Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

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Asian Journal of Plant Sciences 8 (3): 212-217, 2009
ISSN 1682-3974

Bract Size Enlargement and Longevity of *Bougainvillea spectabilis* as Affected by GA_3 and Phloemic Stress

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Abstract: A field experiment was conducted to assess the effect of gibberellic acid (GA_3 100 and 150 ppm), phloemic stress and combination of 100 ppm GA_3 and phloemic stress on *Bougainvillea* bract blooming, expansion, development and bract longevity under exposed sun light condition (400-700 $\mu Em^{-2} sec^{-1}$). A seven-years-old *Bougainvillea* plant was used in this experiment. Fifteen selected brunches were applied with 100, 150 ppm GA_3, phloemic stress, 100 ppm GA_3+phloemic stress and water control. The results showed that 100 ppm GA_3 increased the length of petiole, bract size and shape by 40%. Bract blooming was three days earlier in 100 ppm GA_3 treated branches and 4 days earlier in 150 ppm GA_3 than in water control. Bract longevity (required days from bract initiation to abscission) was higher for 4 in phloemic stress and for 2 days in 100 ppm GA_3+phloemic stress than in water control. However, bract longevity was shorter in 100 and 150 ppm GA_3 than in control. The number of bracts per branch was higher in 100 ppm GA_3+phloemic stress and phloemic stress than the other treatments. Petal size and petiole length were the highest in 100 ppm GA_3. But there were no significant changes in bract size and color development in phloemic stress. Maximum chlorophyll fluorescence was observed in phloemic stress. Quantum yield (F_v/F_m) was higher in phloemic stress and 100 ppm GA_3+phloemic stress than in other treatments. The findings suggested that gibberellic acid played an important role to induce rapid bract blooming and expansion whereas, phloemic stress increased total number of bract and longevity.

Key words: *Bougainvillea*, flowering, gibberellic acid, carbohydrate, bract size

INTRODUCTION

Bougainvillea is a genus of flowering plants belongs to the family Nyctaginaceae native to South America. It includes about 18 species and generally used in the arid landscapes for beatification, horticulture, pharmaceutical industries, agriculture and environmental industries on account of the large flexibility in different agro climatic regions of the world (Suxia *et al*., 2009). It is grown in tropical and subtropical zones and is enriched with different varieties having attractive shades. Considering its vast scope, introduction and alteration of flower color and size are highly desired and sought after traits. In addition, the *Bougainvilleas* bloom cycle is usually 5-6 weeks long and then all bracts fall off (Grodon, 2002). Commercial value can be improved by prolonging the bract longevity and increasing its quality. The shelf life and commercial value of many potted plants are seriously affected for early dropping. Longevity or vase life was an important factor in consumer preference and considerable research has been carried out on the causes of senescence (Hossain *et al*., 2007).

Plant hormones play an integral role in controlling the growth, development, metabolism and morphogenesis of higher plants (Claus, 2008). However, gibberellins are well known plant growth hormones and they differ considerably in their modes of action (Hye and William, 2009). GA_3 is well known for the bolting effect in plants. Ogale *et al*. (2000) observed in *Portulaca grandiflora* that GA_3 induced changes in flower color and size. In strawberry, GA_3 application increased petiole length and leaf area. It reduced the time needed for inflorescence emergence, accelerated flowering and increased the number of flower buds and open flowers in most growing conditions (Khan and Chaudhry, 2006). During flower development, GA_3 was found to be essential for the development of stamens and petals (Claus, 2008). High concentration of GAs showed positive role on flower formation in olive during induction and initiation period (Salih *et al*., 2004). In addition, the application of gibberellic acid (GA_3) has the potential to control growth and flowering and induce earliness. Hossain *et al*. (2006) reported that flower bud percent was a higher when phloemic stress was applied since flowering was closely

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related to starch content and girdling increased leaf sugar content. Carbohydrates may affect vase life of some plant species. You-Min *et al*. (2008) reported that the involvement of carbohydrate was effective in regulating flower development when plants were investigated from floral bud stage to flower senescence. Sucrose extends vase life of rose and gladiolus (Kuiper *et al*., 1995). It shows that sugar like sucrose have a negative effect on the process of cell death leading to petal senescence. Jose (1997) found less vegetative growth in all the treatments of ringing (girdling) in a ringing experiment. Hossain *et al*. (2004) reported that starch content in the bark was higher in samples when it was taken from the upper part of the phloemic stress. However, research on efforts to increase bract size in *Bougainvillea*, to lengthen the bloom cycle and also to develop bract qualities such as color, longevity, expansions and delayed senescence by applying GA_3 and phloemic stress are scanty and hence this study.

MATERIALS AND METHODS

Experimental site: The experimental site was in the Plant Physiology Garden, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia.

Plant material: The experiment was started on 10th June 2008 and ended on 30th August 2008. A seven-years-old *Bougainvillea* plant having selected branches was used in this experiment. The plant was 1.5 m height and canopy length was 2.5 m. The tree consisted of 4 main branches and 20 sub-branches. Treatments were set following completely randomized design. Each treatment was repeated by 3 replications.

Treatment setting

Phloemic stress: The experiment on phloemic stress was carried out using a small sharp knife removing 2 cm length and 0.30 cm width of bark (Fig. 1).

The phloemic stress was applied 15 cm away from shoot apex in a branch. There were three replications for phloemic stress. Another three phloemic stress branches were selected for mixed treatment with 100 ppm GA_3. The six selected branches were sprayed by 100 and 150 ppm GA_3. So, there were total 15 branches used in the experiment including three control branches.

Data collection: Bract development was divided into five stages (1st = initial bud stage, 2nd = bud opening stage, 3rd = initial bract blooming, 4th = partial bract blooming, 5th = full blooming) to measure and compare

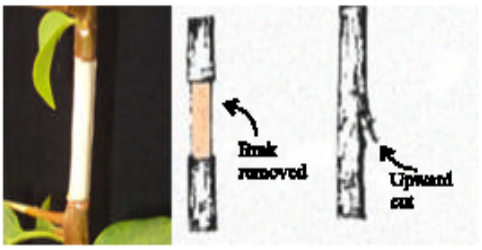

Fig. 1: Application of phloemic stress

bract longevity and weight among the treatments (Table 1). During the experiment, new shoot growth, bract longevity, bract weight, bract length (diameter) and total bract number were measured weekly.

Chlorophyll fluorescence yield measurement: Chlorophyll fluorescence yield was measured by 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 light shine was applied on the leaf, the fluorescence signal was counted for 3 sec and observed the fluorescence yield or photosynthetic yield. It was represented by F_o, F_m, F_v and F_v/F_m (fluorescence yield). Where, F_o is lower fluorescence, F_m is higher fluorescence, F_v is relative variable fluorescence (F_m-F_o), temperature is 27°C, time range is 10 μsec-3 sec.

Chlorophyll content measurement: Chlorophyll content (SPAD value) was measured by chlorophyll meter SPAD-502, Minolta Co., Japan. The leaf was inserted into the meter and the SPAD value measured 5 times from different spots of a single leaf.

Bract weight and length measurement: The weight of bract was taken using the weighing machine Mettle PJ3000 and bract length was measured by Vernier scale.

Statistical analysis: Statistical analysis was performed using STATGRAPHIC plus 3.0. The one way ANOVA was applied to evaluate the significant difference of the parameters studied in the different treatments. LSD ($p = 0.05$) was calculated using the error mean squares of the analysis of variance.

RESULTS AND DISCUSSION

Total number of bracts was found to be 18.66 in water control, while it was 23.33 and 17.33, respectably in 100 and 150 ppm GA_3 (Fig. 2). In the case of phloemic stress,

the number of bracts per branch was 24.66. Most significant difference was expressed when the branch was treated with 100 ppm GA_3+phloemic stress. In this treatment total bract number was 28.66, which was higher than the other treatments.

Results of Fig. 2 and 8 show that 100 ppm GA_3 prolonged bract (petal) length significantly at all stages. At the full blooming stage (5th), the results showed that the length of petiole, bract size increased by 40% increased in 100 ppm GA_3. In the case of 150 ppm GA_3, the petal length was prolonged at all stages, but bract length did not show such a change as in 100 ppm GA_3. In addition, a similar increasing trend was found in the combined treatment of 100 ppm GA_3 and phloemic stress. For the phloemic stress treatment, bract (petal) length was almost similar to control from 1st stage to 5th stage.

Bract longevity was found to be four days higher in phloemic stress and two days higher in 100 ppm GA_3+phloemic stress than in water control (Table 1) whereas bract longevity was two days lesser in GA_3 100 ppm and four days lesser in 150 ppm GA_3 than in water control. However, the required days were 16.8 and 15.3 to reach in full blooming stage in 100 and 150 ppm GA_3 and it was almost 3 and 4 days earlier than in control.

Table1: Effect of different treatments on bract longevity and full blooming days of *Bougainvillea spectabilis* bract

Treatments	Full blooming days	Bract longevity* days
Control	19.33±0.33bc	23.66±1.45bc
GA_3 100 ppm	16.83±0.44d	21.66±1.45bc
GA_3 150 ppm	15.33±0.33de	19.66±0.88de
Phloemic stress	22.00±0.57a	27.33±1.20a
GA_3 (100 ppm) +phlo. Stress	21.16±1.16ab	25.33±1.16ab

Different letter(s) beside columns indicate significant difference among treatments according to $LSD_{0.05}$ test. *Required days from bract initiation to senescence

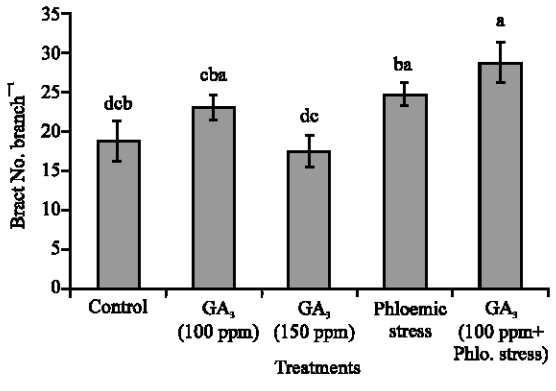

Fig. 2: Bract No. branch^{-1} of *Bougainvillea* plant as affected in different treatments. Values followed by different letter(s) indicate the existence of significant differences according to $LSD_{0.05}$ test

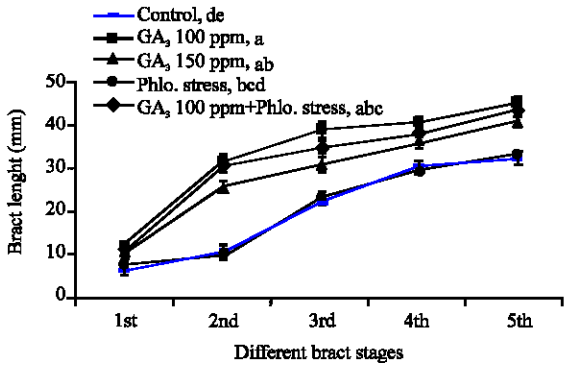

Fig. 3: Effect of different treatments on petal size (mm) at different bract stages of *Bougainvillea spectabilis*. Where, 1st is initial bud stage, 2nd is bud opening stage, 3rd is initial bract blooming, 4th is partial bract blooming and 5th is full blooming. Values followed by a different letter(s) indicate the existence of significant differences to $LSD_{0.05}$ test at 5th stage according

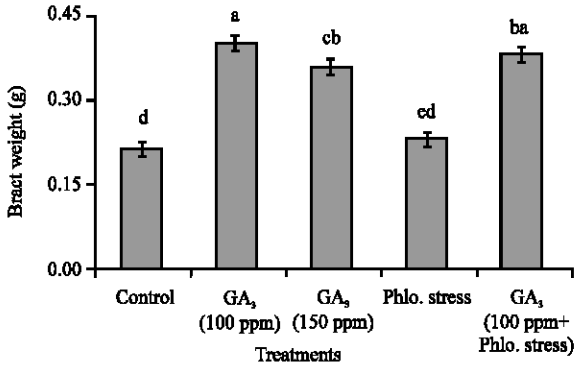

Fig. 4: Bract weight as affected by different treatments on *Bougainvillea spectabilis*. Values followed by a different letter(s) indicate the existence of significant differences according to $LSD_{0.05}$ test

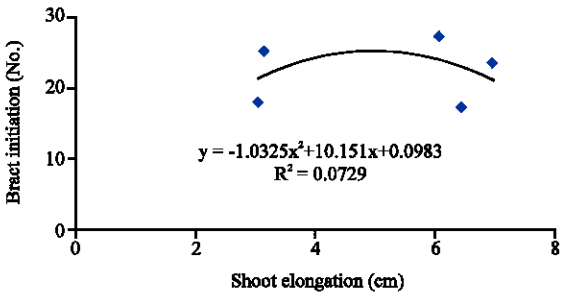

Fig. 5: Polynomial correlation between flower initiation and shoot elongation

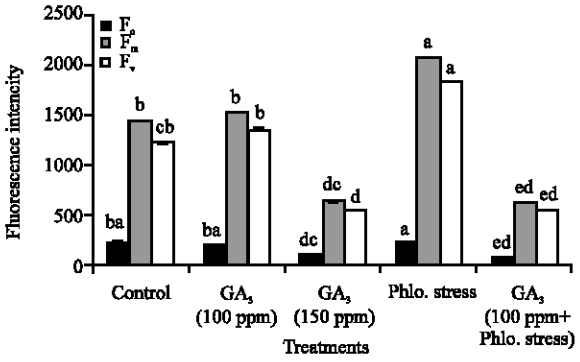

Fig. 6: Measurement of chlorophyll florescence intensity (yield) of leaves on different experiments branches. F_o is lower fluorescence, F_m is higher fluorescence, F_v is relative variable fluorescence. Bars with different letter(s) indicate significant difference according to $LSD_{0.05}$ test

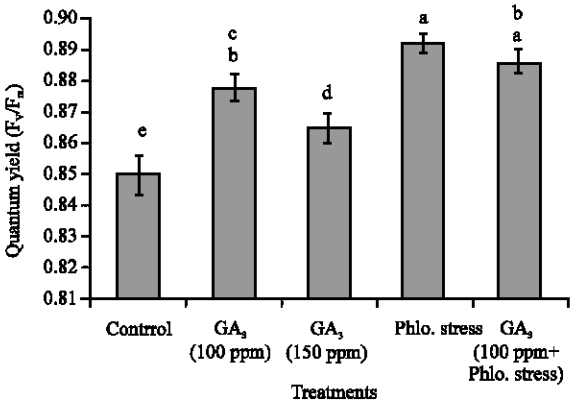

Fig. 7: Measurement of quantum yield (F_v/F_m) of leaves on different experiments branches. F_v is relative variable florescence (F_m - F_o). Bars represent SE (n = 3). Time range: 2 mecs-3 sec. Values followed by different letter(s) indicate the existence of significant differences according to $LSD_{0.05}$ test

In case of 100 ppm GA_3+phloemic stress, the required days were found to be 21.1 days. However, in phloemic stress it was 22.0 days. This was almost 3 days higher than in the control.

Bract weight of all stages showed an increase by applying 100 and 150 ppm GA_3, when compared to the same stage of the control (Fig. 4). The increasing effect of bract weight in 100 ppm GA_3 was significantly better than in 150 ppm GA_3 at the same stage. In the case of phloemic stress, bract weight increased with increase of bract stage, except for stage 1 and 2. Combined treatment of 100 ppm GA_3 and phloemic stress over branch increased

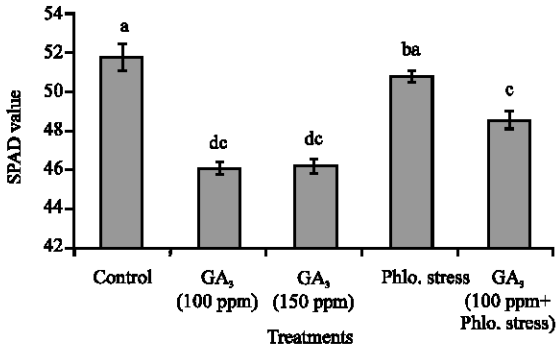

Fig. 8: The SPAD value, chlorophyll (carotinoid) content, of leaves in different treatment branches. Different letter(s) above bars indicate significant difference among treated leaves according to $LSD_{0.05}$ test

the bract weight at all stages. It was found that bark initiation was correlated with shoot elongation. This correlation was variable with shoot bract initiation and elongation (Fig. 5). Bract initiation was related to shoot elongation because of its polynomial correlation.

Chlorophyll florescence intensity (yield) was found to be higher in phloemic stress and 100 ppm GA_3 than in control (Fig. 6). In the case of 150 and 100 ppm GA_3+phloemic stress, chlorophyll florescence intensity (yield) was lower than in control (Fig. 6).

Quantum yield (F_v/F_m) of leaves was significantly lower in the case of control (Fig. 7). Highest quantum yield (F_v/F_m) was observed for phlolemic stress treatments. In the other treatments the values was fluctuated in between the values of these two treatments.

High SPAD value of leaves was found when the branch was treated by water control. The SPAD value, however was extremely affected by 100 ppm GA_3 compared with other treatments (Fig. 8).

In this study it was found that bract number per branch and bract size and weight were greater in the case of the treatments 100 ppm GA_3+phloemic stress, 100 ppm GA_3, 150 ppm GA_3 and phloemic stress when compared with the control (Fig. 9). It might be due to the elongation of bracts induced by the treatments. GA_3 showed positive effect on bract size and expansion but not on bract longevity and this was in accordance with the general concept that larger bracts last lesser. The bract expansion might be due to carbohydrate availability from the leaves to bract. However, bract longevity was prolonged by phloemic stress, whereas both the treatments 100 and 150 ppm GA_3 reduced bract longevity. When combined application of 100 ppm GA_3+phloemic stress was applied higher bract longevity was observed as compared with individual doses of 100 and

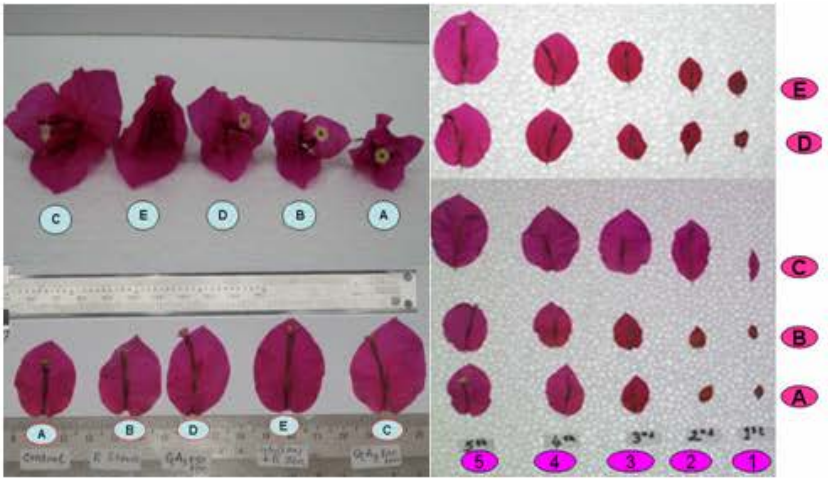

Fig. 9: The petal architecture and size at different stages. Whereas, 1 is 1st, 2 is 2nd, 3 is 3rd, 4 is 4th and 5 is 5th stage. A: Water control, B: Phloemic stress, C: GA_3(100 ppm), D: GA_3(150 ppm), E: GA_3(100 ppm + phloemic stress)

150 ppm GA_3. 100 ppm GA_3 treated branch of *Bougainvillea* exhibited three days earlier full blooming (5th) than in the control (Table 1). This might be due to the well known effect of GA_3 on early cell development. GA_3 bolting effects are well known and have been commercially exploited for agri- and horti-cultural productivity (Ogale *et al*., 2000). Hay and William (2008) reported that in callas, GA_{4+7} had been used to increase total shoot number, flowering shoot number and also to increase flower longevity. Brokking and Chon (2002) observed that plants treated with GA_3 induced earlier flowering and lower leaf number. This is similar to present results. In present results it was observed that bract initiation was also related to the shoot elongation because of polynomial correlation between bract initiation and shoot elongation.

In the present study, the number of bract per branch was higher in 100 ppm GA_3+phloemic stress and phloemic stress than in control branch (Fig. 2). This might be due to the deposit of sufficient carbohydrates and nutrients at the upper side of the phloemic stress region (Mataa *et al*., 1998). Arakawa *et al*. (1997) also found that flowering of Fuji apple was significantly increased by phloemic stress (girdling). It is supposed that *Bougainvillea*, like *Christmas cactus* (Han and Boyle, 1996), might be sensitive to ethylene in early budding stage. Consequently, inhibition of abscission by phloemic stress was blocked by the availability of excessive carbohydrates and nutrients in a branch from bud initiation to full blooming stage. For that, bract number was higher in phloemic stress and 100 ppm GA_3+phloemic stress branches than the other treatments. Earlier studies showed that the ethylene production rate of bracts was significantly higher in the early stages than in the later stages of their development (Chang and Chen, 2000). That is why a lot of buds were abscised at budding stage and also before full blooming stage. This implies that phloemic stress might be more effective in blocking sugar movement and the ethylene production rate may be decreased by these concentrated carbohydrates and nutrients. Whereas, in the control branch the total sugar quantity produced by leaves was uniform at all locations and it moved towards roots through phloem.

CONCLUSION

It is concluded that 100 ppm GA_3 may be more effective than 150 ppm GA_3 to enlarge bract size. Phloemic stress may be applied for bract longevity and large number of bract formation in the branches of *Bougainvillea spectabilis*. The treatment of 100 ppm GA_3+phloemic stress was found to be more effective to prolong bract longevity and size than phloemic stress. Finally, it is summarized that these methods are useful to induce bract enlargement and longevity in *Bougainvillea* which is an important plant in landscaping and environmental beautification. These methods can be used by gardeners and growers for commercial and asthetic values.

ACKNOWLEDGMENTS

The authors are grateful to the University of Malaya IPPP fund and Fellowship Scheme, Malaysia for providing funds for this research and also to Prof. Dr. Amru Nasrulhaq Boyce for valuable suggestion to carryout the experiment.

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