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The Effects of Naphthaleneacetic Acid and Gibberellic Acid in Prolonging Bract Longevity and Delaying Discoloration of *Bougainvillea spectabilis*

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Abstract: In this study, experiments were conducted to investigate the effects of NAA and GA_3 on bract longevity under exposed sunlight conditions and six months of observation. *Bougainvillea* bracts at four different stages of bract development were sprayed with gibberellic acid (100 ppm GA_3), naphthaleneacetic acid (50, 100 and 150 ppm NAA) and mixed GA_3 (100 ppm) and NAA concentrations (50,100 and 150 ppm). Bract longevity was found to be almost 10 days longer in NAA (50, 100 and 150 ppm) than in the water control and in GA_3 (100 ppm) treatment. In the case of GA_3 and NAA (50, 100 and 150 ppm) treatment on alternative days, bract longevity was 30 days longer when compared with the water control. It was also observed that a delay in discoloration and stomata conductance were increased in the presence of GA_3 with low a concentration of NAA. The results indicated that the prolonging effect of low concentrations of NAA at the initial budding stages was more effective compared with its application at other stages of development and at higher concentrations. Maximum bract weight and shoot length were observed in the GA_3 and GA_3 plus NAA treated flowers.

Key words: *Bougainvillea*, hormone, longevity, senescence, flower

INTRODUCTION

1-Naphthaleneacetic acid, commonly abbreviated NAA, is an organic compound with the formula $C_{10}H_7CH_2CO_2H$. NAA is a plant hormone in the auxin family and is an ingredient in many commercial postharvest horticultural products; it is also a rooting agent and used for the vegetative propagation of plants from stem and leaf cutting (Dimitrios *et al*., 2008). On the other hand, another prominent phytohormone, Gibberellic acid (GA_3), has the potential control on growth and flowering process. In addition, GA_3 application increased petiole length, leaf area and delayed petal abscission and color fading (senescence) by the hydrolysis of starch and sucrose into fructose and glucose (Khan and Chaudhry, 2006; Emongor, 2004).

It has been well documented that the commercial value of flowers is very dependent on its longevity (Hye and William, 2008). Other factors that are of importance include flower color and size. In the case of *Bougainvillea*, a plant well known for its beautiful flowers as a potted plant and in landscaping activities, relatively little study has been carried to study its flower potential. *Bougainvillea* is one of the most popular and widely grown tropical vines (Suxia *et al*., 2009) and possesses characteristics that makes the plant attractive to the industry, such as fast growing, variation in type of foliage, the production of many flowering inflorescences on one plant and continuous blooming of flowers with a short production cycle (Grodon, 2002). These plants are sold as an indoor flowering potted plants and planted outdoors all year round. In addition, *Bougainvillea* is extensively used in arid landscapes for beautification on account of its wide adaptability to different climatic environments.

Recently, it has been reported that the shelf life and commercial value of *Bougainvillea* flowers are affected by its early shedding (Hossain *et al*., 2007). Its commercial value can be improved by prolonging flower longevity and increasing its quality in terms of size (Tjosvold *et al*., 1994). Flower longevity is related to flower and leaf senescence and in some flowers it is caused by the plant hormone, ethylene (Van Door, 2002; Arthur and Michael, 1983). The plant hormone, ethylene, is responsible for early senescence in many flowers such as orchids, roses etc. (Leiv and Hans, 2005). For cut flowers shelf life can be improved by delaying senescence using ethylene synthesis and receptor inhibitors such aminooxyacetic acid and silver thiosulfate, respectively. However, in potted plants, flower longevity can be improved by using growth regulating hormones, such as GA, at the different development stages and flowering period (Hye and

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William, 2009). The phenomena of flowering is a complex developmental process consisting of at least four sequential phases, namely, flower induction, initiation, flower opening and flower senescence (Chang and Chen, 2001). Flower induction depends on factors such as nutrient availability, flowering time, light, temperature and day length (Wurr *et al*., 2000).

The purpose of this study was to investigate flower opening and determine how plant hormones, namely GA_3 and NAA, influence the development of flower size and flower longevity in *Bougainvillea spectabilis*, respectively.

MATERIALS AND METHODS

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

Plant material: Seven-year-old Bougainvillea plants were used in this study. The plant was approximately 1.5 m in height with a canopy length of about 2.5 m. The tree consisted of 4 main branches and 30 minor branches. The experiment was conducted between 10th June 2008 and ended on 25th December 2008.

Hormone treatments: Treatments were set following a completely randomized design. Each treatment had 3 replications. Each selected branches bracts were wetted by dropper with 50, 100 and 150 ppm NAA on a 2 days interval. Other nine branches bracts were wetted with 50 ppm NAA+100 ppm GA_3, 100 ppm NAA+100 ppm GA_3, 150 ppm NAA+100 ppm GA_3, respectively. Application was done on alternative two day intervals on selected branches in the case of mixed NAA (50, 100 and 150 ppm) + GA_3 (100 ppm).

Data collection: Bract development was divided into four stages (*1st stage = initial budding, 2nd stage = bud opening, 3rd stage = partial blooming, 4th stage = full blooming, 5th stage = 50% discoloration or flower abscission) to measure and compare bract longevity and weight among the different treatments. Bract longevity, bract weight, bract discoloration were measured weekly. Each treatment had three branches and each branch had all the four bract stages. Bracts of three flowers in the same stage were considered to be a unit. Through out experiment, the plants were placed under exposed sunlight conditions (Chang and Chen, 2001).

Bract longevity: Bract longevity was counted as the number of days from treatment setting stages (1st, 2nd, 3rd and 4th) to 50% discoloration or abscission (5th stage) (Saifuddin *et al*., 2009).

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

Bract weight and length measurements: Bract weight was taken using a Mettle PJ3000 micrometer balance. Bract length was measured by a vernier scale at the 4th stage of every individual treatment; control, GA_3, NAA and combination treatment of NAA and GA_3.

Evaluation of abscission and petal discoloration: Abscission was counted in number of days from the treatment setting stage (1st, 2nd, 3rd and 4th) to the flower abscission (5th) stage. Bract (flower) status was observed everyday. Petal discoloration was also observed by observing petal symptoms:

$$\text{Petal discolored area (\%)} = \frac{\text{Discolored petal area}}{\text{The total petal area}} \times 100$$

Stomatal conductance: Stomatal conductance was measured by a portable porometer (Leaf Porometer, Model SC-1, USA). A leaf clip was attached to one of the leaf and kept in ambient temperature for 10-15 min to maintain sunlight adaptation. Stomata conductances were measured 3 times from different spots of a single leaf.

Statistical analysis: Data were analyzed by the ANOVA procedure, using SPSS software. Statistical significance was judged at $p = 0.05$. When the analysis was statistically significant, the DMR test for separation of means was performed.

RESULTS AND DISCUSSION

As can be seen in Table 1, bract longevity was prolonged after applying NAA (50, 100, 150 ppm) at all stages of flower development. Application of 100 ppm GA_3 showed the shortest duration of (20 days) flower life compared to the other treatments. However, bract longevity in all the stages was prolonged by applying NAA (50, 100, 150 ppm) + GA_3 compared to the control. When single treatments of NAA or GA_3 were compared with combined treatments (NAA + GA_3), the prolonging effect of NAA + GA_3 was significantly better. Among the different concentration of combined treatments of NAA plus GA_3, the best result was exhibited by GA_3 (100 ppm)

Table 1: The effects of NAA and GA3 solutions on bract longevity at different stages of bract development in *Bougainvillea spectabilis*

	Bract longevity (days)			
Treatments	Stage 1	Stage 2	Stage 3	Stage 4
Control	19.33±1.65gh	13.33±0.88f	9.00±1.15gh	6.33±0.33g
50 ppm NAA	28.33±1.76def	23.33±1.2de	20.66±1.76abcdef	14.33±1.20f
100 ppm NAA	30.00±2.08de	27.66±0.88c	22.66±1.2abcde	19.33±0.88bc
150 ppm NAA	30.33±1.2d	25.66±0.33cd	21.33±1.76abcde	18.00±1.15cde
100 ppm GA	20.00±1.15g	12.33±1.2fg	11.33±0.88g	5.33±0.33gh
100 ppm GA+ 50 ppm NAA	52.00±1.52ab	35.66±1.2a	23.33±4.7abc	21.66±1.45ab
100 ppm GA+100 ppm NAA	54.66±1.76a	34.66±1.45ab	26.66±0.88a	23.00±1.15a
100 ppm GA+150 ppm NAA	50.66±1.33abc	32.00±1.15ab	24.66±0.88ab	19.00±0.57bcd

Means followed by different letters within column are statistically different at 5% level of significance, using DMR test

Table 2: Leaf and bract chlorophyll readings (SPAD values) at the different stages of bract development in different NAA and GA3 treatments

	SPAD value				
Treatments	Stage 1	Stage 2	Stage 3	Stage 4	Leaf
Control	7.80±1.00bc	5.86±0.23bd	1.63±0.24c	0	54.20±0.98a
50 ppm NAA	8.83±0.71abc	6.26±0.30abc	2.13±0.20ab	1.26±0.12c	50.46±1.31b
100 ppm NAA	8.86±1.09ab	6.66±0.20a	2.70±0.17a	1.70±0.28ab	48.36±0.63bc
150 ppm NAA	9.63±1.21a	6.40±0.49ab	2.26±0.24abc	2.10±0.26a	45.10±1.05cde
100 ppm GA3	2.23±0.23defg	0.60±0.05f	0	0	46.50±0.25cd
100 ppm GA3+ 50 ppm NAA	2.60±0.35def	0.86±0.08	0	0	44.23±0.31def
100 ppm GA3+100 ppm NAA	3.20±0.32d	1.26±0.17e	0	0	43.30±1.59defg
100 ppm GA3+150 ppm NAA	2.93±0.34de	0.50±0.08fg	0	0	42.46±1.12efgh

Means followed by different letters within columns are statistically different at 5% level of significance, using DMR test

Table 3: The effect of NAA and GA3 treatments on bract discoloration at the different stages of bract development in *Bougainvillea spectablis*

	100% Discoloration (days)			
Treatments	Stage 1	Stage 2	Stage 3	Stage 4
Control	19.57±2.54ef*	13.60±0.75f*	8.00±1.29fg*	7.63±0.29e*
50 ppm NAA	35.33±1.62cd	29.33±2.17de	26.66±1.79cd	20.33±1.50cd
100 ppm NAA	37.00±1.00c	32.54±0.48cd	29.21±1.24b	23.45±1.78ab
150 ppm NAA	39.63±1.30c	29.66±0.93d	24.33±1.65de	20.00±1.18cd
100 ppm GA	21.00±2.26e*	12.33±0.70fg*	11.33±0.74f*	5.33±0.31ef*
100 ppm GA+ 50 ppm NAA	59.00±2.15ab	40.66±0.24a	31.33±4.70a	3.66±2.30ab
100 ppm GA+100 ppm NAA	62.66±2.76a	38.66±2.55ab	30.66±0.45ab	25.00±1.65a
100 ppm GA+150 ppm NAA	60.66±2.52ab	36.00±2.50bc	28.66±0.45bc	21.00±0.87bc

*Abscission stage. Mean separation within columns by analysis of Duncan's multiple range test

+NAA (100 ppm). These results showed that harvest quality of *Bougainvillea* bracts were vastly improved as a result of the combined NAA and GA_3 application throughout the different developmental stages (Table 1).

Table 2 shows the results on the determination of chlorophyll values using the SPAD meter. There was a considerable increase in petal SPAD values in all the NAA treatments when applied singularly (18% in 1st stage). The values were lower in the case of the combined hormonal treatments (NAA and GA_3). At stages 3 and 4, the SPAD value readings were very low in the NAA treatments whilst in the GA_3 and GA_3 plus NAA treatments no SPAD value readings were recordable. The results imply that there was a little effect of NAA on the bract natural color. Consequently, the SPAD values of the bracts at the subsequent stages were affected. Furthermore, it was observed that the bract SPAD values in the GA_3 and GA_3 plus NAA treatments during all the stages were lower than the control. However, as for the leaf SPAD values, they were lower in all the treatments when compared with the control (average 11% in NAA and 20% in NAA plus GA_3). The observation was could possibly be attributed to the effect of NAA and GA_3 on chlorophyll synthesis.

It has been reported that NAA not only inhibit ethylene biosynthesis and contribute to longevity but also slow down natural shoot growth (Paul and Pieter, 1989). The influence of all the hormonal treatments on bract discoloration, observed throughout the experiment by visual observations is shown in Table 3. Study results showed that there was a significant delaying in discoloration in the NAA and GA_3 plus NAA treatments compared to the control and GA_3 treatment. The control and GA_3 treatments showed abscission, whereas the other treatments exhibited a gradual discoloration without abscission. Bract discoloration was delayed when NAA was applied in all the stages compared to the control. However, bract discoloration process was remarkably

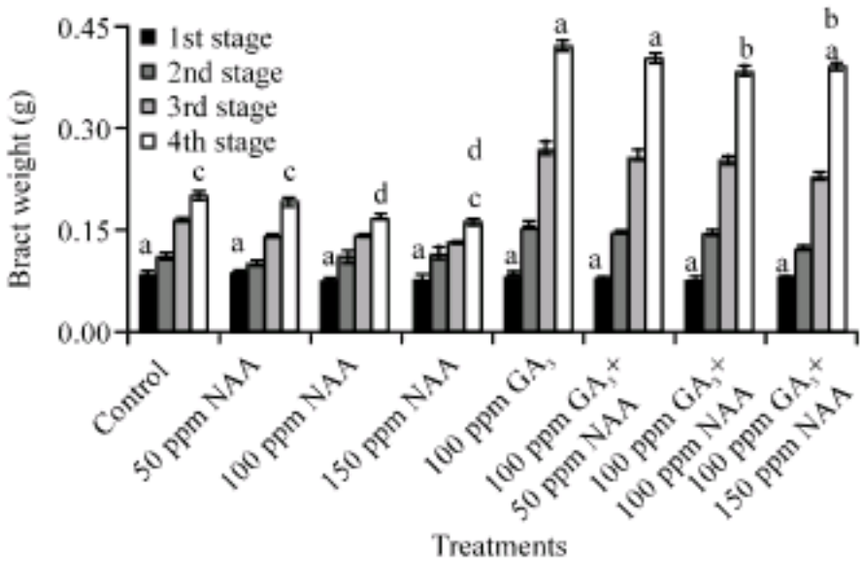

Fig. 1: The effect of NAA and GA_3 treatments on bract weight at different developmental stages. Means followed by different letters above bars are statistically different at 5% level of significance, using DMR test

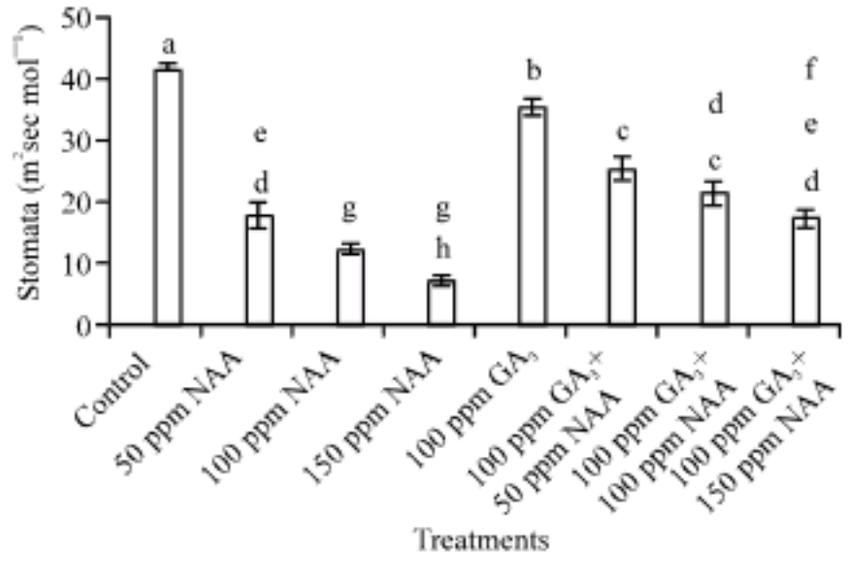

Fig. 2: Stomatal conductance in leaves of branches treated with NAA and GA_3. Means followed by different letters are statistically different at 5% level of significance, using DMR test

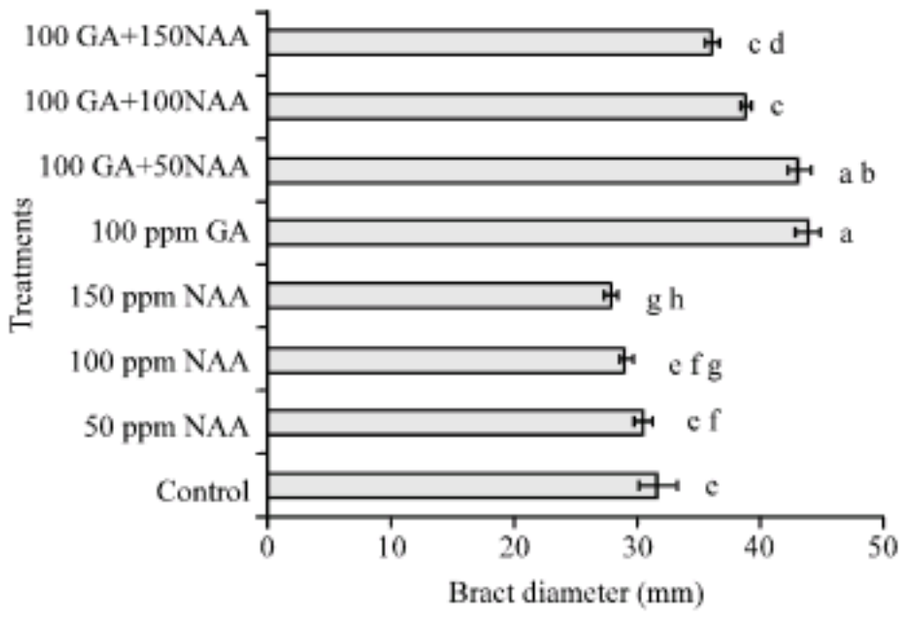

Fig. 3: The effects of the different hormonal treatments on bract diameter and size (mm) at the 4th developmental stage in *Bougainvillea.* Means followed by different letters are statistically different at 5% level of significance, using DMR test

slower in the combined NAA and GA_3 treatments. The results suggest that there was a positive effect of the combined treatments of NAA and GA_3 on the bract discoloration process.

Figure 1 show, bract weight was almost the same in all the treatments at the 1st stage. From the 2nd stage onwards a change was observed and the maximum bract weight was recorded in 100 ppm GA_3 treatment. When the individual treatments of NAA and the combined treatments of NAA plus GA_3 were compared, the bract weight was significantly higher in all the NAA plus GA_3 treatments and bract weight decreased as the NAA concentration increased. At the 4th stage, the highest bract weight was observed in the 100 ppm GA_3 treatment. It is clear that 100 ppm GA_3 had a positive effect on bract weight compared to NAA.

With regard to stomatal conductance (40.3 $m^2sec\ mol^{-1}$) it was found that it was higher in the control treatment compared to all the hormonal treatments (Fig. 2). Amongst the hormonal treatments stomata frequency was significantly higher in the GA treated leaves than in the NAA and NAA plus GA treatments. The difference in stomatal conductance between NAA and NAA plus GA leaf surfaces were probably related to the fact that NAA caused a reduction in stomatal conductance (12 $m^2sec\ mol^{-1}$). The negative effects of NAA on growth are again exhibited here. All the leaves treated with NAA showed a little wrinkle on its surface area.

In case of the bract diameter, the mixed treatments of GA_3 and NAA and the single treatment of GA_3 only, clearly showed a positive effect by enlarging bract size. However, the single NAA treatments showed a slightly negative effect with regard to bract size compared to the control. The diameter of fully open bracts was about 45 mm (Fig. 3) in GA_3. Spraying with low concentrations of NAA plus GA_3 stimulated flower size with diameters ranging from 44 to 39 mm (Fig. 3).

As expected shoot length was significantly increased by the influence of GA_3 probably due to its well known effect on early cell development. However it decreased remarkably in the presence of NAA in all the GA plus NAA treatments. Shoot length was extremely affected by all the NAA treatments when applied singly, compared to the control (Fig. 4).

It is well known and documented that plant hormones play an important role in controlling the growth, development, metabolism and morphogenesis of plants (Claus, 2008). One of these, the gibberellins are plant growth hormones that are well known for their ability to

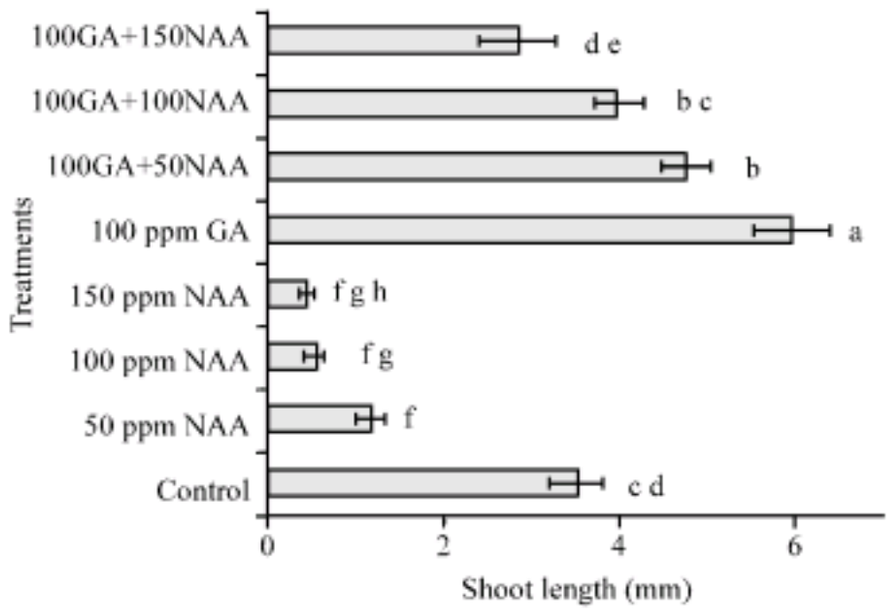

Fig. 4: The effect of NAA and GA_3 treatments on shoot length. Means followed by different letters are statistically different at 5% level of significance, using DMR test

elicit different mode of actions (Hye and William, 2008). Ogale *et al*. (2000) reported that GA_3 induced changes in *Portulaca garndiflora* flower size by ~40% and color from crimson red to complete white. GA_3 has also been reported to reduce the time needed for inflorescence emergence, accelerated flowering and increased the number of flower buds and open flowers in most growing conditions (*Cucumis sativus*) (Khan and Chaudhry, 2006). In addition, the application of Gibberellic Acid (GA_3) has the potential to control growth, flowering and induce earliness meristem. Contrary to GA_3, the effect of the plant hormone NAA on a plant often depends on the stage of the plant's development (Chang and Chen, 2001). NAA have been reported to prevent flowers and fruits from dropping off trees before maturation. NAA has also been used to prevent the undesirable sprouting of stems from the base of ornamental trees. Although, GA_3 is well-known in promoting flower growth and development, its involvement in controlling the delay of senescence is less clear (Rosenvasser *et al*., 2006). There have been reports that GA_3 has little effect as an ethylene inhibitor, inhibiting both climacteric ethylene production and flower senescence (Kun *et al*., 2008). In view of the above reports, it is possible that a combination of both GA and NAA, when applied together, are able to produce larger flowers with good longevity (Luiz *et al*., 2008).

In the present study, it was observed that alternative day treatments of NAA with GA_3 significantly improved flower size, weight and longevity in *Bougainvillea spectabilis*. The improvement of flower longevity, delay in discoloration and flower senescence could possibly be the result of NAA inhibiting ethylene action (Chang and Chen, 2001). The variable effects of GA_3 on bract weight and delay in discoloration is probably due to cultivar differences in soluble carbohydrate level of the flowers. The action of GA_3 with NAA might have been to increase soluble carbohydrates level in the abscission zones. As a result, the soluble sugar content of flower petals are increased at the time of flower opening and reaches a maximum before full opening stage. Consequently, premature abscission was prevented at the different flowering stages (Van Doorn, 2004).

Some studies have suggested that *Bougainvillea*, like *Christmas cactus*, might be sensitive to ethylene in the early flower bud stages resulting in a lot of buds abscising at the budding stage before full bloom (Han and Boyle, 1996). It was also suggested that the bracts were less sensitive to ethylene in the early stages of development (Elgar *et al*., 2003), thereby allowing NAA to inhibit abscission more effectively, as ethylene production was neutralized by NAA in the early stages of bract development. In addition, the variable effects of mixed GA_3 and NAA treatments at different stages of bract development could be due to differences in ethylene production and or sensitivity at different bract maturities. Chang and Chen (2001) reported that NAA delayed bract abscission in *Bougainvillea*. In this study, bract longevity was significantly prolonged by 100 ppm NAA plus 100 ppm GA_3 and in treatments with 100 ppm NAA. However it was observed that the prolonging effect of NAA in this cultivar was more effective in the early stages of bract development and the effects decreased as the bracts matured. These results are in agreement with studies conducted on the *Eustoma* flower (Kazuo *et al*., 1998) and *Easter* cactus (Han and Boyle, 1996).

There was a positive correlation between NAA plus GA_3 treatment and stomatal conductance even though it was lower than the control leaf. The leaf tends to have its stomata closed during severe chemical stress. The stomata adjustment towards progressively severe stress indicates their chemical resistance mechanisms as reported by Yoko (2006). Amongst the numerous theories, this response of the stomata may be regarded as a feedback response where a signal from the NAA is transmitted to the leaves and bracts so that natural growth and cell activity is postponed by NAA to protect ethylene production through ACC path. The effect of NAA was more pronounced when its concentration was increased. Low stomata conductance reduced leaf SPAD value when compared to the control leaves. In the presence of GA_3 stomatal conductance was higher than in the single NAA treatments. Consequently SPAD values were higher in NAA plus GA_3 than in NAA alone. This implies that GA_3 might be effective in blocking NAA activity and stomatal conductance might be increased by the availability of concentrated external nutrients. In the

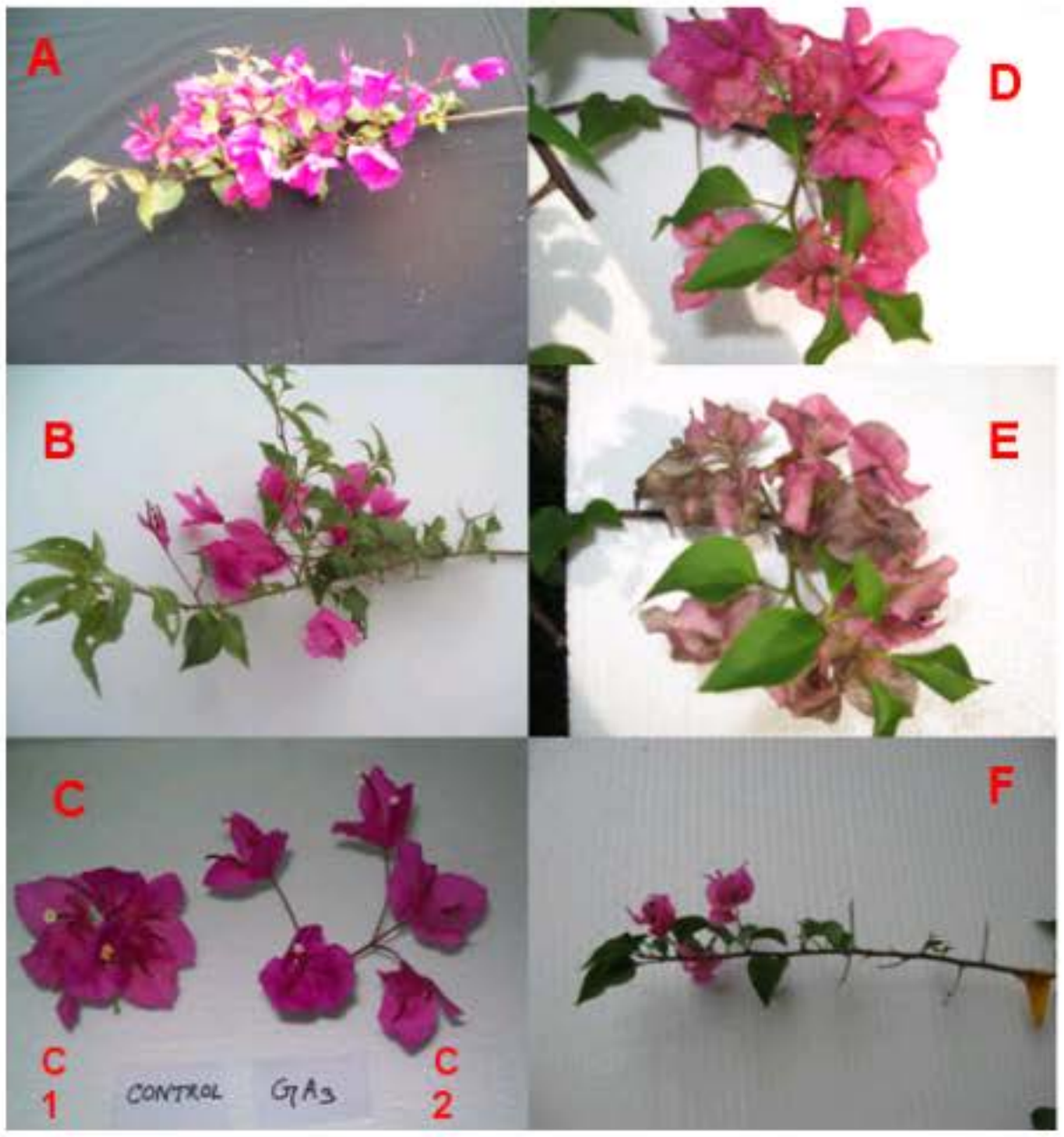

Fig. 5: Photographs show the effects of mixed treatments (100 ppm GA_3 plus 100 ppm NAA) and 100 ppm NAA on the different developmental flowering stages in *Bougainvillea specliblis*. (A) The petal architecture and size after 25 days, (B), Flowers 40 days after applying mixed treatment (100 ppm GA_3 + 100 ppm NAA), (C) Represents the control (C1) and 100 ppm GA_3 (C2) treatments, (D, E) 100 ppm NAA treatment, 25 and 40 days after application, respectively and (F) Control treatment after 25 days

water control, bracts were abscised rapidly, within 20 days. In the presence of GA_3, bract discoloration was delayed in all GA_3 plus NAA treatments. Such an effect, observed presently (Table 3, Fig. 5), seems to be due to exhaustive and rapid nutrient utilization. On the other hand, when NAA is applied singly the bracts showed rapid discoloration (in the 50, 100 and 150 ppm). NAA treatments possibly due to blocking ethylene and delaying growth process together. In addition, the GA_3 treatment increased flower size and weight due to its well known effect on rapid cell elongation. On the other hand, flower size and weight were not increased by NAA due to its delay action in cell elongation processing and low stomatal conductance. Therefore, less photosynthesis process is conducted by these chemical treated branches leaf.

Gibberellins are well known to increase hydrolysis of starch and sucrose into glucose and fructose, which were utilized by the flowers for disc floret opening (Emongor, 2004). The increased reducing sugars in the flower heads and stems of bougainvillea flowers may increase the osmotic potential of the flower bracts and petals, thus improving their ability to absorb nutrients and maintain their turgidity, which may explain the increase of flower longevity (Table 1) in different developmental stages and observed in this study. NAA in presence of GA_3 delayed bract abscission and color fading because of GA_3 increased bract osmotic potential thereby promoted hydrolysis of starch and sucrose into fructose and glucose which delayed petal abscission and color fading (senescence). There is a possibility of NAA treated in the branch interacting with GA and hence delaying the senescence of flower possibly by either altering the sensitivity of the tissue to ethylene or by delaying the natural rise in ethylene production, or both.

CONCLUSION

The experimental results indicate that GA_3 hormone involved more in cell elongation and little response to petal senescence. NAA hormone was increased bract longevity blocking the ethylene biosynthesis path.

Therefore, it should be concluded that spraying containing GA_3 and NAA might be commercial value in enhancing postproduction quality of *Bougainvillea specliblis*. Considering that concentration (100 ppm) of GA_3 plus NAA (100 ppm) could to protected unwanted early senescence and discoloration. Other concentration (50 and 150 ppm NAA) would be less useful for achieving maximum effectiveness. NAA should be applied during the earlier stages of bract development to prolong bract longevity in potted bougainvillea. NAA + GA_3 were more effective than NAA and recommended for exposed sunlight condition and for using on potted *Bougainvilleas* being transported.

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