Delineating the genetic regulation of cannabinoid biosynthesis during female flower development in *Cannabis sativa*

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SUMMARY

- Cannabinoids are predominantly produced in the glandular trichomes on cannabis female flowers. There is little known on how cannabinoid biosynthesis is regulated during female flower development. We aim to understand the rate-limiting step(s) in the cannabinoid biosynthetic pathway.
- We investigated the transcript levels of cannabinoid biosynthetic genes as well as cannabinoid contents during 7 weeks of female flower development. We demonstrated that the enzymatic steps for producing CBG, which involve genes *GPPS*, *PT* and *OAC*, could be rate limit cannabinoid biosynthesis. Our findings further suggest that cannabinoid synthases, *CBDAS* and *THCAS* in a hemp and medical marijuana variety respectively, are not critical for cannabinoid biosynthesis. The cannabinoid biosynthetic genes are generally upregulated during flower maturation, which indicate glandular trichome development.
- MeJA can potentially increase cannabinoid production. We propose that biweekly application of 100 µM MeJA staring from flower initiation would be efficacious for promoting cannabinoid biosynthesis.
- Our findings suggest that the step of CBG production could rate limit the terminal cannabinoid biosynthesis. In addition, different cannabis varieties demonstrated discrete transcriptional regulation of cannabinoid biosynthetic genes.

Key words: *Cannabis sativa*, cannabinoid biosynthesis, CBDAS, GPPS, THCAS, methyl jasmonate, prenyltransferase, transcriptional regulation

INTRODUCTION

Cannabis sativa (cannabis or *C. sativa*) is currently garnering attention for its important chemical constituents known as cannabinoids, which have a number of pharmacological properties, including chronic pain treatment, seizure mitigation, spasticity reduction, and a host of others (Brodie & Ben-Menachem, 2018; Groce, 2018). Female plants of dioecious cannabis varieties are favored for cannabinoid production since the unfertilized flowers produce cannabinoids in the highest concentration in the glandular trichomes of high density. Terminal cannabinoids such as cannabidiolic acid (CBDA), tetrahydrocannabinolic acid (THCA), and cannabichromenic acid (CBCA) are the products of a biosynthesis pathway (Fig. S1).

Although there are published studies on cannabis horticulture, trichome cell biology and the chemistry of its valuable metabolites, little is known on the regulation of cannabinoid biosynthesis in *C. sativa*. We aimed to elucidate (a) how cannabinoid biosynthesis is regulated, (b) which of the enzymatic step(s) in the biosynthetic pathway rate-limit the production of THCA and CBDA, and (c) what factors and developmental programming that occur in the flowers of female plants that could be manipulated to enhance and improve the quality of production and/or prevent occurrences such as unacceptably high THCA generation in hemp varieties cultivated for CBD production. Our experimental work attempted to bring new information and understanding to these questions and aimed to add greater resolution by measuring cannabinoid content and expression of key biosynthesis genes as the female flower matures and generates cannabinoids. Another factor that may be important for cannabinoid production is trichome density. Studies have shown that the exogenous application of the plant hormone, jasmonic acid, increases trichome density and secondary metabolite concentrations in plants containing glandular trichomes (Boughton *et al.*,

2005; Yan *et al.*, 2017). However, little research has been done to evaluate if applications of methyl jasmonate (MeJA) increases cannabinoid content in female flowers of cannabis plants.

In this report, we monitored transcripts of cannabinoid biosynthetic genes during 7 weeks of female flower development in two cannabis varieties, a hemp variety Cherry Wine (CW) and a medical marijuana variety Gorilla Glue (GG). Meanwhile, we measured cannabinoid concentrations at the same time points. In general, cannabinoid biosynthesis showed correlation with the expression of the corresponding gene except for THC and *THCAS* in GG, which was also demonstrated in the Pearson's matrix. In addition, we examined the effect of MeJA on cannabinoid production. The results showed that MeJA increased THC content by about 21% percent compared to control. The rate limiting step(s) of cannabinoid biosynthesis was not understood and is discussed here.

MATERIALS AND METHODS

Plant materials. Cuttings of 5-month old GG mother plants were taken and rooted in an EZ-Cloner Classic ChamberTM (Sacramento, California) under aeroponic conditions. Rooted cuttings were grown under 18 h light/ 6 h darkness for 9 weeks in the closed-loop commercial facility, CTPharma LLC. Plants were then grown under 12 h light/12 h darkness for reproductive growth.

Cuttings of 5-month old CW mother plants grown in the greenhouse at University of Connecticut were taken to produce new clones for experiments. Plants were grown under 18 h light/6 h darkness for vegetative growth. After 3 weeks of vegetative growth, plants shifted to a new cycle of 12 h light/12 h darkness to initiate floral development. The plants were maintained in this environment for 7 weeks.

Sample collection, RNA isolation, and cDNA synthesis. Approximately 0.1 g of flower material was collected and then immediately placed into liquid nitrogen for flash freezing. Samples were then stored in a -80˚C freezer until RNA extraction. Macherey-Nagel Plant and Fungi Mini kit for RNA (Düren, Germany) was used for RNA extraction according to the manufacturer's protocol with modifications. Single-stranded cDNA was synthesized from 1 µg of the isolated RNA using the Bio-Rad iScript™ cDNA synthesis kit (Hercules, California).

Cannabinoid Extraction and HPLC Analysis. Approximately 0.5 g of flower material was added to individual 50 mL Falcon® tubes, then 20 mL of 25°C 9:1 methanol:dichloromethane (v/v) was added to tubes. Each tube was vortexed vigorously for 3 sec, and then tubes were placed on a shaker plate for 90 min. Tubes were then vortexed vigorously again for 3 sec before transferring 1-2 mL of extract into a syringe and pressing the liquid through 0.45 µm filter into a 2 mL microcentrifuge tube. Following filtration, 100 µL of extract was added to 900 µL of methanol in autosampler vials. The extracts were stored at -20℃ until further analysis.

Cannabinoids were resolved on a 1260 Agilent High Pressure Liquid Chromatograph (HPLC) instrument (Santa Clara, California) using the Restek Raptor ARC C18 column (150 x 4.6 mm, 2.7 µm particle size) (Bellefonte, Pennsylvania) and a binary isocratic gradient mobile phase A (water, 5 mM ammonium formate, 0.1% (v/v) formic acid) and mobile phase B (acetonitrile, 0.1% (v/v) formic acid) at 30℃. A UV detector at 228 nm was implemented for the analysis. Peaks on the chromatograms were identified through comparison of retention times of cannabinoid reference RestekTM (Bellefonte, Pennsylvania) standards. Cannabinoid quantification was performed by comparing values to a calibration curve created with cannabinoid reference standards.

Real Time PCR. Reactions were performed with the Bio-Rad iTaq Universal SYBR Green Supermix (Hercules, California). Primers were chosen based off what other authors have used in other publications (Table S1). Ubiquitin (UBQ) was used as a reference gene. RT-qPCR analysis was performed using four biological replicates.

Methyl Jasmonate (MeJA) treatment. Different concentrations of MeJA (Food Grade, Sigma Aldrich) were prepared from a stock 95% MeJA solution: 100 µM, and 500 µM, 1000 µM, and a water control. In CTPharma LLC., MeJA was applied to three biological replicates of 'White Tangy Haze' for each concentration to the point of runoff after they had developed flowers for 2 weeks. Each plant received approximately 1 L of MeJA solution. Weekly samples for cannabinoid analysis were harvested for the next 4 weeks.

RESULTS

The correlation between cannabinoid production and the transcript levels of corresponding biosynthetic genes

To learn cannabinoid production during flowering development, we examined flowers over the course of the 7-week flower development period (Fig. S2). Week 1 is right after the transition to reproductive growth and week 7 flowers are fully matured. Cannabigerol (CBG) levels typically did not rise above 2% in any variety that we studied. In CW, CBG levels increased consistently and peaked in week 4. After week 4, CBG was maintained at a similar level (Fig. 1a, c, black line). In GG, CBG levels also peaked in week 4 but decreased thereafter over the next three weeks (Fig. 1b, d, black line). The enzyme, PT, catalyzes the alkylation of GPP and OA to create the precursor cannabinoid, CBGA. PT1 was initially found to catalyze CBGA biosynthesis (Page & Boubakir, 2012). Recently PT4 was reported to have higher enzymatic activity (Blatt-Janmaat & Qu, 2021). Therefore, we investigated both gene expression of *PT1* and *PT4*. Both genes are enriched in

glandular trichomes and localized in the chloroplast (Page & Boubakir, 2012; Gülck *et al.*, 2020). We examined the expression of both genes to learn how these two genes are transcriptionally regulated during female flower development.

In CW, *PT1* transcripts peaked in the week 5 and this greater level of expression was maintained through the following weeks of flower development (Fig. 1a). In GG, *PT1* transcripts

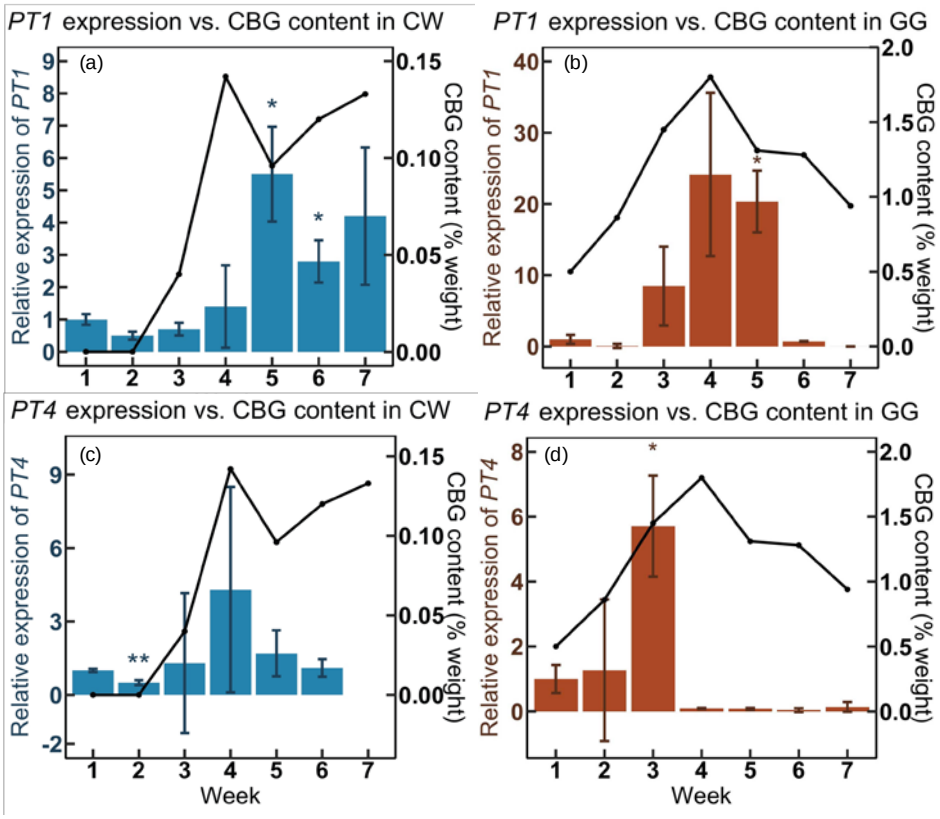

Figure 1. The remained CBG amount and the gene expression of *PT1* and *PT4* over the course of seven-week flower development in CW (a, c) and GG (b, d). y axes on the left show relative expression of *PT1* and *PT4*. y axes on the right show the weight percentage of CBG in wet flower samples. Data are presented as means ± SE (n = 4). Significant difference of $P < 0.05$ (*) and $P < 0.01$ (**) was determined by Student *t*-test.

were upregulated week 3 through week 5, but were abolished in later weeks (Fig. 1b). *PT4* expression in CW showed some interesting fluctuation (Fig. 1c). *PT4* was abruptly increased in week 4 but reduced to the basal level in weeks 5 and 6, while it again surged in week 7. Unlike in CW, *PT4* transcripts in GG were only significantly upregulated in week 3 and then eliminated in

the following weeks (Fig. 1d). In general, *PT1* and *PT4* are similarly regulated during female flower maturation in the same variety but the regulation is temporally different. The superimposition suggests that *PT1* instead of *PT4* more correlates with CBG content, which is not consistent with the recent findings (Blatt-Janmaat & Qu, 2021). Further analysis of these two enzymes will be more explicitly when there are *PT1* or *PT4* cannabis mutants available.

We next examined cannabinoids and their corresponding synthase genes. It is shown that CBD accumulation was steadily increased from week 1 to week 7 in CW (Fig. 2a, black line) even though CBG stopped increasing after week 4 (Figure 1a, black line). *CBDAS* showed a similar expression pattern to that of *PT1* in CW, with the expression in weeks 5 to 7 being plateaued. However, *CBDAS* expression is different from the pattern of CBD accumulation (Fig. 2a), suggesting that CBD production is not dependent on *CBDAS* transcript levels but likely by the available CBG content in the secretory reservoir. In the marijuana variety GG, no CBD was detected despite that *CBDAS* transcripts could still be measured (Fig. 2b). Unlike the upregulation of *CBDAS* in CW, *CBDAS* expression in GG was maintained at similar levels during the whole period of flower development. With *CBDAS* transcripts being detected, CBDAS enzyme should be produced in GG. One possibility for the non-detected CBD is that there are mutations in the *CBDAS* gene that result in a nonfunctional protein. It is also possible that the amount of CBDAS enzyme is very low and the synthesized CBD is under the detectable level.

In CW, THC biosynthesis and *THCAS* gene expression showed a very similar trend to that of CBD production and *CBDAS* gene expression respectively (Fig. 2c). *THCAS* gene expression was significantly increased through week 4-7 in CW (Fig. 2c, while it had a descending trend during flower development in GG (Fig. 2d). Despite of the drastic decrease of CBG after week 4 (Fig. 1b,d, black line), THC production in GG still constantly increased during flower

development, with a slight drop in week 7 (Fig. 2d, black line). The findings on THC production indicate that THC biosynthesis does not rely on the upregulation of *THCAS* gene expression.

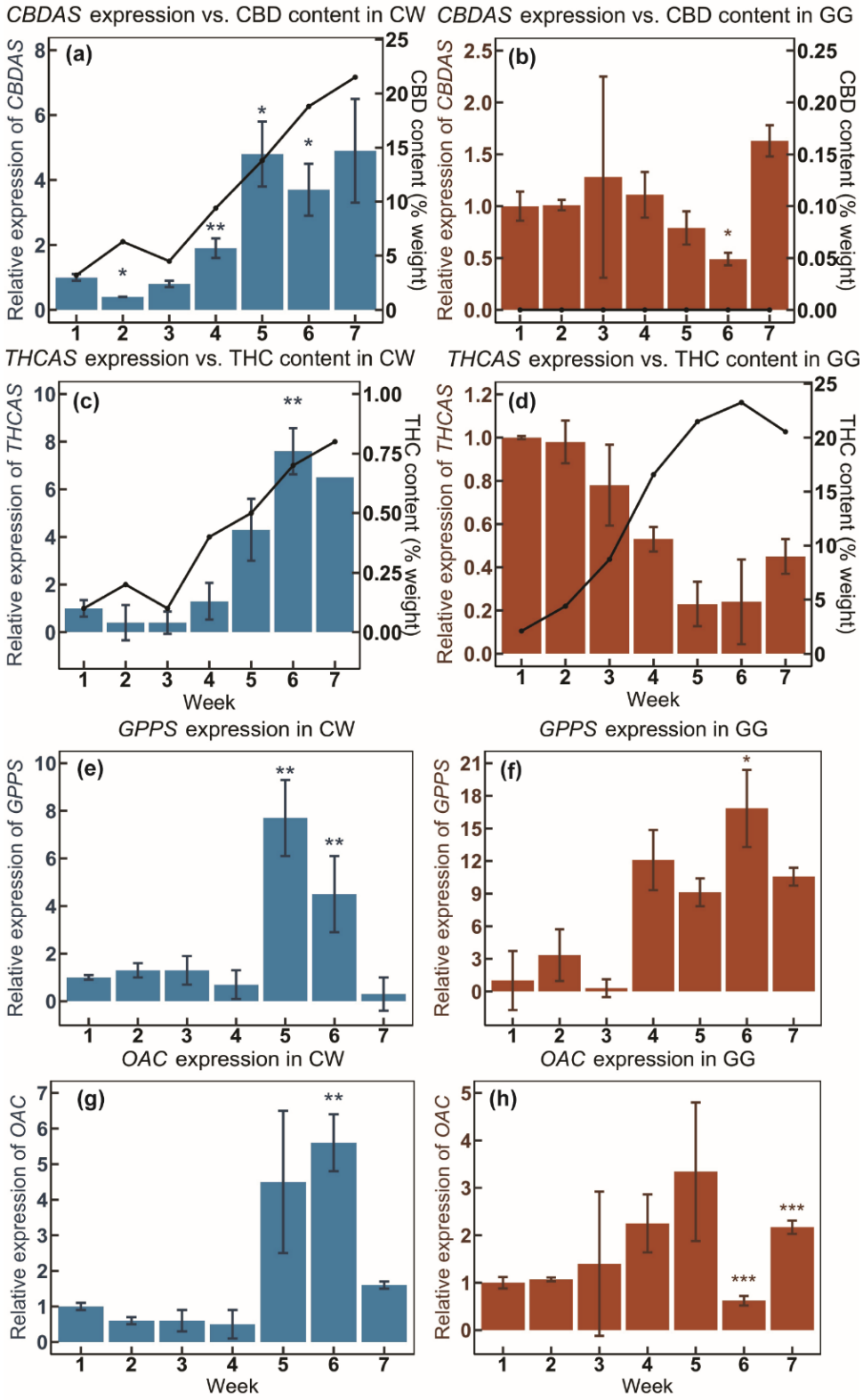

Figure 2. Analysis of CBD and THC accumulation with the expression of genes in the cannabinoid biosynthetic pathway over the course of seven-week flower development. (a, b) Superimposition of CBD content with *CBDAS* expression in CW (a) and GG (b). (c, d) Superimposition of THC content with *THCAS* expression in CW (c) and GG (d). Gene expression of *GPPS* and *OAC* in CW (c, g) and GG (d, h). Data are presented as means ± SE (n = 4). Significant difference of $P < 0.05$ (*), $P < 0.01$ (**) and $P < 0.001$ (***) was determined by Student *t*-test.

Even though CBC possesses very low amount compared to the other two cannabinoids, *CBCAS* was still highly upregulated in both the two varieties (Fig. S3). *CBCAS* was abruptly upregulated 15 folds in week 5 in CW following by dramatic decrease in the last two weeks (Fig. S3a). CBC content did not rise above 0.5% dry weight in CW (data not shown) and there was no marked increase in the rate of production of CBC itself. In GG, there was a steady increase in *CBCAS* gene expression and the high expression was maintained to the last week (Fig. S3b).

Regulation of upstream genes in cannabinoid biosynthetic pathway

GPPS is an enzyme in the plastidial 2-C-methyl-D-erythritol 4 phosphate (MEP) pathway that produces GPP (Fig. S1). In CW, *GPPS* expression levels did not change in week 1 through week 4 but was significantly increased in week 5 and then dropped in week 6 and eventually abolished in week 7 (Fig. 2e). In GG, *GPPS* expression was upregulated abruptly starting week 4 and was maintained through the following weeks (Fig. 2f).

Similar to *GPPS*, *OAC* expression was observed to increase abruptly in week 5 and peaked in week 6, but dramatically decreased to almost basal level in week 7 in CW (Fig. 2g). *OAC* expression levels in GG was not increased as much as that in CW. In GG, there was slight increase of *OAC* in weeks 4, 5 and 7 but a significant decrease in week 6 (Fig. 2h). It is possible that *OAC* transcripts are already abundant in GG so not much upregulation is necessary for making cannabinoids, which is similar to what was observed in the expression of *THCAS*. Because GPP is also needed for producing monoterpenes, greater upregulation of *GPPS* is still required. The results

indicate that *OAC* expression may not be important for cannabinoid production in GG while the upregulation of *OAC* may still play certain roles in CW.

Correlation analysis of cannabinoid biosynthetic genes and cannabinoid production

Overall, the expression of the genes that encode cannabinoid biosynthesis enzymes follow a similar trend during flower development. The gene expression levels increase dramatically relative to the early phases of flower development, and the cannabinoid biosynthesis increases steadily as flowers develop. We analyzed the correlation between the expression of cannabinoid biosynthetic genes and cannabinoid production using Pearson's correlation matrix.

Figure 3 demonstrates the association between genes and cannabinoids in CW and GG respectively. In CW, it is clearly shown that the expression of *PT1* (*AP* in the figure) was positively correlated with the accumulation of CBGA over time. There was a statistically significant correlation between the expression of *PT1* and production of THCA and CBDA. In addition, expression of both *CBDAS* and *THCAS* was positively correlated with the production of CBDA and THCA in CW (Fig. 3a). *GPPS* has positive but weak correlation with cannabinoid production

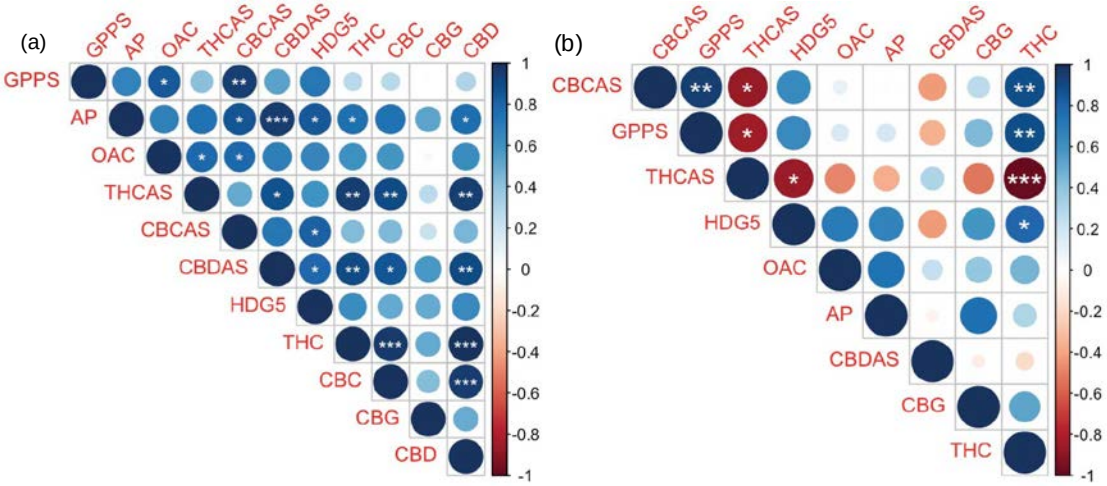

Figure 3. Graphic representation of correlation matrices demonstrates the correlation plot of cannabinoid biosynthetic genes and metabolites for CW (a) and GG (b). Blue indicates a positive correlation, while red indicates a negative correlation. Size and color of circle represent strength of correlation. Figures were generated using the R corrplot package https://github.com/taiyun/corrplot). (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

in CW. Interestingly, in GG, there was a strong negative correlation between the expression of *THCAS* and THCA (Fig. 3b), indicating that THCAS is not the determinant factor for THC production. However, *GPPS* is highly correlated with THCA production in GG, while the positive correlation was much weaker in CW. THCA production has positive correlation with the steps for CBGA production but not with *THCAS*, suggesting that CBGA production is critical for THCA biosynthesis, which is not dependent on *THCAS* transcript level. OA is a special component in cannabinoid biosynthetic pathway, and there is a positive correlation between *OAC* expression level and cannabinoid production in both the two varieties.

Effects of MeJA on cannabinoid production

MeJA has been shown to enhance secondary metabolites production in various plant species (Choi *et al.*, 2005; Kim *et al.*, 2006; Chen *et al.*, 2006; Yan *et al.*, 2017; Jiang *et al.*, 2017).

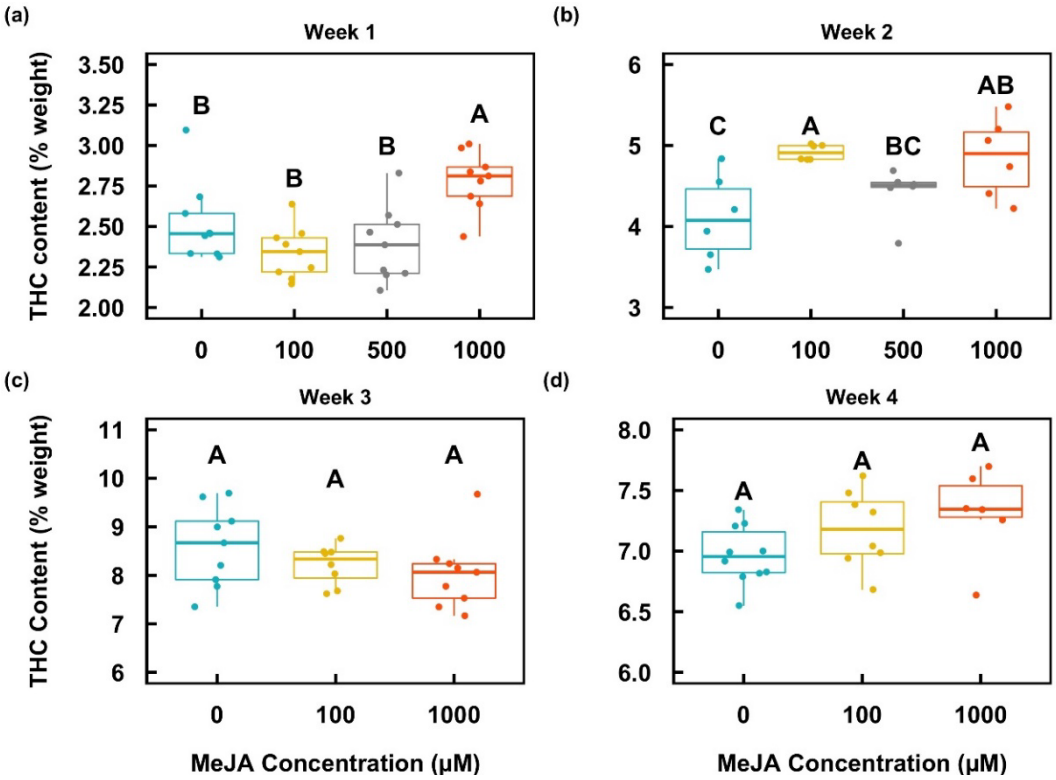

Figure 4. THC content in White Tangy Haze flowers over the period of four weeks (a-d) following application of MeJA. The box whiskers indicate variability outside the upper and lower quartiles (the upper and lower lines of the box) (n = 12). Different letters indicate statistical significance ($P < 0.05$) determined by one-way ANOVA followed by Fisher's LSD.

We examined how MeJA treatment could regulate cannabinoid production in a marijuana variety, 'White Tangy Haze', with different concentrations of MeJA by exogenous foliar spray.

In the first week following application, only plants sprayed with 1 mM MeJA had significantly higher THC (Fig. 4a) and total cannabinoid levels (Figure S4a) than those treated with water. All the treatments in the second week ameliorated THC production compared to the water control. More interestingly, plants treated with 100 µM MeJA produced a similarly high level of THC (Fig. 4b) and total cannabinoid contents (Figure 4b) to those treated with 1 µM MeJA. The effect of MeJA appeared to be subsiding three weeks after application but 1 mM MeJA treatment still showed significantly higher THC production (Fig. 4c). The positive effects of MeJA treatments (100 µM and 1 mM) on cannabinoid production was compromised 4 weeks after treatment (Fig. 4d). The observation suggests that MeJA is efficacious for enhancing cannabinoid production; 100 µM MeJA could be treated biweekly one week after flower induction.

DISCUSSION

CBGA biosynthesis is the rate-liming step in the cannabinoid biosynthesis pathway

We provide the greatest amount of resolution into how the plants generate cannabinoids, and accurately draw a correlation between the terminal cannabinoids and the expression of genes that encode the biosynthetic enzymes. Both the hemp CW and medical marijuana GG showed gene upregulation starting at week 4 while genes were kept at basal level during the first 3 weeks. The findings indicate that the regulation of cannabinoid biosynthetic genes is dependent on trichome development. Livingston *et al.* (2020) showed that cannabinoids are primarily accumulated in the capitate-stalked glandular trichomes, which is abundant during the later stages of flower development. Our gene expression analysis and cannabinoid quantification results are consistent with and support their observation.

This report puts emphasis on potentially rate-limiting aspects of cannabinoid production. In GG, we observed increased THC production as the flowers developed, while *THCAS* expression decreased in the later stages compared to the earliest phase of flower development (Fig. 2d). The findings of THC is consistent with previous reports that *THCAS* was downregulated with the development of flowers despite the increase of THC production (Richins *et al.*, 2018; Liu *et al.*, 2021), which could be due to the upregulation of two transcription factors (TF) that suppresses *THCAS* (Liu *et al.*, 2021). In addition, Muntendam *et al.* (2012) did not find strong correlations between synthase enzymes and their corresponding products. Onofri *et al*. (2015) demonstrated that the expression levels of *CBDAS* or *THCAS* are not associated with cannabinoid production when comparing various cannabis strains together. Richins *et al.* (2018) showed that while cannabinoid levels increased during flower development, transcripts of *CBDAS*, *THCAS*, and *PT* showed a descending trend, suggesting a negative correlation between gene expression and cannabinoid production. Our and other studies imply that the transcript level of the synthase genes is not critical for cannabinoid production.

We further found that *PT*, *GPPS* and *OAC* were also experiencing significant increases in their expression levels in both varieties (Figs. 1, 2e-h). Based on our findings in GG, we propose that THCA production is mainly determined by the expression and activity of *PT* and *GPPS*, the steps that lead to CBG production. It has been shown in several plant species that overexpression or upregulation of GPPS increased monoterpene biosynthesis by enhancing GPP production (Orlova *et al.*, 2009; Xi *et al.*, 2016; Yin *et al.*, 2017; Chuang *et al.*, 2018a,b). Our Pearson's matrix analysis further demonstrated that *GPPS* is highly correlated with THCA in GG (Figure 3b). It is reasonable to propose that elevating GPP production will increase CBG biosynthesis and eventually enhance cannabinoid production in cannabis. In addition, because of the dual function

of GPP, increasing *PT* expression would allow more GPP to be allocated for CBG production. We also included a TF involved in glandular trichome initiation, HDG5, in this report (Fig. 3). *HDG5* expression showed positive correlation with cannabinoids, suggesting the important role of glandular trichome development in cannabinoid production. These genes could be targets for traditional breeding or genetic modification.

Hemp and marijuana differ in transcriptional regulation of cannabinoid biosynthetic genes

We examined transcript levels of cannabinoid biosynthetic genes in two types of cannabis varieties, hemp CW and medical marijuana GG. Although the overall gene expression pattern showed some similarities, there are indeed differences between the two varieties. *PT* expression in CW surged in week 5 and was maintained at a similar level (Fig. 2a,c). *PT* expression in GG gradually increased until weeks 4 and 5 but was completely abolished in later weeks (Fig. 2b,d). The findings suggest that the *PT* genes may be distinctly regulated in hemp and marijuana by TFs. In addition, *GPPS* expression in GG and CW also showed temporally different regulation (Fig. 2e,f). Distinct monoterpene profiling could also be a feedback regulation of *GPPS* expression in the two varieties.

We showed that transcription of *THCAS* in CW is normal, but CW does not produce as much of THC as GG. To learn if there are mutations in *THCAS*, we sequenced CW *THCAS*. The result showed that CW THCAS is 100% identical to the known sequence on NCBI, indicating that it is fully functional. By comparing *THCAS* transcripts in CW and GG, we found that GG *THCAS* expression is hundreds of folds higher than CW *THCAS* (Figure S5), indicating that *THCAS* transcription is vastly suppressed in CW. Despite the differences in gene expression levels in the two varieties, CBD and THC were still produced to the similar levels in CW and GG respectively (~20% of dry weight).

MeJA regulates cannabinoid biosynthesis

MeJA is the plant hormone that plays important roles in secondary metabolite production and glandular trichome development (Chen *et al.*, 2017; Boughton *et al.*, 2005; Wang *et al.*, 2010; Maes *et al.*, 2011; Montiel *et al.*, 2011; Yu *et al.*, 2012; Shen *et al.*, 2016; Yan *et al.*, 2017; Hua *et al.*, 2021). However, there is no publication regarding the effect of MeJA on cannabinoid production. Our findings showed that 100 µM MeJA was as effective as 1 mM MeJA to enhance THC production two weeks after treatment (Fig. 4b). Jiang *et al.* (2017) demonstrated that high MeJA concentrations caused necrosis on cucumber leaves and did not improve monoterpene production. Therefore, for the improvement of cannabinoid production, a biweekly 100 µM MeJA treatment during flower development would be ideal to promote cannabinoid production.

Studies have shown that MeJA controls secondary metabolite biosynthesis by regulating TFs that regulate glandular trichome formation and biosynthetic genes. It is demonstrated in *Artemisia* and tomato that MeJA upregulated TFs that positively regulate glandular trichome formation and increased artemisinin content in *Artemisia* and terpene production in tomato (Yan *et al.*, 2017; Chalvin *et al.*, 2020; Schuurink & Tissier, 2020). Furthermore, JA responsive TFs have been characterized in various plant species that can directly or indirectly regulate transcription of terpene biosynthetic genes (Van Der Fits & Memelink, 2000; Li *et al.*, 2015; Jiang *et al.*, 2016; Shen *et al.*, 2016; Nolan *et al.*, 2017; Chuang *et al.*, 2018a,b; Hua *et al.*, 2021). The alteration of these MeJA upregulated TFs resulted in changes in secondary metabolite levels. Based on our findings, MeJA could also play vital roles in cannabinoid and secondary metabolite biosynthesis in cannabis. It would be critical to identify TFs in cannabis that are responsible for glandular trichome development and transcriptional regulation of cannabinoid biosynthetic genes.

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AUTHOR CONTRIBUTIONS

PA, YM and GAB designed the research; PA and LS performed experiments and analyzed data; PA, YM and GAB wrote the manuscript; GAB and YM supervised the research.

The authors declare no conflict of interest.

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Figure legends

Figure 1. The remained CBG amount and the gene expression of *PT1* and *PT4* over the course of seven-week flower development in CW (a, c) and GG (b, d). y axes on the left show relative expression of *PT1* and *PT4*. y axes on the right show the weight percentage of CBG in wet flower

samples. Data are presented as means ± SE (n = 4). Significant difference of $P < 0.05$ (*) and $P < 0.01$ (**) was determined by Student *t*-test.

Figure 2. Analysis of CBD and THC accumulation with the expression of genes in the cannabinoid biosynthetic pathway over the course of seven-week flower development. (a, b) Superimposition of CBD content with *CBDAS* expression in CW (a) and GG (b). (c, d) Superimposition of THC content with *THCAS* expression in CW (c) and GG (d). Gene expression of *GPPS* and *OAC* in CW (c, g) and GG (d, h). Data are presented as means ± SE (n = 4). Significant difference of $P < 0.05$ (*), $P < 0.01$ (**) and $P < 0.001$ (***) was determined by Student *t*-test.

Figure 3. Graphic representation of correlation matrices demonstrates the correlation plot of cannabinoid biosynthetic genes and metabolites for CW (a) and GG (b). Blue indicates a positive correlation, while red indicates a negative correlation. Size and color of circle represent strength of correlation. Figures were generated using the R corrplot package https://github.com/taiyun/corrplot). (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

Figure 4. THC content in White Tangy Haze flowers over the period of four weeks (a-d) following application of MeJA. The box whiskers indicate variability outside the upper and lower quartiles (the upper and lower lines of the box) (n = 12). Different letters indicate statistical significance ($P < 0.05$) determined by one-way ANOVA followed by Fisher's LSD.

Supplemental Figure legends

Figure S1. Cannabinoid biosynthetic pathway. Purple inscriptions refer to the enzymes, including geranyl pyrophosphate synthase (GPPS), olivetolic acid cyclase (OAC), aromatic prenyltransferase (AP), tetrahydrocannabinolic acid synthase (THCAS), cannabidiolic acid synthase (CBDAS), and cannabichromenic synthase (CBCAS), that catalyze reactions. Green labels indicate the metabolites. A non-enzymatic conversion, which is expedited in the presence

of heat, spurs the generation the decarboxylated cannabinoids: THC, CBD, and CBC. The figure is created using Chemdraw.

Figure S2. Representative pictures show flower development over a period of 7 weeks. Scale bar is 2 cm. Note that there is barely flower tissue developed one week after transition to short day condition.

Figure S3. Real-time PCR analysis of *CBCAS* in two C. sativa varieties, CW (a) and GG (b). Data are presented as means ± SE (n = 4). ** indicates $P < 0.01$.

Figure S4. Total cannabinoid content in White Tangy Haze flowers over the period of four weeks (a-d) following application of MeJA. The box whiskers indicate variability outside the upper and lower quartiles (the upper and lower lines of the box) (n = 12). Different letters indicate statistical significance ($P < 0.05$) determined by one-way ANOVA followed by Fisher's LSD.

Figure S5. The comparison of *THCAS* expression in week 1 between CW and GG. *THCAS* expression is hundreds of folds higher in the medical marijuana variety GG than in the hemp variety CW. Data are presented as means ± SE (n = 4). * indicates $P < 0.05$.