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Size Enlargement and Shorten Longevity of *Hibiscus* Flower Affected by Gibberellic Acid and Aluminium Sulphate Using Dripping Technique

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Abstract: Growth regulator is an important factor to enlarge the flower size in the floriculture industry. Flower growers have a lot of interest in making flower enlargement and shorten longevity to harvest flower earlier and to make it marketable soon. Gibberellic Acid (GA₃) and aluminium sulphate Al₂(SO₄)₃ play significant roles in flower enlargement and development. *Hibiscus* sp. was used in this experiment. The branches were dripped with the respective chemical [GA₃ and Al₂(SO₄)₃ 100ppm] at 3 days intervals for 3 weeks. It had been shown that the 100 ppm GA₃ played major role in developing a bigger size of the flower, production of more leaves, shorter longevity of flower, quicker bloom and greater size and weight of the flower as compared to the Al₂(SO₄)₃ and control. Chlorophyll content (represented by SPAD value) and fluorescence were higher in GA₃ treated branch than in aluminium sulphate treated branch and control. The quantum yield (Fv/Fm) was maximal in GA₃ treated branch. The aluminium sulphate treated branch also showed the similar results but the longevity of the flower had the longer duration in aluminium sulphate treated branch than in GA₃ but shorter than control. The results showed that these two chemicals [GA₃ and Al₂(SO₄)₃ 100ppm] using dripping technique instead of spray were effective for the enlargement of flower size and shorten the flower longevity with less chemical cost and quantity of chemicals without hazarding the environment instead of spray. In addition, it improved the weight and increased the blooming rate and could be acceptable to the flower growers easily and could be harvested for commercial purposes earlier.

Key words: Flower size, chemical, quantum yield, flower longevity

INTRODUCTION

The phenomena of flowering is a complex developmental process consisting of at least five sequential phases namely flower induction, initiation, flower opening, pollination and flower senescence. Flower induction or the influence of flowering time depends on some known factors such as physiological stress, nutrient availability, light, day length and temperature. The timing of the transition from vegetative growth to flowering is of paramount importance in agriculture, horticulture and plant breeding because flowering is the first step of sexual reproduction (Koning, 1982; Bernier *et al.*, 1993). Many studies are carried out to understand how this transition was controlled and have occupied countless physiologists during the past half century and have produced an almost unmanageably large amount of

information. A majority of plant use environmental cues to regulate the transition to flowering because all individuals of a species must flower synchronously for successful out crossing and because all species must complete their sexual production under favorable external conditions. Any environmental variable exhibiting regular seasonal changes is potential factor that control the transition to flowering.

Many environmental factors, such as natural stress and nutrient availability influence flowering time but perhaps the most important are light intensity, day length and temperature (Vaz *et al.*, 2004). It has been also reported that application of phenolic stress at the vegetative stage promotes the flowering (Khandaker *et al.*, 2011). Besides environmental factors, some other factors have been found to induce flower earlier or frequent flowering such as plant growth regulating hormones and

chemicals (De Stigter, 1981; Saifuddin *et al.*, 2009a). The most reliable method of flower promotion has been found to be the application of plant growth regulators such as gibberellins and cytokinins. In most experiments, the best results have been achieved by GA₃ and along with some additional chemicals such as ethanol, 8-hydroxyquinoline sulfate and aluminium sulphate (Hossain *et al.*, 2007; Ichimura and Korenaga, 1998; Ichimura and Ueyama, 1998). Results might vary from year to year, from clone to clone and from experiment to experiment. Because, each species would have particular requirements regarding types, dosage of hormone, types of soil media and timing of treatment for better establishment and economize the use of plants (Moloo *et al.*, 1999; Wahome *et al.*, 2010; Ertekin, 2011). During flower development, GA₃ was found to be essential for the development of stamens and petals (Schwechheimer, 2008; Saifuddin *et al.*, 2009b). High concentration of GAs showed a positive role on flower formation in olive during induction and initiation period. In addition, the application of Gibberellic Acid (GA₃) has the potential to control growth and flowering and induce earliness of meristem (Khan and Chaudhry, 2006; Sharma and Singh, 2009).

Application of GA₃ promotes flowering in a range of plant species. Many species that flower early in response to GA₃ also flower early in response to long days or vernalization (Oka *et al.*, 2001). Ogale *et al.* (2000) inferred that GA₃ spraying changed the mode of action, by increasing the flower size to varying degrees (20- 40%) in all *Portulaca grandiflora* cultivars. Kim and Miller (2009) reported that GA₄₊₇ were a potentially useful compound in horticultural practice to enhance postproduction quality of many cultivars of pot tulips. Due to the high competition in flower market, the basic condition of the acceptance of flowers to buyers is their quality. Research is being carried out throughout the world on how to improve bloom cycle, flower size, flower color and flower longevity. Many techniques have been conducted but there is still a significant lack of knowledge on *Hibiscus* flower enlargement and early blooming. Therefore, the study was undertaken to know the effects of a new technique, dripping of chemical application and growth regulators (100 ppm GA₃ and 100 ppm aluminium sulphate) on the enlargement of flower and shorter longevity as well as the flowering process of *Hibiscus* sp.

MATERIALS AND METHODS

Experimental site and plant materials: The experiment was conducted in the Plant Physiology Garden, Institute of Biological Sciences, University of Malaya. Three year old *Hibiscus* plants having 1.5 m of height and 1.0 m canopy length were used in this experiment. Plant to plant

and row to row distance were maintained 1.0×1.0 m². The tree was consisted of five branches and grown under prevailing conditions (relative humidity 60-90% and temperature 30-32°C). The three branches of each plant were selected for chemical treatment with maintaining same age, leaf number, shoot length, sunlight position and period. For each treatment, three plants were used.

Chemical treatments: Treatments were set in a Completely Randomized Design (CRD). Each treatment was carried out in three replications and dripped on a three days interval for three weeks. The selected branches flower bud (before initiation) were dripped with GA₃ (100 ppm) and aluminium sulphate (100 ppm). The controlled branches were dripped with distilled water.

Quantum yield measurements: Chlorophyll fluorescence was measured by using Plant Efficiency Analyser (Hansatech Instrument Ltd., England). A leaf clip was attached to one of the leaves and kept in dark for 30-45 min to maintain dark adaptation. Then, the leaf clip was oriented with the shutter plate. When the light intensity was applied on the leaf, the fluorescence signal was counted for 3 sec and observed the quantum yield or photosynthetic yield. The maximal Fluorescence (F_m) and minimal Fluorescence (F_o) value was taken from the display pad of Plant Efficiency Analyser machine. The yield of variable Fluorescence (F_v) was calculated as F_m-F_o. Calculation of quantum yield was determined according to the equation F_v/F_m (Temperature = 28°C, Time range = 10 µs-3 sec).

Flower size measurement: Flower size was measured by Vernier scale and flower weight was taken using the balance machine (Mettler, PJ3000).

Evaluation of flower longevity and Full blooming days: Flower status was observed every day. Flower longevity was counted as the number of days from flower initiation to flower abscission and full blooming days was counted from flower initiation to full blooming stage.

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 total carotenoid content: Total carotenoids were determined according to the methods describe in Khandaker *et al.* (2012). The method consisted of repeated acetone extraction, until obtained colorless residue, with a pestle and mortar and filtered over filter

paper. The extracts were made up to 50 mL with acetone. The concentration of carotenoids was measured at 470nm in a Shimadzu UV 160A spectrophotometer.

RESULTS

The influence of all treatments on leaf initiation was observed throughout the experiments. All strategies were able to initiate leaf with respect to experimental periods. In the present work, the number of leaf per branch was higher in GA₃. After fifth week leaf number was observed to be twenty-two in water control, while it was twenty nine and twenty seven in GA₃ and aluminium sulphate respectively (Fig. 1). The influence of all treatments on bud initiation was observed throughout the experiments. The number of bud per branch was one in all treatment at first week. In second week, significant difference was expressed when the branch was treated with the combination of GA₃. In aluminium sulphate treated branch total bud number was five at fifth week, while the bud number was seven in GA₃ treatment which was the highest among the treatments. The new bud initiation was particularly delayed and decreased by control (Fig. 2).

The time from flower initiation to full blooming, required blooming days, varied with treated application. Blooming was quickest in GA₃ and was significantly delayed in water. The required days were three and four to reach full blooming stage in GA₃ and aluminium sulphate respectively. It was almost two and one day earlier than in control. In case of water treatment, the required days for blooming were found to be five days. This was almost 35% higher in GA₃ than in the control. These treatments indicated that external chemicals are an important factor for the flower production and comparatively GA₃ was suitable for early flower production of *Hibiscus* sp. (Fig. 3).

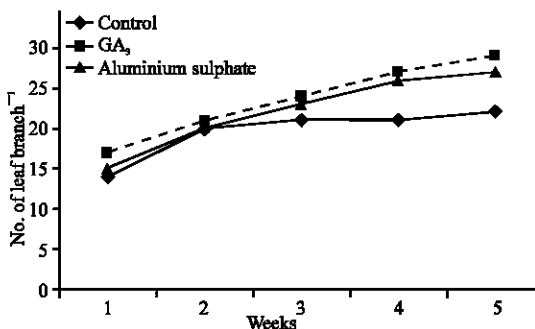


Fig. 1: Leaf number/branch of *Hibiscus* plant as affected by different treatments. Values followed by different alphabets indicate the existence of significant differences according to LSD_{0.05} test

Flower longevity was prolonged by applying water control (Fig. 4). Application of GA₃ showed the shortest duration of (4 days) flower life compared to the other treatments. However, bract longevity was prolonged by

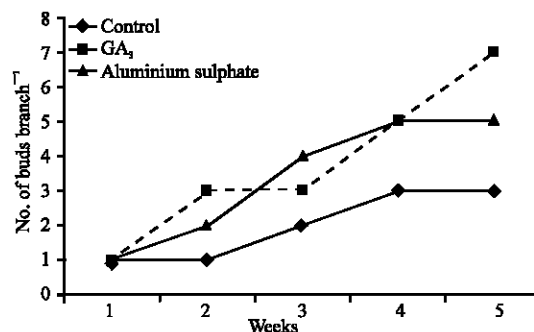


Fig. 2: Effects of different treatments on bud number/branch of *Hibiscus* plant. Different alphabets indicate significant difference among treatments according to LSD_{0.05} test

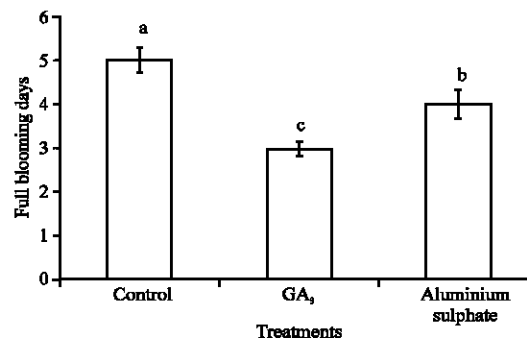


Fig. 3: Effects of different treatments on full blooming days. Different alphabets indicate significant difference among treatments according to LSD_{0.05} test

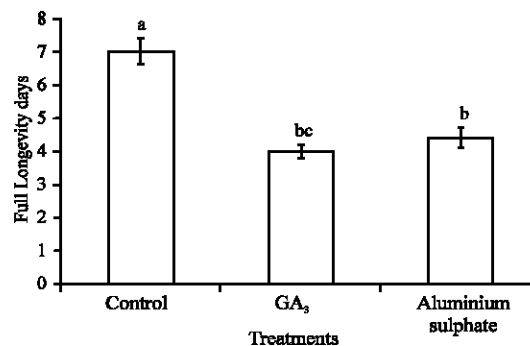


Fig. 4: Flower longevity was affected by different treatments applied to *Hibiscus* sp. Values followed by different alphabets indicate the existence of significant differences according to LSD_{0.05} test

applying Aluminium S. compared to the GA_3 . These observations referred that harvest quality of *Hibiscus* flower life vastly decreased as a result of the activity of GA_3 and aluminium Sulphate throughout the flower

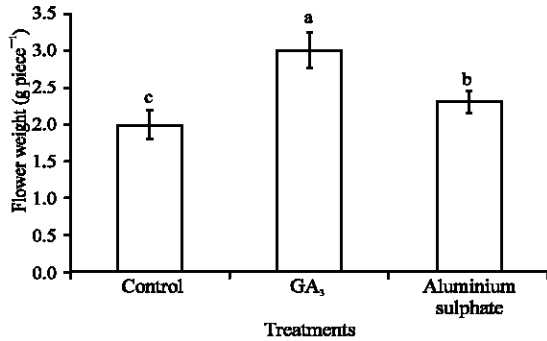


Fig. 5: The effect of GA_3 and aluminium sulphate treatments on flower weight of plants. Means followed by different alphabets above bars are statistically different at 5% level of significance according to $LSD_{0.05}$ test

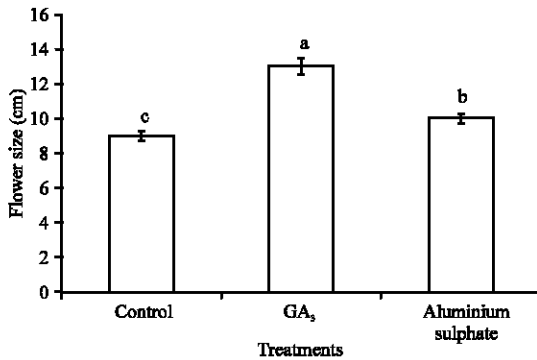


Fig. 6: The effects of GA_3 and aluminium sulphate treatments on flower size at full blooming stage (5% level of significance according to $LSD_{0.05}$ test)

developmental stages. Both chemicals were not preferable for keeping flower fresh for long days. Flower longevity or senescence is distinct from ageing. Senescence may be simply defined as those changes that lead eventually to the death of any organism or flowers. Senescence can be defined as the deteriorative processes those natural causes of death of flower. Hence, a flower lifespan and post production quality is determined by the senescence process (Ichimura and Goto, 2002; Ichimura, 1998). From their findings, it could be deduced that by slowing down the senescence process using suitable chemicals, it would delay the wilting process and therefore, prolong the longevity of the flower.

It is seen in (Fig. 5) flower treated in GA_3 showed the highest percentage of weight compared to other treatments. On the other hand water exhibited lowest weight. The increasing effect of flower weight in GA_3 was significantly better than aluminium sulphate. The weight increment of both chemical treated flower was increased with the advancement of GA_3 and aluminium sulphate chemicals. The effects of GA_3 and aluminium sulphate on flower size, at full blooming stage, were showed in (Fig. 6 and 7).

The quantum yield of dark-adapted leaves determining the maximum efficiency of photosystem was showed (Table 1). The photosystem of dark-adapted leaves, measured by quantum yield showed lower values in control plants. Whereas, significant higher values of quantum yield were observed in GA_3 treated plants. In Aluminium treated plants, the quantum value increased by 1.1% in leaves of flower shoots.

The photosynthetic pigment chlorophyll showed a significant difference with respect to the applied chemical treatments (Table 2). The accumulation of chlorophyll was significantly higher in plants which underwent in GA_3 . Enhanced synthesis of chlorophyll and carotenoid by GA_3 treatment had previously been reported and it has been



Fig. 7: Enlargement of flower in different treatments. Treated with (a) Control, (b) Aluminium sulphate and (c) Gibberellic acid (GA_3)

Table 1: Determination of quantum yield in all type of pruning plant leaves including control leaves

Treatments	Fo (Lower photosynthetic yield)	Fm (Higher photosynthetic yield)	Fv (Variable of photosynthetic yield)	Fv/Fm (Quantum yield)
Control	232	1650	1418	0.85 ^{bc}
GA ₃	205	2010	1805	0.89 ^a
aluminium sulphate	226	1692	1466	0.86 ^b

Values followed by different alphabets indicate the existence of significant differences according to LSD_{0.05} test

Table 2: Determination of carotenoid content and chlorophyll, represented by SPAD value as affected by treatments

Treatments	Carotenoid ($\mu\text{g g}^{-1}$ FW)	Chlorophyll (SPAD)
Control	0.7 ^c	43 ^c
GA ₃	1.9 ^a	57 ^a
aluminium sulphate	1.3 ^b	46 ^b

Values followed by different alphabets indicate the existence of significant differences according to LSD_{0.05} test

suggested that the enhanced synthesis was attributed to the increased hormone activity in rose and bougainvillea plants (Calatayud *et al.*, 2008; Saifuddin *et al.*, 2009a; Hossain *et al.*, 2007).

DISCUSSION

It has been well documented that the commercial value of flowers is very dependent on its early blooming including flower color and size. *Hibiscus* plant is well known for its beautiful flowers and in rising landscaping activities. Its commercial value could be improved through early blooming and increasing its quality in terms of size and weight (Tjosvold *et al.*, 1994). Flower initiation and blooming rate could be improved also by using growth regulating hormones, such as GA₃. Gibberellins are well known to increase hydrolysis of starch and sucrose into glucose and fructose which were utilized by the flowers for floret opening (Emongor, 2004). The increased sugars in the flower heads and stems may increase the osmotic potential of the petals, thus improving their ability to absorb nutrients and maintain their turgidity which may explain the increase of flower weight in different developmental stages and observed in this study too.

In this experiment, the GA₃ took short period for bud formation and flower number/branch, flower size and weight was increased by GA₃ and aluminium sulphate. Saifuddin *et al.* (2009a) have shown that GA₃ increased the supply of total sugar in petal and treated leaf. It might be responsible for stimulating cell division, new leaf formation and ultimately more flower/ branch and frequent flower bud initiation. However, in the case of control treatment, the number of flower/branch was low due to the prolonged vegetative stage for shoot initiation and lack of leaves to utilize in photosynthetic process or lack of contribution of cytokinins from root towards shoot (Calatayud *et al.*, 2004). In addition, plants produced fewer flowering buds in water control than in chemical treatment. This indicated that the switch to early flowering

was maintained at subsequent flower formation under preferable condition. Saifuddin *et al.* (2010) referred a similar report that plants initiate more and larger leaves in order to capture more sunlight and decrease the flowering time. But in control treatment, the plant showed a greater tendency toward vegetative reproduction rather than reproductive growth. The quantum system of this sp declined in control and increased GA₃ chemical. The quantum efficiency in the dark adapted leaf (Fv, Fo and Fm) has been carried out extensively as an indicator of hormonal and chemical stress on leaves or plant morphology (Bibi *et al.*, 2008). However, measuring quantum yield of photosynthesis with the light-adapted test in our studies proved the positive effect of chemical on leaf photosynthesis process. The present value indicated that quantum system of PSII increased significantly in GA₃ treatment. The lowest values were obtained in water treated branch leaves. Maximum efficiency of photosystem of dark-adapted leaves, measured by fluorescence quantum showed lower values in control plant leaf. Fv and Fm reflected the potential quantum efficiency and were used as a sensitive indicator of plant performance, with optimal values of around 0.8 measured for most plant species (Calatayud *et al.*, 2002; Johnson *et al.*, 1993). Values around 0.89 have been obtained in leaves of flower shoots in GA₃ treated plants. The value obtained by fluorescence, indicate that GA₃ treated branch promoted better photosynthetic light reaction than control branch (0.85). This fact can be attributed, among other causes, to a higher radiation intercepted by the leaves to the enhancement of photosynthetic rates in the remaining mature leaves or to changes in photosynthetic capacity of mature leaves (Mediene *et al.*, 2002).

This experiment can also be improved by minimizing the number of bud, flower or leaves drop before reach maturity as much as possible. There a lot of reason why this happened and prevention or extra treatment can be done to control this. One of the most common causes of *Hibiscus* blossoms falling off plants is insect pests, particularly thrips. These tiny insects feed on *Hibiscus* flower buds, causing them to fall off prior to blooming. Using an organic insecticide once a week as directed should help take care of the problem. Gall midge is another common pest affected *Hibiscus* flowers. This insect lays its eggs inside the buds, turning them yellow and

eventually causing them to drop. In addition, *Hibiscus* flower and bud drop can also be the result of a number of other factors such as nutritional deficiencies and environmental conditions, bud drop on *Hibiscus* flowers in oftentimes associated with an underlying issue that can be easily corrected. *Hibiscus* flowers require lots of light, high humidity and moist soil. They also need regular feeding with fertilizer as directed.

CONCLUSION

In a conclusion, based on the current research findings, 100 ppm GA₃ and aluminium sulphate showed the effective growth regulators to induce frequent flower production and increase flower weight and size. These changes finally led to the decreases in the flower longevity. These prominent effects would really be recommended the species studied as landscaping and environmental beautification and commercial purposes. In addition, the results suggested that these growth regulators [GA₃ and Al₂(SO₄)₃ 100ppm] using dripping technique instead of spray were effective for the enlargement of flower size and shorten the flower longevity with less chemical cost and quantity of chemicals without hazarding the environment (causing pollution due to spray) instead of spray. However, It could be harvested for commercial purposes earlier and could be achieved high market value by earlier harvest and attractive larger size.

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