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Research Article
Bougainvillea Bract, Chlorophyll Fluorescence, Anthocyanin and Antioxidant Development as Affected by AOA, Sucrose and Phloem Cut

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Abstract

Background and Objective: *Bougainvillea* is an evergreen plant and blooms all the year round. *Bougainvillea* has decorated value in office, home, yard and medicinal values as the treatment of cough. The flowers and stems are dried, boil in water and drink as tea. The study was carried out to investigate the effects of amino oxyacetic acid (AOA), sucrose and phloem tissue cut stress, on the bract size, pigments, antioxidant, flavonoid and physico-biochemical parameters of *Bougainvillea* bract. **Materials and Methods:** The treatments were of the phloem cut stress in the bark of one year shoot, 100 ppm AOA and 3% sucrose concentration and water control employing swabbing technique. **Results:** The floral bract number was higher in the 15th week in phloem cut stress and 3% sucrose treated bract than in water control and AOA treated bract. Moreover, the size of the bract, weight and longevity were the highest in the physiological stress treated bract. However, it had been shown that the Fo, Fm, Fv and quantum or photo-synthetic yield (Fv/Fm) were found higher in phloem cut stress and 3% sucrose treated bract than in other two treatments. The highest chlorophyll (a and b) and carotene content was recorded in the phloem cut stress treated bract. The anthocyanin, total phenol, total flavonoid and DPPH radical scavenging activity increased in all treated bract compared to the control bract. However, the highest anti-oxidant was found in the phloem cut stress treated bract. **Conclusion:** Finally it seemed that phloem cut stress and 3% sucrose were the effective treatment compared to the AOA and water control.

Key words: Phloem cut, AOA, *Bougainvillea*, sucrose, chlorophyll, anti-oxidant, flavonoid

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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

*Bougainvillea*s are popular ornamental plants and used as official flowers in most areas with warm climates, including Australia, India, Malaysia, the Mediterranean region, Mexico, South Africa, Taiwan and the United States in Arizona, California, Florida, Hawaii and southern Texas[1,2]. The genus *Bougainvillea*, in the Nyctaginacea (Four-o'clock) family of plants have 14 species, with three that are horticulturally important, *B. spectabilis* Willd now, *B. glabra* Choisy and *B. peruviana* Humboldt and Bonpland[3]. It had been reported that *B. Spectabilis* Willd and *B. glabra* choisy were most commonly used in horticulture[4].

Plant development is the process of plant changing from one growth stage to another[5]. Plant growth and development involve the integration of many environmental and endogenous signals that together with the intrinsic genetic program, determine plant form. Fundamental to this process are several growth regulators collectively called the plant hormones or phytohormones. This group includes auxin, cytokinin, the gibberellins (GAs), abscisic acid (ABA), ethylene, the brassinosteroids (BRs) and jasmonic acid (JA), each of which acts at low concentrations to regulate many aspects of plant growth and development[6]. Plants that have flowers are called angiosperms.

Plant hormone has a significant role in the growth, development, metabolism and morphogenesis of plants[7]. However, gibberellins are well known plant growth hormones and Gibberellic acid (GA_3) induced the flower and fruit color and size[8,9]. It had been reported that Gibberellic acid and other plant growth promoters accelerated the flowering and increased the number of flower buds and opened flowers earlier[10]. The senescence of *Bougainvillea* flower was delayed and vase life was longer in AOA 50 ppm and 4% sucrose concentration when compared to the control[9]. Moreover, they also reported that the application of gibberellic acid (GA_3) has the potential to control growth, flowering and induce early flowering. It had been suggested that flower bud percent was a higher when phloem stress (phloem cut) was applied on the flower[11,12]. It was reported that starch content was higher in bark cutting than in control. 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 phloem stress. The objectives of this experiment were to improve the longevity and size of the flower also to investigate the pigments and anti-oxidant of the flower as medicinal value using different treatments.

MATERIALS AND METHODS

Field work: The experiment was conducted in an orchard at the Institute of Biological Science, University of Malaya, Malaysia.

Plant material: Five years old trees were used in this experiment located in the University Malaya Orchard, Kuala Lumpur. The experiment was conducted with completely randomized design using 16 trees. There were 4 replicates were taken for each treatment and the 9 branches were used in the experiment. Total of $4 \times 4 = 16$ trees were used.

Treatments: Phloem cut stress, 100 ppm amino oxyacetic acid (Sigma) and 3% of Sucrose (Sigma) were used as treatments in the experiment. The 100 ppm amino oxyacetic acid and 3% of sucrose were applied at the bud initiation stage. Distilled water was used at 3 days interval for the control plant. For the phloem cut stress, a partial ring was made by removing a 1 cm long strip of bark using the knife, leaving a connecting band of bark 1.0 mm wide as a latest innovative technique (1.0 cm × 1.0 mm) (Fig. 1).

Methods: The trees were directed to sunlight and supplied water at 3 days interval. Then, 3 branches were chosen for each tree. Nine bracts were selected from each tree. The treatments were applied for every 3 days using swabbing technique. Then, the measurement of the flower buds, the size of flower bracts and the flower bract initiation day were taken at the same day when the treatments were applied.

Data collection: Number of flower, flower diameter and length and flower weight were measured.

Parameter: Chlorophyll a and b and carotene determination The chlorophyll and carotene contents of the leaves and fruit were determined using the methods described by Hendry and Price[13].

Total phenol determination: The total phenolic contents (TPC) of wax apple fruits were determined with the Folin-Ciocalteu assay as described by Singleton and Rossi[14].

Anthocyanin determination: The total anthocyanin and carotenoid contents of the hydrophilic extracts were measured using the pH-differential method with cyanidin-3-glucoside used as a standard as described by Rodriguez-Saona *et al*.[15].

(d)

Fig. 3(a-d): Number of flower produced at different treatment, (a) Control, (b) 3% sucrose, (c) Phloemic stress and (d) 100 ppm AOA

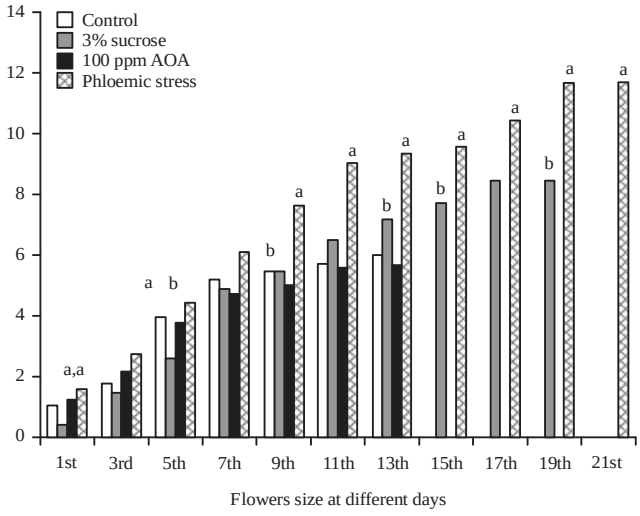

Fig. 4: Bracts size of *Bougainvillea glabra* treated with distilled water (control), 3% of sucrose solution (SIGMA), 100 ppm amino oxyacetic acids (SIGMA) and partial ring (phloemic stress)
Different letters (a, b) showed difference at 5% level of significant by least significant difference (LSD) test

(a) (b) (c) (d)

Fig. 5(a-d): Flower bract size at different treatments, (a) Control, (b) 3% sucrose, (c) Phloemic stress and (d) 100 ppm treated flower

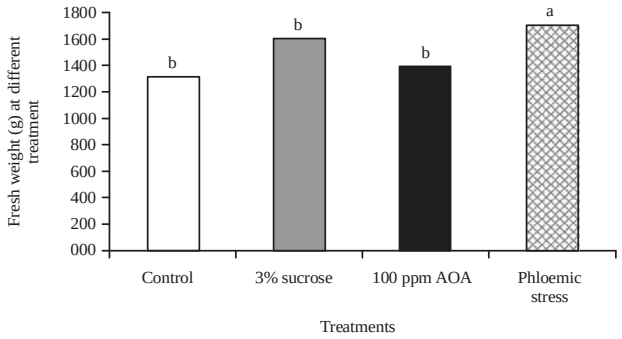

Fig. 6: Fresh weight of the *Bougainville glabra* flowers treated with distilled water (control), 3% of sucrose solution (SIGMA), 100 ppm amino oxyacetic acids (SIGMA) and partial ring (phloem stress)
Different letters (a, b) showed difference at 5% level of significant by least significant difference (LSD) test

Table 1: Stage of flower longevity at different treatments like distilled water (control), 3% sucrose, phloemic stress (partial ringing) and 100 ppm AOA

		Stages			
Treatments	Initiation day	2 day (budding stage)	3 day (Blooming started)	4 day (Full blooming)	5 day (Wilting or abscission)
Control	4+0.0	5+0.0	7+0.1	8+0.0	10+0.12
3% sucrose	3+0.2	6+0.3	9+0.5	13+0.8	16+0.90
Phloemic stress	2+0.1	3+0.1	6+0.1	13+0.2	18+0.10
100 ppm amino oxyacetic acid	2+0.1	3+0.2	9+0.5	11+0.6	12+0.70

Mean+SE (n = 4)

Table 2: Chlorophyll fluorescence yield measurement

Treatments	Fo (Lower fluorescence)	Fm (Higher fluorescence)	Fv (Variable fluorescence)	Fv/Fm (Photo-synthetic or quantum yield)
Control	356[b]	1134[c]	778[d]	0.68[b]
3% sucrose	481[a]	1850[a]	1369[b]	0.74[a]
Phloem stress	474[a]	1920[a]	1446[a]	0.75[a]
100 ppm amino oxyacetic acid	455[b]	1565[b]	1110[c]	0.70[b]

Different letters (a, b) showed difference at 5% level of significant by least significant difference (LSD) test

Table 3: Chlorophyll a, b and carotene content determination

Treatments	Chlorophyll a (μg g^{-1} fw)	Chlorophyll b (μg g^{-1} fw)	Carotene (μg g^{-1} fw)
Distilled water (Control)	1.54[b]	1.6[b]	0.80[b]
3% sucrose	1.72[a]	1.9[a]	1.40[a]
Phloem stress	1.74[a]	2.1[a]	1.50[a]
100 ppm amino oxyacetic acid	1.60[b]	1.83[a]	1.37[a]

Different letters (a, b) showed difference at 5% level of significant by least significant difference (LSD) test

(stage 1), budding stage (stage 2), blooming (stage 3), full blooming (stage 4) and wilting or abscission (stage 5).

From the budding stage to fully blooming stage, the 3% sucrose and phloem cut stress (ringing) showed the high longevity (Table 1). The last stage of longevity of the flower was the wilting or abscission stage. In this stage, the flower dropped and caused senescence. Compared to all treatments, the phloem cut stress exhibited the highest longevity (18) followed by 3% sucrose (16), 100 ppm AOA (12) and control (10) (Table 1). In Table 2, it has been shown that higher and variable chlorophyll fluorescences were found maximum in phloem stress followed by 3% sucrose, AOA and control. The highest quantum or photo-synthetic yield was found in the phloem cut stress. Figure 7 showed the strong and ascending correlation of anthocyanin content and total anti-oxidant where $R^2 = 0.981$.

Biochemical, pigments and phytochemical analysis

Chlorophyll and carotenoid content: Chlorophyll a, b and carotenoid were found higher in phloem cut stress, 3% sucrose and AOA treated flower than the control (Table 3).

Anthocyanin content: As shown in Table 4, the application of phloem stress, 3% sucrose and AOA 100 ppm had significant effects on the anthocyanin in the bract compared to the control. For the phloem stress treatment, the highest amount of anthocyanin was observed.

Table 4: Anthocyanin, total phenol, flavonoid and anti-oxidant determination

Treatments	Anthocyanin (mg/100 g)	Total phenol (mg GAE/100 g)	Total flavonoid (mg/100 g)	Total anti-oxidant (DPPH) (mg/100 g)
Distilled water (control)	2.1±0.20	345.2±4.5	11.56±0.8	12.4±0.23
3% sucrose	3.8±0.30	423.0±4.5	13.80±0.6	14.9±0.15
Phloem stress	4.1±0.31	403.0±5.1	14.90±0.7	15.7±0.25
100 ppm amino oxyacetic acid	3.2±0.25	389.0±4.3	12.50±0.7	14.2±0.30

Mean+SE (n = 4)

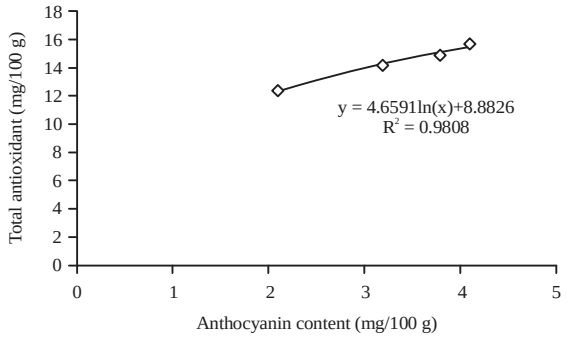

Fig. 7: Correlation between the anthocyanin content and total anti-oxidant (DPPH)

Total phenol and flavonoid content: The total phenol and total flavonoid content were increased using phloem stress followed by 3% sucrose and AOA 100 ppm (Table 4). The 3% sucrose showed the highest total phenol content.

Total anti-oxidant content: The DPPH radical scavenging activity measured and showed in Table 4. The results showed that the DPPH radical scavenging activity was increased in the phloem stress followed by 3% sucrose and AOA 100 ppm. The higher anti-oxidant capacity was observed in phloem stress and 3% sucrose compared to the AOA and control. Control bract showed the least antioxidant capacity.

DISCUSSION

From the results it has been cleared that the longevity of *Bougainvillea* flowers that studied in the field were increased by sucrose and phloem stress and amino oxyacetic acid (AOA) 100 ppm. Among the different types of sugars, sucrose has been found to be the most commonly used sugar in prolonging the vase life of cut flowers[17]. Phloem stress (ringing) gave the best results because as reported by Fishier *et al.*[18], the bark ringing resulted in accumulated carbohydrates above the bark ring. Therefore, it contains lot of food to increase the longevity of the flowers. However, for the control the petal fall was accelerated when there is no preservatives were added to the holding solution[19]. Previous studies were done on peach trees[11]. The result showed that partial ring (phloem stress) caused the longevity and high number of flowers. This is because of the ringing often created an accumulation of carbohydrates above the ring[18,20]. It had been reported that non-structural carbohydrate in stems steadily decreased in trees that were not subjected to bark ringing. However, when bark ringing was performed, stem carbohydrates steadily increased. The increasing carbohydrate concentration caused increasing in flower longevity and also the increased the number of flowers compared to the other treatment. This study was same as Arakawa *et al.*[21] that flowering in the apple trees was significantly increased by ringing. Other previous study showed that the percentage of flower buds was higher in partial ringed trees than control trees. This might be due to deposited sufficient carbohydrates and nutrients in above ringing[11]. Bark ringing was apparently effective in blocking phloem translocation as revealed by the low carbohydrate levels in roots of plants with bark ringin[21]. Previous study proved that ringing tends to increase the size of fruit[22]. It also showed in the study where the bract size and the flower weight were high when the bark ringing was applied.

In this study, the result showed that number of flower that treated with 3% sucrose also showed the highest number of flowers and increased the pigments. Based on Leon and Sheen[23], it showed that sucrose apparently inhibited the ethylene signal pathway through the production of low ABA concentration. Some studies had also shown that the sensitivity of flowers to ethylene was significantly reduced treated with exogenous sugars. It is due to the sucrose supplies the energy and carbon required for bud opening. For flower opening, large amount of soluble carbohydrates is required as the substrate for respiration and synthetic materials as well as osmolytes. Thus, treatment with sucrose was considered to satisfy the supply of such soluble Carbohydrates[24]. The availability of soluble carbohydrate for that purpose was probably partly responsible for the improved opening of the flowers in preservative solution[25]. Exogenous application of sucrose supplied the flower with much needed substrates for respiration and not only prolongs the vase life[26]. Exogenous supply of sugars delayed wilting in many flowers and this effect was due to maintenance in starch and sugar levels in cut flowers[27]. Sucrose also showed the high weight of flowers. It is suggested that sucrose induced the

closure of stomata, eventually reduced the loss of water in gladiolus leaves[28] or rose petals thereby reduced the transpiration and maintained the fresh weight[29].

There were many researchers showed that AOA did prolong the longevity of the flowers such as *Dendrobium* (Heang Beuty) flower[29] and Dendrobium Pompadour[30]. They also reported that the solutions containing AOA analogs offered improved vase life over the controls, even at low (0.01 and 0.08 ppm) ethylene levels. As reported in the previous study AOA treated flowers would had been expected to last longer than controls because it inhibited ethylene synthesis[31,32]. Moreover, the highest chlorophyll, carotenoid, anti-oxidant, flavonoid, phenol and anthocyanin were found in the phloem stress treated flower bract. It might be due to the bract development having increased more nutritive value and pigments.

CONCLUSION

It can be concluded that the size of the bract, longevity, chlorophyll and photo-synthetic yield were found higher in phloem cut stress and 3% sucrose treated bract than in water control and AOA treated bract. The anthocyanin, total phenol, total flavonoid and DPPH radical scavenging activity increased in all treated bracts. In addition, the highest anti-oxidant was found in the phloem stress treated bracts. Finally it has been exhibited that phloem stress and 3% sucrose were the more effective treatment compared to the AOA and water control.

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