EFFECT OF PACLOBUTRAZOL ON mRNA ACCUMULATION OF *ent-kaurene oxidase* AND *GA20-oxidase* GENES AND PLANT HEIGHT OF *Jatropha curcas* L.

Anittaya Kanghae, Sarun Phukhae, Apichet Sangsuriyaroj, Pongmanee Thongbai,

and Siam Popluechai*

School of Science, Mae Fah Luang University, Thasud, Chiang Rai 57100, Thailand

*e-mail: s.popluechai@sci.mfu.ac.th

Abstract

Jatropha curcas L. (*Jatropha*) is a perennial plant that has received attention as a source of biodiesel production. However, the *Jatropha* plants exhibit unfavorable heights. Reducing the plant heights will improve seeds yield and easiness for harvesting. This study aimed to investigate the effect of paclobutrazol (PCB) on *Jatropha* seedlings (21 days after germination). The plant growth and mRNA accumulation of *ent-kaurene oxidase (JcKO)* and *GA20-oxidase (JcGA20ox)* genes were investigated. Height of the PCB treated plants was significantly shorter until the end of the experiment during 17-60 DAT (day after treated). The stem diameters of the treated plants were obviously and significantly thicker than those of the control during 7-19, 25-53 DAT. RT-PCR analysis was performed to determined mRNA accumulations of *JcKO* and *JcGA20ox* of the plant collected from 10, 17, and 22 DAT. The results showed that the mRNA accumulations of *JcKO* from the treated plants were not significantly different from those of the control at 17 and 22 DAT. The mRNA accumulations of *JcGA20ox* of the treated plant were not significantly different from those of the control at 10, 17 and 22 DAT.

Keywords: *Jatropha curcas* L., paclobutrazol, plant height, *ent-kaurene oxidase*, *GA20-oxidase*, RT-PCR analysis

Introduction

Jatropha curcas L. (*Jatropha*), or physic nut, is a perennial monoecious plant belongs to the Euphorbiaceae family, the same as castor bean, cassava and rubber tree. *Jatropha* has been planted widely in South, Central America, Africa and Asia, on good and degraded soil, in and high rain fall (Heller, 1996). Its leaves and stems are toxic to animals, including phorbol esters, but the seeds or seed cake can be used as an animal feed after treatment (FACT Foundation 2006; Makkar et al, 2001). It is reported to be high tolerance to drought, heat and photo-insensitive and has been attracting increase attention as an alternate source of biodiesel, as the seed of *Jatropha* content of up to 48% oil (Kochhar et al, 2008). The oil can also be used to manufacture candles, high-quality soap, cosmetics, pesticides and anti-cancer medicine. In addition, *Jatropha* also help in carbon sequestration by increasing carbon stocks both above and below the ground (B&T, 2008; Joshi et al, 2011). However, as a fast-growing deciduous bush,

Jatropha can sometimes reach over 8 m in height with stem diameter ranging from 20 to 30 cm, made it difficult to harvest and reducing plant height was suggested to be a major trait for varietal improvement (Sujarta et al, 1995; Thongbai et al, 2007, 2009).

Plant height is controlled by apical dominant phytohormones namely auxin and gibberellins (Chebotar&Chebotar, 2011). Gibberellin (GA) is collectively referred to a group of diterpenoid acids, some of which act as phytohormones (Jan&Komatsu, 2006). Besides control stem elongation, GAs is essential to other plant growth, affecting seed germination, flower induction, anther development and seed and shell growth (Crozier, 1983; Koornneef et al, 1980; Subidi et al, 2003; Itoh et al, 2004). Lacking GA is the plant could lower the scrub and promote growth of lateral buds (Hopkin, 1995; Kende&Zeevaart, 1997; Shani et al, 2006). In *Jatropha*, Thongbai et al. (2006) found that using GA inhibitors could reduce plant height through reducing internode length but not the number of nodes, which was more effective than inhibiting auxin by cytokinin. The effect of GA inhibitor on plant height was further reported by Thongbai et al, (2007); Hadiwijaya and Thongbai. (2009); and Ghosh et al. (2010) found that applying GA inhibitor PCB could increase seed yield. In the gene level, it has been reported that PCB and uniconazole increased ent-kaurene oxidase (*KO)* expression in *Arabidopsis thaliana* (Swain et al 2011). Song et al (2011) also reported that the relative mRNA accumulation of *KS1 and KO* decrease in maize during seed embryos at 32 hrs after PCB treatment, while *GA20ox1* and *GA20ox5* are up-regulated by PCB. The *KO* is a multifunctional cytochrome P450 enzyme that catalyzes the three intermediate steps of the GA pathway *from ent-kaurene to entkaurenoic acid* (Swain et al 2011). *GA 20ox* is a multifunctional enzyme that catalyzes the sequential oxidation of GA53 to GA20. The product of the reaction catalyzed by *GA20ox, GA20*, is then hydroxylated by *GA 3b-hydroxylase (GA 3b-hy)* to produce the active *GA, GA1*. *GA20ox* is encoded by a small multigene family whose members are differentially regulated (Phillips et al 1995; Rebers et al 1999) related with GA biosynthesis pathway. However, here is still no report on this effect on accumulation of mRNA involved GA biosynthesis, which could be useful information for improving semi-dwarf *Jatropha*. In this study, the effect of PCB in growth of *Jatropha* plants and levels of the mRNA accumulation of the *JcKO* and *JcGA20ox* gene were characterized.

Methodology

Plant preparation

Jatropha seeds were soaked in water for 24 hours. The soaked seeds were then incubated at 38 °C for 8 hours, after which the seeds are growth in black plastic bags filled with 500 g soil. At 21 days after planting, 50 ml of 600 ppm of PCB was sprayed to 10 seedlings, 3 times every alternate days, while 50 ml of pure water was sprayed to another 10 seedlings as control. Height and stem diameter of all plants were measured and 1 cm of the shoot tips were sampling for RNA extraction at 10, 17 and 22 DAT.

RNA extraction

Total RNA was extracted from the shoot following method according to Chaudhary et al. (2011). The pellet was air-dry and dissolved with 20 µl DEPC treated water. The total RNA was quantified with a nanoDrop spectrophotometer at 230, 260 and 280 nm and stored at -20 ^{o}C.

RT-PCR analysis

First stand cDNA synthesis is performed with 1 µg of total RNA samples using the RevertAid^{TM} Frist Stand cDNA Synthesis Kit (Fermentas$^{®}$) following the manufacturer's instruction. The PCR reaction mixture of 20 µl contained 2 µl of cDNA template, 10 µM of forward and reverse primers, 10 µl of 2x PCR master (Intron$^{®}$, Korea). Amplifications were performed in a programmed for an initial incubation at 94 ^{o}C for 2 min, 40 cycles of 94 ^{o}C for 30 sec, 55 ^{o}C for 30 sec and 72 ^{o}C for 30 sec. The sample was incubated at 72 ^{o}C for 5 min and hold at 4 ^{o}C.

The amplification of Actin was used as an internal control. The experiment was repeated twice. Amplified products were analyzed by 2% agarose gel electrophoresis. The gels were stained with 0.5 µg/ml Ethiduim Bromide solution and vistualized on a UV transilluminator (Bio-Rad). The 100 bp DNA ladder (Invitrogen$^{®}$) was used as a size marker. The PCR products intensity was quantified using the GelDoc 2000 image analysis program (Bio-Rad$^{®}$). The optical density was determined for bands of interested genes and Actin primers and a ratio of the interested genes band intensity to Actin band intensity was calculated and compared.

Result and Discussion

Spraying with GA inhibitor PCB on *Jatropha* plants had pronounced effects on its growth. Figure 1 and Figure 2 shows that PCB inhibited the vegetative growth of *Jatropha* plants. Height of the PCB treated plants was non-significantly shorter than those of control since 1-13 DAT, and becomes significantly shorter until the end of the experiment during 17-60 DAT. Stem diameters of the treated plants were obviously and significantly thicker than control during 7-19, 25-53 DAT. These results are similar to numerous works, such as Tsegaw et al, (2005). Berova and Zlatev, (2000) found that in Potato PCB treatment resulted in shorter and thick stems compared to the control plant. In *Arabidopsis* and *Calendula officinalis* L*,* Swain et al, (2005) found that PCB treatment resulted in shorter stem than in control. The difference in height was probably due to the inhibited GA effect from PCB on the cell elongation and hence, retarding the internodes length. The most noticeable effect of PCB is internodes compression resulting in compact and short plants (Berova and Zlatev, 2000; Yeshitela et al, 2004), which have been previously shown in *Jatropha* by Thongbai et al, (2007) and Hadiwijaya and Thongbai. (2009).

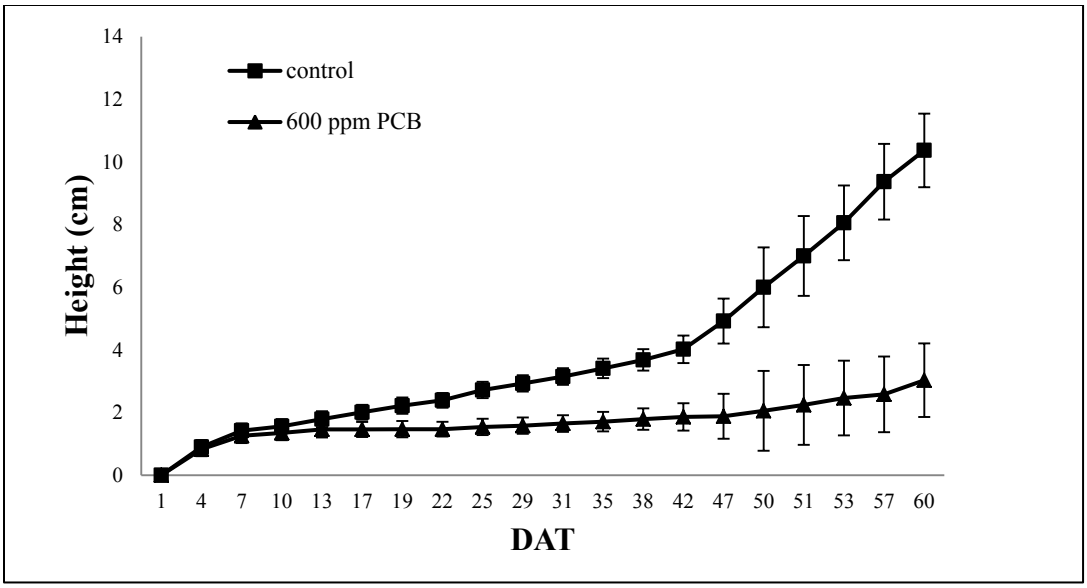

Figure 1. Plant height of *Jatropha* seedlings treated with PCB and in control

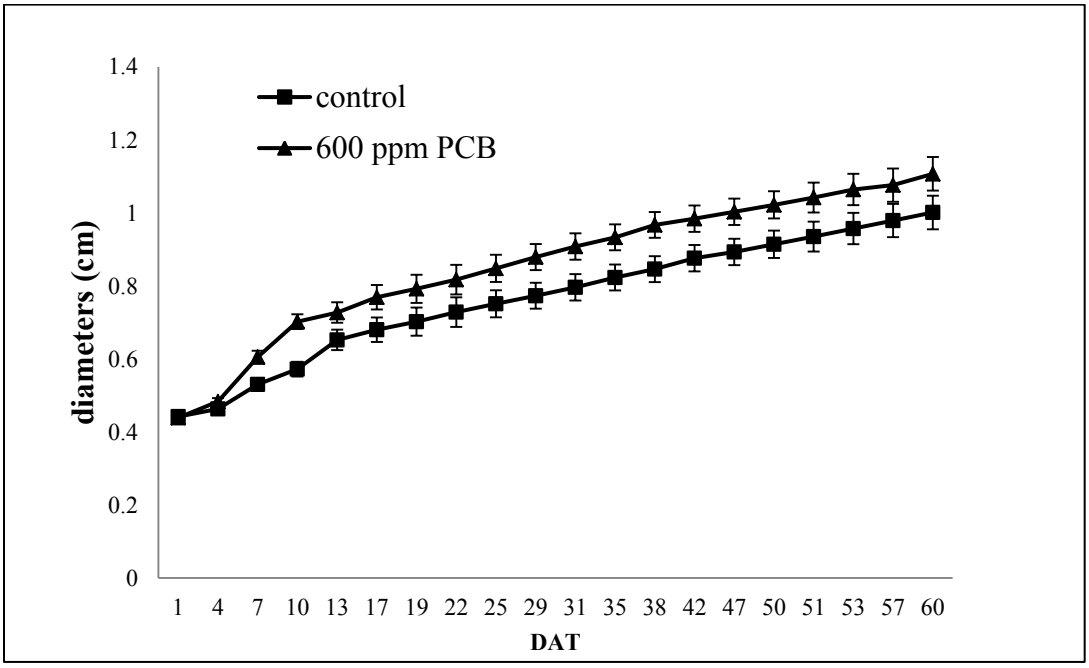

Figure 2. Stem dimeters of *Jatropha* seedlings treated with PCB in control

The level of mRNA accumulation of *JcKO* and *JcGA20ox* gene by RT-PCR analysis, shown in Figure 3, Figure 4 and Figure 5, were different between the control and PCB treated seedling. The relative mRNA accumulation of *JcKO* of the treated plants was not significantly different from control at 17 and 22 DAT. The absent of mRNA accumulation of *JcKO* in control at 10 DAT might be due to the long retention period of the sample collection of *JcKO*.

Although non-significantly different, the relative mRNA accumulation of *JcGA20ox* of the treated plants was slightly higher than control ones at 10 and 17 DAT. These results are similar to numerous works, such as in maize reported that the relative mRNA accumulation of *KS1* and *KO* decrease in seed embryos at 32 hrs after paclobutrazol treatment. This is similar to the report. Song et al (2011). Recently, it was reported that treatment of the GA-deficient *ga1-2* mutant of Arabidopsis with GA resulted in an increase in mRNA content for two GA2ox genes (Thomas et al 1999).

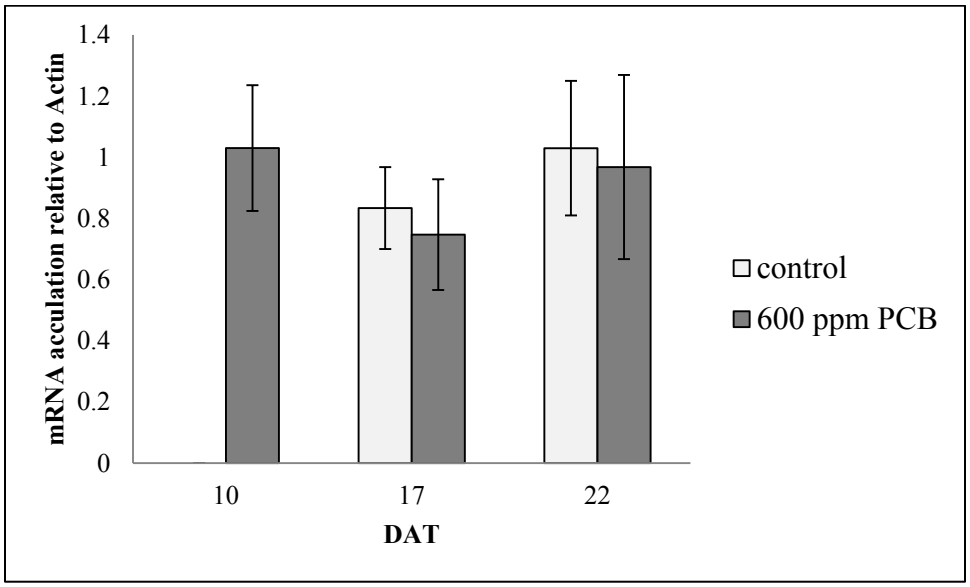

Figure 3 Expression analysis by RT-PCR of the *Jatropha* at 10, 17 and 22 DAT of *JcKO*

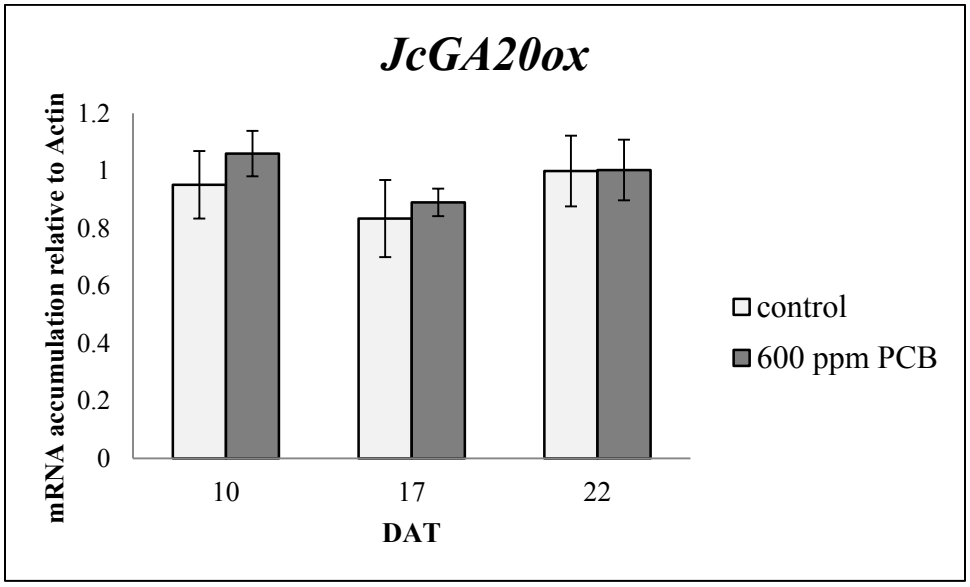

Figure 4 Expression analysis by RT-PCR of the *Jatropha* at 10, 17 and 22 DAT *JcGA20ox* genes

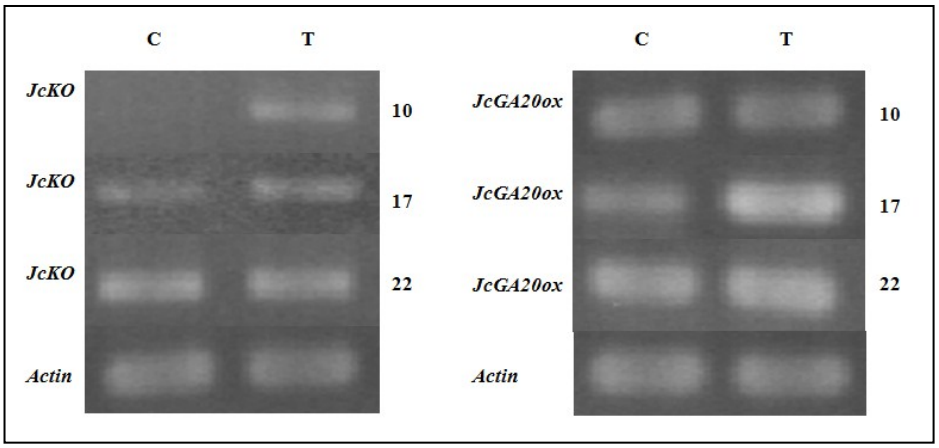

Figure 5 RT-PCR analysis of mRNA accumulation pattern of *JcKO* and *JcGA20ox* gene in Control (C) the PCB treated (T) in the *Jatropha* at 10, 17, and 22 DAT

Conclusion

In this study, we demonstrated that PCB reduced plants height. However, the result for mRNA accumulation of *JcKO* and *JcGA20ox* gene is still inconclusive and need further study. It is recommended that more frequent sampling should be done immediately after treating PCB to be able to detect changes in the accumulated mRNA of *JcKO* and *JcGA20ox* gene.

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