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Effect of Plantlux Light on Coloration and Flavonoid Accumulation in Commercial Lisianthus Flowers (*in vitro*)

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Summary

Flowers from detached buds of three lisianthus cultivars, 'Asuka no Asa', 'Mickey Rose' and 'Asuka no Kurenai', were examined *in vitro* to investigate the influence of the partial UV-A (380-400 nm) and blue light (400-470 nm) spectra of visible light on petal coloration and flavonoid accumulation. The saturation and hue of flower color changed with time after anthesis under conditions of darkness, control irradiation (380-700 nm) and 380-470 nm cut irradiation (470-700 nm). The chromaticity of lisianthus flowers was highest in the control, but the hue angles of flower petal color did not differ among the three conditions in any of the cultivars. Anthocyanin biosynthesis was enhanced by partial UV-A and blue light as well as 470-700 nm of visible light, whereas flavonol biosynthesis did not proceed without partial UV-A and blue light. Although the biosynthesis of pigments might start before flower opening and partial UV-A and blue light spectra were considered to be a factor for accelerating the synthesis after anthesis, minimum petal flavonoids aggregated even in darkness where the quantity retained a similar level to that found before treatment. The wavelengths 380-470 nm are required for the expression of vivid flower colors as well as for the accumulation of flavonoids without changing lisianthus flower hue.

Key words: anthocyanidin, *Eustoma grandiflorum*, flavonol, flower color, Gentianaceae, ultra violet (UV)

Introduction

Most plants with green leaves must absorb sunlight, especially the red and blue components, in order to grow and flower. Plantlux provides high levels of blue and red radiation. Plantlux, a newly designed fluorescent lamp that stimulates plant growth, as well as the photosynthesis and production of chlorophyll in locations with insufficient daylight, provides a correct balance of blue and red energy.

Photo-biological induction of flavonoids formed by UV-B has been studied in flowers of *Nymphaea* (300-550 nm) [9]. UV-radiation induces the synthesis of anthocyanins in *Petunia hybrida* (from 310 nm to far-red light) [8]. In many other flowers, including *Geranium, Phlox* and *Antirrhinum majus*, UV-radiation has no effect on anthocyanin synthesis, specifically the proportion and composition of anthocyanins [6]. Regulation of the biosynthesis and the accumulation of petal

anthocyanins has been studied at different visible light intensities in lisianthus [5, 7, 14]. Several distinct "inductive" and "synergistic" UV/blue photo-transduction pathways regulate chalcone synthase (*Chs*) gene transcription and transcript accumulation in *Arabidopsis* leaf tissue [2].

Flowers for commercial use are normally harvested with many buds. The buds of a cut flower often do not grow normally and do not show the same pigmentation as mature flowers. The reason for this is considered to be the lack of a biological response to accumulated petal anthocyanins. UV-A/blue and visible irradiation might correspond with flower color to increase flower color intensities. The objective of this study is to assess the role of Plantlux light in flower coloration and flavonoid accumulation *in vitro* in three lisianthus cultivars.

Materials and Methods

Plant materials

Seeds of lisianthus cultivars were purchased from Sakata seed Corp., Yokohama, Japan. Seedlings of lisianthus cultivars were grown in a greenhouse at Kagoshima University Experimental Farm, using standard greenhouse practices established for lisianthus [12]. The cultivars 'Asuka no Asa' (bluish violet), 'Mickey Rose' (reddish purple) and 'Asuka no Kurenai' (reddish) were used, as representative for the petal anthocyanidins, delphinidin (Dp), cyanidin (Cy), and pelargonidin (Pg), respectively.

Phytotron condition

Plantlux lamps (Toshiba, FL20SSBRN/18) were used in partial UV and visible light chambers (380-700 nm) as a control. Irradiation 380-470 nm cut film was placed in a neighboring chamber to cut the irradiation below 470 nm (only visible light, 470-700 nm). Non-transparent black sheets were used for the third condition (darkness).

Condition of cut-flower cultures

The temperature of the artificially illuminated chambers was maintained *via* air conditioning units at 25 °C with 80% relative humidity. The chambers were illuminated with the same flux density (approximately 3,500 lx) for 24 hr everyday. Buds were obtained as described in a previous report [14]. They were collected 5 days before flower opening, and placed in vials containing 0.125 M sucrose solution.

Petal color measurement

Petal colorization, L^*, a^*, and b^*, was measured using a handy-type color analyzer (Nippon Denshoku, NR-3000) based on the CIELab (Commission Internationale de l'Éclairage) scale [1, 11]. $L^*a^*b^*$ consists of a lightness component (L^*), corresponding to the vertical axis, and two chromatic components: a^* (from green to red) and b^* (from blue to yellow). C^* (Chroma; brightness) and hue angle (h, degree) were calculated using the equations, $C^* = (a^{*2}+b^{*2})^{0.5}$ and $h_{ab} = \tan^{-1}(b^*/a^*)$, respectively [3]. Hue angle represents the angle from the axis $+a^*$ to display a locus of color on a hue diagram; a direction of color (hue).

Total anthocyanins (TA) and flavonols (TF) in petals

Petal slices were macerated in 5 ml of acidic methanol solution (1% HCl-MeOH) in a test tube and allowed to equilibrate overnight at 4 °C. Concentrations of anthocyanins and flavonols in

different samples were calculated quantitatively from a simple linear regression using cyanidin 3,5-di-*O*-glucoside for TA and quercetin 3-*O*-rutinoside for TF as standards, respectively [6]. The optical density (O.D.) of individual samples was measured with a UV spectrophotometer (Toshiba, Spectra; Model SPM-60A) at 525 nm and 370 nm for TA and TF, respectively, and calculated in mg/100mg fresh petals (mg%).

Statistical Analysis

SAS [13] was used to determine the analysis of variance (ANOVA) to examine the observed anthocyanin concentration as well as flavonol concentration under different light conditions in lisianthus petals. The mean and standard deviation (three replicates) were calculated for each of the samples.

Results

Chromatic characterization after anthesis

Hue (h_{ab}), chroma (C^*) and lightness (L^*) showed significant variation under the specified light conditions in all the cultivars (Fig. 1). One day after anthesis (DAA), petal C^* in all cultivars was highest under irradiation at 380-700nm (Plantlux). At 1-2 DAA, C^* values did not show any extreme changes in color saturation, however, a rapid increase tended to occur through to 5 DAA. C^* values were highest in the control flowers for all the cultivars throughout and were lowest in flowers kept in darkness after 5 DAA. L^* tended to decrease along with DAA and the flowers became bright, whereas colors were vivid under 380-700 nm light for all the cultivars.

Flower hue in each cultivar decreased rapidly in the primary stage of anthesis through to 2 DAA, but was a constant value at 4 DAA (Fig. 2). From 5 to 7 DAA, 'Asuka no Asa', 'Mickey Rose' and 'Asuka no Kurenai' retained hue angles ranging between -40 and -43, -22 and -24, -8 and -10 degrees, respectively.

Petal pigmentation

The accumulation of pigments in fresh petals from detached buds differed under the three conditions (Fig. 3). Specifically, 'Asuka no Kurenai' showed a significant difference in anthocyanin synthesis from the other two cultivars. Amounts of anthocyanins in the three cultivars were similar and lowest in the dark condition, whereas maximum amounts accumulated with irradiation at 380-700 nm.

Darkness and the 380-470 nm cut condition (470-700 nm) did not produce any significant variations, but a significant effect caused by irradiation at 380-700 nm (control) was observed for flavonol synthesis ($P<0.05$, Fig. 3). The cultivar 'Mickey Rose' produced the most flavonols under all conditions.

Discussion

The concentration of anthocyanin was markedly low when irradiation at 380-470nm was cut in comparison with irradiation at 380-700nm, due to the lack of partial UV-A (380-400 nm) and blue light (400-470 nm) spectra of visible light. The synthesis of anthocyanin was evident in the dark condition, probably due to a lack of light intensities suitable for synthesizing pigments [14]. Prior to harvesting, a signal might have already occurred to start synthesizing pigments in buds since the

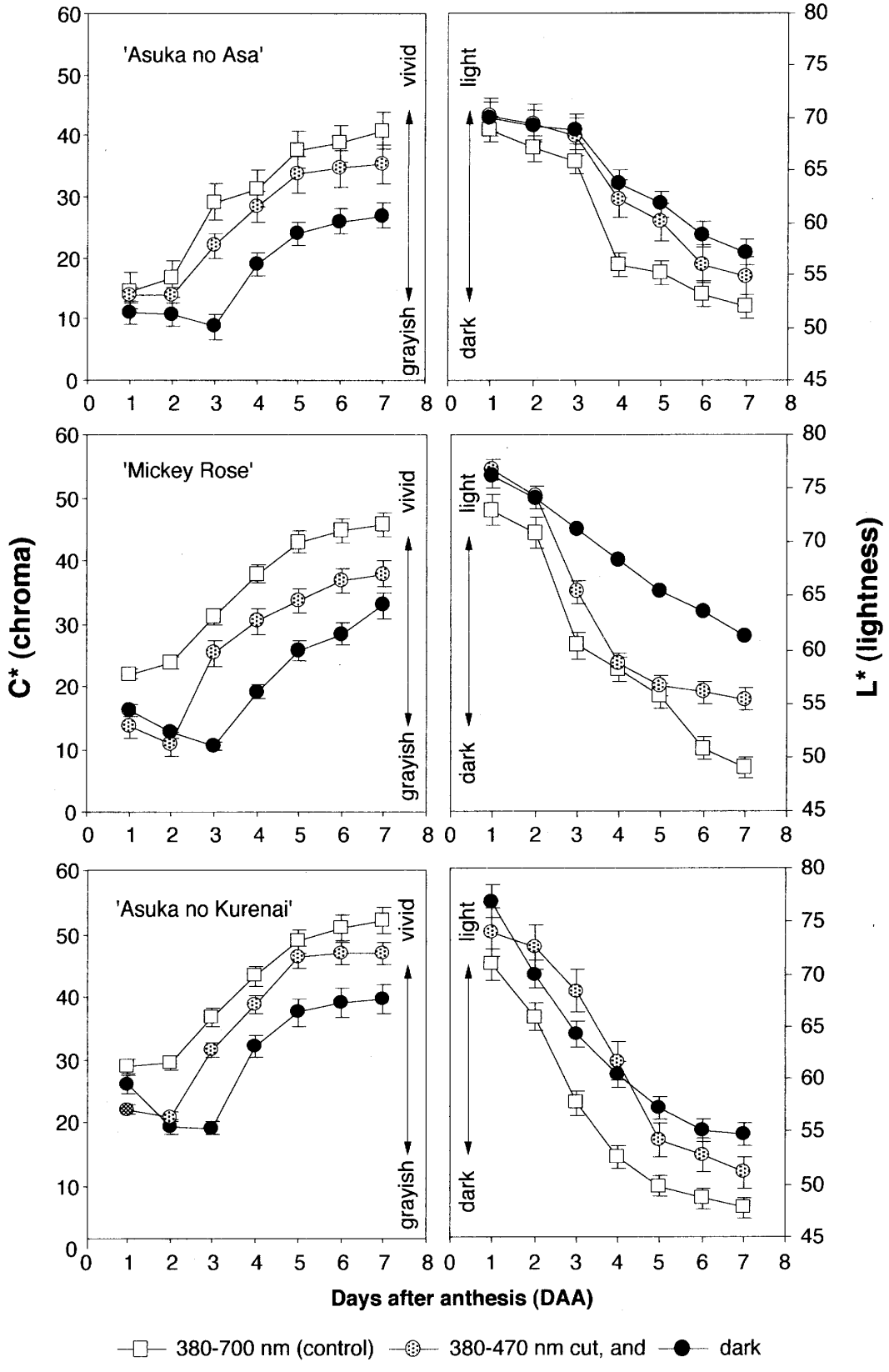

Figure 1. Variation in C* and L* values of three lisianthus flowers for seven days after anthesis due to light quality. Vertical line represents SD for three replicates.

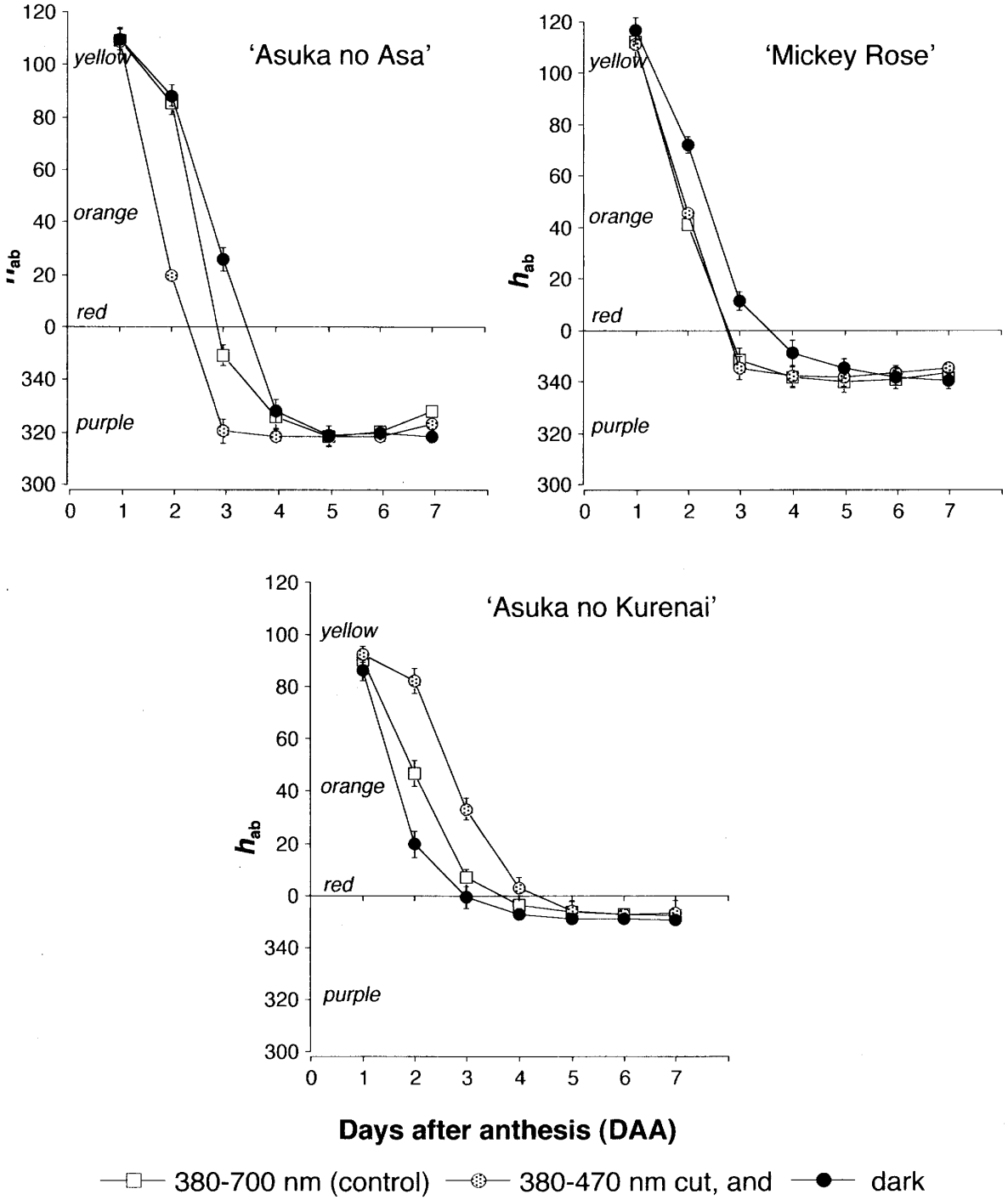

Figure 2. Variation in hue angle of three lisianthus flowers for seven days after anthesis due to light quality. Vertical line represents SD for three replicates.

lowest concentration was observed in the dark-treated samples whose buds were incubated without partial UV-A or visible light.

The irradiation at 380-470 nm has a direct photo-biological effect on the synthesis of both anthocyanins and flavonols. The pigmentation in cultivars of lisianthus proved to be extensive with

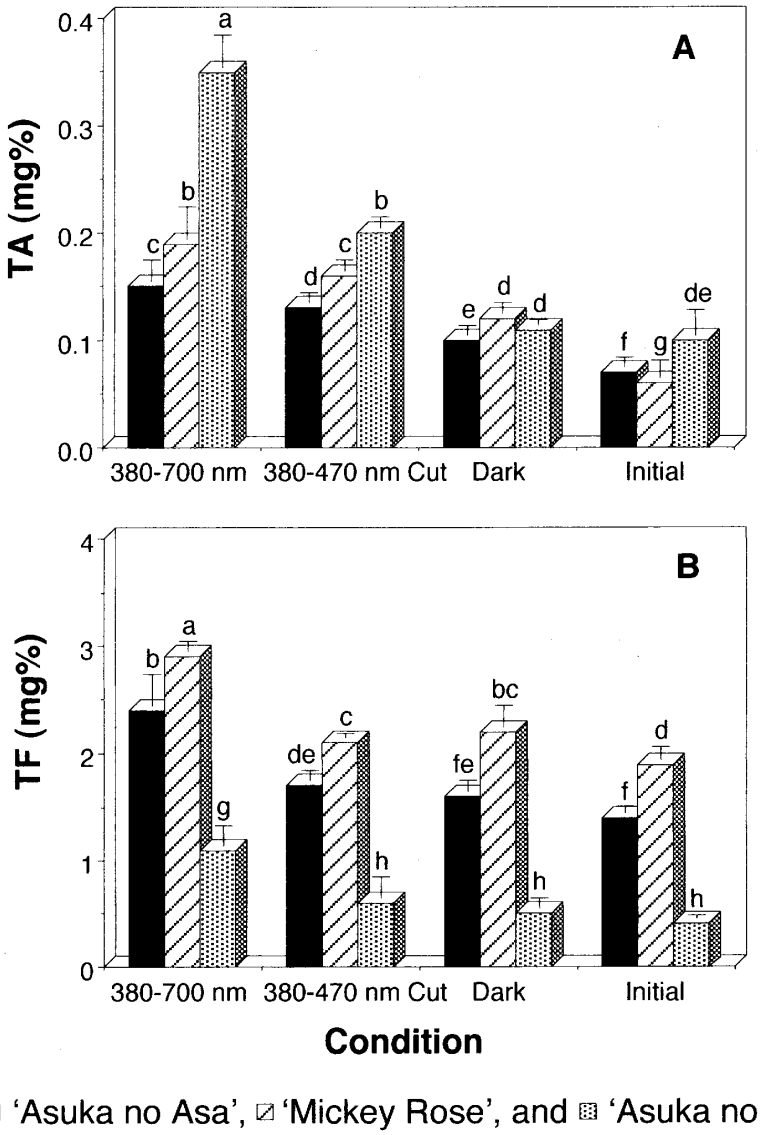

Figure 3. Production of petal (**A**) anthocyanins and (**B**) flavonols in three lisianthus flowers at 5 DAA. Vertical line shows SD for three replicates (mg% = mg/[100mg fresh petal]). Initial: data on the 1st day without treatment. Within each cultivar and bar, means followed by a different letter are significantly different ($P<0.05$).

the most vivid coloration obtained in the presence of irradiation at 380-470 nm. The accumulation of pigments is important to the color of a flower. The colors that are perceived by the human eye often depend upon the types of pigments in the cells of flowers, the vacuolar pH, or chemical interactions such as co pigmentation [4]. In these three lisianthus cultivars, petal anthocyanidin proportions did not change during culture *in vitro* under different light intensities and with sucrose-containing media [14]. None of the flowers in the present study showed a change in petal anthocyanidin proportions with or without UV or in darkness. Thus, the biosynthesis of anthocyanins is well controlled and in balance even under different light conditions. The three cultivars 'Asuka no Asa', 'Mickey Rose' and 'Asuka no Kurenai' contained the anthocyanidins delphinidin, cyanidin and pelargonidin at over 80%, respectively, and the hues of their flowers

resulted in purple, reddish purple and red at maturity with average hue angles of -41, -23 and -9 degrees, respectively (Fig. 2). It was suggested that the petal anthocyanidins defined flower color [15], however, petal chromaticity depended on the 380-470 nm light spectra that unequivocally produced vivid flower colors.

The study demonstrated that a part of the UV-A and blue light spectra of visible light enhanced flower pigmentation confirming that irradiation at 380-470 nm is required for normal petal pigmentation in lisianthus flowers (Fig. 3). Furthermore, both the UV and visible light spectra increased the amount of petal anthocyanins, but only the 380-470 nm spectra increased the amount of petal flavonols. Since different regulators control different parts of the biosynthetic pathway of flavonoids, the control of petal color appears to be vested primarily in the transcription of genes responsible for anthocyanin biosynthesis [10].

The spectra 380-470 nm induced the production of anthocyanins in petals, and the increased concentration produced more vivid colors. Irradiation with Plantlux lights can ensure a vivid petal color and would increase the high market price of commercial cut-flowers.

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