

CHAPTER 5

Bract size enlargement of *Bougainvillea spectabilis* as affected by gibberellic acid and phloemic stress

5.1 Introduction

Gibberellins (GA_s), a large family of tetracyclic, diterpenoid plant hormones, play an important role in regulating diverse processes throughout plant development. Gibberellic acid (GA₃) has been reported to influence vegetative growth, flowering, fruiting and various disorders in many fruit crops including strawberry (Paroussi *et al.*, 2002). Similarly, gibberellic acid has been reported to enhance pollen germination (Kappel and MacDonald, 2007) and may also affect colour development in many flower and fruit plant species.

In strawberry, GA₃ 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; Kuiper *et al.*, 1995). During flower development, GA₃ 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 has the potential to control growth and flowering and induce earliness. In addition to GA₃ effect, Hossain *et al.* (2004) also reported that flower bud percent and sugar content was a higher when phloemic stress was applied in treated plants.

Jose (1997) found that less vegetative growth in all the ringing (girdling) treatments. Hossain *et al.* (2004) reported that starch content in the bark was higher in peach trees when it was taken from the upper part of the phloemic stress. Gibberellic acid

delayed petal abscission and color fading because of GA₃ decreased flower head and stem dry matter of flowers thereby promoted hydrolysis of starch and sucrose into fructose and glucose which delayed petal abscission and color fading. The objective of this experiment was to undertake the effect of GA₃ hormone and phloemic stress on flower enlargement (bract length and weight), chlorophyll content and floescence yield in *Bougainvillea spectabilis*.

5.2 Materials and Methods

5.2.1 Experimental Site and Plant Materials

The experiment was conducted in the Plant Physiology Garden, Institute of Biological Sciences, University of Malaya. A seven-year-old bougainvillea plant having selected sub-branches (fifteen) was used in this experiment. The plant was 1.5 m in height and canopy length was 2 m. The tree consisted of four main branches & twenty five sub-branches. Treatments were set in completely randomized design (CRD). Each treatment was repeated by three replications in the sub branches with maintaining same age, leaf number, shoot length and sunlight position and period.

5.2.2 Treatment Setting

The phloemic stress was applied 15 cm away from shoot apex in a branch removing bark from wood (Fig. 5.1). The phloemic stress (PS) was done by using a small sharp knife to remove a 2 cm in length of partial ring (70%) leaving a 2 mm in width (thickness) of a connecting strip (bridge) (Hossain and Fusao, 2008; Yoshiko *et al.*, 1999). There were

three replications for each treatment. The treatments were control (no GA₃ and phloemic stress), phloemic stress, 100 ppm GA₃, 150 ppm GA₃ and a combination of 100 ppm GA₃ and phloemic stress. So there were total of fifteen branches used in the experiment.

5.2.3 Data Collection

Bract (flower) 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} in order to measure and compare bract longevity and weight among the treatments. During the experiment, new shoot growth, bract longevity, bract weight, bract length (diameter) and total bract number were recorded weekly.

5.2.4 Bract number, weight and Length

The fresh weight of 5th stage of bract was taken using the balance machine (Mettler, PJ3000) and bract length was measured by Vernier scale.

5.2.5 Bract longevity and Full blooming days

Bract longevity was counted days from bract initiation to abscission and full blooming days was counted from bract initiation to 5th bract stage.

5.2.6 Chlorophyll Fluorescence Measurements

Chlorophyll fluorescence was measured as described earlier (3.2.3).

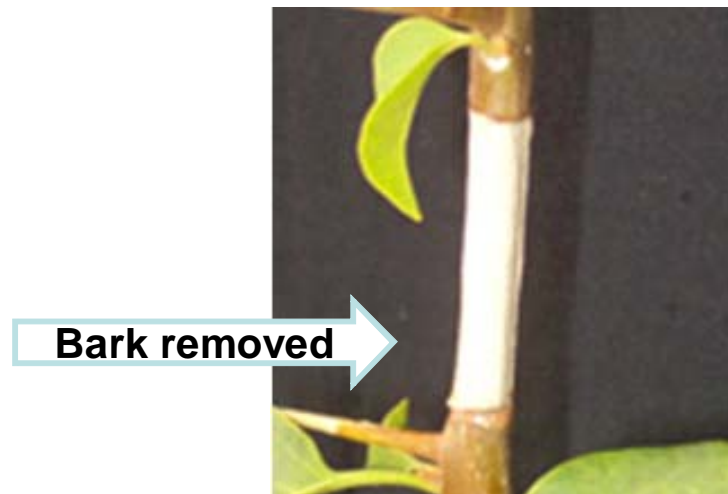


Figure 5.1: Photographs show phloemic stress (70%) and trunk circumference of bougainvillea plant branch.

5.2.7 Chlorophyll Content

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

5.2.8 Statistical Analysis

Statistical analysis was evaluated as described earlier (3.2.10).

5.3 Results

Bracts number was observed to be eighteen in water control, while it was twenty three and seventeen in 100 ppm GA₃ and 150 ppm GA₃, respectively (Fig. 5.2). In the case of phloemic stress, the number of bracts per branch was twenty four. The most significant difference was expressed when the branch was treated with the combination of 100 ppm GA₃ and phloemic stress. In this treatment total bract number was twenty eight, which was the highest among the treatments.

Bract length was significantly prolonged by 100 ppm GA₃ at all stages (Fig. 5.3 and Fig. 5.7). At the full blooming stage (5th), the results showed that the length of petiole, bract size increased by 40% in 100 ppm GA₃. In the case of 150 ppm GA₃, the petal length was prolonged at all stages, but bract length did not show much difference as those in 100 ppm GA₃. In addition, a similar increasing trend was found in the combined treatment of 100 ppm GA₃ and phloemic stress. In the case of phloemic stress treatment, bract (petal) length was almost similar to those in control from 1st to 5th stage. The time from flower initiation to full blooming varied with treated application. Blooming was quickest in GA₃ and was

significantly delayed in phloemic stress. The required days were sixteen and fifteen to reach full blooming stage in 100 ppm GA₃ and 150 ppm GA₃, respectively. It was almost three and four days earlier than in control. In case of 100 ppm GA₃ + phloemic stress, the required days for blooming were found to be twenty one days. This was almost 13% higher in phloemic stress than in the control. However, bract longevity was found to be four days higher in phloemic stress and two days higher in 100 ppm GA₃ + phloemic stress than in water control (Table 5.1). Whereas, bract longevity was two days less in GA₃ 100ppm and four days less in 150 ppm GA₃ than in water control. Bract weight showed an increase by applying 100 ppm GA₃ and 150 ppm GA₃, when compared to the control (Fig. 5.4). The increasing effect of bract weight in 100 ppm GA₃ was significantly better than in 150 ppm GA₃. In the case of phloemic stress, bract weight increased with increase of bract stage. Combined treatment of 100 ppm GA₃ and phloemic stress over branch increased the bract weight. The quantum yield of dark-adapted leaves determining the maximum efficiency of photosystem was showed (Fig. 5.5). The present value indicated that quantum yield of PSII decreased significantly with increasing GA₃ concentration. The lowest values were obtained in 150 ppm GA₃ treated branch leaves. The maximum values were obtained in all types of leaves in phloemic stress with significant difference. In the presence of GA₃, a negative effect was observed in combined treatment of GA₃ and phloemic stress. The chlorophyll content at the end of the experiment was higher with significant differences in control branches leaf. The chlorophyll content value was affected by GA₃ chemical compared with other treatments (Fig. 5.6). Where, the content of chlorophyll was similar in both 100 ppm GA₃ and 150 ppm GA₃. However the negative effect is being normalized by treating the plant with phloemic stress. The chlorophyll content was 5-6% lower in combination of 100 ppm GA₃ and phloemic stress treated leaves than control.

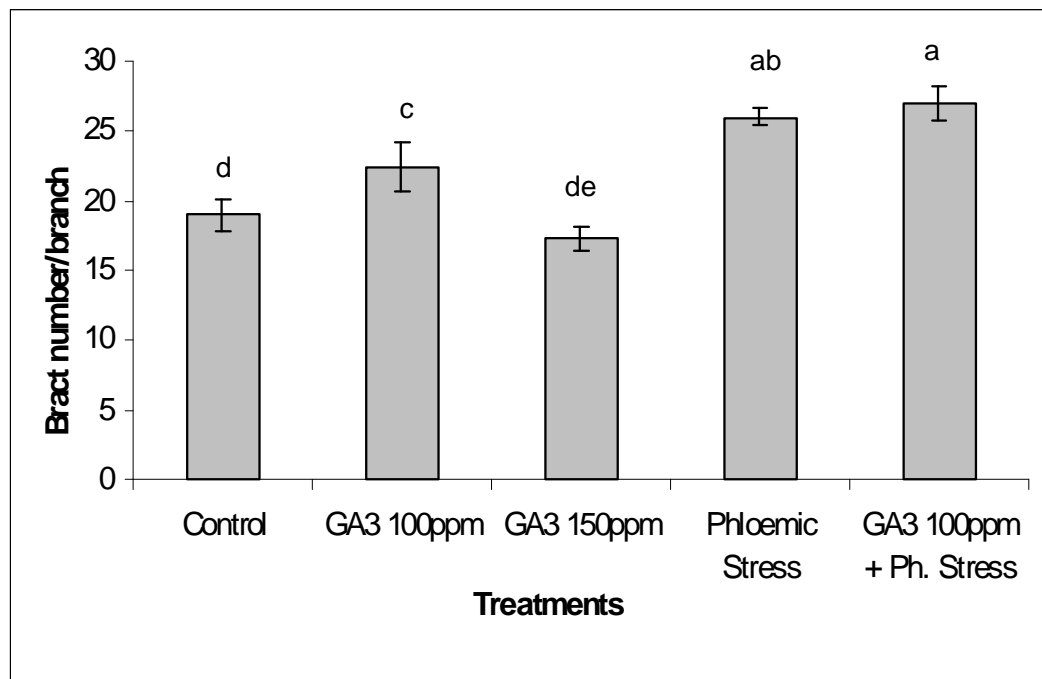


Figure 5.2: Bract number/branch of bougainvillea plant as affected by different treatments. Values followed by different alphabets indicate the existence of significant differences according to LSD $_{0.05}$ test.

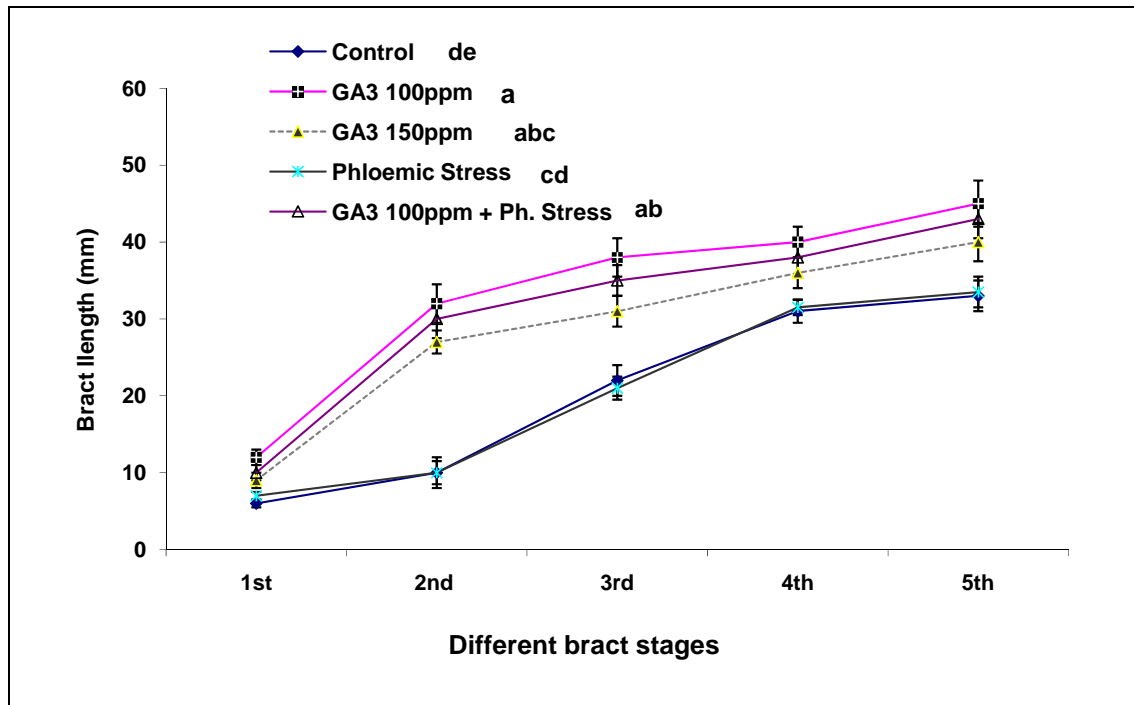


Figure 5.3: Effects of different treatments on petal size (mm) at different bract stages of *Bougainvillea* sp. Where, 1st : initial bud stage, 2nd : bud opening stage, 3rd : initial bract blooming, 4th : partial bract blooming, 5th : full blooming. Values followed by different alphabets indicate the existence of significant differences at 5th stage according to LSD 0.05 test.

Table 5.1: Effects of different treatments on bract longevity and full blooming days of *Bougainvillea* sp bract. Different alphabets indicate significant difference among treatments according to LSD_{0.05} test.

Treatments	Full blooming days	Bract longevity* (days)
Control	19.33±0.33 bc	23.66±1.45 bc
GA ₃ 100 ppm	16.83±0.44 d	21.66±1.45 cd
GA ₃ 150 ppm	15.33±0.33 de	19.66±0.88 de
Phloemic stress	22.00±0.57 a	27.33±1.20 a
GA ₃ (100 ppm)+Phlo.Stress	21.16±1.16 ab	25.33±1.16 ab

*Required days from bract initiation to senescence.

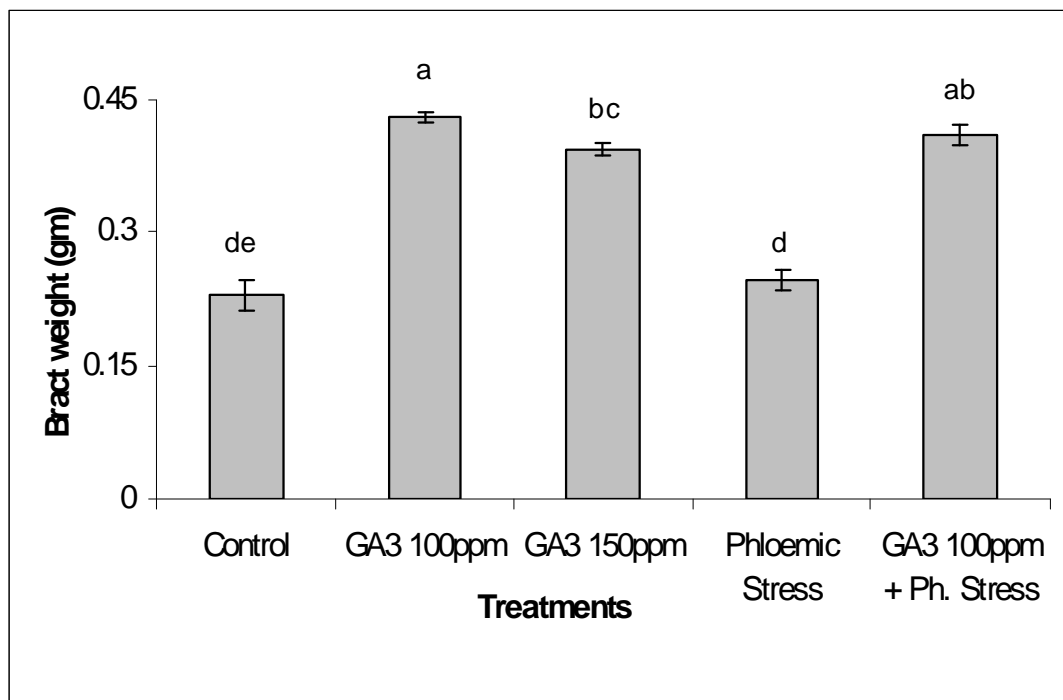


Figure 5.4: Bract weight as affected by different treatments applied to *Bougainvillea* sp. Values followed by different alphabets indicate the existence of significant differences according to LSD_{0.05} test.

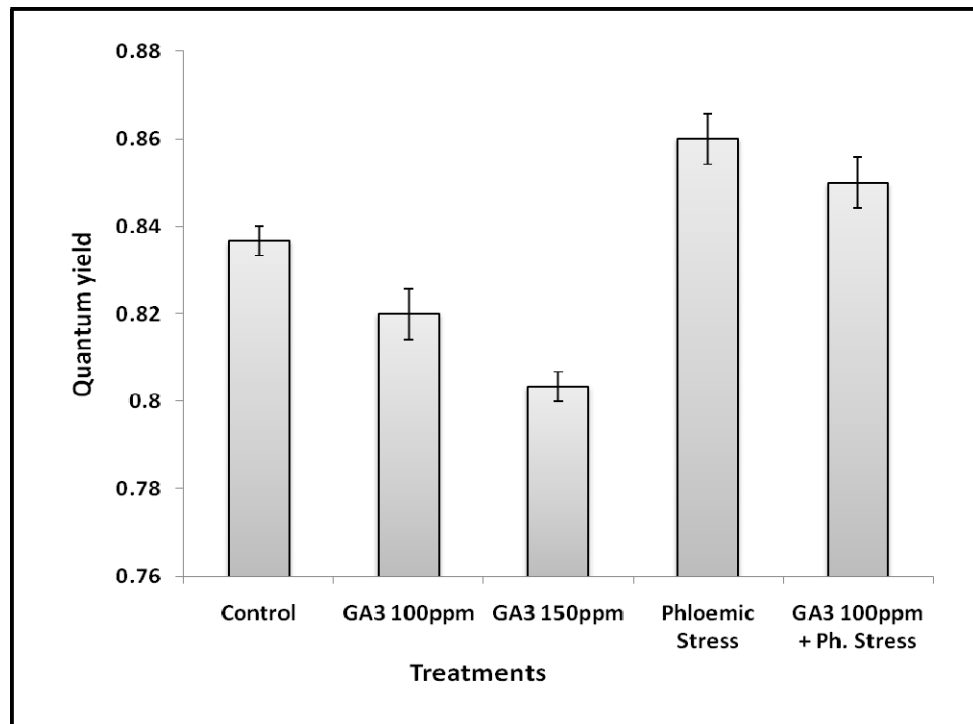


Figure 5.5: Determination of quantum yield in different treated branches. Bars with different alphabets indicate significant difference according to $LSD_{0.05}$ test.

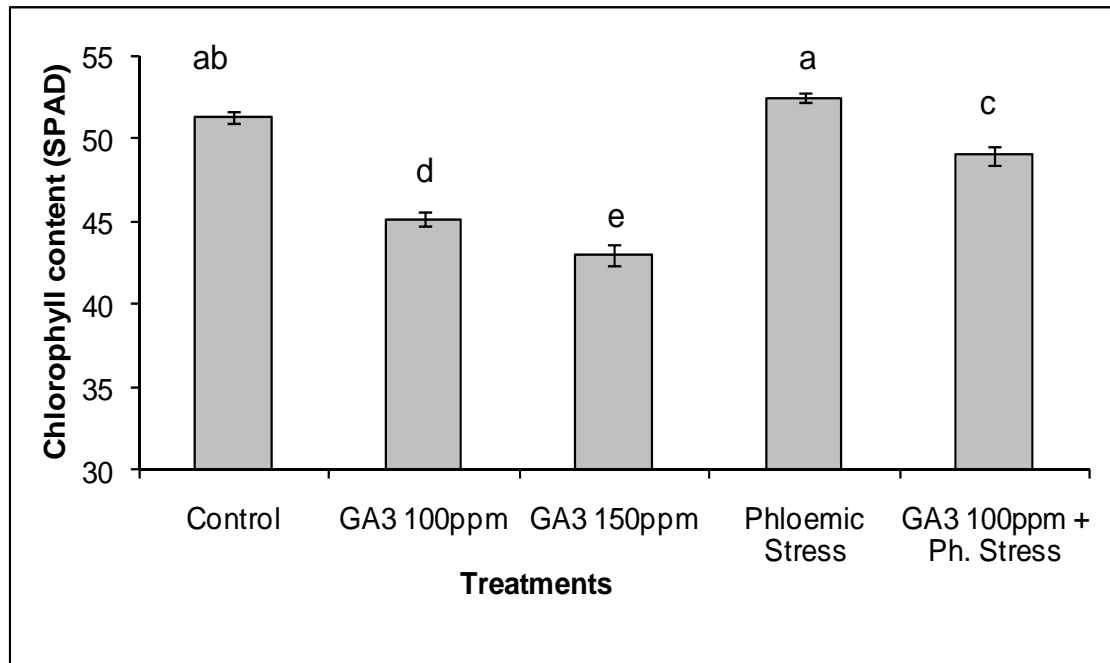


Figure 5.6: The SPAD value of leaves in different treatment branches. Reading was taken 5 times from different spots of a single leaf. Different alphabets above bars indicate significant difference among treated leaves according to LSD $_{0.05}$ test.

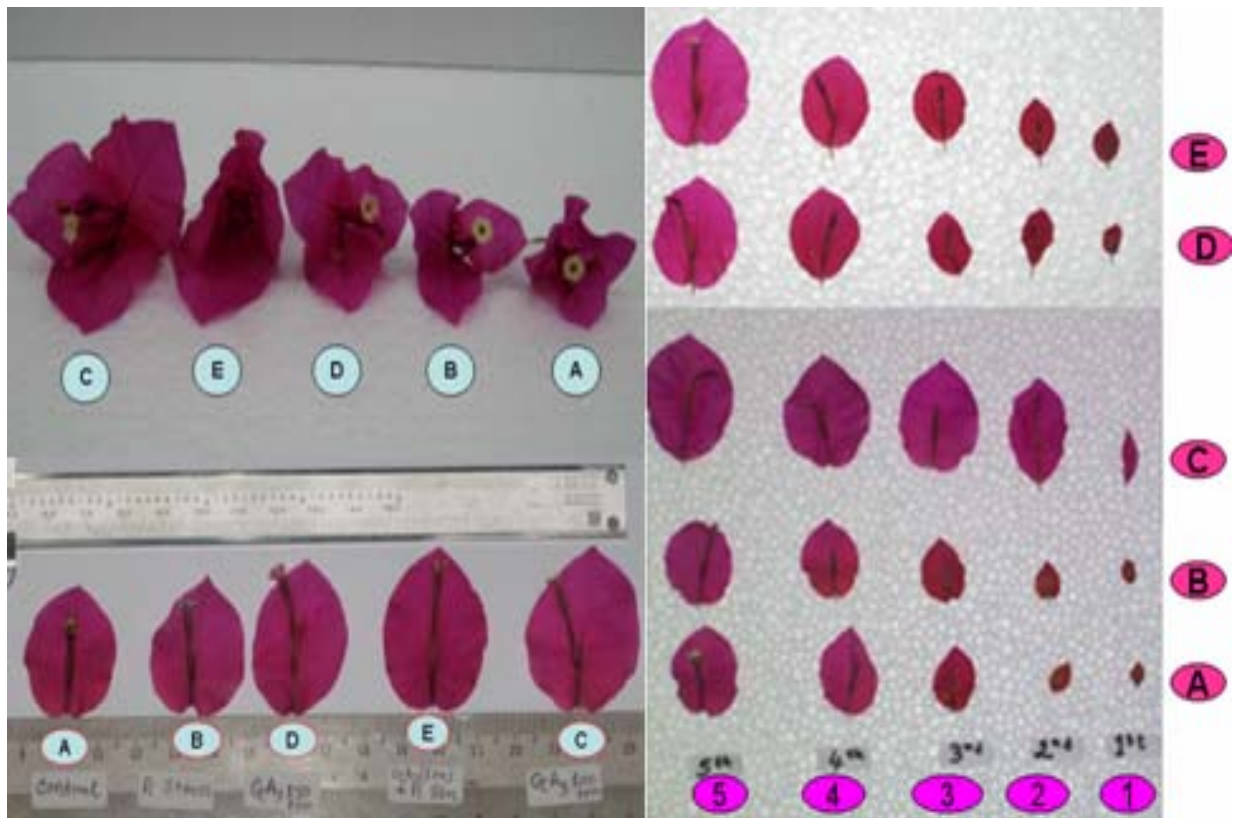


Figure 5.7: Photo graphs show the petal architecture and size at different stages. Where, 1: 1st, 2: 2nd, 3: 3rd, 4: 4th and 5: 5th stage. A: Water control, B: Phloemic stress, C: GA₃ (100 ppm), D: GA₃ (150 ppm), E: GA₃ (100 ppm) + phloemic stress.

5.4 General Discussion

In this study, it was found that bract number per branch, bract size and weights were greater in 100 ppm GA₃ + phloemic stress, 100 ppm GA₃ and phloemic stress compared to the control. It might be due to the elongation of bracts induced by the treatments. GA₃ showed a positive effect on bract size and expansion (Fig. 5.7), however, it did not affect on bract longevity and this is the general concept that larger bracts last short life. The bract expansion might be due to carbohydrate availability from the leaves to bract. However, bract longevity was prolonged by phloemic stress, but, both the treatments 100 ppm and 150 ppm GA₃ reduced bract longevity. When 100 ppm GA₃ + phloemic stress was applied, higher bract longevity was observed as compared with individual doses of 100 ppm GA₃ and 150 ppm GA₃. The 100 ppm GA₃ treated branch of bougainvillea exhibited three days earlier full blooming (5th) compared to control (Table 5.1). This is because of the well known effect of GA₃ on early cell development (Hay and William, 2008). The GA₃ bolting effects were well known and have been commercially exploited for agri- and horti-cultural productivity (Sharma and Room, 2009; Ogale *et al.*, 2000). Hay and William (2009) reported that GA₄₊₇ had been used to increase total shoot number, flowering shoot number, and also to increase flower longevity.

In the present work, the number of bract per branch was higher in 100 ppm GA₃ + phloemic stress and phloemic stress than in control branch (Fig. 5.2). This might be due to the deposition of sufficient carbohydrates and nutrients at the upper side of the phloemic stress region or better quantum efficiency of leaves (Mataa, *et al.*, 1998). Arakawa *et al.* (1997) also found that flowering of Fuji apple was significantly increased by phloemic stress (girdling). Hossain and Fusao (2008) found that phloemic stress increased the flower

bud compared to the control. 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. Bract number was higher in phloemic stress and 100 ppm GA₃ + phloemic stress branches than the other treatments. Previous 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, 2001). 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 internal sugar movement.

The ethylene production rate might be decreased by these concentrated carbohydrates and nutrients. Whereas, in the controlled branch, the total sugar quantity produced by leaves was uniform at all locations and it moved towards roots through phloem. The quantum yield of PSII for this cultivar declined in a linear pattern with increasing GA₃ concentration. The quantum efficiency of PSII measured with the dark adapted test (Fv/Fm) has been used widely as an indicator of hormonal stress and as a screening method for resistance in plants (Bibi *et al.*, 2008); however, measuring quantum yield of photosynthesis with the light-adapted test in our studies proved the negative effect of high concentration on plant photosynthesis process. In addition, plant senescence induced by nutrient deficit or less induction of photosystem apparatus of leaves (Yang and Zhang, 2005). GA₃ supplied or contain enough nutrients though, it showed early senescence (Table 5.1). In spite of this availability of nutrients, the limited utilization of photosystem, CO₂ fixation or lower chlorophyll content (Fig. 5.6) in leaves, could result in faster senescence in GA₃ treated branches bract.