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Full Length Article

Effects of Shading Treatments on Pigmentation and Inflorescence Quality of *Calathea crotalifera* Bracts

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Abstract

The pigment composition and colouration of inflorescence bracts of *Calathea crotalifera* were evaluated in this study. The concentration of anthocyanin and chlorophyll were quantified during bract development in two selected cultivars (Red and Yellow). The results of this study indicate that the concentration of different pigments (chlorophyll a and b, carotenoids and anthocyanins) varied according to bract maturity stage. The photosynthetic pigments of chlorophylls were increased at the early stage of inflorescence development and significantly decreased during the fully pigmented stage simultaneous with an increase in either carotenoids or anthocyanins. The chlorophyll contents start to increase when the inflorescence showed a discolouration of red and yellow pigments in the bracts. The major pigments in the fully pigmented yellow bract were carotenoids while the main composition pigments in red bracts were anthocyanins and carotenoids. In the investigation of shading treatments, we found that shading significantly reduced chlorophylls, carotenoids and anthocyanins contents in the bracts. The highest pigment contents were recorded in the control treatment (without shading) followed by shading at 40% and 80%. Relatively, control treatment gave the best result for growth and development of *C. crotalifera* cv. 'Red' inflorescence in terms of colour, number and size of bract, and inflorescence yield. The inflorescence length, diameter and colouration decreased significantly in shading treatments (40% and 80%) as compared with control. Overall, these works highlight the positive effects of control treatments on the development and quality of cut flowers of *C. crotalifera* under tropical climate in Malaysia.

Keywords: Anthocyanins; *Calathea crotalifera*; Carotenoids; Chlorophyll; Developmental stages; Shading treatments

Introduction

Calathea crotalifera is one of the important ornamental plants belonging to Marantaceae family. This rhizomatic plant species can produce a very attractive inflorescence and have been used in landscaping especially for screening and indoor plants. The other species in *Calathea* genus was also widely used in the horticulture industry due to its attractive foliar colours and variegation patterns (Yang and Yeh, 2008; Perera *et al*., 2009). The exotic appearance of *C. crotalifera* inflorescence that known as the rattle shaker flower makes it more popular in cut flower industry (Meon, 2013). The inflorescence has attractive bracts (modified leaf) that can be found in four different colours as red, white, green or yellow with a few conspicuous flowers will peek out from the bract as they matured. The vase life of the inflorescence can vary considerably (15 to 30 days) like the other exotic tropical ornamentals such as *Heliconia* sp., *Strelitzia* sp. and *Alpinia* sp. (Paull and Chantrachit, 2001). Various cultivars of *C. crotalifera* have been introduced in the floriculture business mainly differentiated by the size and pigments colouration of bracts. However, there is limited information about the relationships between bract colour and pigment composition during the inflorescence development in this ornamental rhizomatic species.

Theoretically, the presence of pigments in the vacuoles of the epidermal cells was contributed to a broad spectrum of plant tissues colours. The major classes of these natural plant pigments include anthocyanins, betalains and photosynthetic pigment of carotenoids and chlorophylls. The combination of anthocyanin and/or carotenoid, or betalains alone can be attributed to the flower colouration in the range of yellow, orange-red to red as reported in the genera *Euphorbia sp.* (Slatnar *et al.*, 2013), *Rosa sp.* (Schmitzer *et al.*, 2010), and *Gerbera sp.* (Meng *et al.*, 2004). A similar pattern of flower colour and pigmentation have also been reported in a few ornamental rhizomatic plants. Mangave *et al.* (2014) reported the analysis of carotenoid during the post-harvest study of heliconia, and Pirone *et al.* (2010) demonstrated the presence of carotenoids, anthocyanin and bilirubin (an animal pigment) in the floral and aril parts of *Strelitzia* sp. In general, these natural plant pigments (anthocyanins, carotenoids and betalains) act as visible signals to attract pollinators and seed dispersers and also protect plants against abiotic and biotic stresses (Tanaka *et al.*, 2008).

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Study on pigment contents during the inflorescence development had been reported in several important ornamental species like poinsettia (Slatnar *et al*., 2013), rose (Dela *et al*., 2003; Schmitzer *et al*., 2010), orchid (Tatsuzawa *et al.*, 2010), daisy (Meng *et al*., 2004), and petunia (Moscovici *et al.*, 1996) and found that anthocyanin biosynthesis and pigmentation are regulated by environmental stresses and flower developmental stage (Martin and Gerats, 1993). The effect of transient high temperature and progression of flower development on the concentration and composition of anthocyanins in rose petals have been previously reported (Dela *et al*., 2003; Schmitzer *et al*., 2010). The impact of different quality and intensity of light on flower pigmentation and anthocyanin accumulation in horticulture field have been demonstrated in daisy (Meng *et al*., 2004) and red pears (Qian *et al*., 2013), respectively. Furthermore, Mol *et al.* (1996) and Petroni and Tonelli (2011) have reviewed that the biosynthesis of anthocyanin is also regulated by nutrient supply. Based on these findings, it seems that pigment biosynthesis in *C. crotalifera* bracts is similarly regulated.

In this study, we attempt to investigate the pigments accumulation in the inflorescence bracts of two different cultivars of *C. crotalifera* (cv. Red and cv. Yellow) during mature or fully pigmented bracts. The comparative study of this work will be used to determine the different pigmentation patterns between red cultivar and yellow cultivar, the total content of anthocyanin, and also photosynthetic pigments with the progression of flower development. This study will also determine the effect of shading on pigments accumulation in the bracts of red cultivar inflorescence by using three levels of shading (non-shade, 40% and 80%). This project is a pioneering study aimed to develop a better understanding of inflorescence quality of *C. crotalifera* grew in Malaysia as influenced by shading treatments.

Materials and Methods

Plant Material

The mother plants of yellow and red cultivar of *C. crotalifera* were obtained from a commercial nursery located in Kuantan, Pahang, Malaysia. All of these plants were cultivated in the open field with 90% of sunlight at mid-day. The plants were maintained under natural environment and natural photoperiod. The plants were irrigated with the tap water twice a day and fertilized with organic fertilizer (chicken manure) once a month to induce and maintain the plant growth. The flower inducing fertilizer with NPK ratio at 15:15:15 was used to induce flowering of the plant during a hot season started from April 2014 until August 2014. The collection of inflorescences was begun in June 2014.

Determination of Different Stage of Inflorescence Development

Two distinct cultivars of *C. crotalifera* (Red and Yellow) were used in this study. The different stages of development of inflorescence were determined as shown in Table 1 and Fig. 1. Three inflorescences from each plant (15 per cultivar) were analysed for pigment identification at four different stages within eight weeks. Extraction of chlorophyll, carotenoid and anthocyanin pigments in the inflorescence bract were analysed on each week. The differences in measured parameters between both cultivars were analysed in fully pigmented bracts at Stage S3 as shown in Fig. 2.

Effect of Shading Treatment

The *C. crotalifera* cv. Red was used in this study. The plants were put under natural sunlight at three different level of shading. The experimental site of each shading level were covered entirely with high density polyethylene black shading net (Brand: Tengfei; ISO standard quality) as follows the treatments: T1 (80% shading with four layers of shading net), T2 (40% shading with two layers of shading net) and T3 (without shading net as control). The experiments consist of three treatments including control with three plants for each treatment. The plants were maintained under natural environment and natural photoperiod. The plants were irrigated with the tap water twice a day and fertilized with organic fertilizer (chicken manure) every three weeks to induce and maintain the plant growth. Three inflorescences at Stage S3 (5-6 weeks) from each plant (15 per cultivar) were analysed for pigment identification at different shading treatments. The development and quality of the inflorescence were determined by measure the size and counting the total number of an inflorescence produced at the time of harvesting.

Extraction and Determination of Photosynthetic Pigments

The photosynthetic pigment including chlorophyll a (Ch *a*), chlorophyll b (Ch *b*) and carotenoid (Cr) in the bract were determined according to the method by Wellburn (1994). The fresh bract as shown in Fig. 2 was ground in the mortar with dimethyl sulphoxide (DMSO) solution at ratio 1:10 with 1 g of $CaCO_3$ powder. Another half volume of DMSO solution was added and mashed again for better extraction. The samples were poured into the test tube and incubated in the water bath at 50°C in the dark for 1 h. The samples were filtered using Whatman qualitative filter paper Grade 1. The collected supernatants were centrifuge at 1700 *g* for 5 min. The clear supernatant was decanted into the cuvette vessel and the adsorption was analysed using UV-spectrophotometer (UV-VIS Spectrophotometer Model

Libra SII) at 648 nm (Ch *b*), 666 nm (Ch *a*) and 480 nm (Cr). The concentration of each pigment was calculated using Wellburn Equation (Wellburn, 1994) as follows:

Chlorophyll *a* (µg/mL), Ch $a = 10.91A_{666} - 1.2A_{648}$
Chlorophyll *b* (µg/mL), Ch $b = 16.36A_{648} - 4.57A_{666}$
Carotenoid (µg/mL), Cr = $(1000A_{480}–1.42Ca–46.09Cb)/202$.

Extraction and Determination of Anthocyanin Pigments

The total anthocyanin concentration was determined using a pH differential method. The pigment concentration was expressed as cyaniding-3-glucoside (cyd-3-glu) equivalents in mg/L (Lee *et al*., 2005). The pigment was extracted from 1.0 g of fresh inflorescence bracts were ground in the mortar with 20 mL of 70% acetone containing 0.01% HCl. The samples were poured into the test tube and incubated in the water bath at 50°C in the dark for 1 h. The samples were filtered using Whatman filter paper Grade 1. The collected supernatants were centrifuge at 1700 *g* for 5 min. The clear supernatant was decanted into the cuvette vessel, and the adsorption was analysed using UV-spectrophotometer (UV-VIS Spectrophotometer Model Libra SII). The appropriate dilution factor was determined by diluting the sample with pH 1.0 buffer (0.025*M* KCl) until absorbance at 520 nm was within the linear range of the spectrophotometer (Lee *et al*., 2005). Two dilutions of the sample were prepared with pH 1.0 buffer and pH 4.5 buffer, respectively by using this dilution factor. The absorbance of the sample (diluted with the buffer) was determined at both 520 and 700 nm. The cuvette filled with distilled water was used as a blank. The anthocyanin pigment concentration was expressed as cyd-3-glu equivalents, as follows (Lee *et al*., 2005):

Anthocyanin pigment (cyd-3-glu equivalents, mg/L) = $(A \times MW \times DF \times 10^3) / \varepsilon \times 1$

A = $(A_{520} – A_{700})$ pH 1.0 – $(A_{520} – A_{700})$ pH 4.5

MW = molecular weight, 449.2 g/mol for cyanidin-3-glucoside

DF = dilution factor

1. = path length in cm

ε = 26 900 molar extinction coefficient, in L x mol^{-1} x cm^{-1}, for cyd-3-glu

10^3 = factor for conversion from g to mg.

Statistical Analysis

Statistical analyses were performed using software of SPSS Version 17.0. The data were analysed with one-way analysis of variance (ANOVA). Differences between means were tested with Duncan's Multiple Range Test (DMRT). Mean with different letters in the same column differs significantly at $p \leq 0.05$.

Results

Pigment Analysis during Inflorescence Development

Four different stages of inflorescence development were identified in *C. crotalifera* within eight weeks of flowering (S1, S2, S3 and S4). Fully pigmented bracts were observed in S2 and S3 stages within week 3 and week 6 for both cultivars. Based on Fig. 1, bract discolouration was observed after six weeks. However, bracts of *C. crotalifera* cv. Red showed discolouration at the end of the S3 stage (after six weeks) while bract discolouration in *C. crotalifera* cv. Yellow occurred in week 7. The flowers bloomed and emerged from the bracts at the early S2 stage within three weeks for both cultivars. The bracts were continuously producing flowers until S4 stage with most of the flowers wilting and browning at the end of week 7. No flower production had been observed after week 8 for both cultivars. These observations indicate that inflorescence development in Red and Yellow cultivars are quite similar but with different bract discolouration period. Fig. 3 showed the chlorophyll pigments accumulation pattern in the bract during inflorescence development. Both, concentration of chlorophyll a and chlorophyll b were increased in partially coloured bracts. Chlorophyll a was higher and predominated over chlorophyll b in all stages as shown in Table 2. The concentration of chlorophyll pigments were found higher in Yellow cultivar compared to Red cultivar inflorescence (Table 2). In general, chlorophyll pigments for both cultivars started to decrease after four weeks of development during the early stage of fully pigmented bracts. Thus, the concentration of chlorophyll pigments was low in the fully pigmented bract of S3 stage (Table 2). However, *C. crotalifera* cv. Yellow showed an increasing of chlorophyll pigments after six weeks in S4 stage when the bracts showed discolouration effects (Fig. 3). The concentration of carotenoids pigment in the bract of Yellow cultivar was higher than the Red cultivar throughout the developmental stages of inflorescence (Fig. 3 and Table 2). Fig. 3 showed the highest concentration of carotenoids were observed in week 5 with 12.468 ± 0.090 µg/mL (Yellow cultivar) and 7.224 ± 0.001 µg/mL (Red cultivar). The inflorescence bracts of Red cultivar contain higher anthocyanin concentration compared to Yellow cultivar (Fig. 3). The highest concentration of anthocyanin in Red cultivar was recorded within S2 and S3 stage (after four weeks) as demonstrated in table 2 and Fig. 3. Anthocyanin pigments start to accumulate in the bracts of Yellow cultivar inflorescence after five weeks with a very low concentration. Our result indicates that the red colouration of bracts in Red cultivar was dominated by anthocyanins. The yellow colouration of bracts in Yellow cultivar was mainly due to the concentration and composition of carotenoids. Study on fully pigmented bracts showed that the accumulation of pigments in the fully pigmented bracts occur in two different parts as shown in Fig. 2. The fully pigmented part is the part that exposed to the sunlight during the development while the non-fully pigmented part did not expose to the sunlight. These two condition significantly affected the photosynthetic pigments and

Table 1: Stage of inflorescence development in *C. crotalifera* cv. 'Red' and 'Yellow'

Stage	Phase	Conditions	Age
S1	Young	Partially coloured bracts	1-2 weeks
S2	Pre-mature	Fully pigmented bracts	3-4 weeks
S3	Mature	Fully pigmented with discoloration	5-6 weeks
S4	Old	Bract discoloration	7-8 weeks

Table 2: Pigment concentration of chlorophyll a, chlorophyll b, carotenoids and anthocyanins measured at four developmental stages in inflorescence bract of 'Red' and 'Yellow' cultivars of *C. crotalifera*

Cultivar	Stage	Bract colour	Pigment concentration (µg/mL)			
			Chlorophyll a	Chlorophyll b	Carotenoids	Anthocyanins
Red	1	Orange to red	5.591 ± 0.009b	1.776 ± 0.023a	3.873 ± 0.141a	2.050 ± 0.189b
	2	Orange to red	8.033 ± 0.067bc	2.803 ± 0.058b	6.014 ± 0.302b	4.169 ± 0.190d
	3	Red	7.324 ± 1.132bc	2.797 ± 0.302b	5.910 ± 0.588b	3.507 ± 0.320c
	4	Red to green	3.129 ± 0.641a	1.887 ± 0.159a	3.871 ± 0.317a	0.320 ± 0.069a
Yellow	1	Green to yellow	10.420 ± 1.158a	4.634 ± 0.788a	5.729 ± 0.373a	0.000 ± 0.000a
	2	Green to yellow	14.800 ± 0.170b	3.122 ± 0.694a	8.704 ± 0.693b	0.016 ± 0.009a
	3	Yellow	9.563 ± 0.889a	3.247 ± 1.047a	11.863 ± 0.280c	0.124 ± 0.009c
	4	Yellow	9.287 ± 0.229a	5.667 ± 0.653a	8.780 ± 0.412b	0.908 ± 0.009b

Values are mean with SE (n = 15). Means in a column followed by the same letter are not significantly different at $p \leq 0.05$ according to DMRT

Table 3: Pigment accumulation in two different parts of fully pigmented bracts of 'Red' and 'Yellow' cultivars of *C. crotalifera*

Cultivar	Part of bract	Colour	Pigment concentration (µg/mL)		
			Total chlorophyll a + b	Carotenoids	Anthocyanins
Red	Exposed part	Red	14.610 ± 0.131	8.931 ± 0.051	19.607 ± 0.719
	Overlapped part	Yellow	5.845 ± 0.118	3.299 ± 0.302	0.518 ± 0.028
Yellow	Exposed part	Yellow	9.028 ± 0.078	11.395 ± 0.147	0.134 ± 0.011
	Overlapped part	Yellow to green	20.656 ± 0.043	6.897 ± 0.012	0.042 ± 0.015

Values are mean with SE. (n = 15)

Table 4: Effects of different shading treatments on pigments accumulation in bracts and inflorescence development of *C. crotalifera* cv. Red

Treatment	Shading Level	Pigment composition (µg/mL)			Total number of bract	Diameter of inflorescence (cm)	Length of inflorescence (cm)
		Total Chlorophyll	Carotenoid	Anthocyanin			
T1	Mean ± SE	7.347 ± 0.042a	3.770 ± 0.212a	2.064 ± 0.056a	22.00 ± 1.15a	4.23 ± 0.15a	13.60 ± 0.58a
T2	Mean ± SE	12.616 ± 1.030b	7.199 ± 0.207b	4.096 ± 0.214b	27.33 ± 0.67b	5.47 ± 0.18b	17.00 ± 0.35b
T3	Mean ± SE	13.894 ± 0.225c	7.991 ± 0.119c	4.517 ± 0.202b	29.33 ± 1.33b	5.87 ± 0.22b	17.67 ± 0.35b

Values are mean with SE (n = 15). Means in a column followed by the same letter are not significantly different at $p \leq 0.05$ according to DMRT

anthocyanin accumulation in the inflorescence bracts. Based on Table 3, the concentration of chlorophylls, carotenoids and anthocyanin pigments were found higher in the exposed part compared to the overlapped part. However, the concentration of chlorophylls in the overlapped part of Yellow cultivar bracts was higher than the exposed part. The photosynthetic pigments of carotenoids were predominated over chlorophylls pigments in the exposed part of Yellow cultivar bracts.

Effects of Shading Treatments on Inflorescence Pigmentation and Development

The two different parts of colouration pattern in the fully pigmented bracts, as shown in Fig. 2 revealed that the pigments accumulation in the bracts were significantly affected by bracts shading. In this study, we attempted to demonstrate the effects of different shading treatments on pigments accumulation in the bracts as it will determine the quality of inflorescence. This study showed the significant different of shading levels on the bracts pigmentation in *C. crotalifera* cv. Red (Fig. 2). Based on Table 4, shading treatments at 80% (T1), and 40% (T2) reduced the concentration of chlorophylls, carotenoids and anthocyanin pigments significantly compared to the control treatment (T3). The highest concentration of chlorophylls (13.894 ± 0.225 µg/mL), carotenoids (7.991 ± 0.119 µg/mL) and anthocyanin (4.517 ± 0.202 µg/mL) pigments were recorded in control treatment (without shading) followed by shading at 40% and 80%. Thus, 80% shading treatment produced the lowest concentration and composition of pigments in the bracts. The intensity of pigments colouration of inflorescence were increased, and more illuminated as the light

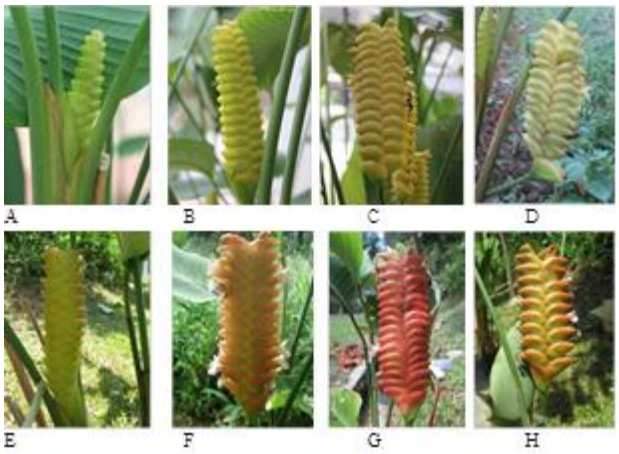

Fig. 1: Developmental stage of inflorescence of *C. crotalifera* cv. 'Yellow' and 'Red' (A and E) Stage 1. (B and F) Stage 2. (C and G) Stage 3. (D and H) Stage 4

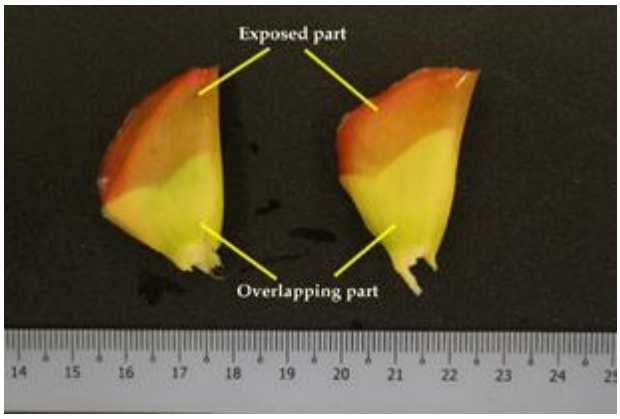

Fig 2: Colouration pattern in fully pigmented bract of *C. crotalifera* cv. Red

intensity decreased. The development and quality of the inflorescence were significantly affected by shading treatments (Table 4, Fig. 4 and 5). All shading treatments decreased the total number of bracts when compared to the control treatment (T3). The total number of bracts in T3 was 29.33 ± 1.33 while the number of 27.33 ± 0.67 and 22.00 ± 1.15 bracts recorded in T2 (40%) and T1 (80%) shading treatments respectively. The inflorescence size was also significantly altered by shading treatments (Table 4 and Fig. 4). All shading treatments decreased the diameter and length of inflorescence when compared to the control treatment. The inflorescence length in the control treatment (T3) was 17.67 ± 0.35 cm followed by 17.00 ± 0.35 cm and 13.60 ± 0.58 cm in T2 and T1 shading treatment, respectively. The diameter of the inflorescence was longer in the control treatment (5.87 ± 0.22 cm) compared to T2 (5.47 ± 0.18 cm) and T1 (4.23 ± 0.15 cm). As shown in fig. 5, shading treatments had adverse impacts on the final yield of inflorescence production. The highest number of the inflorescence was obtained in the control treatment (4.0 ± 0.32 inflorescences) followed by T2 (2.6 ± 0.51 inflorescences) and T1 (1.2 ± 0.37 inflorescences). The reduction in number of inflorescences produced was about 35% in T2 treatment and about 70% in T1 treatment when compared with the control treatment.

Discussion

Pigment Analysis during Inflorescence Development

In general, chlorophyll pigments for both cultivars started to decrease after four weeks of development during the early stage of fully pigmented bracts. The result is in agreement with the findings of Kannangara and Hansson (1998) and Slatnar *et al*. (2013) for chlorophyll accumulation during the bracts development of poinsettia. Similarly, significant decrease of total chlorophyll pigments was also observed in the early stage of bract pigmentation of poinsettia (Slatnar *et al*., 2013). The presence of anthocyanins and carotenoids pigments colouration have been demonstrated in the inflorescence bracts of other ornamental rhizomatic plants like heliconia (Mangave *et al*., 2014) and bird-of-paradise (Pirone *et al*., 2010). The similar red colouration of inflorescence by anthocyanin also had been demonstrated in *Telopea* sp. (Martyn *et al.*, 2007) and *Hippeastrum* sp. (Byamukama *et al.*, 2006). In addition, Tatsuzawa *et al*. (2010) reported that both anthocyanins and carotenoids significantly influenced the flower colour in different cultivars of *Disa* orchids that resulted in orange-red and red flowers. Our result showed that the increase in anthocyanin contents was affected the photosynthetic pigment accumulation in the bracts during early pigmentation. These observations indicate that photosynthetic pigments were synthesized in the first stage of inflorescence development before replaced by the different phenolic compounds. The observation is in agreement with the finding of Slatnar *et al*. (2013) in poinsettia inflorescence bracts. Kannangara and Hansson (1998) reported that the loss of protein content and enzymes during fully pigmentation stage in the chlorophyll biosynthesis pathway could affect the chlorophyll formation in the inflorescence bracts. In general, anthocyanin and carotenoid content increased with bract development in both analysed cultivars. The present results demonstrated that the accumulation and composition of anthocyanin and carotenoids significantly affect the colour of Red and Yellow cultivars of *C.* crotalifera, respectively. Our results revealed that the direct exposure of bracts to the sunlight could induce the production and accumulation of pigments (Mol *et al*., 1996; Qian *et al*., 2013). Also, Meng *et al*. (2004) reported that the red colouration in ray floret of *Gerbera hybrid* is influenced and controlled by light. The low accumulation and composition of carotenoids and anthocyanin pigments in the overlapped part is due to the shading effect on the bracts (Cortell and Kennedy, 2006; Azuma *et al.*, 2012).

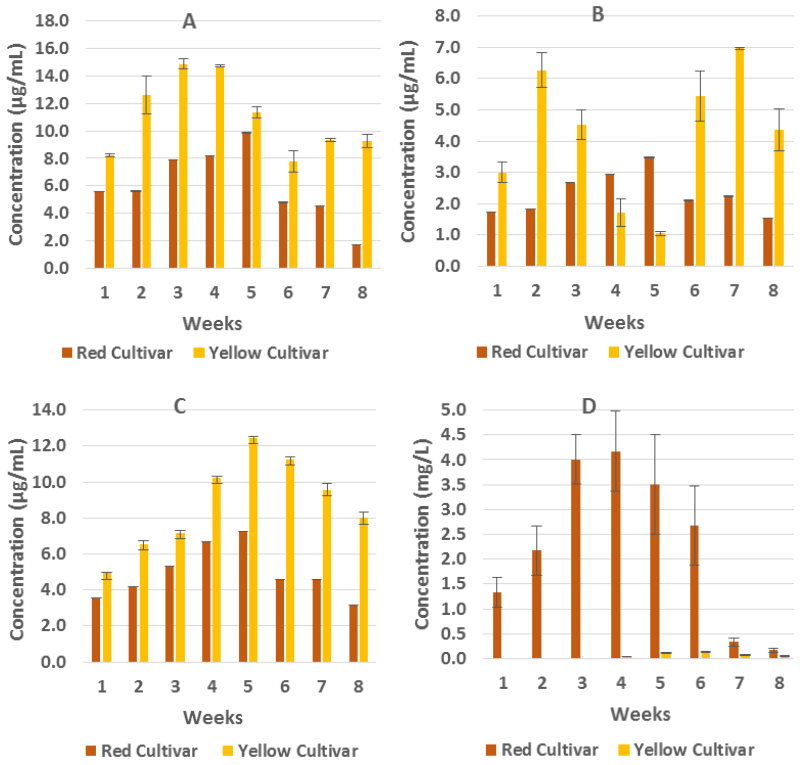

Fig. 3: Concentration of chlorophyll a (A), chlorophyll b (B), carotenoids (C) and anthocyanin (D) pigments in 'Red' and 'Yellow' cultivars of *C. crotalifera* within eight weeks of inflorescence development. Data are means ± standard errors (*n*=15)

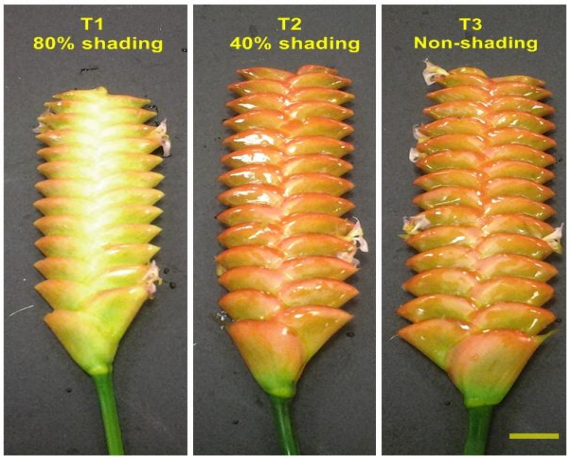

Fig. 4: Effect of different levels of shading on inflorescence morphology of *Calathea crotalifera* cv. 'Red'. (bar = 2 cm)

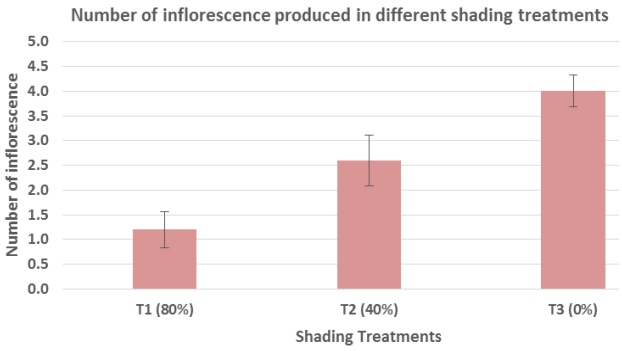

Fig. 5: Number of inflorescence produced by *C. crotalifera* cv. 'Red' as influenced by different shading treatments. Data are means ± standard errors (*n*=15)

Effects of Shading Treatments

Shading treatments were significantly affected the pigmentation patterns and inflorescence development of *C. crotalifera* cv. Red. Similar results were also reported in grape berry (Jeong *et al*., 2004), maize (Singh *et al.*, 1999), bougainvillea (Saifuddin *et al*., 2010), petunia (Albert *et al*., 2009) and daisy (Meng *et al*., 2004). Cortell and Kennedy (2006) have reported the effects of shading on the accumulation of anthocyanins and flavonoids in the *Vitis vinifera* L. fruit. Several researchers have reviewed that the environmental stimuli like light and temperature play a key role in the regulation of anthocyanins and carotenoid synthesis pathway in the fruits and also flowers (Meng *et*

al., 2004; Guo and Wang, 2010; Azuma *et al*., 2012). Alebidi (2013) similarly reported that shading severely suppressed anthocyanin accumulation in the Malaysian wax apple skin. The control treatment (without shading) could enhance the red colouration of wax apple skin compared to the shading treatments. Furthermore, it has been previously reported that the phenylalanine ammonia-lyase (PAL) activity during the flavonoids and anthocyanins biosynthesis is regulated and stimulated by environmental stresses including UV light, drought, high temperature and chilling (Lafuente *et al.*, 2003; Guo *et al*., 2008; Guo and Wang, 2010). Dash *et al.* (2012) have reported that severe shading could reduce the growth development of apple by decreasing the production and expansion of the cells. Several studies have reported that different light intensity through shading treatments could affect the plant development including flowering, physiology and morphology of leaf, and colouration in several ornamental plants (Brand, 1997; Hlatshwayo and Wahome, 2010; Stanton *et al*., 2010). The study result is in agreement with Hlatshwayo and Wahome (2010), which demonstrated that the diameter of carnation flower increased under low shading treatment. In addition, Morandi *et al.* (2011) have indicated that shading decreases the growth rate of fruit by reducing their phloem import. Our results revealed that sunlight exposure is essential to maximize inflorescence development in *C. crotalifera* cv. Red. However, some ornamental species could not tolerate with the high light intensity and the response to light can vary among genotypes within a species (Martyn *et al*., 2007; Stanton *et al*., 2010). Our results are in agreement with the study by Hlatshwayo and Wahome (2010) which indicated that shading intensity resulted in a significant decrease in yield of carnation cut flowers. The study on the carnation flowers demonstrated that the number of flowers at 0% shading was more than double that of plants under 70% shading. The similar results on reduction in number of inflorescences produced also have been reported in begonia (Jeong *et al.*, 2007) and meadowsweet shrubs (Stanton *et al*., 2010). It can be suggested that to maintain healthy growth and production of inflorescence, it is required to expose plants to appropriate sunlight.

Conclusion

This study provided a better understanding of the process of various pigment accumulation in these two different cultivars of exotic ornamental plants. It is important to investigate the relationships between flower colour and pigment, and their inheritance that could stimulate more efficient breeding of this genus. Further analysis of the anthocyanins and carotenoids contents in the inflorescence is essential to study the chemotaxonomic and phylogenetic in other cultivars. To our knowledge, this work is the first to report the effects of shading treatments on the production and quality of cut flowers of *C. crotalifera* under tropical climate in Malaysia.

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