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Study on Basic Physiological Response of Different Cultivars of *Bougainvillea glabra* with various Ages to Natural Cooling

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Table of Contents **Page**

List of Tables

List of Figures **Page**

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Abstract

Bougainvillea glabra is a characteristic ornamental plant popular in the world and native in South America. Temperature is one of the important factors limiting the northward movement of *Bougainvillea glabra*. This research chooses 7 kinds of *Bougainvillea glabra* with 2 ages, set up the facility cultivation test, and observed the differences of *Bougainvillea glabra* leaves in chlorophyll content, relative electricity conductance and photosynthesis parameters under natural temperature conditions. The test results show that: 1. the reponse of 7 kinds of *Bougainvillea glabra* to natural cooling was different. For relative electric conductivity, three years old cultivars are not affected as REC value is not higher than the first experiment, and A cultivar is affected most, followed by F, C, D and B respectively in two years old. The photosynthesis pigment content of all Bougainvillea cultivars decreased. Among two years old cultivars, E, G, C and F decreased more. Compared with two-year-old *Bougainvillea glabra* cultivars, the photosynthesis pigment contents of the three-year-old *Bougainvillea glabra* decreased by a small amount. 2.. There are significant differences in photosynthetic parameters of different *Bougainvillea glabra* cultivars under natural cooling conditions. Under natural cooling conditions, net photosynthesis (Pn), stomatal conductance (Gs), transpiration (Tr) of each cultivar decreased, but the net photosynthetic rate of cultivars B increased. The net photosynthetic rate of three-year-old and two-year-old *Bougainvillea glabra* cultivars both decreased under natural cooling conditions, but the decline rate of the three-year-old was smaller.

Keywords: *Bougainvillea glabra*, Natural cooling, Electric conductivity, Photosynthesis, Chlorophyll content

အပူချိန်လျော့နည်းခြင်းဖြင့် *Bougainvillea glabra* အပင်များ၏ အခြေခံဇီဝကမ္မဗေဒဆိုင်ရာ တုံ့ပြန်မှုများအား လေ့လာခြင်း

လင်းအောင်၊ ဦးစီးအရာရှိ
ပတ်ဝန်းကျင်ထိန်းသိမ်းရေးဦးစီးဌာန

စာတမ်းအကျဉ်း

Bougainvillea glabra သည်တောင်အမေရိက၏ ဒေသမျိုးစိတ်တစ်ခုဖြစ်ပြီး ထူးခြားသော အလှပန်းပင်တစ်မျိုးဖြစ်ပါသည်။ *Bougainvillea glabra* များကို အအေးပိုင်း ဒေသသို့ ပြောင်းရွှေ့စိုက်ပျိုးရာတွင် အပူချိန်သည် ထည့်သွင်းစဉ်းစားရမည့် အရေးကြီးသော အကြောင်းအရာ တစ်ခုဖြစ်ပါသည်။ ထို့ကြောင့် ဤစာတမ်းတွင် ၂နှစ်သားအရွယ်နှင့် (၃)နှစ်သား အရွယ်အပင် (၇)မျိုးအား ရွေးချယ်၍ အပူချိန် လျော့နည်းလာမှုကြောင့် ၎င်းတို့၏ chlorophyll content, electric conductance နှင့် photosynthesis parameters များအပေါ် သက်ရောက်မှုအား စမ်းသပ်တိုင်းတာခဲ့ပါသည်။ အပင်အားလုံးသည် အပူချိန်လျော့နည်းခြင်းအပေါ် ကွဲပြား ခြားနားစွာ တုံ့ပြန်ကြောင်း တွေ့ရှိရပါသည်။ ၃နှစ်သား အပင်များတွင် Electric conductivity သည် သက်ရောက်မှု မရှိဘဲ ၂နှစ်သားအပင်များတွင် သက်ရောက်မှုရှိကြောင်း၊ chlorophyll contentနှင့် photosynthesis rate တွင် ၂နှစ်သားအပင်များသည် ၃နှစ်သား အပင်များထက် လျော့နည်းသွားကြောင်း တွေ့ရှိရပါသည်။

Study on Basic Physiological Response of Different Cultivars of *Bougainvillea glabra* with various Ages to Natural Cooling

1. Introduction

1.1. Background

Bougainvillea, native in South America is a genus of flowering plant belonging to the family of Nyctaginaceae with lots of species such *as B. berberidifolia, B. buttiana, B. campanulata, B. glabra, B. herzogiana, B. infesta, B. lehmanniana, B. lehmannii, B. malmeana, B. modesta, B. pachyphylla, B. peruviana, B. pomacea, B. praecox, B. spectabilis, B. spinosa, B. stipitata, and B. trollii* and it is used in the arid landscapes beautification, horticulture, pharmaceutical industries, agriculture and environmental industries on account of the large flexibility in different agro climatic regions of the world. Bougainvillea flowers are hermaphrodite, tubular in shape with a constriction in the middle and borne in clusters of three, each flower subtended by a brightly coloured bract for cross pollination. Its flowers have in red, pink, white, yellow, scarlet purple, and lavender. Bougainvillea cultivars are diversed among themselves by many factors such as foliage variegation, floral tube and bract leaf. Bougainvillea is a popular ornamental plant among parks, home and institutional gardens as it can be used as shrub, climber, pot plant and bonsai. Moreover, bougainvillea is a pollution tolerant plant and it can be used in industrial places. So, this plant was highly recommended for plantation where particulate is a problem. It can grow best in well drained acidic soil (pH 5.5-6.0) with full sun and needs high light intensity for flowering. Among the Bougainvillea species, *Bougainvillea glabra*, paper flower is the horticulturally important species that can more tolerate cold stress and thrive well at higher altitudes.

There are many abiotic factors such as extreme temperatures, drought, salinity, chemical toxicity and oxidative stress that limit the plant growth, survival and distribution across large area of the world and low temperature (cold and freezing) is one of the factors among abiotic (Boyer, 1982)(Karimzadeh et al., 2006)(Guy et al., 2008). Consequently, plants have different adaptive mechanism for low temperature(Bressan et al., 2009) and also involve a number of physiological and biochemical changes by means of increasing of soluble sugar and proline level as well as enzyme activities(Holaday et al., 1992). However, The temperature below the freezing point can occur annually for about two third of the world's landmass(Larcher, 2001).Therefore, the impacts of cold stress on plants have been studied thoroughly in the world.

Bougainvillea is grown in tropical and subtropical zones and is enriched with different varieties having attractive shades. Bougainvillea is an evergreen vine which is spreading horizontally downwards and upwards. The plants will be under stress when they are subjected to less-than-ideal growing conditions. Stress factors can affect growth, survival and crop yields. Plant screening for cold and freezing tolerance, in tradition, is based on visual observations in the field. However, this is subjective and subordinate to other influential factors such as wind, air humidity, exposure, and water status and health conditions of plants that could distort the final result(Mancuso, 2000). Moreover, cold resistance is related to physiological and biochemical changes in plants. So, an understanding of the physiological and biochemical mechanisms of cold hardiness is a prerequisite for efficient breeding as well as the introduction and management of plants into cold or freezing environments.

1.2. Physiological and Biochemical Study *Bougainvillea glabra* Plants

Physiological and biochemical damages can be occurred in most of the plants by higher or lower temperature than their optimal growth(Lyons, 1973) (Grace et al., 1998). Low temperature stress affects the whole plant by causing damages and adaptation reactions(Beck et al., 2007). Each plant has a relevant set of temperatures for its properly growth. The required temperature may differ from plants to plants. So, the native plants to warm conditions may be stressed under the low temperature and its injury signs rely on the plant sensitivity to cold stress. The different phenotypic symptoms such as reducing leaf expansion, chlorosis and necrosis can be occurred when the plants are under stress. Plants have a variety of efficient mechanism such as biological and physiological changes to adapt the adverse environmental conditions. Photosynthetic capacity, plant anatomy, and carbohydrate contents in plants altered after exposure to low temperature stress (Ensminger et al., 2006)(Muller et al., 2014)(Cohu et al., 2014)(Adams et al., 2016).These changes are of great interest so as to understand adaptation mechanism for low temperature stress.

Plasma membrane damage, due to cold stress induced dehydration,(Steponkus, 1984) is major adverse effect of cold stress. The loss of membrane integrity which is leading to solute leakage, the integrity of intracellular organelles, reduction and impairing the photosynthesis, protein assembly and general metabolic processes are also affected by cold stress exposure. Many species can enhance the antioxidant enzyme activities connected to higher proline content when they are subjected to abiotic stress conditions(Ashraf & Foolad, 2007) but, with the cold stress, enzyme and chemical reaction is resisted(Wang et al., 2009)(Distelbarth et al., 2013).

Plants have defense system against reactive oxygen species (ROS) which are formed in stressed and unstressed cells. The defense system can be overwhelmed under stressed conditions with increasing of ROS formation but oxygen formation and removal are in equal under unstressed. In the stressed conditions, plants respond to a rise in ROS when the defense system can't remove the increase enzymatic and non-enzymatic antioxidant processes. Superoxide dismutase (SOD) performs in the first line of defensing to ROS within a cell and it is important to remove the oxygen radical in the compartments where oxygen radicals are formed.

Physiological processes such as water regime, respiration, metabolism and photosynthesis face disturbance with cold stress. In chilling sensitive plants, wilting occurs as a result of water loss (Vernieri et al., 1991)(Boese et al., 1997)(Bloom et al., 2004) and photosynthesis rate also decreases with decreasing temperature and lengthening of chilling period(Janda et al., 1994)(Boese et al., 1997) (Gesch & Heilman, 1999)(Allen & Ort, 2001)(Van Heerden et al., 2003)(X. G. Li et al., 2004)(Strauss et al., 2007). Under the low temperature conditions, Bougainvillea leaves are swelling from the nodes, becoming yellow of leaves as a result of inhibition of photosynthesis (chlorosis), curling, wilting and then fall off.

2. Objectives

Cold tolerance in plants is primarily related to the activity of antioxidants in conjunction with biochemical and physiological mechanisms and compatible solutes, antioxidant enzymes, membrane damages are recently used to identify cold tolerance(Sakai & Larcher, 1987)(Bridger et al., 1994)(Bettaieb et al., 2007). Depends on these factors, plant differs the tolerance for stress. However, there have been only a few studies comparing the activity of photosynthesis, chlorophyll contents and relative electric conductivity (REC) in bougainvillea cultivars exposed to cold stress under the natural decreasing air temperature. Thus, the objectives of this study are: 1) to explore the response of physiological characteristics such as photosynthesis, chlorophyll and REC of different kinds and age *Bougainvillea glabra* plants under natural cooling conditions. 2) to provide the theoretical basis for the cultivation and management of these different bougainvillea cultivars under natural cooling conditions.

3. Materials and methods

3.1 Plant Materials and Growth Conditions

Seven potted Bougainvillea cultivars of two years old, labeled as A for Brazilian violet, B for Mosaic red, C for Green cherry, D for Yunnan violet, E for Pearl white, F for Lemon yellow, G for China red; and three potted Bougainvillea cultivars of three years old, labeled as H for Lemon yellow, I for Green cherry, J for Yunnan violet, are used to analyze and test relative electric conductivity, photosynthesis, chloroplast ultra-structure and chlorophyll. Among them, varieties C and I, D and J, F and H are the same respectively. Two years old are potted in plastic pots with the size of (18 cm high, diameter 21 cm at the top and 13 cm at the bottom) and three years olds are with of (21 cm high, diameter 31 cm at the top and 19 cm at the bottom). Their plantlets were produced by cuttings of different Bougainvillea species, and then, grown in nurseries until they reached to two and three years old. Watering the plants are taken regularly when the soil appeared to dry. One variety has thirty plants and each experiment was made by five replications. The experiment was conducted at the Zhejiang Institute of Subtropical crops in Wenzhou, China.

The simulation of low temperatures was performed in natural condition in Zhejiang Institute of Subtropical crops of Wenzhou, China and also daily temperature was recorded. Groups of thirty plants of the same varieties were submitted under natural decreasing process of air temperature and leaf samples were collected totally five times (12 November 2019 to 30 January 2020) every 20 days for physiological testing. Daily maximum and minimum temperature as shown in figure-1 at each experiment is recorded by the temperature measuring device.

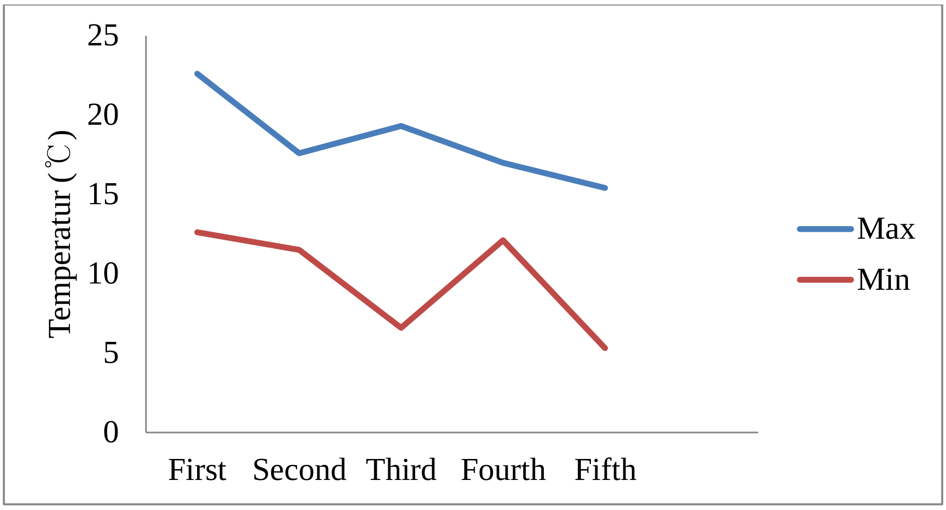


Figure.1 Maximum and Minimum Temperature under Natural Cooling at each Five Experiments

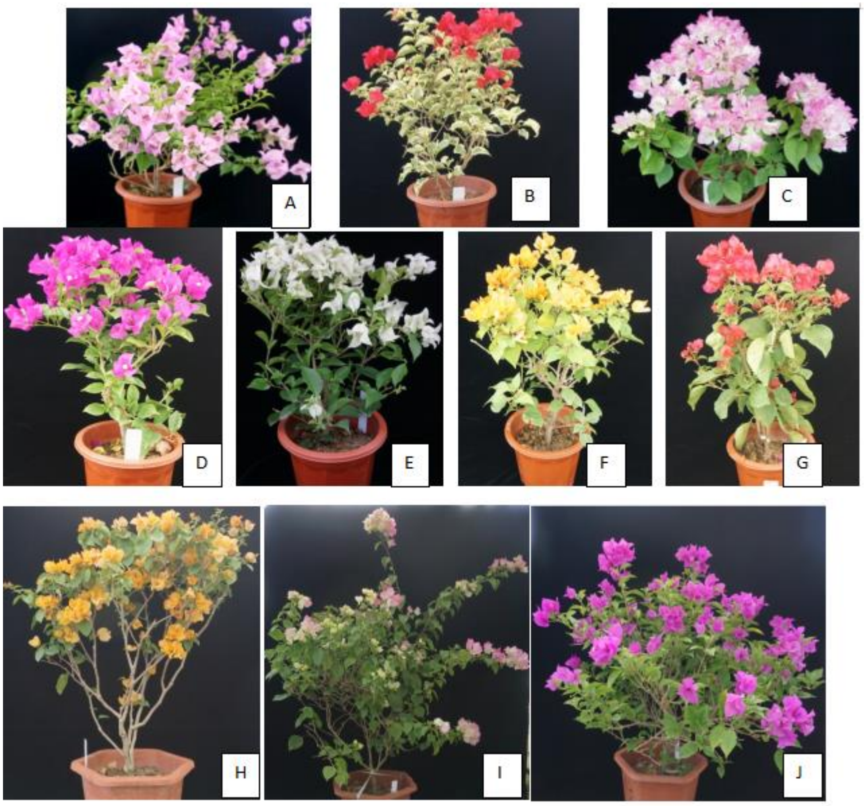


Figure 2. First phenotypic characteristics conditions of different cultivars

3.2 Determinations of Relative Electric Conductivity (REC)

To measure the electric conductivity, sample leaves are excised and washed with deionized water. After drying with filter paper, 0.1 g of sample leaves with five replications were cut into small pieces about 1cm^2 and them immersed with 8 ml distilled water at 25°C for 30 minutes, and the electrical conductivity (EC1) was measured by conductivity bridge. The same samples tubes are placed in 100°C boiling water for 30 minutes to completely kill the tissuse and release all electrolytes. The final electrical conductivity (EC2) was measured after cooling at room temperature. The electrolyte leakage (EL) was calculated as following formula EL= (EC1/EC2) x 100%.

3.3 Determination of Photosynthesis and chlorophyll concentration

Photosynthesis on healthy and fully expended leaves from a randomly selected branch were evaluated after cold stress of natural condition using an LI-6400 XT portable photosynthesis system equipped with an 6400-05 conifer chamber at concentration of 400 μmol mol^{-1} CO_2, 21% O_2, and 50% relative humidity with a natural light source. Measurements were conducted on sunny day from 8:30 to 11:30 according to (Pan et al., 2016).

A total of 0.2 g of leaf discs was weighed, placed into a test tube, and then repeatedly extracted with 8 mL of 95% (V/V) ethanol. The photosynthetic pigments were extracted at room temperature for 24 h in the darkness. The light absorption of the extracted solution was measured at different wavelengths; 470, 649 and 665 nm with a spectrophotometer (UV-2550, Shimadzu). Then, the content of four pigments, chlorophyll a, chlorophyll b, carotenoids and total chlorophyll concentration was determined by the following four equations according to the slightly modification method of (Lichtenthaler, 1987) and expressed as mg g^{-1} fresh weight (FW)

$$\text{Chl a} = (13.36A_{665} – 5.19A_{649}) \times [8 / (1000 \times 0.2)] \quad (1)$$

$$\text{Chl b} = (27.43A_{649} − 8.12A_{665}) \times [8 / (1000 \times 0.2)] \quad (2)$$

$$\text{Car} = [1000A_{470} − 2.13C_a − 97.64C_b/209] \times [8 / (1000 \times 0.2)] \quad (3)$$

$$\text{Total Chl} = (22.24A_{649} + 5.24A_{665} \times [8 / (1000 \times 0.2)] \quad (4)$$

Whereas, Chl is the chlorophyll a content, Chl b is the chlorophyll b content, Car is the carotenoid content, and Total Chl is the total chlorophyll content. A665, A649, and A470 represent the absorbance at 665, 649, and 470 nm, respectively. .

4. Statistical analysis

The experiment was completely randomized with a factorial design, consisting of ten cultivars and five minimum and maximum temperatures, with five replicates composed of one plant each for REC, three replications for photosynthesis and chlorophyll contents. For all recorded variables, an analysis of variance (ANOVA) was conducted on the data, and the means were compared using LSD post-hoc test with $p<0.05$ significance level.

5. Results

5.1 Changes of Relative Electrolyte Leakage under Natural Decreasing Process of Air Temperature

Cell membrane penetrability damaged by cold stress was evaluated by the relative conductance of the cell membrane under cold stress (Steponkus et al., 1990)(Rohde et al., 2004). The degree of cellular membrane damaging was worse with the continuity of low temperature stress(Steponkus et al., 1990)and can be reflected by intracellular electrolyte leakage rate. The relative conductance value is one of the effective indicators to know, indirectly, the plant response to cold stress (Steponkus, 1984)and electrolyte leakage, an estimator of cell membrane, has been used as a key parameter (L. Hu et al., 2010)(Zhao et al., 2011). The electrolyte leakage test was performed to compare membrane integrity (MURRAY et al., 1989). So, plants were subjected to natural decreasing low temperature. The relative electrolyte leakage of the cultivars was increased greatly with the time of low temperature stress in second, third and fifth experiments of cultivars A, B, C, D and F but decreased in fourth. However, REC is only increased in third experiment of cultivar E. Conversely, REC is decreased in all experiments of three-year-old cultivars and G compare with first experiment as shown in figure-3.

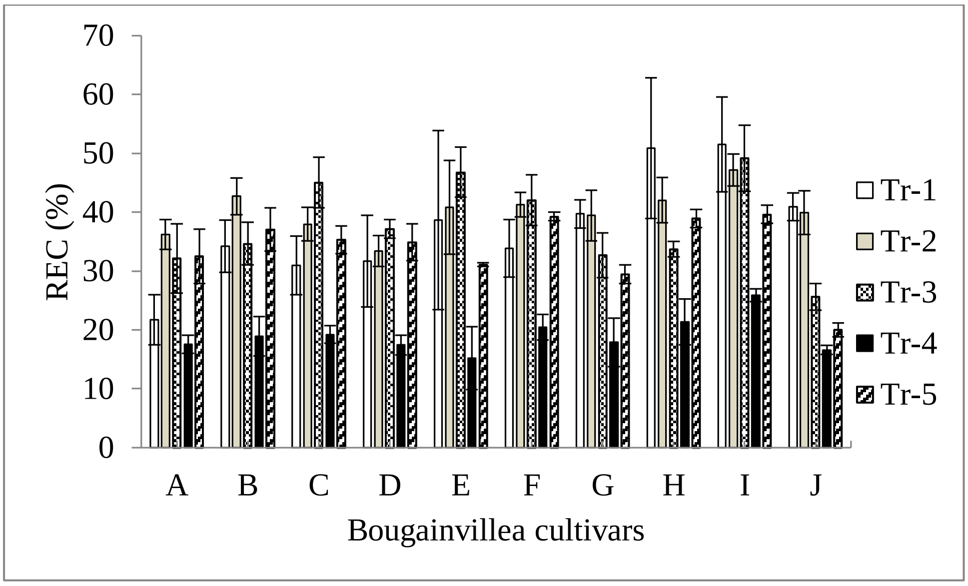


Figure 3. Effects of cold stress on Relative Electric Conductivity for five experiments of *Bougainvillea glabra* choisy. Mean value ±Standard deviation are showed.

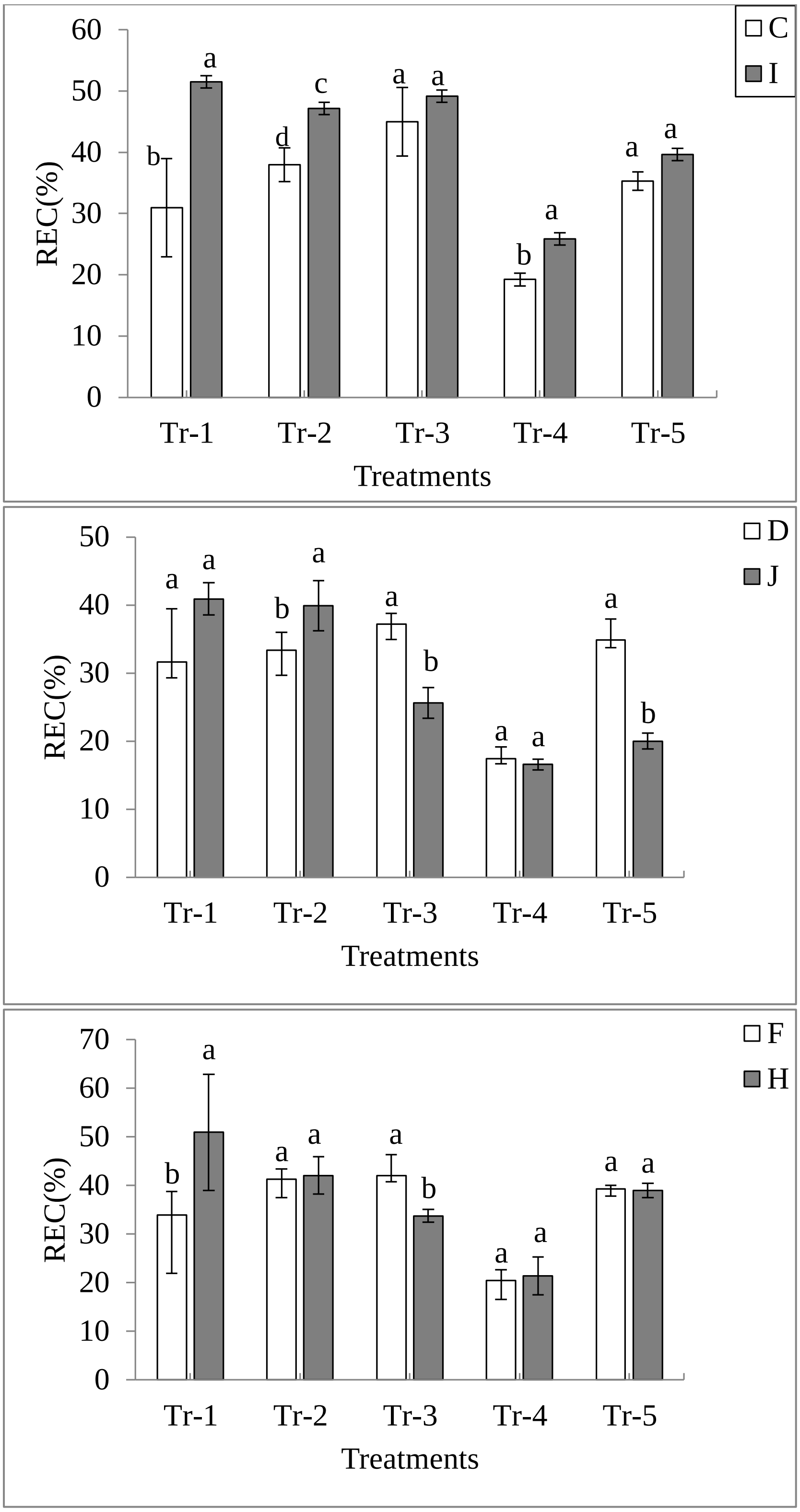


Figure 4. Effects of cold stress on Relative Electric Conductivity for five experiments of same *Bougainvillea glabra* cultivars. Mean value ±Standard deviation are showed. Different letters indicate significant difference between treatments of REC (p‹0.05 LSD test)

Table 1. Effect of Low Temperature Stress on Relative Electric Conductivity of all *Bougainvillea glabra* cultivars. Mean value ±Standard deviation are showed. Different letters within a row indicate significant difference between treatments of Relative Conductivity (p‹0.05 LSD test)

	Relative Electric Conductivity of all Cultivars (%)				
	Tr-1	Tr-2	Tr-3	Tr-4	Tr-5
A	21.73±4.27d	36.2±2.56a	32.14±5.88ca	17.57±1.55d	32.52±4.6ba
B	34.21±4.47b	42.72±3.12a	34.66±3.64b	18.92±3.35c	37.09±3.67b
C	30.97±4.99c	37.97±2.86bc	45±4.29a	19.22±1.49d	35.31±2.32ca
D	31.67±7.79b	33.38±2.64b	37.19±1.58ab	17.45±1.68c	34.89±3.09b
E	38.66±15.2a	40.83±7.96a	46.79±4.25a	15.2±5.32b	31.08±0.29ac
F	33.85±4.86d	41.29±2.09b	42.02±4.3ab	20.44±2.15e	39.27±0.71cb
G	39.71±2.41a	39.42±4.31a	32.68±3.82b	17.91±4.12d	29.47±1.58cb
H	50.89±11.94a	42.03±3.86b	33.7±1.31d	21.35±3.93e	38.93±1,52bcd
I	51.49±11.94a	47.14±3.86a	49.17±1.31a	25.87±3.93c	39.63±1.52b
J	40.91±8.05a	39.89±2.74a	25.63±5.61b	16.59±1.08d	20±1.53c

5.2 Changes of Chlorophyll Content under Natural Decreasing Process of Air Temperature

Chlorophyll is included especially in the green areas of leaf, flower, stem and root (Hasanuzzaman et al., 2013)(Srichaikul et al., 2011). Chlorophyll is a critical biomolecule in photosynthesis with function of light absorbance and light energy transformation (Xu et al., 2000) and it also plays crucial role in plant physiology. In the study, variation in photosynthesis pigments levels such as Chlorophyll a, Chlorophyll b, carotenoids and total chlorophyll were investigated. With the prolongation of low temperature stress, the chlorophyll a in cultivars B, C, E, F, G and J was decreased significantly about 2.27%, 16.84%, 47.17%, 11.11%, 36.73% and 16.13% at the last experiment. Cultivars H and I was also decreased about 5.22% and 6.35% although the results show not significantly. Chlorophyll b is also significantly decreased in all cultivars by mean of 11.76% in B, 19% in C, 20% in D, 50.22% in E, 9.68% in F, 33.33% in G, 10% in I, 10.32% in J cultivar except A and H cultivars. The content of carotenoid in cultivars A and B remains the same while F was increased its amount. Moreover, the total chlorophyll content was not change in cultivar A. The cultivar D is totally different with the significantly increase of 17.91% in chlorophyll a, 19.23% in chlorophyll b, 43.33% in carotenoid and 20% in total chlorophyll content. In comparison of same species, cultivar C is more decreased significantly all chlorophyll contents than I. However, the carotenoid of cultivar F is slightly increased while H has remained the same.

Table 2. Effect of Low Temperature Stress on Pigment Contents of *Bougainvillea glabra* cultivar A. Mean value ±Standard deviation are showed. Different letters within a row indicate significant difference between treatments of Pigment Contents (p‹0.05 LSD test)

Pigment Contents of cultivar A (mg/g)

	Tr-1	Tr-2	Tr-3	Tr-4	Tr-5
Chl a	0.43±0.11c	0.48±0.02c	1.12±0.03a	0.52±0.01bc	0.43±0.02bc
Chl b	0.16±0.05d	0.21±0.01c	0.4±0.01a	0.21±0.01bc	0.15±0.01d
Car	0.21±0.06b	0.22±0.01b	0.47±0.01a	0.23±0.01b	0.21±0.01b
Total Chl	0.59±0.16c	0.68±0.03c	1.53±0.04a	0.72±0.02bc	0.59±0.03c

Table 3. Effect of Low Temperature Stress on Pigment Contents of *Bougainvillea glabra* cultivar B. Mean value ±Standard deviation are showed. Different letters within a row indicate significant difference between treatments of Pigment Contents (p‹0.05 LSD test)

	Pigment Contents of cultivar B (mg/g)				
	Tr-1	Tr-2	Tr-3	Tr-4	Tr-5
Chl a	0.44±0.04c	0.59±0.04a	0.47±0.03c	0.57±0.03ba	0.43±0.04bc
Chl b	0.17±0.02c	0.24±0.02a	0.18±0.01c	0.21±0.01b	0.15±0.01d
Car	0.22±0.02c	0.27±0.02a	0.24±0.01c	0.27±0.01ba	0.22±0.01c
Total Chl	0.61±0.06c	0.83±0.06a	0.65±0.04c	0.77±0.04ba	0.58±0.05cd

Table 4. Effect of Low Temperature Stress on Pigment Contents of *Bougainvillea glabra* cultivar C. Mean value ±Standard deviation are showed. Different letters within a row indicate significant difference between treatments of Pigment Contents (p‹0.05 LSD test)

	Pigment Contents of cultivar C (mg/g)				
	Tr-1	Tr-2	Tr-3	Tr-4	Tr-5
Chl a	0.95±0.08a	0.88±0.08a	0.99±0.04ac	0.92±0.03ba	0.79±0.02b
Chl b	0.35±0.03a	0.37±0.03a	0.38±0.01a	0.36±0.01a	0.28±0.01b
Car	0.45±0.05a	0.42±0.04a	0.46±0.01ac	0.46±0.01ad	0.39±0.01ba
Total Chl	1.29±0.01a	1.25±0.11a	1.37±0.05ac	1.28±0.03a	1.08±0.03b

Table 5. Effect of Low Temperature Stress on Pigment Contents of *Bougainvillea glabra* cultivar D. Mean value ±Standard deviation are showed. Different letters within a row indicate significant difference between treatments of Pigment Contents (p‹0.05 LSD test)

	Pigment Contents of cultivar D (mg/g)				
	Tr-1	Tr-2	Tr-3	Tr-4	Tr-5
Chl a	0.67±0.01d	0.90±0.08b	0.48±0.03e	0.99±0.03a	0.79±0.01c
Chl b	0.26±0.01d	0.37±0.02b	0.19±0.01e	0.38±0.01ab	0.31±0.01c
Car	0.29±0.00d	0.39±0.02c	0.21±0.01e	0.44±0.01a	0.41±0.01b
Total Chl	0.93±0.02d	1.27±0.10b	0.67±0.04e	1.37±0.05a	1.1±0.02c

Table 6. Effect of Low Temperature Stress on Pigment Contents of *Bougainvillea glabra* cultivar E. Mean value ±Standard deviation are showed. Different letters within a row indicate significant difference between treatments of Pigment Contents (p‹0.05 LSD test)

	Pigment Contents of cultivar E (mg/g)				
	Tr-1	Tr-2	Tr-3	Tr-4	Tr-5
Chl a	0.53±0.04b	0.55±0.01b	0.69±0.03a	0.46±0.02c	0.28±0.01d
Chl b	0.23±0.02b	0.23±0.01b	0.27±0.01a	0.18±0.01c	0.11±0.02d
Car	0.24±0.02b	0.24±0.00b	0.29±0.01a	0.21±0.01c	0.14±0.01d
Total Chl	0.75±0.05b	0.78±0.02b	0.96±0.05a	0.64±0.03c	0.39±0.03d

Table 7. Effect of Low Temperature Stress on Pigment Contents of *Bougainvillea glabra* cultivar F. Mean value ±Standard deviation are showed. Different letters within a row indicate significant difference between treatments of Pigment Contents (p‹0.05 LSD test)

	Pigment Contents of cultivar F (mg/g)				
	Tr-1	Tr-2	Tr-3	Tr-4	Tr-5
Chl a	0.81±0.04b	0.83±0.05b	0.96±0.02a	0.73±0.03c	0.72±0.03dc
Chl b	0.31±0.01c	0.35±0.02b	0.36±0.01a	0.28±0.01d	0.28±0.01ed
Car	0.36±0.01b	0.38±0.02b	0.44±0.01a	0.36±0.01b	0.37±0.01b
Total Chl	1.13±0.05b	1.18±0.06b	1.32±0.03a	1.01±0.04c	0.99±0.04dc

Table 8. Effect of Low Temperature Stress on Pigment Contents of all *Bougainvillea glabra* cultivar G. Mean value ±Standard deviation are showed. Different letters within a row indicate significant difference between treatments of Pigment Contents (p‹0.05 LSD test)

	Pigment Contents of cultivar G (mg/g)				
	Tr-1	Tr-2	Tr-3	Tr-4	Tr-5
Chl a	0.98±0.04b	0.85±0.05c	1.11±0.04a	0.95±0.04b	0.62±0.03d
Chl b	0.36±0.01b	0.33±0.02c	0.44±0.01a	0.36±0.02b	0.24±0.01d
Car	0.45±0.02b	0.37±0.02c	0.51±0.01a	0.46±0.02b	0.31±0.02d
Total Chl	1.34±0.05b	1.18±0.07c	1.55±0.05a	1.31±0.05b	0.86±0.05d

Table 9. Effect of Low Temperature Stress on Pigment Contents of all *Bougainvillea glabra* cultivar H. Mean value ±Standard deviation are showed. Different letters within a row indicate significant difference between treatments of Pigment Contents (p‹0.05 LSD test)

	Pigment Contents of cultivar H (mg/g)				
	Tr-1	Tr-2	Tr-3	Tr-4	Tr-5
Chl a	1.15±0.13a	1.08±0.05a	0.94±0.03c	0.97±0.02bc	1.09±0.03a

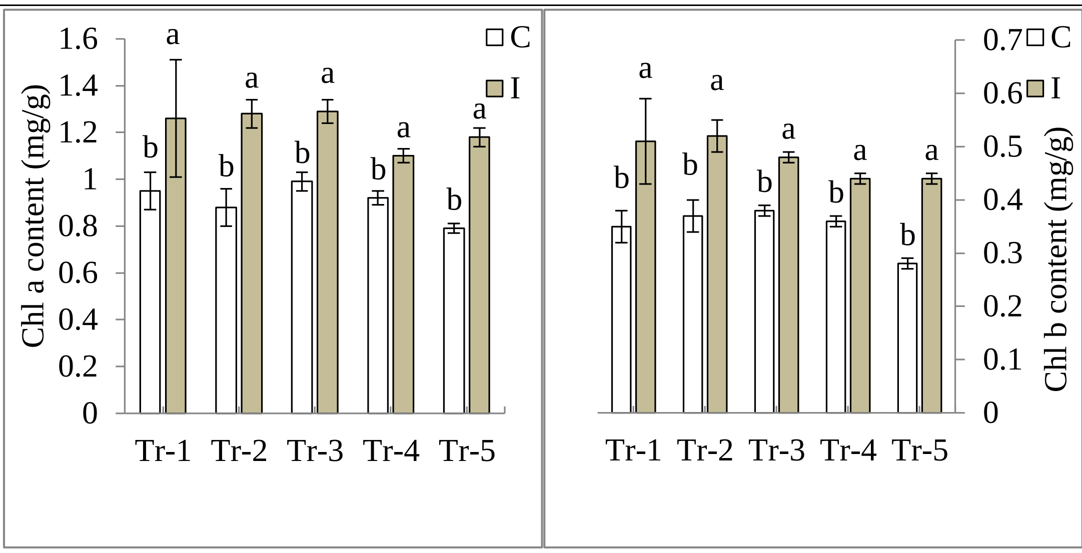
Chl b	0.44±0.05a	0.43±0.02a	0.37±0.01c	0.38±0.01bc	0.43±0.01a
Car	0.53±0.06a	0.49±0.02a	0.43±0.01c	0.48±0.01ba	0.53±0.01a
Total Chl	1.59±0.17a	1.51±0.07a	1.31±0.04c	1.35±0.03bc	1.52±0.04ad

Table 10. Effect of Low Temperature Stress on Pigment Contents of *Bougainvillea glabra* cultivar I. Mean value ±Standard deviation are showed. Different letters within a row indicate significant difference between treatments of Pigment Contents (p‹0.05 LSD test)

	Pigment Contents of cultivar I (mg/g)				
	Tr-1	Tr-2	Tr-3	Tr-4	Tr-5
Chl a	1.26±0.25a	1.28±0.06a	1.29±0.05a	1.10±0.03b	1.18±0.04ab
Chl b	0.51±0.08a	0.52±0.03a	0.48±0.01a	0.44±0.01ba	0.44±0.01ca
Car	0.58±0.13a	0.55±0.03a	0.54±0.02a	0.51±0.01ba	0.55±0.02a
Total Chl	1.77±0.32a	1.79±0.09a	1.77±0.06a	1.54±0.04b	1.62±0.05ab

Table 11. Effect of Low Temperature Stress on Pigment Contents of *Bougainvillea glabra* cultivar J. Mean value ±Standard deviation are showed. Different letters within a row indicate significant difference between treatments of Pigment Contents (p‹0.05 LSD test)

	Pigment Contents of cultivar J (mg/g)				
	Tr-1	Tr-2	Tr-3	Tr-4	Tr-5
Chl a	0.93±0.12a	0.97±0.04a	0.99±0.02a	0.98±0.03a	0.78±0.07b
Chl b	0.34±0.04d	0.39±0.02a	0.38±0.01ca	0.39±0.01ba	0.3±0.02e
Car	0.41±0.04a	0.39±0.01ab	0.42±0.01a	0.43±0.01ac	0.38±0.03b
Total Chl	1.27±0.17a	1.36±0.06a	1.37±0.03a	1.37±0.04a	1.09±0.09b



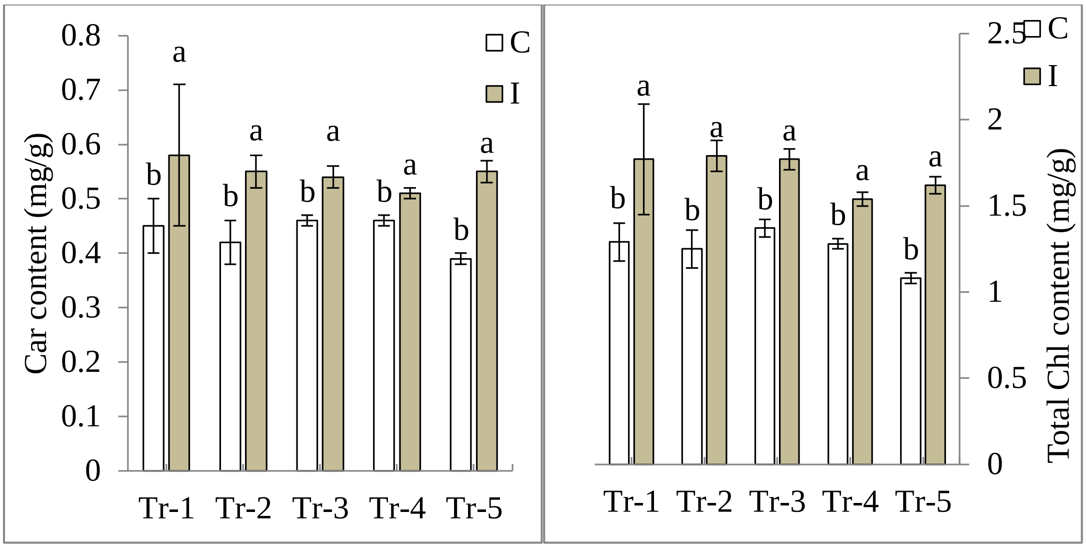


Figure 5. Effects of cold stress on Pigment contents of same cultivars (C & I) *Bougainvillea glabra*. Mean value ±Standard deviation are showed. Different letters indicate significant difference between treatments of Pigment contents (p‹0.05 LSD test)

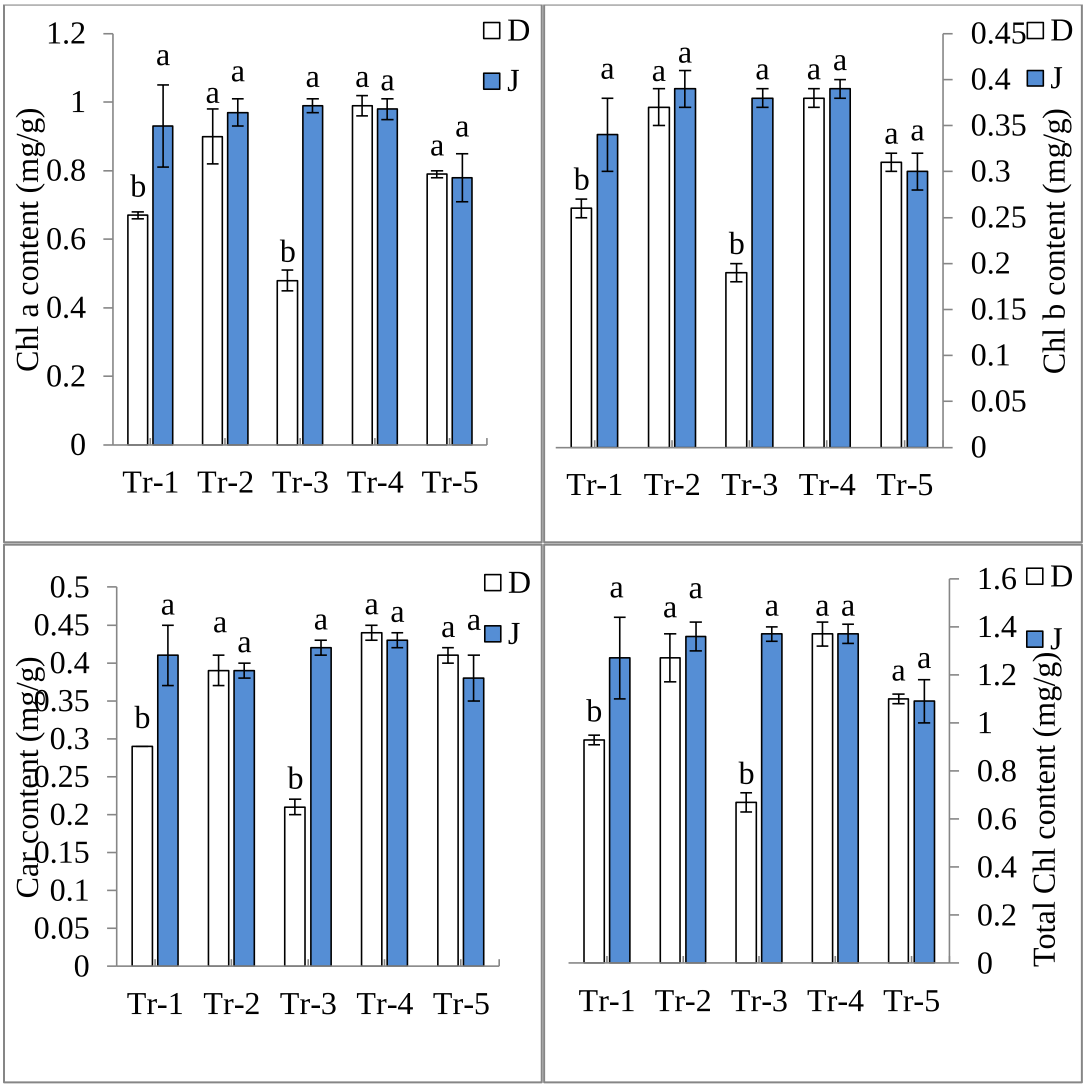


Figure 6. Effects of cold stress on Pigment contents of same cultivars (D & J) *Bougainvillea glabra*. Mean value ±Standard deviation are showed. Different letters indicate significant difference between treatments of Pigment contents (p‹0.05 LSD test)

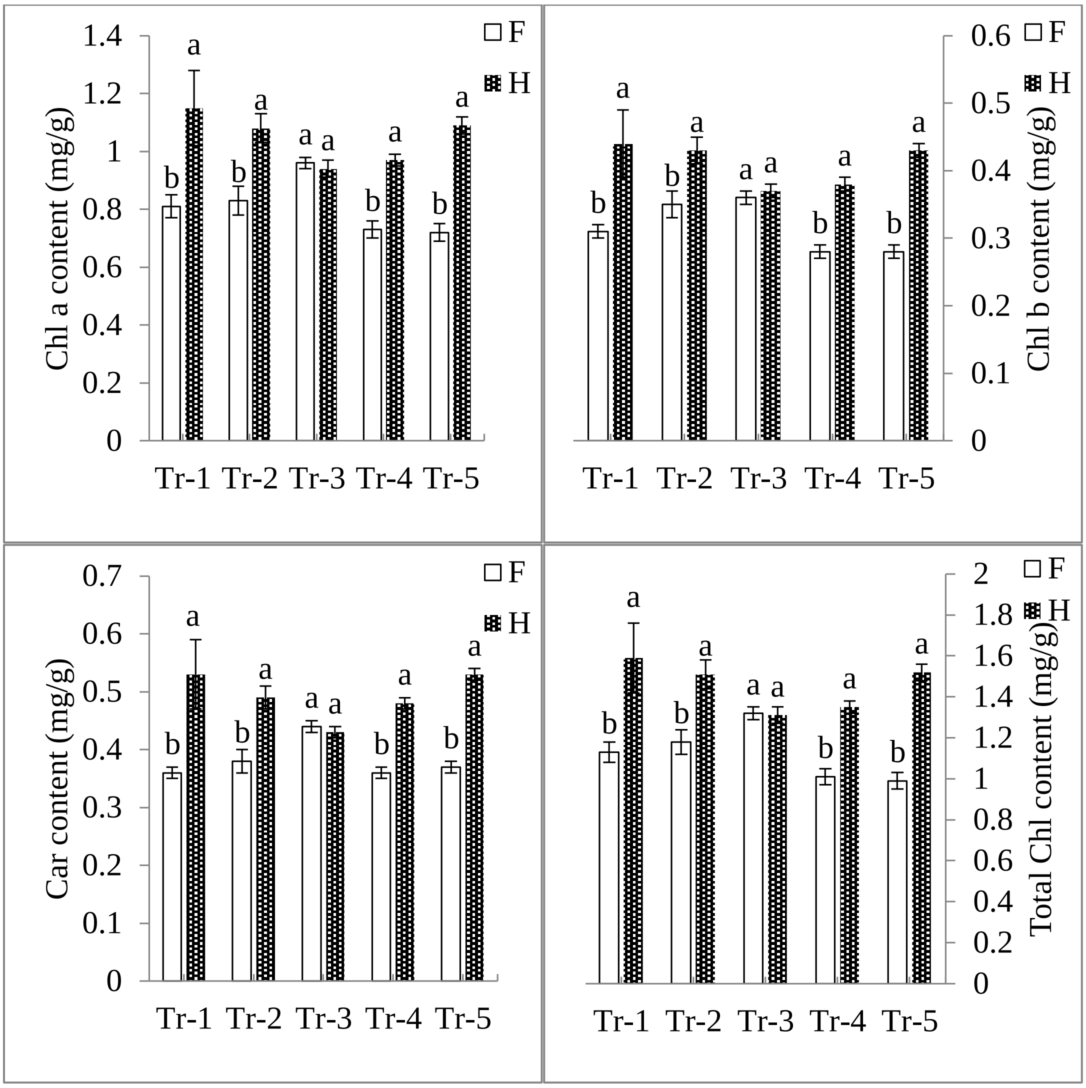


Figure 7. Effects of cold stress on Pigment contents of same cultivars (F and H) *Bougainvillea glabra*. Mean value ±Standard deviation are showed. Different letters indicate significant difference between treatments of Pigment contents (p‹0.05 LSD test)

5.3 Changes of Photosynthesis under Natural Decreasing Process of Air Temperature

Cold stress can affect the plant photosynthesis and decrease the light utilization (Glaszmann et al., 1990)(Wu et al., 1997). In this research, i measured the parameters of photosynthesis under natural decreasing process of air temperature. Photosynthesis rate is slightly decreased 17.39% in cultivar A, 24.02% in C, 33.69 % in D, 15.95% in E, 35.5% in F, 32.69% in G, 73.71% in H, 54.58% in I, 42.31% in J of each last experiment. On the contrary, photosynthesis rate of cultivar B was increased 42.86% compare with first experiment. The photosynthesis rate (Pn), stomatal conductance (Gs) and transpiration rate (Tr) values of cultivars A, C, D, E, F, H, I and J were decreased in last experiment compared to first experiment after low temperature stress. The Gs and Tr values were also decreased about 33.33% (P >0.05) and 70% (P >0.05) in cultivar C, 56.86% (P <0.05) and 75.49% (P <0.05) in I, 71.43% (P <0.05) and 86.36%(P <0.05) in D, 63.16% (P <0.05) and 9.72% (P >0.05) in J, 72.13% (P >0.05) and 85.44% (P <0.05) in F, 85.87% (P <0.05) and 92.12% (P <0.05) in H respectively. Moreover, the low temperature stress declines in Gs and Tr rates of all cultivars except cultivar G. After low temperature stress, photosynthesis rate of same cultivars did not show significantly different although the rate of each same cultivars decreased about 24-35% in two years old and 42-73% in three years old.

Table 12. Effects of cold stress on net photosynthesis rate (Pn), stomatal conductance (Gs), and transpiration (Tr) of Bougainvillea glabra (A). Mean value ±Standard deviation are showed. Different letters within a row indicate significant difference between treatments of Photosynthesis (p‹0.05 LSD test)

	Gas exchange parameters of cultivar A (μmol $m^{-2}s^{-1}$,mmol $m^{-2}s^{-1}$,mmol $m^{-2}s^{-1}$)				
	Tr-1	Tr-2	Tr-3	Tr-4	Tr-5
Pn	2.3231±0.52a	3.33±0.98a	2.26±0.76a	3.29±1.46a	1.96±0.63a
Gs	0.0027±0.00a	0.0034±0.00a	0.0004±0.00ab	0.0051±0.00ad	0.0004±0.00ac
Tr	0.075±0.02a	0.089±0.03a	0.009±0.00ab	0.129±0.08a	0.007±0.00ac

Table 13. Effects of cold stress on net photosynthesis rate (Pn), stomatal conductance (Gs), and transpiration (Tr) of *Bougainvillea glabra* (B). Mean value ±Standard deviation are showed. Different letters within a row indicate significant difference between treatments of Photosynthesis (p‹0.05 LSD test)

	Gas exchange parameters of cultivar B (μmol $m^{-2}s^{-1}$,mmol $m^{-2}s^{-1}$,mmol $m^{-2}s^{-1}$)				
	Tr-1	Tr-2	Tr-3	Tr-4	Tr-5
Pn	2.1077±0.57b	2.2181±0.88b	1.4944±0.99b	4.4819±1.69a	3.0523±0.28ba
Gs	0.0035±0.01a	0.0028±0.00a	1.4187±2.46a	0.0097±0.00a	0.0012±0.00a
Tr	0.0993±0.08a	0.0751±0.05b	0.006±0.00cb	0.2464±0.03a	0.0187±0.02b

Table 14. Effects of cold stress on net photosynthesis rate (Pn), stomatal conductance (Gs), and transpiration (Tr) of *Bougainvillea glabra* (C). Mean value ±Standard deviation are showed. Different letters within a row indicate significant difference between treatments of Photosynthesis (p‹0.05 LSD test)

	Gas exchange parameters of cultivar C (μmol $m^{-2}s^{-1}$,mmol $m^{-2}s^{-1}$, mmol $m^{-2}s^{-1}$)				
	Tr-1	Tr-2	Tr-3	Tr-4	Tr-5
Pn	4.3247±0.69b	5.9081±1.11a	1.5131±0.22c	3.73±0.53b	3.2911±0.49b
Gs	0.0036±0.00b	0.0053±0.00b	2.9258±2.55a	0.0083±0.00b	0.002±0.00b

Tr	0.1012±0.02b	0.143±0.08b	0.0021±0.00c	0.2159±0.04ab	0.0324±0.00bdc

Table 15. Effects of cold stress on net photosynthesis rate (Pn), stomatal conductance (Gs), and transpiration (Tr) of *Bougainvillea glabra* (D). Mean value ±Standard deviation are showed. Different letters within a row indicate significant difference between treatments of Photosynthesis (p‹0.05 LSD test)

	Gas exchange parameters of cultivar D (μmol $m^{-2}s^{-1}$,mmol $m^{-2}s^{-1}$,mmol $m^{-2}s^{-1}$)				
	Tr-1	Tr-2	Tr-3	Tr-4	Tr-5
Pn	4.686±2.09a	2.7196±1.10a	2.5984±1.04a	4.9549±0.95ab	3.1102±0.13a
Gs	0.0079±0.00a	0.0029±0.00b	0.0003±0.00d	0.0068±0.00a	0.0022±0.00c
Tr	0.2294±0.10a	0.0786±0.05b	0.0075±0.00db	0.1798±0.08ab	0.0346±0.00cb

Table 16. Effects of cold stress on net photosynthesis rate (Pn), stomatal conductance (Gs), and transpiration (Tr) of *Bougainvillea glabra* (E). Mean value ±Standard deviation are showed. Different letters within a row indicate significant difference between treatments of Photosynthesis (p‹0.05 LSD test)

E	Gas exchange parameters of cultivar E (μmol $m^{-2}s^{-1}$,mmol $m^{-2}s^{-1}$,mmol $m^{-2}s^{-1}$)				
	Tr-1	Tr-2	Tr-3	Tr-4	Tr-5
Pn	3.265±1.23a	3.3055±0.8a	2.407±0.42a	3.6427±2.62a	2.7445±0.51a
Gs	0.0043±0.00b	0.0024±0.01b	0.8176±1.42b	0.0058±0.01b	0.0013±0.00b
Tr	0.1303±0.03a	0.0651±0.02a	0.0044±0.00a	0.1544±0.15ab	0.0202±0.01a

Table 17. Effects of cold stress on net photosynthesis rate (Pn), stomatal conductance (Gs), and transpiration (Tr) of Bougainvillea glabra (F). Mean value ±Standard deviation are showed. Different letters within a row indicate significant difference between treatments of Photosynthesis (p‹0.05 LSD test)

	Gas exchange parameters of cultivar F (μmol $m^{-2}s^{-1}$,mmol $m^{-2}s^{-1}$,mmol $m^{-2}s^{-1}$)				
	Tr-1	Tr-2	Tr-3	Tr-4	Tr-5
Pn	4.9328±2.39a	4.8012±1.39a	2.1826±0.04a	5.2952±1.63ab	3.1771±1.57a
Gs	0.0061±0.00b	0.0039±0.00b	0.9905±1.72b	0.0089±0.00b	0.0017±0.00b
Tr	0.1861±0.12a	0.1023±0.03a	0.0035±0.00ca	0.2363±0.03ad	0.0271±0.03bca

Table 18. Effects of cold stress on net photosynthesis rate (Pn), stomatal conductance (Gs) and transpiration (Tr) of *Bougainvillea glabra* (G). Mean value ±Standard deviation are showed. Different letters within a row indicate significant difference between treatments of Photosynthesis (p‹0.05 LSD test)

	Gas exchange parameters of cultivar G (μmol $m^{-2}s^{-1}$,mmol $m^{-2}s^{-1}$,mmol $m^{-2}s^{-1}$)				
	Tr-1	Tr-2	Tr-3	Tr-4	Tr-5
Pn	6.0235±1.56a	5.8671±0.18a	4.7713±1.66a	5.2134±0.29a	4.0546±2.93a
Gs	0.0062±0.00b	0.0038±0.00b	0.0024±0.00b	0.0063±0.00b	61.3386±106.23b
Tr	0.1924±0.06d	0.0994±0.02d	0.0583±0.05d	0.1679±0.02d	0.2392±0.29d

Table 19. Effects of cold stress on net photosynthesis rate (Pn), stomatal conductance (Gs), and transpiration (Tr) of *Bougainvillea glabra* (H). Mean value ±Standard deviation are showed. Different letters within a row indicate significant difference between treatments of Photosynthesis (p‹0.05 LSD test)

	Gas exchange parameters of cultivar H (μmol $m^{-2}s^{-1}$,mmol $m^{-2}s^{-1}$, mmol $m^{-2}s^{-1}$)				
	Tr-1	Tr-2	Tr-3	Tr-4	Tr-5
Pn	8.5556±3.09a	8.7662±1.26a	4.6634±2.21a	7.1324±3.19a	2.2458±1.77ba
Gs	0.0092±0.00a	0.0085±0.00a	0.0024±0.00b	0.0094±0.00a	0.0013±0.00b
Tr	0.2882±0.11a	0.2229±0.41a	0.056±0.05b	0.2568±0.08ba	0.0227±0.02cb

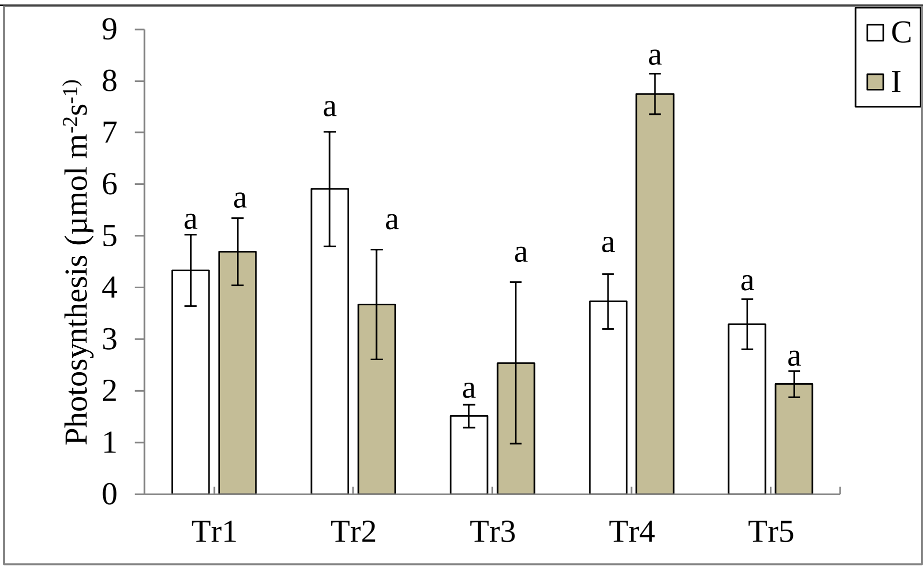
Table 20. Effects of cold stress on net photosynthesis rate (Pn), stomatal conductance (Gs), and transpiration (Tr) of *Bougainvillea glabra* (I). Mean value ±Standard deviation are showed. Different letters within a row indicate significant difference between treatments of Photosynthesis (p‹0.05 LSD test)

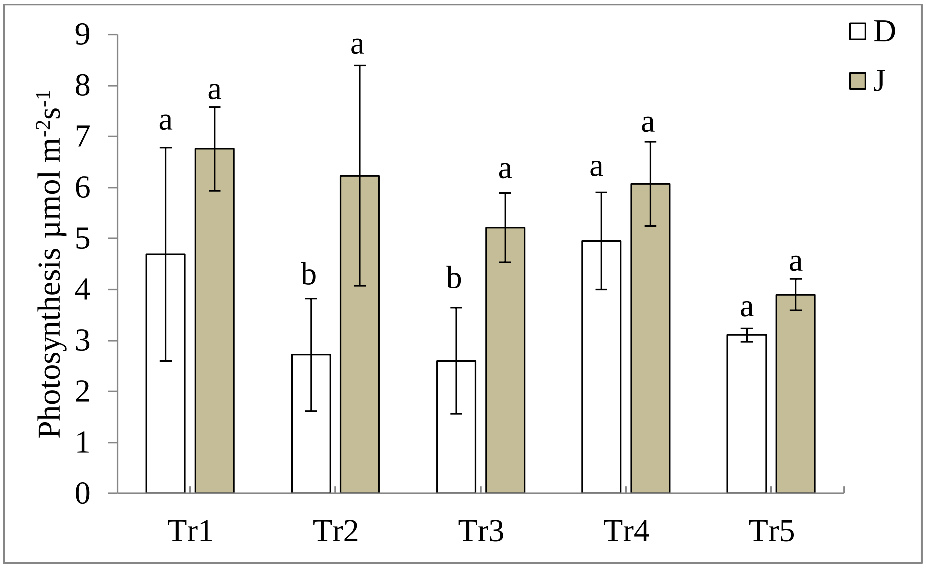
	Gas exchange parameters of cultivar I (μmol $m^{-2}s^{-1}$,mmol $m^{-2}s^{-1}$,mmol $m^{-2}s^{-1}$)				
	Tr-1	Tr-2	Tr-3	Tr-4	Tr-5
Pn	4.6856±0.65b	3.6714±1.06b	2.5393±1.56cb	7.7466±0.39a	2.1254±0.25db
Gs	0.0051±0.00b	0.0028±0.00c	0.0005±0.00e	0.0119±0.00a	0.0022±0.00dc
Tr	0.1702±0.02b	0.0749±0.02c	0.0116±0.00e	0.3281±0.03a	0.0417±0.01dce

Table 21. Effects of cold stress on net photosynthesis rate (Pn), stomatal conductance (Gs) and transpiration (Tr) of *Bougainvillea glabra* (J). Mean value ±Standard deviation are showed. Different letters within a row indicate significant difference between treatments of Photosynthesis (p‹0.05 LSD test)

Gas exchange parameters of cultivar J (μmol $m^{-2}s^{-1}$,mmol $m^{-2}s^{-1}$,mmol $m^{-2}s^{-1}$)

	Tr-1	Tr-2	Tr-3	Tr-4	Tr-5
Pn	6.7561±0.82a	6.2306±2.16a	5.2058±0.68a	6.0699±0.83a	3.9032±0.31ba
Gs	0.0095±0.00a	0.0092±0.00a	0.0012±0.00c	0.0075±0.00a	0.0035±0.00bc
Tr	0.322±0.06b	0.2463±0.09b	0.0261±0.00a	0.2101±0.05a	0.2907±0.36b





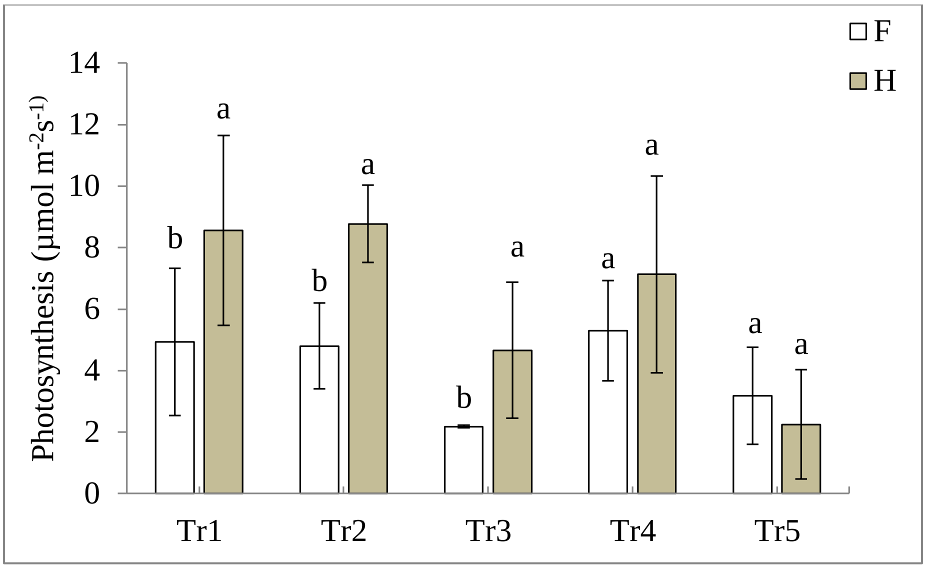


Figure 8. Effects of cold stress on Photossynthesis rate (Pn) of same cultivars *Bougainvillea glabra* cultivars. Mean value ±Standard deviation are showed. Different letters within a row indicate significant difference between treatments of Photosynthesis (p‹0.05 LSD test)

6. Discussion

Bougainvillea glabra is used in landscapes beautification, horticulture, pharmaceutical industries and environmental industries. Moreover, this can more tolerate cold stress among other bougainvillea species. Natural cooling stress affects virtually all aspects of cellular function in plants. Membrane disintegration is one major influence of cold stress-induced dehydration and such changes caused by low temperature stress adversely affect the growth and development of plants. According to(Bray et al., 2000), plant's resistance and sensitivity depends on the genotype, species and age of the plants. In this study, the results of natural cooling among *Bougainvillea glabra* species are compared on the physiological parameters between treatments and species. The main finding was that under natural cooling conditions three years old cultivars are not affected as REC value is not higher than the first experiment in Figure-3. Moreover, the results show that REC of three year old cultivars is decreased 23.5% , 23.03%, 51.11% in H, I, J while two year old cultivar is increased 49.66%, 8.41%, 14.02%, 10.19%, 15.99% in A, B, C, D, F within the same cultivars as shown in Figure(4). Among the same cultivars of different age, cultivar D and J are significantly different as shown in Figure (4). Although cultivars F and H, C and I are not significantly different, the values of REC respond like the same of others. For other two year old cultivars, REC is increased about 47.9-66.6% in A, 1.3-24.9% in B, 14-45% in C, 5-17% in D and 15.9-24.1% in F cultivar with the low temperature stress although the results show mostly not significant. So, the results were consistent with previous studies that the electrolyte leakage in leaves gradually increased under low temperature stress which means the damage of low temperature on cell membrane increased gradually(Liu et al., 2013). Therefore, in two years old cultivars, A cultivar is affected most, followed by F, C, D and B respectively. However, studying on abiotic stress alone can be misleading, although it is useful, as the plants response to individual stress and abiotic stress are not the same (Chinnusamy et al., 2004). Low temperature stress often causes damage to cell membranes. Therefore, the cell-membrane penetrability is tested. Cell membrane penetrability was assessed by the relative conductance of the cell membrane under low temperature stress(Rohde et al., 2004)(Steponkus et al., 1990). The electrolyte leakage test was performed to compare membrane integrity (MURRAY et al., 1989). For that experiment, plants were subjected to naturally low temperature conditions.

Chlorophyll is an extremely important and critical biomolecule in photosynthesis with function of light absorbance and light energy transformation(Xu et al., 2000). Low temperature stress can influent plant photosynthesis and decrease the utilization of light (Glaszmann et al., 1990)(Wu et al., 1997). Moreover, reduction in photosynthetic pigments content caused by cold stress may contribute to photosynthesis decreasing. The chlorophyll content and photosynthesis rate is complicated with the natural air temperature condition. In this research, the contents of chlorophyll were evaluated under naturally low temperature stress condition. Compared with the first experiment, the chlorophyll content (chlorophyll a, chlorophyll b, carotenoids and total chlorophyll) in seedling leaves of last experiment in cultivars B, C, E, G, I and J under low temperature was decreased. But, chlorophyll a and carotenoid of cultivar A, carotenoid in F and H were increased while cultivar D was significantly increased 17.91% in chlorophyll a, 19.23% in chlorophyll b, 43.33% in carotenoid and 20% in total chlorophyll content. Under natural cooling stress condition, total chlorophyll content of almost all cultivars was decreased. This is consistent with the finding by (Liu et al., 2013) that the chlorophyll content in seedling leaves of Avena nuda L., also named naked oats under low temperature was lower than that under room temperature. Maximum decreased was recorded in cultivar E, followed by G, C, F and B. In comparison with the same cultivars of different age, all chlorophyll contents are significantly different in cultivar C and I, D and J. Moreover, this research shows that younger age more suffer for decreasing the content of total chlorophyll.

Low temperature is one of the major factors that may limit the activity of photosynthesis. Since the cold affects thylakoid electron transport, control of stomatal conductance and carbon fixation, decreasing of photosynthesis rates caused by low temperature is a well-known response of chilling sensitive plants (Yadegari et al., 2007). Photosynthesis is interrupted just above the freezing points as the susceptibility of thylakoids with cold in low temperature sensitive plants. Plants contain pigments like chlorophyll and carotenoid to absorb light and provide energy for photosynthesis(Blackburn, 1998).Chlorophyll is the main nitrogenous substance which can be used to measure the plant growth(Xue & Yang, 2009) (Kochubey & Kazantsev, 2007). (Kerdnaimongkol et al., 1997)observed significant reductions in net CO_2 assimilation rates and gs in chilled tomato (Solanum lycopersicum) plants compared to the nonchilled transplants. This current research is consistent with this finding as Gs is also decreased in cultivars C, I, D, J And H. (W. H. Hu et al., 2010) reported a chill-induced inhibition of photosynthesis (lower Pn and gs) in cucumber leaves. Net photosynthetic rate (Pn) is reduced by stomatal and non-stomatal limitations or both (Saibo et al., 2009). A decline in intercellular CO_2 concentration (Ci) indicates that the main cause of decline in leaf photosynthetic rate is a decrease in stomatal conductance (Gs) (Pessarakli et al., 2001)(Jones, 2013)(Xu and Shen, 2001;Jones, 2014). The

Pn, Gs and Tr values of cultivars A, C, D, E, F, H, I and J in last experiment after low temperature stress were decreased compared to first experiment while cultivar B is increasing its photosynthesis rate and G is also at it's stomatal conductance. The leaf Pn, Gs, Tr of zoysiagrass remarkably declined with the temperature reduction(S. Li et al., 2018). The results of current research are in good agreement with this finding.

7. Conclusion

Bougainvillea glabra cultivars showed different response to low temperature stress probably due to the level of low temperature tolerance. Other uncontrollable factors may affect on plants' resistance to low temperature stress under natural conditions. The low temperature stress depends on the tolerance of the species, exposure time, chilling rate and other related factors. In the present study, the basic physiological response in *Bougainvillea glabra* cultivars were investigated under natural cooling. According to this current research, the following conclusion can be drawn; three years old Bougainvillea glabra is more resistant to naturally low temperature stress than the two years old. Therefore, this study provided theoretical basis and positive contribution to the cultivation and management of these different *Bougainvillea glabra* cultivars under natural cooling conditions.

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9. References

Adams, W. W., Stewart, J. J., Cohu, C. M., Muller, O., & Demmig-Adams, B. (2016). Habitat temperature and precipitation of Arabidopsis Thaliana ecotypes determine the response of foliar vasculature, photosynthesis, and transpiration to growth temperature. *Frontiers in Plant Science*. https://doi.org/10.3389/fpls.2016.01026

Allen, D. J., & Ort, D. R. (2001). Impacts of chilling temperatures on photosynthesis in warm-climate plants. In *Trends in Plant Science* (pp. 121–129). https://doi.org/10.1016/S1360-1385(00)01808-2

Ashraf, M., & Foolad, M. R. (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and Experimental Botany, 59*, 206–216. https://doi.org/10.1016/j.envexpbot.2005.12.006

Beck, E. H., Fettig, S., Knake, C., Hartig, K., & Bhattarai, T. (2007). Specific and unspecific responses of plants to cold and drought stress. *Journal of Biosciences*. https://doi.org/10.1007/s12038-007-0049-5

Bettaieb, T., Mahmoud, M., Galarreta, J. I. R. de, & Jardin, P. Du. (2007). Relation between the low temperature stress and catalase activity in gladiolus somaclones (Gladiolus grandiflorus Hort.). *Scientia Horticulturae, 113*, 49–51. https://doi.org/10.1016/j.scienta.2007.01.007

Blackburn, G. A. (1998). Spectral indices for estimating photosynthetic pigment concentrations: A test using senescent tree leaves. *International Journal of Remote Sensing*. https://doi.org/10.1080/014311698215919

Bloom, A. J., Zwieniecki, M. A., Passioura, J. B., Randall, L. B., Holbrook, N. M., & St. Clair, D. A. (2004). Water relations under root chilling in a sensitive and tolerant tomato species. *Plant, Cell and Environment*, *27(8)*, 971–979. https://doi.org/10.1111/j.1365-3040.2004.01200.x

Boese, S. R., Wolfe, D. W., & Melkonian, J. J. (1997). Elevated CO2 mitigates chilling-induced water stress and photosynthetic reduction during chilling. *Plant, Cell and Environment*, *20(5)*, 625–632. https://doi.org/10.1111/j.1365-3040.1997.00082.x

Boyer, J. S. (1982). Plant productivity and environment. *Science, 218*, 443–448. https://doi.org/10.1126/science.218.4571.443

Bray, E. A., Bailey-Serres, J., & Weretilnyk, E. (2000). Responses to abiotic stresses. *Biochemistry and Molecular Biology of Plants*. https://doi.org/10.1016/j.ibmb.2010.02.006

Bressan, R., Bohnert, H., & Zhu, J. K. (2009). Perspective: Abiotic stress tolerance: From gene discovery in model organisms to crop improvement. *Molecular Plant*, *2(1)*, 1–2. https://doi.org/10.1093/mp/ssn097

Bridger, G. M., Yang, W., Falk, D. E., & McKersie, B. D. (1994). Cold Acclimation Increases Tolerance of Activated Oxygen in Winter Cereals. *Journal of Plant Physiology*, *144*, 235–240. https://doi.org/10.1016/S0176-1617(11)80549-3

Chinnusamy, V., Schumaker, K., & Zhu, J. K. (2004). Molecular genetic perspectives on cross-talk and specificity in abiotic stress signalling in plants. *Journal of Experimental Botany*. https://doi.org/10.1093/jxb/erh005

Cohu, C. M., Muller, O., Adams, W. W., & Demmig-Adams, B. (2014). Leaf anatomical and photosynthetic acclimation to cool temperature and high light in two winter versus two summer annuals. *Physiologia Plantarum*. https://doi.org/10.1111/ppl.12154

Distelbarth, H., Nägele, T., & Heyer, A. G. (2013). Responses of antioxidant enzymes to cold and high light are not correlated to freezing tolerance in natural accessions of Arabidopsis thaliana. *Plant Biology*, *15*, 982–990. https://doi.org/10.1111/j.1438-8677.2012.00718.x

Ensminger, I., Busch, F., & Huner, N. P. A. (2006). Photostasis and cold acclimation: Sensing low temperature through photosynthesis. In *Physiologia Plantarum* (pp. 28–44). https://doi.org/10.1111/j.1399-3054.2006.00627.x

Gesch, R. W., & Heilman, J. L. (1999). Responses of photosynthesis and phosphorylation of the light-harvesting complex of photosystem II to chilling temperature in ecologically divergent cultivars of rice. *Environmental and Experimental Botany*, *41(3)*, 257–266. https://doi.org/10.1016/S0098-8472(99)00008-8

Glaszmann, J. C., Kaw, R. N., & Khush, G. S. (1990). Genetic divergence among cold tolerant rices (Oryza sativa L.). *Euphytica*, *45(2)*, 95–104. https://doi.org/10.1007/BF00033276

Grace, S. C., Logan, B. A., & Adams, W. W. (1998). Seasonal differences in foliar content of chlorogenic acid, a phenylpropanoid antioxidant, in Mahonia repens. *Plant, Cell and Environment*, *21(5)*(1998), 513–521. https://doi.org/10.1046/j.1365-3040.1998.00282.x

Guy, C., Kaplan, F., Kopka, J., Selbig, J., & Hincha, D. K. (2008). Metabolomics of temperature stress. In *Physiologia Plantarum* (pp. 220–235). https://doi.org/10.1111/j.1399-3054.2007.00999.x

Hasanuzzaman, M., Nahar, K., Alam, M. M., Roychowdhury, R., & Fujita, M. (2013). Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. In *International Journal of Molecular Sciences* (pp. 9643–9684). https://doi.org/10.3390/ijms14059643

Holaday, A. S., Martindale, W., Aired, R., Brooks, A. L., & Leegood, R. C. (1992). Changes in activities of enzymes of carbon metabolism in leaves during exposure of plants to low temperature. *Plant Physiology*, *98(3)*, 1105–1114. https://doi.org/10.1104/pp.98.3.1105

Hu, L., Wang, Z., Du, H., & Huang, B. (2010). Differential accumulation of dehydrins in response to water stress for hybrid and common bermudagrass genotypes differing in drought tolerance. *Journal of Plant Physiology*, *167*, 103–109. https://doi.org/10.1016/j.jplph.2009.07.008

Hu, W. H., Wu, Y., Zeng, J. Z., He, L., & Zeng, Q. M. (2010). Chill-induced inhibition of photosynthesis was alleviated by 24-epibrassinolide pretreatment in cucumber during chilling and subsequent recovery. *Photosynthetica*. https://doi.org/10.1007/s11099-010-0071-y

Janda, T., Szalai, G., Kissimon, J., Paldi, E., Marton, C., & Szigeti, Z. (1994). Role of irradiance in the chilling injury of young maize plants studied by chlorophyll fluorescence induction measurements. *Photosynthetica*, *30(2)*, 293–299.

Jones, H. G. (2013). Plants and microclimate: A quantitative approach to environmental plant physiology. In *Plants and Microclimate: A Quantitative Approach to Environmental Plant Physiology*. https://doi.org/10.1017/CBO9780511845727

Karimzadeh, G., Sharifi-Sirchi, G. R., Jalali-Javaran, M., Dehghani, H., & Francis, D. (2006). Soluble proteins induced by low temperature treatment in the leaves of spring and winter wheat cultivars. *Pakistan Journal of Botany*, *38(4)*, 1015–1026.

Kerdnaimongkol, K., Bhatia, A., Joly, R. J., & Woodson, W. R. (1997). Oxidative stress and diurnal variation in chilling sensitivity of tomato seedlings. *Journal of the American Society for Horticultural Science*. https://doi.org/10.21273/jashs.122.4.485

Kochubey, S. M., & Kazantsev, T. A. (2007). Changes in the first derivatives of leaf reflectance spectra of various plants induced by variations of chlorophyll content. *Journal of Plant Physiology*. https://doi.org/10.1016/j.jplph.2006.11.007

Larcher, W. (2001). Ökophysiologie der Pflanzen: Leben, Leistung und Streßbewältigung der Pflanzen in ihrer Umwelt. *Zeitschrift Für Pflanzenernährung Und Bodenkunde*, 302. https://doi.org/10.1038/ng.520.New

Li, S., Yang, Y., Zhang, Q., Liu, N., Xu, Q., & Hu, L. (2018). Differential physiological and metabolic response to low temperature in two zoysiagrass genotypes native to high and low latitude. *PLoS ONE*. https://doi.org/10.1371/journal.pone.0198885

Li, X. G., Wang, X. M., Meng, Q. W., & Zou, Q. (2004). Factors limiting photosynthetic recovery in sweet pepper leaves after short-term chilling stress under low irradiance. *Photosynthetica*, *42(2)*, 257–262. https://doi.org/10.1023/B:PHOT.0000040598.48732.af

Lichtenthaler, H. K. (1987). Chlorophylls and Carotenoids: Pigments of Photosynthetic Biomembranes. *Methods in Enzymology*. https://doi.org/10.1016/0076-6879(87)48036-1

Liu, W., Yu, K., He, T., Li, F., Zhang, D., & Liu, J. (2013). The low temperature induced physiological responses of avena nuda L., a cold-tolerant plant species. *The Scientific World Journal*. https://doi.org/10.1155/2013/658793

Lyons, J. M. (1973). Chilling Injury in Plants. *Annual Review of Plant Physiology*, *24(1)*(1973), 445–466. https://doi.org/10.1146/annurev.pp.24.060173.002305

Mancuso, S. (2000). Electrical resistance changes during exposure to low temperature measure chilling and freezing tolerance in olive tree (Olea europaea L.) plants. *Plant, Cell and Environment*, *23*, 291–299. https://doi.org/10.1046/j.1365-3040.2000.00540.x

Muller, O., Stewart, J. J., Cohu, C. M., Polutchko, S. K., Demmig-Adams, B., & Adams, W. W. (2014). Leaf architectural, vascular and photosynthetic acclimation to temperature in two biennials. *Physiologia Plantarum*, *152(4)*(2014), 763–772. https://doi.org/10.1111/ppl.12226

MURRAY, M. B., CAPE, J. N., & FOWLER, D. (1989). Quantification of frost damage in plant tissues by rates of electrolyte leakage. *New Phytologist*, *113(3)*, 307–311. https://doi.org/10.1111/j.1469-8137.1989.tb02408.x

Pan, S., Liu, H., Mo, Z., Patterson, B., Duan, M., Tian, H., Hu, S., & Tang, X. (2016). Effects of Nitrogen and Shading on Root Morphologies, Nutrient Accumulation, and Photosynthetic Parameters in Different Rice Genotypes. *Scientific Reports*. https://doi.org/10.1038/srep32148

Pessarakli, M., Shen, Y.-K., & Xu, D.-Q. (2001). Photosynthetic Efficiency and Crop Yield. In *Handbook of Plant and Crop Physiology*. https://doi.org/10.1201/9780203908426.pt10

Rohde, P., Hincha, D. K., & Heyer, A. G. (2004). Heterosis in the freezing tolerance of crosses between two Arabidopsis thaliana accessions (Columbia-0 and C24) that show differences in non-acclimated and acclimated freezing tolerance. *Plant Journal*, *38(5)*, 790–799. https://doi.org/10.1111/j.1365-313X.2004.02080.x

Saibo, N. J. M., Lourenço, T., & Oliveira, M. M. (2009). Transcription factors and regulation of photosynthetic and related metabolism under environmental stresses. In *Annals of Botany*. https://doi.org/10.1093/aob/mcn227

Sakai, A., & Larcher, W. (1987). Frost survival of plants: responses and adaptation to freezing stress. In *Ecological studies*.

Srichaikul, B., Bunsang, R., Samappito, S., Butkhup, L., & Bakker, G. (2011). Comparative Study of Chlorophyll Content in Leaves of Thai Morus alba Linn. Species. *Plant Sciences Research*, *3*, 17–20. https://doi.org/10.3923/psres.2011.17.20

Steponkus, P. L. (1984). Role of the Plasma Membrane in Freezing Injury and Cold Acclimation. *Annual Review of Plant Physiology*, *35*, 543–584. https://doi.org/10.1146/annurev.pp.35.060184.002551

Steponkus, P. L., Lynch, D. V., Uemura, M., Heber, U., & Pearce, R. S. (1990). The Influence of Cold Acclimation on the Lipid Composition and Cryobehaviour of the Plasma Membrane of Isolated Rye Protoplasts [and Discussion]. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *326(1237)*, 571–583. https://doi.org/10.1098/rstb.1990.0032

Strauss, A. J., Krüger, G. H. J., Strasser, R. J., & Van Heerden, P. D. R. (2007). The role of low soil temperature in the inhibition of growth and PSII function during dark chilling in soybean genotypes of contrasting tolerance. *Physiologia Plantarum*, *131(1)*, 89–105. https://doi.org/10.1111/j.1399-3054.2007.00930.x

Van Heerden, P. D. R., Krüger, G. H. J., Loveland, J. E., Parry, M. A. J., & Foyer, C. H. (2003). Dark chilling imposes metabolic restrictions on photosynthesis in soybean. *Plant, Cell and Environment*, *26(2)*, 323–337. https://doi.org/10.1046/j.1365-3040.2003.00966.x

Vernieri, P., Pardossi, A., & Tognoni, F. (1991). Influence of Chilling and Drought on Water Relations and Abscisic Acid Accumulation in Bean. *Functional Plant Biology*, *18(1)*, 25–35. https://doi.org/10.1071/pp9910025

Wang, X., Peng, Y., Singer, J. W., Fessehaie, A., Krebs, S. L., & Arora, R. (2009). Seasonal changes in photosynthesis, antioxidant systems and ELIP expression in a thermonastic and non-thermonastic Rhododendron species: A comparison of photoprotective strategies in overwintering plants. *Plant Science*, *177*, 607–617. https://doi.org/10.1016/j.plantsci.2009.08.009

Wu, J., Lightner, J., Warwick, N., & Browse, J. (1997). Low-temperature damage and subsequent recovery of fab1 mutant arabidopsis exposed to 2°C. *Plant Physiology*, *113(2)*, 347–356. https://doi.org/10.1104/pp.113.2.347

Xu, W., Rosenow, D. T., & Nguyen, H. T. (2000). Stay green trait in grain sorghum: Relationship between visual rating and leaf chlorophyll concentration. *Plant Breeding, 119(4)*, 365–367. https://doi.org/10.1046/j.1439-0523.2000.00506.x

Xue, L., & Yang, L. (2009). Deriving leaf chlorophyll content of green-leafy vegetables from hyperspectral reflectance. *ISPRS Journal of Photogrammetry and Remote Sensing*. https://doi.org/10.1016/j.isprsjprs.2008.06.002

Yadegari, L. Z., Heidari, R., & Carapetian, J. (2007). The influence of cold acclimation on proline, malondialdehyde (MDA), total protein and pigments contents in soybean (Glycine max) seedlings. *Journal of Biological Sciences*. https://doi.org/10.3923/jbs.2007.1436.1441

Zhao, Y., Du, H., Wang, Z., & Huang, B. (2011). Identification of proteins associated with water-deficit tolerance in C4 perennial grass species, Cynodon dactylon×Cynodon transvaalensis and Cynodon dactylon. *Physiologia Plantarum*, *141*, 40–55. https://doi.org/10.1111/j.1399-3054.2010.01419.x