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Effect of Light and Temperature through Poly Film Covers on Anthocyanin Content in Rose Cut Flowers

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Quality is the most important attribute in rose cut flowers for both export and domestic market. Quality in cut flowers may be defined by many attributes however, among the most important is colour. A group of pigments commonly known as anthocyanins determine colour in plants. Anthocyanins play a significant role by ameliorate the effect of high irradiance in plants under stressful environment. They also play a key role in delaying senescence hence enhancing the cut flower vase life. Despite the advantages anthocyanins are affected by the preharvest conditions mainly light and temperature interfering with their stability. An experiment was set up to investigate the effect of light and temperature through selected coloured poly film covers on rose petal

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anthocyanin content. The greenhouse structure was covered by poly films of different colours that were compartmentalized i.e. UV-A clear, IR504 with yellow tint and UV-A 205/N with green tint replicated three times. Two rose cultivars Red calypso and Furiosa were established and maintained, upon maturity the flower heads were plucked and oven dried at 60℃ to constant weight. 5 g of the crushed petals was used in anthocyanin extraction. The anthocyanins were extracted and quantified in comparison with commercial standards using HPLC machine. The data obtained from the chromatogram as peak areas was subjected to analysis of variance (ANOVA) using SAS statistical package (SAS Inst., Inc., Cary, NC) at P = .05. Where there were treatment differences, mean separation was done using Tukey's procedure. Poly films significantly affected the quantity and quality of anthocyanin accumulation in rose petals. Cyanidin 3-0-glucoside was the most prevalent anthocyanin across all poly film covers and it was noted to be high under the UV-A 205/N (110.95±8.26 µg ‒ 5 g–1 DW) and IR504 (109.69±8.26 µg ‒ 5 g–1 DW) compared to UV-A clear (84.56± 8.26 µg ‒ 5 g–1 DW). The quantity of anthocyanins was low under the UV-A clear poly film that was characterized by high light transmission and day temperature. Combination of high irradiance and temperature affect the quality and quantity of anthocyanin in rose cut flowers.

Keywords: Rose cultivars; temperature; light; anthocyanin; poly film.

1. INTRODUCTION

Light affects productivity and quality of ornamental plants besides growth and development. Plants response to light is influenced by the fluctuating environmental conditions and more so light properties like duration, intensity and quality [1] Plant growth attributes such as height, leaf area and leaf length decrease in response to UV-B radiation [2]. Red and far red wave band influence the phytochrome pigment which initiates photomorphogenic changes in plants [3]. This wave band varies from one poly film to the other depending on colour and gauge. Previous studies have shown that absorption of the far-red light of the poly films increase with the concentration of the dye [4,5,2]. Intensity of light in the far-red region affects morphological plant responses [6] which may have positive or negative impact on the physiochemical processes of the plants. Depending on the quality of light transmitted plant quality may be jeopardized affecting the colour of the flowers.

Anthocyanin content in cut flowers is affected by pre- harvest conditions to which the plant is subjected. A wide range of colours is not only insured by substrates accumulation but also other factors such as co-pigments, vacuole pH and cell shape [7]. Environmental factors such as elevated temperature received during growth reduce anthocyanin content of flower petals [8]. In other studies, it has been argued that plant growth and development is determined by the genomic characteristics of the plant that affect fundamental components like the flower colour [9]. Depending on the changes that occur during biosynthesis different molecules are formed resulting to diverse types of anthocyanins.

Peonidin type of anthocyanins is biosynthesized from cyanidin, while petunidin and malvidin are biosynthesized from delphinidin type of anthocyanins [10]. Anthocyanin produced varies from one species to another depending on their genetic constitution. Anthocyanin stability and catabolism is quite dynamic and its concentration in plants is bound to vary from time to time [11]. Rose and carnation the major cut flowers for example are only able to produce anthocyanin based on pelargonidin and cyanidin. Cut flower consumers may prefer rose stem in which the leaves have deep green colour that blends well with other hues of the bloom. Therefore, it is important to study and maintain all factors that will enhance flower colour.

2. MATERIALS AND METHODS

2.1 Experimental Layout and Crop Establishment

The research was carried out under a split plot experiment laid down in a completely randomized block design. The main treatment involved poly film covers with different colours denoted as; G0 = UV-A clear (control), G1= IR 504 (green tint) and G2 = UV- A 205/N (Yellow tint) of similar gauge 200 microns. The greenhouse was divided into three sections of $44M^2$. Each section was covered with a different poly film cover as described above and replicated three times. Two rose cultivars; Red calypso and Furiosa were established and maintained till maturity for data collection.

2.2 Light Spectra: Poly Film Transmission Properties

The transmission and absorbance properties of the selected poly film covers were tested in the laboratory. Measurements were carried out on samples taken before the installation of the poly films on the greenhouse and subsequent years during the production period. A strip of the poly film was cut from the extra ends of greenhouse. The strip was carefully cut in a rectangular shape and locked in a jig before insertion in the cuvette holder to ensure an upright position is maintained perpendicular to the source of light. The poly films were then scanned at wavelengths (190-1000 nm) using UV- 1800 Shimadzu spectrophotometer. Care was taken to ensure that the light beam from the spectrophotometer entered through the outer surface of the poly film and left through the inner surface.

Relative humidity, temperature and photosynthetic active radiation (PAR) were monitored using watch dog mini data logger and weather station. The machine was plugged into the port on the watch dog while in the field for data collection under the ambient solar radiation. The machine was fixed firmly on a support, with the sensor head being at the level of the plant heads. Data was downloaded at the end of every flush using a specware soft ware. Although data was collected for the entire day, 0800 hr, 1200 hr and 1600 hr sampling times were used as a baseline for omparison of PAR among the treatments. Temperature and relative humidity were monitored both day and night and averaged over 7 day period.

2.3 Quantitative and Qualitative Anthocyanin Analysis

Five grams (5 g) of dry ground rose petals were weighed into 250 ml conical flasks and covered with aluminum foil paper. The ground petals were mixed with 50 ml methanol (MeOH) and formic acid at a ratio of (99:1v/v) and magnetically stirred at 900 rpm for 4 hours at room temperature. The resultant solution was filtered and evaporated to remove as much methanol as possible using a rotary evaporator (Buchi Rotavapour R-300, Switzerland) under reduced pressure at 35℃. The concentrated extract was dissolved in 10 ml distilled water and passed through a membrane filter 0.45 µM. Anthocyanin purification was then done by passing the extracts through reverse phase (RP) C18 solid phase extraction (SUPELCO, SPE) (Sigma–Aldrich, USA) catridge previously activated with 10% MeOH followed by 0.01% HCl v/v in distilled water. Anthocyanins get adsorbed onto the column while sugars, acids and other water-soluble compounds are washed out using 0.01% HCl in distilled water. Anthocyanins are recovered using acidified methanol (10% Formic acid v/v). The catridges were washed with ethyl acetate (Fischer Scientific, UK) to remove phenolic compounds other than anthocyanins. The purified extracts were stored at -10℃ until further analysis.

2.4 Quantification of Anthocyanins

The anthocyanins in the rose petals were characterised by HPLC using a Shimadzu LC 20 AT HPLC system fitted with a SIL 20A auto sampler and a SPD-20 UV–Visible detector with a class LC10 chromatography work station. UV detection was set at 520 nm using a Gemini C18 ODS (4.0 mm _ 4.6 mm i.d.) (Phenomenex Inc. Torrance CA, USA) fitted with a Gemini C6 ODS column (4.0 mm _ 3.0 mm i.d.) (PhenomenexInc. Torrance CA, USA) guard. The column temperature was at 35 ± 0.5℃. The eluents were mobile phase A water/acetonitrile/formic acid-87/3/10 v/v/v) and mobile phase B (100% HPLC grade Acetonitrile). The chromatographic conditions were: 3% B in A at the time of injection, at 45 min; 25% B in A, at 46 min; 30% B in A and at 47 min; 3% B in A (initial conditions). The flow rate of the mobile phase was1 ml/min and injection volume of 20 µl. The anthocyanin cyanidin chloride and pelargonidin chloride were used as standards for the identification and quantification of anthocyanin fractions in dried rose flower petals. Other types of anthocyanins (Cyanidin 3-0 glucoside, peonidin, delphinidin and Cyanidin 3-0 galactoside) were quantified using the calibrated standard curves in tea research Institute of Kenya Laboratories.

2.5 Data Collection and Analysis

Data collection involved quantification of the peak areas from the chromatograms. The collected data was subjected to analysis of variance (ANOVA) using SAS statistical package (SAS Inst., Inc., Cary, NC) at $P \leq 5\%$. Where there were treatment differences, mean separation was done using Tukey's procedure.

3. RESULTS AND DISCUSSION

3.1 Effect of Poly Film Covers on Greenhouse Microclimate and PAR

3.1.1 Light transmission through selected poly films

The poly films varied in their spectral properties including PAR transmission. It was observed that the quantity of PAR transmitted was dependent on sampling time and prevailing weather conditions. Average PAR values ranged from; 222 µmol m^{-2} s^{-1} at 0800 hrs, to 1613 µmol m^{-2} s^{-1} at 1200 hrs and 115 µmol m^{-2} s^{-1} at 1600 hrs for UV-A clear poly film cover. The intensity of transmission was significantly higher for UV-A clear poly film compared to IR-504 that transmitted, 245 µmol m^{-2} $s^{-}1$, 1063 µmol m^{-2} s^{-1} and 40 µmol m^{-2} s^{-1} at 0800 hrs, 1200 hrs and 1600 hrs sampling time respectively. Comparison of the PAR values at 0800 hrs for UV-A clear and IR 504 poly film indicated low PAR values for UV-A clear. This could have been caused by the water condensate that formed on the UV-A clear poly film during the night hours thus hindering maximum transmission in the early morning hours. The quantity of PAR transmitted was noted to fluctuate from time to time in line with the prevailing natural environmental conditions (Figs. 1 and 2).

Transmission properties of the poly films were consistent in both flushes I and II. The least PAR but high temperature was recorded under UV-A 205 cover. In previous work, Copinet et al. [12]

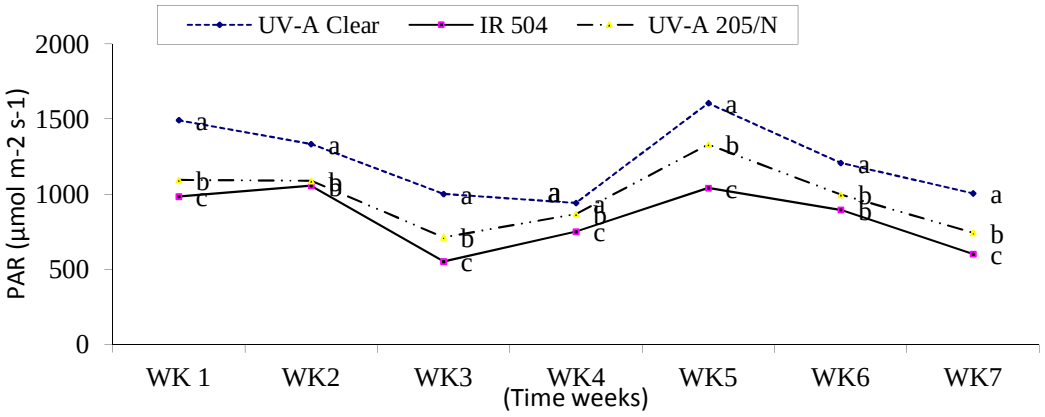

Fig. 1. Effect of selected poly film on transmission of photosynthetic active radiation (flush 1). Values presented are weekly averages (n=3) of PAR sampled at mid-day

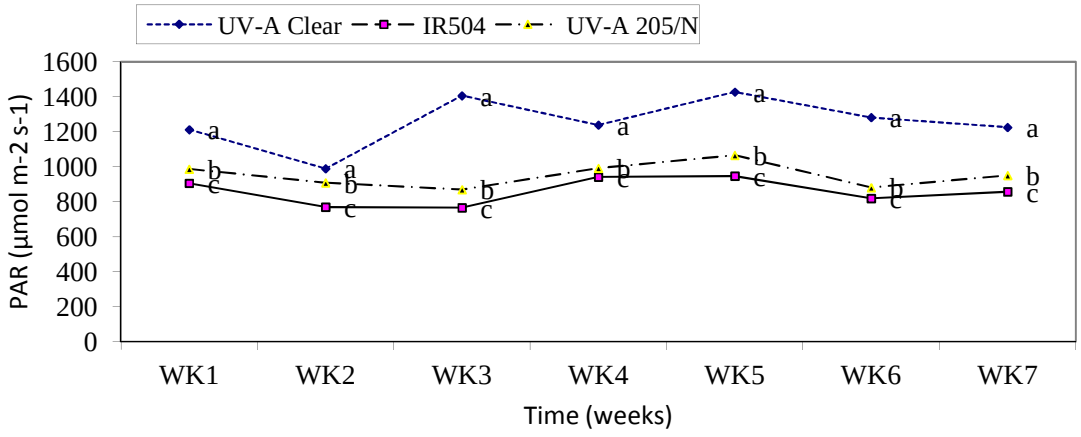

Fig. 2. Effect of selected poly film on photosynthetic active radiation during flush II The values presented are weekly averages (n=3) of PAR sampled at mid-day

studied the influence of temperature (30℃, 45℃ and 60℃) and relative humidity (RH) (30%, 50% and 100%) on the degradation of poly-lactic acid and established that degradation increased with increase in temperature and relative humidity. Copinet et al. [12] further examined effect of UV- at 315 nm wavelength in the same study and reported that UV- accelerated degradation process. This could be the reason as to why the UV-A clear poly film under high temperature and low relative humidity exhibited higher transmission. Part of the findings of this study state that increased humidity levels increase photo degradation of ester-based polymers through increased water absorption and hydrolytic reactions initiated by UV radiation, thus photo degradation is enhanced by presence of relative humidity. UV-A clear poly film allowed higher light transmission and higher relative humidity during the night hours which enhanced the degradation process.

3.1.2 Poly film covers, greenhouse temperature and relative humidity

Relative humidity was higher under UV-A clear poly film at 0400 hrs. The lowest values were recorded under the same cover during at 1200 hrs. Relative humidity was noted to be inversely proportional to the temperature with the lowest values being recorded at 1200 hrs. Relative humidity of 69.2%, 76.1% and 71% with temperatures of 31.2℃, 31.1℃ and 30.9℃ was recorded under UV-A 205/N, UV-A clear and IR504 poly films respectively at 1200 hrs. Higher values of relative humidity 93.1%, 96.5% and 93.3% and low temperature of 8.5℃, 8.9℃ and 9.2℃ was recorded at 0400 hrs under the UV-A 205/N, UV-A clear and IR504 respectively (Figs. 3 and 4).

The IR treated poly film showed tendency of forming condensate during night hours. This could be attributed to the fact that the IR treated poly film was slightly warmer during the night hours as opposed to the cool air outside the greenhouse. Temperature fluctuation between 1600hrs and 0400 hrs was similar across all covers. The highest difference in temperature was noted at 1200 hrs where by the UV-A clear recorded 2.5℃ and 1.3℃ higher than UV-A 205/N and IR504 respectively. The poly film covers constituted different additives which had potential impact on microclimate. Conventionally, under conditions of elevated temperatures farmers have adopted whitening of the greenhouse roof to reflect excess light and reduce temperature within the structure. Influence of whitening of greenhouse microclimate was studied and observed to reduce the transmission coefficient of solar radiation from 0.62 to 0.31 as a result changing air temperature drastically [13]. Apparently, the UV-A clear poly film that was permissive to high transmission accumulated higher temperature during the day although it could not be retained at night. Holcman and Sentelhas, [14] evaluated microclimate under different shading screens among them being red, blue and black. The reflective shade screen used as the control

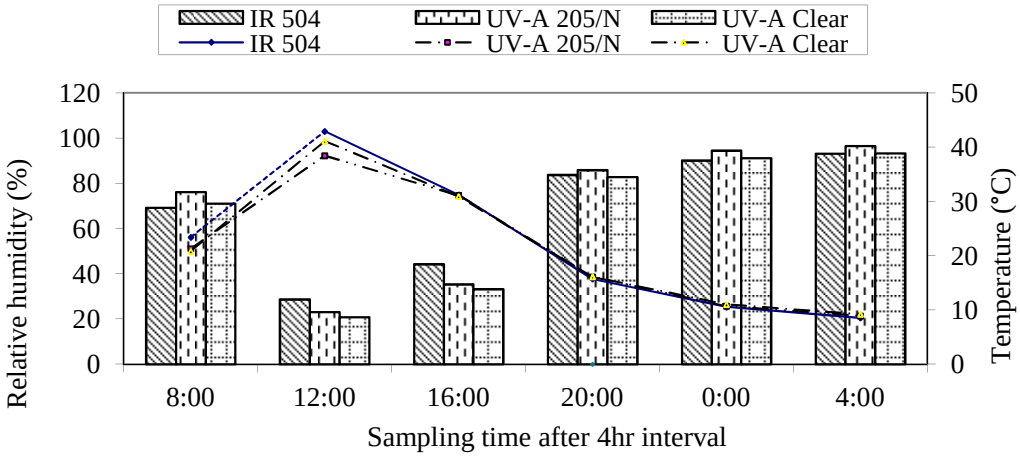

Fig. 3. Effect of selected poly film covers on percent relative humidity and air temperature (flush 1). Bar graphs and line graphs represent relative humidity and temperature respectively. Values presented are means over a growth period of 42 days at different sampling time; 0800 hrs, 1200 hr and 1600 hr

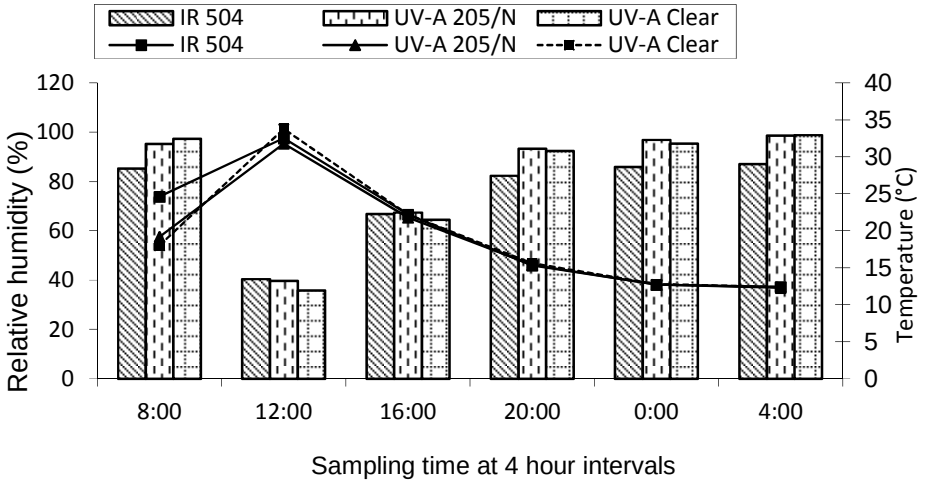

Fig. 4. Effect of selected poly film covers on percent relative humidity and air temperature (Flush 1I). Bar graphs and line graphs represent relative humidity and temperature respectively Values presented are means over a growth period of 42 days at different sampling time; 0800hrs, 1200hrs and 1600hrs

transmitted 56.3% light followed by the red screen which transmitted 27%, while black recorded the least transmission of 10.4%. Temperature also varied drastically, with the blue screen recording 1.3℃ higher temperature than the external conditions. The colour of the greenhouse cover therefore, effects greenhouse microclimate and the extent vary among different colours as observed in this study.

The structures in the current study had higher temperature than normal outdoor temperature, which could be attributed to the small size of the structure. The bigger the greenhouse the better the air flow thus size greatly affect air temperature within a structure. According AlHelal and Abdel-Ghany, [15] they observed that greenhouse air temperature is affected by more than one single factor including; solar radiation, level of greenhouse venting and the size of the greenhouse. Structural units used in his experiment were relatively small precisely 0.0048Ha in size and about two-thirds the height of normal greenhouse (6 M) as a result high temperature was recorded consistently. Holcman and Sentelhas, [14] pointed out that besides climate, greenhouse design, size and height affects the internal microclimate. Greater height of the greenhouse structure enhanced air circulation minimizing heat buildup within the greenhouse. Conventionally, reduction in air temperature is a key objective of greenhouse use in the tropics and as observed here it may be limited by height of the structure. The smaller the greenhouse size the higher the temperature and the bigger the greenhouse in width and height the lower the temperature.

Several modifications have been adopted in the plastic industry, including use of shade screens, cladding of the poly films with different colours and treatment with UV- and IR additives among others. According to AlHelal and Abdel-Ghany, [15] colourful screens have the capacity to alter the spectral properties which in turn influence the microclimate by lowering temperature. In the current study, it was observed that the UV-A clear poly film transmitted more light compared to the coloured ones. The findings in this study concurs with the work done by Shahak, [16] who reported that the darker the colour of the poly film the less the amount of light transmitted. This explains why the control poly film (UV-A clear) recorded the highest light transmission and the highest temperature at 1200 hrs compared to IR504 and UV-A 205/N.

The influence of the poly films on microclimate was consistent in both flushes I and II although the levels varied with the prevailing weather conditions at the time of the experiment. The poly films had significant effect on relative humidity and corresponding temperature within the greenhouses structures which also varied with the specific sampling time. Greenhouse relative humidity directly influences the plant water relations and indirectly affect plant growth and development. High temperature, as was

the case under UV-A clear cover translated to low relative humidity at 1200 hrs which demonstrates that relative humidity is highly affected by temperature. Although the experiment was conducted under same natural conditions, the results showed variations in temperature in addition to relative humidity. Mean temperature values of 41℃, 35.2℃ and 32.8℃ were recorded at 1200 hrs during the hottest part of the day and 12.3℃, 13.1℃ and 12.1℃ at 0400 hrs during the coolest part of the day under UV-A clear, IR-504 and UV-A205/N covers respectively. It is evident that during the day UV-A clear recorded the highest temperature compared to UV-A 205/N, while the mean night temperatures between the two poly films were not significantly different. However, it is also important to note that, while IR504 poly film recorded the lowest mean day temperature than the control it was significantly higher than the UV-A 205/N. IR504 had the highest mean night temperature (13.1℃) being 0.8℃ and 1.0℃ higher than UV-A clear and UV-A 205/N respectively. It is argued that poly film colour is an integral part of greenhouse microclimate. For example, Kitta et al. [1] maintains that microclimate under different shading screens is dependent on the colour of the greenhouse cover material used. It is apparent therefore, that colour of the covering material affects the microclimate under the growth structures.

3.1.3 Poly films and flower petal anthocyanin content

Poly films significantly affected the quantity and quality of anthocyanin accumulation in rose petals. Cyanidin 3-0-glucoside was the most prevalent anthocyanin across all poly film covers and it was noted to be high under the UV-A 205/N (110.95±8.26) and IR504 (109.69±8.26) compared to UV-A clear (84.56± 8.26) that exhibited high irradiance. The quantity of cyanidin 3-0-glactoside and cyanidin chloride were statistically similar under the IR504 and UV-A205/N poly films compared to UV-A clear. Interestingly pelargonidin chloride was relatively stable across all the poly film treatments. Peonidin chloride was relative low under the UV-A clear poly film compared to IR504 and UV-A205/N (Tables 1 and 2). The anthocyanin quantities were higher in the cultivar Red calypso compared to Furiosa.

Cyanidin 3-0- glucoside and cyanidin 3-0-galactoside were high in Red calypso and lower in Furiosa implying that species of same plant accumulate different quantities of similar anthocyanins. Apparently, the quantities of delphinidin chloride and pelargonidin chloride were not significantly different among the cultivars used in the study. The major anthocyanins were cyanidin 3-0-glucoside and cyanidin 3-0-galactoside while the minor anthocyanins were the chlorides of peonidin and pelargonidin. Previous works have established that the amount of coloured material in leaves or petals depend on cultivars, plant habitat and the climatic conditions encountered during the growing season [17].

Anthocyanin accumulation was substantially high in flush II compared to flush I which probably reflects influence of changes in the environmental conditions. Flush I was characterized by high irradiance consequently low anthocyanin content was observed in rose petals whereas low irradiance was recorded in flush II where petals were noted to accumulate more anthocyanins. Further differences were observed in specific anthocyanins, whereby peonidin, cyanidin and pelargonidin based

Table 1. Effect of cover and cultivar on rose petal anthocyanin content flush I

Flush 1	Anthocyanins [(mean ± SE) (μg ⁻ 5 g–1 DW)]				
Poly film	**Cya 3-0-glu**	**Cya-3 -0-gla**	**Cya-cl**	**Pela-cl**	**Peo-cl**
UV-A clear	84.56 ±8.26b[1]	14.88±1.23b	2.99±0.23b	5.49±0.51b	0.97±0.17b
IR504	109.69±8.26a	20.58±1.23a	4.50±0.23a	7.89±0.51 a	1.90±0.17a
UV-A205/N	110.95±8.26a	20.07±1.23ab	3.98±0.23a	7.72 ±0.51a	2.09±0.17a
Red calypso	142.93± 6.74a	23.11±1.0a	4.40±0.18a	8.92±0.41a	2.18±0.14a
Furiosa	60.54±6.74b	13.91±1.0b	3.26±0.18b	5.14±0.41 b	1.13±0.14b

[1]*Means followed by different letter(s) along the column for or poly film cover and cultivars are significantly different at 5% level of significance according to Tukeys' HSD procedure.*

Where; letters 'a' and 'b' denote mean separation. While; Cya 3-0-glu, Cya 3-0-gla, Cya-cl, Pela-cl and Peo-cl are Cyanidin-3-0-glucoside, Cyanidin 3-0-galactoside, Cyanidin chloride, Pelargonadin chloride and Peonidin chloride respectively

Table 2. Effect of cover and cultivar on rose petal anthocyanin content flush II

Flush II	Anthocyanins	[(mean ± SE) (μg ⁻ 5g–1 DW)]			
Poly film cover	**Cya 3-0-glu**	**Cya-3 -0-gla**	**Cya-cl**	**Pela-cl**	**peo-cl**
UV-A clear	110.16 ±13.02b[1]	19.84±1.77b	4.32±0.43b	6.76±1.40b	5.85±0.72a
IR504	140.07±13.02a	28.44±1.77a	7.60±0.43a	9.82±1.4 a	4.39±0.72b
UV-A205/N	139.54±13.02a	29.65±1.77a	7.58±0.43a	9.59±1.4a	4.84±0.72b
Red calypso	192.60± 10.63a	29.23±1.45a	7.56±0.35a	9 .01±1.14a	4.11±0.59a
Furiosa	67.25± 10.63b	22.72±1.45b	5.45±0.35b	8.46±1 .14a	4.61±0.59a

[1]*Means followed by different letter(s) along the column for or poly film cover and cultivars are significantly different at 5% level of significance according to Tukeys' HSD procedure.*

Where; letters 'a' and 'b' denote mean separation. While; Cya 3-0-glu, Cya 3-0-gla, Cya-cl, Pela-cl and Peo-cl are Cyanidin-3-0-glucoside, Cyanidin 3-0-galactoside, Cyanidin chloride, Pelargonadin chloride and Peonidin chloride respectively

anthocyanin increased with decrease in temperature. 30.3% more cyanidin-3-0-glucoside was recorded in flush II compared to flush I while cyanidin-3-0-galactoside increased by 33.3% in flush II compared to flush I. This observation supports the finding of Laleh et al. [18] who studied the effect of temperature on anthocyanin among other factors and established a positive relationship between temperature and anthocyanin. What is not clear from this study is the effect of other microclimatic factors like relative humidity on the anthocyanin since it was difficult to quantify their effect singly under field conditions.

A wide range of flavonoids and phenolic compounds are produced in plants in response to UV- radiation related stresses. Plants produce and accumulate these secondary products to protect themselves against UV light damage [19]. Other studies have shown that where both wavelengths UV-A and UV-B are excluded anthocyanin reduced drastically implying that both UV-A and UV-B are essential in anthocyanin photo-induction. This observation could also explain the reason why there were less anthocyanins under the UV-A clear poly film cover that had high irradiance with substantial amount of UV-A and UV-B radiation transmitted.

Temperature may impact negatively or positively on anthocyanin production. Dela et al. [8], demonstrated that anthocyanins are induced by low temperatures and reduced by high temperatures. Several authors have observed and reported that the expression of genes for anthocyanin biosynthesis pathway increased at low temperature [20,21,22]. Furthermore, Ban et al. [23] studied the effect of temperature on apples and established that high temperature caused a decline in accumulation of cyanidin the major anthocyanin in Rosaceae family causing fluctuations in skin colour. When the same apples were subjected to cooler temperatures there was renewed synthesis and improvement in colour [22]. Low temperature cause high levels of ABA that act as a signal to influence the expression of the main genes involved in the anthocyanin biosynthesis pathway, resulting in an increase in anthocyanin levels [24]. This explains why we had more anthocyanin accumulation in flush II compared to flush I. Anthocyanins are highly influenced by temperature and have been observed to be stable at 0℃ whether in light or darkness, implying that temperature has greater influence on anthocyanin stability both in the absence and presence of light. The combination of light and temperature therefore could have accelerated the anthocyanin degradation process. The effect of temperature could further be attributed to the hydroxylation of 3- glycoside structure which confers protection to the unstable anthocyanin structure. low temperatures on the other hand increase both anthocyanin content and the expression of genes of the anthocyanin biosynthetic pathway while high temperature affect the activity of the phenylpropanoid pathway responsible for anthocyanin biosynthesis [20,22] resulting in less accumulation.

4. CONCLUSION

Anthocyanin quality and quantity is highly dependent on the environmental conditions. Low temperatures enhanced anthocyanin accumulation in rose flower petals. Clear poly films therefore, may not be the best in areas with high irradiance as they exhibit high temperature jeopardizing quality. Growers should therefore adopt appropriate poly films in relation to the

environmental conditions to obtain cut flowers with best quality for export.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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