EFFECT OF GIBBERELLIN IN INDUCING ALTERATION OF FLOWER SEX RATIO IN *Jatropha curcas*

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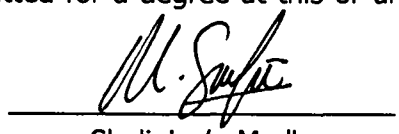

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ABSTRACT

A field experiment was conducted at the School of Sustainable of Agriculture in Universiti Malaysia Sabah, Sandakan, Sabah to determine the effect of gibberellins concentration on the flower sex ratio and yield of *Jatropha curcas*. The experimental design was using Completely Randomized Design (CRD). This study was done using four levels of treatments, 0 ppm (control), 50 ppm, 100 ppm and 150 ppm of gibberellin at which each treatment were replicated 6 times. The spraying was done for three times with the interval of five days on the Jatropha flower buds. Data was collected from the day of last spray on the flower buds until the fruit development. The results were analyzed using various analyses such as One Way ANOVA, Independent-Samples T-test, and also descriptive analysis through SPSS version 21 software. Although the study showed that increase in gibberellin effect increase the mean number of female flowers which lead to alteration on the flower sex ratio but could not be proven through statistical analysis as it shows not significant result at 5% of significant level. Whereas, result on flower sex ratio showed that gibberellin has caused alterations. However, the alteration did not increase the ratio but reduced after being treated with gibberellin. This is due to large difference between the mean values of male and female flowers on treated plants lead to reduction in the flower sex ratio being reflected. Application of 50 ppm and 100 ppm of gibberellin shows good effect on the number of flowers and fruits development. Application of 150 ppm of gibberellin increased mean number of female flowers but does not translate to higher fruit yield compared to fruit yield produced form concentrations of 50 ppm and 100 ppm. Therefore, treating with gibberellin would be efficient in increasing flower and fruit yield, although increase in number of female flower and number of fruits could not be proven through statistical analysis. This study could be tried with other types of plants to find out whether this result would be applicable for them.

ABSTRAK

Satu kajian telah dijalankan di Sekolah Pertanian Lestari di Universiti Malaysia Sabah, Sandakan, Sabah untuk menentukan kesan kepekatan gibberellin terhadap nisbah seks bunga dan hasil Jatropha curcas aksesi. Reka bentuk eksperimen yang telah digunakan adalah Rekabentuk rawak lengkap (CRD). Kajian ini dilakukan dengan menggunakan empat peringkat rawatan, 0 ppm (kawalan), 50 ppm, 100 ppm dan 150 ppm gibberellin di mana setiap rawatan direplikasi 6 kali. Penyemburan dilakukan selama tiga kali dengan selang lima hari pada tunas bunga Jatropha curcas. Data telah dikumpulkan bermula daripada hari terakhir semburan pada tunas bunga sehingga buah matang. Keputusan telah dianalisis dengan menggunakan pelbagai analisis seperti ANOVA satu hala pada aras keertian 0.05, Bebas ujian Sampel T, dan juga analisis deskriptif melalui perisian SPSS versi 21. Walaupun kajian menunjukkan bahawa peningkatan dalam kesan gibberellin meningkatkan min bilangan bunga betina yang membawa kepada perubahan pada nisbah jantina bunga, perkara ini tidak dapat dibuktikan melalui keputusan statistik kerana analisis tidak menunjukkan keputusan yang signifikan pada aras keertian 0.05. Manakala, hasil pada nisbah jantina bunga menunjukkan gibberellin telah menyebabkan perubahan. Walaubagaimanapun, perubahan yang berlaku tidak menunjukkan peningkatan tetapi telah menunjukkan pengurangan setelah dirawat dengan gibberellin. Hal ini kerana wujudnya perbezaan yang amat besar antara nilai min bunga jantan dan bunga betina pada pokok yang dirawat dengan gibberellin yang menyebabkan wujudnya pengurangan pada nisbah jantina bunga. Penggunaan rawatan 50 ppm dan 100 ppm gibberellin menunjukkan kesan yang baik kepada bilangan bunga dan perkembangan buah. Penggunaan rawatan 150 ppm gibberellin meningkatkan min bilangan bunga betina tetapi tidak meningkatkan hasil buah yang dihaslikan berbanding dengan hasil buah yang dihasilkan melalui kepekatan 50 ppm dan 100 ppm. Oleh itu, penggunaan rawatan gibberellin berkesan dalam meningkatkan bunga dan hasil buah walaupun perkara tersebut tidak dapat dibuktikan melalui keputusan statistik. Kajian ini boleh dibuat pada pokok-pokok yang lain untuk mengetahui sama ada keputusan ini berkesan kepada pokok-pokok tersebut.

TABLE OF CONTENTS

LIST OF TABLE

LIST OF FIGURES

LIST OF SYMBOLS, UNITS AND ABBREVIATIONS

%	Percent
°C	Degree Celsius
ANOVA	Analysis of Variance
BA	6-benzyladenine
cm	Centimetre
DEGJSP	DEG Jatropha Support Programme
FAO	Food and Agriculture Organisation
FACT	Fuels from Agriculture in Communal Technology
ICBF	International Conference on Bio-fuel Crop Production and Development
g	Gram
g/L	Gram per litre
GA	Gibberellins
H_2O_2	Hydrogen peroxide
IAA	Indole-3-acetic acid
IBA	Indole butyric acid
ICBF	International Conference on Bio-Fuel Crop Production and Development
kg	Kilogram
m	Metre
µg/L	Microgram per litre
mg/L	Milligram per litre
ml	Millilitre
mm	Millimetre
NAA	1-naphthaleneacetic acid
ng	Nanogram
OH	Hydroxyl group
ppm	Part per million
SLDB	Sabah Land and Development Board
SO_2	Sulphur dioxide

CHAPTER 1

INTRODUCTION

1.1 Background

Biofuel is a type of fuel that is made from the organic materials such as plants, fruits or seeds. This fuel is an alternative way to the fossil fuel as fossil fuel is a non-renewable energy. Biofuel can be categorized into two generation. First generation is also termed as conventional biofuel as they are made from plants being cultivated for the purpose of fuel production. The second generation is usually made from the wastes of agriculture site. Plants that can be used for the purpose of biofuel production are sunflower, soybeans, canola, cotton, groundnuts, *Jatropha curcas*, oil palm, avocado, croton, and castor. Currently, *Jatropha curcas, Croton megalocarpus* and castor become the main focus of the world in producing biodiesel.

Jatropha curcas also known as Jatropha is a perennial deciduous shrub which belongs to the family Euphorbiaceae. Possible origin of Jatropha is in Central America and it has been widely distributed in the tropics and subtropics (Fairless, 2007; Carels, 2009; Makkar and Becker, 2009). Portuguese had established Jatropha plant in Asia and Africa as one of the oil producing plant. Jatropha has been widely cultivated in parts of India like Madhya Pradesh, Maharashtra Rajasthan, Gujarat, Andhra Pradesh, and Tamil Nadu (Augustus *et al.,* 2002). There have been many investors investing on researches and cultivation of Jatropha since the year of 2005. Moreover, in the year of 2008 there is much properly organized Jatropha cultivation in over 25 countries.

Jatropha has been estimated to be cultivated commercially over 900, 000 hectare all over the world. Above 85 % of Jatropha plantation has been established in Asia, mainly in Myanmar, India, China, and Indonesia. Africa covers about 12 %; which is approximately 120, 000 hectare mainly in Madagascar, Zambia also in

Tanzania and Mozambique. Jatropha cultivation in Latin America is about 20, 000 hectare which covers mostly in Brazil. In future years, Indonesia expected to be the largest producer in Asia for Jatropha plant with 5.2 million hectare, followed by Ghana and Madagascar together will have 1.1 million hectare in Africa. Brazil will be the largest producer in Latin America with 1.3 million hectare (FAO, 2010).

There are 200,000 acres of land in Malaysia has been allocated for the cultivation of Jatropha. This area expected to be expanded to 750,000 acres at the end of 2008. Whereas, Passion Masters Resources Sdn Bhd., which is a growing plantation company in Malaysia plans to cultivate 809 hectares of Jatropha in Belawai, Jerijeh, Tanjung Manis and the Rajang area in Mukah division. This plantation expects to plant two million seedlings in 2009 (BioZio, 2011).

Cultivation of Jatropha is highly favourable in Malaysia due to adequate land and good climatic condition of the country. Many challenges have been taken by Malaysia to increase the production of Jatropha through the partnership between the government agencies and the private sectors. This can lead Malaysia to be the global alternative fuels industry.

Jatropha considered as a valuable multi-purpose crop which used as alternative for petrol-diesel to produce biodiesel. Cultivating Jatropha in commercial way for biodiesel production depends on the market level. Government supports through provision of clearer policies and concern towards developing framework for renewable energy are needed. Supports includes in a way of policies, Research and Development (R & D), incentives, land availability, investments, education, promotions and marketing. All these supports need to be collaborated with the help from several ministries and agencies. (SLDB, 2012).

Gibberellin (GA3) is a type of plant hormone which can be used for the regulation of various development processes. This includes stem elongation, germination, dormancy, flowering, sex expression, enzyme induction, and leaf and fruit senescence. There are more than 100 forms of GA which has been identified from the day of its discovery. This hormone is used in the natural process of dormancy breaking and other aspect of germination (Fordham *et al.*, 2000).

1.2 Justification

Generally, cost of edible oil is much higher than the petroleum diesel. Edible oil is mainly used for the production of vegetable oil. Usage of edible oil in order to produce biodiesel has lead to food oil crisis. Therefore, alternative way has been chosen at which using the much cheaper and low cost level of non edible oil for the production of biodiesel. Among the non-edible oil sources, Jatropha curcas is identified as potential biodiesel source and comparing with other sources, which has added advantages as rapid growth, higher seed productivity, suitable for tropical and subtropical regions of the world. Jatropha biodiesel is ideal solution to meet out higher diesel demand and oil imports.

However, Jatropha cultivation especially in Malaysia also has its limitation as production of seed yield per area is considered low. Most important reason for its poor seed yield is due to less female flower production and low percentage of fruit set by the plant. Several studies are ongoing in different parts of the world to optimize, enhance and exploit the growth, fruiting cycle and different developmental stages of the plant so that the economic yield of the plants can be utilized to the fullest limits.

Therefore, it is important to increase the female flowers in order to increase the yield production of the plant. According to research, phytohormones seem to be the most important factors known to alter sex ratio in plants. The mechanisms by which phytohormones alter sex ratio differ in different plant species. There are many types of hormones such as auxin, ethrel, 6-benzyladenine and gibberellin which could be used to alter the sex ratio in Jatropha plant. According to previous research, 6-benzyladenine is much effective compared to gibberellin on inducing alteration of flower sex ratio. However, handling and application method of gibberellin seem to be much simpler and easier than applying 6-benzyladenine to Jatropha plant. Simple method will be effective and considerable for the farmers to understand them and to apply the knowledge that they have gained.

The aim of this study is to determine the effect of various gibberellin concentrations on the flower sex ratio of Jatropha plant. Applying hormones is a common method to induce alteration in flower sex ratio of plants. However, there is no report stating that this application has been commercialized widely in Jatropha

cultivation. This study might help to increase awareness to use hormones to increase the fruit yield which is a much convenient.

1.3 Objective

Objective of this research is to determine the effect of gibberellins concentration on the flower sex ratio and yield of *Jatropha curcas*.

1.4 Hypothesis

Hypothesis that will be tested in this research is as follows:

H_o: There is no significant difference on the effect of various concentrations of gibberellins on flower sex ratio and yield of *Jatropha curcas*.

H_a: There is significant difference on the effect of various concentrations of gibberellins on flower sex ratio and yield of *Jatropha curcas*.

CHAPTER 2

LITERATURE REVIEW

2.1 Classification of *Jatropha curcas*

Taxonomy classification of Jatropha curcas starts with the kingdom of the plant, which namely called as Plantae. The Division of the plant is Embryophyta while the Class to which the plant belongs to is Spermatopsida. The Order of the plant is Malpighiiales. The Family to which the plant belongs to is Europhorbiaceae. The Genus of the plant is Jatropha and Species is Curcas. The binomial name of the plant is called as *Jatropha curcas* which was established by the scientist, Linnaeus in the year 1753.

2.1.1 Botanical of *Jatropha curcas*

Jatropha plant has smooth and thickened terminal branch. Jatropha tree forms a straight trunk with bark that is gray or reddish in colour. The leaves are arranged in alternate pattern. Jatropha undergoes dormant stage when there is variation in rainfall, temperature, and light. However, simultaneous respond do not occur to all the trees.

Their branches consist with latex which is white in colour. This latex creates brown staining which could be difficult to be removed. Seeds usually produce five roots which consist of one tap root and four lateral roots. Lateral roots could be found on plant that develops from cutting propagation.

Inflorescences could be seen to appear on the bottom most of the branches. Their seeds are black in colour. Outermost layer of the pericarp remain in fleshy condition even when the seed reach the maturity stage. Figure 2.1 shows the different parts of Jatropha plant.

Jatropha is a type of plant that can adapt to arid condition. It is unresponsive to the day length, flowering does not depend on the latitude factor, and can produce flowering at any time in a year (Heller, 1996). Leaf shedding occurs in the dry seasons.

Normally Jatropha varieties have two harvesting stages whereas hybrid varieties can have three harvesting stages in a year. The life span of Jatropha is in the range of 35 to 40 years. The plant could survive for more to 50 years if there is no contact between the root zone and the rising water table.

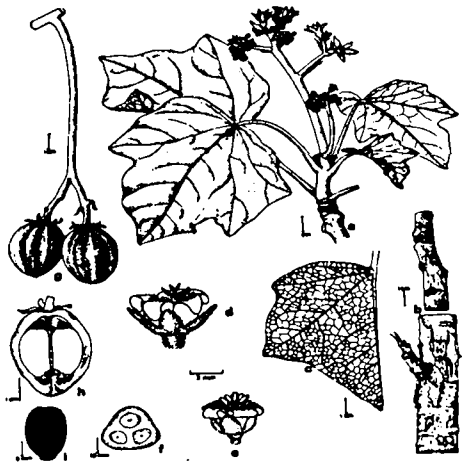

Figure 2.1 Different part of *Jatropha curcas* plant

Source: Verma *et al.*, 2009

2.1.2 Agronomic Requirements of *Jatropha curcas*

Jatropha needs at least 600 mm of rainfall in order for it to flower and set fruit. The optimum rainfall for seed production is considered between 1,000 and 1,500 mm (FACT, 2007), which corresponds to sub-humid ecologies. Rain will aid flowering process after a short (a month) duration of drought. Therefore, it is said to be that flowering process could be manipulated by the irrigation system (FACT, 2007). However, it is crucial to clearly aware that excessive amount of irrigation could lead to increase in the total biomass production at the loss of seed yield. Higher amount of rainfall might lead to fungal attack and halt the growth of root system in all soils but most significant in free-draining soils.

Optimum temperatures for the plant are between 20°C and 28°C at which too high temperature depress yield production. Jatropha is not being able to withstand waterlogged conditions at which usage of heavy clay soils are not preferred. Jatropha

has the ability to grow in alkaline soils but the suggested soil pH should be within 6.0 to 8.0/8.5 (FACT, 2007). Jatropha grow well on soils like aerated sands and loams at a depth of 45 cm (Gour, 2006).

Jatropha requires low nutrient as it can adapt itself to poor condition of soil. However, production of quality crop needs appropriate fertilizer application. Recommended supply of fertilizer for each seedling would be 1 kg of farmyard manure or added with 100 g of Neem waste with a recommendation of 2,500 plants per hectare. This will bring to about 2.5 tonne organic fertilizer per hectare. After the transplantation and beginning stage of plant development, fertilizer such as N, P and K should be applied. Annual application of Twenty gram urea + 120 g Single Super Phosphate (SSP) and 16 g Muriate of Potash (MOP) needed for plant growth and development.

Seedlings planted directly in soil need refilling with top soil and organic manures (500 g farmyard manure, 100 g Neem cake or Jatropha oil cake, and 100 g Super Phosphate) into the pits before planting. Besides organic manure application, there is need for the application of NPK fertilizer (Nitrogen, Phosphorus, and Potassium) for the growth and development of the plant. The NPK ratio is 46: 48: 24 kg per hectare respectively. Application of 20 g of urea, 120 g of Single Super Phosphate and 16 g of Muriate of Potash is recommended for the planting pits.

Normally Jatropha plants are planted about 1,100 to 2,500 plants per hectare. The wider the space between the plants, the higher would be the yield per tree. However, there would be reduction in the yield per hectare (Achten, 2008). Spacing is determined based on the environmental factors. Areas like semi-arid, low-input system need wider spacing such as 3.0 x 2.0, 3.0 x 2.5 or 3.0 x 3.0 metres.

Standard cultural practices are timely weeding which is about 4 times a year (Centre for Jatropha Promotion, 2004). Additional irrigation is not needed if they are planted during the onset of rain. Fallen leaves of Jatropha can be used as mulch around the base of the plant. The organic matter contained in the fallen leaves aids the growth and the activity of earthworms in the soil which near the root zone of the plants. This enhances the fertility level of the soil.

Pruning and trimming is important to give shape to the plant. In the first five years time, the plant need to be trimmed (February to March) and a single pruning to reach the height of 1.5 m. Secondary branches can be induced by pinching the terminal growing branches. Secondary and tertiary branches need to be pruned in the end of first year for a minimum of 25 branch growth in the end of second year.

There is no significant threat to Jatropha by any pest or disease due to the toxic characters in all over the plant. However, there are cases reported at which pest and disease to occur in monoculture plantation. Known disease such as collar rot, leaf spots, root rot and damping-off, can be reduced by applying techniques like fungicide application, and avoiding waterlogged condition. Pest like larvae of the moth *Pempelia morosalis* can affect the flower and young fruits, termites cause damages to young plant. Other common pest in Jatropha plantation are the bark-eating borer *Indarbela quadrinotata*, the blister miner *Stomphastis thraustica*, the semi-looper *Achaea janata*, and the flower beetle. Insecticide application is crucial in critical condition (Brittaine *et al.*, 2010).

Mycorrhizal soil fungi help in enhancing the plant capability in taking up mineral nutrients and water from the soil also increasing resistance level of the plant towards drought and disease. The Energy and Resources Institute (TERI) in India has developed mycorrhizal inoculations for Jatropha that improve germination and give earlier fruiting and higher yields.

In Brazil, studies on mycorrhizal inoculation of Jatropha are also showing promise in improving uptake of Phosphorus and Potassium (Carvalho, 2008, cited Parsons, 2008). Insertion of mycorrhizal fungi to the *Jatropha curcas* plants cause plant to adapt well and encourage the young seedling to grow fast with adequate amount of nutrient uptake. This will mean the plants will be more likely to survive stressful environmental conditions and give higher yields.

Fungi helps to increase the soil volume at which nutrients are gathered by increasing resistance towards drought condition and reduces time to outplanting. Mycorrhizal fungi also facilitate other nutrient uptake which is essential for plant growth. Mycorrhizal fungi have the ability to take up unavailable forms of nutrients like Phosphates for the plant use.

2.1.3 Propagation of *Jatropha curcas* plant

Jatropha cultivation can be carried through methods like nursery, direct sowing in field, and also through vegetative propagation. The most suggested method in cultivating Jatropha is through nursery-raised plants from cuttings and seeds.

For commercial cultivation of Jatropha, seeds are preferred. As the first step in the cultivation through seed propagation, the seeds need to be soaked in solution containing cow dung for 12 hours. The seeds are then kept under the wet gunny bags for 12 hours. Good germinate seed need hot and humid weather condition. Seeds that have germinated are planted in the polybags (15 cm X 25 cm) which are filled with potting mix of soil, sand, and farm yard manure in the ratio of 1:1:1 respectively.

Seeds and cuttings of Jatropha also can be planted directly in the field. However, pre-rooted cuttings are best to be planted in the polybags before transplanting into the field for better yield production. When considering a piece of land for planting Jatropha, one or two times of ploughing will be required. This process is depending on the nature of the soil. Direct planting of seeds or cuttings in the field need spacing of 3 m X 2 m. Ploughing could be impossible in the areas like hilly areas and pits with the size of 30 cm X 30 cm X 30 cm need to be dug with recommended spacing.

Heller (1996) found that Jatropha grown from cutting propagation of at least 30 mm diameter gave earlier and higher initial yields than plants grow from seed. There is little or no yield difference was seen for later harvests. There are various cuttings types of Jatropha available as shown in Figure 2.2. Recommended length of the cuttings ranges between 25 to 120 cm. Highest survival rate seem to be on the cuttings taken from the mid - lower part of one year-old branches (Kaushik and Kumar, 2006, cited Achten, 2008).

These cuttings can be planted in polyethylene bags, planted direct into the soil, or in shaded nursery beds by inserting 10 to 20 cm into the soil. Before inserting the cuttings, soil need to be filled in the polybags.

John Innes who established the John Innes Horticulture Institute in 1910 had proposed sterile growing media in order to gain consistent results when conducting experiments. The potting composts were not produced commercially in the institute but the formulae were exposed to the public and used by manufacturers and growers. There are many type of potting mixture established by John Innes. Standard potting that is normally used is in the ratio of 3:2:1 (Top soil: Sand: Organic).

The potting mix used for stem cuttings is 1:1:1 (mixed soil and farm yard manure). Polybags used must be with the holes at the bottom. The sizes of cuttings could vary and could be as wide as six inches (15 cm). Cuttings of Jatropha will follow as the mother plant and can be considered as the genetic clone of the mother plant.

Besides, cuttings are also preferred than the seeds as the cuttings have higher survival rate compared to seeds. Jatropha cuttings can grow root at fast rate and develop into a productive tree. Survival rate of cuttings could reach closely to 100 % and can reach to height of six to eight inches (20.2 to 30.5 cm) within two to three weeks with the development of eight to twelve secondary leaves.

Root formation takes within two to three months and cuttings should be planted near the rainy season. Rooting could occur as soon as the rain starts off. Rooting could be enhanced with the usage of well drained and aerated rooting media. Besides, rooting could start to form within 30-45 days after planting with the application of IBA (indole butyric acid).

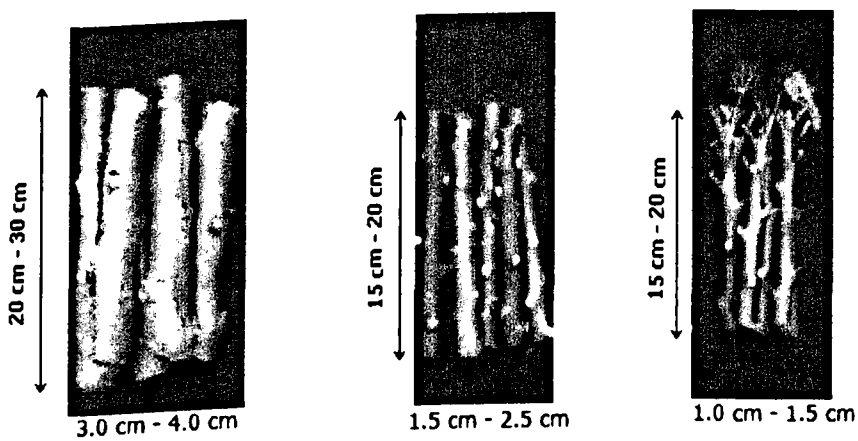

Figure 2.2 Different types of stem cuttings of *Jatropha curcas* plant
Source: Aminul *et al.*, 2010

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