

CHAPTER 3

Pruning impacts on root-shoot growth and development, biochemical and physiological changes of *Bougainvillea glabra*

3.1 Introduction

Pruning is a useful management technique for a variety of ornamental plants under natural exposed sunlight or greenhouse condition (Sarkka and Erikson, 2003). Generally the purpose of pruning of a plant is to observe and control the plant growth and development, to facilitate growing operations, or for commercial reason, i.e. timing fluctuations in demand for flowers and fruits during different seasons or days or all year round. Many investigators have emphasized the importance of pruning on flowering and its effects on the subsequent fruit growth and quantity as well as its quality (Calatayud *et al.*, 2007). But in the ornamental flowering plants, pruning usually focused on the frequent flowering by creating or increasing the availability of metabolic sinks. Meanwhile, for the non-pruned plants, the expansion of new and older shoots may cause a temporary depletion of stored metabolites from older shoots, causing a decrease in flower and yield production (Zieslin and Mor, 1981). After a prolonged growth period, the plant can recover its reserves, partially or completely, and restore their floral production. The degree of plant recovery would depend on pruning-position, pruning-height and the period of time the plants are kept from flowering (Zieslin and Mor, 1981; Li *et al.*, 2003). Therefore, the base of hedges is suggested to be wider than the top, so that lower branches will get enough light to utilize its leaves as a potential part. In addition, most ornamental plants are pruned to preserve their natural shape as much as possible. This is accomplished, in some cases, by

removing old branches of shrubs at ground level; by cutting back some of the branches, by removing dead, diseased, weak, broken and other wood; and by thinning out areas of the plant that become over-crowded. These methods are more difficult and time consuming than shearing, but the results improve flowering, natural beauty, and generally plant health (Calatayud *et al.*, 2008; Calatayud *et al.*, 2007).

The movement of nutrients and their accumulation in different parts of plants are affected by environmental conditions and pruning treatments. By means of shoot pruning, light penetration and distribution within the canopy are improved (Admasu and Struikb, 2000). Pruning of the bent shoots in plants can modify the light interception in internal leaves and change their photosynthetic reactions (Stitt *et al.*, 1990). Before pruning, internal leaves were acclimated to low light intensity and after pruning; internal and external leaves received the same light intensity (Angeles *et al.*, 2008). Other effects on some physiological activities of a plant may be influenced by the age of leaf, leaf position within the canopy and leaf position in different directions of branches (Nabi *et al.*, 2000). Hossain and Fusao (2008) reported that flower bud, sugar content and N, P, K content in leaf were higher in pruned trees than unpruned peach trees.

Pruning generally increased stem potential, induced canopy transpiration and improved water status. Therefore, even under dry condition a potential effect of pruning on fruit growth was observed. However, in wet and cloudy years the status may not be limited and carbohydrate supply and demand effects may be more dominant (Lin and Hsu, 2004; Manuel *et al.*, 1998). Higher stem water potential was reported to be beneficial to increase flower number, fruit growth and quality (Roberto and Jonathan, 2000). In addition, leaf photosynthetic rate and water status has been shown to vary with potassium status in several herbaceous crop species (Bednarz and Oosterhuis, 1999) and woody ornamental species (Egilla and Davies, 1995). Potassium deficiency can reduce net CO₂ assimilation

rate, increase leaf respiration and control photosynthetic efficiency at the stomatal and/or biochemical levels (Onguso *et al.*, 1994).

Thus, this experiment was aimed to study the effects of differing pruning on 1) the new shoot and root initiation, 2) the flowering process under exposed sunlight condition and 3) the content of chlorophyll a & b, quantum yield, biomass, sugar and potassium.

3.2 Materials and Methods

3.2.1 Experimental Site & Plant Materials

The experiment was carried out in the Plant Physiology Garden, Institute of Biological Sciences, University of Malaya, Kuala Lumpur, Malaysia. In this experiment, two-year-old (total of plants 12) bougainvillea plants were grown in a small polythene bag having 12 cm in diameter and 20 cm in length. The bags were placed under prevailing conditions (temperature 21-32°C, maximum PAR 2100 $\mu\text{E m}^{-2} \text{s}^{-1}$ and relative humidity of 60-90%). The plants were irrigated twice a day, to avoid water stress condition. The average height of each plant was 50 cm and having 50 cm row to row distance and 50 cm plant to plant distance. The plant consist (control) of eight primary branches and five secondary branches. The nutrient, N: P: K (12:12:17), at the rate of 5 gm per plant was applied at fifteen days interval.

3.2.2 Pruning Treatments

Effects of different pruning treatments were analyzed following a completely randomized design, having three replications per treatment. The experiment was started on the 28th August, 2008. There were four treatments. The control plant was not pruned within six months. Partial pruning was done by pruning all branches except one branch. Complete pruning was performed by completely cutting all primary and secondary branches. Frequent pruning was carried out by pruning all branches, 4 cm from shoot apex, at 30 days interval throughout the experiment (Fig. 3.1).

3.2.3 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 minutes 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 seconds 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 μ s- 3 sec).

3.2.4 Relative Water Content (RWC)

The Weatherly and Slatyer (1962) method was used. Three youngest fully expanded leaves per plant were taken randomly from each treatment. The leaves were weighed immediately to determine the fresh weight (FW). They were then floated in

distilled water (in petri dishes) under a fluorescent lamp supplying compensation light intensity for 12 hours. Next, the leaves were blotted gently with tissue paper and weighed to determine the turgid weight (TW). The leaves were then oven dried (80°C) to constant dry weight (DW). The relative water content (RWC) was calculated by $RWC = [(FW - DW) / (TW - DW)] \times 100$.



Figure 3.1: Different types of pruning have been executed at the beginning phase which has shown above; Non-pruning or Control (T1); Partial pruning (T2); Complete pruning (T3); and Frequent pruning (T4)

3.2.5 Photosynthetic Pigments

The photosynthetic pigments chlorophyll *a*, chlorophyll *b* and Total chlorophyll were estimated after homogenizing 0.5 gm of the fresh leaf sample in 80 percent ethanol. The absorbance was measured at 663 and 645 nm and the chlorophyll content was measured using the following formula (Asare - Boamah *et al*, 1986).

$$\text{Chl } a \text{ (mg g}^{-1}\text{)} = [(12.7 \times A_{663}) - (2.6 \times A_{645})] \times V \text{ mg/ gm Fresh Weight}$$

$$\text{Chl } b \text{ (mg g}^{-1}\text{)} = [(22.9 \times A_{645}) - (4.68 \times A_{663})] \times V \text{ mg / gm Fresh Weight}$$

3.2.6 Estimation of Total Soluble Sugars

In order to estimate the total soluble sugars (Ts), one gm of leaf was homogenized in 4 ml of 0.5 N of sodium hydroxide and grind in a mortar with a pestle and then centrifuged at 3,500 g for 20 min at 4°C. The pellet was discarded and the supernatant was neutralized with 0.5 N Acetic acid. The resulting solution was made up to 40 ml and was stored at 4°C till use. These extracts were then used in the estimation of total soluble sugars according to the Phenol-Sulphuric method by Dubois *et al.* (1956). One ml of the leaf extract was placed in a test tube and 1ml of Phenol [5% w/v] was added followed by 5 ml of concentrated sulphuric acid and the contents were mixed and left at room temperature for 10 minutes. Spectrophotometers reading were taken at 490 nm absorbance. The sugar concentration was obtained by referring to the standard glucose graph. The assay for this standard glucose graph was carried out by adding phenol and sulphuric acid to a standard glucose solution with concentrations varying between 0-100 µg/ml. Total soluble sugars was expressed in mg/100gm leaf fresh weight.

3.2.7 Stomatal Conductance

Stomatal conductance was measured using a portable porometer (Leaf Porometer, Model SC-1, USA). A leaf clamber was attached to one of the leaf and kept in ambient temperature for 10-15 min to maintain sunlight adaptation. A stomatal conductance was measured in 3 replicates from different spots of a single leaf.

8.2.8 Potassium Estimation

The most recent fully expanded same age and relative position on the plant leaves were taken from each treatment. For potassium estimation, one gram leaf was homogenized in 5 ml distilled water in a mortar with a pestle and then centrifuged at 3,500 rpm for 20 min. The 3 to 5 drops of the supernatant liquid were transpired onto the calibrated sensor pad (Cardy Potassium Meter, Model-2400, USA). A sampling paper was also placed on the sensor and saturated with the liquid. After the value has stabilized (30 to 45 seconds), reading (ppm) was taken from the display pad.

3.2.9 Bract Weight and Length and Plant Biomass Measurements

The weight of bract recorded at different (1st and 3rd) flower blooming cycles (FBC) and biomass was measured at the end of experiment using the weighing machine (Model-Mettler PJ3000, Japan) and bract length was measured by Vernier scale. Branch initiation was calculated by following formula:

$$\text{Branch initiation (\%)} = \frac{\text{Final branch number} - \text{Initial branch number}}{\text{Final branch number}} \times 100$$

3.2.10 Statistical Analysis

Statistical analysis was performed using SPSS software. 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.

3.3 Results

The results showed that bract length, weight and numbers/plant were significantly decreased by complete pruning in the 3rd flower blooming cycle (Table 3.1). Bract length and weight was almost similar in all treatments in 1st flower blooming cycle. In the 3rd flower blooming cycle, the highest bract length and weight was observed in the frequent pruning as compared with control plants. It was clear that frequent pruning had a positive effect on bract length and weight compared to non-pruning and other types of pruning. While complete pruning reduced the bract number/plant in both first and final seasons by 40% and 35%, respectively.

The influence of all treatments on branch initiation was observed throughout the experiments. All strategies were able to initiate primary and secondary branch with respect to experimental periods. The most effective strategies to increase 100% secondary branches/plant were partial and complete pruning (Fig. 3.2). In the case of secondary branch, lower branch initiation (38%) was observed in frequent pruning. However, the most effective strategy for increasing primary and secondary branch/plant was in the partial pruning. In this experiment, tertiary branch initiation was observed only in non-pruning

treatment. In contrast, the maximum branch width was observed in partial pruning and the minimum was observed in complete pruning plants (Table 3.2).

In addition, biomass measurements obtained at the end of the experiment. The influence of different styles of pruning on biomass was easily observed here and discussed in pruning studies. There has been little effort to explain biomass responses to pruning on the basis of tree physiology. The results of above treatments showed that partial pruning has a great influence to increase on fresh weight and dry weight of branches. The fresh weight of complete pruned plants decreased by 60% as a result of the lower branches present, smaller size and lower width of the plant canopy. Another related cause was that complete pruning stimulated less vegetative growth. The new branch initiation was particularly delayed and decreased by complete pruning. It might be due to the sudden shock to the plant and that is way, it might take time to initiate new branches (Table 3.2).

The difference in plant biomass among the different types of pruned and non-pruned treatments was evaluated (Table 3.3). It was perceptible that partial pruning improved root length as well as root weight than complete and frequent pruning. Root growth depends on the strategy of shoot distribution or pruning tactics or cutting position. Therefore, different type of pruning had shown different root length, though little similar results were observed between non-pruned and partial pruning. The highest reduction in root weight by 61% as a result of complete pruning may be attributed to lower root length or less vegetative growth (Table 3.4). These findings led to the proposal of root growth based on the remaining shoots or vegetative growth. The photosystem of dark-adapted leaves, measured by quantum yield showed lower values in non-pruned plants. Whereas, significant higher values of quantum yield were observed in all types of leaves in pruned plants (Fig. 3.3). In frequent pruning, the quantum value increased by 4% in leaves of flower shoots. Among the pruned plants, the lowest values were obtained in complete pruning plant leaves.

Table 3.1: Measurement of ornamental characteristics (bract length, weight and bract number) as affected by different type of pruning. Values are means of 3 measurements \pm SE. Values followed by different alphabets indicate the existence of significant differences according to LSD $_{0.05}$ test. ¹Non-pruning or Control (T1); Partial pruning (T2); Complete pruning (T3); and Frequent pruning (T4)

Treatments ¹	Bract Length (mm)		Bract weight (gm)		Bract number/ plant	
	1 st FBC*	3 rd FBC	1 st FBC	3 rd FBC	1 st FBC	3 rd FBC
T1	44.33 \pm 0.42	41.6 \pm 0.32d	0.53 \pm .006	0.370 \pm .02d	44.66 \pm 2ab	34.66 \pm 1.4b
T2	44.66 \pm 0.24	43.1 \pm 0.26ab	0.53 \pm .006	0.438 \pm .02b	36.66 \pm 2bc	33.3 \pm 2.7bc
T3	44.83 \pm 0.14	42.7 \pm 0.37bc	0.53 \pm .007	0.403 \pm .01bc	26.6 \pm 2.3d	22.66 \pm 1.7d
T4	44.53 \pm 0.35	44.4 \pm 0.52a	0.53 \pm .007	0.530 \pm .01a	53.0 \pm 2.8a	50.33 \pm 1.4a

*Flower Blooming Cycle (FBC)

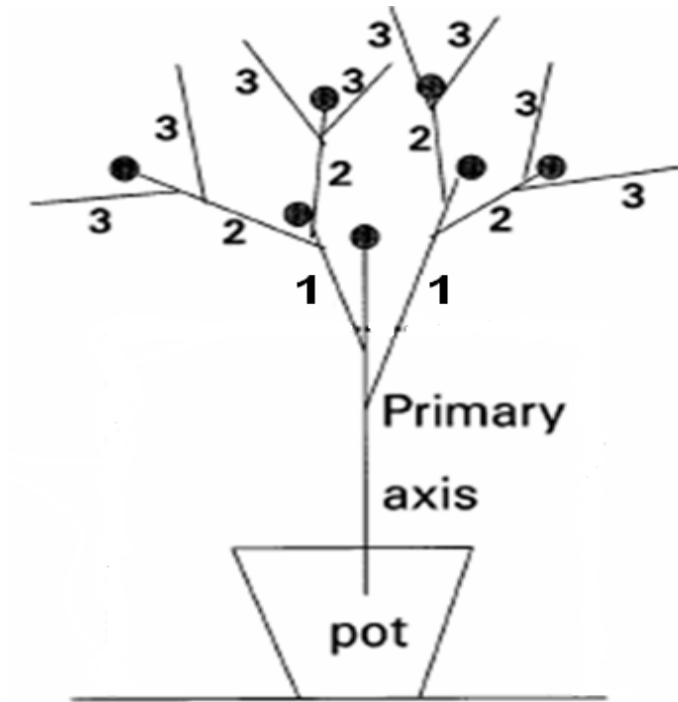


Figure 3.2: Bougainvillea plant structure containing different types of branches. Number 1, 2 and 3 represents the primary, secondary and tertiary branch, respectively, at the end of experiments.

Table 3.2: Different types of branch initiation were affected by pruning. Values are means of 3 measurements \pm SE. Values followed by different alphabets indicate the existence of significant differences according to LSD $_{0.05}$ test. *Non-pruning or Control (T1); Partial pruning (T2); Complete pruning (T3); and Frequent pruning (T4)

Treatment*	Primary Branch Initiation	Secondary Branch Initiation	Tertiary Branch Initiation	Branch width
T1	52%	80%	100%	45.9 \pm 1.30b
T2	91%	100%	0%	55.32 \pm 1.80a
T3	100%	100%	0%	28.84 \pm 0.90c
T4	38%	75%	0%	23 \pm 60 \pm 0.50d

Table 3.3: Biomass measurement (of branches) at the end of experiment. Values are means of 3 measurements \pm SE. Values followed by different alphabets indicate the existence of significant differences according to LSD $_{0.05}$ test. *Non-pruning or Control (T1); Partial pruning (T2); Complete pruning (T3); and Frequent pruning (T4)

Treatments*	Total Branch Fresh Weight (gm)	Total Branch Dry Weight (gm)	Ratio (Fw/Dw)
T1	36.54 \pm 1.20a	21.45 \pm 0.90a	1.70 \pm 0.03
T2	34.65 \pm 1.05ab	18.84 \pm 0.34b	1.84 \pm 0.08
T3	14.43 \pm 1.03d	7.96 \pm 0.46d	1.90 \pm 0.24
T4	22.35 \pm 1.20c	11.72 \pm 0.37c	1.91 \pm 0.13

Table 3.4: Measurement of root biomass at the end of experiment. Values are means of 3 measurements \pm SE. Values followed by different alphabets indicate the existence of significant differences according to LSD $_{0.05}$ test. *Non-pruning or Control (T1); Partial pruning (T2); Complete pruning (T3); and Frequent pruning (T4)

Treatments*	Root Fresh Weight (gm)	Root Dry Weight (gm)	Fw/Dw	Root Length (cm)
T1	7.68 \pm 0.53a	4.63 \pm 0.37a	1.67 \pm 0.19	20.27 \pm 0.74a
T2	6.83 \pm 0.37ab	4.16 \pm 0.28ab	1.66 \pm 0.21	19.86 \pm 0.79ab
T3	2.93 \pm 0.40cd	1.86 \pm 0.20d	1.56 \pm 0.06	13.21 \pm 0.38cd
T4	3.29 \pm 0.35c	2.44 \pm 0.17c	1.30 \pm 0.13	16.57 \pm 0.56c

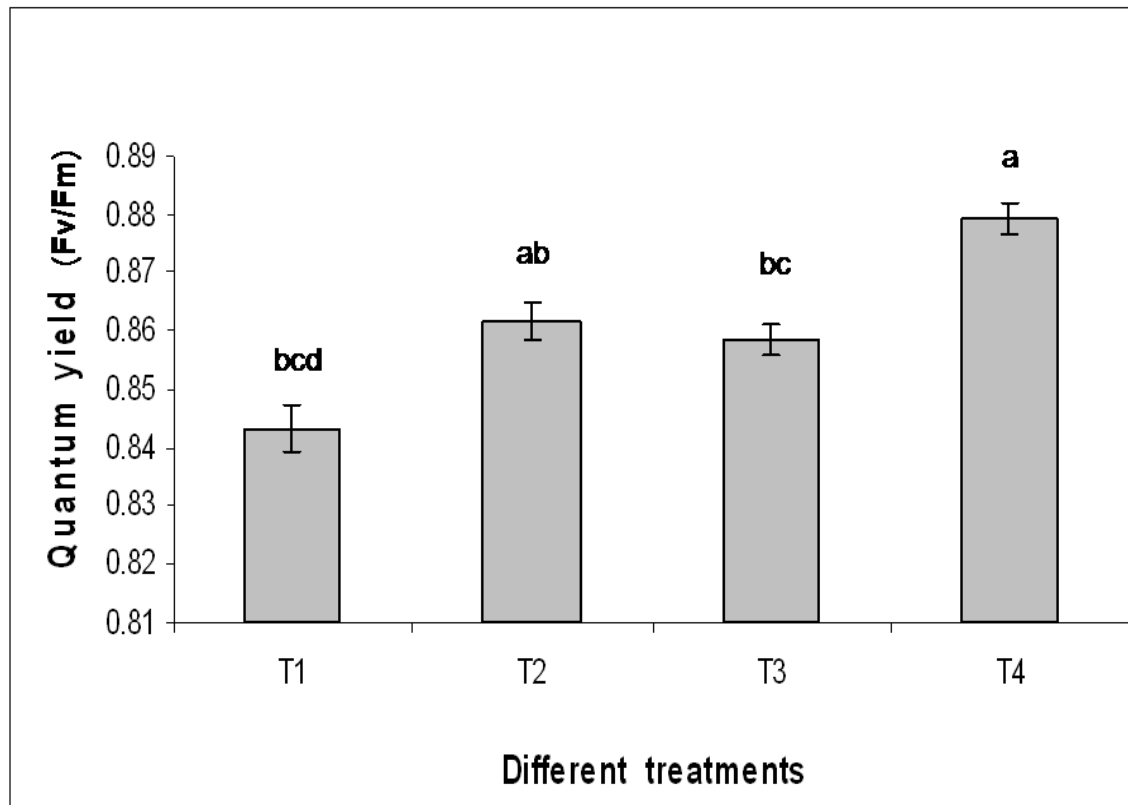


Figure 3.3: Determination of quantum yield in all type of pruning plants leaf including control leaves. Values are means of 3 measurements \pm SE. Values followed by different alphabets indicate the existence of significant differences according to LSD $_{0.05}$ test.

With regard to stomatal conductance, it was found that the initial conductance value was higher in control treatment compared to all pruning treatments (Fig. 3.4). Amongst the pruning treatments, frequent pruning leaves exhibited the highest value of stomatal conductance. With passing months, the stomatal conductance was gradually increased except those leaves of the control or non-pruned. The difference in stomatal conductance between complete and partial pruning was probably related to the fact that complete pruning caused a reduction in stomata frequency, thus to maintain the physiological coherence or leaf age or due to slow growth rate. That means in a vegetative stage, leaf exhibited low stomatal conductance. Nabi *et al.* (2000) showed that the stomatal conductance was closely related to leaf age and position. With the increase of leaf age, the stomatal conductance improved up to a certain value which differed from plant species to species. In case of complete and partial pruning, stomatal conductance increased rapidly beyond the second month of observation.

The decline in relative water content (RWC) of complete pruning application was due to less plant growth and less capability to maintain photosynthesis process or less nutrient supply through root profile. The effect of partial pruning on relative water content (RWC) observed little higher than complete pruning. RWC improved by frequent pruning by 15% (Fig. 3.5). Therefore, higher quantum yield and higher flower number/branch was observed in this pruning and similar result was proved on carambola (*Averrhoa carambola L.*) trees by Roberto and Jonathan (2000).

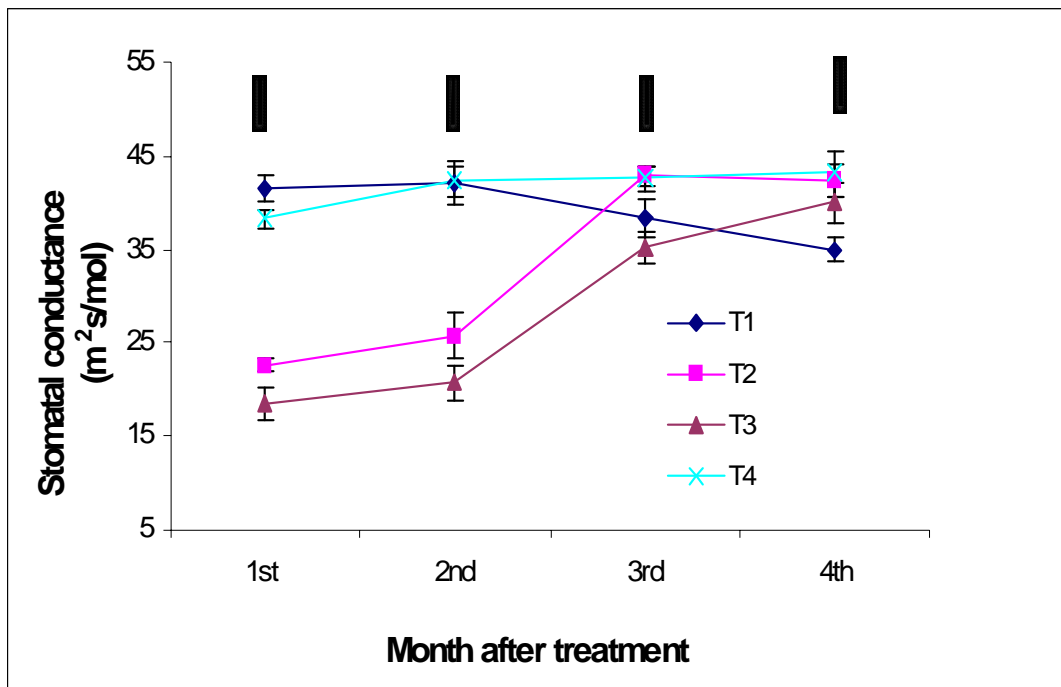


Figure 3.4: Stomatal conductance value was affected by different pruned treatments. Values are means of 3 measurements \pm SE. Values followed by a line indicates the existence of significant differences according to LSD $_{0.05}$ test.

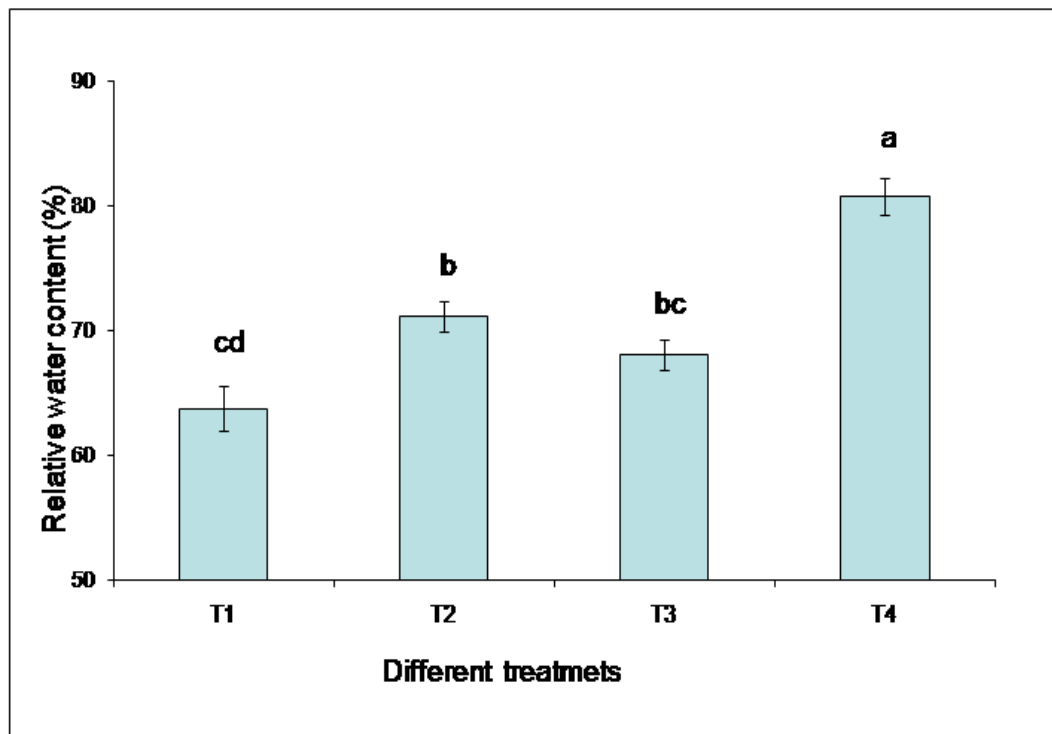


Figure 3.5: Relative water content of different treatments in bougainvillea plant leaves. Bars with different alphabets indicate significant difference according to LSD_{0.05} test.

The photosynthetic pigment chlorophyll *b* showed a significant difference with respect to the applied pruning treatments. The accumulation of chlorophyll *b* was significantly higher in plants which underwent in frequent pruning. Enhanced synthesis of chlorophyll pigment by pruning treatment had previously been reported and it has been suggested that the enhanced synthesis was attributed to the increased cytokinin activity in rose plants (Angeles *et al.*, 2008) (Fig. 3.6). Similarly, chlorophyll *a* also showed a significant difference in both pruning and non-pruning treatments. The lowest amount of both chlorophyll *a* and *b* was observed in the control treatment. Hence, it was visualized that frequent pruning once per month was the optimum rate for bougainvillea to maintain the highest chlorophyll content. Thus finding of the present study agrees with the reports on the enhancement of the photosynthetic pigments through pruning (Angeles *et al.*, 2008).

Potassium content was increased by frequent pruning. So this was the effective strategies for increasing potassium content in plant leaf. The potassium content was lower in partial pruning than in control treatment. But the significant and effective treatment for reducing plant potassium concentration was complete pruning (Fig. 3.7).

Total soluble sugars at the end of the experiment were significantly higher in the non-pruned plants, where the contents in Ts were similar in partial and non-pruned plants. Soluble sugars contents were 50% higher in non-pruned plants. The minimum total soluble sugar was obtained in complete pruning compared to all pruning treatments and it was about 40% lower in frequent pruning (Fig. 3.9).

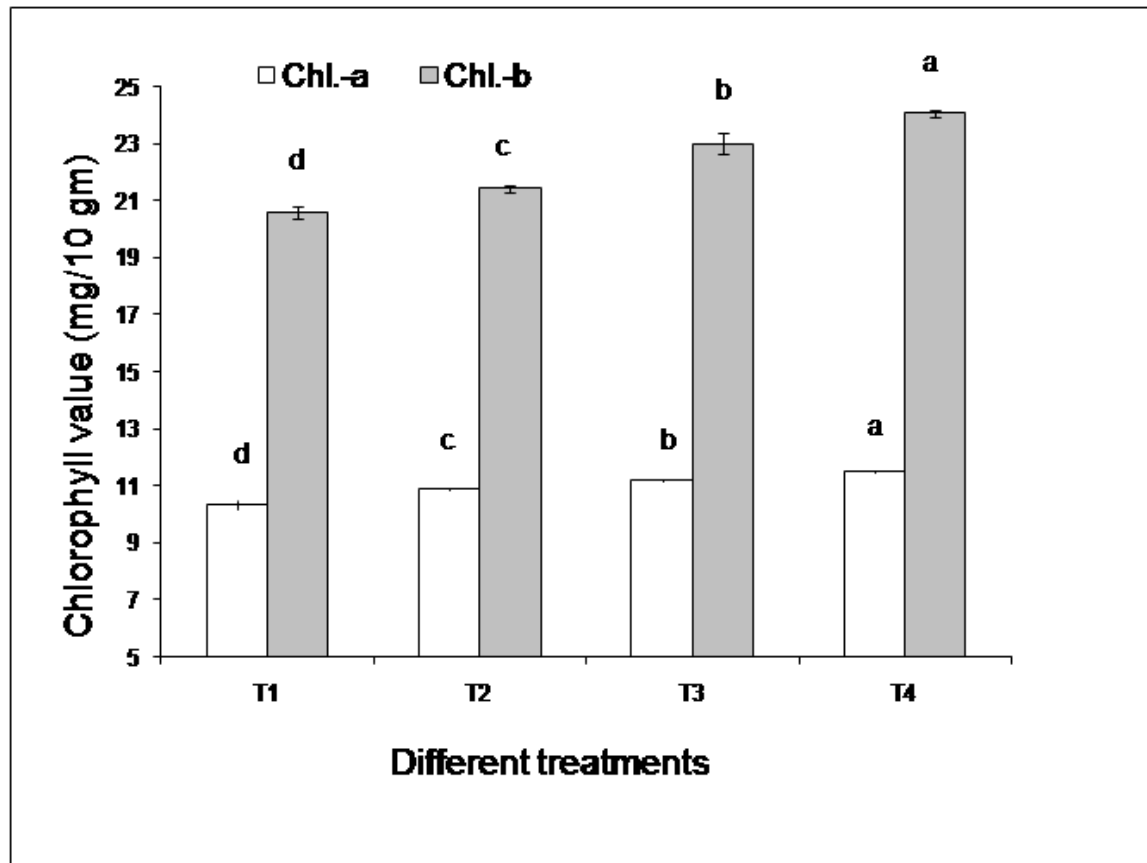


Figure 3.6: The effect of different pruning treatments on photosynthetic pigment, chlorophyll *a* & *b* value of *Bougainvillea* sp. leaf. Values followed by different alphabets indicate the existence of significant differences according to LSD $_{0.05}$ test.

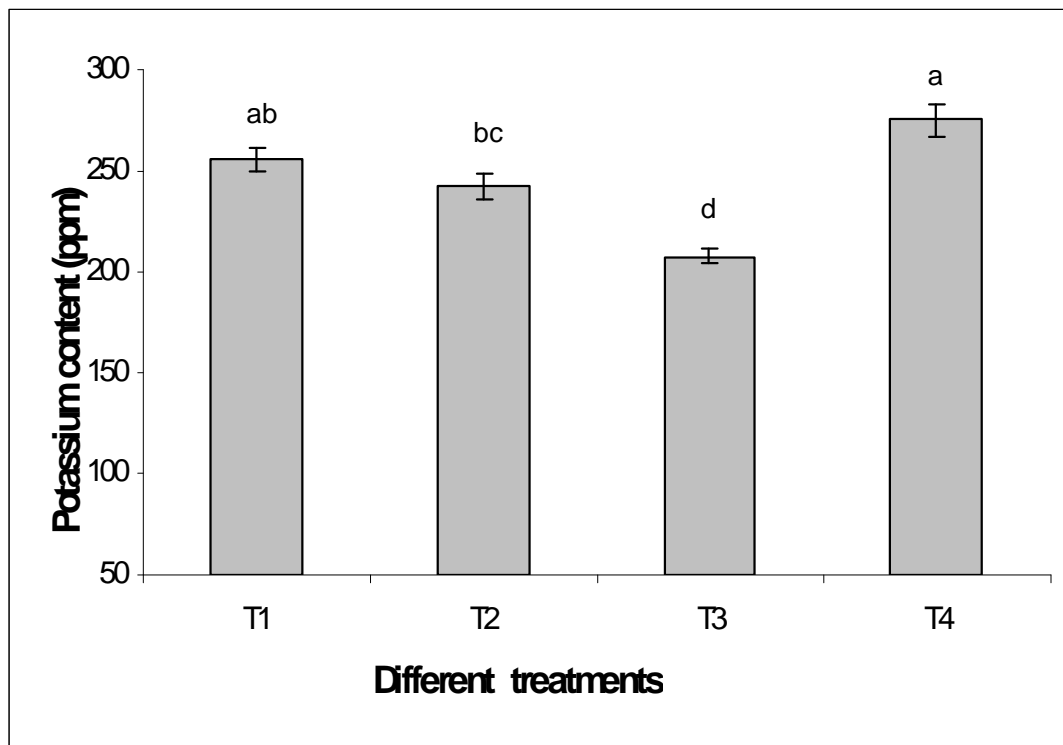


Figure 3.7: Potassium concentration of different treated bougainvillea plants leaf. Values are means of 3 measurements \pm SE. Values followed by different alphabets indicate the existence of significant differences according to LSD $_{0.05}$ test.



Figure 3.8: Flower number per branch in different types of pruning.

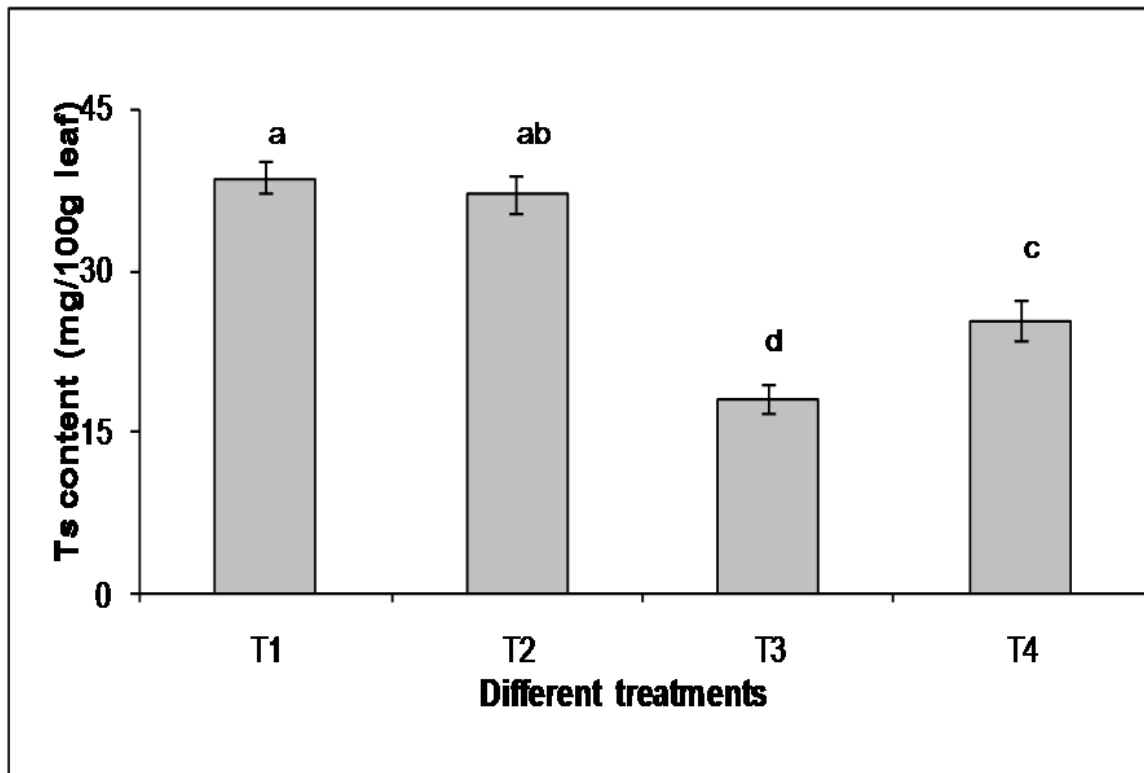


Figure 3.9: Total soluble sugars (Ts) in leaf under different pruned treatments. Values are means of 3 measurements \pm SE. Bars with different alphabets indicate significant difference according to LSD_{0.05} test.



Figure 3.10: Plant architecture containing branch and root profile in different types of pruning. Where, T1, T2, T3 and T4 are Non-pruning, Partial pruning, Complete pruning and frequent pruning, respectively.

3.4 General Discussion

In this work, the analysis and discussion on some physiological aspects of pruning on bougainvillea plants were presented. Until recently, research about pruning was only focused on physiological aspects, mainly on yield and carbohydrates studies of many fruits plant. This research is focused on offering a wide view about physiological mechanism related to pruning, a common cultural practice under natural sunlight growing condition of flowering plant.

In this current study, it has been discovered that pruning is an effective method to increase quantum yield and chlorophyll (*a and b*), also to maintain bract length, bract number/plant and relative water content of leaf.

3.4.1 Quantum Yield

Maximum efficiency of photosystem of dark-adapted leaves, measured by fluorescence quantum yield (F_v/F_m) showed lower values in non-pruned plants. F_v/F_m reflected the potential quantum efficiency and was 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 0.88 have been obtained in leaves of flower shoots in frequent pruning plants (Fig. 3.3). The value obtained by fluorescence, indicate that pruned plant promoted better photosynthetic light reaction than non-pruned plant (0.84). 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).

3.4.2 Total Soluble Sugar and Shoot Length

The analysis of total soluble sugar (Ts) in the leaves of the different types of pruned and non-pruned plants showed that non-pruned plant leaves exhibited higher TSS than pruned plants. It can be explained that pruning might create a large number of metabolic sinks with the expansion of new flower shoots (Fig. 3.8). Therefore, the reductions of stored carbohydrates of plant parts or shoots were observed due to the translocation of sugar to the developing flower shoots. Flower primordium development is a major sink for assimilation. Other reasons, pruning reduces the total mass of the photosynthesizing vegetation (Fig. 3.2) and decreases the production of new carbohydrate reserves (Fig. 3.9) (Li *et al.*, 2003). Stitt *et al.* (1990, 2002) have shown that the accumulation of carbohydrates over a time-span of weeks, leads to both an inhibition of photosynthesis and an increase of respiration.

3.4.3 Root and Shoot Growth

With full expansion of the shoot and leaves, the first priority in the shoot is the deposition of carbohydrate behind the new buds. Shoot storage is always filled with carbohydrate from the tip downward. There should be a positive correlation between shoot and root. Both shoot and root must effectively operate and share resources to ensure tree success (Coder, 1997). Therefore, pruning plants has received an effort to balance shoot mass and root mass by controlling photosynthesis and nutrients uptake (Pinkard *et al.*, 1998). Complete pruning has adjusted the mass of roots or shoots through the deficiency of potassium and stomatal conductance. Whereas, partial pruning showed extensive shoot

range due to the availability for utilizing the remaining branch leaves in photosynthetic process and nutrient uptake by excessive root (Table 3.4).

In the case of complete pruning treatment, shoot initiation and branch length was lower than in partial pruning and non-pruning. This decrease could have been due to the less root length to up take nutrient (potassium) or the lack of photosynthesis devices (leaf) or vice versa. Therefore, less root and shoot development was observed throughout the experimental period. It has been observed that the number of shoot length and number of shoot/plant was correlated to root length and weight in all treatments. This hypothesis was described by Coder (1997) that shoot growth was dependent upon root length, water and mineral nutrient uptake systems, while root growth was depend upon the above shoot part or the plant for carbohydrates stored amount. Tertiary branch initiation as a result of non pruning may be attributed to high Ts or capability in photosynthesis through existing primary and secondary branches leaves (Table 3.2 and Fig. 3.10). Whereas, tertiary branch was not initiated by the pruned plants due to its need to re-grow and existing less number of shoot that was pruned off at the beginning of experiment.

3.4.4 Stomatal Conductance

The effect of pruning on stomatal conductance was clearly significant throughout the experiment. Initially, stomatal conductance was higher in the non-pruned plants then the value was gradually decreased except in the leaves of pruning plants. This reason was described by Poni and Intrieri (1996) that in matured field grown vines, the photosynthesis reached a maximum at 30 days after unfolding and then declined with leaf age. Nabi *et al.* (2000) and Ferree *et al.* (1993) also found that leaves from the younger branch or middle

age had a higher rate of photosynthesis and high stomatal conductance than the leaves of older branches.

3.4.5 Relative Water Content, Potassium, Flower and Chlorophyll

Potassium content was higher in frequent pruning plant than the non-pruned plants and potassium deficiency was reduced by complete pruning (Cheng and Fuchigami, 2000). These values might be caused by younger leaf of complete pruning. In addition, relative water content was increased by all types of pruning treatments. This might be due to the higher capability to accumulate chlorophyll *a* and *b* (Fig. 3.6) by pruning plants. Therefore, higher chlorophyll allowed more photosynthetic to take place and maintain higher RWC, producing new branches and leaves, allowing the plant to maintain same quality, size and number of its flower. Roberto and Jonathan (2000) also reported similar results which showed that as the water potential increased, the flower numbers, growth and quality also increased.

3.4.6 Flower Initiation

Avner and Staden (1983) have shown that pruning increased the supply of cytokinins from the roots, measured as increased concentration in the remaining above-ground tissue. The increase of hormone levels is probably responsible for stimulating cell division, new shoot formation and ultimately more flower/ branch and frequent flower bud initiation. In this experiment, the frequent pruning took short period to induce flower by stimulating continuously cytokinins for bud formation and therefore flower number/branch, bract length, and weight was remain unchanged from the first to final season (Table 3.1).

However, in the case of partial and complete pruning, the number of flower/branch was low in first and final seasons 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, 2004).