HAYATI Journal of Biosciences September 2014
Vol. 21 No. 3, p 111-120
EISSN: 2086-4094

Available online at:
http://journal.ipb.ac.id/index.php/hayati
DOI: 10.4308/hjb.21.3.111

Increasing Hermaphrodite Flowers using Plant Growth Regulators in Andromonoecious *Jatropha curcas*

DASUMIATI[1,4], MIFTAHUDIN[2], TRIADIATI[2*], ALEX HARTANA[2], DIBYO PRONOWO[3]

[1]Study Program of Plant Biology, Graduate School, Bogor Agricultural University, Darmaga Campus, Bogor 16680, Indonesia
[2]Department of Biology, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University, Darmaga Campus, Bogor 16680, Indonesia
[3]Research Institute for Industrial and Beverages Crops, Sukabumi 43357, Indonesia
[4]Department of Biology, Faculty of Sciences and Technology, UIN Syarif Hidayatullah, Jakarta 15412, Indonesia

Received September 12, 2013/Accepted May 21, 2014

***Jatropha curcas* (JC) is a crop with potential for use in biodiesel. Production of biodiesel requires plant seed as raw material, so the viability of JC for use in biodiesel will dependent greatly on the plant's production of flowers. Generally, this plant is monoecious, meaning it has both male and female flowers. However, very rarely JC plants may be andromonoecious. Andromonoecious specimens of JC produce hermaphrodite and male flowers in the same plant. The number of hermaphrodite flowers per inflorescence is generally low compared to the number of male flowers. The aim of this study was to increase the proportion of hermaphrodite flowers by using plant growth regulators (PGRs) in andromonoecious JC. Our experiment was conducted in Randomized Block Design (RBD) with 9 treatments, namely kinetin, GA_3, and IAA with concentrations of 0 ppm as a control, 50 and 100 ppm of each PGRs. The treatments were applied to stem cuttings from each plant and repeated 4 times. PGRs were applied by spraying the leaves within the buds of each plant. Applications took place weekly beginning when the plants entered flower initiating phase, until inflorescence produced. Observations were conducted during the treatment period (10 weeks). Results showed that plants treated with IAA, GA_3, and kinetin at 50 and 100 ppm produced increased inflorescence per plant. The increases measured were 155.4 and 92.9% of (IAA), 120.4 and 151% (GA_3), 96.6 and 51.7% (kinetin) respectively. In addition, we found that application and GA_3 at concentrations of 50 and 100 ppm, and kinetin at 50 ppm, increased the number of hermaphrodite flowers per inflorescence by 50%, and increased the number of hermaphrodite flowers per plant by 275.6 and 183.1% (IAA), 219.5 and 254.1% (GA_3), 162.9 and 103.1% (kinetin) respectively. As would be expected, the number of fruit per plant increased in those specimens treated with IAA, GA_3, and kinetin at 50 and 100 ppm. The increases measured were 301.7 and 167.4% (IAA), 211.7 and 257.0% (GA_3), 162.5 and 101.4% (kinetin) respectively.**

Key words: GA_3, IAA, inflorescence, *Jatropha curcas*, kinetin

INTRODUCTION

Jatropha curcas (Euphorbiaceae) is a plant of commercial importance due to its potential to produce biodiesel from it seeds, which have high oil content. (Sharma *et al.* 2009; Wang & Ding 2012). Production of *J. curcas* (JC) biodiesel requires seeds as raw material. Thus, indirectly, production of biodiesel using JC is highly dependent on production of JC flowers, because the seed is produced by hermaphrodite or female flowers.

Generally, JC plant produces both male and female flowers on the same plant (monoecious) (Alam *et al.* 2011); however sometimes JC might also produce hermaphrodite flowers (Jones & Csurhes 2008; Yi *et al*. 2010). JC specimens that produce hermaphrodite and male flowers, but not female flowers, are called andromonoecious (Dellaporta & Urrea 1993). Andromonoecious JC rarely found. The JC flower is the unlimited compound flower type (*racemosa*) with a *dichasial* branch pattern, where female flowers are located between two male flower branches, on monoecious JC plants (Raju & Ezradanam 2002). In monoecious JC, there are about five female flowers in one inflorescence (Wijaya *et al.* 2009). The ratio of female to male flowers is about 1:20 (Wu *et al.* 2011) to 1:29 (Raju & Ezradanam 2002). The low proportion of female flowers in monoecious JC limits fruit production. The low level of fruit production not only results in low seed production, but also causes lack of seed continuity. This problem is the focus of JC improvement efforts (Wahyudi & Wulandari 2007).

Andromonoecious plants were a great opportunity to produce much fruit (Miller & Diggle 2007).

*Corresponding author. Phone/Fax: +62-251-8622833, E-mail: adiatiipb@gmail.com

In andromonoecious JC, as in other plants, the number of fruit is dependent on the number of hermaphrodite flowers. Based on previous research, andromonoecious JC has the number of hermaphrodite flowers per inflorescence which is low in comparison to male flower (1:15). Nonetheless the potential for high fruit yield is strong. Andromonoecious JC flowers throughout the year and hermaphrodite flowers have high rates of fruit set, reaching 100%. High numbers of hermaphrodite flowers will produce more fruit. Therefore, it is very important to improve hermaphrodite flower production in andromonoecious JC.

The enhancement of hermaphrodite flower production does not only increase the yield of fruits and seeds, but also improves seed quality. One study showed that pollination of hermaphrodite flowers occurs simultaneously with flower bloom, so that hermaphrodite flowers have a tendency to self-pollinate. Therefore, the seed produced from hermaphrodite flowers are nearly uniform, and have properties similar to the parent.

An increased number of hermaphrodite flowers on andromonoecious JC can be achieved by application of Plant Growth Regulators (PGRs), because PGRs influence the developmental process of flowers. PGRs are organic compounds naturally occurring in plants that affect physiological processes. PGR levels may impact the sex ratio of plants, since PGRs are one of the factors which determine sex in plants, aside from genetic and environmental factors (Meagher 2007). Ahoton and Quenum (2012) also suggested the use of plant growth regulator on female parent to increase the success of hybridization in JC. Several studies have attempted to influence sex in plants by using PGRs. Auxins, such as indol acetic acid (IAA), affected sex expression in hermaphrodite and andromonoecious plants of *Cucumis sativus* L. (Galun *et al.* 1964). Gibberellin (GA_3) increased the female to male ratio in spinach (*Spinacia oleracea*) (Komai *et al.* 1999) and also increased the number of female flowers in JC (Makwana *et al.* 2010). Application of benzylaminopurin (BA), triiodobenzoic acid (TIBA), and maleat hidrazide through the leaves can increase the number of female flowers and of fruits per plant in monoecious JC (Abdelgadir *et al.* 2009). However, the use of kinetin has not been studied yet for its potential to increase the number of hermaphrodite flowers on JC. Kinetin treatment has been shown to increase the number of flowers per plant in lentil crops (*Lens culineris*) (Khalil *et al*. 2006).

We found no research related to the improvement of hermaphrodite flower production for JC. Given the favorable properties of andromonoecious JC with their hermaphrodite flowers, high fruit set, and continuous flowering throughout the year, as well as the known effect of growth regulators to increase flowering and influence the sex of flowers produced, we conducted research that aims to enhance hermaphrodite flower producing by application of PGRs (kinetin, GA_3, and IAA) on andromonoecious JC. An increased number of hermaphrodite flowers in andromonoecious JC will increase the productivity and development of JC.

MATERIALS AND METHODS

Plant Material. The plant materials used were stem cuttings of three mother plants of andromonoecious JC Dompu accession. PGRs used as treatments consisted of kinetin, GA_3 and IAA. This study was conducted from March-November 2010 in Cibeureum, Dramaga, Bogor, and in the green house of the Department of Biology, Bogor Agricultural University.

Experimental Design. This study used a Randomized Block Design (RBD) with 9 treatments, namely kinetin, GA_3, and IAA with concentrations of 0 ppm as a control, 50 ppm, and 100 ppm of each PGRs. Each treatment was repeated 4 times over the course of 10 weeks.

PGRs Applications and Observations. PGRs treatments were carried out after the plant material had grown in the field to more or less a month old. At that point in time, the plant had flower initiation occurred in several plants. Foliar application of PGRs was conducted by spraying onto the leaves in the buds, until the leaf surface (abaxial and adaxial) was wet. Spraying took place once a week between 05:00 and 6:00 am. We chose a pre-dawn application time because IAA is light sensitive and easily oxidized. Spray treatment was discontinued when inflorescences appeared. The full observation period lasted for 10 weeks.

The parameters monitored were: number of hermaphrodite flowers per inflorescence, number of male flowers per inflorescence, percentage of hermaphrodite to male flowers, number of inflorescences per plant, number of hermaphrodite flowers per plant during 10 weeks of observation, number of fruit per inflorescence (fruit set per inflorescence), fruit size (length and width), number of seeds per fruit, seed size (length and width), dry weight of seed per inflorescence, and weight per 100 seeds.

Data Analysis. Data were analyzed by using SPSS version 16. The difference between PGR treatments and concentrations were shown in variation analysis

by using analysis of variance (ANOVA) followed by Duncan multiple range test (DMRT) at 5% level.

RESULTS

Effect of PGRs to Flower Development of Andromonoecious JC. All stem cuttings from the three andromonoecious JC mother plants used in this study, produced male and hermaphrodite flowers. The flower of andromonoecious JC has a compound flower structure (*Inflorescentia racemosa*). The number of inflorescence per plant measured over 10 weeks of observation did not differ between treatment modes: kinetin, GA_3, and IAA ($P>0.05$). However, the concentration of PGRs did significantly influence the number of inflorescences ($P<0.05$). Treatment with each of the PGRs (kinetin, GA_3, and IAA) at 50 and 100 ppm significantly increased the number of inflorescence per plant compared to controls. However, plants treated at concentrations of 50 ppm had a higher number of inflorescences per plant than those treated at 100 ppm (except GA_3 treatment) ($P<0.05$). Plants treated with GA_3 at 100 ppm produced a higher number of inflorescences than plants treated with GA_3 at 50 ppm, but this difference was not significant (Figure 1). Plants treated with kinetin at 50 and 100 ppm produced a higher number of inflorescences compared to the control group, with increases of 51.7 and 96.6% respectively ($P<0.05$). Plants treated with GA_3 at 50 and 100 ppm showed about 120.4 and 151% increases in numbers of inflorescence, respectively, compared to the control. Plants treated with IAA at 50 ppm produced the highest number of inflorescences. Compared to control groups, plants treated with IAA at 50 and 100 ppm showed an increase in the number of inflorescences of about 155.4 and 92.9% respectively.

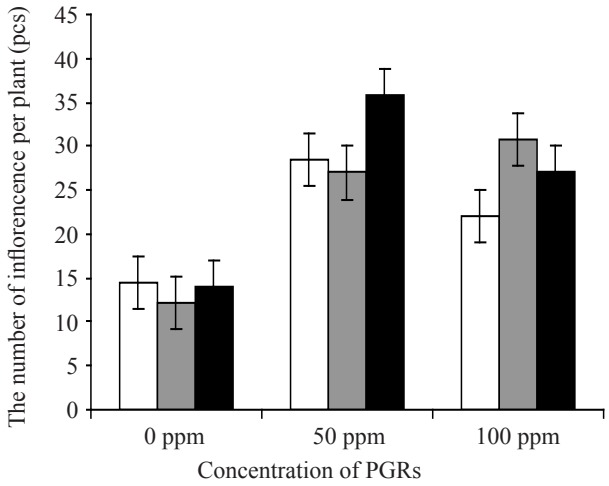

Figure 1. Effect of application of kinetin, GA_3, and IAA to andromonoecious Jatropha curcas on the number of inflorescence per plant during 10 weeks of observation. Vertical lines on bars represent SE (standard error). □ Kinetin, ▩ GA_3, ■ IAA.

The inflorescence of andromonoecious JC consists of two main or primary branches, each of which produces secondary branches, which in turn produce tertiary branches. Generally, one of the main branches produces two secondary branches, while the other primary branch produces six secondary branches. Each secondary branch produces one hermaphrodite flower. The tertiary branches produce male flowers (Figure 2A,B). PGR treatment (kinetin, GA_3, and IAA) at 50 and 100 ppm seemed to affect the pattern of inflorescence branching. The number of secondary and tertiary branches increased, and the plants developed a further level of quaternary branches (Figure 2C,D).

The number of hermaphrodite flowers per inflorescence was not affected by the type of PGR applied in treatment (kinetin, GA_3, and IAA) ($P>0.05$); however, it was affected by the concentration of PGRs used ($P<0.05$). PGR treatments at 50 and 100 ppm significantly increased the number of hermaphrodite flowers per inflorescence by approximately 9 flowers per inflorescence compared to control. The increase in number of hermaphrodite flowers in plants treated with GA_3 and IAA at 50 ppm compared to controls was similar to the increase in plants treated with100 ppm. Plants treated with kinetin at 50 ppm produced more inflorescences than those treated at 100 ppm

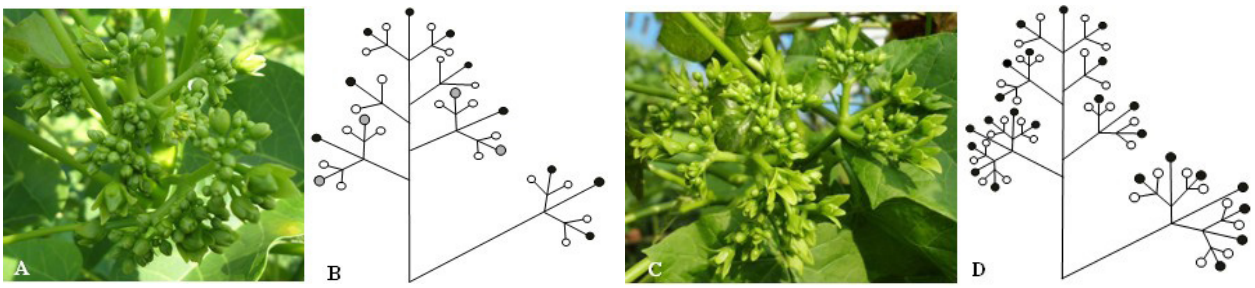

Figure 2. Effect of PGR treatments on branching of inflorescences in andromonoecious *Jatropha curcas*. Inflorescences without PGRs treatments (A and B), inflorescences with PGR treatments (C and D) (●= hermaphrodite flowers, ♁ = cluster of male flowers, ● = male flower).

but this was not significant (P>0.05) (Figure 3A). Plants treated with IAA and GA_3 at 50 and 100 ppm, and kinetin at 50 ppm, showed significant increases (P<0.05) of about 50% in the number of hermaphrodite flowers per inflorescence. Plants treated with kinetin at 100 ppm showed an increase of about 33.3% in the number of hermaphrodite flowers per inflorescence compared to control (P<0.05) (Figure 3A). Observation of the number of hermaphrodite flowers per inflorescence and the number of inflorescences total, during 10 weeks observation, indicated that application of kinetin at 50 and 100 ppm significantly increased number of hermaphrodite flowers per plant by about 162.9 and 103.1% respectively compared to control. The number of hermaphrodite flowers per plant in the groups treated with GA_3 at 50 and 100 ppm increased about 219.5 and 254.1% respectively compared to control. Finally, plants treated with IAA at 50 and 10 ppm showed the highest increase, about 275.6 and 183.1% respectively (Figure 3B).

The number of male flowers per inflorescence was not affected by either the type or concentration of PGRs (P>0.05) (Figure 3C). The percentage of hermaphrodite to male flowers showed the ratio of hermaphrodite to male flowers per inflorescence. The percentage of hermaphrodite to male flowers was not affected by type of PGRs (P>0.05), while affected by concentration of PGRs (P<0.05) except kinetin treatment (Figure 3D). PGRs treatment of both 50 and 100 ppm significantly increased the percentage of hermaphrodite flowers to male flowers. The percentage of 6% or 1:16 in the control increased to 9% or 1:11 in the treatment of GA_3 and IAA.

Effect of PGR Treatment on Fruit Development in Andromonoecious JC. The number of fruit per inflorescence was not influenced by the type of PGR (kinetin, GA_3, and IAA) (P>0.05), but was affected by the concentration of PGRs (P>0.05). PGR treatment at concentrations of both 50 and 100 ppm increased the number of fruit per inflorescence approximately 50% (Figure 4A). The number of hermaphrodite flowers per inflorescence very nearly matched the number of fruit per inflorescence in all treatment groups, so that this increase was the same as for fruit set; i.e. 50% (P>0.05). The number of fruit per inflorescence produced by PGR treated plants ranged from 3-25 fruits, compared to 2-13 fruit per inflorescence for the control groups. The number of fruit per plant measured during 10 weeks

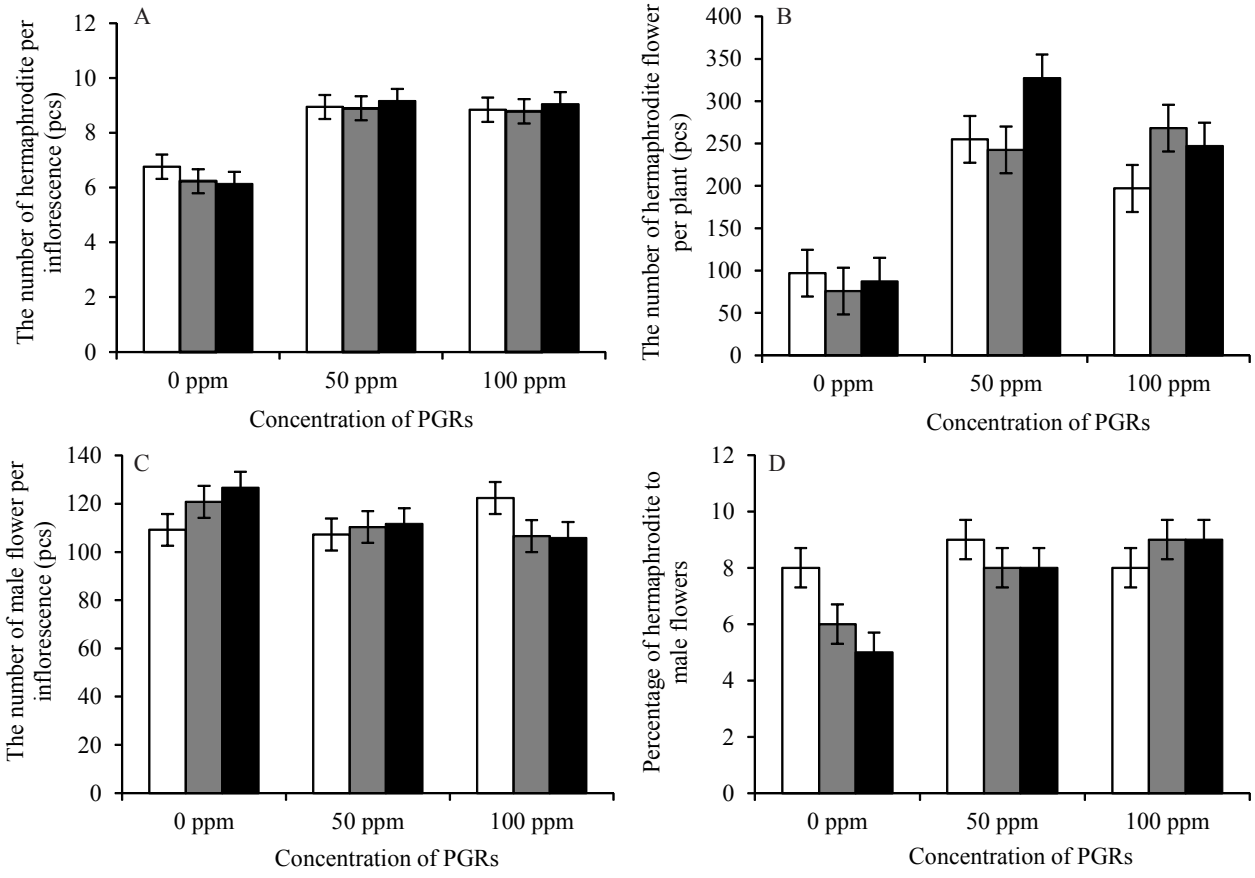

Figure 3. Effect of kinetin, GA_3, and IAA treatment on Jatropha curcas flowers. The number of hermaphrodite flowers per inflorescence (A), the number of hermaphrodite flowers per plant (B), the male flowers per inflorescence (C), the percentage of hermaphrodite to male (D). Vertical lines on bars represent SE (standard error). □ Kinetin, ▩ GA_3, ■ IAA.

of observation was not affected by the type of PGR (P>0.05), but it was significantly affected by the concentration of PGRs (P<0.05). Treatment with IAA at 50 ppm produced a significantly higher number of fruit per plant compared to other treatments. Increases in the number of fruit per plant for groups treated with IAA at 50 and 100 ppm, were 301.7 and 167.4% respectively. For plants treated with kinetin at 50 and 100 ppm, the increases were 162.5 and 101.4% respectively, and for plants treated with GA_3 at 50 and 100 ppm, 211.7 and 257.0% respectively (Figure 4B). Fruit size was nearly the same in each treatment group. Fruit set for all treatments were approximately 100% for early and 95% for final fruit harvested. Fruit length ranged from 3.3-4.0 cm with an average of 3.7 cm; width ranged from 3.0-3.7 cm with an average of 3.5 cm.

The fruit of JC has 3 loculars. Generally, each locular contains 1 seed (Figure 5A,B), but occasionally some loculars do not produce seeds. It is very interesting to note that in this study, in plants treated with PGRs, approximately 2% of fruit had 4, rather than 3 loculars (Figure 5C,D). In andromonoecious JC fruit with 4 loculars, there will be 2-4 seeds per fruit. Generally, fruit with 4 loculars have large fruit (3.7 cm wide and 3.9 cm long) but seed size (1.1 cm wide and 2.2 cm long) remains similar to that of untreated fruit. This was borne out

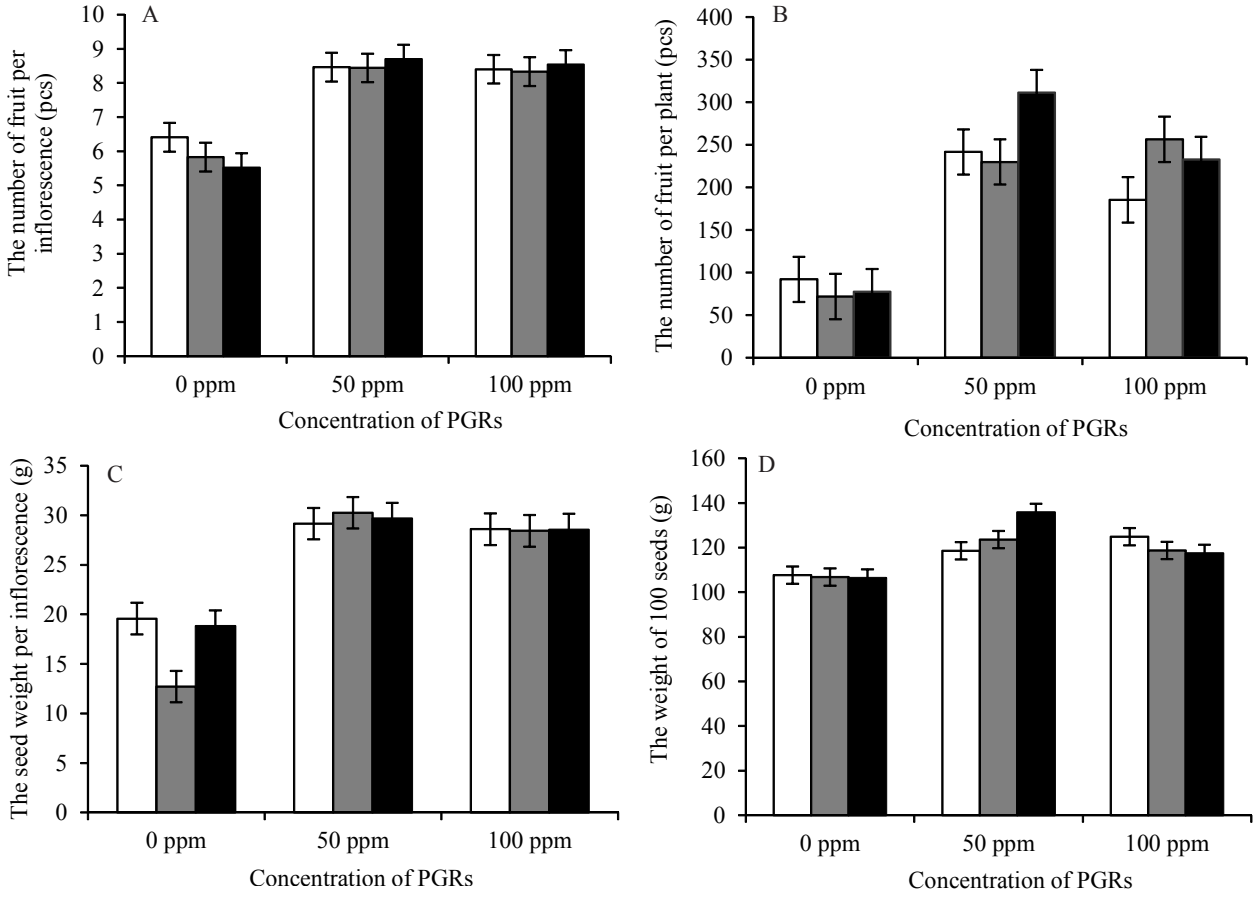

Figure 4. Effect of kinetin, GA_3, and IAA to Jatropha curcas fruits. The number of fruit per inflorescence (A), the number of fruit per plant (B), the seed weight per inflorescence (C), the weight of 100 seeds (D). Vertical lines on bars represent SE (standard error). □ Kinetin, ▩ GA_3, ■ IAA.

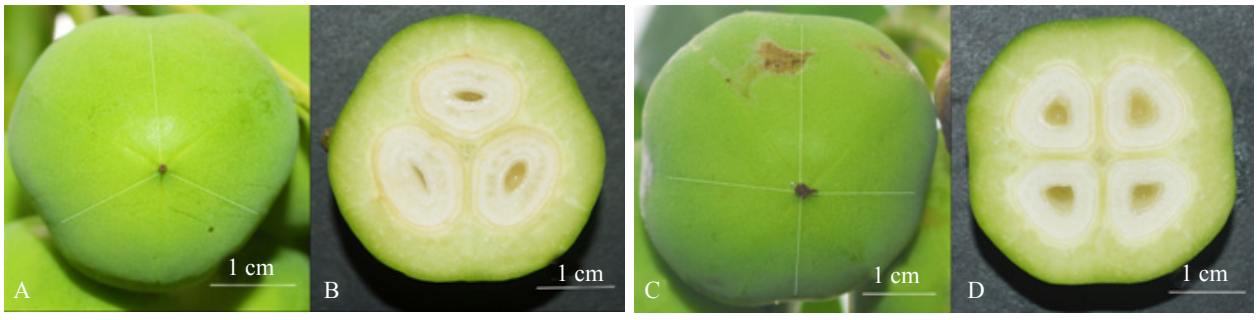

Figure 5. Fruit of andromonoecious *Jatropha curcas* with three loculars per fruit (A and B), with four loculars (C and D).

in our results, with plants treated by PGRs having an increased number of seeds per inflorescence ($P<0.05$), but with seed size almost identical for each treatment. Seed length ranged from 2.0-2.6 cm with an average 2.4 cm, and seed width ranged from 0.9-1.3 cm with an average 1.2 cm.

Seed weight per inflorescence was significantly influenced by PGR treatments ($P<0.05$). PGR treatment significantly increased seed weight per inflorescence compared to control, but there was no significant difference in seed weight per inflorescent in groups treated with PGR at 50 ppm compared to 100 ppm (Figure 4B). Plants treated with PGRs produced between 8.1-79.0 g in seed per inflorescence, compared to control plant which produces only 4.0-35.4 g of seed per inflorescence. This increase in seed weight per inflorescence was reflected in a corresponding increase in weight per 100 seeds ($P<0.05$) (Figure 4C). The weight of 100 seeds produced by plants treated with PGRs ranged between 102.7-183.6 g, whereas that of untreated plants ranged from 90.4-115.6 g.

DISCUSSION

Flower Development in Andromonoecious JC. Type of PGR used in treatment (kinetin, GA_3, and IAA) produced no significant difference in flowering character. However, the concentration of each of the PGR (50 and 100 ppm) greatly influenced the development of flowers in andromonoecious JC, from the number of inflorescences to the number of flowers, and the sex type of flowers in andromonoecious JC. Foliar application of kinetin, GA_3, and IAA at 50 and 100 ppm increased the growth and development of andromonoecious JC flowers.

The number of inflorescence per plant produced during 10 weeks of observation increased in groups treated with kinetin, GA_3, and IAA at 50 and 100 ppm. Kinetin, GA_3, and IAA may have this effect via an increase in flowering meristem activity, stimulating initiation of flowering in the apical meristem and meristem side buds on nodes. Kinetin, GA_3, and IAA each works via a different mechanism to increase the number inflorescence per plant.

In our study, treatment with kinetin increased flowering meristem activity, especially at 50 ppm concentration, thus increasing the number of flowers buds per plant or inflorescence. Similar effects have been found in other studies. Kinetin at 10, 20, and 40 mg/l has been shown to increase the number of flowers per plant on lentil crops (Khalil *et al*. 2006). Treatment with paklobutrazol on durian plants (*Durio zibeinus*) increased the content of kinetin naturally occurring in the plants, and the number of flowers per plant. The increase in kinetin content and in the number of flowers generated, corresponded to an increased dose of paklobutrazol, i.e. greater in trees treated at 4 g per tree than 2 g per tree and 0 g per tree (Sakhidin & Suparto 2011). Exogenous cytokinin application has been shown to increase inflorescence meristem activity and promote floral initiation in several species (Wang & Li 2008).

GA_3 also works to increase the number of inflorescence per plant by increasing flowering meristem activity. Application of higher doses of GA_3 results in a higher number of inflorescences. Thus plants treated with GA_3 at 100 ppm produced a higher number of inflorescence per plant than those treated at 50 ppm. GA_3 produced the most important effects with regard to increased plant flowering, indicating that GA_3 treatment may increase flowering meristem activity (Li-Jun *et al.* 2008) IAA works to increase the number of inflorescences not only by increasing flowering meristem activity, but also by increasing the number of branches. The number of secondary branches produced in plants treated with IAA treatment averaged 23 branches, while the number of branches that developed in plants treated with GA_3 and kinetin numbered only 18 and 13 branches respectively. Each secondary branch produced an inflorescence, so that IAA treatment especially at 50 ppm concentration resulted in a higher number of inflorescences per plant than treatment with GA_3 and kinetin.

Treatment with IAA and GA_3 both increased the number of inflorescences per plant. GA_3 treatments, however, also increased the content of endogenous auxin, so that the effect of GA_3 and IAA treatments was quite similar. Treatment with IAA and GA_3 both increased flower formation induction. Isenberg *et al.* (1974) and Kojima (1996) also stated that flower formation induction has been shown to increase by using gibberellin and auxin. GA_3 treatments to the plant will increase the amount of auxin through proteolitic enzyme formation, which will produce tryptophan as the auxin precursor (Kusumawati *et al.* 2009). In addition, auxin serves as a cellular signal to control growth rate and other aspects of plant growth (Silvaci & Yacin 2008). Therefore, Gaudinova *et al*. (2009) found that the amount of endogenous auxin also increased along the flower formation; however, it was not the same with cytokinin in blanka's plants. In addition to increasing the number of inflorescences,

PGR treatments have also been shown to increase inflorescence branching. The flowers of andromonoecious JC have the same inflorescence branching pattern as monoecious JC: the *dichasial* type (Wang & Ding 2012). In this pattern, an inflorescence branch consists of main branches (primary), that split off into secondary, and then tertiary branches. In our study, PGR treatments (kinetin, GA_3, and IAA) increased the number of secondary and tertiary branches developed, and caused the formation of an additional level of quaternary branches. The increased number of branches and number of flowers per inflorescence in these plants can be attributed to the effect of PGR treatment on plant growth and development. PGRs act as growth regulators that can increase cell division and differentiation (Taiz & Zeiger 2010). But each PGRs has different role in improving the growth and development of the flower.

In this research, kinetin acted to stimulate the development of buds in each inflorescence, therefore this may have increased the number of branches inflorescence. Kinetin works by stimulating cell division which results in more cells that eventually become buds. Thus, application of cytokinin would be expected to result in the growth of axillary buds (Taiz & Zeiger 2010).

IAA is a compound from the class of auxin hormones, that affects the development of the meristem into a flower or leaf. Development of the floral meristem depends on auxin transport from sub apical tissue. Without transport of auxin, the meristem will be deficient in this hormone, and phyllotaxis and flower development will be disturbed (Kuhlemeier & Reinhardt 2001). Normally, auxin causes apical dominance in plants. The influence of apical dominance can be seen in the development of inflorescence branches on JC flowers which take the form of *Infloresentia racemosa* (infinitely compound flowers). At the beginning of inflorescence, two main branches develop, followed by secondary and tertiary branches. Secondary branching develops after the terminal flower forms at the end of the primary, and tertiary branching develops after the terminal flower forms at the end of the secondary branches. In the present research, we found that application of IAA may reduce the impact of apical dominance on inflorescence, so that secondary, tertiary, and quaternary inflorescence branches were more numerous and formed faster.

Increasing the number of inflorescence branches would influence the number of hermaphrodite flowers, because each branch terminates in a hermaphrodite flower, especially primary and secondary branches. However, the sex of a terminal flower can be affected by treatment with PGRs. Generally, the terminal flower of tertiary branches is male, but in plants treated with PGRs, the tertiary branches produced hermaphrodite terminal flowers. In this case, PGRs seems to have affected the sex of terminal flowers by influencing the formation of the structure of flowers. PGRs affected apical cell differentiation of JC flowers. Apical cells from each terminal flower differentiated to form carpels and staments. Carpel did not develop in the male flowers, abortion of stamens occurred in the female flowers, while carpel developed and abortion of stamens did not occurred in the hermaphrodite flowers. The process of sex differentiation of monoecious JC flowers is described in Wu *et al.* (2011). In that study, in the early phase, there was no differentiation between male and female flowers. All flowers had identical parts: sepal primordial, petal, glandule, and stamen. In the next phase, the apical meristem of inflorescence branches elongated and differentiated to form carpels, while the stamens were aborted, so that the fully developed terminal flower was female. In cases where the apical meristem does not differentiate, the flower will retain the stamens and the terminal flower will be male when fully developed. Thus, PGR treatments in our research may stimulate differentiation of the apical meristem cells to form carpels in the branch terminal flowers, while retaining the existing stamens, thus resulting in hermaphrodite terminal flowers.

In addition to affecting apical cell differentiation, treatment with PGRs can influence the amount and proportion of growth regulators found in treated plants. Changes in PGR content can affect the development of flower parts, and influence the sex of the flowers. PGRs influence the formation and the abortion of pistils or stamens in female and male flowers (Khryanin 2002). Kojima (1996) suggested that changes in the PGR content of plants can affect the development of flower parts and early fruit growth.

Kinetin treatments in this research affected the development and sex of terminal flowers in every inflorescence branch. Generally, terminal flowers are male, but were replaced by hermaphrodite flowers in our study. In this case, kinetin caused a change of sex from male to hermaphrodite flowers. This phenomenon has been described in several studies. Replenishment of cytokinin (BAP) 67 μM in the regenerating media for male spinach plants resulted

in expression of 11.1% of plants as andromonoecious (Komai *et al.* 1999). In monoecious JC, application of benzyladenine (BA) at a concentration of 320 mg/L produced 3.1% hermaphrodite flowers (Pan & Xu 2011).

In the present research, treatment with GA_3 also influenced flower sex, especially of terminal flowers in the tertiary branches. The terminal flowers in these inflorescence branches are usually male, but plants treated with GA_3 nearly all produced hermaphrodite flowers on their tertiary branches. We suspect that GA_3 treatments affect the expression of genes that control the sex of flowers or plants. GA_3 works to enhance flower growth and formation by activating genes in the flower meristem. It does this by producing a protein which induces the expression of the gene to form flower parts, such as petals, sepals, stamens and pistils (Kusumawati *et al*. 2009), thus hermaphrodite flower, a complete flower, would be induced. In the gibberellin pathway, gibberellin affected the formation of flowers and flower parts through the expression of *AGAMOUS-LIKE 20* and *LEAFY* genes, furthermore the expression of *APETALA 3* gene have a role in formation of petals and stamens (Taiz & Zeiger 2010). In other studies, spinach plants replenished with GA_3 at 10 µM for regeneration were able to express 2.10% gymonoecious from female plants and 42.20% andromonoecious from male plants. Addition of GA_3 at 144 µM was able to express 51.90% andromonoecious from male plants (Komai *et al.* 1999). GA_3 treatment has also been shown to increase the ratio of females in spinach plants (Culcafic & Neskovic 1980). The result of Makwana *et al.* (2010) showed an increase of 125.97 and 184.78%, in the number of female flowers in JC plants that were treated with GA_3 at 100 and 1000 ppm. The female to male flower ratio in this treatment group was 1:15 and 1:13. However, a higher proportion of female flowers does not always increase fruit production, because not all female flowers are able to develop into fruit. Finally, some studies show non-linear effects of GA_3 treatment. Treatment of JC plants with GA_3 at 200 ppm increased the number of total flower and of female flowers but higher doses of GA_3 at 400 and 600 ppm caused a decrease in these measures (Kusumawati *et al.* 2009). Replenishment of exogenous auxin affects the amount of auxin within the plant so as to affect the sex of its flowers. We see this in our treatment of andromonoecious JC with IAA, which caused flowers to change sex from male to hermaphrodite on tertiary inflorescence branches. In *C. sativus,* differences in auxin content result in differential expression of plant sex as well. *C. sativus* grown with IAA at 1.91 µg in 100 g material will express hermaphrodite sex, and plants grown with 0.95 µg will express andromonoecious sex (Galun *et al.* 1964).

Production of Andromonoecious JC. In general, fruit set can be affected by many factors, including variations in plant population, age, climate and soil conditions (Luo *et al*. 2007). Fruit set for andromonoecious JC flowers in our study was not affected by treatment with PGRs, but rather determined by the number of hermaphrodite flowers produced on each inflorescence. Early fruit set for andromonoecious JC can reach 100%. Inflorescences which contain many hermaphrodite flowers (>15 pcs per inflorescence), tend to have a lower final fruit set of around 85%. In this case, factors such as nutrient limitations or photosynthate competition among flowers or fruits may also affect fruit set. Flowers that bloom and experience early conception will absorb more nutrients, faster than flowers that bloom later. The fruit that comes from hermaphrodite flowers that bloomed later, tended not survive until harvest if the number of hermaphrodite flowers in the inflorescence was very high.

Application of GA_3 at 50 and 100 ppm seems to increase the number of hermaphrodite flowers and fruits per inflorescence, according to our results. Foliar application of GA_3 is not recommended in high concentrations, as it may trigger a process of cell death in the flower stalks or developing fruit, so that fruit may fall before it can be harvested. Steffens and Sauter (2005) stated that treatment of GA_3 of plants can lead to the release of hydrogen peroxide, and cause cell death. The same result was found by Makwana *et al*. (2010) i.e. that monoecious JC plants treated with GA_3 at 1000 ppm developed more female flowers than other plants, but produced less fruit set than average, as a result of production of hydrogen peroxide (H_2O_2).

In our study, plants treated with PGRs showed an increase in the number of fruit per inflorescence, the number of seeds per inflorescence, seed weight per inflorescence, and weight of 100 seeds. Based on these results, it can be stated that the production of andromonoecious JC can be increased by the treatment with kinetin, GA_3, and IAA. This result is in line with findings by Nurnasari and Djumali (2011), that paclobutrazol, GA_3, NAA, mepiquat chloride, and 2.4-D treatments can increase the number of female flowers and number of fruits in monoecious JC.

We can assess the effect of PGR treatment on yield at 10 weeks after flowering, by calculating the number of flowers, inflorescence, and fruit. We found an increased yield of 301.7 and 257.0% in plants treated with a 50 ppm concentration of IAA and 100 ppm concentration of GA_3, respectively. An increased yield of fruit will lead to increased seed yield, with each fruit containing 3-4 seeds.

From our results we conclude that foliar application to andromonoecious JC plants, of kinetin, GA_3, and IAA at 50 and 100 ppm, increases the number of hermaphrodite flowers per inflorescence, number of inflorescences per plant, number of fruit per inflorescence, and seed dry weight. The PGR treatments with the best results using this technique were IAA and GA_3 at 50 ppm concentration. Foliar applications of PGRs began after the plants entered the process of flower initiation.

ACKNOWLEDGEMENT

We are truly grateful to the Agricultural Development and Research Center, Ministry of Agriculture for their financial support for this study through participation in the KKP3T program in 2009. We also offer thanks to Adi Supardi and Abdul Hakim Hidayatullah for their excellent technical assistance.

REFERENCES

Abdelgadir HA, Johnson SD, Staden JV. 2009. Effect of foliar application of plant growth regulators on flowering and fruit set in *Jatropha curcas*–A potential oil seed crop for biodiesel. *South Afr J Bot* 27:1.

Ahoton LE, Quenum F. 2012. Floral biology and hybridization potential of nine accessions of physic nut (*Jatropha curcas* L.) originating from three continents. *Tropicultura* 30:193-198.

Alam NCN, Abdullah TL, Nur APA. 2011. Flowering and fruit set under Malaysian climate of *Jatropha curcas* L. *Am J Agric Biol Sci* 6:142-147. http://dx.doi.org/10.3844/ajabssp.2011.142.147

Culafic L, Neskovic M. 1980. Effect of growth substances on flowering and sex expression in isolated apical buds of *Spinacia oleracea*. *Plant Physiol* 48:588-591. http://dx.doi.org/10.1111/j.1399-3054.1980.tb03310.x

Dellaporta SL, Urrea AC. 1993. Sex determination in flowering plants. *Plant Cell* 5:1241-1251. http://dx.doi.org/10.1105/tpc.5.10.1241

Galun E, Izhar S, Atsmon D. 1964. Determination of relative auxin content in hermaphrodite and andromonoecious *Cucumis sativus* L. *Plant Physiol* 40:321-326. http://dx.doi.org/10.1104/pp.40.2.321

Gaudinova A, Malbeck J, Dobrev P, Kubelkova D, Spak J, Vankova R. 2009. Cytokinin, auxin, and abscisic acid dynamics during flower development in white and red currants infected with Blackcurrant reversion virus. *Physiol Mol Plant Pathol* 73:119-125. http://dx.doi.org/10.1016/j.pmpp.2009.03.004

Isenberg FMR, Thomas TH, Pendergrass M, Rahman MA. 1974. Hormone and histological differences between normal and mallic hydrazide treated onions stored over winter. *Acta Hort* 38:95.

Jones MH, Csurhes S. 2008. *Pest Plant Risk Assessment Jatropha (Jatropha curcas)*. Bio Security Queensland Department of Primary Industries and Fisheries. Queensland.

Khalil S, El-Saeid HM, Shalaby M. 2006. The role of kinetin in flower abscission and yield of lentil plant. *J Appl Sci Res* 2:587-591.

Khryanin VN. 2002. Role of phytohormones in sex differentiation in plants. *Russ J Plant Physiol* 49:545-551. http://dx.doi.org/10.1023/A:1016328513153

Kojima K. 1996. Changes of abscisic acid, indole-3-acetic acid and Gibberellin-Like substances in the flowers and developing fruitlets of citrus cultivar 'Hyuganatsu'. *Sci Hort* 65:263-272. http://dx.doi.org/10.1016/0304-4238(96)00882-5

Komai F, Masuda K, Ishizaki T, Harada T. 1999. Sex expression in plants regenerated from the root callus of female and male spinach (*Spinacia oleracea*). *Plant Sci* 146:35-40. http://dx.doi.org/10.1016/S0168-9452(99)00090-4

Kuhlemeier C, Reinhardt D. 2001. Auxin and Phyllotaxis. *Trends Plant Scie* 6:187-189. http://dx.doi.org/10.1016/S1360-1385(01)01894-5

Kusumawati A, Hastuti ED, Setiari N. 2009. Pertumbuhan dan pembungaan tanaman jarak pagar setelah penyemprotan GA3 dengan konsentrasi yang berbeda. *J Penelitian Saintek* 10:18-29.

Li-Jun A, Liang J, Chun-qin Y, Tian-hong L. 2008. Effect and functional mechanism of the action of exogenous gibberellin on flowering of peach. *Agric Sci China* 7:1324-1332. http://dx.doi.org/10.1016/S1671-2927(08)60181-9

Luo CW, Kun L, You C, Sun YY. 2007. Floral display and breeding system of *Jatropha curcas* L. *Forest Studies China* 9:114-119. http://dx.doi.org/10.1007/s11632-007-0017-z

Makwana V, Shukla P, Robin P. 2010. GA application induces alteration in sex ratio and cell death in *Jatropha curcas*. *Plant Growth Regul* 61:121-125. http://dx.doi.org/10.1007/s10725-010-9457-x

Meagher TR. 2007. Linking the evolution of gender variation to floral development. *Ann Bot* 100:165-176. http://dx.doi.org/10.1093/aob/mcm035

Miller JS, Diggle PK. 2007. Correlated evolution of fruit size and sexual expression in andromonoecious Solanum sections Acanthophora and Lasiocarpa (Solanaceae). *Am J Bot* 94:1706-1715. http://dx.doi.org/10.3732/ajb.94.10.1706

Nurnasari E, Djumali. 2011. Respon tanaman jarak pagar (*Jatropha curcas* L.) terhadap lima zat pengatur tumbuh (ZPT). *Bul Tan Tembakau, Serat & Minyak Industri* 3:71-79.

Pan BZ, Xu ZF. 2011. Benzyladenine treatment significantly increases the seed yield of the biofuel plant *Jatropha curcas*. *Plant Growth Regul* 30:166-174. http://dx.doi.org/10.1007/s00344-010-9179-3

Raju AJS, Ezradanam V. 2002. Pollination ecology and fruiting behaviour in a monoecious species, *Jatropha curcas* L. (*Euphorbiaceae*). *Curr Sci* 83:1395-1398.

Sakhidin, Suparto SR. 2011. Kandungan giberelin, kinetin, dan asam absisat pada tanaman durian yang diberi paklobutrazol dan etepon. *J Hort Indones* 2:21-26.

Sharma DK, Pandey AK, Lata. 2009. Use of *Jatropha curcas* hull biomass for bioactive compost production. *Biomass Bioenergy* 33:159-162. http://dx.doi.org/10.1016/j.biombioe.2008.05.002

Sivaci A, Yalcin I. 2008. The seasonal changes in endogenous levels of indole-3-acetic acid, gibberellic acid, zeatin and abscisic acid in stems of some apple varieties (*Malus sylvestris* Miller). *Asian J Plant Sci* 7:319-322. http://dx.doi.org/10.3923/ajps.2008.319.322

Steffens B, Sauter M. 2005. Epidermal cell death in rice is regulated by ethylene, gibberellin, and abscisic acid. *Plant Physiol* 139:713-721. http://dx.doi.org/10.1104/pp.105.064469

Taiz L, Zeiger E. 2010. *Plant Physiology*. 5rd ed. Sinauer Associates. p 423-515.

Wahyudi A, Wulandari S. 2007. Potensi permasalahan dan pengembangan agribisnis jarak pagar di Indonesia. *Info Tek Jarak Pagar* 2:30.

Wang XR, Ding GJ. 2012. Reproductive biology characteristic of *Jatropha curcas* (*Euphorbiaceae*). *Rev Biol Trop* 60:1525-1533. http://dx.doi.org/10.15517/rbt.v60i4.2070

Wang YH, Li JY. 2008. Molecular basis of plant architecture. *Annu Rev Plant Biol* 59:253-279. http://dx.doi.org/10.1146/annurev.arplant.59.032607.092902

Wijaya A, Susantidiana, Harun MU, Hawalid H. 2009. Flower characteristics and the yield of Jatropha (*Jatropha curcas* L.) accessions. *HAYATI J Biosci* 16:123-126. http://dx.doi.org/10.4308/hjb.2009.16.4.123

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

Yi C, Zhang S, Liu X, Bui HTN, Hong Y. 2010. Does epigenetic polymorphism contribute to phenotypic variances in *Jatropha curcas* L.? *BMC Plant Biol* 10:259. http://dx.doi.org/10.1186/1471-2229-10-259