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# Variation in betalain content and factors affecting the biosynthesis in *Portulaca* sp. 'Jewel' cell cultures

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## Abstract

Sixteen colored cell lines of *Portulaca* sp. 'Jewel' forming betalain were established, seven cultured on modified Murashige and Skoog (mMS) medium and nine on modified Girod and Zrÿd (mJ1). Each line was different in term of betalain content. Both betacyanin and betaxanthin widely varied in quality and quantity from line to line. A magenta-colored phenotype contained the maximum betacyanin and an orange-colored phenotype contained the maximum betaxanthin. Modified MS medium was optimal for betacyanin synthesis whereas modified J1 medium synthesized more betaxanthin. Betacyanin and betaxanthin both accumulated in a positive correlation to the cell growth in our culture system, and the highest contents were recorded during the logarithmic phase. The addition of natural auxin, indole-3-acetic acid (IAA), to both types of culture media instead of synthetic auxin, 2,4-dichloro-phenoxyacetic acid (2,4-D) inhibited the growth and reduced betalain synthesis. But a few subcultures after transfer to IAA-containing medium, two cell lines were established one producing betacyanin and one betaxanthin. Different cell phenotypes (either magenta or yellow colored cells) exhibit same responses in their requirement of light. Cellular betacyanin and betaxanthin drastically increased under continuous illumination, particularly with blue light irradiation along with the increasing number of growth cycles.

**Key words:** Betalain, biosynthesis, blue light, culture media, IAA, *Portulaca* sp. 'Jewel' (cultured cell lines).

## Introduction

The major pigments responsible for plant color are the flavonoids, carotenoids, chlorophyll and the taxonomically restricted betalains. The betalains, water-soluble chromoalkaloids, exhibit yellow, orange, red and magenta in the floral tissue of only a few plant species, being restricted to the Caryophyllales and pilei of some higher fungi. *Portulaca grandiflora*, a model plant for the study of the biochemistry and genetics of betalain synthesis (Trezzini and Zrÿd, 1990), and its inbred lines JR and JM (Adachi *et al.*, 1985) contain a lot of betalain pigments in floral and stem parts. Betalain accumulates in the *in vitro* culture as a secondary metabolite. This type of pigment is classified as betacyanin and betaxanthin depending on the conjugation of betalamic acid (the chromophore) with *cyclo*-DOPA (*cyclo*-L-3, 4-dihydroxyphenylalanine) derivative or free amino acid/amine (**Fig. 1**). Betacyanin is a conjugation product of betalamic acid and *cyclo*-DOPA, which shows red-violet color. On the other hand, when betalamic acid condenses with amino acid or amine, yellow betaxanthin is formed (Schliemann *et al.*, 1999). Betacyanin-producing culture (magenta/red color) was reported in *Portulaca* (Kishima *et al.*, 1991) and has also been observed in *Chenopodium rubrum* (Berlin *et al.*, 1986), *Phytolacca americana* (Sakuta *et al.*, 1986) and *Beta vulgaris* (Girod and Zrÿd, 1991). Betaxanthin-producing culture (yellow/ orange color) was also reported in *Portulaca grandiflora* (Böhm and Böhm, 1996; Noda and Adachi, 2000). Betalains are used as natural colorants in the food industry, pharmaceutical products and cosmetics. Recently betalain has gained attraction because betanin, the main betacyanin, has shown anti-

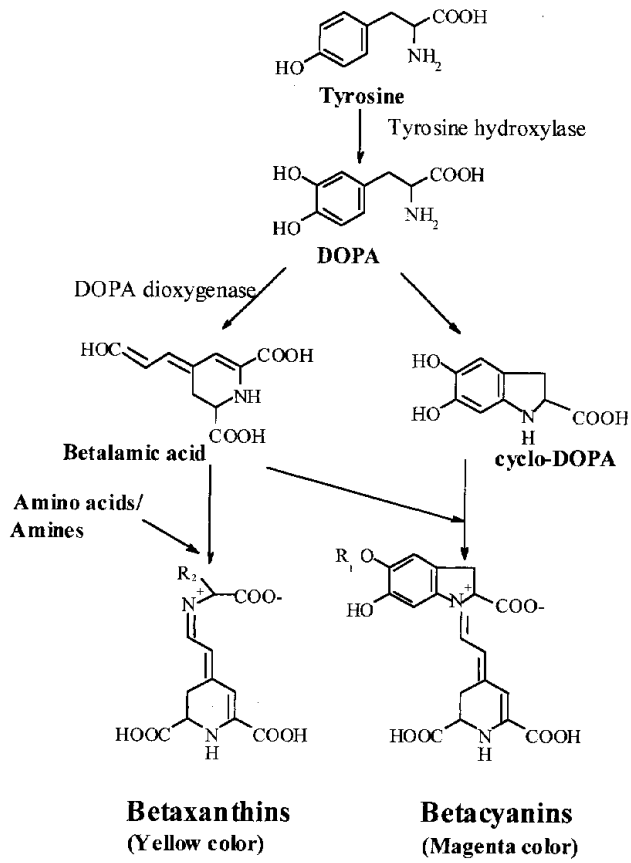


**Fig. 1** Biosynthetic pathway of betalain. (DOPA: 3, 4-dihydroxyphenylalanine; $R_1$: glucose in case of betanin and H in case of betanidin; $R_2$: lateral chain of amino acid or amine compound, i.e. glutamine is the responsible amino acid in case of Vulgaxanthin I and $R_2$ is $-(CH_2)_2-CO-NH_2$).

microbial activity (Delgado-Vargas *et al.*, 2000) and also betaxanthin has been suggested as an essential dietary colorant that contains different types of amino acids (Leathers *et al.*, 1992).

Plant cell culture has been a very useful tool in the study of various aspects of biochemistry, enzymology, genetics and biosynthesis of secondary metabolites. It is well known that the biosynthesis of betalain is markedly affected by various factors in the cell culture such as growth regulators, nutrients, light etc. (Jiménez-Aparicio and Gutiérrez-lópez, 1999). The effects of different nutrients such as nitrogen in *Phytolacca americana* (Sakuta *et al.*, 1987), zinc and sucrose in *Beta vulgaris* (Akita *et al.*, 2000) have been investigated on the growth and betalain biosynthesis in cell culture. Also, the effects of growth regulators on *Phytolacca americana* (Sakuta *et al.*, 1991) and light on *Portulaca* (Kishima *et al.*, 1991) have been reported for betalain accumulation. However, relatively little attention has been paid to the factors related to betalain biosynthesis in *Portulaca* sp. 'Jewel' cell culture.

Here, we report on the variations in betalain content among 16 different cell lines of *Portulaca* sp. 'Jewel' established on modified Murashige and Skoog (mMS) and modified Girod and Zryd (mJ1) culture medium. We also discuss some factors like culture medium, IAA and light condition, which affect the biosynthesis of betalain in cell culture.

## Materials and Methods

*Plant material and culture conditions*

*In vitro* seedlings of *Portulaca* sp. 'Jewel' (inbred line JR, red flowering genotype, cf. Adachi 1972) were excised and transferred onto MS medium supplemented with 30 $g\,l^{-1}$ sucrose, 9 $\mu$M 2,4-D and 2.5 $g\,l^{-1}$ Gellan Gum for the induction of magenta colored callus (Kishima *et al.*, 1991). These calli have maintained high productivity of betacyanins for about 10 years and stably exhibited purple to magenta pigmentation when selectively subcultured every 25 days. Yellow and orange callus lines were spontaneously produced by two magenta colored lines (JR4 and JR12) five years after initiation (Noda and Adachi, 2000). We are maintaining these callus lines under 16-h per day white light at 25 ℃ by selective subculture every 25 days and 21 days on mMS and mJ1 solidified medium (Noda and Adachi, 2000), respectively.

*Addition of IAA*

The natural auxin, IAA (Indole 3-acetic acid), was added to the autoclaved medium in different concentrations (4.5, 6, 9 and 20 $\mu$M) with filter (0.45 $\mu$m) sterilization instead of 2,4-D. The effects of IAA were observed with N12 and N4Y-cell lines. IAA treated cells were collected from the culture after 25 days and 21 days of initiation from mMS and mJ1 medium, respectively. In a control experiment, 2,4-D was added to mMS (9 $\mu$M) and mJ1 (4.5 $\mu$M) culture medium.

*Light conditions*

Effect of light conditions on the betalain contents were observed by using two-cell lines exhibiting magenta (N4M) and yellow (N4Y) color. All cell lines were maintained under 16-h per day white light conditions except N4M and N4Y. These two cell lines were maintained under 16 hour white light, continuous white light (photon flux density = 32.5 $\mu mol\ m^{-2}\ s^{-1}$), and continuous blue light (photon flux density = 22 $\mu mol\ m^{-2}\ s^{-1}$) conditions.

*Growth rate of the cell cultures*

The fresh weight of the cultures was measured and averaged for at least three cultures. Cell growth rate was defined as W/W0, where W0 and W denote

fresh weight at the initiation and harvest time of defined culture period, respectively.

*Extraction and analysis of betalain*

Cells were collected and stored at -30 ℃ . For betalain analysis, frozen cells were thawed and suspended with ultra-pure (0.22 $\mu$m filter unit) distilled water [200 mg (200 $\mu$l)$^{-1}$]. The extract was centrifuged at 12,000 rpm for 5 min and the supernatant was filtered through 0.45 $\mu$m filter unit and immediately injected to HPLC column (Shimadzu VP ODS-II column, 150 x 4.6 mm i.d.). All extracts were maintained at 5-10 ℃ before injection and each injection was 10 $\mu$l in volume. For HPLC, solvent system A was 5% acetic acid and solvent system B was 50% acetonitrile. Betalains were eluted using a linear gradient from 0 to 40% B at 2% B per minute with a flow rate of 1 ml min$^{-1}$. Betacyanin and betaxanthin were detected at 540 nm and 470 nm, respectively. HPLC system was equipped with an Intelligent pump (L-6200, Hitachi), an UV-VIS detector (L-4200, Hitachi), a chromato-integrator (D-2000, Hitachi) and a photodiode array detector (SPD-M10AVP, Shimadzu). Betaxanthin identification was achieved through co-chromatography using semi-synthetic betaxanthins (Trezzini and Zrÿd, 1991). The content of betacyanins and betaxanthins were calculated using the standard calibration curve of purified beet powder that was determined by HPLC and photodiode array detector operated under the same condition.

*Determination of DOPA and betalamic acid*

DOPA ( $\lambda_{max}$, 280 nm) and betalamic acid ( $\lambda_{max}$, 409 nm) were determined by taking spectrophotometric (UVIDEC-320, Jasco) absorbance from extracted pigment of all cell lines. DOPA and betalamic acid were quantified from their respective molar extinction coefficients (DOPA, $3 \times 10^6$ cm$^2$ mol$^{-1}$; BA, $25 \times 10^6$ cm$^2$ mol$^{-1}$).

## Results and Discussion

*Betalain status in different cell lines*

Yield improvement is a major initial objective for the practical use of plant tissue culture for the commercial production of useful metabolites (Havkin-Frenkel *et al.*, 1997). For this purpose, different callus lines were established on two types culture media to evaluate the optimum medium requirements for accumulation of maximum betacyanin and betaxanthin contents as well as to select the high betalain producing line. Seven callus lines (N2, N4, N7, N12, N18, N21, N22) and nine callus lines (N4M, N4R, N4Y, N4Or, N10, N12M, N12Or, N18M, N22M) were established on mMS and mJ1 culture medium, respectively, as shown in **Table 1**. The betacyanin and betaxanthin contents were greatly varied from line to line. Total betacyanin and total betaxanthin ranged from 0.05 mg (g FW)$^{-1}$ (N4Y, yellow color) to 4.8 mg (g FW)$^{-1}$ and from 0.06 mg (g FW)$^{-1}$ (N18M, magenta color) to 3.8 mg (g FW)$^{-1}$, respectively. Among the 16 lines, the N12 line, a magenta color phenotype, synthesized the highest amount of betacyanin [4.8 mg (g FW)$^{-1}$]. On the other hand, the N12Or line, an orange color phenotype, accumulated the maximum content of total betaxanthin [3.8 mg (g FW)$^{-1}$]. The average amount of total betacyanin was 3.23 mg (g FW)$^{-1}$ and 1.86 mg (g FW)$^{-1}$ in the mMS and mJ1 medium cell lines, respectively. The average amount of total betaxanthin was 0.26 mg (g FW)$^{-1}$ and 1.2 mg (g FW)$^{-1}$ in the mMS and mJ1 medium cell lines, respectively. In all cultured lines, betanin was the main component of betacyanin while vulgaxanthin I was the main component of betaxanthin. The highest betanin accumulating line was N18, a magenta color phenotype and highest vulgaxanthin I accumulating line was N12Or, an orange color phenotype. The acylated form of betacyanin was also observed and determined except in the N7, N4Or, N4Y and N12Or cell lines. Other betacyanins (i.e. isobetanin, betanidin) and betaxanthins, which accumulated as minor components in our culture system, were identified and showed a wide variation among cell lines (data not shown). The cell growth also varied in a range from 3.5 ± 0.86 to 9.4 ± 1.1. The best growth was observed in cell line N2.

Some secondary metabolite production by plant tissue and cell cultures is generally disappointingly low compared with whole plants (Tabata and Fujita, 1985). But interestingly, the biosynthetic potential of cultured *Portulaca* cells has been observed to change during subculturing. Many factors are involved in this type of rapid change. Since the biochemical capabilities of metabolites producing cultured cells have been shown to vary widely among strains or cell clones, a possible remedy for these problems might be the selection of stable and high-producing strains by plating or cloning techniques. Clonal variations in the content of several other metabolites have been reported, such as anthocyanin in sweet potato cell culture lines (Nozue *et al.*, 1987), shikonin in *Lithospermum* callus lines (Mizukami *et al.*, 1978), etc. Selection of cultured cells is very effective in improving the production potentials of cultured cells, even though it is not known of the variation in pigment production among cell lines is due to a gene mutation or to

**Table 1.** Betalain composition and growth rate of different cell lines of *Portulaca* sp. 'Jewel' on modified MS and modified J1 medium[1-5].

Modified MS culture medium

| Cell line | Growth rate ($W/W_0$) | TBc mg (g FW)$^{-1}$ | Betanin mg (g FW)$^{-1}$ | AcBc mg (g FW)$^{-1}$ | TBx mg (g FW)$^{-1}$ | Vul. I mg (g FW)$^{-1}$ | DOPA nmol (g FW)$^{-1}$ | BA nmol (g FW)$^{-1}$ |
|---|---|---|---|---|---|---|---|---|
| N2 | 9.40 ± 1.10 | 3.51 | 3.13 | 0.20 | 0.19 | 0.10 | 493 | 96 |
| N4 | 4.80 ± 0.45 | 0.60 | 0.40 | 0.15 | 0.50 | 0.17 | 90 | 14 |
| N7 | 9.30 ± 1.15 | 2.81 | 2.70 | - | 0.23 | 0.03 | 330 | 70 |
| N12 | 7.50 ± 0.91 | 4.80 | 4.13 | 0.60 | 0.20 | 0.04 | 616 | 162 |
| N18 | 5.80 ± 1.02 | 4.65 | 4.40 | 0.12 | 0.50 | 0.08 | 483 | 91 |
| N21 | 3.50 ± 0.86 | 1.90 | 1.12 | 0.18 | 0.12 | 0.02 | 436 | 167 |
| N22 | 9.40 ± 1.65 | 4.20 | 2.80 | 1.02 | 0.11 | 0.02 | 583 | 114 |

Modified J1 culture medium

| Cell line | Growth rate ($W/W_0$) | TBc mg (g FW)$^{-1}$ | Betanin mg (g FW)$^{-1}$ | AcBc mg (g FW)$^{-1}$ | TBx mg (g FW)$^{-1}$ | Vul. I mg (g FW)$^{-1}$ | DOPA nmol (g FW)$^{-1}$ | BA nmol (g FW)$^{-1}$ |
|---|---|---|---|---|---|---|---|---|
| N4M | 7.70 ± 0.95 | 2.02 | 1.81 | 0.10 | 0.45 | 0.16 | 490 | 111 |
| N4R | 4.97 ± 0.60 | 2.80 | 2.60 | 0.10 | 1.00 | 0.62 | 336 | 162 |
| N4Or | 5.40 ± 0.50 | 0.60 | 0.44 | - | 2.80 | 1.30 | 210 | 195 |
| N4Y | 6.60 ± 0.35 | 0.05 | 0.05 | - | 1.80 | 0.90 | 196 | 188 |
| N10 | 3.97 ± 0.57 | 2.90 | 2.70 | 0.04 | 0.10 | 0.02 | 400 | 104 |
| N12M | 7.70 ± 1.10 | 3.40 | 1.30 | 0.50 | 0.50 | 0.32 | 496 | 136 |
| N12Or | 7.40 ± 0.70 | 0.72 | 0.50 | - | 3.80 | 1.70 | 253 | 287 |
| N18 | 6.36 ± 0.84 | 1.70 | 1.60 | 0.05 | 0.06 | 0.03 | 356 | 38 |
| N22 | 6.70 ± 0.96 | 2.60 | 2.05 | 1.20 | 0.61 | 0.24 | 466 | 206 |

[1]All the values are the mean of three replicates.

[2]Cells were harvested at 25 and 21 days after inoculation on mMS and mJ1 medium, respectively.

[3]Betanin and vulgaxanthin I are the main compound of betacyanin and betaxanthin, respectively.

[4]TBc = Total betcyanin, AcBc = Acylated betacyanin, TBx = total betaxanthin, Vul.I = Vulgaxanthin I, DOPA = 3,4-dihydroxyphenylalanine, BA = Betalamic acid.

[5]$W_0$ = fresh weight at the initiation and W = fresh weight at the end of culture period, growth rates are presented in mean ± SD value ; n = 3.

extra-chromosomal variation. Since culture lines of *Portulaca* sp. 'Jewel' are not of single cell origin, many heterogeneous cells are likely to be contained in any single culture line. Thus, it may be possible to isolate single cell clones having higher pigment synthesizing capabilities by the cell cloning method.

Moreover, in our culture system, the variations in betalain content among different cell lines on the same medium are probably due to the causes of the genotypic differences of explants or the differences in gene expression of enzymes involved in betalain biosynthesis in cell culture.

*Effects of culture medium on growth and betalain synthesis*

As shown in **Fig. 2**, the growth and betalain accumulation of the N12 cell line was monitored every week on the mMS and mJ1 medium. Growth gradually increased and maximum growth was reported at 25 days on mMS and at 21 days on mJ1. Also, the highest betacyanin and betaxanthin accumulation was observed at 25 days on mMS and at 21 days on mJ1 medium, and after that both betalain content and growth declined. This means that in *Portulaca* sp. Jewel, the betalain accumulation was parallel with cell growth during the logarithmic phase. The observation of this study suggests that betalain synthesis in cell culture is closely related to cell division or cell growth. A similar relationship between cell growth and betacyanin content has been observed in *Phytolacca Americana* (Sakuta *et al.*, 1986) and *Chenopodium rubrum* (Berlin *et al.*, 1986) cell culture. The growth rate was slightly higher on the mJ1 medium, while betacyanin accumulation was significantly higher on the mMS medium. For betaxanthin accumulation, mJ1 me-

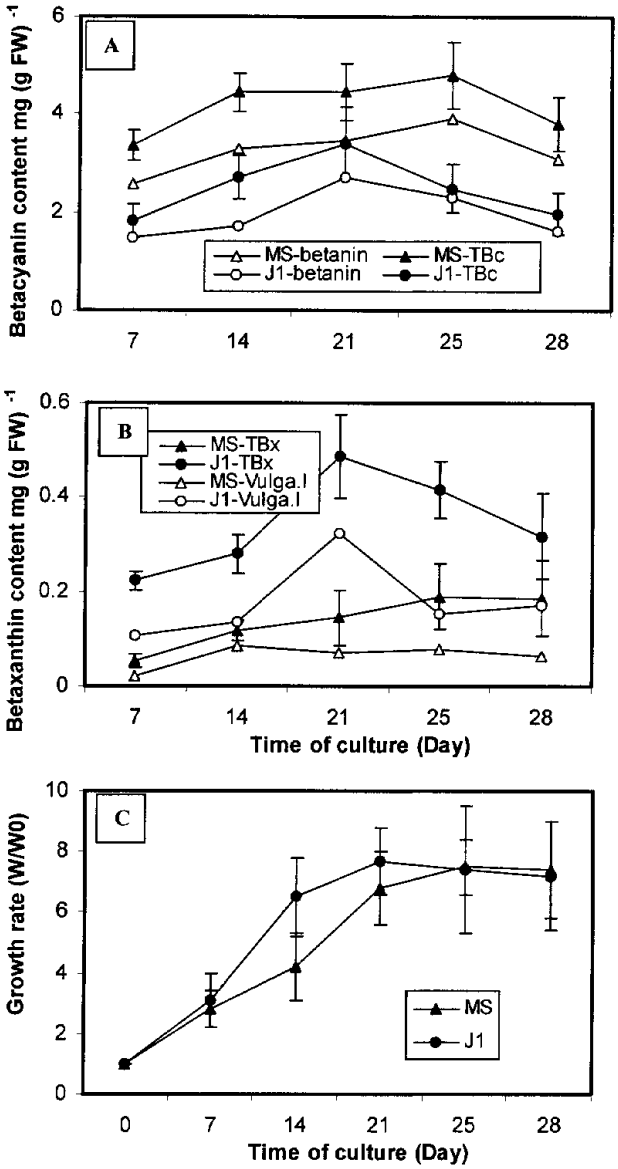


**Fig. 2** Effect of modified MS and modified J1 culture media on betacyanin (A), betaxanthin (B) synthesis and growth rate (C) of N12 cell line. TBc = Total betacyanin, TBx = Total betaxanthin, Vulga.I = Vulgaxanthin I. TBc and TBx are presented in mean ± SD of three replicates. Betanin, the main betacyanin and Vulgaxanthin I, the main betaxanthin are presented in mean value of three replicates.

dium was superior to mMS medium. In addition, it was observed that the cell lines cultured on mMS medium synthesize more betacyanin (in average) in comparison with the cell lines cultured on mJ1 medium. Whereas in the cell lines cultured on mJ1 medium the average betaxanthin content was higher than those on mMS medium.

Variation in medium ingredients and growth regulator concentrations of mMS and mJ1 medium are probably the causes of such differences. In a previous study on *Beta vulgaris*, it was shown that the modification of growth regulator caused changes in cell phenotypes (Girod and Zryd, 1991). In this study, the mJ1 medium contains half amount of macro and micro salts of mMS medium and contains some vitamins such as folic acid, calcium panthothenate and biotin, which are lacking in the mMS medium. In addition, the mJ1 medium contains half the concentration of 2,4-D that the mMS medium does. The modified J1 medium ingredients, including macro, micro, vitamins and 2,4-D concentratrion, may influence the direction of biosynthetic pathway intermediates or enzymes activity. Although the enzymology of betalain is not well understood, we can assume that mJ1 medium ingredients play some role in the activity of DOPA dioxygenase to make betalamic acid available, because an increase in the level of betalamic acid would result in an increased synthesis of betaxanthin. After that the betalamic acid bonds with amines or amino acids (the site of this reaction is not yet confirmed, Schliemann *et al.*, 1999) and produce betaxanthins within the vacuole (**Fig. 1**). It is also possible that three vitamins such as folic acid, calcium-panthothenate and biotin facilitate the availability of amino acids within the cells cultured on the mJ1 medium, because these vitamins are essential for many amino acid syntheses. These are the possible reasons why more betaxanthins accumulate within the cells cultured on the mJ1 medium than those on the mMS medium. On the other hand, the mMS medium supplements may increase the level of cyclo-DOPA, thereby increasing betacyanin synthesis. From these, it may be concluded that when an appropriate environmental stimuli is given, such as growth regulator or medium composition, the *Portulaca* cells appear to respond by switching off or on a series of reactions which commit the cells to a specific developmental pathway, either betacyanin or betaxanthin synthesis. Another explanation is that the medium composition modulates the activity of gene(s) involved in the control of betacyanin or betaxanthin biosynthesis. From these observations, we can suggest that mMS and mJ1 culture medium are suitable for betacyanin and betaxanthin synthesis of *Portulaca* cells, respectively.

*Effects of natural auxin (Indole 3-acatic acid, IAA) on growth and betalain synthesis*

All culture lines were initially established by using the synthetic auxin 2,4-D. But 2,4-D is an herbicide that is not appropriate for production of food colorant, so we tested the effect of IAA on cell growth and betalain synthesis instead. Different concentrations of IAA were added to the N12

(magenta) and N4Y (yellow) cell lines. Among the concentrations tested, 4.5 and 6 $\mu$M IAA showed significant promotion of cellular betacyanin and betaxanthin over the control in the N12-cell line during the first growth cycle (**Fig. 3**). Interestingly, betacyanin and betaxanthin both suddenly decreased during the 2nd growth cycle of the line treated at four levels of IAA. During the 3rd subculture, only 4.5 $\mu$M again slightly increased the amounts of betacyanin and betaxanthin in the N12 cell line. Cell growth rate was also markedly decreased during the 2nd cycle at all concentrations of IAA but increased again slightly during the 3rd cycle at 4.5 and 6 $\mu$M IAA.

For the N4Y cell line, the amount of betacyanin and betaxanthin increased significantly for all concentrations except 4.5 $\mu$M IAA during first growth cycle (**Fig. 4**). At the time of 2nd growth cycle the betaxanthin level suddenly decreased in cells treated with 6, 9 and 20 $\mu$M IAA, although the betacyanin level increased at any concentration of IAA. The betaxanthin level increased slightly for 4.5 $\mu$M IAA during the 2nd growth cycle. During the 3rd subculture, the cellular betacyanin level increased gradually at all concentrations of IAA. Due to the increasing amount of betaxanthin during the 3rd growth cycle in N4Y-cell line by the

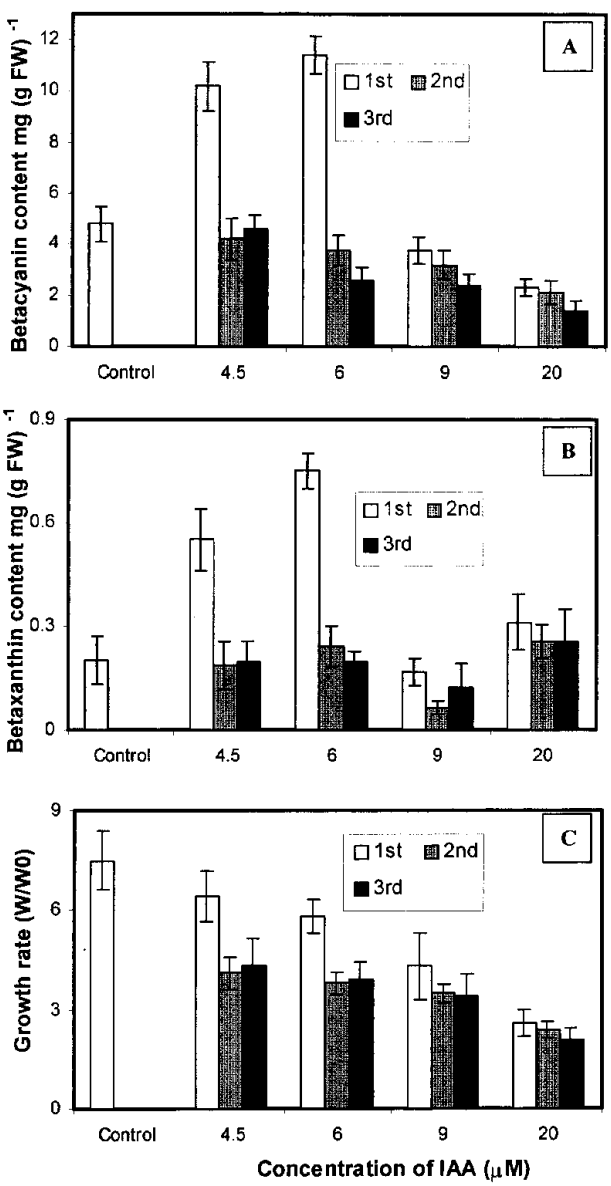


**Fig. 3** Effects of IAA on betacyanin (A), betaxanthin synthesis (B) and growth (C) of N12 (magenta phenotype) cell line for continuous three growth cycles. 1st , 2nd and 3rd represent the number of growth cycle. All values are the mean ± SD of three replicates.

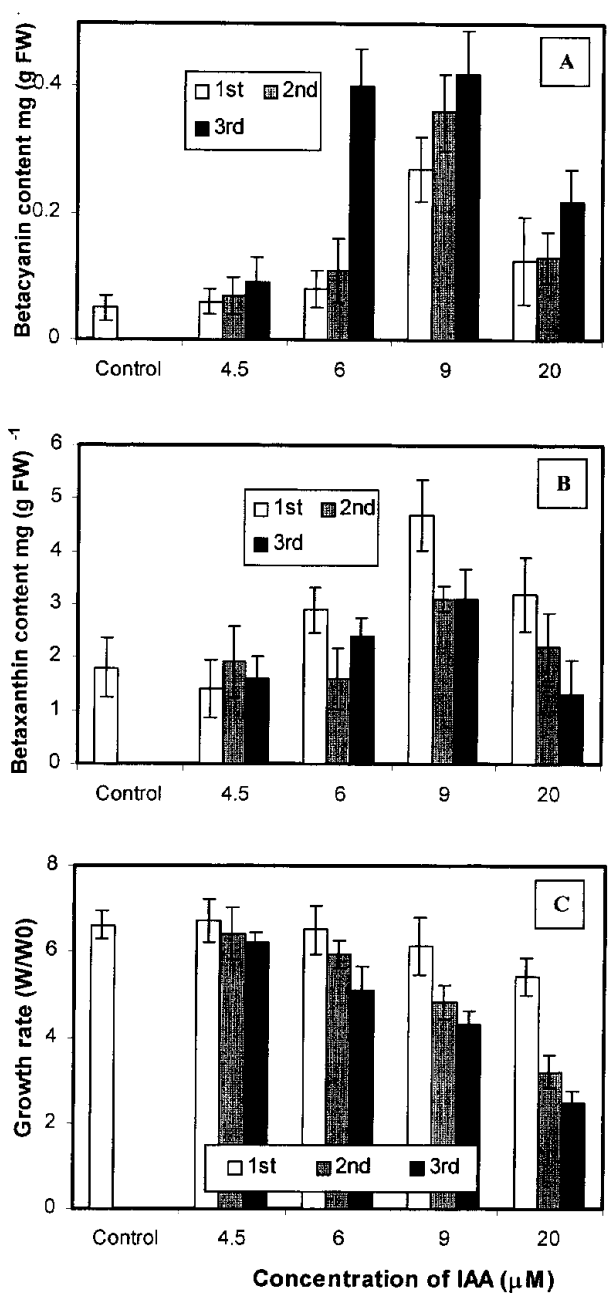


**Fig. 4** Effects of IAA on betacyanin (A), betaxanthin synthesis (B) and growth (C) of N4Y (Yellow phenotype) cell line for continuous three growth cycles. 1st, 2nd and 3rd represent the number of growth cycle. All values are the mean ± SD of three replicates.

addition of 6, 9 and 20 $\mu$M IAA, the color of cells turned yellow to orange. Although the cellular level of betaxanthin and betacyanin did not increased significantly by 4.5 $\mu$M IAA, it assists the cells to remain color stable. As shown in **Fig. 4C**, the growth rate was also affected after the addition of different concentrations of IAA. The cellular growth of both lines was less with higher concentrations of IAA than those in lower concentration. However, the growth of the N12 line was more affected than that of the N4Y line.

Many investigators have reported the effects of auxins, especially 2,4-D, on growth and betacyanin accumulation (Rodríguez *et al.*, 1996, Noda and Adachi, 2000) but the effects of IAA on growth and betalain accumulation are not available. It will be interesting to elucidate the interaction between endogenous and exogenous IAA for betalain induction in our culture system. We are now maintaining these two cell lines with IAA (4.5 $\mu$M) supplemented medium instead of 2,4-D. The IAA-mediated both callus lines will be good sources for establishment of betacyanin and betaxanthin producing cell suspension cultures.

*Effect of light conditions on different cell phenotypes*

Light has been shown to be an important regulator for betalain formation in the cell culture. Kishima *et al.* (1995) reported that *Portulaca* cells (red-violet phenotype) were not able to synthesize betalain in the dark. Cells grown in the dark were white, the accumulation of betalain started again after exposure to light. In addition, they showed that blue light was more effective for the production of betalain than other lights. But it is not yet known whether the different colored callus phenotypes exhibit differences in their requirement of light (Leathers *et al.*, 1992).

In our experiment, one magenta (N4M) color and another yellow (N4Y) color callus lines were treated with 16-h per day white light (16HWL), continuous white light (CWL) and continuous blue light (CBL). We observed that both cell lines cultured under CBL significantly increased the cellular contents of betacyanin and betaxanthin compared to those cultured under CWL or 16HWL (**Fig. 5**). The CWL also slightly increased the contents of betacyanin and betaxanthin more than 16HWL in both lines. To compare the rate of pigment synthesis in both colored lines under three light conditions along increasing number of subculture (growth cycle) time, we continued the treatments of both lines with three light conditions up to seventh subculture. We found that after the seventh subculture in CBL, both betacyanin and betaxanthin synthesized rapidly and reached a high level. In the magenta line, N4M, total betacyanin increased 4.2-fold and total betaxanthin content increased 4.5-fold (data not shown) over the first subculture under 16HWL. On the other hand, N4Y line cells synthesized 4.7-fold higher total betacyanin (data not shown) and 4.8-fold higher total betaxanthin than the content accumulated during the first growth cycle of 16HWL condition. Betalain content also increased after the seventh subculture of both lines under CWL. However, the promotion of both type of betalain synthesis in both lines was negligible under 16HWL after the seventh growth cycle. Extended blue light exposure did not cause any damage or necrosis on cell growth in both colored lines.

Therefore, we can conclude that different cell phenotypes, either magenta (rich in betacyanin) or yellow (rich in betaxanthin), show similar responses in their requirement of light. Moreover, continuous light irradiation, particularly blue light, is more effective for both betacyanin and betaxanthin pro-

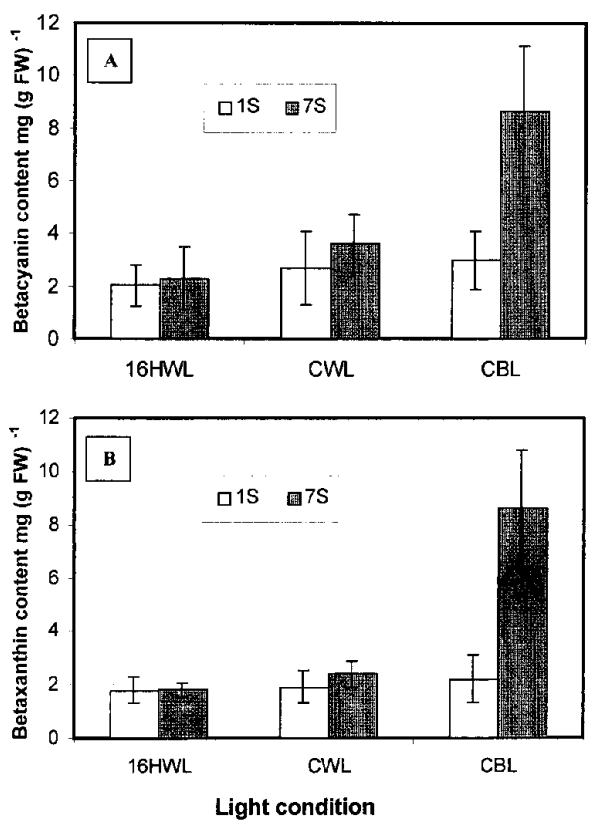


**Fig. 5** Effect of different light conditions on betacyanin (A) and betaxanthin (B) accumulation along cell growth cycles in a magenta (N4M) and a yellow (N4Y) phenotypes, respectively. 16HWL = 16 h/day white light, CWL = continuous white light, CBL = continuous blue light, 1S = 1st growth cycle, 7S = 7th growth cycle. Data presented in mean ± SD of three replicates.

duction in *Portulaca* cells and cellular pigments drastically increased along with the cell growth cycles. From the above findings it can be assumed that continuous light, especially blue light, may stimulates the genes related to betalain synthesis more rapidly than that of other light conditions.

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