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Research note

Evidence that blue light induces betalain pigmentation in *Portulaca* callus

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Abstract

The wavelength range that activates betalain pigmentation has been studied following selection of light inducible betalain producing callus lines originating from *Portulaca* sp. 'Jewel' seedlings. Light sources with different wavelengths were used to irradiate the callus, resulting in blue light being effective in inducing betalain pigmentation. In addition, when UV light was combined with blue light, some calluses from this cell line showed high production of the pigment. This is a first report that betalain pigmentation in callus was induced by blue and blue/UV lights.

Abbreviations: UV – ultra violet

Plant responses to red, blue and UV light have been employed to evoke different phenotypic expressions via signal perception and transduction pathways (Kendrick & Kronenberg 1993). Flavonoid synthesis induced by UV light in suspension cell culture of parsley (Wellmann 1975) or carrot (Gleitz & Seitz 1989) has been well-studied, and provided useful systems for investigating secondary metabolite synthesis and the control of relevant gene expression. However, we have little information about betalain pigmentation responses to specific light sources.

Betalain is one of the plant pigments as is anthocyanin, although their two chemical structures are completely different. Indeed the two pigments stem from distinct biosynthetic pathways. Moreover, anthocyanin pigments have not been detected in most of the families belonging to the Centrospermae where betalains are expressed (Piatelli 1981). Thus, it is of interest to explore whether betalain biosynthesis can be induced by common stimuli, especially light, as observed in the UV induction of anthocyanins (Chappell & Hahlbrock 1984).

We have previously established a betalain pigmentation system in callus cultures that originated from seedlings of *Portulaca* sp. 'Jewel' (Kishima *et al.* 1991). The pigment can be induced by light and rapid synthesis can occur within 24 h after irradiation, but disappear in the dark due to pigment degradation and proliferation of white cells. Most in vitro studies have shown evidence for some effects of light on the accumulation of betalain pigments in cultured cells (Bianco-Colomas & Hugues 1990; Boehm *et al.* 1991), although the details of the light responses have not yet been clarified. To examine light specificity for induction of the pigmentation, we have exposed calluses to several light sources with different wavelengths. The results obtained show that the betalain pigmentation in the callus of *Portulaca* was strongly stimulated by blue light.

Calluses originating from seedlings of JR line flowers in *Portulaca* sp. 'Jewel' having a red colour were selected as described previously (Kishima *et al.* 1991). These calluses were divided into small pieces (2.5 mm diameter) and grown at 25 °C under continuous white light (1 W m^{-2}). Subculture was performed every four weeks. The light exposure experiments were preceded by a decolorization treatment in darkness for two weeks that caused pigment degradation in the callus-

es and the production of white cell clusters through callus proliferation. All calluses used in the exposure experiments were cultured and irradiated in glass Petri dishes (64 mm diameter) which intercept wavelengths below 300 nm. Control calluses from JW line with white petals, which is near isogenic to JR (Adachi *et al.* 1985), were induced from seedlings and cultured by the same methods as the JR line (Kishima *et al.* 1991).

White light (15 W m^{-2}) obtained from cool white fluorescent lamps (Mitsubishi-Osram; FCL32HF-S/32 and FCL30HF-S/28), which consisted of three major wavelength regions around 450 nm, 550 nm and 620 nm, were filtered through 3 inch square filters, Kodak Wratten Gelatin Filter No. 2B, 9, 25 and 89B. These filters eliminated all wavelengths below 390 nm, 460 nm, 590 nm and 680 nm, respectively and gave light intensities of 11 W m^{-2}, 7 W m^{-2}, 3 W m^{-2} and 1 W m^{-2}, respectively. Dark-grown, decolorized JR calluses were exposed to seven days of continuous irradiation either with or without these filters.

Red, green and blue light sources were as follows: red light (Mitsubishi-Osram; FL10-P), 610 nm to 700 nm, 33 μmol m^{-2} s^{-1} at λ max 660 nm; green light (Mitsubishi-Osram; FL10-G), 490 nm to 590 nm, 27 μmol m^{-2} s^{-1} at λ max 530 nm; and blue light (Mitsubishi-Osram; FL10-B) 360 nm to 550 nm, 22 μmol m^{-2} s^{-1} at λ max 430 nm. These lights were used to irradiate calluses derived from both JR and JW lines for periods of either 24 h or 5 days, respectively.

UV light was obtained from a Mitsubishi-Osram FL10-BL 360 lamp (16 μmol m^{-2} s^{-1} at λ max 360 nm) which provided sufficient UV-B (below 320 nm) to induce UV-B specific responses. Calluses were subjected to UV irradiation for half a day. A continuous exposure of UV treatment over one hour induces necrosis of *Portulaca* callus.

The betalain pigment was extracted from approximately 0.5 g fresh weight callus (15–20 mm diameter) soaked in 5 ml 80% ethanol for 24 h at -20 °C. The content of betalain whose molecular weight is 550 was calculated using the molar extinction coefficient of betalain 5.66×10^4 at 536 nm) according to Piatelli *et al.* (1969). Total chlorophyll in JW callus (0.5 g) was extracted in 80% acetone and measured by spectrophotometric absorbance at 652 nm as described by Bruinsma (1963). The mean values were calculated from independent light-treated calluses.

To determine the wavelength of light specificity required for pigmentation in *Portulaca* callus, the calluses were irradiated with light of various wavelengths. Figure 1 shows the changes of betalain content of the calluses under white cool light treated with four different cut-off filters. Betalain which is detected as single compound in irradiated *Portulaca* calluses (Kishima *et al.* 1991) can be separated into two accumulation patterns. Light above 390 nm and non-filtered light both raised the level of pigment accumulation which correlated with the exposure period. Both exhibited parallel curves with only slight differences. However, irradiance above 460, 590 and 680 nm failed to induce the pigment. The results suggest that callus pigmentation requires wavelengths ranging from 390 nm to 460 nm, which correspond with the spectrum of blue light (UV-A waveband).

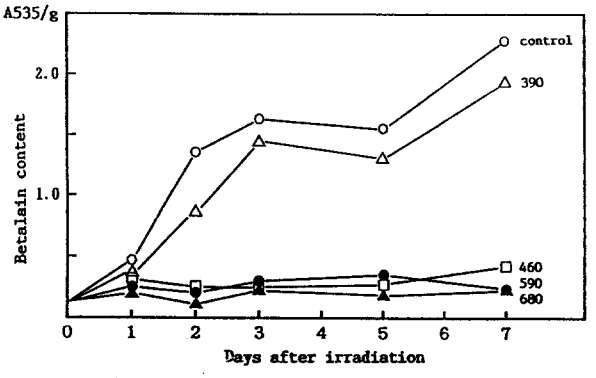

Fig. 1. Effect of continuous irradiation with filtered light on the accumulation of betalain pigment in *Portulaca* JR callus. The calluses were kept in darkness for two weeks after subculture. Light source was Mitsubishi-Osram FCL32HF-S/32 and FCL30HF-S/28. Light was filtered through four different cut-off filters which cut off all wavelengths below 390 nm, 460 nm, 590 nm and 680 nm, resulting in energies of 11 W m^{-2}, 7 W m^{-2}, 3 W m^{-2} and 1 W m^{-2}, respectively. No filter was applied as control (15 W m^{-2}). The data are means of four different trials with standard errors.

To confirm the above results, we carried out further experiment and exposed the calluses to red, green and blue light sources. Table 1 shows the betalain contents in JR calluses under continuous illumination from the three light sources and the corresponding chlorophyll contents. Blue light was most effective at inducing betalain pigmentation with accumulation averaging 8.5 ng g^{-1} after 24 h expose (Table 1). On the other hand, under red light pigment accumulation was significantly lower than with blue light. In contrast to betalain accumulation in JR callus, preferential accumulation of chlorophyll in JW callus was observed under red light, whereas we were not able to detect any chlorophyll in blue light treatments. This results clearly illustrate that betalain synthesis in JR callus has a different light

Table 1. Effect of light quality on the accumulation of betalain pigment in *Portulaca* JR callus and chlorophyll in JW callus[1]

Pigment in callus	Pigment content under light sources ng g^{-1} (f.w.)		
	Red (660 nm^2)	Green (530 nm)	Blue (430 nm)
Betalain in JR	4.5±1.5	5.5±2.0	8.5±1.8
Chlorophyll in JW	21.0±2.0	10.0±2.5	0

[1]Betalain and chlorophyll contents were measured after 24 h and 5 days of illumination, respectively. Each value represents the means and standard errors for five independent experiments.
[2]λ max of the light source.

requirement from chlorophyll synthesis in JW callus. The callus growth has not been affected by any of light treatments.

To test whether the pigmentation in the callus can be induced by UV light, we undertook UV irradiation experiments with decolorized *Portulaca* calluses. After five days of repeated UV treatment, all calluses failed to accumulate significant betalain pigment (Fig. 2A). However, when 30 min UV irradiation was combined with continuous blue light for 6 h per day, the pigment accumulation was recovered (Fig. 2C). Betalain contents of individual calluses, from the combined blue and UV light treatment showed a relatively broad distribution, compared to treatment without UV (Fig. 2C, 2B). In particular, some calluses showed an ability for high betalain production under blue/UV light, and in those betalain synthesis might be activated by UV irradiation. All calluses in the 6 h red light treatment, however, had quite low level of betalain and were unaffected by UV treatment (data not shown). Thus, the callus pigmentation in UV light appeared only where combined with blue light. In UV treatment, unless necrosis happened, the callus growth seemed to be normal.

We have demonstrated in this paper that betalain pigmentation can be induced by blue or blue/UV light. It has been recognized that photo-morphogenesis in higher plants is under the control of at least three different photo-transduction systems involving phytochrome, blue light and UV photoreceptors (Kendrick & Kronenberg 1993). In our experiments, betalain synthesis in JR callus irradiated with blue light was most efficient among the three light sources employed here, and was distinguished from a light responsive system of phytochrome-mediated chlorophyll synthesis (Furuya 1993). Thus, betalain pigmentation in our callus could be activated through blue light signal transduction where flavin-like photoreceptors are involved in the initiation affecting certain gene expression (Ahmad & Cashmore 1993).

In addition to the blue light effect, continuous red light irradiation of JR callus was also able to induce betalain pigment up to the average of 4.5 ng g^{-1}. This fact suggests that phytochrome may be stimulated by red light to produce low amount of the pigment. Furthermore, the existence of high betalain accumulating callus following irradiation of blue/ UV light probably represents a coaction for transduction via blue and UV photoreceptors (Mohr 1986). Therefore, while blue light is essential for an efficient induction of betalain pigmentation, both red and UV light might be also involved as minor factors in betalain synthesis in *Portulaca* callus.

Blue light has been shown previous to be more effective than red light in inducing betalain in seedlings of *Amaranthus* (Obrenovic 1985), and these are agree with our results. The betalain pigmentation in *Portulaca* callus could be convenient system for further investigation on blue light effects.

We conclude that betalain pigmentation in our callus is committed to one of the blue light responses. And it is noteworthy that to date, no paper has reported UV-induction of betalain pigment, neither did this pigmentation appear to be a defense response against UV as observed by the cultured cells with anthocyanin pigmentation (Chappell & Hahlbrock 1984).

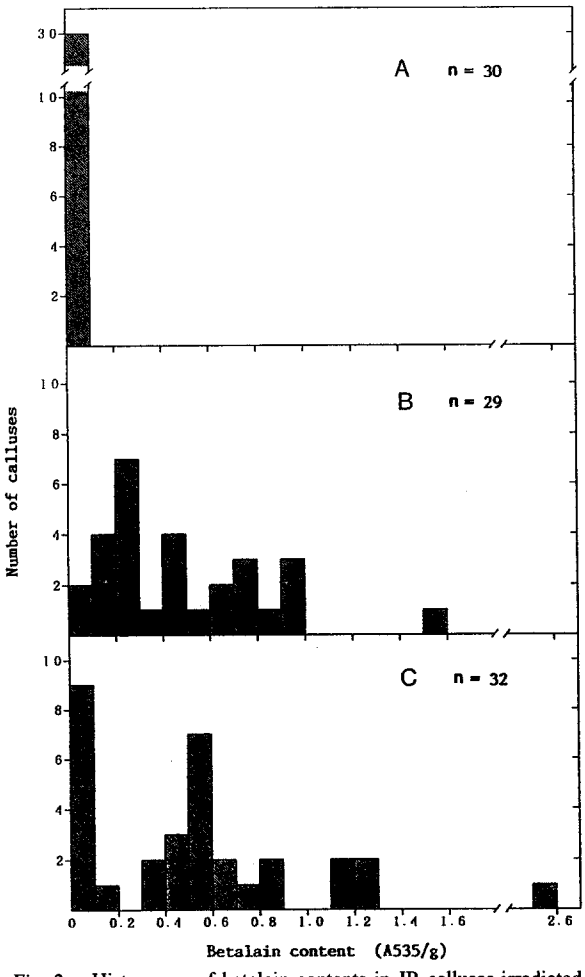

Fig. 2. Histograms of betalain contents in JR calluses irradiated with UV (*A*), blue light (*B*) and UV/blue light (*C*). Light treatments per day were as follows: (*A*) was given 30 min UV; (*B*) was given 6 h blue light; (*C*) was given 30 min UV + 6 h blue light. The calluses were analyzed 5 days after continuous treatment. UV and blue light intensities were 16 μmol m^{-2} s^{-1}, and 22 μmol m^{-2} s^{-1}, respectively.

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