Gibberellin Inhibits Floral Initiation in the Perennial Woody Plant *Jatropha curcas*

Jialong Li¹ · Bang-Zhen Pan¹ · Longjian Niu¹ · Mao-Sheng Chen¹ · Mingyong Tang¹ · Zeng-Fu Xu¹

Received: 7 June 2017 / Accepted: 27 February 2018 / Published online: 8 March 2018
© The Author(s) 2018. This article is an open access publication

Abstract

Gibberellin (GA) is an important phytohormone and has been reported to promote flowering in annual and biennial plants, but may play an opposite role in woody perennial plants. In this study, the promoting or inhibiting effect of GA on floral initiation was investigated in *Jatropha curcas* L., a perennial woody plant, which has great potential as renewable energy plant due to the high oil content in the seed. We found that exogenous application of gibberellic acid (GA₃) inhibited flowering, whereas GA biosynthesis inhibitors promoted flowering. The promotive effect of the GA biosynthesis inhibitor paclobutrazol on floral initiation could be diminished by GA₃ treatment. The expression levels of GA biosynthesis genes *Jatropha GA 3-oxidase 3* (*JcGA3ox3*) and the GA receptor genes, *Jatropha GA-INSENSITIVE DWARF 1C* (*JcGID1C*) were decreased, whereas a GA catabolism gene, *Jatropha GA 2-oxidase 8* (*JcGA2ox8*), was elevated during floral transition. These results suggest that GA inhibits floral initiation in *J. curcas*.

Keywords Gibberellin · Gibberellin biosynthesis inhibitor · Paclobutrazol · Floral transition · Flowering · Biofuel · Physic nut

Jialong Li and Bang-Zhen Pan have contributed equally to this work.

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s00344-018-9797-8>) contains supplementary material, which is available to authorized users.

✉ Zeng-Fu Xu
zfxu@xtbg.ac.cn; zengfu.xu@gmail.com

Jialong Li
10710129@gmail.com

Bang-Zhen Pan
pbz@xtbg.org.cn

Longjian Niu
niulongjian@126.com

Mao-Sheng Chen
chenms@xtbg.org.cn

Mingyong Tang
tangmingyong@xtbg.ac.cn

¹ Key Laboratory of Tropical Plant Resources and Sustainable Use, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Menglun, Mengla 666303, Yunnan, China

Introduction

Floral initiation occurs at a certain point after plants switch from the vegetative to the reproductive phase (Boss and others 2004). Floral formation is controlled strictly by the physiological state of the plant and environmental factors (Wellmer and Riechmann 2010). Gibberellin (GA) has been reported to control flowering in many plant species (Campos-Rivero and others 2017; Conti 2017). The ability of GA to promote floral initiation in plants has been established primarily for long-day and biennial species under conditions that would not normally permit flowering (Lang 1957). The exogenous application of GAs accelerates flowering in wild-type *Arabidopsis*, particularly during short days (Langridge 1957). The increase in bioactive GAs in the shoot apex contributes to the transition to reproductive development in, for example, the long-day grass *Lolium temulentum* (King and others 2006) and biennial species of sugar beet (Sorce and others 2002).

However, GA application did not result in the promotion of flowering in short-day plants (Lang 1957), and even has the opposite effect in woody perennial plants. For example, GA application inhibits floral initiation in avocado (Salaazar-Garcia and Lovatt 1998), citrus (Muñoz-Fambuena

and others 2012), sweet cherry (Lenahan and others 2006), peach (García-Pallas and others 2001), and Japanese plums (Gonzalez-Rossia and others 2006), whereas GA biosynthesis inhibitors have promoted reproductive development in mango (Winston 1992) and lychee (Menzel and Simpson 1990). Before the flowering of *Mangifera indica*, the concentration of GA in the shoot apical meristem is reduced (Tongumpai and others 1989). Boss and Thomas (2002) reported that in a dwarf, GA-insensitive mutant of grapevine, tendrils were converted to inflorescences, demonstrating that GA inhibited flowering in the grapevine. These studies indicated that GA plays different roles in annual, biennial and perennial plants. However, a recent report demonstrated that GA promotes the transition from vegetative to inflorescent development but inhibits flower formation in *Arabidopsis* (Yamaguchi and others 2014). The authors claimed that these results may help to explain the paradoxical observation that GA promotes flowering in most annual plants but inhibits floral initiation in some woody plants. However, the molecular mechanism of the GA-mediated inhibition of flowering in perennial woody plants is unknown.

Jatropha curcas L. (hereafter referred to as *Jatropha*) is a perennial and woody plant species belonging to the Euphorbiaceae family. *Jatropha* is widely distributed in tropical and sub-tropical regions and has great potential as renewable energy plant due to the high oil content in the seed (Koh and Mohd Ghazi 2011; Pandey and others 2012). However, the poor yield of this plant is an obstacle for its industrialization (Edrisi and others 2015; Sanderson 2009). Previous studies have tried to improve the seed yield by increasing female flower number per inflorescence or branching through the application of exogenous plant growth regulators and breeding transgenic plants (Abdelgadir and others 2010; Chen and others 2014; Ni and others 2015; Pan and others 2016; Pan and Xu 2011). However, another highly probable reason for the poor yield of this plant is that *Jatropha* has vigorous vegetative growth, whereas the reproductive growth is relatively poor (Ghosh and others 2010; Kochhar and others 2005). A feasible way to improve the seed yield is by promoting flowering.

Previous studies have reported that the GA biosynthesis inhibitor paclobutrazol (PAC) can effectively promote floral initiation of *Jatropha* (Ghosh and others 2010, 2011; Song and others 2013; Xu and others 2013). However, the effects on flowering of *Jatropha* by direct GA treatment and GA co-applied with a GA biosynthesis inhibitor have not been reported. In this study, we treated 4-year-old pruned and/or non-pruned *Jatropha* plants with GA and GA biosynthesis inhibitors separately or together to investigate the role of GA in the floral initiation of *Jatropha*. The expression profiles of GA biosynthesis, catabolism, and receptor genes in the shoot apex and inflorescence bud before and after floral initiation were also determined. The results in this study

demonstrated that the exogenous application of GA inhibited flowering in *Jatropha*, while GA biosynthesis inhibitors promoted flowering. The results in this study could shed light on the mechanism of floral transition in *Jatropha* and/or other woody plants.

Materials and Methods

Plant Material

Four-year-old *Jatropha* plants were grown at the Xishuangbanna Tropical Botanical Garden (XTBG, 21°54'N, 101°46'E, 580 m in altitude) of the Chinese Academy of Sciences located in Mengla County, Yunnan Province, southwest China. The dry season lasts from November to April, and the rainy season lasts from May to October. The average rainfall, temperature, and relative humidity in April to July 2013, when the experiments were conducted, were 187.6 mm, 25.8 °C, and 85.8%, respectively (data from Xishuangbanna Station for Tropical Rain Forest Ecosystem Studies). The trees were planted 2.5 m × 2.5 m apart. Treatments were performed on pruned and non-pruned *Jatropha* plants. Pruning was conducted in January 2013 during the period of dormancy.

Plant Treatments and Data Collection

Application of GA₃ to Pruned and Non-pruned *Jatropha* Plants

When the new shoots of the pruned plants were 20 cm in length and the newly emerged leaves of the non-pruned plants had expanded, gibberellic acid (GA₃, Bio Basic Inc., Toronto, Ontario, Canada) was applied as a foliar spray to the entire pruned and non-pruned *Jatropha* trees at concentrations of 0, 50, 100, 200, 400, 800, 1200, and 1600 μM using a sprayer. Each GA₃ working solution contained an equal volume of alcohol, which was used as a solvent for GA₃. A surfactant, Tween-20 (Polysorbate-20, Shanghai Sangon Biological Engineering Technology & Services Co., Ltd., China), was added to all the spray solutions at a concentration of 0.05%. The spraying was consecutively conducted three times at 10-day interval. Phenotypic observations and data collection were performed 2 months after the treatment. For the non-pruned *Jatropha* plants, we randomly marked 30 branches per plant to estimate the total number of inflorescences and infructescences per branch. However, because the pruned *Jatropha* plants produced few flowers at that time, the ratio of the number of reproductive branches to the total number of branches was determined to represent the percentage of reproductive branches.

Application of GA Biosynthesis Inhibitors to Pruned *Jatropha* Plants

GA biosynthesis inhibitors, chlormequat chloride (CCC), mepiquat chloride (*N*-dimethylpiperidinium chloride, DPC) and PAC, were supplied as wettable powders (products of Zhengzhou Century Technology Co., Ltd., China), the purities of which were 98, 97, and 15%, respectively. When the new shoots of the pruned plants were 20 cm in length, dilute suspensions of CCC, DPC, and PAC were sprayed onto the entire pruned *Jatropha* plants at concentrations of 500, 1500, 3000, and 4500 mg/l with Tween-20 at a concentration of 0.05%. Control plants were sprayed with distilled water containing 0.05% Tween-20. The spraying was consecutively conducted twice with a 10-day interval. Phenotypic observations and data collection were performed 2 months after the treatments. The percentages of reproductive branches were then determined.

Co-application of GA and PAC to the Pruned *Jatropha* Plants

The soil application of PAC (8 g per plant) was carried out during the period of dormancy according to Ghosh's method (Ghosh and others 2010). Various concentrations of GA₃ were sprayed onto PAC-treated plants when the new shoots of the pruned plants were 20 cm in length. The GA₃ treatments were consecutively conducted three times at 10-day interval. Phenotypic observations and data collection were performed 2 months after the treatments. We randomly marked 30 branches per plant to estimate the total number of inflorescences and infructescences per branch. The percentages of reproductive branches were also determined.

RNA Extraction and Quantitative Real-Time PCR (qRT-PCR)

For the expression profile analysis of GA biosynthesis and catabolism genes, the shoot apex and inflorescence bud before and after floral initiation of 4-year-old *Jatropha* plants were sampled and immediately frozen in liquid nitrogen and then maintained at −80 °C. Total RNA was isolated according to the method reported by Ding and others (2008). The RNA was quantified using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, USA). Approximately one µg of total RNA was used for cDNA synthesis according to the method described in the Takara PrimeScript™ RT Reagent Kit (Takara Biotechnology, Dalian, China). qRT-PCR was performed with the LightCycler 480 II (Roche, Switzerland) using three independent biological replicates and three technical replicates for each sample. The data were analyzed using the $2^{-\Delta\Delta C_T}$ method as described by Livak and Schmittgen (2001). The transcript levels of specific genes were normalized using *JcACTIN* (Zhang and

others 2013). The primers used are listed in Supplementary Table S1.

Statistical Analysis

The data were analyzed using the Statistical Product and Service Solutions software (SPSS Inc., Chicago, IL, USA, version 16.0). Differences among the means were determined using a one-way ANOVA with Tukey's or Tamhane's post hoc tests ($p < 0.05$). Graphs were drawn with SigmaPlot (version 12.5; Systat Software, Point Richmond, CA).

Results

GA₃ Treatments Decreased the Percentages of Reproductive Branches and the Total Number of Inflorescence and Infructescence

To study the effect of GA treatment on *Jatropha* floral initiation, different concentrations of GA₃ were applied to pruned and non-pruned *Jatropha* plants. The results showed that GA₃ treatment profoundly inhibited the floral initiation of *Jatropha* in proportion to the concentration of GA₃ (Fig. 1). In the pruned plants, the percentages of reproductive branches were significantly reduced in four treatments (100, 800, 1200, 1600 µM), but no significant difference was found among the four treatments (Fig. 1c). In the non-pruned plants, the total number of inflorescences and infructescences per branch was significantly decreased from 1.7 (control) to 0.5 (800 µM GA₃), which was not significantly different from that of the GA₃ concentrations > 800 µM (Fig. 1b, d).

Treatment with GA Biosynthesis Inhibitors Increased the Percentages of Reproductive Branches

Three GA biosynthesis inhibitors, CCC, DPC, and PAC were applied at various concentrations to the pruned *Jatropha* plants. The results indicated that all three inhibitor treatments can improve flowering in *Jatropha*. The percentage of reproductive branches was significantly increased after treatments with PAC and DPC compared to the control plants and was not different among the four concentrations in each group. In the CCC treatment group, the maximum value was obtained at the concentration of 1500 mg/l, which was significantly higher than that obtained at the concentrations of 500 mg/l and 4500 mg/l (Fig. 2). The percentage was 8.2% in the controls and was increased to 53.8, 43.5, and 45.0% respectively, after the PAC (3000 mg/l), DPC (1500 mg/l), and CCC (1500 mg/l) treatments (Fig. 2). The highest percentage among the three groups of treatments was obtained at 3000 mg/l in PAC treatment group (the purity

Fig. 1 GA_3 inhibits flowering in *Jatropha*. **a** Control plant; **b** GA_3 -treated plant (800 μM); **c** effect of GA_3 on percentage of reproductive branches in pruned *Jatropha* plants ($n=21-30$); **d** effect of GA_3 on total number of inflorescence and infructescence per branch

in non-pruned *Jatropha* plants. Values are means $\pm$ standard deviations ($n=15-20$ plants). Different letters above the error bar in (c) and (d) indicate significant difference for $p < 0.05$

Fig. 2 GA biosynthesis inhibitors promote flowering in *Jatropha*. PAC paclobutrazol, DPC mepiquat chloride, CCC chlormequat chloride. Values are means $\pm$ standard deviations ($n=15-20$ plants). Different letters above the error bar indicate significant difference for $p < 0.05$

of the PAC compound was 15%), demonstrating that PAC is more active than DPC and CCC.

GA_3 Treatment Diminished the Promotional Effect of PAC on Floral Initiation

To further confirm the inhibition of floral initiation by GA_3 in *Jatropha*, the co-application of GA_3 and the GA biosynthesis inhibitor (PAC) was conducted in *Jatropha* plants. The pruned plants, which are more sensitive to PAC than non-pruned plants (Ghosh and others 2010; Song and others 2013), were chosen for these experiments. The percentage of reproductive branches was profoundly increased from 10.3% in the control plants to 54.7% after PAC treatment alone (Fig. 3a, b, e). The ability of PAC to improve flowering was diminished by the exogenous application of GA_3 (Fig. 3c, d, f). The percentage of reproductive branches was reduced with increasing concentrations of GA_3 , reaching the lowest at 1600 μM of GA_3 (6.9%), which was not significantly different from the control plants (Fig. 3e). The total number of inflorescences and infructescences per branch exhibited the same trend (Fig. 3f), and was increased profoundly from 0.15 in the controls to 0.60 per branch after PAC treatment alone but decreased significantly to 0.05 per branch after the exogenous application of GA_3 at 1600 μM (Fig. 3f).

Fig. 3 GA₃ reverses the promotive effect of PAC on flowering in *Jatropha*. **a** Control plant; **b** PAC-treated plant; **c** plant treated with PAC+GA₃ (400 μM); **d** plant treated with PAC+GA₃ (1600 μM); the percentage of reproductive branches (**e**) and the total number of

inflorescences and infructescences per branch (**f**) were decreased by GA₃ treatment in PAC-treated plants; values are means ± standard deviations ($n = 30$ plants). Different letters above the error bar in (**e**) and (**f**) indicate significant difference for $p < 0.05$

Expression Analysis of GA Biosynthesis, Catabolism and Receptor Genes During Floral Initiation

To determine probable changes in the level of endogenous GA during floral initiation, the expression profiles of GA biosynthesis, catabolism and receptor genes (Hedden and Phillips 2000) were analyzed in the shoot apex and inflorescence buds before and after floral initiation. Sequences of *Jatropha* homologues of genes encoding two GA biosynthesis genes GA 20-oxidase 1 (*JcGA20ox1*) and GA 3-oxidase 3 (*JcGA3ox3*), four catabolism genes GA 2-oxidase 2/4/6/8 (*JcGA2ox2/4/6/8*), and two GA receptor genes GA-INSENSITIVE DWARF1B (*JcGID1B*) and *JcGID1C*, were indentified from the *Jatropha* Genome Database (<http://www.kazusa.or.jp/jatropha/>, (Hirakawa and others 2012)), and were used for phylogenetic analysis together with those of the *Arabidopsis* genes (The *Arabidopsis* Information Resources; <http://www.arabidopsis.org>) (Figure S1). qRT-PCR analysis (Fig. 4) revealed that *JcGA3ox3*, which catalyze the final step of GA biosynthesis (Hedden and Phillips 2000) was down-regulated in the inflorescence bud after floral initiation compared to their levels in the shoot apex before floral initiation, whereas the expression level of another biosynthetic gene *JcGA20ox1* showed no significant difference. Among the four GA catabolism genes *JcGA2ox2*, *JcGA2ox4*,

Fig. 4 Quantification of the expression of GA biosynthesis, catabolism and receptor genes during floral transition by qRT-PCR. *JcGA20ox1*, *Jatropha* GA 20-oxidase 1; *JcGA3ox3*, *Jatropha* GA 3-oxidase 3; *JcGA2ox2*, *Jatropha* GA 2-oxidase 2; *JcGA2ox4*, *Jatropha* GA 2-oxidase 4; *JcGA2ox6*, *Jatropha* GA 2-oxidase 6; *JcGA2ox8*, *Jatropha* GA 2-oxidase 8; *JcGID1B*, *Jatropha* GA-INSENSITIVE DWARF1B; *JcGID1C*, *Jatropha* GA-INSENSITIVE DWARF 1C. The levels of the detected amplicons were normalized using the amplified product of *JcACTIN*. Values are means ± standard deviations ($n = 3$). *Statistically significant at the 5% level

JcGA2ox6, and *JcGA2ox8*, only the expression of *JcGA2ox8* was up-regulated during floral initiation (Fig. 4). We also detected the expression levels of two GA receptor genes (Murasu and others 2008) encoding *Jatropha* *JcGID1B* and *JcGID1C* (Ni and others 2017), which undergo a conformational changes after the reception of bioactive GA and then binding to DELLA to form the GA–GID1–DELLA complex, which stimulates the degradation of DELLAs by the 26S proteasome, thereby initiating the signal transduction cascade (Sun 2011). *JcGID1C* was down-regulated during the floral transition, whereas the expression level of *JcGID1B* was not significantly affected (Fig. 4).

Discussion

GA has been previously reported to possess an inhibitory role in the floral transition of certain perennials (Wilkie and others 2008). In this study, the results suggested that the exogenous application of GA inhibited floral initiation in *Jatropha*. Consistently, we found that the expression level of a GA biosynthesis gene *JcGA3ox3* was reduced, whereas that of *JcGA2ox8*, a GA degradation gene was elevated after floral initiation (Fig. 4), indicating a decrease in endogenous GA contents during floral transition. And the down-regulated expression of the GA receptor gene *JcGID1C* after floral initiation (Fig. 4), which could result in a decreased GA sensitivity, may further reinforce the regulation of GA-responsive genes involved in floral initiation (Campos-Rivero and others 2017; Conti 2017; Davière and Achard 2013). The result is in agreement with previous studies of other woody plant species. In *M. indica*, the GA content in shoot apical meristem (SAM) was decreased during the floral transition (Chen 1987). In rose, a decrease in the GA content in SAM was a prerequisite for floral transition (Guo and others 2017; Roberts and others 1999). Furthermore, the variation of GA content was correlate with the expression of GA metabolism genes in rose during floral transition (Guo and others 2017; Remay and others 2009). In both non-recurrent and recurrent blooming rose, GA biosynthesis gene *RoGA20ox* was transiently repressed before floral initiation, whereas the expression of GA degradation gene *RoGA2ox* increased (Remay and others 2009). Recently, Guo and others (2017) also showed that in recurrent flowering *Rosa chinensis* ‘Old Blush,’ a common ancestor of modern roses, GA biosynthesis genes, such as *ent-copalyl diphosphate synthase (CPS)*, *ent-kaurene oxidase (KO)*, *GA20ox1*, and *GA3ox*, were down-regulated from vegetative meristem to pre-floral meristem, while *GA2ox* was up-regulated. However, the expression of GA receptor gene *RoGID1* in rose was regulated differently between the non-recurrent and the recurrent blooming rose (Remay and others 2009). In non-recurrent rose whose flowering was inhibited by exogenous

applications of GA₁ and GA₃ (Roberts and others 1999), *RoGID1* was expressed at the same level during the floral process; whereas in recurrent rose whose flowering was not inhibited by exogenous applications of GA (Roberts and others 1999), *RoGID1* was up-regulated after the floral initiation (Remay and others 2009). In this study, *JcGID1C*, one of the two GA receptor genes in *Jatropha* whose flowering was inhibited by exogenous applications of GA₃ as shown above, was down-regulated during the floral transition, whereas another member *JcGID1B* remained stable (Fig. 4). Therefore, the molecular mechanism of GA-mediated inhibition of flowering in woody plants remains elusive.

In *Arabidopsis*, the elevated levels of endogenous GA₄ or exogenous application of GA₃ promoted the termination of vegetative phase and increase the expression of *LEAFY* (*LFY*), which induces the expression of the GA catabolism enzyme *EUI-LIKE P450 A1 (ELA1)*, resulting in a decrease in GA content (Yamaguchi and others 2014). The subsequent reduction in the GA levels allows the DELLA protein to bind SPL, inducing *API* expression and promoting the transformation of the lateral primordia into flowers (Yamaguchi and others 2014). The ability of GA to promote the transition from the juvenile to the adult growth phase was observed in several species (Mutasa-Göttgens and Hedden 2009). A previous study also has reported that GA promoted the flowering of *Arabidopsis* by activating the *LEAFY (LFY)* promoter (Blázquez and others 1998). However, in *Jatropha*, the expression of *Jatropha LFY (JcLFY)* was not induced but rather was repressed between 3 and 48 h after GA application both in the shoot apex and the inflorescence bud (Tang and others 2016a). Overexpressing *JcLFY* in *Arabidopsis* causes significant early flowering while the overexpression of this gene in *Jatropha* causes slight early flowering, only 2 months earlier than the WT plant (Tang and others 2016a), which has also been observed in apple (Flachowsky and others 2010) and poplar (Rottmann and others 2000). In addition, a transgenic *Jatropha* with co-suppressed *JcLFY* did not exhibit significantly late flowering (Tang and others 2016a). These results suggested that *JcLFY* is not a key flowering promoter in *Jatropha*. Thus, although GA application repressed *JcLFY* expression, the GA-mediated inhibition of flowering in *Jatropha* may be unrelated to *JcLFY*. Moreover, the overexpression of *JcAPI* did not cause early flowering in *Jatropha* but did cause early flowering in *Arabidopsis* (Tang and others 2016b), as has also been observed in aspen (Azeez and others 2014). Thus, the molecular mechanism of the GA-regulated floral transition in woody plants, at least in *Jatropha*, is complicated and may be different from that in *Arabidopsis*, which may involve multiple factors (Albani and Coupland 2010).

Our results in this report provide strong evidence that GA inhibits flowering in woody *Jatropha*. However, additional experiments including detection of the GA contents during

floral initiation by new sensitive methods for GA quantification (Deng and others 2017; Li and others 2016, 2017), expression analysis of *DELLA* and other genes in the GA signaling pathway (Davière and Achard 2013), and modulation of GA contents in shoot apex by transgene expression, are required to elucidate the cellular and molecular mechanisms underlying GA regulation of floral initiation in *Jatropha*. In addition, several studies with adult *Jatropha* plants showed that GA biosynthesis inhibitor PAC, rather than GA, promoted reproductive development (Ghosh and others 2011, 2010; Song and others 2013; Xu and others 2013), which seems contrary to the role of GA in promoting the transition from vegetative to reproductive development in *Arabidopsis* (Yamaguchi and others 2014). Therefore, further studies with *Jatropha* seedlings are required to investigate the role of GA in the transition from juvenile to adult phase.

Acknowledgements The authors gratefully acknowledge the Xishuangbanna Station for Tropical Rain Forest Ecosystem Studies for granting permission to use the data presented in this study and the Central Laboratory of the Xishuangbanna Tropical Botanical Garden for providing research facilities.

Author Contributions Z-FX, JL, and B-ZP designed experiments. JL and B-ZP analyzed the data and drafted the work. JL and LN carried out experiments, Z-FX, M-SC, and MT critically revised the manuscript.

Funding This work was supported by funding from the National Natural Science Foundation of China (31500500 and 31370595), the Natural Science Foundation of Yunnan Province (2016FB033), and the Program of Chinese Academy of Sciences (ZSZC-014, 2017XTBG-T02).

Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

- Abdelgadir H, Jäger A, Johnson S, Van Staden J (2010) Influence of plant growth regulators on flowering, fruiting, seed oil content, and oil quality of *Jatropha curcas*. *S Afr J Bot* 76:440–446
- Albani MC, Coupland G (2010) Comparative analysis of flowering in annual and perennial plants. *Curr Top Dev Biol* 91:323–348
- Azeez A, Miskolczy P, Tylewicz S, Bhalarao RP (2014) A tree ortholog of *APETALA1* mediates photoperiodic control of seasonal growth. *Curr Biol* 24:717–724
- Blázquez MA, Green R, Nilsson O, Sussman MR, Weigel D (1998) Gibberellins promote flowering of *Arabidopsis* by activating the *LEAFY* promoter. *Plant Cell* 10:791–800
- Boss PK, Thomas MR (2002) Association of dwarfism and floral induction with a grape ‘green revolution’ mutation. *Nature* 416:847–850
- Boss PK, Bastow RM, Mylne JS, Dean C (2004) Multiple pathways in the decision to flower: enabling, promoting, and resetting. *Plant Cell* 16:S18–S31
- Campos-Rivero G, Osorio-Montalvo P, Sánchez-Borges R, Us-Camas R, Duarte-Aké F, De-la-Peña C (2017) Plant hormone signaling in flowering: an epigenetic point of view. *J Plant Physiol* 214:16–27. <https://doi.org/10.1016/j.jplph.2017.03.018>
- Chen W-S (1987) Endogenous growth substances in relation to shoot growth and flower bud development of mango. *J Am Soc Hortic Sci* 112:360–363
- 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
- Conti L (2017) Hormonal control of the floral transition: can one catch them all? *Dev Biol* 430:288–301. <https://doi.org/10.1016/j.ydbio.2017.03.024>
- Davière J-M, Achard P (2013) Gibberellin signaling in plants. *Development* 140:1147–1151. <https://doi.org/10.1242/dev.087650>
- Deng T, Wu D, Duan C, Yan X, Du Y, Zou J, Guan Y (2017) Spatial profiling of gibberellins in a single leaf based on microscale matrix solid-phase dispersion and precolumn derivatization coupled with ultraperformance liquid chromatography-tandem mass spectrometry. *Anal Chem* 89:9537–9543. <https://doi.org/10.1021/acs.analchem.7b02589>
- Ding L-W, Sun Q-Y, Wang Z-Y, Sun Y-B, Xu ZF (2008) Using silica particles to isolate total RNA from plant tissues recalcitrant to extraction in guanidine thiocyanate. *Anal Biochem* 374:426–428
- Edrisi SA et al (2015) *Jatropha curcas* L.: a crucified plant waiting for resurgence. *Renew Sustain Energy Rev* 41:855–862
- Flachowsky H, Hättasch C, Höfer M, Peil A, Hanke M-V (2010) Overexpression of *LEAFY* in apple leads to a columnar phenotype with shorter internodes. *Planta* 231:251–263
- García-Pallas I, Val J, Blanco A (2001) The inhibition of flower bud differentiation in ‘Crimson Gold’ nectarine with GA 3 as an alternative to hand thinning. *Sci Hortic* 90:265–278
- Ghosh A, Chikara J, Chaudhary DR, Prakash AR, Boricha G, Zala A (2010) Paclobutrazol arrests vegetative growth and unveils unexpressed yield potential of *Jatropha curcas*. *J Plant Growth Regul* 29:307–315
- Ghosh A, Chikara J, Chaudhary DR (2011) Diminution of economic yield as affected by pruning and chemical manipulation of *Jatropha curcas* L. *Biomass Bioenergy* 35:1021–1029. <https://doi.org/10.1016/j.biombioe.2010.11.019>
- Gonzalez-Rossia D, Juan M, Reig C, Agusti M (2006) The inhibition of flowering by means of gibberellic acid application reduces the cost of hand thinning in Japanese plums (*Prunus salicina* Lindl.). *Sci Hortic* 110:319–323
- Guo X et al (2017) Transcriptome of the floral transition in *Rosa chinensis* ‘Old Blush’. *BMC Genomics* 18:199. <https://doi.org/10.1186/s12864-017-3584-y>
- Hedden P, Phillips AL (2000) Gibberellin metabolism: new insights revealed by the genes. *Trends Plant Sci* 5:523–530
- Hirakawa H et al (2012) Upgraded genomic information of *Jatropha curcas* L. *Plant Biotechnol* 29:123–130
- King RW et al (2006) Regulation of flowering in the long-day grass *Lolium temulentum* by gibberellins and the *FLOWERING LOCUS T* gene. *Plant Physiol* 141:498–507. <https://doi.org/10.1104/pp.106.076760>
- Kochhar S, Kochhar V, Singh S, Katiyar R, Pushpangadan P (2005) Differential rooting and sprouting behaviour of two *Jatropha* species and associated physiological and biochemical changes. *Curr Sci* 89:936–939
- Koh MY, Mohd Ghazi TI (2011) A review of biodiesel production from *Jatropha curcas* L. oil. *Renew Sustain Energy Rev* 15:2240–2251
- Lang A (1957) The effect of gibberellin upon flower formation. *Proc Natl Acad Sci USA* 43:709–717

- Langridge J (1957) Effect of day-length and gibberellic acid on the flowering of *Arabidopsis*. *Nature* 180:36–37
- Lenahan OM, Whiting MD, Elfving DC (2006) Gibberellic acid inhibits floral bud induction and improves ‘Bing’ sweet cherry fruit quality. *HortScience* 41:654–659
- Li D, Guo Z, Chen Y (2016) Direct derivatization and quantitation of ultra-trace gibberellins in sub-milligram fresh plant organs. *Mol Plant* 9:175–177. <https://doi.org/10.1016/j.molp.2015.07.001>
- Li D, Guo Z, Liu C, Li J, Xu W, Chen Y (2017) Quantification of near-attomole gibberellins in floral organs dissected from a single *Arabidopsis thaliana* flower. *Plant J* 91:547–557. <https://doi.org/10.1111/tpj.13580>
- Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* 25:402–408
- Menzel C, Simpson D (1990) Effect of paclobutrazol on growth and flowering of lychee (*Litchi chinensis*). *Aust J Exp Agric* 30:131–137
- Muñoz-Fambuena N, Mesejo C, González-Mas MC, Iglesias DJ, Primo-Millo E, Agustí M (2012) Gibberellic acid reduces flowering intensity in sweet orange [*Citrus sinensis* (L.) Osbeck] by repressing *CiFT* gene expression. *J Plant Growth Regul* 31:529–536
- Murase K, Hirano Y, Sun T-p, Hakoshima T (2008) Gibberellin-induced DELLA recognition by the gibberellin receptor *GID1*. *Nature* 456:459–463
- Mutasa-Göttgens E, Hedden P (2009) Gibberellin as a factor in floral regulatory networks. *J Exp Bot* 60:1979–1989
- Ni J, Gao C, Chen MS, Pan BZ, Ye K, Xu ZF (2015) Gibberellin promotes shoot branching in the perennial woody plant *Jatropha curcas*. *Plant Cell Physiol* 56:1655–1666
- Ni J, Zhao M, Chen M, Pan B, Tao Y, Xu Z (2017) Comparative transcriptome analysis of axillary buds in response to the shoot branching regulators gibberellin A3 and 6-benzyladenine in *Jatropha curcas*. *Sci Rep* 7:11417
- 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
- Pan BZ, Luo Y, Song L, Chen MS, Li J, Xu ZF (2016) Thidiazuron increases fruit number in the biofuel plant *Jatropha curcas* by promoting pistil development. *Ind Crops Prod* 81:202–210
- Pandey VC, Singh K, Singh JS, Kumar A, Singh B, Singh RP (2012) *Jatropha curcas*: a potential biofuel plant for sustainable environmental development. *Renew Sustain Energy Rev* 16:2870–2883
- Remay A, Lalanne D, Thouroude T, Le Couviour F, Hibrand-Saint Oyant L, Foucher F (2009) A survey of flowering genes reveals the role of gibberellins in floral control in rose. *Theor Appl Genet* 119:767–781. <https://doi.org/10.1007/s00122-009-1087-1>
- Roberts AV, Blake PS, Lewis R, Taylor JM, Dunstan DI (1999) The effect of gibberellins on flowering in roses. *J Plant Growth Regul* 18:113–119. <https://doi.org/10.1007/pl00007058>
- Rottmann WH et al (2000) Diverse effects of overexpression of *LEAFY* and *PTLF*, a poplar (*Populus*) homolog of *LEAFY/FLORICAULA*, in transgenic poplar and *Arabidopsis*. *Plant J* 22:235–245
- Salazar-Garcia S, Lovatt CJ (1998) GA3 application alters flowering phenology of ‘Hass’ avocado. *J Am Soc Hortic Sci* 123:791–797
- Sanderson K (2009) Wonder weed plans fail to flourish. *Nature* 461:328
- Song J, Chen M-S, Li J-L, Niu L-J, Xu Z-F (2013) Effects of soil-applied paclobutrazol on the vegetative and reproductive growth of biofuel plant *Jatropha curcas*. *Plant Divers Resour* 35:173–179
- Sorce C, Stevanato P, Biancardi E, Lorenzi R (2002) Physiological mechanisms of floral stem elongation (bolting) control in sugar beet (*Beta vulgaris* ssp. *vulgaris* L.). *Agroindustria* 1:87–91
- Sun T-p (2011) The molecular mechanism and evolution of the GA–GID1–DELLA signaling module in plants. *Curr Biol* 21:R338–R345
- Tang MY, Tao YB, Fu QT, Song YL, Niu LJ, Xu ZF (2016a) An ortholog of *LEAFY* in *Jatropha curcas* regulates flowering time and floral organ development. *Sci Rep* 6:37306. <https://doi.org/10.1038/srep37306>
- Tang MY, Tao YB, Xu ZF (2016b) Ectopic expression of *Jatropha curcas APETALA1 (JcAPI)* caused early flowering in *Arabidopsis*, but not in *Jatropha*. *PeerJ* 4:e1969. <https://doi.org/10.7717/peerj.1969>
- Tongumpai P, Jutamanee K, Sethapakdi R, Subhadrabandhu S (1989) Variation in level of gibberellin-like substances, during vegetative growth and flowering of mango cv. Khiew Sawoei. *III Int Mango Symp* 291:105–108
- Wellmer F, Riechmann JL (2010) Gene networks controlling the initiation of flower development. *Trends Genet* 26:519–527
- Wilkie JD, Sedgley M, Olesen T (2008) Regulation of floral initiation in horticultural trees. *J Exp Bot* 59:3215–3228
- Winston E (1992) Evaluation of paclobutrazol on growth, flowering and yield of mango cv. Kensington Pride. *Aust J Exp Agric* 32:97–104
- Xu G, Luo R, Yao Y (2013) Paclobutrazol improved the reproductive growth and the quality of seed oil of *Jatropha curcas*. *J Plant Growth Regul* 32:875–883. <https://doi.org/10.1007/s00344-013-9353-5>
- Yamaguchi N, Winter CM, Wu M-F, Kanno Y, Yamaguchi A, Seo M, Wagner D (2014) Gibberellin acts positively then negatively to control onset of flower formation in *Arabidopsis*. *Science* 344:638–641
- Zhang L, He L-L, Fu Q-T, Xu Z-F (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. <https://doi.org/10.3390/ijms141224338>