Establishment of callus and cell suspension culture of *Scrophularia striata* Boiss.: an in vitro approach for acteoside production

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Abstract In the present study, a protocol was optimized for establishment of callus and cell suspension culture of *Scrophularia striata* Boiss. as a strategy to obtain an in vitro acteoside producing cell line for the first time. The effects of growth regulators were analyzed to optimize the biomass growth and acteoside production. The stem explant of *S. striata* was optimum for callus induction. Modified Murashige and Skoog medium supplemented with 0.5 mg/l naphthalene acetic acid + 2.0 mg/l benzyl adenine was the most favorable medium for callus formation with the highest induction rate (100 %), the best callus growth and the highest acteoside content (1.6 µg/g fresh weight). Incompact and rapid growing suspension cells were established in the liquid medium supplemented with 0.5 mg/l naphthalene acetic acid + 2.0 mg/l benzyl adenine. The optimum time of subculture was found to 17–20 days. Acteoside content in the cell suspension was high during exponential growth phase and decreased subsequently at the stationary phase. The maximum content of acteoside (about 14.25 µg/g cell fresh weight) was observed on

the 17th day of the cultivation cycle. This study provided an efficient way to further regulation of phenylethanoid glycoside biosynthesis and production of valuable acteoside, a phenylethanoid glycoside, on scale-up in *S. striata* cell suspension culture.

Keywords Acteoside · Callus · Cell suspension culture · Phenylethanoid glycoside · *Scrophularia striata* Boiss.

Introduction

Scrophularia striata Boiss. (Scrophulariaceae) is one of the native plants in Iran (Grau 1981). It is a traditional medicinal plant with the local name Tashne Dari and has been reported to have many pharmaceutical effects such as analgesic (Sofiabadi et al. 2012), anticancer (Arde-shiry Lajimi et al. 2010), antimicrobial (Bahrami and Valadi 2010), nephroprotective (Zaheri et al. 2011) and nitric oxide suppressive (Azadmehr et al. 2009) properties. Additionally, ethanolic extract of *S. striata* was shown to have inhibitory effect against matrix metalloproteinase (MMP) (Hajiaghvae et al. 2007).

The presence of phenylethanoid glycoside (PeG) acteoside in the aerial parts of *S. striata* has been reported (Monsef-Esfahani et al. 2010). Acteoside (Fig. 1) is a well-studied PeG that consists of several chemical groups, including caffeic acid, 3,4-dihydroxyphenylethanol, glucose, and rhamnose (Hwang et al. 2010). It has been reported that acteoside has extensive biological

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Fig. 1 Acteoside structure (Pereira et al. 2008)

activities including antioxidant (Chiou et al. 2004; He et al. 2000), anti-inflammatory (Lee et al. 2005, 2006), hepatoprotective (Xiong et al. 1999; Zhao et al. 2009), cell apoptosis regulation activities (Ohno et al. 2002; Xiong et al. 1999; Yang and Pu 2006) and cytotoxicity against various tumor cells (Kunvari et al. 1999; Lee et al. 2007). It may be developed into a promising medication (He et al. 2011). Acteoside is generally contained only at low amount in plants (Imakura et al. 1985; Kitagawa et al. 1988).

In the search for alternatives to produce desirable medicinal compounds from plants, biotechnological approaches, specifically, plant tissue cultures, are found to have potential as a supplement to traditional agriculture in the industrial production of bioactive plant metabolites (Giri and Narasu 2000; Ramachandra and Ravishankar 2002). Plants and cultured cells metabolize foreign compounds in qualitatively similar ways (Hellwig et al. 2004), and there are successful examples of tissue cultures for several medicinal plants where an increased content of the bioactive secondary metabolites has been achieved compared to that from wild plants (Estrada-Zúñiga et al. 2009). In vitro cultures represent a potential source for producing phenylpropanoids instead of using wild plants (Estrada-Zúñiga et al. 2009). Taking into account the increasing demand for these products, plant cell culture provides an attractive alternative source that can overcome the limitations of extracting useful metabolites from limited natural resources. Currently, in vitro culture is widely being employed as a model system to investigate the production of specific secondary metabolites as it offers experimental advantages to both basic and applied research and to the development of models with scale-up potential

(Buitelaar and Trapner 1992; Chang and Sim 1995; Mukherjee et al. 2000).

Among the *Scrophularia* species, generation of callus has been described only in *S. nodosa* (Sesterhenn et al. 2007) and there is no information available with respect to *S. striata*. Realizing the importance of *S. striata* and its extract in medicine, we attempted to apply this strategy to establish callus and cell suspension cultures of *S. striata* acting as a source for the production of valuable secondary metabolites. In the present paper, the initiation of callus and cell suspension culture of *S. striata* and their characteristics are described. This provides a tool for investigation on PeGs biosynthesis pathway and its up-regulation in the future.

Materials and methods

Plant material

Scrophularia striata Boiss. plants were collected in July 2008 from Aseman-abad region, Jan Jan village, Ilam, Iran. The plant material was identified by Dr. Shahrokh Kazempour and a voucher specimen was deposited at the Herbarium of Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran.

Seedling obtainment

Scrophularia striata seeds were washed thoroughly under running tap water for 5 min while few drops of mild detergent were added and followed by dipping in 70 % (v/v) ethanol for 70 s. Seeds were put into 30 % (v/v) H₂O₂ for 10 min and rinsed three times in sterile

distilled water under laminar flow cabinet then immersed in an antiseptic solution (2 % sodium hypochlorite) for 15 min. Seeds were rinsed with autoclaved distilled water for three times (5 min each) and transferred into 70 % (v/v) ethanol solution for 70 s rinsed with sterile distilled water 3 times. Sterilized seeds of *S. striata* were treated overnight with gibberellic acid (GA3) (750 mg/l) at 4 °C. For germination, sterile seeds were transferred to solid free hormone MS media (Murashige and Skoog 1962) and incubated in darkness for 3 days at 25 °C. Then they were transferred to a phytotron (16 h light/8 h dark photoperiod, at 25 °C). Using this method, seed germination increased to 82 %.

Callus induction

To induce callus formation, 1–2 cm pieces of stem and leaf explants lacking nodules were isolated from 15 day-old seedlings. The explants were placed on MS medium containing 3 % (w/v) sucrose, 0.7 % (w/v) agar supplemented with different levels of naphthalene acetic acid (NAA) (0.0, 0.25, 0.5, 2.0 and 4.0 mg/l) and of benzyl adenine (BA) (0.0, 0.25, 0.5, 2.0 mg/l) and combinations of them, pH 5.8. They were incubated in darkness at 25 °C. For each treatment, 3 explants were placed onto Petri dishes (7 cm diameter) as one replicate, with 3 replicates performed and experiments were repeated twice independently. The calli were emerged from the explants and were subcultured every 2 weeks on MS medium with the same plant growth regulators supplementation. After 1 month of explants inoculation, data were recorded including callus induction rate (%) (Zeng et al. 2009), growth score (Hazeena and Sulekha 2008), callus index (CI) (Hazeena and Sulekha 2008), callus fresh weight and callus status. In addition, relative growth rate of callus (RGR) (Mini and Sankaranarayanan 2013) and acteoside content were measured after 2 months of explants inoculation. Optimum callus culture media was determined according to CI, RGR and acteoside content of the calli.

Cell suspension culture

Cell suspension culture was established by transferring 3 g of white and friable callus into 250 ml flasks containing 100 ml of fresh MS liquid medium supplemented with NAA (0.5 mg/l), BA

(2.0 mg/l) and sucrose (30 g/l) lacking agar, pH 5.8. The suspension cultures were subcultured in the MS liquid medium at 17–20 day-intervals agitated on a rotary shaker (110 rpm, 25 °C) and kept in darkness.

Characterization of cell suspension culture

The growth measurement of the cells was performed to determine the best period for the cell suspension to be used. For the preparation of cell suspension culture stocks, 3 g of the established callus (i.e., 15 day-old), was added into 100 ml of fresh medium in 250 ml Erlenmeyer flasks and subcultured every 17–20 days. The suspension cultures were shaken at 110 rpm, maintained at 25 °C in darkness. After 2 months cells were homogenized. For determination of cell suspension growth curve, the stock cells were separated from the medium by filtration under suction. Then 1 ± 0.05 g fresh cells were inoculated into 50 ml of fresh liquid medium in a 100 ml Erlenmeyer flask. Growth of cell suspension culture, cell viability and acteoside content were monitored with sets of flasks harvested at 2 day-intervals from the day of subculture (day 0) up to 29 days. Readings were taken from three flasks for each parameter. Cells were separated from the medium by filtration using nylon mesh and weighed as fresh weight.

Cell viability

Cells viability was determined by the Evan's blue staining test (Rodríguez-Monroy and Galindo 1999). Two ml samples from each flask were incubated in 0.25 % Evan's blue stain for 5 min and then at least 500 cells were counted, and this was repeated twice ($n = 6$).

Acteoside determination

Acteoside was extracted using 2 g of callus by 90 % (v/v) methanol aqueous solution (10 ml) at room temperature. The homogenate was centrifuged at 5,000 rpm and supernatant was collected, air-dried and re-dissolved in 500 μ l methanol. Then centrifuged at 13,000 rpm prior to HPLC analysis. The isolated acteoside was analyzed by HPLC using a (Knauer, Berlin, Germany) HPLC system with a C-18 column (Perfectsil Target

Table 1 Effect of different levels of NAA and BA on *S. striata* stem explants in MS medium

NAA (mg/l)	BA (mg/l)	1st month					2nd month		
		Callus induction rate (%)	Growth score	Callus index (CI)	Fresh weight (g)	Callus status	relative growth rate of callus (g/day)	Fresh weight (g)	Callus status
0	0	0	1	0	0.00 ^h	–	0	0.00 ^h	–
	0.25	0	1	0	0.00 ^h	–	0	0.00 ^h	–
	0.5	0	1	0	0.00 ^h	–	0	0.00 ^h	–
	2	0	1	0	0.00 ^h	–	0	0.00 ^h	–
0.25	0	–	–	–	–	Organogenetic	–	–	Organogenetic
	0.25	100	3.66	366	0.20 ± 0.04 ^a	Yellowish	0.05	1.55 ± 0.09 ^{ab}	Clear
	0.5	100	3.72	372	0.14 ± 0.02 ^{bcd}	Slightly opaque	0.05	1.54 ± 0.29 ^{ab}	Obvious and clear
	2	100	4	400	0.13 ± 0.02 ^{cd}	Clear	0.05	1.48 ± 0.06 ^{abc}	Yellowish
0.5	0	–	–	–	–	Organogenetic	–	–	Organogenetic
	0.25	100	3.66	366	0.09 ± 0.01 ^{efg}	Slightly opaque	0.04	1.33 ± 0.09 ^{de}	Obvious and clear
	0.5	100	3.55	355	0.17 ± 0.01 ^{ab}	Clear	0.04	1.29 ± 0.14 ^c	Fragile and clear
	2	100	4	400	0.18 ± 0.02 ^{ab}	Yellowish	0.05	1.62 ± 0.09 ^a	Obvious and clear
2	0	–	–	–	–	Organogenetic	–	–	Organogenetic
	0.25	100	3	300	0.16 ± 0.06 ^{bc}	Clear	0.04	1.46 ± 0.12 ^{bcd}	Fragile and clear
	0.5	100	4	400	0.12 ± 0.02 ^{de}	Yellowish	0.04	1.37 ± 0.08 ^{cde}	Yellowish
	2	100	4	400	0.17 ± 0.01 ^{ab}	Yellowish	0.04	1.35 ± 0.15 ^{cde}	Yellowish
4	0	–	–	–	–	Organogenetic	–	–	Organogenetic
	0.25	100	3.33	333	0.06 ± 0.02 ^g	Clear	0.02	0.52 ± 0.13 ^g	Fragile and clear
	0.5	100	3.77	377	0.09 ± 0.02 ^{fg}	Clear	0.02	0.73 ± 0.14 ^f	Clear
	2	100	3.44	344	0.12 ± 0.00 ^{def}	Watery and clear	0.02	0.84 ± 0.04 ^f	Obvious and clear

Data represent mean ± SD, n = 6; with each different levels of NAA and BA as a treatment. Means sharing the same letter in each column do not differ significantly at $P \leq 0.05$ (Duncan's test)

ODS-3 (5 µm), 250 × 4.6 mm) MZ-Analysentechnik, Mainz, Germany). Mobile phases were 0.4 % (v/v) acetic acid aqueous solution (A) and pure methanol (B). The applied gradient was as follows: 35 % (B)-2 min-39 % (B)-28 min-60 % (B)-5 min-35 % (B). The flow rate was 1 ml/min and during the last 5 min it was 1.5 ml/min (this method was set up by the authors and data are under publication). Chromatography was performed at 25 °C. Components were identified at 333 nm using a UV detector (PDA, Malvern Instruments, Herrenberg, Germany). Acteoside standard was purchased from Sigma-Aldrich (Seelze, Germany). Identification of acteoside was performed by comparison of

retention times and UV spectral peaks of the sample with authentic standard. Quantitative estimation of acteoside was done based on the peak area of specific concentrations of the sample and the standard.

Statistical analysis

All data were analyzed by analysis of variance (ANOVA) using MSTAT-C software. Duncan's multiple range tests were used to measure statistical differences between treatment methods and controls. $P \leq 0.05$ was considered significantly.

Table 2 Effect of different levels of NAA and BA on *S. striata* leaf explants in MS medium

NAA (mg/l)	BA (mg/l)	1st month					2nd month		
		Callus induction rate (%)	Growth score	Callus index (CI)	Fresh weight (g)	Callus status	relative growth rate of callus (g/ day)	Fresh weight (g)	Callus status
0	0	0	1	0	0.00 ^d	–	0	0.00 ^g	–
	0.25	0	1	0	0.00 ^d	–	0	0.00 ^g	–
	0.5	0	1	0	0.00 ^d	–	0	0.00 ^g	–
	2	0	1	0	0.00 ^d	–	0	0.00 ^g	–
0.25	0	–	–	–	–	Organogenetic	–	–	Organogenetic
	0.25	100	2.22	222	0.16 ± 0.02 ^b	Yellowish	0.02	0.77 ± 0.01 ^f	Compact and clear
	0.5	100	1.33	133	0.09 ± 0.02 ^c	Clear	0.04	1.40 ± 0.15 ^c	Clear
	2	100	2.11	211	0.27 ± 0.03 ^a	Clear	0.03	1.29 ± 0.11 ^{cd}	Fragile and clear
0.5	0	–	–	–	–	Organogenetic	–	–	Organogenetic
	0.25	100	2	200	0.14 ± 0.01 ^{bc}	Opaque	0.04	1.32 ± 0.10 ^{cd}	Clear
	0.5	100	1.44	144	0.14 ± 0.01 ^{bc}	Opaque	0.05	1.54 ± 0.27 ^b	Clear
	2	33	1.11	36.63	0.15 ± 0.01 ^c	Opaque	0.04	1.22 ± 0.01 ^d	Clear
2	0	–	–	–	–	Organogenetic	–	–	Organogenetic
	0.25	100	2	200	0.09 ± 0.02 ^c	Opaque	0.02	0.80 ± 0.04 ^{ef}	Compact and clear
	0.5	100	2	200	0.09 ± 0.02 ^c	Watery and opaque	0.02	0.67 ± 0.01 ^f	Clear
	2	100	4	400	0.27 ± 0.02 ^a	Yellowish	0.06	2.09 ± 0.10 ^a	Compact and clear
4	0	–	–	–	–	Organogenetic	–	–	Organogenetic
	0.25	55	2	110	0.14 ± 0.02 ^{bc}	Opaque	0.02	0.80 ± 0.11 ^f	Opaque
	0.5	100	2	200	0.18 ± 0.02 ^b	Opaque	0.04	1.37 ± 0.02 ^c	Opaque
	2	66	2	132	0.12 ± 0.02 ^{bc}	Opaque	0.03	0.94 ± 0.01 ^e	Opaque

Data represent mean ± SD, n = 6; with each different levels of NAA and BA as a treatment. Means sharing the same letter in each column do not differ significantly at $P \leq 0.05$ (Duncan's test)

Results and discussion

Callus induction

Many studies have investigated the effects of plant growth regulators on callus growth of different plant species. In the present study, to determine the optimum level of plant growth regulators in *S. striata*, different concentrations of NAA and BA (mg/l) were used in MS medium for callus induction in stem and leaf explants (Table 1 and 2). Callogenesis was observed in both types of the explants. Similar results were reported in callus induction of *Corydalis saxicola* (Cheng et al. 2006) and *Orthosiphon stamineus* (Lee and Chan

2004), in which callus could be induced successfully from leaf and stem explants.

We found that calli were successfully generated in all combinations of NAA and BA (Tables 1, 2). Similar results were supported by other investigators (Duangporn and Siripong 2009; Nabi et al. 2002; Tanveer et al. 2012), they found that the highest percentage of callus cultures derived from explants grown on NAA and BA combinations than NAA or BA alone. Ahmad and Spoor (1999) have shown that both NAA and BA were necessary for good callus initiation and callus growth. Also, Nurazah et al. (2009) reported that NAA and BA at various concentrations led to a faster growth of callus in *Cananga odorata*. No callus was induced on basal

Fig. 2 Establishment of *Scrophularia striata* callus and cell suspension culture. **a** callus from stem explants 1 month after inoculation, **b** callus from stem explants 2 months after inoculation, **c** *S. striata* callus in optimized medium, **d** *S. striata*

suspension culture grown in flask, **e** cell suspension culture showing viable cells (v) and non viable cells (nv) ($\times 40$), **f** photomicrograph of round (r) and elongated (e) shape cells ($\times 40$)

MS medium. Many studies found that explants cultured on MS medium without hormones did not produce any callus (Mathur and Shekhawat 2013; Ray et al. 2011). Our results indicated that exogenous hormone was essential to callus formation of *S. striata*. Among the explants used, stem was more responsive for callus induction than leaf explants. The stem explants swelled up and calli started growing from the cut surfaces. Calli were successfully generated at all combinations of NAA and BA 3–7 days after inoculation. In this treatments, callus induction was 100 % and callus index was over 300 except in samples that had no hormone in their media as well as those samples in which organogenesis occurred (Table 1). The exclusive presence of BA in the medium, regardless of its concentration, was less satisfactory for callus initiation. According to Nurazah et al. (2009), no callus induction was obtained without the addition of NAA. This phenomenon suggests that NAA plays a more important role in callus formation from stem explants than BA. Two months after stem explants inoculation, the highest

callus fresh weight was achieved in the media containing 0.25 mg/l NAA + (0.25, 0.5, 2.0) mg/l BA and 0.5 mg/l NAA + 2.0 mg/l BA (callus RGR, 0.05 g/day). In these explants, better callus texture, color and total fresh weight were obtained when MS medium was supplemented with 0.5 mg/l NAA + 2.0 mg/l BA (Table 1; Fig. 2a, b). Ray et al. (2011) reported on *Solanum melongena* stem explants that the percentage of callus induction and callus weight was highest on MS medium containing 0.5 mg/l NAA + 2.0 mg/l BA. Also, the best callus induction was observed when leaf segments of *Solanum nigrum* were cultured on MS medium supplemented with 0.5 mg/l NAA + 2.0 mg/l BA (Jahan and Hadiuzzaman 1996). Increasing NAA levels caused calli become gelatinous and brownish and overall fresh weight reduction was noticed. In addition, organogenesis occurred in samples that had just NAA in the medium.

In leaves explants, callus induction was very slow and occurred after 10–20 days. The rate of callus induction varied depending on the combination of

applied plant growth regulators. Callus initiation was observed to develop from margins of leaf explants and petiole. During the time, NAA alone induced root formation while callogenesis was absent for explants grown on medium containing only BA. The calli observed with higher BA and NAA concentrations were brown and exhibited excessive necrosis, indicating toxic effects. The darkening of callus was probably due to the production and oxidation of phenolic compounds released by explants (Monacelli et al. 1995). MS medium supplemented with 2.0 mg/l NAA + 2.0 mg/l BA was the most effective, providing callus for 100 % of leaf explants associated with the highest CI (400), callus fresh weight (2.09 g) and RGR of callus (0.06 g/day) (Table 2). Explants grown in this medium for 2 weeks formed callus at margins. Ahmad and Spoor (1999) also indicated that when auxin and cytokinin were in balance (i.e. NAA/BPA = 1) callus growth was enhanced.

A plant growth regulator is a key factor responsible for callus initiation and development in plant cell cultures (Cheng et al. 2006). However, optimal concentration of these compounds may depend on many factors, such as genotype of the original plant, explants origin, peculiarities of the strain etc. (Mathur and Shekhawat 2013). In addition, difference between stem and leaf explants response to NAA and BA concentrations in the medium could be a reflection of probable differences in endogenous growth regulator levels in the explant sources or different tissue sensitivities to these plant growth regulators (Lisowska and Wysokinska 2000).

It was observed that in these treatments the stem callus produced were clear and friable unlike the leaf callus which presented compact.

Calli acteoside content

Acteoside production has been reported in callus cultures (Estrada-Zúñiga et al. 2009; Inagaki et al. 1991; Shoyama et al. 1986). In the present study, trace amount of acteoside was detected in the calli formed on the applied NAA and BA concentrations (data are not shown) except in stem calli subjected to 0.5 mg/l NAA + 2.0 mg/l BA when it reached up to 0.0016 mg/g callus fresh weight. El-Mawla et al. (2005) reported that acteoside concentration was 0.0076 mg/g callus fresh weight in *Barleria cristata*. In addition, acteoside content in different species calli was reported to be between 0.63 and 6.33 % of dry

Fig. 3 Growth curve of suspension-cultured *S. striata* cells during 29 days of incubation in MS medium containing 0.5 mg/l NAA + 2.0 mg/l BA and acteoside content during different growth stages

weight (Inagaki et al. 1991). This may be explained by complexity of differential expression of secondary metabolism genes, physiological states and ability of precursor synthesis in different plant tissues (Zeng et al. 2009). We found that using 0.5 mg/l NAA + 2.0 mg/l BA is more compatible with acteoside accumulation in calli. Increase in the PeG contents in the callus may be due to the stimulation of PeG biosynthesis by plant growth regulators (Ouyang et al. 2003). Auxin and cytokinin in the medium may control the division and differentiation of the callus and cytokinins can stimulate the rate of protein synthesis and inhibit cell enlargement, thereby affecting the balance between cell division, cell expansion and secondary metabolite formation (Sahai and Shuler 1984).

Stem explant of *S. striata* have a great callogenesis potential for acteoside production, however the response is highly sensitive and directly related to the combinations of exogenous growth regulators in the culture medium. Therefore, the homogenous calli selected from cultures were maintained in MS medium containing 0.5 mg/l NAA + 2.0 mg/l BA in dark condition for a long repeated subculture process. Development of fast-growing homogeneous callus was achieved after about 1 year.

Cell suspension culture

Friable and clear calli clumps (Fig. 2c) which had been grown on 0.5 mg/l NAA + 2.0 mg/l BA were used for initiating a cell suspension culture (Fig. 2d). Cell growth was demonstrated in liquid MS medium

Fig. 4 Cell viability of suspension-cultured *S. striata* cells during 29 days of incubation in MS medium containing 0.5 mg/l NAA + 2.0 mg/l BA

supplemented with 0.5 mg/l NAA + 2.0 mg/l BA by recording the fresh weight of the cells every 2 days (Fig. 3). The growth curve of suspension cultures indicated that the growth rate of cells was initially slow during first 3 days (lag phase). However, as the cultures proceeded it showed a marked increase from day 6 onwards and accumulated great amount of fresh weight over a period of 11 days (exponential phase). Maximum increase in fresh weight was reached on day 15 that was about 20 fold over the initial fresh weight. The rate of growth was stable for 10 days (Stationary phase). The stationary phase was followed by a gradual reduction in cell density (Fig. 3).

Based on the growth curve, the need for subculturing to new fresh media was between days 15 and 17 of incubation, the end of exponential growth phase. Stafford and Warren (1991) mentioned that it was better to subculture cells at the end of exponential growth phase. After linear growth stage, the medium became exhausted and toxic substances were produced by the cells (Bhojwani and Razdan 1983). Furthermore, the cell viability as shown in Fig. 2e was around 83 % throughout the 15 days of culture (Fig. 4). When cell viability remained around 50 %, it is considered that the suspension culture establishment has failed (Qui et al. 2009). For maintenance of the fine suspension culture, it is necessary to subculture them because the cultures tend to form cell clusters of a few cells to aggregate (Soomoro and Memon 2007). The suspension consisted of two types of cells with a round and an elongated shape (Fig. 2f). Usually in durations longer than 7 days (exponential phase), the number of large round shaped cells in the culture was increased. These results point out that the cells have changed their shape from elongated to round during the culture. This fact has

important implication on the establishment of *S. striata* cell suspension culture when scaling up to bioreactor level. These results are in corroboration with Curtis and Emery (1993), Trejo-Tapia and Rodríguez-Monroy (2007) who reported that the morphology of different plant cell suspension cultures affected the rheology of plant cell broths during bioreactor culture. These results confirm that the *S. striata* cell suspension culture has been successfully established.

Dynamics of acteoside production in suspension culture

Production of acteoside has been reported in cell suspension cultures (Chen et al. 2007; Estrada-Zúñiga et al. 2009; Saimaru and Orihara 2010). Higher acteoside production was reported in suspension cultures than in the callus of *Aphelandra* sp. (Nezbedová et al. 1999). In *Leucosceptrua japonicum* f. *barbinerve* callus, acteoside content was 3.16 % of dry weight otherwise in cell suspension culture the acteoside content reached 11.97 % of dry weight. Similar results were obtained for *Syringa josikaea*, and *S. vulgaris* (Inagaki et al. 1991). Dynamics of acteoside accumulation in *S. striata* cell suspension culture, during its cultivation cycle is shown in Fig. 3. The maximum content of acteoside (about 14.25 µg/g cell fresh weight) was observed on the 17th day of cultivation cycle. These results are in agreement with earlier studies (Estrada-Zúñiga et al. 2009; Nezbedová et al. 1999), that reported acteoside production increased parallel to cell growth, and reached a maximum several days after maximum cell growth. In addition, Ouyang et al. (2005) demonstrated at the end of lag phase the cells of *Cistanche deserticola* started to grow rapidly and the biosynthesis of PeG was activated. Our cultures showed the variation between 4.24 and 14.25 µg/g cells fresh weight in acteoside content within the growth cycle. It is essential to produce as much metabolically active biomass per unit volume by cell suspension culture as possible in order to achieve the higher possible production of bioactive compounds.

Conclusion

In conclusion, callus and cell suspension cultures of *S. striata* were achieved for the first time and provided a homogenous material for the acteoside biotechnological

production. The stem explants of *S. striata* were optimum for callus induction. The best callus growth and the highest acteoside production were obtained under the culture in MS basal medium containing 0.5 mg/l NAA + 2.0 mg/l BA. MS liquid medium supplemented with 0.5 mg/l NAA + 2.0 mg/l BA is favorable for establishment of incompact and rapid growing suspension cells. By suspension culture, not only large amount of calli could be obtained within a short period of time but also the acteoside concentration could be increased in the cells. The paper provides a new and efficient way to produce *S. striata* secondary metabolites. This work may be beneficial for further regulation of phenylethanoid biosynthesis and enhanced production of valuable PeGs on a large scale. In addition, application of these methodologies in bioreactors could give rise to the commercial production of these valuable metabolites. Further studies are required to investigate its potential for enhanced production of acteoside through precursor feeding, elicitation and biotransformation which is of potential research and development value in the field of bioactive pharmaceutical compounds.

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