

Anthocyanin production in a callus line of *Panax sikkimensis* Ban

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Abstract A root-derived callus line of *Panax sikkimensis* that stably accumulates anthocyanins was established by small cell aggregate selection method. The selected line showed a growth index of 221.36 and an anthocyanin content of 2.76 mg/g fw (7.076% dw) in 50–60 d of growth on a modified MS medium containing 4.5 μ M 2,4-dichlorophenoxy acetic acid and 1.2 μ M kinetin under 16-h light and 8-h dark photoperiodic conditions. Incubation under continuous light increased the growth index to 435.57 but led to a marginal dilution of anthocyanin content to 2.192 mg/g fw (6.928% dw). The purple-red pigment had absorption maximum at 528 nm. The selected callus line has shown sustained growth and productivity for more than 6 yr now. Interestingly, pigment accumulation in the selected line did not hinder the ginsenoside production in the callus tissue (0.9–1.2% fw).

Keywords *Panax sikkimensis* · Callus cultures · Cell aggregate selection · Anthocyanin production

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Introduction

Anthocyanins are colorful members of the flavonoid group of phytochemicals valued as natural food colorants. Anthocyanins show a wide range of biological activities like free-radical scavenging, antioxidant capacity, estrogenic supplements, antiinflammatory, anticancer, and antilipid peroxidation agents (Kang et al. 2003; Liu 2003; Lila 2004). Anthocyanins are normally found in low concentration in aqueous cell sap of fruits and flowers and chemically consist of an anthocyanidine moiety and a glycosidic residue. The former undergoes hydroxyl and methyl substitutions at various molecular sites to provide an array of bluish to reddish pigmentation at different cellular pH (Seitz and Hinderer 1988). Cultured cells and callus tissues of a large number of plant species have been shown to synthesize and accumulate anthocyanins under controlled *in vitro* environment (Cállebáut et al. 1990; Shuler et al. 1990; Kobayashi et al. 1993; Zhong et al. 1993; Zubko et al. 1993; Hirner et al. 2001; Mori et al. 2001; Konczak-Islam et al. 2003, 2000; Grusak et al. 2004). Cell cultures of *Vitis*, *Ipomaea*, *Glehinia*, and *Vaccinum* have been reported to accumulate anthocyanins in amounts higher than in their *in vivo* grown plant parts (Cormier and Do 1993; Kandil et al. 2000; Lila 2004). Because of their easy extraction and identification, good understanding of biogenesis, pathway enzymes, genes and transcription factors, and amenability to biotic and abiotic elicitation, anthocyanins are the focus of metabolic engineering efforts today (Verpoorte and Alfermann 2000).

The genus *Panax* (Family Araliaceae), commonly known as ginseng, has long been in use in traditional herbal medicines and the health food industry (Choi 1988; Haughton 1999). The most common species of *Panax* in the herbal trade are *Panax ginseng*, *Panax quinquefolium*,

Panax pseudoginseng, and *Panax japonicus*. The dried roots of these ginseng species are rich source of a variety of saponins (ginsenosides) having antiaging, adaptogenic, and immunomodulatory actions (Ngan et al. 1999; Nocerino et al. 2000). Our laboratory has been engaged in biotechnological studies in American and Indian species of *Panax* for the past several years (Shukla and Thakur 1988; Mathur et al. 1993, 1994, 1999, 2003). While screening for cell lines rich in various ginsenoside fractions from callus cultures of an Indian species *P. sikkimensis*, we encountered an anthocyanin-rich purple pigmented cell cluster that has subsequently been cloned through repeated cell-aggregate selection procedure. This communication briefly describes the growth kinetics and factors controlling *in vitro* anthocyanin productivity of this unique callus line, which is not only rich in anthocyanin but also produces characteristic immunomodulatory ginsenosides. We believe this to be the first report of *in vitro* anthocyanin production in the genus *Panax*.

Materials and Methods

P. sikkimensis Ban. (Bennet and Sharma 1983; Mehta and Haridasan 1992) roots were collected from Lachung area of Sikkim (1,500–3,000 m, altitude). A fast-growing callus line was induced from root explants on a modified agar-gelled Murashige and Skoog's (1962) medium containing 3.0% sucrose, 0.01% myoinositol, 0.33 μM thiamine hydrochloride, 2.5 μM pyridoxine hydrochloride, 4.0 μM nicotinic acid, 4.5 μM 2,4-dichlorophenoxy acetic acid (2,4-D), and 1.2 μM kinetin (Kn; Mathur et al. 1994). The fragile, pale-green callus was multiplied through regular sub-culturing every 6th week. The callus line was characterized by a growth index of 272.2% and 0.95% crude ginsenoside content in 40–45 d of growth on fresh weight basis. The major ginsenoside fractions included Rb₂ (20.32%), Rd (13.72%), and Rf (15.23%) as reported earlier (Mathur et al. 1999).

Selection of anthocyanin-producing callus line. The anthocyanin-producing callus line was isolated by selectively sub-culturing a variant purple-red pigment-containing cell cluster that appeared spontaneously in one of the stock culture of the wild callus line. The cell aggregate cloning method was repeatedly applied for seven to eight generations until a uniformly pigmented callus tissue was obtained. Non-radiative resonance energy transfer procedure of Sakamoto et al. (1994) using fluorescein isothiocyanate staining of the cells confirmed the presence of anthocyanins in them. Anthocyanin-producing cell line was sub-cultured every fourth wk onto the fresh medium for eight consecutive passages before subjecting to growth and production studies.

The pH of all the medium combinations was adjusted to 5.8 ± 0.03 before autoclaving at 1.04 kg/cm² pressure (121°C) for 15–20 min. Unless otherwise stated, the cultures were incubated under diffused light (15 $\mu\text{E m}^{-2}\text{s}^{-1}$) provided by cool white fluorescent light, 16:8-h light/dark photoperiod, $25 \pm 3^\circ\text{C}$, and 60–70% relative humidity.

Measurement of callus growth. For all growth measurement experiments, 4.0 g fresh weight of callus was inoculated onto 40 ml of the fresh agar medium in 100-ml Erlenmeyer flask, and the biomass gain was monitored at 10/20-d interval over an 80–90-d culture cycle. The fresh weight of the cultured tissue was measured by carefully removing the adhered agar from callus tissue. The biomass increase on a fresh weight basis is expressed as the percent increment over the initial inoculum (growth index) that was calculated as follows:

$$\text{Growth index (GI)} = \frac{\text{Final biomass wt.} - \text{Initial inoculum wt.}}{\text{Initial inoculum wt.}} \times 100$$

A minimum of three replicates were run for all the treatments and the experiments were repeated thrice.

Extraction and spectral analysis of the pigment. Fresh callus tissue (approx. 5.0 g) was homogenized in 10 ml of 0.1% (v/v) HCl–methanol mixture and filtered. The clear supernatant (1.0 ml) was diluted 3-fold with the same acidic methanol solution. The absorbance of the methanolic solution was measured at 535 nm using UV/VIS spectrophotometer (Perkin-Elmer, Lambda Bio-20). The total anthocyanin concentration in the extract was measured using the extinction coefficient $E 1\% = 98.2$ at 535 nm for cranberry anthocyanin extracted with the same solvent (Cormier and Do 1993). To chemically characterize the anthocyanin in *P. sikkimensis* callus line, freshly harvested tissue was separately extracted using acetone or 0.1% HCl–MeOH. The acetone and acidic methanol extracts, thus obtained, were concentrated at 40–50°C, and the concentrate was applied directly onto a Whatman filter paper no.3. Descending paper chromatography was carried out at room temperature for 10–15 h using the solvent system butanol/acetic acid/water 4:1:5. The discrete anthocyanin bands obtained from the two extracts were cut and eluted with methanol, vacuum-concentrated, and subjected to spectrophotometry to determine the absorption spectra in the visible range.

Statistical analysis. Quantitative data were analyzed using two-way factorial randomized complete block designs with three replications. The effect of (a) five levels of hormone treatments on growth index and anthocyanin content at four levels of culture duration, (b) three levels of light conditions

on growth index and anthocyanin content at nine levels of culture duration, and (c) seven levels of sucrose concentration on growth index and anthocyanin content at two levels of culture duration along with their possible interaction effects were compared using critical difference (CD) values of $P < 0.05$ and $P < 0.01$ levels of significance.

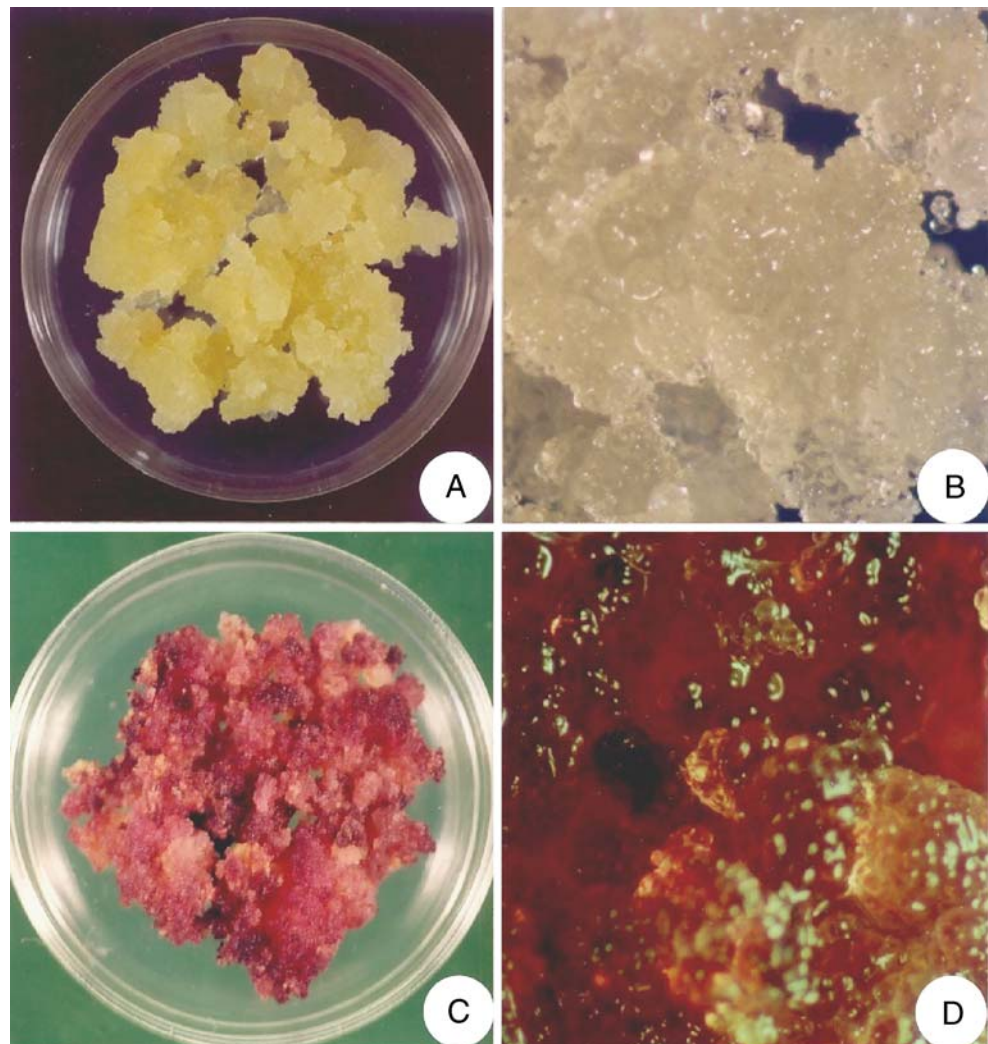
Results

Isolation of anthocyanin-producing callus line. *P. sikkimensis* callus initiated from root explants was light greenish and friable in nature (Fig. 1A, B). During the continuous *in vitro* cultivation of this callus line on $4.5\mu\text{M}$ 2,4-D and $1.2\mu\text{M}$ kinetin containing medium, a purple-red-colored cell cluster appeared spontaneously in one of the wild line cultures. This sector was selectively excised and sub-cultured onto the fresh medium. The frequency of pigmented cells was gradually increased with each subculture cycle. Following

this continuous cell aggregate selection technique, a more or less uniformly stained, dark purple-pigmented callus line was established (Fig. 1C, D). The variant callus line was subjected to detailed characterization for growth and pigment accumulation and results are summarized below.

Growth and anthocyanin production pattern under different sets of culture conditions. Growth and pigment production in the selected callus line as a function of various phytohormone treatments, light/dark conditions, and varying sucrose levels in the medium was monitored at 10–20-d interval (Tables 1, 2, and 3). For these characterization studies, the inoculum to medium ratio of 1:10 (4.0 g inoculum in 40 ml of agar medium in 100-ml Erlenmeyer flask) was kept constant. Among the six hormonal combinations tested (Table 1), highest growth index (261.77) was obtained on callus induction and maintenance medium (MS+ $4.5\mu\text{M}$ 2,4-D+ $1.2\mu\text{M}$ Kn) followed by on medium where 2,4-D level was reduced to $2.25\mu\text{M}$ (growth index=206.67). Media combinations consisting of Kn with

Figure 1. Cell line selection in *P. sikkimensis* root callus cultures: (A) wild callus line, (B) its magnified view, (C) selected anthocyanin-producing line, (D) its magnified view.



indole-3-acetic acid (IAA) or α -naphthalene acetic acid (NAA) did not support growth of this line in comparison to 2,4-D/Kn fortified medium wherein the biomass increment became evident even from the 20th day of incubation. Anthocyanin content in callus grown on these six medium combinations (Table 1) was found to be highest (3.81 mg/g fw; 9.76% dw) in cells grown on MS+2.5 μ M NAA+1.2 μ M Kn for 40 d. The pigment level progressively declined with further advancement of age on this medium (3.2% and 1.45% dw after 60 and 80 d of growth, respectively). Callus growth on NAA/Kn medium was, however, extremely slow, and GI values of only 55.41, 55.66, and 53.78 could be registered after 40, 60, and 80 d of the growth. In comparison, medium with 2.25 μ M 2,4-D and 1.2 μ M Kn or 4.50 μ M 2,4-D and 1.2 μ M Kn supported maximum anthocyanin accumulation in callus harvested on 60th day of incubation (7.59% and 7.08% dw, respectively). These cultures were therefore better in terms of net pigment yield when their high biomass was also taken into account. Subsequent characterization experiments were hence carried out on medium containing 4.5 μ M 2,4-D and 1.2 μ M Kn as phytohormone supplementation.

Influence of photoperiod on growth and pigment accumulation (Table 2) was also studied under three sets of light and dark regimes: (a) 16-h light/8-h dark, (b) 24-h dark, and (c) 24-h light. A time course study at 10-d intervals under these three photoperiod conditions indicated that selected callus line attained maximum biomass gain on the 50th d of culture under continuous light conditions (GI=435.57). Rapid biomass gain in these cultures became evident between 20th and 50th d, followed by slight decline around 60th day, and then a steady state up to 90th day of the culture cycle. The pattern of growth under 16-h light/8-h dark conditions followed a similar trend but biomass gain (GI=421.14) was evident only between 60 and 70 d of culture followed by short decline between 80 and 90 d (GI=242.76). Incubation in complete dark resulted in poor growth during the initial 50 d of culture. Maximum growth index in these cultures was recorded after 60 d (GI=375.31). Light, was however, found to be a critical factor for pigment accumulation in the selected line (Table 2). Continuous exposure to light consistently favored more anthocyanin accumulation with the maximum found at 50 d (2.192 mg/g fw; 6.928% dw). Cultures exposed to 16-h light/8-h dark photoperiod had (4.025% dw) anthocyanin in 60-d-old cultures. Incubation under continuous dark resulted in 5–12-fold less pigment content in the cells. Maximum anthocyanin in case of dark grown tissue was present in 10-d-old cultures (0.678 mg/g fw; 1.15% dw), which might be a carry-over content of the initial inoculum itself.

Anthocyanin productivity at two stages of growth (50 and 70 d) was also assessed at seven concentrations of sucrose

Table 1. Effect of hormone combination on the growth and anthocyanin production in the selected callus line of *P. sikkimensis*

Hormone treatment	Growth index				Anthocyanin concentration ^z			
	20d	40d	60d	80d	20d	40d	60d	80d
6.0 μ M IAA+1.2 μ M Kn	39.42 ^y ± 1.27 ^x	51.17 ± 2.14	52.68 ± 0.97	64.62 ± 0.59	2.54 ± 0.11 (6.51)	2.66 ± 0.05 (6.82)	1.27 ± 0.12 (3.26)	0.69 ± 0.01 (1.76)
3.0 μ M IAA+1.2 μ M Kn	37.85 ± 2.26	36.90 ± 2.50	56.06 ± 2.72	39.31 ± 1.77	2.98 ± 0.09 (7.64)	2.57 ± 0.15 (6.59)	1.72 ± 0.04 (4.40)	0.63 ± 0.03 (1.61)
5.0 μ M NAA+1.2 μ M Kn	34.47 ± 1.20	46.92 ± 0.34	59.26 ± 0.95	81.71 ± 1.29	0.58 ± 0.06 (1.48)	2.23 ± 0.02 (5.72)	1.65 ± 0.04 (4.24)	0.58 ± 0.06 (1.48)
2.5 μ M NAA+1.2 μ M Kn	37.35 ± 2.11	55.41 ± 2.72	55.66 ± 3.71	53.78 ± 1.89	2.66 ± 0.11 (6.82)	3.81 ± 0.06 (9.77)	1.25 ± 0.06 (3.21)	0.57 ± 0.03 (1.45)
2.25 μ M 2,4-D+1.2 μ M Kn	59.19 ± 0.45	107.41 ± 0.41	96.89 ± 2.64	206.67 ± 2.47	1.28 ± 0.13 (3.27)	2.33 ± 0.08 (5.97)	2.96 ± 0.29 (7.59)	0.50 ± 0.07 (1.29)
4.5 μ M 2,4-D+1.2 μ M Kn (control)	51.42 ± 1.68	177.14 ± 1.03	221.36 ± 3.39	261.77 ± 5.82	0.98 ± 0.08 (2.52)	2.43 ± 0.01 (6.23)	2.76 ± 0.05 (7.08)	0.55 ± 0.04 (1.41)

Data were subjected to two-way factorial RBD analysis. CD (interaction) at 5% level=0.304; CD (interaction) at 1% level=0.4066

^z mg/g fw; values in parentheses indicate concentration on % dw basis

^y Each value represents average of three replicates

^x Standard deviation

Table 2. Effect of light conditions on growth and anthocyanin concentration in the selected callus line of *P. sikkimensis* over a 90-d culture passage

Light condition	10d	20d	30d	40d	50d	60d	70d	80d	90d
Growth index									
16-h light/ ^y	19.11 ^z ±0.49 ^y	88.76±4.29	183.22±1.70	346.38±11.15	368.81±8.46	421.14±3.06	415.33±3.82	324.04±8.29	242.76±14.40
8-h dark									
24-h light	12.24±0.73	74.72±0.89	184.29±3.29	375.13±5.68	435.57±11.60	411.82±4.29	347.90±3.55	339.58±2.95	321.61±9.61
24-h dark	14.05±1.50	84.59±5.30	140.59±3.34	295.19±14.63	349.61±6.42	375.31±5.36	342.80±9.05	337.93±7.57	341.39±7.36
Anthocyanin concentration^x									
16-h light/ ^y	0.54 ^z ±0.02 ^y (1.04)	0.59±0.002 (1.23)	0.62±0.002 (1.37)	1.77±0.03 (5.18)	1.41±0.01 (3.99)	1.54±0.02 (4.03)	0.62±0.02 (2.08)	0.56±0.004 (1.71)	0.38±0.01 (0.80)
8-h dark									
24-h light	0.73±0.02 (1.15)	0.58±0.01 (1.11)	1.21±0.05 (2.78)	2.07±0.03 (6.09)	2.19±0.02 (6.93)	1.83±0.02 (5.37)	1.08±0.03 (1.77)	1.09±0.20 (1.60)	0.72±0.03 (1.01)
24-h dark	0.68±0.02 (1.14)	0.35±0.002 (0.78)	0.18±0.002 (0.43)	0.17±0.01 (0.42)	0.20±0.01 (0.60)	0.16±0.01 (0.60)	0.17±0.01 (0.42)	0.11±0.01 (0.29)	0.08±0.000 (0.17)

Data were subjected to two-way factorial RBD analysis. CD (interaction) at 5% level=19.84586 for GI and 0.2086752 for anthocyanin concentration; CD (interaction) at 1% level=27.15229 for GI and 0.285509 for anthocyanin concentration

^z Each value represents average of three replicates

^y Standard deviation

^x mg/g fw; values in parentheses indicate concentrations on % dw basis

(1–13% w/v) under continuous illumination (Table 3). Data showed that the biomass increase in selected callus line was highest at 3% level of sucrose after 50 d of culture (GI=572.74). Sucrose levels less than 3.0% or more than 5.0% slowed down the callus growth up to Day 50. Cultures grown on medium with 1.0% sucrose indicated a further drastic fall in biomass in the next 20 d (GI=74.20 in comparison to GI=474.79 and 467.57 at 3.0% and 5.0% sucrose level, respectively). Sucrose concentration at >5.0% though supported marginal improvement in growth after Day 50 but it was again substantially less in comparison to cultures grown on 3.0% or 5.0% sucrose containing media. Pigment production, on the other hand, was found to be positively correlated with increasing sucrose concentration in the medium up to 7.0% after 50 d and up to 9.0% after Day 70 of growth. The highest anthocyanin content of 7.36% and 9.10% dw was recorded in tissues cultured on 7.0% and 9.0% sucrose for 50 and 70 d, respectively. When data on biomass gain and anthocyanin contents were extrapolated in terms of net pigment yield, it was found that cultures grown in presence of 3.0–7.0% sucrose supplementation had almost the same level of anthocyanin until Day 50 of the culture cycle. However, if the culture cycle was extended to 70 d, then cultures on medium with 7–9% sucrose were better yielders of the pigment.

Spectral properties of the pigment. The pigment from freshly harvested callus tissue upon extraction in acetone or HCl–MeOH followed by descending paper chromatography gave four discrete bands with Rf values 0.45, 0.39, 0.32, and 0.27 and gave two discrete bands with Rf values 0.40 and 0.73, respectively. The major purified fraction of acetone extract (Rf=0.39) gave an absorption maxima peak at 528.64 nm (Fig. 2), whereas the major (Rf=0.40) and minor (Rf=0.73) fractions of 0.1% HCl–MeOH extract had an absorption maxima at 528.99 and 536.51 nm, respectively. Though not structurally verified, spectral characteristics of the pigment produced by the selected callus line indicates the possibility of it being a “peonidine” type of anthocyanin structure.

Discussion

Plant cell and tissue-based production of anthocyanins and other natural colorants is now being viewed with renewed interest (Callebaut et al. 1990; Mori et al. 2001; Konczak-Islam et al. 2003; Yousef et al. 2004). According to Lila (2004), the special attributes of such alternate production methods include (a) quick and easy isolation; (b) lack of interference that is normally encountered at whole plant level due to co-extraction of pectin, polysaccharides, and

Table 3. Effect of sucrose on growth and anthocyanin concentration in the selected callus line of *P. sikkimensis*

Sucrose (% w/v)	Growth index		Anthocyanin concentration (mg/g fw)	
	50d	70d	50d	70d
1.0	254.89	74.20	0.16 [0.38] ^x	0.01 [0.02]
3.0 (control)	572.74	474.79	1.69	0.81
5.0	434.54	467.57	2.13	1.56
7.0	269.94	383.72	3.11	2.91
9.0	145.32	323.04	2.63	3.03
11.0	75.97	200.48	2.04	3.22
13.0	20.47	39.05	1.17	2.03
CD at 5%	1.502	1.979	0.125	0.256
1%	2.109	2.779	0.175	0.359

Values in parentheses denote transformed data on square root basis, to which CD and SE values are applicable

^z Values significant at 1% level

^y SE

^x anthocyanin concentration on% dw basis

^w Values significant at 5% level

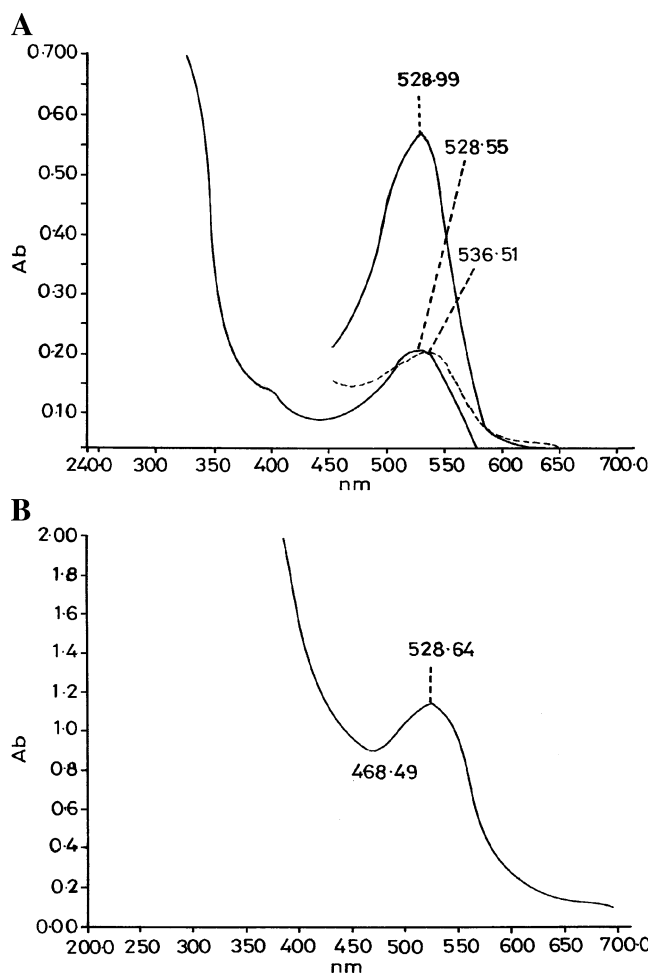


Figure 2. UV Absorption spectrum of methanol/HCl (A) and acetone (B) extracts of *P. sikkimensis* root callus cultures. (A) major (λ 528.99) and minor (λ 536.51) purified compounds in methanol/HCl extract obtained after descending paper chromatography and the total extract (λ 528.55); (B) major purified compound in the acetone extract (λ 528.64).

other enzymes; (c) amenability for biotic or abiotic elicitation to provoke higher accumulation than in intact plant organs; and (d) a possibility of bio-labeling to trace post-ingestion metabolism in the human body. Callus and cell suspension cultures of a large number of plant species have now been demonstrated to accumulate anthocyanin *in vitro* (Cállebáut et al. 1990; Shuler et al. 1990; Cormier and Do 1993; Zubko et al. 1993; Konczak-Islam et al. 2000, 2003; Hirner et al. 2001). The highest anthocyanin content reported in cultured cells was 13% dw in *Vitis* (Yamakawa et al. 1983).

The anthocyanin-producing callus line selected in the present study is the first among the *Panax* species. The growth and production characteristics of the selected line of *P. sikkimensis* match well with many previously reported pigment-accumulating cell cultures of other plant species. For example, pigment accumulation in the callus line

selected in our study was concurrent with a depression in callus growth as was the case in cell lines of *Ajuga reptans*, *Vitis vinifera*, and *Ipomaea batatus* (Cállebáut et al. 1990; Cormier and Do 1993; Konczak-Islam et al. 2000). Since anthocyanin accumulation is a cytodifferentiation process, it has been shown to be strongly influenced by culture environment and medium variables. In this respect, *P. sikkimensis* line has shown an age-dependent influence of various auxins. While NAA supplementation in the medium brought about an early peak of pigment accumulation in about 40 d of growth, it was delayed by 20 d when 2,4-D was used as an auxin in the medium. Supplementation of 2,4-D was, however, more critical for cell proliferation and biomass accumulation and hence the net pigment yield per unit time. Auxins have been reported to favor anthocyanin synthesis and can substitute for light in such cultures (Kobayashi et al. 1993; Kandil et al. 2000; Konczak-Islam et al. 2000, 2003; Mori et al. 2001). Though growth of the selected line of *P. sikkimensis* was marginally affected under three different photoperiod conditions tested, continuous irradiation significantly favored more pigment synthesis throughout the culture passage up to 90 d. Dark incubation of the selected line consistently accounted for poor anthocyanin accumulation (5–12-folds less than under 24-h light or 16-h light/8-h dark conditions) as was shown in cell lines selected in *Haplopappus*, *Hibiscus*, strawberry, *Populus*, cranberry, and *Ajuga* (Cállebáut et al. 1990; Cormier and Do 1993). The callus line of *I. batatus* was, however, found to accumulate anthocyanins under dark incubation condition (Konczak-Islam et al. 2000).

The role of sugars in promoting pigment accumulation in cultured cells and tissues has been well documented (Cormier et al. 1990; Payne et al. 1992; Vitrac et al. 2000; Hirner et al. 2001). Besides their role as an energy source and structural components, sugars affect cytodifferentiation via their influence as a cytoplasmic and/or vacuolar osmoticum in the cells and as physiological signals in the global expression of pathway genes (Lila 2004). Our finding on the positive effect of high concentrations of sucrose on anthocyanin production in the selected line are in agreement with previous reports on *Vitis* (Cormier et al. 1990; Vitrac et al. 2000) and *Ajuga* (Cállebáut et al. 1990) where a 12–15-fold increase in pigment accumulation was observed when sucrose was present at higher levels during the active growth phase. Sucrose concentrations between 3.0% and 7.0% (w/v) were found optimal for growth and pigment production in *P. sikkimensis* cultures but sucrose levels higher than 5.0% did not support growth during the initial period of the culture cycle probably due to time required for metabolic adjustments to high osmotic stress in the medium. Negative effect of high sucrose level (>9.0%) on growth and anthocyanin yield due to non-supportive osmotic status of the media was

also observed by Meyer and Van Staden (1995) for *Oxalis linearis* and Suzuki (1995) for grape cell lines.

The selection of high anthocyanin yielding (2.0–3.0 mg/g fw; 6.0–8.0% dw) callus line of *P. sikkimensis* is a valuable addition to the genus *Panax* whose species are otherwise valued for their immunomodulatory saponins present in the roots and cultured cells. The presence of natural pigment in the cells without compromising the content of characteristic ginsenosides may be exploited in ginseng-based candies, cakes, pastries, and health drinks. The selected line has stably maintained its growth and anthocyanin productivity for more than 6 yr now. Efforts to develop co-extraction methods to isolate pigment and ginsenosides from the same tissue are underway.

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