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Research Article
Effects of Thinning, Physiological Stress and Gibberellic Acid on *Hibiscus* Flower Growth and Development

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Abstract

Background and Objectives: *Hibiscus* plant (*Rosa sinensis)* is an important in the field of ornamental plant. Its beautiful blooms make its perfect in the environmental beautification. The study was undertaken to evaluate the effects of thinning, phloem cut stress (represented by bark ringing) and hormone application (Gibberellic acid, GA_3) on the flowers development and the longevity. **Materials and Methods:** Excess leaves were discarded from the branches in the range of 20 cm. A 3 cm length of bark was removed from the trunk leaving 4 mm width connecting bark band. The GA_3 of 20 mg L^{-1} concentration was sprayed to the branches twice a week. Number of buds and leaves, leaf size, flower size, petal diameter, flower weight, chlorophyll a, b and total carotenoid contents were examined. **Results:** The result showed that GA_3 application was most effective in delaying senescence followed by phloemic stress and thinning. The GA_3 application to the flowers induced highest number of buds and leaves, biggest size of flowers, leaves and also showed a delay in flower wilting and discoloration. Chlorophyll b and carotenoid contents were also highest in flowers treated with GA_3. However, flowers with no treatment (control) exhibited highest content of chlorophyll a. **Conclusion:** These results indicate that hormone application (GA_3) was the best treatment to extend the longevity of *Hibiscus rosa-sinensis*. The use of this hormone would be of a great help in flower industry.

Key words: *Hibiscus* thinning, phloem cut stress, longevity, growth, development, chlorophyll contents

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The genus *Hibiscus*, classified under the Malvaceae family and the order of Malvales includes a wide range of flowering plants. *Hibiscus* flowers grow in many forms. Their petals may be flared, cut or fringed, single flowers or doubles and colors can run the gamut, from whites to reds and yellows to orange. Size of blossoms varies with species, as do plant height, leaf size and shape[1]. The common *Hibiscus rosa-sinensis* known colloquially as the Chinese hibiscus, is an evergreen flowering shrub native to East Asia. It is also known as China rose and shoe flower[2].

Hibiscus is easy to grow with a few basic requirements for successful cultivation. It is normally propagated through layering, grafting or cutting as seeds are not set due to the absence of pollination[1]. Thus, it is widely grown as an ornamental plant throughout the tropics and subtropics[2]. The environmental factors like; physical (light, temperature and gravity), chemical (water, air, minerals) and biological components (microbes, larger organisms) affects the plant growth and development[3].

It was reported by Forshey[4] that thinning increased the fruit size. They also reported that thinning had an excessive crop that made the results in a high percentage of small fruit. These negative effects of an excessive crop were due to an unsatisfactory leaf/fruit ratio. Moreover, the leave through the process of photosynthesis produced all the carbohydrates that were used in fruit growth and that contribute to fruit quality. In addition, fruit thinning improved the leaf/fruit ratio by increasing the leaf area available to each of the persisting fruits as well as the growth and development of flowers size.

It was reported by Hossain *et al.*[5] that when performed properly, bark ringing tends to dwarf the tree, induce flower bud formation and promotes fruiting in peach trees. The increase in trunk circumference above the ring might be caused by a swelling of the trunk due to the accumulation of carbohydrates[6]. It is also reported that sugar and starch levels were higher above the ring than below the ring. Breaking the flow of assimilates and bioregulators in the phloem of the tree trunk induced flower bud setting. This event occurred irrespective of whether the break was caused by grafting a bark ring from a dwarf rootstock[7] or by girdling[8].

Plant growth regulators such as; auxins, gibberellins and abscisic acid are used in agricultural industry for stimulation and synchronization of flowering and fruit setting, promotion of rooting, reduction of vegetative growth, reduction of lodging of agronomic crops or defoliation[9]. On the other hand, plant growth retardants such as; ancymidol, daminozide, paclobutrazol, chlormequat chloride and uniconazole are used specifically to reduce vegetative growth and control plant size and shape[10]. Gibberellins (GAs) mediate many responses in plants from seed germination to senescence[11]. The most widely available compound is Gibberellic Acid (GA_3), which induces stem and internodes elongation, seed germination, enzyme production during germination and fruit setting and growth[10].

Its beautiful blooms make it perfect in the environmental beautification. Thus, it is essential to find ways to increase its longevity and eventually enhance the use of *Hibiscus rosa-sinensis* in flower industry. However, these beautiful blooms last only 12 h, a setback to its being useful in the cut flower industry[1]. Hence, researchers nowadays are looking for the alternatives for prolonging the longevity of *Hibiscus rosa-sinensis*.

The study was undertaken to evaluate the effects of thinning, phloem cut stress (represented by bark ringing) and hormone application (Gibberellic acid, GA_3) on the flowers development and the longevity. In addition, to determine the biochemical level, chlorophyll content of the leaves and carotenoid content in the flowers.

MATERIALS AND METHODS

Study area: Completely Randomized Design (CRD) was applied in the methodology. The experiment was carried out in a garden in the Department of Biological Science, University of Malaya located in Kuala Lumpur, Malaysia (April-September, 2015) and laboratory analysis was done in the Department of Biological Science, University of Hail, KSA (October-January, 2016).

Plant materials: *Hibiscus rosa-sinensis* plant was used in this experiment.

Treatment setting: The treatments were control, thinning, phloemic stress and hormone gibberellic acid (GA_3). Thinning was done by removing excess leaves on the branches. A partial ring was made by removing a 3 cm long strip of bark from around the circumference of the trunk with a knife (thin razor blade type) leaving a connecting band of bark of 4 mm wide. Hormone gibberellic acid (GA_3 of 20 mg L^{-1} concentration) was applied twice a week by dipping. The experiment was conducted in a completely randomized design. There were three replications and 4 treatments (including control) used in the experiment. A total of 12 branches were used in the experiment from 4 trees.

Data collection: Number of buds, leaves, flower size, petal diameter and flower weight were counted at 7 days interval for 4 weeks. Flower buds and leaves were measured per 20 cm of shoot.

Longevity: Stage of flowering was observed everyday to determine its longevity. There were four stages of flowering; (i) Bud stage, (ii) Partially bloom stage, (iii) Full bloom stage and (iv) Senescence stage. Vase life of the flower begins with the bud stage until it was dropped from the plant. The start of senescence was indicated by initial browning and discoloration of the petals.

***In vivo* measurement of leaf chlorophyll:** The chlorophyll meter SPAD-502 (Minolta Co. Japan) was used for determination of chlorophyll in leaves. The SPAD-502 determines the relative amount of chlorophyll present by measuring the absorbance of the leaf in two wavelength regions.

Determination of chlorophyll a, b and total carotenoid content: Total carotenoids and chlorophylls were determined according to the methods[12]. The method consisted of repeated acetone extraction, until obtained colorless residue with a pestle and mortar and filtered over filter paper (Whatman No. 1). The extracts were made up to 50 mL with acetone. The concentration of carotenoids was measured at 470 nm, chlorophyll a at 666 mm and chlorophyll b at 653 nm in a Shimadzu UV 160A spectrophotometer. The amount of chlorophyll a, b and total carotenoid content were calculated[12].

Statistical analysis: Least Significant Different Test (LSDT) was used in this study.

RESULTS

All treatments showed an increase in the number of buds (Fig. 1), but subsequently remained unchanged at 3rd and 4th week. In fact, both control and hormone application treatments remained unchanged since, the 2nd week of treatment. Branches treated with GA_3 induced the highest number of buds (6.00±2.65) followed by thinning (5.00±2.00) and phloemic stress (4.00±0.58). Control exhibited the least number of buds among the four treatments. Figure 2 showed that initially, the number of leaves for all treatments were lower than that of control, however, they increased after 2nd week of treatments. Branches treated with GA_3 application showed an obvious increase in the number of leaves. This means that GA_3 was capable of inducing leaves formation. However, not much difference could be seen in the number of leaves between the treatment of thinning and phloemic stress. As shown in Fig. 3, leaf size with the treatment of hormone was much higher compared to other treatments. However, this was due to its initial leaf size which

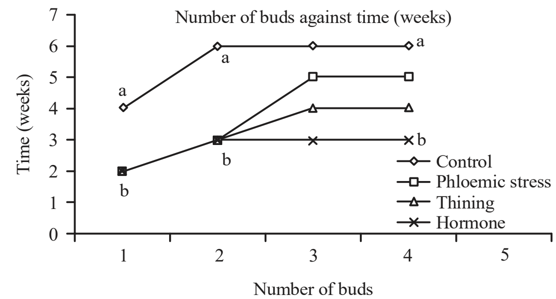

Fig. 1: Number of buds for *Hibiscus rosa-sinensis* at 1-4 week after treatments
Means followed by the common letters are not significantly different at 5% level by Least Significant Different Test (LSDT)

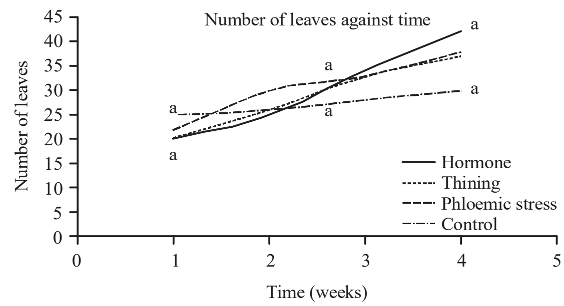

Fig. 2: Number of leaves for *Hibiscus rosa-sinensis* at 1-4 week after treatments
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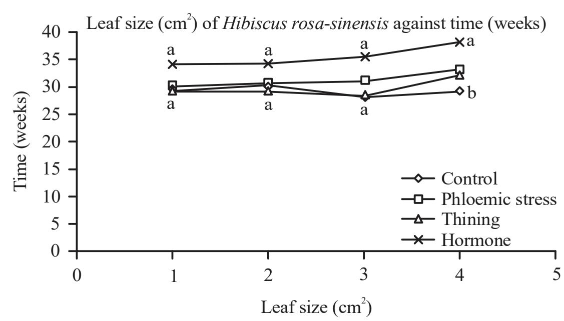

Fig. 3: Leaf size of *Hibiscus rosa-sinensis* at 1-4 week after treatments
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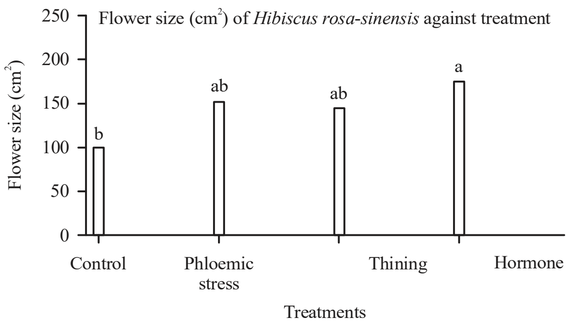

Fig. 4: Flower size (cm^2) of *Hibiscus rosa-sinensis* after treatments

Means followed by the common letters are not significantly different at 5% level by Least Significant Different Test (LSDT)

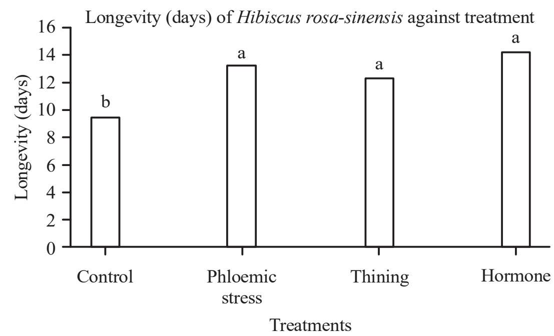

Fig. 7: Longevity (days) of *Hibiscus rosa-sinensis* after treatments

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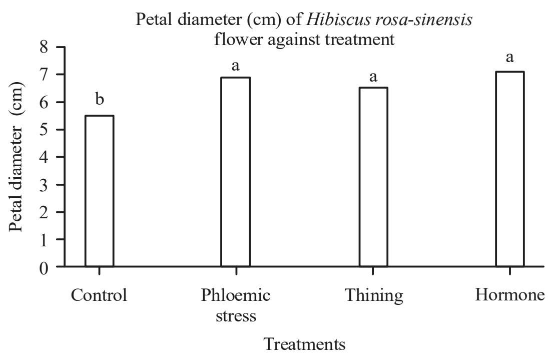

Fig. 5: Petal diameter (cm) of *Hibiscus rosa-sinensis* flower for treatments

Means followed by the common letters are not significantly different at 5% level by Least Significant Different Test (LSDT)

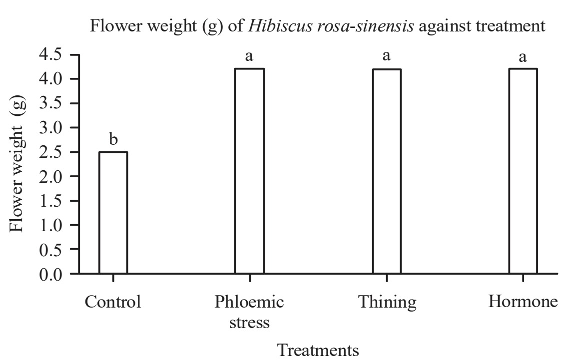

Fig. 6: Flower weight (g) of *Hibiscus rosa-sinensis* after treatments

Means followed by the common letters are not significantly different at 5% level by Least Significant Different Test (LSDT)

is the biggest of all. Its increment in leaf size was not so obvious in the first two weeks, but subsequently increased afterward, thus, resulting in the highest leaf size among all treatments (38.00±19.15). Leaves with no treatment (control) showed a drop in leaf size at week 3 (27.45±8.63), but slightly increased at week 4 (28.20±13.88).

Based on Fig. 4, the flower size of *Hibiscus rosa-sinensis* was more or less the same for all treatments. However, the biggest size of flower can be seen in the flower sprayed by hormone GA_3 (176±2.46), whilst the smallest was shown in control. This is meant that GA_3 application was effective in flower growth and development. Petal diameter was the highest in the flower treated with hormone GA_3 (7.13±0.31) and the least in the flower with no treatment (control) (Fig. 5). However, petal diameter for both phloemic stress and thinning treatment were only slightly lower than that of hormone application. This showed that all three treatments managed to induce flowers with high petal diameter.

As shown in Fig. 6, all treated flowers exhibited higher flower weight compared to control. The highest flower weight could be seen in the flowers applied with hormone GA_3, phloemic stress and thinning. This result showed that flowers treated with GA_3 contained highest water content and thus, it is believed that it is to be able to delay the onset of senescence. The longevity of *Hibiscus rosa-sinensis* flowers were determined by petal discoloration and senescence. As shown in Fig. 7, hormone GA_3 was most effective in prolonging the longevity of the flowers from 14-15 days. The average longevity of phloemic stress was slightly higher than thinning.

From the results shown in Fig. 8, it could be seen that there were no significant differences between control and thinning and also between phloemic stress and hormone application. Chlorophyll content value for *Hibiscus rosa-sinensis* leaves were the highest in leaves treated with hormone GA_3. The absorbance properties of photosynthetic pigments allowed the amounts of

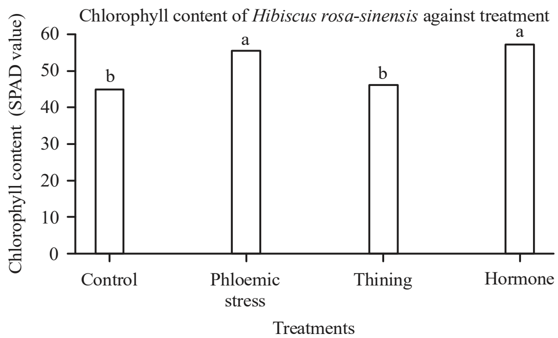

Fig. 8: Chlorophyll content of *Hibiscus rosa-sinensis* (SPAD value) for control, phloemic stress, thinning and hormone application
Means followed by the common letters are not significantly different at 5% level by Least Significant Different Test (LSDT)

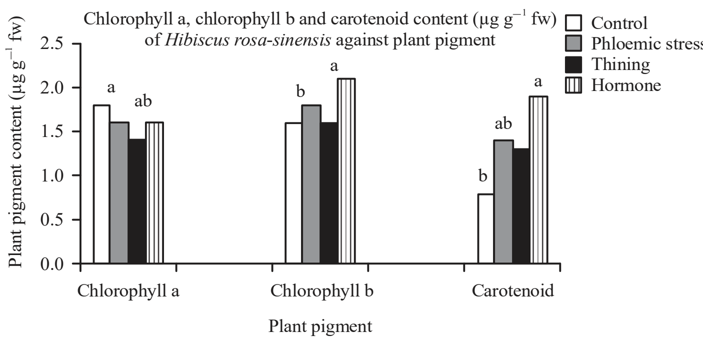

Fig. 9: Chlorophyll a, b and content of *Hibiscus rosa-sinensis* (SPAD value) for control, phloemic stress, thinning and hormone application
Means followed by the common letters are not significantly different at 5% level by Least Significant Different Test (LSDT)

chlorophyll a, b and carotenoid to be determined spectrometrically. As shown in Fig. 9, the amount of chlorophyll b was higher than chlorophyll a in *Hibiscus rosa-sinensis* flowers. This was followed by carotenoid contents, which showed the lowest content. From the result, the control treatment showed the highest content of chlorophyll a, while, GA_3 treated flowers contained highest amount of chlorophyll b and carotenoid.

DISCUSSION

Finding of this study showed that GA_3 application induced the highest number of buds and leaves in *Hibiscus rosa-sinensis* plant. These results showed that GA_3 was capable of breaking the dormancy in plants. It was reported by Szalai *et al.*[13] that the exogenous application of Gibberellic Acid (GA) enhanced dormancy breakage, despite the fact that the levels of endogenous gibberellins did not increase prior to visible spouting of the tubers in tuber plants. This was further supported by Hossain *et al.*[14], including the correlation between gibberellins and germination. Gibberellins encouraged the germination by breaking the dormancy. During flower bud opening, various events takes place in a well defined sequences representing all aspects of plant development, such as; cell division, cellular differentiation, cell elongation or expansion and a wide spectrum of gene expression[15]. It is reported that GA_3 involved in the stimulation of cell division at the shoot apex by shortening the cell cycle[16]. Gibberellins stimulate cell division and extension in buds and the synthesis of α-amylase in the aleurone layer of wheat[17].

Thinning induced the second highest number of buds in this study. However, phloem cut stress produced higher number of leaves compared to thinning. Carbohydrates are necessary for the growth of any plant art as carbohydrates provide energy and the building blocks for growth processes. Flowers usually do not have chlorophyll and therefore, cannot carry out photosynthesis to produce carbohydrates for their needs. In addition, flowers have very rapid growth rates that require large amounts of carbohydrates. For these reasons, flowers are dependent upon other parts, especially leaves, for their carbohydrate supply[18].

Gibberellins have been shown to stimulate cell division by shortening the cell cycle. As for stimulation of cell growth, it is done by increasing the hydrolysis of starch and other food materials. Exogenous application of GA induced an increase in the concentration of reducing sugars[19], while it was observed[16] an increase in glucose concentration in the tissues near the buds of GA-treated tubers prior to visible sprouting. The increase in starch hydrolysis and the concentration of soluble carbohydrates can also be seen. In this study, phloem cut stress was done via partial ringing in which part of the bark was removed from around its periphery, thereby blocking the downward translocation of photosynthates and metabolites through the phloem[17,20]. It was reported by Janssen[21] that when bark ringing was done, the flow of manufactured food in plants was stopped. The roots starved for lack of food and the plant eventually died.

Thinning (pruning) is the judicious removal of leaves, flowers, branches, twigs and shoots of roots to increase the usefulness of plants[10]. The principal mode of action for thinning is to reduce the competition between plants. It was reported by Janssen[21] and Jones and Woodson[22] that the ones that remain have room to grow and access to the sunlight, water and nutrients they need to grow and produce to their full potential. As was shown in the results, GA_3 treated flowers were the heaviest among all treatments and also was effective in prolonging the longevity of *Hibiscus rosa-sinensis* up to 14-15 days compared to 9 days in control.

Petal wilting is due to loss of turgor which is mainly caused by the degradation of carbohydrates, proteins, lipids and nucleic acids in petal tissues. On the other hand, withering is a color change resulting from degradation of anthocyanin pigments and lowered water uptake[23]. It was reported by Gomes *et al*.[9] that water deficit also led to accumulation of abscisic acid (ABA), another of type growth inhibitor hormone. The ABA accumulation showed the same trend as ethylene biosynthesis and endogenous concentration was highest in short-lived hibiscus flowers. This may act by increasing ethylene sensitivity as observed by exogenous applications of ABA in hibiscus flowers[24]. Physiological studies during hibiscus flower senescence showed that ethylene production and 1-aminocyclopropane-1-carboxylic acid (ACC) content increased in petals during flower development and senescence. Exposure to ethylene accelerated the onset of senescence as indicated by petal in-rolling and stimulated ethylene production. Senescence was also hastened by basal application of ACC[25].

Light and phytohormones both influence leaf senescence. The effect of light on leaf senescence has been proposed to act on photosynthesis[25] and a photomorphogenic effect involving phytohormones[26]. Phytohormones like cytokinins and in some system gibberellins delay the loss of chlorophyll whereas ethylene and ABA enhance the rate of chlorophyll loss[27]. In this study, leaves treated with GA_3 contained the highest content of chlorophyll. This result is also similar to those reported by Dai and Paull[11] who found that loss of chlorophyll in leaves of alstroemeria cut flowers is strongly delayed by GA_3.

Chlorophyll pigments are accompanied by carotenoid ones, usually, from yellow to orange and is frequently masked by green chlorophylls[28]. Carotenoids are important nutritive and biological constituents common in the plant world. In this study, chlorophyll a, b and total carotenoid contents in *Hibiscus rosa-sinensis* flowers were determined spectrophotometrically. Generally, pigments contained in flowers are much lower compared to that in the leaves.

CONCLUSION

From this study, it can be concluded that the application of GA_3 to *H. rosa-sinensis* was the best treatment in delaying the flowers senescence compared to other different treatments as it exhibited the least petal discoloration and wilting. Furthermore, it induced the highest number of buds and leaves and also biggest size of flowers and leaves. In addition, GA_3 treated flowers had higher full bloom duration which is 2-3 days compared to only one day for control. Thus, GA_3 application was the most effective method in prolonging the longevity of *Hibiscus rosa-sinensis* flowers.

SIGNIFICANCE STATEMENT

From this study, it can be recommended that the administration of GA_3 to *H. rosa-sinensis* was the best treatment (dipping method) in delaying the flower senescence compared to other different method of treatments like spray and sprinkle etc. Thus, GA_3 application was the most effective scientific method to prolong the longevity of *Hibiscus rosa-sinensis* flowers. This study will help researchers to uncover the potential of GA_3 and possible use in longevity of *H. rosa-sinensis*.

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