Role of Photoreceptor- and Sugar-mediated Reactions in Light Dependent Anthocyanin Production in Lily and Stock Flowers

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Summary

Low light intensity causes poor flower pigmentation possibly by two mechanisms: 1) the reaction mediated by photoreceptors located in the petal, 2) the reaction mediated by the sugar supply from leaves or stems. We investigated which mechanism is more important in the pigmentation of the oriental hybrid lily, 'Acapulco' and stock, 'Pigmy Rose'. Shading flowers by cheesecloth (PPFD < 17 μmol·m⁻²·sec⁻¹) or by aluminum foil (0 μmol·m⁻²·sec⁻¹) reduced anthocyanin concentration in both species, suggesting that anthocyanin production is a photoreceptor-mediated reaction. When whole plants were shaded by cheesecloth (< 17 μmol·m⁻²·sec⁻¹) in lily, anthocyanin concentration became lower than that of flower-shaded plants. This treatment reduced total sugar concentration from 3% to 1.6%, suggesting that limited sugar supply caused poor anthocyanin production in lily. In contrast, in stock, whole plant shading did not reduce anthocyanin concentration as compared with flower-shaded plants, suggesting that the amount of available sugar is not a limiting factor for anthocyanin production. Although this treatment reduced total sugar concentration from 4.7% to 3.7%, it was still higher than that of the control lily plants. When detached flowers of stock were placed on sucrose solutions, the anthocyanin concentration declined as hexose concentration in the petal decreased, especially under 2%. These results indicate that, although soluble sugars in petals affected anthocyanin production, their high concentration prevented fading of flower color, even under low light conditions in stock.

Key Words: anthocyanin, light, Lilium, Matthiola incana, sugar.

Introduction

Anthocyanin biosynthesis is known to be affected by light intensity. Flowers which open under low light intensity often have faded color, as in roses (Biran and Halevy, 1974; Shida and Takano, 1964), petunia (Moscovici et al., 1996; Weiss and Halevy, 1989, 1991), lisianthus (Griesbach, 1992; Halevy and Kofranek, 1984; Kawabata et al., 1999), carnation (Koyama and Uda, 1994; Maekawa, 1975), snapdragon (Sang et al., 1991; Toki et al., 1987), and kangaroo paw (Ben-Tal and King, 1997). In cut-flower production, pale flowers are regarded as of low quality. In contrast to the above, stocks are hardly influenced by light conditions during the flowering period. Our interest is why some plants are sensitive to light conditions, whereas others are not.

There are two possible mechanisms of light-dependent anthocyanin biosynthesis: 1) the reaction mediated by photoreceptors located in flower petals, such as UV photoreceptors, cryptochromes, and phytochromes (Mancinelli, 1983, 1985), 2) the reaction mediated by the sugar supply from leaves and stems. Sucrose is known to promote anthocyanin biosynthesis in petunia (Weiss and Halevy, 1989, 1991; Moscovici et al., 1996), antirrhinum (Sang et al., 1991), and lisianthus (Kawabata et al., 1999). A low light condition reduces photosynthesis, which results in a low sugar supply to flowers and reduced anthocyanin biosynthesis.

In our previous study in lisianthus, shading flowers had no effect on flower pigmentation, whereas shading leaves and stems significantly reduced it (Kawabata et al., 1999), indicating that pigmentation in lisianthus flower depends solely on sugar transport from leaves or stems to flowers, and not on photoreceptor-mediated reaction. Therefore, we assumed that some plants develop faded colors under low light condition on account of insufficient sugar supply and some plants do so because of insufficient photoreceptor-mediated reaction. Incorporating traits of sugar-insensitive anthocyanin production and photoreceptor unmediated production into a single species theoretically may lead to a light-insen-
Material and Methods

Effect of light intensity on pigmentation in lily

Pre-chilled bulbs of the Oriental hybrid lily (Lilium species ‘Acapulco’) were planted on 1:2 mixture (v/v) of commercial medium Soil Mix (Sakata) and Engei Baido (Kureha) in a greenhouse with supplemental light by halogen lamp (60 $\mu$mol·m$^{-2}·$sec$^{-1}$) from 6 am to 8 pm on February 1998. The greenhouse was kept above 15°C at night; it was ventilated when the temperature reached 23°C during the day. When the length of the flower buds reached 75–80 mm, the flowers began to open and show color. Plants, having flowers at this stage, were chosen and exposed to the following treatments: 1) in some plants, flowers were enclosed in acrylic frames surrounded by two layers of cheesecloth or aluminum foil, while other whole plants were surrounded by two layers of cheesecloth. Although the temperature of the perianth was not measured directly, air temperatures were not different between inside and outside the shaded box. During the experiment, the light intensity in the greenhouse was 130–340 $\mu$mol·m$^{-2}·$sec$^{-1}$ at noon. The transmittance of the two layers of cheesecloth was 5%, so that the light intensity under the cheesecloth was lower than 17 $\mu$mol·m$^{-2}·$sec$^{-1}$. Between 8 and 10 shaded flowers and unshaded control flowers were collected at anthesis and the inner perianths were analyzed for soluble sugar, anthocyanin, and color.

Effect of light intensity on pigmentation in stock

Stock plants (Matthiola incana ‘Pygmy Rose’) were transplanted to 10 cm plastic pots containing 1:1 mixture of Soil Mix (Sakata) and Engei Baido (Kureha) in a greenhouse on April 2000. The greenhouse was ventilated when the temperature reached 23°C. When the bolting started, plants were transferred to a growth chamber kept at 23°C with 14 hr photoperiod at a light intensity of 310 $\mu$mol·m$^{-2}·$sec$^{-1}$ supplied by a metal halide lamp. When the petal margins of the second florets began to show slight pigmentation, the inflorescences or whole plants were shaded with cheesecloth as described for lily. The light intensity inside the shading box was 16 $\mu$mol·m$^{-2}·$sec$^{-1}$. The second florets were collected at anthesis.

Effect of exogenous sucrose on pigmentation in detached stock florets

Stock plants (Matthiola incana ‘Pygmy Rose’) were transplanted to plastic pots as described above and grown in a growth room kept at 20°C and under natural lights. Florets with 5 mm peduncles were detached from the inflorescence when pigmentation started on the petal margins. The florets were placed in vials with their peduncles in 0, 0.005, 0.01, 0.025, 0.05, 0.075, 0.1, or 0.2 M sucrose solution and incubated in a chamber controlled at 25°C, 14 hr photoperiod at an intensity of 70 $\mu$mol·m$^{-2}·$sec$^{-1}$ supplied by cool-white fluorescent lamp or in the dark.

Measurements

Immediately after the sampling, one of the petals or inner perianths were selected for colorimetric and anthocyanin measurements. Lightness (L*) was measured with a colorimeter CR-221 (Minolta) as an index of pigmentation at the well-pigmented part of the petals in stock and near the center of the perianths that did not include the darkly pigmented spots in lily. The anthocyanin was extracted from the same above perianths by soaking the samples in 1% HCl–methanol at 4°C over night. Anthocyanin content was measured as absorbance at 510 nm. Comparable petals or perianths were freeze-dried for soluble sugar analysis. Dried samples were ground into fine powder, extracted with 80% ethanol at 85°C for 1.5 hr. The extracts were filtered through Whatman GF/F filter and the filtrates were dried in a rotary evaporator. The residue was dissolved in distilled water and centrifuged at 15,000 rpm for 10 min. The soluble sugar concentration of the supernatant was determined enzymatically. Glucose and fructose concentrations were measured, using F-Kit glucose (Roche), coupled with phosphoglucone isomerase according to the manufacturer’s instruction (Roche). Prior to sucrose analysis, glucose in the sample solution was treated with 6 U·ml$^{-1}$ glucose oxidase and 1,600 U·ml$^{-1}$ catalase in a buffer (73 mM triethanolamine–HCl, 1mM MgSO$_4$, pH 7.6) at 37°C for 30 min with vigorous shaking. The solution was heated at 100°C for 5 min. After centrifugation at 3,000 rpm for 15 min, 20 $\mu$l of the supernatant was mixed with 80 $\mu$l of 320 mM citrate buffer (pH 4.6), containing 100 U·ml$^{-1}$ invertase and incubated at 37°C for 10 min. The resultant glucose after the hydrolysis was analyzed, using B-Test (Wako) to calculate sucrose concentration.

Results

Effect of light intensity on pigmentation in lily

Both flower shading treatments (95% shading by cheesecloth and 100% shading by aluminum foil) reduced pigmentation. The anthocyanin concentration of perianths became lower, while L* of flowers in 100% shading became significantly higher than control L* (Table 1). Anthocyanin concentration of perianths was significantly reduced when whole plant were under 95% shading, compared with flowers receiving the same
Table 1. Effects of shading flowers or whole plants on flower fresh weight, perianth lightness (L*) and concentrations of anthocyanins, glucose, fructose and sucrose in perianths of lily.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fresh weight (g)</th>
<th>L*</th>
<th>Anthocyanin ( (A_{500} \cdot \text{cm}^{2}) )</th>
<th>Glucose (%FW)</th>
<th>Fructose (%FW)</th>
<th>Sucrose (%FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.52a(^2)</td>
<td>58.7b</td>
<td>0.404a</td>
<td>1.55a</td>
<td>1.18a</td>
<td>0.22a</td>
</tr>
<tr>
<td>95% shading of flowers</td>
<td>1.53a</td>
<td>62.5ab</td>
<td>0.351b</td>
<td>1.57a</td>
<td>1.09a</td>
<td>0.24a</td>
</tr>
<tr>
<td>100% shading of flowers</td>
<td>1.52a</td>
<td>63.1a</td>
<td>0.330b</td>
<td>1.50a</td>
<td>1.06a</td>
<td>0.23a</td>
</tr>
<tr>
<td>95% shading of whole plants</td>
<td>1.44a</td>
<td>65.8a</td>
<td>0.274c</td>
<td>0.89b</td>
<td>0.56b</td>
<td>0.13b</td>
</tr>
</tbody>
</table>

\(^2\) Means followed by same letters within a column are not significantly different at 5% by LSD test, n=8 – 10.

Table 2. Effects of shading inflorescence or whole plant on floret fresh weight, petal lightness (L*) and concentrations of anthocyanins, glucose, fructose and sucrose in petals of stock.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fresh weight (g)</th>
<th>L*</th>
<th>Anthocyanin ( (A_{500} \cdot \text{gFW}^{-1}) )</th>
<th>Glucose (%FW)</th>
<th>Fructose (%FW)</th>
<th>Sucrose (%FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.758a(^2)</td>
<td>34.2b</td>
<td>153a</td>
<td>1.65a</td>
<td>3.01a</td>
<td>0.050a</td>
</tr>
<tr>
<td>95% shading of inflorescence</td>
<td>0.660ab</td>
<td>39.9a</td>
<td>116b</td>
<td>1.58ab</td>
<td>2.91a</td>
<td>0.077a</td>
</tr>
<tr>
<td>95% shading of whole plants</td>
<td>0.574b</td>
<td>38.0a</td>
<td>117b</td>
<td>1.39b</td>
<td>2.24b</td>
<td>0.045a</td>
</tr>
</tbody>
</table>

\(^2\) Means followed by same letters within a column are not significantly different at 5% by LSD test, n=8 – 10.

treatment. Since the extent of shading was the same between flower and whole plant shadings, the difference shows the effect of shading leaves and stems on anthocyanin synthesis.

All shading treatments did not influence perianth fresh weight (Table 1). Whereas flower shading did not influence sugar concentrations, whole plant shading significantly reduced the concentrations of glucose, fructose, and sucrose.

**Effect of light intensity on pigmentation in stock**

Shading flowers by cheesecloth (95%) increased L* (Table 2), which indicated paler flower color, as reflected by a decreased anthocyanin concentration of petals (Table 2). Unlike the results in lily, no differences in L* and anthocyanin concentration were observed between 90% flower shading and whole plant shading. Therefore, shading of leaves and stems did not affect anthocyanin concentration in stock.

Stock petals contained mainly fructose (Table 2); the glucose concentration was about half that of fructose, whereas that of sucrose was very low. Shading of whole plants decreased flower fresh weight and concentration of fructose. The total sugar concentration in control plants was 4.7%, which was much higher than that of lily. Even if whole plants were shaded, the concentration was 3.7%, which was still higher than that of control lily plants.

**Effect of exogenous sucrose on pigmentation in detached stock florets**

To test the effect of sugar on the anthocyanin production in stock, detached florets of stock were placed in various concentrations of sucrose solutions. Since the petals of stock contain mainly hexose and a very small level of sucrose, the concentration of hexose was measured in this experiment. After the florets of stock opened in the sucrose solutions, hexose concentration in the petals increased as the sucrose concentration of the medium increased (Fig. 1A). While the concentration of hexose was 0.76% at 0 M sucrose, it reached 7.2% at 0.2 M sucrose. Petal pigmentation and anthocyanin concentration were lower below 0.025 M sucrose than at higher sucrose concentrations, as indicated by higher L* (Fig. 1B, C). The florets which opened in the dark contained less anthocyanin and hexose than those under light. There was a positive correlation between hexose and anthocyanin concentrations in petals (Fig. 2). The dark treatment reduced anthocyanin concentration, regardless of hexose concentration in the petals (Fig. 2).

**Discussion**

Many studies have related the effect of light on pigmentation in flowers but only a few sought the basis of photoreceptor-mediated fading of their color under low light intensities. Most experiments dealt with whole plants so that the fading may have resulted from restricted photosynthesis. In other studies, detached flowers were used to test the effect of light (Kawabata et al., 1999; Maekawa, 1974; Moscovici et al., 1996; Weiss and Halevy, 1989, 1991). Irradiation stimulated sugar uptake from the medium in detached flowers by hastening solute uptake by transpiration (Kawabata et al., 1999). Therefore, the enhanced pigmentation may be due to increased sugar uptake. Klein (1990) showed that supplemental UV radiation did not enhance flower color.
in several ornamentals including stock, whereas Maekawa (1975) found that anthocyanin content in carnation did not differ between plants grown under the UV-absorbing and UV-transmitting films. In lisianthus, shading flowers did not influence anthocyanin production (Kawabata et al., 1999). Therefore, fading of flower color under low light intensity seems to be attributable to a photoreceptor-mediated reaction only in a limited number of species.

When flowers or whole plants were shaded to seek the effect of low light intensity on different parts of plants on pigmentation, shading the flowers reduced pigmentation in both lily and stock. This indicates that low light intensity caused poor pigmentation through photoreceptor-mediated reactions in both flowers. In detached florets of stock, the dark treatment reduced anthocyanin concentration, regardless of hexose concentration in the petals, which suggests that pigment formation is photoreceptor-mediated. This is in contrast to lisianthus, in which irradiation led to increased anthocyanin production because of enhanced sugar uptake by the detached flowers (Kawabata et al., 1999).

Light intensity perceived by the shaded part of the plant was lower than 7 μmol·m⁻²·s⁻¹ during the day, which do not occur under normal commercial production. Low light intensity, higher than 7 μmol·m⁻²·s⁻¹, may not cause photoreceptor-mediated fading of the petals but the reaction may be still important in greenhouse culture of some species. Glass and many plastic films used for greenhouse do not transmit UV-B light which promotes anthocyanin production in primula (Kashiwagi et al., 1977), rose (Maekawa et al., 1980; Nakamura et al., 1980), kalanchoe (Hoffman, 1999), and kangaroo paw (Ben-Tal and King, 1997).

Whereas whole plant shading reduced pigmentation as compared with flower shading in lily, it did not influence pigmentation in stock, which indicates that pigmentation depends on photosynthates in lily but not in stock. There are two possible explanations for this sugar-independence for anthocyanin production in stock: 1) anthocyanin synthesis is not promoted by sugars in stock, 2) the petals contained enough sugars to promote anthocyanin synthesis even under a low light condition, since sugar concentration is always high. When these possibilities were tested in detached florets, exogenous sucrose concentration of less than 0.025 M reduced floret pigmentation and anthocyanin accumulation was significantly lower than at higher sucrose concentrations. The significant correlation between pigmentation concentration and hexose concentration in the petals
indicates that anthocyanin synthesis is dependent on soluble sugars when the concentrations in the petal is below 2%. In intact stock florets, hexose concentration was 4.7% in unshaded plants and 3.7% in shaded plants, which is more than the minimal 2%, so that sufficient soluble sugars are present in petals to prevent petal fading even under low light conditions.

In our previous study with lisianthus (Kawabata et al., 1999) and this one, we found three types of flowers with respect to light–dependency in anthocyanin production: 1) flowers, such as lisianthus, depend mainly on light intensity perceived by leaves and stems, and not directly by flowers; 2) in types, such as stock, flower color depends on light intensity perceived by flowers but not necessarily that perceived by leaves and stems; and 3) this type, including lily, flower color depends on light perceived by both flowers and photosynthetic organs. Photoreceptor-mediated reactions may not play important roles in fading of flower color under low light intensities in the commercial production, except for UV sensitive flowers under protected production, because only extreme shading inhibits pigment formation. Thus, sugar availability is critical for anthocyanin production for lisianthus and lily, whereas sugar accumulation by stock prevents fading under a low light condition.

**Literature Cited**


ユリおよびストックの光依存的なアントシアニン合成における光受容体と糖の役割について

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摘要

弱光下において花が着色不良を起こすメカニズムとして1)花弁に存在する光受容体を介した反応、2)茎葉部からの糖の供給の減少による反応の二通りが考えられる。本研究では、いずれの要因がより重要であるのかをオリエンタル系ユリ 'アカプルコ' とストック 'ピグミーローズ' を用いて調べた。花のみを寒冷凍 (PPFD <17 μmol・m^{-2}・sec^{-1}) またはアルミホイル (PPFD 0 μmol・m^{-2}・sec^{-1}) により遮光した場合、いずれの植物でもアントシアニン濃度が低下した。この結果は、花弁の光受容体を介した反応を示す。ユリでは、植物体全体を寒冷凍によって遮光すると (PPFD <17 μmol・m^{-2}・sec^{-1}), 花のみを寒冷凍で遮光した場合よりさらにアントシアニン濃度が低下した。花弁の全糖濃度は無処理区で3%であったが、この処理によって1.6%まで低下した。従って、糖の供給不足もまた、ユリのアントシアニン合成を抑制することを示した。それに対してストックでは、植物体全体の遮光処理の効果は、花のみの遮光処理の効果と差がなかった。従って糖の供給不足はアントシアニン合成の制限要因ではないと考えられた。花弁の全糖濃度は植物体全体遮光処理によって4.7%から3.7%まで低下したが、この濃度はユリの遮光区における全糖濃度より高かった。ストックの花弁を花びら部で切り落し、スクロース溶液に入れて開花させたところ、花弁中のヘキソース濃度が2%を下回るとアントシアニン濃度の低下がみられた。これらの結果は、ストックにおいても花弁中の糖濃度はアントシアニン合成に影響するものの、弱光下における糖濃度が高いために着色低下を防いていることを示す。

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