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Research Article

In vitro propagation of ceylon spinach (*Basella rubra* L.)

Tanikan Pumchaosuan* and Sasitorn Wongroung

Department of Biotechnology, Faculty of Agro-Industry, Chiang Mai University, Chiang Mai 50100 Thailand.

*Author to whom correspondence should be addressed, email: tanikan_bio107@hotmail.com

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Abstract

The pigments found in plants play important roles in plant metabolism and visual attraction in nature. Anthocyanins, a major group of plant pigments, have been revealed to have strong antioxidant activity with potential use as chemotherapeutics. In this report, *in vitro* culture of Ceylon spinach (*Basella rubra* L.) was preliminarily studied for anthocyanin production. Two types of explants including stem and leaf were used for callus induction. The method for sterilization and concentration of plant growth regulators were optimized. It was found that sterilization by 7% (v/v) Clorox for 15 min and placed on Murashige and Skoog (MS) medium containing 0.1 μML^{-1} 2,4-D and 5 $\mu M\ L^{-1}$ BA gave a result of 100% callus production from the stem type explant. The callus was also subjected to cell culture study. The effect of sucrose concentration on cell culture of Ceylon spinach was investigated and it was found that 0.5070 g $L^{-1}d^{-1}$ growth rate can be obtained at 3% sucrose. Anthocyanin production of the callus was induced by exposing Ceylon spinach callus to UV light for 0, 5, 10, 20 and 30 min and culturing on LS medium with 2,4-D and BA at 3 $\mu M\ L^{-1}$ for 2 weeks. The result showed that callus treated with UV for 20 min and 30 min produced anthocyanin at 1.293 and 1.157 mg /100 g. fresh cell weight respectively, whereas anthocyanin from the control callus was found at 1.001 mg/100 g. fresh cell weight.

Keywords: anthocyanin, plants, tissue culture, Thailand

Introduction

Increasing attention is being paid by consumers to the health and nutrition aspects such as vitamin content, mineral elements and antioxidants of horticultural products [1](Scalzo *et* *al*., 2005). Numerous types of fruit and vegetables contain significant levels of biologically active components that impart health benefits beyond basic nutrients [2] (Oomah and Mazza, 2000). Ceylon spinach (*Basella rubra* L.) ise known for being rich in β-carotene and vitamin A [3](Eliana et. *al*., 2007). Interest in the role of antioxidants in human health has promoted research in the field of horticulture and food science to evaluate fruit and vegetable antioxidants and to determine how their content and activity can be maintained or even improved [4](Ayala-Zavala et. al., 2004).

This preliminary research explores the potential for metabolite production from callus. The objectives of this study were to propagate Ceylon spinach (*B. rubra* L.) under *in vitro* conditions, to study the effect of sucrose on cell suspension culture of Ceylon spinach and to study the effect of UV on anthocyanin production.

Materials and Methods

Induction and culture of callus

Basell rubra L. was procured from the Study Centre for Sufficiency Economy, Chiang Mai, Thailand. Leaf and stem of *B. rubra* L. were rinsed in 70% (v/v) ethanol for 1 second, sterilized by immersion for 15 minutes in 7% (v/v) sodium hypochlorite solution containing Tween 80, and rinsed three times in sterilized distilled water. The sterilized leaves (5 x 5 mm.) and stems 2 – 3 mm in thinness were cut into pieces or blocks and placed on Murashige and Skoog medium (MS medium) supplemented with 3% sucrose, various concentration (0, 0.1, 0.5, 1, 2, 3, 4 and 5μM L^{-1}) of 2,4-dichlorophenoxyacetic acid (2,4-D) and BA. The medium was solidified by 0.7 – 0.8% agar. The cultures were incubated in 16 hr. light and 8 hr. dark at 25^{o}C under cool white fluorescent light and regeneration was observed.

Effect of sucrose concentration on cell suspension

Fourteen-day-old callus was transfer to 25 ml. of MS medium containing different concentrations 3%, 6% and 9% (w/v) of carbon source to induce the anthocyanin production in 125-ml. flask. They were incubated on a reciprocal shaker (150 rpm.) in 16 hr. light and 8 hr. dark at 25^{o}C under cool white fluorescent light. Cell growth was estimated by measurement of fresh cell weight. Cells were separated from the culture medium by vacuum filtration and weighed as a fresh cell weight (fcw).

Measurement of anthocyanin content

Anthocyanin was extracted overnight at 4^{o}C from 1 g. of fresh cells with 10 ml. of ethanolic solution containing 0.1% HCL. After removing the cell debris by centrifugation, absorbance at 535 nm of the clear supernatant was measured and anthocyanin content was calculated [5](Pasqua *et al*., 2005)

Effect of UV on anthocyanin production in tissue culture

Treatments consisted of callus placed on the LS medium containing 0.6 μML^{-1} 2,4-D and BA, the sample without light treatment was used as a control. Callus was subjected to 4 UV illumination durations (5, 10, 20 and 30 min). After UV illumination, control and illuminated callus were stored for 16 hr. light and 8 hr. dark at 25^{o}C under cool white fluorescent light. Samples were taken after 14 day incubation.

Results and Discussion

Callus induction

Stem of Ceylon spinach was cultured on MS medium supplemented with various concentrations of 2,4-D in combination with BA. Within 4 weeks of inoculation in media supplemented with 0.1 µM L^{-1} 2,4-D in combination with 5 µM L^{-1} BA, stem explants formed 100% callus (Table 1). Morphological variation of the induced callus was observed and they were categorized as friable callus, red compact and green compact (Figure. 1). The highest dry weight of callus (125.50 ± 7.85) was recorded at MS media supplemented with 0.1 µM L^{-1} 2,4-D combination with 5 µM L^{-1} BA.

Figure 1. Characteristic of Callus initiation from stem explants culture on MS medium with 2, 4-D and BA.

(A.) Friable callus (0.1 μML^{-1} 2,4-D + 5 μML^{-1} BA.)
(B.) Red compact (0.1 μML^{-1} 2,4-D + 3 μML^{-1} BA.)
(C.) Green compact (0.5 μML^{-1} 2,4-D + 1 μML^{-1} BA.)

Table 1. Callusing (%) and dry weight (mg.) of Ceylon spinach on MS media containing 2, 4-D in combination with BA.

2,4-D (μML^{-1}) / BA (μML^{-1})	0	0.1	0.5	1	2	3	4	5
0	95 / 39.50	95 / 35.00	95 / 30	100 / 40	100 / 45	100 / 65	100 / 77.50	100 / 82.75
0.1	100 / 85.25	100 / 95	100 / 85.25	100 / 100	100 / 90	100 / 98.25	100 / 100	100 / 125.50
0.5	85 / 60	85 / 63	80 / 46.25	80 / 65	80 / 55	80 / 37.50	80 / 65	80 / 47.50
1	72 / 35	72 / 47.50	72 / 30	60 / 30	50 / 55.25	50 / 51.75	50 / 50.25	50 / 47.25
2	25 / 50.50	0 / 53.50	0 / 40.75	0 / 48.25	0 / 51.50	0 / 51.25	0 / 40.75	0 / 35.75
3	0 / 56.25	0 / 60.50	0 / 58.75	0 / 62.75	0 / 57.50	0 / 55.50	0 / 60.25	0 / 50.25
4	0 / 56.25	0 / 63.75	0 / 56.75	0 / 47.50	0 / 54.75	0 / 46.50	0 / 53.75	0 / 65.25
5	0 / 51	0 / 55	0 / 65	0 / 46.75	0 / 45	0 / 51.25	0 / 49.75	0 / 60

Effect of sucrose concentration on cell suspension

Cell-suspensions in 3, 6 and 9% sucrose were compared as shown in Figure. 2. The log phase of cell-suspension was 6 to 10 days after inoculation at 3% and 6% sucrose medium respectively. Cell-suspension in 9% sucrose was slow-growing during the first 9 days of inoculation. Growth rate of cell-suspension is shown in Table 2. Plant cell, tissue or organ culture normally requires the incorporation of a carbon source to the culture medium [6](Vespasiano and Wanger, 2003) and each species requires different concentrations. There are many reports on the effect of sucrose concentration on cell suspension for metabolite production. For example, *Morinda elliptica* (Rubiaceae) cell suspension culture was established for the production of anthraquinones on MS medium and it was found that

8% (w/v) sucrose was the best condition for enhancing both cell growth and anthraquinone [7](Abdullah *et. al*., 1998).

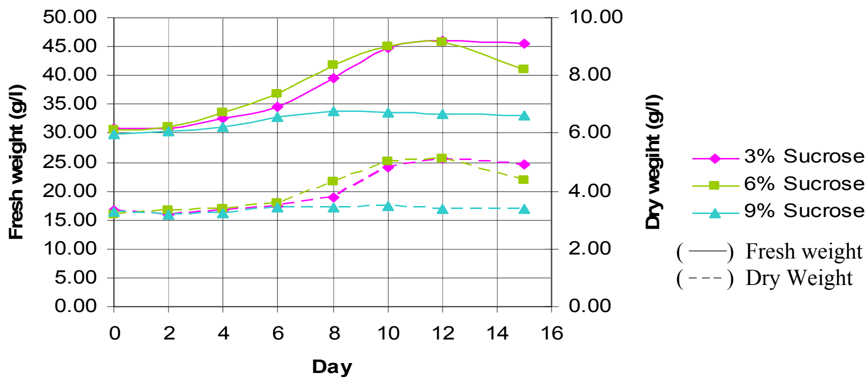

Figure 2. Growth rate of cell suspension on MS medium containing 3, 6 and 9% (w/v) sucrose.

Table 2. Effect of sucrose concentration (3, 6 and 9% w/v) of Ceylon spinach on MS medium.

Sucrose concentration (%w/v)	Growth rate ($gL^{-1}d^{-1}$)
3	0.5070
6	0.4056
9	0.0546

Measurement of anthocyanin content

Measurement of anthocyanin content was compared between 3 types of explant and callus for *Basella alba* L. and *Basella rubra* L. (Table 3.)

Table 3. Anthocyanin content of *Basella alba* L. and *Basella rubra* L.

Type of explant	Anthocyanin content (mg/100 g. Fresh weight)	
	***Basella alba* L.**	***Basella rubra* L.**
Leaf	6.06	6.30
Stem	1.57	2.25
Fruit	72.12	79.63
Callus	0.14	0.98

Effect of UV on anthocyanin production in tissue culture

The results in this study showed that UV illumination effects anthocyanin production in the callus of Ceylon spinach. Callus treated by UV for 20 and 30 min produced anthocyanin at 1.293 and 1.157 mg./100 g. fresh cell weight respectively (Table 4). Similar findings concerning the relationship between anthocyanin production and UV treatment were described by Mustafa *et. al*., [8](2008). The report concluded that UV illumination was the best condition for enhancing anthocyanin accumulation in strawberry.

Table 4. Effect of UV on anthocyanin production in callus of Ceylon spinach after 14 day incubation.

UV illumination (min)	Anthocyanin content (mg./ 100 g. fresh weight)
0	1.001
5	0.953
10	0.856
20	1.293
30	1.157

Conclusions

Stems of Ceylon spinach gave the highest callusing on MS medium supplemented with 0.1 µM L^{-1} 2,4-D and 5 µM L^{-1} BA. Cell-suspension in 3% sucrose medium produced a maximum of fresh weight and dry weight after 15 days. Anthocyanin content varied in different types of explant. Callus treated with UV illumination effected anthocyanin production. For this experiment, the highest anthocyanin production was obtained in calluses treated with 20 min. UV- illumination.

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