

Original Research Article

Biosynthesis of Total Bacosides in the callus culture of *Bacopa monnieri*. L. Pennel from North-east India

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A B S T R A C T

Keywords

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In vitro callus was induced from shoot tip explants of *Bacopa monnieri* in MS medium supplemented individually with different auxins viz. IAA, NAA and 2,4-D. Degree of callus induction was highest in 2,4-D- 2 mg/l concentration where as moderate callusing was recorded in the treatment containing IAA and NAA. Biosynthesis of Bacosides, A tetracyclic triterpenoid saponin was observed in the callus tissue grown in MS medium. Bacoside which is considered to constitute the major drug is used as nervine tonic. Bacosides from dried powdered callus was isolated through continuous hot solvent extraction in a soxhlet distillation at 40° C. Identification of total bacoside was done with TLC followed by quantitative estimation by HPLC using standard bacoside compound, which showed 1.53 % of total bacoside content.

Introduction

Bacopa monnieri, commonly and widely called as brahmi, belongs to the family Scrophulariaceae. The plant is a small herb, prostrate, succulent, with branches spreading or ascending and rooting at the nodes. Leaves are ovate-oblong or spatulate and grows upto a height of about 18 cm. Flowers are white in color, companulate, solitary, axillary, short or long pedicilate and blossoms during the month of August and continues till the month of October. *B. Monnieri* mainly grows in the humid and watery areas and mainly distributed in damp and marshy

tracts in the subtropical region upto 1200 m elevation. In India it is found to grow in almost all the states of the North-east India Viz., Assam, Arunachal Pradesh, Nagaland, Manipur Mizoram Nagaland and Tripura and also in the states of West Bengal, Himachal Pradesh and other Himalayan regions. It requires a well drained, moist, sandy loamy soil, rich in organic matter at a temperature 30° C to 40 ° C and plenty of rainfall. The whole herb is used in medicinal preparation in Ayurveda, Unani and Sidha system of medicines. It is used as a nerve tonic,

diuretic, astringent intellect promoting, carminative and a blood purifier. The active principle of brahmi is an alkaloid Brahmine, Herpestine, Saponine Bacosides –A and Bacoside- B, besides Hirsaponin, Betuloic acid, b- Sitosterol, Stigmasterol etc. are also found to be present (Bose and Bose, 1956). The present study was undertaken to develop an efficient protocol for *in vitro* biosynthesis and production of total bacosides through callus and tissue culture.

Materials and Methods

Callus induction

For callus induction, leaf and nodal segments of healthy brahmi plant were collected afresh from the Departmental garden. MS medium (Murashige and Skoog, 1962) was used for callus induction as well as maintenance of the culture. Auxins viz. IAA, IBA, NAA, 2,4-D in the concentration ranging from 0.2 mg/l to 5.0 mg/l, individually supplemented with MS medium was used for callus induction. However the treatment MS+2,4-D (2 mg/l) was used for regular subculture for production of callus biomass.

The plant parts were initially washed with Tween-20, followed by surface sterilization with the solution of mercuric chloride (0.1%) for four minutes. After rinsing several times with sterile distilled water, the explants were trimmed into desirable size and inoculated into the prepared MS medium. The whole operation was done aseptically under a Laminar-air flow cabinet. The inoculated tubes were transferred into a culture chamber and maintained at temperature of $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and fluorescent light intensity

of 3000 lux. Observations for callus initiation and growth were recorded in terms of fresh weight of the callus biomass after 4 and 8 weeks of growth. Data were the representation of five replication.

Extraction and Estimation of Total Bacoside

Matured callus biomass was harvested and then dried in a hot air oven at 50°C . Dried callus was powdered manually with mortar and pestle and then extracted with 70 % alcohol in a Soxhlet distillation unit for 6 hours. The extract was evaporated in a vacuum under reduced pressure at 40°C . The resinous matter so obtained was dissolved in absolute alcohol and further dried with spray drying. Sample preparation and detection of Bacoside in the callus was done by the method given by Rajpal (2002). Presence of Bacoside in the extract was determined by TLC using Toluene: Ethyl acetate: Glacial acetic acid: Methanol in the ratio 3:4:1:3, which gives dark pink spots on spraying with 20 % H_2SO_4 in methanol.

Estimation of Total Bacosides was determined by HPLC with standard Bacoside. Preparation of standard Bacoside and sample Bacoside for HPLC analysis was done by the method used by Rajpal (2002). HPLC analysis of the Bacoside was carried out with Shimadzu model. 20 μl of the prepared samples were injected for the analysis. The chromatographic system was fitted with ODS C-18 column (Sigma Aldrich Hypersil), 2.5 x 4.6 x 5 μm with flow rate of 1.0 ml per minute. Mobile phase comprised of water and methanol. Detection wavelength and temperature were adjusted to 205 nm, and 40°C , respectively.

Results and Discussion

In vitro callus induction

Callus was induced from the nodal and leaf explants of brahmi plant, which was found to be quite effective, when grown in MS medium. Data recorded after 4 weeks and 8 weeks of culture have shown that out of the various treatments with Auxins, 2,4-D, supplemented with MS medium was found to be the best in terms of callus initiation and growth. Fresh weight of callus was found to be highest in the treatment MS+2,4-D-(2.0 mg/l) which recorded 15.20 g after 4 weeks and 35.60 g after 8 weeks of culture respectively (Table 1). However callus was also found to be induced in the treatments with NAA. Lower concentration of NAA in MS medium was found to have better callus growth compared to the higher concentrations of NAA. As the concentrations of NAA was increased, decreasing trend in the callus biomass production was observed which was contrary to that of the callus grown in 2,4-D supplemented medium. In 2,4-D supplemented MS media increase in callus biomass was recorded with increase in the concentrations of the 2,4-D from 0.2 mg/l to 2.0 mg/l except in the case of 5.0 mg/l where callus weight was somewhat less. (Table 1., Graph-1). Treatments with IAA also showed callus initiation and growth on moderate scale. Lower concentration of IAA, IBA and basal media showed no callus initiation at all. Callus induction was found to occur from the basal end of the nodal explants and from the cut end of the leaf explants. The calli were light green to greenish white in colour but later on turn somewhat brownish after continuous sub-culture. The callus was initially semi hard after initiation, but gradually turns friable due to continuous sub-culture in the same medium.

In vitro studies and micropropagation on *B. monnieri* was carried out by various workers like, Tiwari *et al.* (1998), Tejavathi *et al.* (2006), Srivastava and Rajani (1999). Mahapatra and Rath (2006), reported micropropagation of *B. monnieri* which was achieved on MS medium as well as B5 medium from leaf and nodal explants which supports suitability and response of the explants in the media. Similar explants were also used successfully in our study to induce callus. *In vitro* callus induction in *B. monnieri* was reported by few workers like Jain *et al* (2013), Vijayakumar *et al* (2010) and Parale *et al* (2010). Jain *et al* (2012), reported establishment of suspension culture and production of callus biomass using Gamborgs B5 and MS media for enhanced production of bacosides. Similarly Tiwari *et al* (1998), reported induction of callus from nodal explants in MS medium supplemented with 2,4-D-(0.5 mg /l), which support our findings of callus induction with 2,4-D in MS medium and from nodal explants. However we found vigorous callus growth at higher concentration of 2,4-D (2 mg/l) as compared to low concentrations of 2, 4-D as reported by Tiwari *et al* (1998).

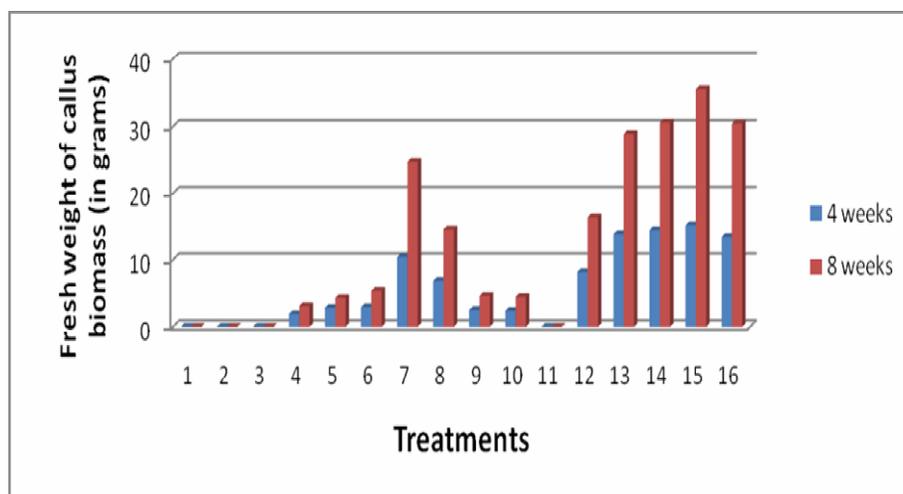
Biosynthesis of bacoside

Callus culture and suspension culture in artificial nutrient media offers an alternative and efficient way for the production of metabolites of interest in comparison to the traditional process of extraction from the plants grown in field. Several compound of medicinal importance have been obtained through these processes in several plants as reported by various workers in the past. Among the most significant compound produced through cell and suspension

Table.1 Effect of Auxins in callus initiation and growth of *B. monnieri* after 4 and 8 weeks of culture.

S.No	Treatments MS +	Callus growth (g) 4 weeks	Callus growth (g) 8 weeks
1.	Basal	--	--
2	IAA (0.2 mg/l)	--	--
3	IAA (0.5 mg/l)	-	--
4	IAA (1.0 mg/l)	1.89	3.05
5	IAA (2.0 mg/l)	2.80	4.26
6	IAA (5.0 mg/l)	2.90	5.30
7	NAA (0.2 mg/l)	10.40	24.67
8	NAA (0.5 mg/l)	6.90	14.60
9	NAA (1.0 mg/l)	2.47	4.56
10	NAA (2.0 mg/l)	2.36	4.40
11	NAA (5.0 mg/l)	--	--
12	2,4-D (0.2 mg/l)	8.22	16.40
13	2,4-D (0.5 mg/l)	13.90	28.90
14	2,4-D (1.0 mg/l)	14.50	30.59
15	2,4-D (2.0 mg/l)	15.20	35.60
16	2,4-D (5.0 mg/l)	13.50	30.45

Graph.1 Effect of various auxins in the growth of fresh callus biomass after 4 and 8 weeks of culture



culture and has been commercialized are Shikonin from *Lithospermum erythrorhizon* (Fujita, 1988), Ajmalicine/Roserpine from *Rauwolfia serpentine*, Diosgenin from *Dioscorea deltoidea*, Taxol from *Taxus bacata* and so on (Vijayashree *et al*, 2010; Oksman and Inze, 2004).

Bacoside synthesis in the callus was detected mainly in the matured callus., which was initially detected with TLC and with standard Bacoside. Synthesis of Total Bacoside in the matured callus harvested after 8 weeks of culture have shown encouraging result. 100 grams of dried powdered callus, grown in the MS medium with 2,4-D was extracted with soxhlet distillation. The purified extract showed 1.53 % of total Bacoside content estimated with HPLC. However it was seen that the concentration of Bacosides in the *in vitro* grown callus tissue is considerably more than that extracted directly from the field grown plants which was recorded at 1.02 %. This proves that enhanced production of bacoside can be obtained by callus culture against that obtained from *in vivo* grown plants. Deepak *et al* (2005), reported presence of bacosides in the concentration range of 0.14-0.85 % bacoside-A and 0.12-0.60 % of bacoside –B which he studied by HPLC in different samples of *Bacopa* collected from different parts of India. Studies on phytochemical analysis of the extract of *B. monnieri* from the plants and also from callus was studied by Binita *et al* (2005). The study demonstrated successful micropropagation of *Bacopa* from leaf and nodal segment using IAA and BAP in MS medium and the *in vitro* grown shoots, when subjected to HPLC analysis, demonstrated similar phytochemical profile to that of the naturally grown plant. Similar studies, carried out by Praveen *et al* (2009), reported clonal propagation of

Bacopa with Kn and TDZ in MS medium and resulted maximum amount of Bacoside -A in the shoots regenerated in liquid medium (11.92 mg/ g DW). Rahman *et al* (2002), carried out studies on the *in vitro* synthesis of Bacosides in the Cell suspension cultures of *B. monnieri*, in modified MS medium, which grew some 5–6 fold over 40 days and produced the important saponin, bacoside, up to 1 g/100 g dry weight. 166 % increase in total saponin bacoside content was obtained from suspension culture established from callus biomass was reported by Jain *et al* (2013). There are several other methods described by various workers on the synthesis and study of active chemical ingredients like bacopa using HPLC (Pal *et al*. 1998; Ganzera *et al*, 2004), Spectrophotometry, (Pal and Sarin, 1992), HPTLC (Shrikumar *et al*. 2004), as reported in the literature for quantification of bacosides in plant extracts and cell cultures.

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