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Effects of Intensity and Quality of Light on the Coloring of Leaves on a Succulent Plant, *Graptopetalum paraguayense* 'Bronze'

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Abstract

Graptopetalum paraguayense 'Bronze' is a small succulent plant with reddish-bronze colored leaves. This plant contains anthocyanins as pigments. Therefore, the effects of light intensity and quality of light on the color and anthocyanin content of leaves in 'Bronze' were investigated. The leaf color became more reddish as the light intensity increased. The a* values of the leaves under the green and red light decreased with increasing of treatment days, but the values hardly changed under white and blue light. The anthocyanin content in the leaf epidermis was highest under white light containing blue light component, but the total polyphenol content was highest under blue light. From the above results, it is considered that anthocyanin and polyphenol synthesis in 'Bronze' leaves are promoted by blue light.

INTRODUCTION

Plant groups called succulent plants have been attracting attention from especially young peoples in Japan in recent years due to their distinctive form and color of leaf and stem. Recently, *Graptopetalum paraguayense* (N.E.Br.) E. Walther (Crassulaceae) plants had gained popularity due to its ease of leaf cutting propagation and easy cultivating management. Among them, one of the cultivars, named 'Bronze' is particularly popular because the green leaves turn beautifully red color at the beginning of autumn in outdoor cultivation (Matsui, 1988). It has the characteristic that leaves spread in a rosette shape at the top of the stem. Already, the authors have revealed that the red pigment in 'Bronze' leaves was anthocyanin (Noguchi, 2019).

Anthocyanins are thought to play a role in plant sunscreen and scavenging reactive oxygen species (Chalker-Scott, 2002; Karageorgou and Manetas, 2006; Hatier and Gould, 2009; Lev-Yadun and Gould, 2009; Llorens et al., 2015). The production of anthocyanin pigment is expected to be affected by cultivation conditions, particularly light. Therefore, the effects of light intensity and light quality during cultivation on the surface (epidermis) color and anthocyanin contents of the 'Bronze' leaf were investigated.

MATERIALS AND METHODS

Potted plants of *Graptopetalum paraguayense* 'Bronze' with about 25 leaves of uniform size were selected and used in the following experiments.

Experiment 1. Effect of light intensity on the leaf color and anthocyanin content

White light emitting diode (LED) lamps (LLM0172A, Stanley Electric Co., Ltd., Yokohama, Japan: 460nm peak blue LED covered with Yttrium Aluminum Garnet (YAG) phosphor) were installed at the top of the plant so that the photosynthetic photon flux density (PPFD) was 60 or 600μmol·m^{-2}·s^{-1}, and three plants were arranged for each light intensity. All plants were subjected to 24-hr photoperiod and room temperature kept at 23±2 °C.

Experiment 2. Effect of light quality on the leaf color and pigment contents

Light quality treatment was performed in a temperature-controlled room at 24±2°C. Three monochromatic LED lights (blue, green and red LEDs had peak wavelengths of 470, 530 and 630 nm respectively) and a combined white LED light (blue: green: red = 1: 1: 1) put on 24-hr photoperiod. Each of treatments was screened with black cloth to preclude extraneous light. The PPFD of each LED was adjusted to 100 μmol·m^{-2}·s^{-1} at the top of plants. Three plants were placed in each light quality treatment area for 7 or 14 days.

Analysis of leaf color and pigment content in leaf epidermis

For the analysis of leaf color, three leaves from the fifth to the seventh counted from the top were sampled. The measurement points were the apex part (within 5mm from the tip) and the center of leaf lamina. L*, a*, and b* values of those leaves were measured with a color meter (ZE6000; Nippon Denshoku Industries Co., Ltd., Tokyo, Japan).

For the measurement of anthocyanin or total polyphenol contents, epidermis of whole three leaves from the fifth to the seventh counted from the top were sampled. The epidermis samples were dipped in 1% HCl-methanol for 24 hours for anthocyanin extraction. The absorbance of each extract was measured at 530 nm by a spectrophotometer (U-2000, Hitachi, Ltd., Tokyo, Japan). Anthocyanin content was expressed as mg of cyanidin chloride (Wako Pure Chemical Industries, Ltd. Tokyo, Japan) equivalent per gram fresh weight of epidermis. Using other epidermis samples, total polyphenol content was analyzed by the Folin–Ciocalteu reagent method (Singleton et al., 1999). Gallic acid (Acros organics, Thermo Fisher Scientific Inc., USA) was used as the standard for the calibration curve, and the total polyphenol contents were expressed as mg gallic acid equivalent per gram fresh weight of epidermis. The means values were compared using ANOVA followed by Tukey's multiple range tests at the 1 % level.

RESULTS AND DISCUSSION

Experiment 1. Effect of light intensity on the leaf color and anthocyanin content

The leaf color became reddish as the light intensity increased. The a* value of the leaves and anthocyanin content of epidermis after 14 days of treatment were 3.16 ± 0.9 and 0.17 ± 0.02 mg/g for 600µmol·m^{-2}·s^{-1} PPFD, and -3.20 ± 1.4 and 0.04 ± 0.00 mg/g for 60µmol·m^{-2}·s^{-1} PPFD respectively. There was a statistically significant difference between the values of 600 and 60µmol·m^{-2}·s^{-1} PPFD at the 1 % level (Table1). On the seventh days from the start of treatment, this difference had already been made visible. It was assumed that the leaves of 'Bronze' were very sensitive to light intensity.

Table 1. Effect of light intensity on the a* value and anthocyanin content of *Graptopetalum paraguayense* 'Bronze' after 14 days from the start of treatment.

Treatment period (Days)	PPFD (µmol·m^{-2}·s^{-1})	a* value	Anthocyanin content (mg/gFW epidermis)
0^{z}	-	3.02a	-
14	60	-3.20b	0.04b
	600	3.16a	0.17a

z: Anthocyanin content was not measured.

n=3, Different letters indicate a significant difference at the 1% level by Tukey's test.

Experiment 2. Effect of light quality on the leaf color and pigment contents

The a* values of the leaves under the green and red light decreased with increasing of treatment days. On the other hand, the values hardly changed under white and blue light (Figure 1).

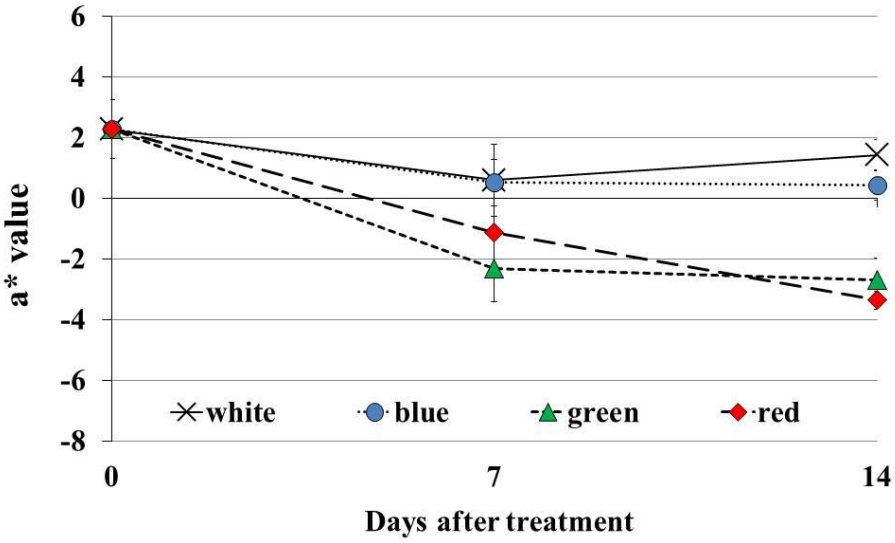

Figure 1. Effect of light quality on the a* value of *Graptopetalum paraguayense* 'Bronze' leaf lamina.

The a* values of the leaves tended to be higher at the apex of the leaves than at the center of the leaf lamina. However, the green light treatment showed the lowest value at both parts (Figure 2).

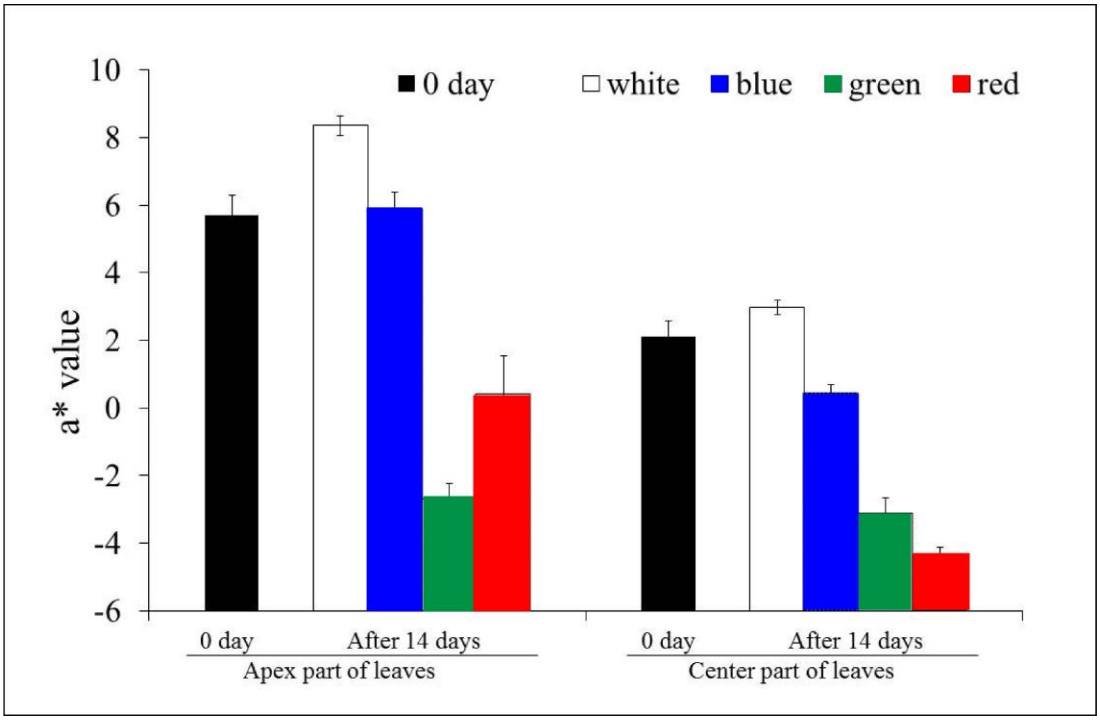

Figure 2. Effect of light quality and the position of leaf lamina on the a* value of *Graptopetalum paraguayense* 'Bronze' leaf lamina after 14 days from the start of treatment.

The content of anthocyanins in the epidermis after 14 days from the start of treatment was about 2 times higher under the white light than under the green and red light (Table 2), and there was a very high correlation (r = 0.93) between the anthocyanin contents and the a* values (data not shown).

Table 2. Effect of light quality on the content of anthocyanin and total polyphenol of *Graptopetalum paraguayense* 'Bronze' leaf epidermis after 14 days.

Treatment period (Days)	Light quality	Anthocyanin content (mg/gFW epidermis)	Total polyphenol content (mg/gFW epidermis)
0	-	0.42 ab	30.55 b
14	white	0.47 a	25.17 c
	blue	0.32 abc	34.88 a
	green	0.24 bc	28.78 b
	red	0.17 c	18.67 d

n=3, Different letters indicate a significant difference at the 1% level by Tukey's test.

Besides, the value of total polyphenol content was the highest under the blue light, and significantly increased from the start of treatment. There are some reports that the coloration of strawberry and apple fruits is improved by blue light irradiation (Yunting et al., 2018; Kokalj et al., 2019). In the leaves of 'Bronze' as well, it is considered that the blue light irradiation promotes anthocyanin and polyphenol synthesis and maintains the anthocyanin content in the leaf epidermis.

Anthocyanin biosynthesis was found to be differentially modulated by environmental and biological factors such as light, temperature, sugar content, and plant hormones. In light, it has been clarified that UV, blue, and red lights are signals for anthocyanin synthesis (Gu et al., 2019). High light intensity stimulates anthocyanin production in many plant species (Maier and Hoecker, 2015). Also, leaves that receive direct light are more intense anthocyanin pigmentation than leaves that shaded (Mazzucato et al, 2013). In 'Bronze', the higher the light intensity, the higher the a* value, especially in the leaf apex parts that are more likely to receive light (Table 1 and Figure 2), indicating that anthocyanin biosynthesis is controlled by light as in previous reports. On the other hand, the degree of change in the a* value was larger for the mixed white light of 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD (Figure 1 and 2) than for the YAG white LED of 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD (Table 1).

In addition, under the irradiation of red or green monochromatic light, the anthocyanin content decreased with the lapse of the treatment time (Figure 2). From these results, it is considered that the light quality that promotes anthocyanin synthesis is blue light, and the involvement of red light is low in 'Bronze'. However, the anthocyanin content was the highest in the mixed white light containing blue rather than blue monochromatic light (Table 2). From this result, it is expected that the light quality of the irradiation light is involved not only in the biosynthesis of anthocyanins but also in the metabolism of sugar as a substrate, the accumulation of anthocyanins in cells and the subsequent metabolism.

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