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Relationship of Light Quantity and Anthocyanin Production in *Pennisetum setaceum* Cvs. Rubrum and Red Riding Hood

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Pennisetum setaceum cvs. Rubrum and Red Riding Hood are purple-pigmented ornamental grasses when grown in high-light environments. In low-light environments, foliage appears light purple or green, and as a result, aesthetic appeal is reduced. The impact of light on anthocyanin pigmentation was compared for *P. setaceum* Rubrum foliage and flowers and Red Riding Hood foliage grown under different light intensities and light sources. Light environments included UV supplemental light in the greenhouse, high-pressure sodium supplemental light in the greenhouse, cool-white fluorescent light in a growth chamber, and full sun outside. Anthocyanins in two cultivars of *P. setaceum* were analyzed by HPLC and characterized by ^{1}H and ^{13}C NMR spectral experiments. Two anthocyanins, cyanidin 3-glucoside and cyanidin 3-rutinoside, were identified in the leaves and flowers of both cultivars and quantified by HPLC analysis. The major anthocyanin in both cultivars was cyanidin 3-glucoside and had highest concentration (0.199% fresh weight) in Rubrum leaves grown under fluorescent lights in the growth chamber with a photoperiod of 24 h and a daily light integral (DLI) of 13.3 mol m^{-2} day^{-1} and in Rubrum and Red Riding Hood leaves and flowers (0.097 and 0.12% fresh weight) from plants grown outside in full sun with a photoperiod ranging from 15 to 13.5 h and DLI of 42 mol m^{-2} day^{-1}. The minor anthocyanin, cyanidin 3-rutinoside, had the highest quantity in plants grown in low-light-intensity greenhouse environments with a photoperiod ranging from 15 to 13.5 h and DLI of 2.3−7.0 mol m^{-2} day^{-1}. The functional significance of anthocyanins in *P. setaceum* Rubrum is discussed.

KEYWORDS: ***Pennisetum setaceum*; Rubrum; Red Riding Hood; ornamental grass; purple fountain grass; anthocyanins; light intensity; antioxidant**

INTRODUCTION

Ornamental grasses have become increasingly popular in recent years. The growing diversity of grasses available to gardeners has emerged through the efforts of botanists, breeders, and greenhouse producers (*1*). Ornamental grass foliage has a wide variety of colors, such as greens, blues, silvers, and reds. *Pennisetum setaceum* is a native African, mound-forming perennial grass with green foliage (*2*). *P. setaceum* Rubrum and Red Riding Hood are cultivars that have been selected for their ornamental purple foliage. *P. setaceum* Rubrum is commonly known as "purple fountain grass" in reference to its dark purple, arching foliage. *P. setaceum* Red Riding Hood is more compact than Rubrum with narrow, light purple leaves. Both *Pennisetum* cultivars produce tall purple flower spikes in late summer and are nonhardy above USDA plant hardiness zones 9. The purple color observed in Rubrum and Red Riding Hood foliage is attributed to anthocyanins.

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Anthocyanins accumulate in vegetative tissues when developmental or environmental factors cause plants to become more sensitive to their surroundings (*3*). Generally, anthocyanins are induced by light, in the form of visible and ultraviolet (UV) light (*4*). Anthocyanins are induced to a lesser extent by other factors including low temperature, nutrient stress, and pathogen attack (*3*).

Anthocyanin pigmentation in vegetative tissues has been related to light exposure and may play a role in reducing oxidative stress in high-light environments (*5*). Photoinhibition occurs when energy capture occurs at a rate faster than electron transport in the photosynthetic apparatus (*3*). This results in repression of photosynthesis, which causes the production of reactive oxygen species and subsequent damage to the photosynthetic tissues (*6*). Anthocyanins may provide a "shield" by absorbing excess radiation, providing protection to the photosynthetic apparatus (*4*). This shield is most effective when anthocyanins are located in the epidermis (*7*). Anthocyanins may function as a protective shield to plants exposed to UV light. Certain species such as *Cotinus coggygria* cv. Royal Purple increase anthocyanin production when exposed to UV light (*8*).

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However, other UV-absorbing compounds, such as flavonols, flavones, hydroxycinnamic acids, and carotenoids, may contribute to UV absorption (*7*). The amount of UV damage to foliage depends on the length and intensity of exposure. In addition, the level of visible light also affects how plants respond to UV stress (*3*).

Like flower color, pigmented foliage in ornamental plants is an important attribute for marketability and consumer preference (*9*). Environmental factors, such as low temperatures and UV light, are tools used to increase color in the ornamental crops *C. coggygria* Royal Purple (*10*) and *Kalanchoe* × *hybrida* cv. Colorado (*11*). Purple fountain grass is valued as an ornamental garden plant because of its dark, plum-purple leaves and flowers. Therefore, determining the environmental conditions for maximum pigmentation is valuable to the plant industry.

Anthocyanins have been characterized in various grasses, such as *Festuca rubra* and *Panicum melinis* (*12*). However, anthocyanins in *P. setaceum* Rubrum and Red Riding Hood have not been identified. Furthermore, the relationship between anthocyanin content and light environment has not been fully understood. In this study, anthocyanins in two cultivars of *P. setaceum*, Rubrum and Red Riding Hood, were characterized. Also, the analyses of anthocyanins in *P. setaceum* Rubrum leaves in plants growing under four different light environments were compared: outdoors in full sun, in a greenhouse with supplemental high-pressure sodium light or supplemental UV light, and in a growth chamber with cool-white fluorescent light.

MATERIALS AND METHODS

Plant Materials. All plant materials used in our study were propagated from mature plants by three-tiller rooted divisions, placed in 72-cell plug trays, and grown in a misted propagation house for 2 weeks with air and medium temperatures of 23 °C. Divisions were transplanted into 5-in. pots in a peat and perlite mixture (Sure-Mix, Michigan Grower Products, Galesburg, MI) and grown in a glass greenhouse at 20 ± 3 °C under 16-h photoperiod (natural and supplemental high-pressure sodium lighting) until the start of experiments.

Plants Grown under Growth Chamber Conditions. *P. setaceum* Rubrum grasses were grown in a greenhouse during October and November 2002 for 8 weeks after leaving the propagation house. Fifteen individual green leaf sections (5 cm at the base of the leaf) were selected from nonflowering tillers and covered with two sheets of opaque paper. The paper shields were removed from the leaf 2 weeks later, and the leaves appeared green. Plants were immediately placed in a controlled growth chamber, and green leaves were attached to a horizontal board ~21.5 cm under six white fluorescent lamps (Phillips F96712/CW/Vtto, 215 W). Control leaves were attached to the board with paper shields still in place. The chamber remained at 20 °C (day/night) and had a 24-h photoperiod. Each leaf received an average daily light integral (DLI) of 13.3 mol m^{-2} day^{-1} (154 μmol m^{-2} s^{-1}) in the chamber. Average leaf temperature was 24.5 °C. After 7 days, leaf sections were sampled by extracting 0.4 g of tissue in (2 mL) acidic methanol (pH 4).

Plants Grown under Outdoor Conditions. Young, nonflowering *P. setaceum* Rubrum and Red Riding Hood were planted in the Michigan State University gardens in early June 2002. Rubrum leaves and flowers and Red Riding Hood leaves were collected from the Michigan State University garden on September 2, 2002, and September 25, 2002, respectively. The leaves were cut from the plant at the leaf base, placed into plastic bags, and stored at −20 °C until extraction. From June 2002 until August 2002, the outdoor plants received an average DLI of 42.0 mol m^{-2} day^{-1}. (Light data were collected from the Horticulture Research Farm, Michigan State University).

Plants Grown under Greenhouse Conditions. *P. setaceum* Rubrum grasses were grown in a 20 °C greenhouse for 8 weeks during October and November 2003 after leaving the propagation house. Leaf samples were taken from the mid-section of leaves on the top, middle, and bottom of the canopy. Leaf samples taken from the middle of the canopy received ~50% less light from plant self-shading. The leaves at the bottom of canopy received ~67% less light from plant self-shading. Leaf cuttings (0.5 g) were taken from the middle of the leaf (6 cm from the base of the leaf), divided into 2 mm sections, and extracted with acidic methanol (2 mL, pH 4). Samples were kept at −20 °C until extraction. Greenhouse temperature was 20 °C (day/night), and the photoperiod was 16 h (supplemental high-pressure sodium lamps and sunlight). Plants received an average DLI of 7.0 mol m^{-2} day^{-1}.

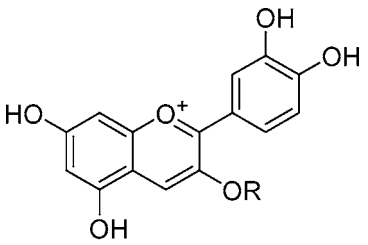
General Experimental. ^{1}H and ^{13}C NMR spectra were recorded on a Varian INOVA 500 MHz spectrometer. Compound was dissolved in CD_3OD/DCl and is reported in δ (parts per million) based on δ residual of CD_3OD at 3.30 for ^{1}H NMR and 49.15 for ^{13}C NMR. Coupling constants, *J*, are in hertz. C_{18} silica gel used for medium-pressure liquid chromatography (MPLC) was purchased from DyChrom (San Jose, CA). All organic solvents used were of ACS reagent grade (Spectrum Chemical Co.). Two experiments were conducted in the same controlled growth chamber (Environmental Growth Chambers, Inc., model 2614-3, Chagrin Falls, OH).

Extraction and Purification of Anthocyanins. *P. setaceum* Rubrum grass leaves from outdoor conditions (992 g) were blended with acidic MeOH (1 L, pH 3) in a Waring blender (Dynamics Corp. of America, New Hartford, CT). The residue was further extracted with acidic MeOH (1 L × 4, 24 h). The combined MeOH extract (5 L) was concentrated under vacuum at 35 °C to 1 L and partitioned between hexane and MeOH. The MeOH soluble portion was evaporated to dryness and yielded a reddish powder (31 g). An aliquot of this powder (5 g) was dissolved in acidic MeOH (50 mL), subjected to C_{18} MPLC, and eluted with a MeOH/H_2O gradient, increasing the MeOH concentration from 10 to 100%. The volumes of eluting solvents used were MeOH/H_2O 1:9 (500 mL), MeOH/H_2O 2:8 (500 mL), MeOH/H_2O 3:7 (500 mL), MeOH/H_2O 4:6 (500 mL), MeOH/H_2O 1:1 (500 mL), and MeOH (500 mL). Thirteen aliquots of 200-mL fractions, 1−13, were collected, and fractions 2−4 were combined to yield pure cyanidin 3-glucoside (**1**, 290 mg).

Cyanidin 3-glucoside (1): ^{1}H NMR (500 MHz) δ 8.96 (H-4, d, J = 0.5 Hz), 8.21 (H-6′, dd, J = 8.50, 2.50 Hz), 8.00 (H-2′, d, J = 2.50 Hz), 6.99 (H-5′, d, J = 8.50 Hz), 6.89 (H-8, J = 2.00, 1.00 Hz), 6.65 (H-6, d, J = 2.00 Hz), 5.30 (H-1″, d, J = 8.00 Hz), 3.68 (H-2″, dd, J = 9.50, 8.00 Hz), 3.57 (H-3″, dd, J = 9.50, 9.00 Hz), 3.46 (H-4″, dd, J = 9.50, 9.00 Hz), 3.58 (H-5″, ddd, J = 9.50, 5.50, 2.00 Hz), 3.92 (H-6″a, dd, J = 12.00, 2.00 Hz), 3.73 (H-6″b, dd, J = 12.00, 5.50 Hz); ^{13}C NMR (125 MHz) 170.4 (C-7), 164.0 (C-2), 159.1 (C-5), 157.6 (C-9), 155.7 (C-3), 147.3 (C-3′), 145.6 (C-4′), 136.7 (C-4), 128.4 (C-6′), 121.2 (C-1′), 118.4 (C-2′), 117.4 (C-5′), 113.3 (C-10), 103.6 (C-6), 103.4 (C-1″), 95.2 (C-8), 78.7 (C-5″), 78.1(C-3″), 74.8 (C-2″), 71.1 (C-4″), 62.3 (C-6″). The NMR data of **1** were identical to the published spectral data of cyanidin 3-glucoside (*13*, *14*).

Cyanidin 3-rutinoside (**2**) was detected by HPLC in fractions 5 and 6. The presence of **2** was confirmed by co-injection of an authentic sample of cyanidin 3-rutinoside under the same conditions as used for cyanidin 3-glucoside, compound **1**. The quantity of cyanidin 3-rutinoside isolated was too small to perform NMR spectral studies.

Sample Preparation for HPLC Analyses. *P. setaceum* Rubrum grass leaves and flowers and *P. setaceum* Red Riding Hood leaves from outdoors (5 g) were blended separately in 3 × 30 mL of acidic MeOH (1% HCl) for 2 min in a Waring blender and filtered. The residue was extracted again with acidic MeOH (3 × 20 mL, 1% HCl). Extracts were combined and evaporated under reduced pressure at 35 °C to yield the solvent-free extracts of each grass sample and stored at −20 °C until analysis. Each extract prepared from the grass grown outdoors was dissolved in 2% aqueous H_3PO_4/CH_3CN (84:16, 20 mL), the mobile phase used for HPLC analysis. The resulting solution (200 μL) was diluted with the same mobile phase to 2.0 mL and filtered through a 0.22-μm membrane. The samples from the top, middle, and lower canopy of plants grown under greenhouse conditions were cut into small pieces (1−2 mm in length), weighed, and extracted for 24 h with 1% HCl in MeOH (2 mL). The extracts prepared from the greenhouse grass were filtered through a 0.22-μm membrane before HPLC analysis.



R

1 Glucose

2 Rutinose

Figure 1. Structure of anthocyanins in *P. setaceum* cvs. Rubrum and Red Riding Hood.

HPLC Quantification of Anthocyanins. All samples were filtered (0.22 μm) and analyzed by HPLC on an XTerra (Waters Corp., Milford, MA) Rp-18 column (250 × 4.6 mm, 5 μm) at 25 °C. The mobile phase, 2% aqueous H_3PO_4/CH_3CN (84:16), was used under isocratic conditions at a flow rate of 0.75 mL/min. Anthocyanins were detected at 520 nm using a PDA detector (Waters Corp.). Quantification of anthocyanin was accomplished using Enpower software (Waters Co.). Pure cyanidin 3-glucoside (1 mg) was dissolved in the mobile phase (1 mL) to yield a stock solution (1 mg/mL). Further serial dilution of the stock solution afforded 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625, and 0.0078 mg/mL concentrations, respectively. Each sample was analyzed in triplicate, and a calibration curve was prepared by plotting the mean peak area against the amount of the anthocyanin injected. Grass samples were analyzed in triplicate, and the mean peak area of anthocyanin was used to determine the quantities of anthocyanin in both cultivars of *P. setaceum*.

Anthocyanin Production under Supplemental UV Light. *P. setaceum* Rubrum was propagated from stock plants in December 2001 at Michigan State University greenhouses. After 4 weeks, divisions were potted into 4.5-in. round pots and placed under UV lamps for treatments, which included 1-, 2-, and 4-h UV-A and 1-, 2-, and 4-h UV-B and control (no UV). All plants were grown under a 16-h photoperiod (natural light and high-pressure sodium lamps) from 6:00 a.m. to 10:00 p.m. UV lamps (Q-Panel Lab Products, UV-B-313 and UV-A-340) were on for 1-, 2-, or 4-h periods beginning at 6:00 p.m. and ending at 12:00 a.m. Five nonflowering *P. setaceum* Rubrum leaves per plant were sampled on days 8 and 20 after the start of the experiment. Leaf samples (6 cm in length) were collected at 6 cm from the base of the plant. Samples were cut into five 16-mm sections and placed in scintillation vials containing HCl/MeOH (5 mL, 1:100). Samples were stored in the dark at 5 °C for 48 h. The anthocyanin concentration was determined by measuring optical density (OD) at 530 nm.

Anthocyanin Production under Growth Chamber Conditions. *P. setaceum* Rubrum grasses were grown for 8 weeks from March to May 2002. Leaves on nonflowering shoots were selected, and the base of the leaf was covered with 2.5 cm of black felt and secured with laboratory tape. After 10 days, plants were placed in a controlled growth chamber with a 12-h photoperiod at 20 °C (day/night) and covers were removed. The pots were lined along the bench, and leaves were secured to the incline. Control leaves were left covered and placed in the light. Six white fluorescent lamps (Phillips F96712/CW/Vtto, 215 W) hung directly above the incline and light levels ranged from 0.6 to 6.5 mol m^{-2} day^{-1} (3−160 μmol m^{-2} s^{-1}). Chromametric a^* values were taken daily for 9 days (Minolta color meter).

RESULTS AND DISSCUSION

Anthocyanins in the leaves and flowers of cv. Rubrum and leaves of cv. Red Riding Hood were analyzed by HPLC. Two anthocyanins were detected by HPLC, which were cyanidin 3-glucoside and cyanidin 3-rutinoside (**Figure 1**). Cyanidin 3-glucoside, the major anthocyanin, was purified from the leaves of Rubrum and characterized by ^1H and ^{13}C NMR spectral experiments and by comparison with published data (*13*). It was further confirmed by HPLC analysis using an authentic sample. Cyanidin 3-rutinoside was present in all grass samples analyzed, as identified by HPLC, and confirmed by co-injection with an authentic sample of cyanidin 3-rutinoside. Both cultivars showed identical anthocyanins as confirmed by HPLC. The major anthocyanin was cyanidin 3-glucoside, in Rubrum leaves and flowers and in Red Riding Hood leaves. The amount of cyanidin 3-glucoside was 0.075, 0.097, and 0.12% for fresh leaves of Red Riding Hood and fresh leaves and flowers of Rubrum grown outdoors, respectively.

The HPLC chromatograms for *P. setaceum* Rubrum plants grown under different light environments are shown in **Figure 2**. Under low-light environments in the greenhouse (2.3−7.0 mol m^{-2} day^{-1}) and higher light environments in the growth chamber and outdoors (13.3−42.0 mol m^{-2} day^{-1}), both anthocyanins were present in Rubrum leaves; peaks 1 and 2 refer to cyanidin 3-glucoside and cyanidin 3-rutinoside, respectively (**Figure 2**). The quantity of cyanidin 3-rutinoside was less in *P. setaceum* Rubrum plants grown under high-light environments in the growth chamber and outdoors, which received 2 and 6 times more daily light than grasses grown in the greenhouse (**Figure 3**).

Purple fountain grass pigmentation is highly dependent on light for induction and can range in color from pale green in low-light environments to dark purple in high-light environments. *P. setaceum* Rubrum plants grown outdoors had dark purple foliage and flower spikes, and the purple coloration covered the entire leaf from base to tip throughout the canopy. Rubrum plants grown in the greenhouse had light-purple foliage from mid-leaf to tip and were most purple at the top of the canopy. Leaves in the bottom and middle of canopy had low anthocyanin contents and appeared mostly green in color (**Figure 3A**,**B**). Plants grown in the chamber under fluorescent lamps developed dark purple leaf color after a week. The anthocyanin pigmentation developed along the leaf margin and veins during the first 48 h. By day 3, pigment evenly covered the leaf surface. Control leaves were shielded from the light and remained green. *P. setaceum* Red Riding Hood plants were grown only outdoors and had light purple foliage compared to leaves of Rubrum. Red Riding Hood and Rubrum flowers started as dark purple and faded to brown during senescence.

The impact of light on anthocyanin production was evaluated for *P. setaceum* plants growing in three light environments. The amount of anthocyanin, in percent fresh weight (% FW), increased as DLI increased from 2.3 to 7.0 mol m^{-2} day^{-1} in the greenhouse **(Figure 3)**. Leaves on the top of the canopy received the highest light (7 mol m^{-2} day^{-1}). Leaves sampled from the middle and lower sections of canopy received ∼50−67% less light than those on the top of the canopy. The photosynthetic photon flux was relatively similar for plants grown in the chamber (159 μmol m^{-2} s^{-1}) and for plant leaves located in the upper canopy of greenhouse-grown plants (215 μmol m^{-2} s^{-1}). However, grasses grown in the chamber received a 24-h photoperiod, whereas those in the greenhouse received a 16-h photoperiod; therefore, daily light quantity was greater in the chamber (13.3 mol m^{-2} day^{-1}). In addition, grasses grown in the chamber were attached to a board and received direct light without any shading from neighboring plants. *P. setaceum* Rubrum plants grown outdoors received the highest light quantities; however, anthocyanin content was greater for plants grown under fluorescent light (**Figure 3**). The other cultivar,

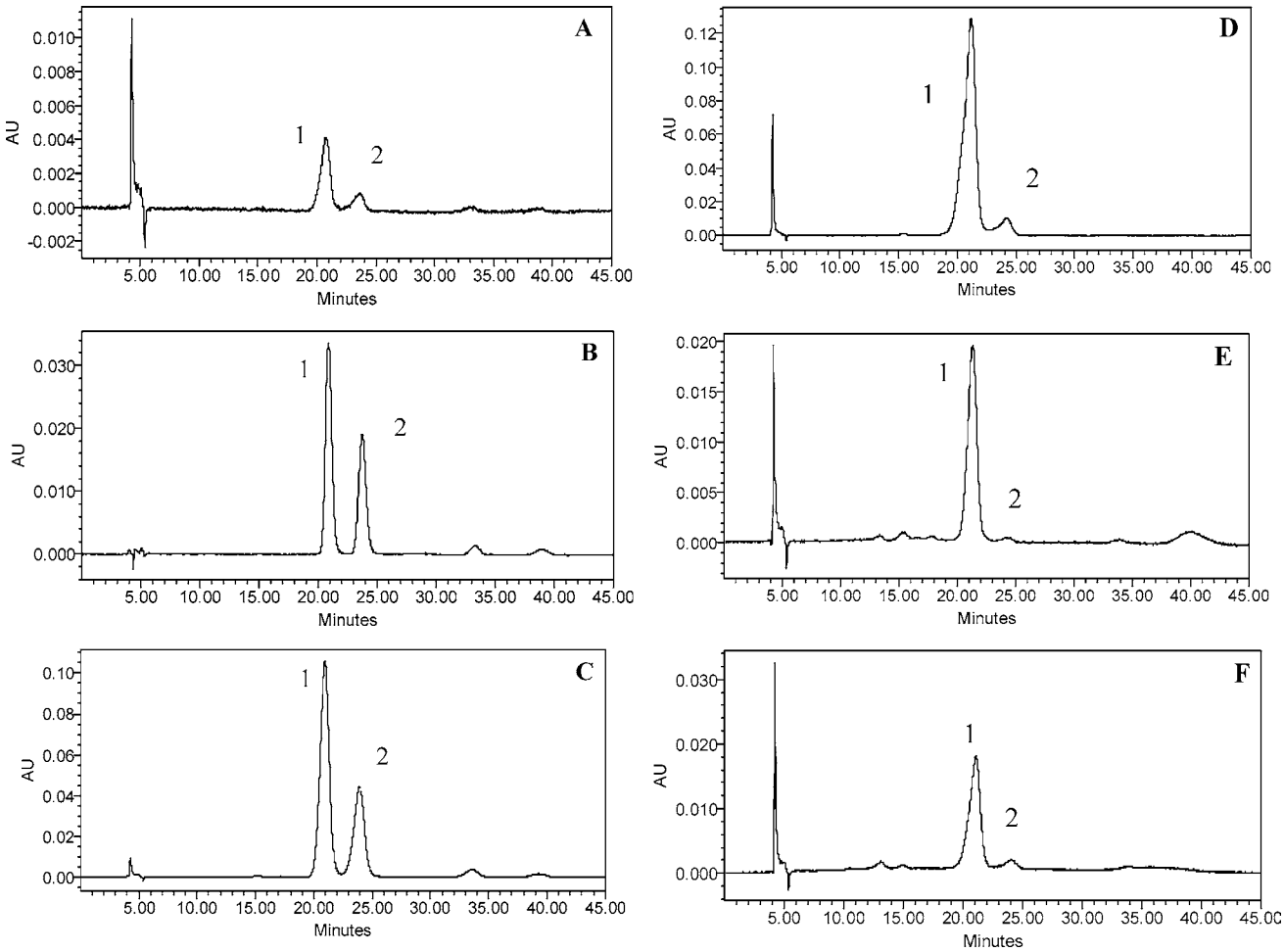


Figure 2. HPLC profiles of anthocyanins in grass samples collected from *P. setaceum* plants grown under different light conditions: (**A**) base of canopy of Rubrum leaves grown in the greenhouse (natural and HPS supplemental, 16-h photoperiod, 2.3 mols m^{-2} day^{-1}); (**B**) middle canopy of Rubrum leaves grown in the greenhouse (natural and HPS supplemental light, 16-h photoperiod, 3.5 mol m^{-2} day^{-1}); (**C**) top canopy of Rubrum leaves grown in the greenhouse (natural and HPS supplemental light, 16-h photoperiod, 7.0 mol m^{-2} day^{-1}); (**D**) leaves of Rubrum grown under fluorescent light in a growth chamber (24-h photoperiod, 13.3 mol m^{-2} day^{-1}); (**E**) Rubrum leaves grown outdoors (photoperiod ranged from 15 to 13.5 h, 42 mol m^{-2} day^{-1}); (**F**) Rubrum flowers grown outdoors (photoperiod ranged from 15 to 13.5 h, 42 mol m^{-2} day^{-1}).

P. setaceum Red Riding Hood, was grown outdoors and contained less anthocyanin than Rubrum grown outdoors (**Figure 3**).

Anthocyanins might protect the plant by quenching free radicals that have formed in the chloroplast or mitochondria and leaked into the vacuole (*14*). The anthocyanidins are more powerful antioxidants compared to anthocyanins (*15*). In general, fewer sugar moieties attached to the anthocyanidin moiety facilitated stronger antioxidant efficacy (*15*). This suggests that anthocyanins may function as antioxidants in *P. setaceum* Rubrum and is supported by the premise that cyanidin 3-rutinoside is converted into cyanidin 3-glucoside in high-light environments (**Figure 4**). The rhamnose moiety may be cleaved from the rutinoside moiety to yield the better antioxidant, cyanindin 3-glucoside (*19, 22*), to provide the maximum amount of antioxidant protection to the plant.

Anthocyanins in plant foliage were induced by blue, UV-A, and UV-B light in a majority of plants (*4, 11, 14, 16*). *P. setaceum* Rubrum plants grown under supplemental UV-A or UV-B light for 1-, 2-, or 4-h durations concurrent with high-pressure sodium (HPS) light delivered during 6:00 p.m. to 12:00 a.m., did not increase anthocyanin production in foliage compared to control plants (**Figure 5**). Plants grown under supplemental UV-A and UV-B light appeared to be "washed out", with little purple color. Under the longer duration UV-B treatments, anthocyanin content decreased over time. This suggests that the anthocyanin pool may have become depleted under the long duration of UV-B due to high turnover of anthocyanins in the high-stress environment. Likewise, the *P. setaceum* Rubrum plants grown outdoors had less anthocyanin (% FW) than plants grown indoors under 13.3 mol m^{-2} day^{-1} fluorescent light (**Figure 3**). This may have resulted from a depletion of the anthocyanin pool due to the high-turnover rate in the high-light environment under outdoor conditions. Also, an increase in colorless flavonoids may have occurred during anthocyanin depletion to increase antioxidant activity (*17*), but this was not measured in this experiment.

In this study, all growing environments provided UV and white light in different quantities. Fluorescent lamps emitted wavelengths ranging between 300 and 800 nm with peaks in the 400−700-mm range and contained small amounts of UV light. Similarly, high-pressure sodium wavelengths ranged between 300 and 800 nm (*16*). Sunlight also contained ~7% UV light (*18*).

To determine the effect of color development in leaves over time, color was measured with a Minolta color meter for *P. setaceum* Rubrum foliage grown under a continuous fluorescent light gradient. The chromametric a^* value (change from green to red) increased as light quantity increased in the growth chamber under fluorescent light (**Figure 6**). Supplemental

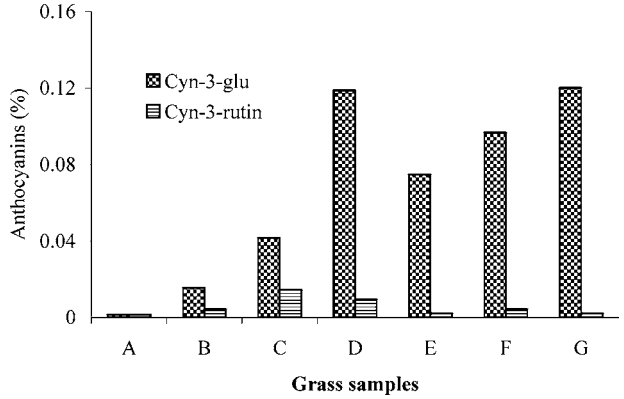


Figure 3. Cyanidin 3-glucoside and cyanidin 3-rutinoside concentrations in two cultivars of *P. setaceum* received various light intensities in a growth chamber, a greenhouse, and outdoors: (**A**) leaves sampled from the lower canopy of greenhouse Rubrum grass (natural and HPS supplemental, 16-h photoperiod, 2.3 mol m^{-2} day^{-1}); (**B**) leaves sampled from the middle canopy of greenhouse Rubrum grass (natural and HPS supplemental, 16-h photoperiod, 3.5 mol m^{-2} day^{-1}); (**C**) leaves sampled from the top canopy of greenhouse Rubrum grass (natural and HPS supplemental, 16-h photoperiod; 7.0 mol m^{-2} day^{-1}); (**D**) leaves sampled from Rubrum grass grown in a chamber (fluorescent light, 24-h photoperiod, 13.3 mol m^{-2} day^{-1}); (**E**) Red Riding Hood leaves grown outdoors (photoperiod ranged from 15 to 13.5 h, 42 mol m^{-2} day^{-1}); (**F**) Rubrum leaves grown outdoors (photoperiod ranged from 15 to 13.5 h, 42 mol m^{-2} day^{-1}); (**G**) Rubrum flowers grown outdoors (photoperiod ranged from 15 to 13.5 h, 42 mol m^{-2} day^{-1}).

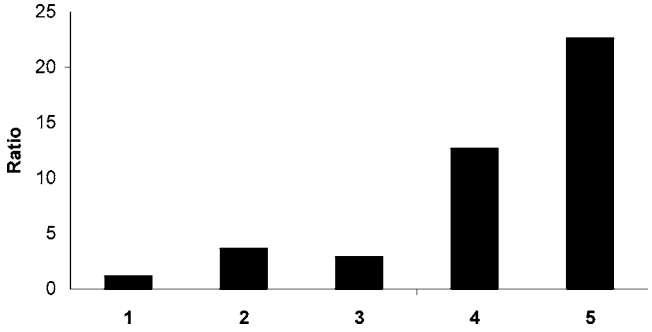


Figure 4. Ratio of cyanidin 3-glucoside to cyanidin 3-rutinoside in *P. setaceum* Rubrum leaves sampled from (**1**) the lower canopy of plants grown in the greenhouse (natural and HPS supplemental, 16-h photoperiod, 2.3 mol m^{-2} day^{-1}), (**2**) the middle canopy of plants grown in the greenhouse (natural and HPS supplemental, 16-h photoperiod, 3.5 mol m^{-2} day^{-1}), (**3**) the top canopy of plants grown in the greenhouse (natural and HPS supplemental, 16-h photoperiod; 7.0 mol m^{-2} day^{-1}), (**4**) plants grown in the growth chamber (fluorescent light, 24-h photoperiod, 13.3 mol m^{-2} day^{-1}), and (**5**) plants grown outdoors during the months of June through August (photoperiod ranged from 15 to 13.5 h, 42 mol m^{-2} day^{-1}).

fluorescent lighting in the greenhouse may be useful for improving the color of *P. setaceum* Rubrum to improve the aesthetic value of this ornamental crop.

Anthocyanins to yield pleasing colors are an important aspect of the ornamental plant industry. Also, they are regarded as health supplements. As a garden plant, a high anthocyanin content in *P. setaceum* Rubrum increases its aesthetic value. Just as anthocyanins have a putative function as a protective compound in plants, anthocyanins benefit human health as well. Various studies have shown that the antioxidant activity of anthocyanins and their therapeutic effect ameliorate inflammation, cardiovascular disease, and cancer (*19*−*22*). For example,

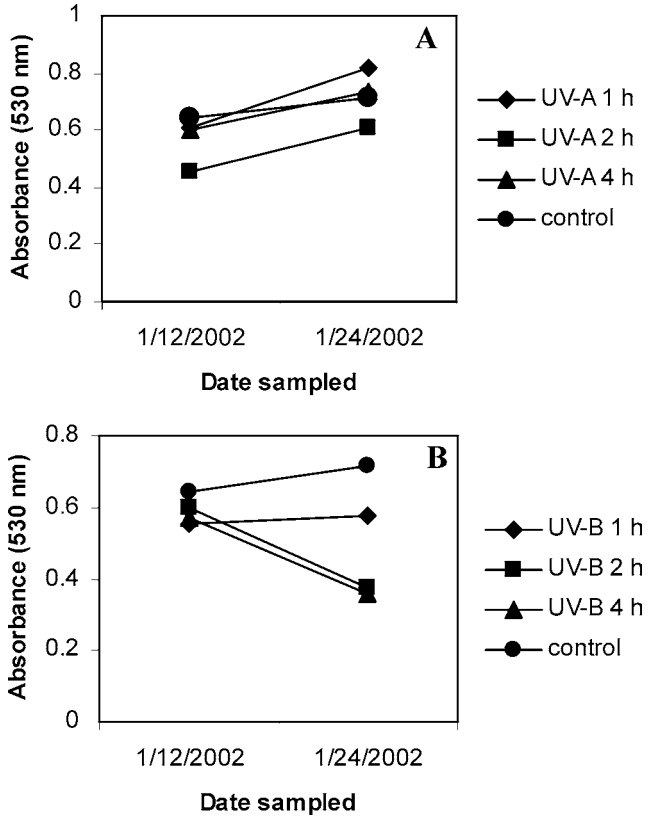


Figure 5. Anthocyanin production in *P. setaceum* Rubrum leaves (6–12 cm from base of leaf), sampled on days 8 and 20, exposed to (**A**) UV-A (1, 2, and 4 h) and (**B**) UV-B (1, 2, and 4 h). Plants were grown under a 16-h photoperiod with supplemental HPS light from 6:00 a.m. to 10:00 p.m. UV lamps (Q-Panel Lab Products, UV-B-313 and UV-A-340) were on for 1, 2, or 4 h periods beginning at 6:00 p.m. and ending at 12:00 a.m.

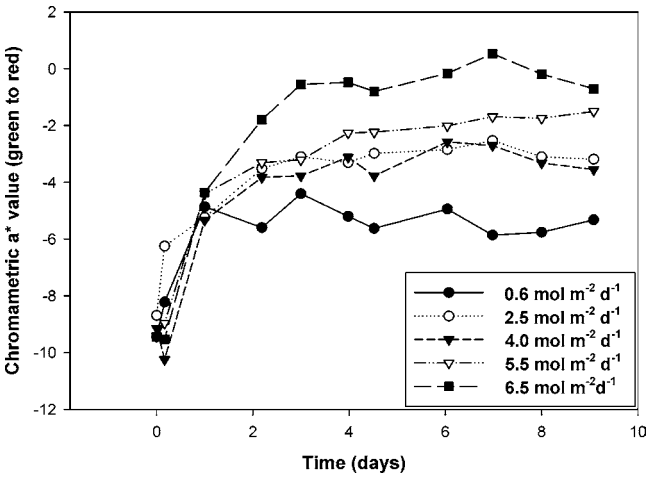


Figure 6. Anthocyanin production in *P. setaceum* Rubrum grown under fluorescent light as determined by chromametric *a** value over time (Minolta color meter). Air temperature was 20 °C, and leaf temperature ranged from 21 to 23 °C.

sour cherry (*Prunus cerasus*) is consumed primarily for its anthocyanins to reduce inflammation caused by arthritis (*19*). In addition to its popular use as an ornamental landscape plant, *P. setaceum* Rubrum may be a potential source of anthocyanins for production and consumption as dietary supplements. This may encourage the ornamental industry to increase the production of *P. setaceum* Rubrum grass for processing anthocyanins for use as phytomedicines.

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