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Red light stimulates flowering and anthocyanin biosynthesis in American cranberry

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Abstract

Morphological responses of American cranberry (*Vaccinium macrocarpon* Ait, Ericaceae) to different light conditions (red, far-red, white light and sunlight) were examined. Root growth and development, stem elongation, leaf enlargement, de-etiolation of stem and leaf, flower bud formation, and flowering of American cranberry were measured under each light condition and in the dark. It was found that red light promotes development of roots and leaves, flowering, and de-etiolation of stem and leaf of American cranberry. Stem elongation and etiolation of stem and leaf were shown in far-red light and dark. Anthocyanin biosynthesis as phytochemical response in cranberry plants was most sensitive to red light. Estimation of anthocyanin levels in different parts of cranberry plant suggested that anthocyanins were present only in red fruit skins, and not in peeled fruits, green fruits, green leaves, green stems, roots and seeds.

Introduction

American cranberry (*Vaccinium macrocarpon* Ait, Ericaceae) is a nondeciduous perennial woody plant, the fruits of which have commercial value. Fruit color is the determining factor of their quality (Craker 1971). The red color of cranberry fruit is due to the presence of anthocyanins, the largest subclass of flavonoids (Harborne and Grayer 1988). Anthocyanins are water-soluble pigments found in many colorful flowers and fruits. Anthocyanins have been found to have important therapeutic values, including antitumor (Kamei et al. 1995; Koide et al. 1996), antiulcer (Cristoni and Magistretti 1987), antioxidant and anti-inflammatory activities (Wang et al. 1999). Therefore, understanding the factors affecting the anthocyanin content is important to the agriculture and food industry.

Among external environmental factors, light is the most critical factor influencing plant development at all phases of its life cycle (Kircher et al. 1999; Neff and Chory 1998). Plants detect the spectral quality, intensity and direction of light with different photoreceptor systems: the phytochromes, the blue-light (B) receptors, the ultraviolet A (UV-A) receptors, and the UV-B receptors (Quail et al. 1995). The most extensively researched photoreceptors are phytochromes, which respond to red-light (660 nm) and far-red-light (730 nm). Phytochromes direct plant gene expression by switching between the red-absorbing form (Pr) and the far-red absorbing form (Pfr). Pr is the biologically inactive form within plants and readily converts to Pfr. Pfr is the biologically active form within plants, and controls transcription level of various genes (Batschauer 1999; Chalker 1999; Ni et al. 1999; Smith 1999).

There are a few ways currently being investigated to enhance the color content of cranberry fruits (Farag et al. 1992; Sapers et al. 1986). However, light, a major factor, has been virtually ignored in cranberry research. In this paper, we describe the effects of light on the development of roots, stems, leaves, flower buds and flowering, and on anthocyanin biosynthesis of American cranberry. Red light was found to be the

most effective light condition for enhancing root development, leaf enlargement, and de-etiolation of stem and leaf. Red light was especially effective in promoting flowering and anthocyanin biosynthesis of cranberry.

Material and methods

Plant material

Cranberry (*Vaccinium macrocarpon* Ait, Ericaceae) used in this study was of 'Early Black' variety from the Cranberry Experiment Station of the University of Massachusetts, East Wareham, MA. A cranberry bed was dug up from the cranberry bog. This bed was divided and separated into smaller sections. Each section was putted in a pot and watered once every two days without fertilizing.

Light sources

Red light, at a photon fluence rate of 12 μM m^{-2} s^{-1}, was obtained from six 40-w fluorescent tubes (F48T12/R-660/HO, Red, General Electric Company, USA) filtered through a red plastic sheet (Roscolux color filter # 27, ROSCO Laboratories, Port Chester, NY). Far-red light, at a photon fluence rate of 5 μM m^{-2} s^{-1}, was obtained from brilliant white light 500 w halogen double ended quartz FCL bulbs (Osram Sylvania Products Inc., Winchester, KY) filtered through 3 mm far-red plastic (type FRF700, West lakes Plastics, Lenni, PA). White light at a photon fluence rate of 31 μM $m^{-2}s^{-1}$ was the same as for far-red light, without the far-red plastic filter. Green light, at a photon fluence rate of 1 μM m^{-2} s^{-1}, was obtained from two 20-w fluorescent tubes (F20T12CW, General Electric Company, USA) filtered through Roscolux color filter # 877 and 874 (ROSCO Laboratories). Short UV and long UV lights were obtained from a 254 nm UV lamp and a 365 nm UV lamp (Model UVLS-28, 115 V ~ 60 Hz, 0.16 Amps, UVP, Inc., Upland, CA). Light sources in each case were kept at 0.8 meters from the plants. All light measurements were made with a Model IL1400A Radiometer/Photometer (International Light, Inc., Newburyport, MA).

Experimental conditions for plant development

PYREX bottles (250 ml) were filled with tap water then covered with two layers of parafilm to prevent evaporation. Eight-centimeter pieces of cranberry plant stems were placed into the bottle through holes in the parafilm four plants per bottle, and 2.5 cm of each stem was under the water. Bottles were randomly divided into five groups. One group consisted of three bottles (12 plants). Four groups were placed in a temperature monitored dark room (20 °C), and each group was arranged under separated different light condition. Among the four groups, one group received continuous red light, the second group received continuous far-red light, third group received continuous white light, and the fourth group received no light (control). The fifth group was placed near the window inside a 20 °C room, and received sunlight. This set of experiments was repeated three times.

Measurement and analysis of growth

After eight weeks, the lengths of the root and stem were measured to the nearest 0.1 cm with a ruler. Root length was measured between the end of stem and the root tip (Figure 1). Only new stems which had grown after the experiment began were used for stem length measurements. Leaf growth and development and de-etiolation of stem and leaf were viewed using a digital camera (Sony). Flower buds and opened flowers per stem were separately counted.

Anthocyanin accumulation

A cranberry bed (0.9 m × 0.6 m) was dug up from the cranberry bog in August 1999. This bed was divided and separated into nine smaller sections (0.3 m × 0.2 m). Each section was putted into a pot and placed under different light conditions. Over 30 cranberry plants bearing green or light red fruits were present in each section, and each section was considered as a separated group. One group of plants was placed in nursery area outside the laboratory, and the remaining eight groups were placed in a temperature monitored dark room (20 °C). One group in the dark room received no light, and six other groups received 30 min of white, far-red, red, green, long UV and short UV light per day, respectively. The eighth group of plants received 30 min of red or far-red light on alternative days, which were otherwise kept in the dark. All fruits were picked from each of the plants

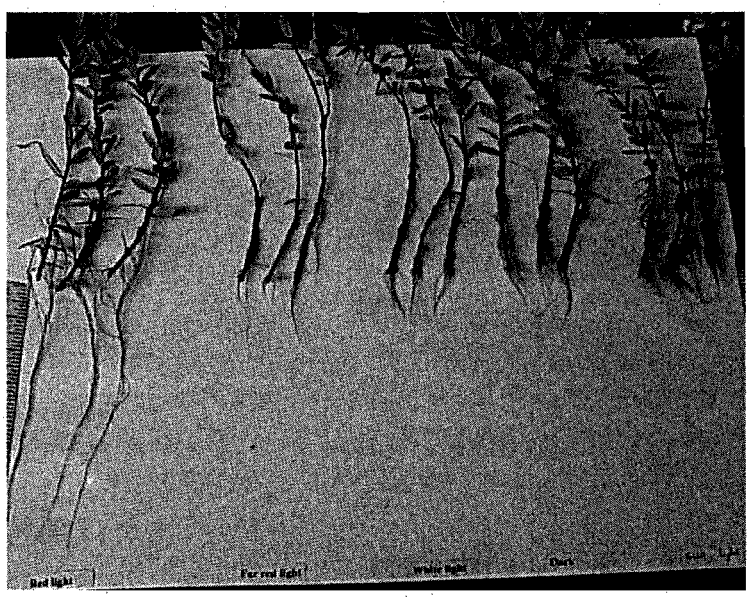

Figure 1. Root growth and development of cranberry in different light conditions. Roots were grown for eight weeks under red, far-red, white light, dark and sunlight. Stems receiving sunlight developed the greatest number of roots, and roots receiving continuous red light were longer in length.

and mixed well after eight days. Five grams were weighted and homogenized in 1% HCl-methanol (1:50) to extract the anthocyanins overnight at 4 °C.

Examination of anthocyanin level in different parts of cranberry

Whole green berries, whole red berries, red berry skins, peeled red berries, green leaves, green stems, roots and seeds from plants grown under natural conditions in September 1999, were weighed and homogenized in 1% HCl-methanol (1:50) and incubated overnight at 4 °C to extract the anthocyanins. Five-gram tissue was used for each sample, and extracted with 10 ml of 1% HCl-methanol.

Analysis of anthocyanin by HPLC

HPLC analyses were carried out on a Waters 515 dual pump HPLC system, equipped with 996-photodiode-array detector and a C_{18} column (4.6 × 150 mm) with 5 μm particle size (Waters Corp.). Elution was carried out using a mobile phase formed by a linear gradient of (A) H_2O-acetic acid (10:1), and (B) MeOH-acetic acid (10:1), with 100% A at 0 min to 40% A and 60% B at 20 min. The flow rate was fixed at 0.2 ml/min. All reagents and solvents were HPLC grade from Fisher Scientific (Pittsburgh, PA).

The sample extracts were filtered through 0.2 μM filters before injection, and anthocyanin elutions were detected by monitoring absorbance at 535 nm.

Results

Root growth and development

Roots were visible on cut stems within 10 days of growth under red light and sunlight conditions. Plants receiving sunlight developed more roots in number (Figure 1), whereas plants receiving continuous red light developed the longest roots (Figure 1, Table 1). Red light increased the root length by 2.0-fold and 3.5-fold, compared to far-red light and dark treatments, respectively.

Stem elongation and leaf development

New stems produced on cranberry stem explants grew longer under far-red light or in the dark than those under red light, white light, or sunlight (Table 1). The new stems on explants in the dark were 2.2-fold, 1.7-fold, 2.6-fold and 3.0-fold longer than those of plants

Table 1. Effects of different light conditions on root and stem growth, flower bud formation, and flower opening of cranberry explants.

	Red light	Far-red light	White light	Dark	Sunlight
Root[a]	7.4 ± 2.2	3.7 ± 1.3	3.0 ± 1.2	2.1 ± 0.5	3.7 ± 1.7
Stem[a]	4.9 ± 2.0	6.5 ± 2.2	4.2 ± 1.6	10.8 ± 5.1	3.6 ± 1.2
Bud[b]	0.8 ± 0.2	1.2 ± 0.2	1.0 ± 0.1	0.6 ± 0.1	0.8 ± 0.1
Flower[b]	2.0 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 0.2

[a] = cm; [b] = number. Root length was measured between the end of the stem and the root tip. Stem length indicates length of new stems that had grown after the experiment began. Flower buds and opened flowers per stem were separately counted. Each value represents the mean ± SE (n = 12).

Figure 2. Cranberry leaf development and etiolation of stems and leaves under different light conditions. Leaves of cranberry explants exposed to red light or sunlight were enlarged and developed. In contrast, leaves of explants exposed to far-red light or kept in the dark were not developed.

kept in red, far-red, white light and sunlight, respectively.

Leaves of cranberry plants exposed to red light or sunlight were enlarged and developed, but almost no leaf development was observed under far-red light condition and in the dark. Typical examples are shown in Figure 2. Stems and leaves on explants growing in red light or sunlight were de-etiolated, whereas under far-red light and in the dark stems and leaves remained etiolated.

Flower bud formation and flowering

There were no significant differences in the number of flower buds formed under the different light conditions tested (Table 1). However, the developments of buds into mature open flowers were clearly affected by light quality. Open flowers developed only under red light and sunlight conditions, but not under far-red light, white light, and dark conditions.

Table 2. Effects of different light conditions on anthocyanin accumulation in cranberry fruits.

Anthocaynin content (mg/g. Fr. Wt.)	
Control	0.0308 ± 0.0020
Sunlight	0.1377 ± 0.0107
White light	0.1019 ± 0.0111
Dark	0.0308 ± 0.0039
Far-red light	0.1134 ± 0.0087
Red light	0.1983 ± 0.0055
Red light → Far-red light	0.1322 ± 0.0027
Green light	0.0871 ± 0.0057
Long UV	0.0821 ± 0.0015
Short UV	0.0452 ± 0.0063

Values are expressed as mean ± SE (n = 3).

Effect of light on anthocyanin levels in fruits

Total anthocyanins were estimated referring an ϵ of 98.2 as reported by Francis (Francis 1982). Anthocyanin levels in fruits picked from cranberry plants exposed to different light conditions (sunlight, white light, far-red light, red light, red light followed by far-red light, green light, long UV light and short UV light) are shown in Table 2. Control experiments were carried out with fruits obtained from plants just before their exposure to a given light condition or from plants that received no light exposure. The anthocyanin levels of fruits picked from cranberry plants kept in dark remained the same as the anthocyanin levels in fruits from plants before their exposure to any light condition. Anthocyanin biosynthesis in fruits exposed to different lights was enhanced by every light-sunlight, white, far-red, red, red followed far-red, green, long UV, and short UV light. The anthocyanin level of fruits from plants given a red light treatment was 1.7-fold, 1.5-fold, 2.4-fold, 4.4-fold and 6.4-fold higher than fruits from plants under far-red light, red light followed far-red light, long UV, short UV and dark, respectively.

Anthocyanin levels in various tissues of the cranberry plants

Anthocyanin levels in different tissues of the cranberry plant are shown in Table 3. Anthocyanins were present only in red fruit skins, and not in peeled berries, green berries, green leaves, green stems, roots or seeds.

Table 3. Anthocyanin levels in various tissues of the cranberry plants.

Anthocyanin level (mg/g. Fr. Wt.)	
Whole green berries	0.0 ± 0.0
Whole red berries	0.1231 ± 0.0018
Red berry skins	0.3288 ± 0.0229
Peeled red berries	0.0 ± 0.0
Green leaves	0.0 ± 0.0
Green stems	0.0 ± 0.0
Roots	0.0 ± 0.0
Seeds	0.0 ± 0.0

Values are expressed by mean ± SE (n = 4).

Discussion

Factors affecting the development of cranberry plants from root growth to flowering need examination for several reasons: cranberry bog development (from planting to the first fruits) takes over five years under current traditional practice; there are only a few geographical areas (e.g., northern United States and southern Canada) which seem suitable for cranberry culture; very little analysis has been carried out to understand the effects of environmental factors on the growth and development of cranberry plants.

Among many factors, the effects of water, temperature and plant hormones on the cranberry plant physiology have been examined to a certain extent (Bewick et al. 1988; Craker 1971; DeMoranville et al. 1996; Eck 1972; Rigby et al. 1972). However, the effect of light has been virtually ignored in previous studies, and the role of a well-known photoreceptor, phytochrome in cranberry development and fruit quality is largely unknown. Therefore, we have initiated a comprehensive study to examine the effect of light on root development, stem elongation, leaf development, flower bud formation and flowering, and anthocyanin biosynthesis.

Results presented in this report strongly indicate that red light enhances cranberry plant growth, including root development, leaf development and de-etiolation, stem de-etiolation, especially flowering, and anthocyanin biosynthesis in fruits.

Red light is perhaps the most important factor affecting cranberry production, not only because it affects the growth of its root, shoot and leaves but also because of its apparent affect on flowering, which is essential in fruit development. Many environmental and endogenous signals have divergent effects on flowering of different plant species (Blazquez and

Weigel 2000). For example, in the model plant, *Arabidopsis*, far-red light and blue light promote flowering, but red light is often inhibitory (Lin 2000). In contrast, red light appears to promote flower opening in cranberry.

Anthocyanin accumulation in plants is influenced by many external environmental factors, among which light is the most important one (Grisebach 1982). Light-dependent anthocyanin biosynthesis significantly depends on plant species and experimental conditions (Mancinelli et al. 1991). As an example, anthocyanin production by strawberry cells depends on both of light intensity and the light/dark cycle operation (Kurata et al. 2000). Under our experimental condition, anthocyanin biosynthesis in cranberry fruits was more responsive to red light than to far-red light and UV light. Far-red light, on the other hand, enhanced processes of cranberry plant stem elongation and increased the stem length by 1.4-fold more than the red light, and the stem length under red light and far-red light was significantly different by *t* test ($0.05 > p > 0.02$). In contrast to red light, leaves and stems in far-red light remained etiolated (Figure 2).

In contrast to red, far-red, white light and sunlight, cranberry stem explants kept in the dark developed the longest new stems, and the shortest roots. Similar observations have been made with other plant systems, such as potato (*Solanum tuberosum*) and mustard seedings (Mohr and Schopfer (1995a, 1995b)). Dark conditions completely inhibited greening of leaf and stem, as well as flower opening and anthocyanin biosynthesis of cranberry, consistent with the observations of other studies with several plant systems, such as bean, mustard seeding, *Arabidopsis* and apple (Galston 1994; Mohr and Schopfer 1995b; Yanovsky et al. 2000).

Plant physiological responses to red and far-red light are mediated by phytochrome. Anthocyanin biosynthesis is also mediated by phytochrome in several plants such as tomato, cabbage, spirodela and mustard (Mancinelli 1985). However, anthocyanin biosynthesis may not be solely regulated by phytochrome. For example, anthocyanin biosynthesis in sorghum is mediated by both of phytochrome and UV-B photoreceptor (Shichijo et al. 1993). A UV photoreceptor does not appear to be regulating anthocyanin production in cranberry fruit as much as phytochrome as evidenced by the lack of response to the both of long UV and short UV lights (Table 2).

Our above experimental results show, for the first time, the effect of selective light quality on cranberry plant development, flowering and anthocyanin biosynthesis. Red light affects root growth and development, de-etiolation of stem and leaf, leaf enlargement and flowering of cranberry plant. Without red light, leaf development and flowering of cranberry plant are interrupted. Red light seems to be a key component to trigger opening of flower buds in cranberry plants. Red light selectively promotes anthocyanin biosynthesis in cranberry fruits; and red light dependent responses appear to be mediated-through phytochrome.

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References

Batschauer A. 1999. Light perception in higher plants. CMLS, Cell. Mol. Life Sci. 55: 153–165.

Bewick T.A., Binning L.K. and Yandell B. 1988. A degree day model for predicting the emergence of swamp dodder in cranberry. J. Amer. Soc. Hort. Sci. 113: 839–841.

Blazquez M.A. and Weigel D. 2000. Integration of floral inductive signals in *Arabidopsis*. Nature 404: 889–892.

Chalker S.L. 1999. Environmental significance of anthocyanin in plant stress responses. Photochem. and Photobio. 70: 1–9.

Craker L.E. 1971. Postharvest color promotion in cranberry with ethylene. HortScience 6: 137–139.

Cristoni A. and Magistretti M.J. 1987. Antiulcer and healing activities *of Vaccinium myrtillus* anthocyanosides. IL Farmaco. Edizione Pratica 42: 29–43.

DeMoranville C.J., Davenport J.R., Patten K., Roper T.R., Strik B.C., Vorsa N. et al. 1996. Fruit mass development in three cranberry cultivars and five production regions. J. Amer. Soc. Hort. Sci. 121: 680–685.

Eck P. 1972. Cranberry yield and anthocyanin content as influenced by ethephon, SADH, and malathion. J. Amer. Soc. Hort. Sci. 97: 213–214.

Farag K.M., Palta J.P. and Stang E.J. 1992. Ethanol enhances the effectiveness of ethephon on anthocyanin production in cranberry fruits in the field. HortScience 27: 411–412.

Francis F.J. 1982. Analysis of anthocyanins. In: Markakis P. (ed.), Anthocyanin as Food Colors. Academic Press, New York, pp. 181–207.

Galston A.W. 1994. Life Processes of Plants. Scientific American Library, A division of HPHLP, New York, pp. 64.

Grisebach H. 1982. Biosynthesis of anthocyanins. In: Markakis P. (ed.), Anthocyanins as Food Colors. Academic Press, New York, pp. 67–92.

Harborne J.B. and Grayer R.J. 1988. The anthocyanins. In: Jeffrey B.H. (ed.), The Flavonoids: Advances in Research Since 1980. Chapman and Hall, New York, pp. 1–20.

Kamei H., Kojima T., Hasegawa M., Koide T., Umeda T., Yukawa T. et al. 1995. Suppression of tumor cell growth by anthocyanins *in vitro*. Cancer Invest. 13: 590–594.

Kircher S., Kozma B.L., Kim L., Adam E., Harter K., Schafer E. et al. 1999. Light quality-dependent nuclear import of the plant photoreceptors phytochrome A and B. Plant Cell 11: 1445–1456.

Koide T., Kamei H., Hashimoto Y., Kojima T. and Hasegawa M. 1996. Antitumor effect of hydrolyzed anthocyanin from grape rinds and red rice. Cancer Biother. Radiopharm. 11: 273–277.

Kurata H., Mochizuki A., Okuda N., Seki M. and Furusaki S. 2000. Intermittent light irradiation with second- or hour-scale periods controls anthocyanin production by strawberry cells. Enzyme Microb Technol. 26: 621–629.

Lin C.T. 2000. Photoreceptors and regulation of flowering time. Plant Physiology 123: 39–50.

Mancinelli A.L. 1985. Light-dependent anthocyanin synthesis: a model system for the study of plant photomorphogenesis. The Botanical Review 51: 107–157.

Mancinelli A.L., Rossi F. and Moroni A. 1991. Cryptochrome, phytochrome, and anthocyanin production. Plant Physiol. 96: 1079–1085.

Mohr H. and Schopfer P. 1995a. Plant Physiology. Springer-Verlag Berlin Heidelberg, New York, pp. 314.

Mohr H. and Schopfer P. 1995b. Plant Physiology. Springer-Verlag Berlin Heidelberg, New York, pp. 353.

Neff M.M. and Chory J. 1998. Genetic interactions between phytochrome A, phytochrome B, and cryptochrome 1 during Arabidopsis development. Plant Physiol. 118: 27–36.

Ni M., Tepperman J.M. and Quail P.H. 1999. Binding of phytochrome B to its nuclear signalling partner PIF3 is reversibly induced by light. Nature 400: 781–784.

Quail P.H., Boylan M.T., Parks B.M., Short T.W., Xu Y. and Wagner D. 1995. Phytochromes: photosensory perception and signal transduction. Science 268: 675–680.

Rigby B., Dana M.N. and Binning L.K. 1972. Ethephon sprays and cranberry fruit color. HortScience 7: 82–83.

Sapers G.M., Graff G.R., Phillips J.G. and Deubert K.H. 1986. Factors affecting the anthocyanin content of cranberry. J. Amer. Soc. Hort. Sci. 111: 612–617.

Shichijo C., Hamada T., Hiraoka M., Johnson C.B. and Hashimoto T. 1993. Enhancement of red-light-induced anthocyanin synthesis in sorghum first internodes by moderate low temperature given in the pre-irradiation culture period. Planta 191: 238–245.

Smith H. 1999. Tripping the light fantastic. Nature 400: 710–711.

Wang H., Nair M.G., Strasburg M., Chang Y.C., Booren A.M., Gray J.I. et al. 1999. Antioxidant and antiinflammatory activities of anthocyanins and their aglycon, cyanidin, from tart cherries. Journal of Natural Products 62: 294–296.

Yanovsky M.J., Whitelam G.C. and Casal J.J. 2000. *fhy3-1* retains inductive responses of phytochrome A. Plant Physiol. 123: 235–242.