Le Gourrierec J, Wenkel S, Adrian J et al (2006) *Arabidopsis* SPA proteins regulate photoperiodic flowering and interact with the floral inducer CONSTANS to regulate its stability. *Development* 133:3213–3222. <https://doi.org/10.1242/dev.02481>
- Lawlor DW (2002) Carbon and nitrogen assimilation in relation to yield: mechanisms are the key to understanding production systems. *J Exp Bot* 53:773–787. <https://doi.org/10.1093/jexbot/53.370.773>
- Lea PJ, Robinson SA, Stewart GR (1990) The enzymology and metabolism of glutamine, glutamate, and asparagine. In: Miflin BJ, Lea PJ (eds) *The biochemistry of plants*. Academic Press, New York, pp 121–159
- Lefebvre S, Lawson T, Zakhleniuk OV, Lloyd JC, Raines CA, Fryer M (2005) Increased sedoheptulose-1,7-bisphosphatase activity in transgenic tobacco plants stimulates photosynthesis and growth from an early stage in development. *Plant Physiol* 138:451–460. <https://doi.org/10.1104/pp.104.055046>
- Liu L, Zhou Y, Szczerba MW, Li X, Lin Y (2010) Identification and application of a rice senescence-associated promoter. *Plant Physiol* 153:1239–1249. <https://doi.org/10.1104/pp.110.157123>
- Ma Q, Longnecker N, Atkins C (1998) Exogenous cytokinin and nitrogen do not increase grain yield in narrow-leaved lupins. *Crop Sci* 38:717–721. <https://doi.org/10.2135/cropsci1998.0011183X003800030017x>
- Macknight R, Duroux M, Laurie R, Dijkwel P, Simpson G, Dean C (2002) Functional significance of the alternative transcript processing of the *Arabidopsis* floral promoter *FCA*. *Plant Cell* 14:877–888. <https://doi.org/10.1105/tpc.010456>
- Maes WH, Achten WMJ, Reubens B, Raes D, Samson R, Muys B (2009) Plant-water relationships and growth strategies of *Jatropha curcas* L. seedlings under different levels of drought stress. *J Arid Environ* 73:877–884. <https://doi.org/10.1016/j.jaridenv.2009.04.013>
- Miflin BJ, Habash DJ (2002) The role of glutamine synthetase and glutamate dehydrogenase in nitrogen assimilation and possibilities for improvement in the nitrogen utilization of crops. *J Exp Bot* 53:979–987. <https://doi.org/10.1093/jexbot/53.370.979>
- Motyka V, Vanková R, Čapková V, Petrásek J, Kamínek M, Schmölling T (2003) Cytokinin-induced upregulation of cytokinin oxidase activity in tobacco includes changes in enzyme glycosylation and secretion. *Physiol Plant* 117:11–21. <https://doi.org/10.1034/j.1399-3054.2003.1170102.x>
- Nagel L, Brewster R, Riedell WE, Reese RN (2001) Cytokinin regulation of flower and pod set in soybean [*Glycine max* (L.) Merr.]. *Ann Bot* 88:27–31. <https://doi.org/10.1006/anbo.2001.1423>
- Pan BZ, Xu ZF (2011) Benzyladenine treatment significantly increases the seed yield of the biofuel plant *Jatropha curcas*. *J Plant Growth Regul* 30:166–174. <https://doi.org/10.1007/s00344-010-9179-3>
- Pan BZ, Chen MS, Ni J, Xu ZF (2014) Transcriptome of the inflorescence meristems of the biofuel plant *Jatropha curcas* treated with cytokinin. *BMC Genomics* 15:974. <https://doi.org/10.1186/1471-2164-15-974>
- Pan BZ, Luo Y, Song L, Chen MS, Li JL, Xua ZF (2016) Thidiazuron increases fruit number in the biofuel plant *Jatropha curcas* by promoting pistil development. *Ind Crops Prod* 81:202–210. <https://doi.org/10.1016/j.indcrop.2015.11.054>
- Pazhouhandeh M, Molinier J, Berr A, Genschik P (2011) MSI4/FVE interacts with CUL4-DDB1 and a PRC2-like complex to control epigenetic regulation of flowering time in *Arabidopsis*. *Proc Natl Acad Sci USA* 108:3430–3435. <https://doi.org/10.1073/pnas.1018242108>
- Peleg Z, Reguera M, Tumimbang E, Walia H, Blumwald E (2011) Cytokinin-mediated source/sink modifications improve drought tolerance and increase grain yield in rice under water-stress. *Plant Biotechnol J* 9:747–758. <https://doi.org/10.1111/j.1467-7652.2010.00584.x>
- Prat L, Botti C, Fichet T (2008) Effect of plant growth regulators on floral differentiation and seed production in *Jojoba* (*Simmondsia chinensis*). *Ind Crops Prod* 27:44–49. <https://doi.org/10.1016/j.indcrop.2007.07.001>
- Qin H, Gu Q, Zhang JL, Sun L, Kuppu S, Zhang YZ, Burow M, Payton P, Blumwald E, Zhang H (2011) Regulated expression of an isopentenyltransferase gene (*IPT*) in peanut significantly improves drought tolerance and increases yield under field conditions. *Plant Cell Physiol* 52:1904–1914. <https://doi.org/10.1093/pcp/pcr125>
- Raines C (2011) Increasing photosynthetic carbon assimilation in C3 plants to improve crop yield: current and future strategies. *Plant Physiol* 155:36–42. <https://doi.org/10.1104/pp.110.168559>
- Ratcliffe OJ, Kumimoto RW, Wong BJ, Riechmann JL (2003) Analysis of the *Arabidopsis* MADS affecting flowering gene family:

- MAF2 prevents vernalization by short periods of cold. *Plant Cell* 15:1159–1169. <https://doi.org/10.1105/tpc.009506>
- Renau-Morata B, Nebauer SG, Sanchez M, Molina RV (2012) Effect of corm size, water stress and cultivation conditions on photosynthesis and biomass partitioning during the vegetative growth of saffron. *Ind Crops Prod* 39:40–46. <https://doi.org/10.1016/j.indcrop.2012.02.009>
- Schmid M, Uhlenhaut N, Godard F, Demar M, Bressan R, Weigel D, Lohmann JU (2003) Dissection of floral induction pathways using global expression analysis. *Development* 130:6001–6012. <https://doi.org/10.1242/dev.00842>
- Schomburg FM, Patton DA, Meinke DW, Amasino RM (2001) *FPA*, a gene involved in floral induction in *Arabidopsis*, encodes a protein containing RNA-recognition motifs. *Plant Cell* 13:1427–1436. <https://doi.org/10.1186/s12870-014-0309-0>
- Schroder F, Lisso J, Obata T, Erban A et al (2014) Consequences of induced brassinosteroid deficiency in *Arabidopsis* leaves. *BMC Plant Biol* 14:309. <https://doi.org/10.1186/s12870-014-0309-0>
- Song CF, Lin QB, Liang RH, Wang YZ (2009) Expressions of ECE-CYC2 clade genes relating to abortion of both dorsal and ventral stamens in *Opithandra* (Gesneriaceae). *BMC Evol Biol* 9:244. <https://doi.org/10.1186/1471-2148-9-244>
- Song J, Clemens J, Jameson PE (2011) Expression of floral identity genes in *Clanthus maximus* during mass inflorescence abortion and floral development. *Ann Bot* 107:1501–1509. <https://doi.org/10.1093/aob/mcr035>
- Sood A, Chauhan RS (2015) Regulation of FA and TAG biosynthesis pathway genes in endosperms and embryos of high and low oil content genotypes of *Jatropha curcas* L. *Plant Physiol Biochem* 94:253–267. <https://doi.org/10.1016/j.plaphy.2015.06.015>
- Sood A, Chauhan RS (2017) Comparative NGS transcriptomics unravels molecular components associated with mosaic virus infection in a bioenergy plant species, *Jatropha curcas* L. *Bioenergy Res* 10:129–145. <https://doi.org/10.1007/s12155-016-9783-6>
- Tamoi M, Nagaoka M, Miyagawa Y, Shigeoka S (2006) Contribution of fructose-1,6-bisphosphatase and sedoheptulose-1,7-bisphosphatase to the photosynthetic rate and carbon flow in the Calvin cycle in transgenic plants. *Plant Cell Physiol* 47:380–390. <https://doi.org/10.1093/pcp/pcj004>
- Tapia-Lopez R, García-Ponce B, Dubrovsky JG, Garay-Arroyo A, Pérez-Ruiz RV, Kim SH, Acevedo F, Pélaz S, Alvarez-Buylla ER (2008) An *AGAMOUS*-related MADS-box gene, *XAL1* (*AGL12*), regulates root meristem cell proliferation and flowering transition in *Arabidopsis*. *Plant Physiol* 146:1182–1192. <https://doi.org/10.1104/pp.107.108647>
- Torti S, Fornara F (2012) *AGL24* acts in concert with *SOC1* and *FUL* during *Arabidopsis* floral transition. *Plant Signal Behav* 7:1251–1254. <https://doi.org/10.4161/psb.21552>
- Vigeolas H, Möhlmann T, Martini N, Neuhaus HE, Geigenberger P (2004) Embryo-specific reduction of ADP-Glc pyrophosphorylase leads to an inhibition of starch synthesis and a delay in oil accumulation in developing seeds of oilseed rape. *Plant Physiol* 136:2676–2686. <https://doi.org/10.1104/pp.104.046854>
- Walters RG, Ibrahim DG, Horton P, Kruger NJ (2004) A mutant of *Arabidopsis* lacking the triose-phosphate/phosphate translocator reveals metabolic regulation of starch breakdown in the light. *Plant Physiol* 135:891–906. <https://doi.org/10.1104/pp.104.040469>
- Wang CY, Ma Y, Zhou S (1996) Study on effects of soil drought stress on winter wheat senescence. *Acta Agric Univ Henan* 30:309–313
- Wang C-Q, Guthrie C, Sarmast MK, Dehesh K (2014) BBX19 interacts with CONSTANS to repress *FLOWERING LOCUS T* transcription, defining a flowering time checkpoint in *Arabidopsis*. *Plant Cell* 26:3589–3602. <https://doi.org/10.1105/tpc.114.130252>
- Werner T, Motyka V, Strnad M, Schmülling T (2001) Regulation of plant growth by cytokinin. *Proc Natl Acad Sci USA* 98:10487–10492. <https://doi.org/10.1073/pnas.171304098>
- Xu G, Huang J, Yang Y, Y-a Yao (2016) Transcriptome analysis of flower sex differentiation in *Jatropha curcas* L. Using RNA sequencing. *PLoS ONE* 11:e0145613. <https://doi.org/10.1371/journal.pone.0145613>
- Yashima Y, Kaihatsu A, Nakajima T, Nakamura T, Kokubun M (2005) Effects of source/sink ratio and cytokinin application on pod set in soybean. *Plant Prod Sci* 8:139–144. <https://doi.org/10.1626/pp.8.139>
- Yeh S-Y, Chen H-W, Ng C-Y et al (2015) Down-regulation of cytokinin oxidase 2 expression increases tiller number and improves rice yield. *Rice* 8:36. <https://doi.org/10.1186/s12284-015-0070-5>
- Yong J, Ng Y, Tan S, Chew A (2010) Effect of fertilizer application on photosynthesis and oil yield of *Jatropha curcas* L. *Photosynthetica* 48:208–218. <https://doi.org/10.1007/s11099-010-0026-3>
- Zalewski W, Galuszka P, Gasparis S, Orczyk W, Nadolska-Orczyk A (2010) Silencing of the *HvCKX1* gene decreases the cytokinin oxidase/dehydrogenase level in barley and leads to higher plant productivity. *J Exp Bot* 61:1839–1851. <https://doi.org/10.1093/jxb/erq052>
- Zhao J, Bai W, Zeng Q, Song S, Zhang M et al (2015) Moderately enhancing cytokinin level by down-regulation of *GhCKX* expression in cotton concurrently increases fiber and seed yield. *Mol Breed* 35:60. <https://doi.org/10.1007/s11032-015-0232-6>
- Zhu XG, de Sturler E, Long SP (2007) Optimizing the distribution of resources between enzymes of carbon metabolism can dramatically increase photosynthetic rate: a numerical simulation using an evolutionary algorithm. *Plant Physiol* 145:513–526. <https://doi.org/10.1104/pp.107>