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The Effect of Subculture on the Bacoside A Content in Adventitious Shoot Cultures of *Bacopa monnieri* (L)

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ABSTRACT

Brahmi (*Bacopa monnieri*) is an important medicinal plant mainly used for the improvement of intelligence, memory and revitalization of sensory organs. Recent investigations revealed that bacoside A is major chemical component shown to be responsible for memory facilitating action. In the present study the effect of subculture on adventitious shoot regeneration, biomass and bacoside a accumulation in *Bacopa monnieri* was carried out. The leaf explants were cultured on Murashige and skoog (MS) medium supplemented with 2.0 mg/L kinetin (Kin) and further they were subcultured in the same medium, and subcultured for every 2 months up to ten subcultures. There was gradual increase in the shoot number, biomass and bacoside content from parental culture and attained optimum at 6th subculture, thereafter decrease in the shoot number. Optimum number of adventitious shoots (79.00 shoots per explant), fresh weight (2.800 g), dry weight (0.190 g) and highest production of bacoside content (13.750 mg/g DW) was recorded on 6th subculture.

Keywords: Adventitious shoot**,** *Bacopa monnieri*, Bacoside A, Biomass, Subculture

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INTRODUCTION

Bacopa monnieri (L) Wettst (Plantaginaceae) also referred as Brahmi or Jalabrahmi is an important ayurvedic system of medicinal herb, found throughout the Indian subcontinent in wet, damp and marshy areas. It is an important ayurvedic medicine for the improvement of intelligence, memory and revitalization of sensory organs [1, 2]. Alcoholic/hydro-alcoholic extracts of the whole plant have been shown to possess nootropic activity [3, 4]. The brahmi extract is known to possess anticancer and antioxidant properties [5, 6]. The pharmacological properties of brahmi are mainly due to the presence of major bioactive saponins called 'bacosides'. Bacoside A is a major chemical entity shown to be responsible for memory-facilitating action of brahmi [3]. The composition of bacoside A has been established very recently as a mixture of tri-glycosidic saponins, bacoside A_3, bacopaside II, jujubogenin and bacopasaponin C [7]. In addition to memory boosting activity, it is also claimed to be useful in the treatment of cardiac, respiratory and of neuropharmocological disorders like insomnia, insanity, depression, psychosis, epilepsy and stress [8]. Increasing the bacoside A content in *Bacopa monnieri* would have a number of benefits in pharmaceutical and industrial applications.

In plant tissue culture most of the studies were focused on the influence of nutrient media, including composition of micro and macro elements, carbohydrates content and type/concentration of plant growth regulators. The influence of repeated subculturing on shoot multiplication and growth received less attention in literature [9]. Generally, subculture effect on multiplication rate of *in vitro* cultures varies from one species to another. A decrease in multiplication potential during long-term growth and repeated subculturing of shoots on medium of constant hormonal composition was reported in six ornamental species and cultivars of Rosaceae [10], two cultivars of *Potentilla fruticosa* [11], various decorative plants [12] and pineapple [13]. Subculture also affects the cell biomass and artemisinin content in a cell suspension culture of *Artimisia annua* [14]. The objective of present study was to assess the effect of subculture frequencies of the *Bacopa monnieri* on shoot multiplication, biomass and bacoside content on media with constant hormonal composition.

MATERIALS AND METHODS

Plant material and surface sterilization

Young leaves of *Bacopa monnieri* (L.) were collected from medicinal plant garden in University of Agricultural Sciences, Dharwad, India and were washed in sterile water thrice, and surface sterilized in 2 % aqueous solution of sodium hypochlorite solution for 5 min followed by washing in sterile distilled water. Subsequently, explants were treated with 0.1 % (w/v) mercuric chloride for 2 min followed by washing in sterile distilled water three times.

Culturing of Explant

Leaf sections (5×5 mm) were cultured (abaxial surface down) into test tubes (Borosil, India) each containing 15 mL of Murashige and Skoog [15] medium (0.8 % agar, 2 % w/v sucrose) supplemented with 2.0 mg/L kinetin (Kin). After one month, the explants were transferred to magenta boxes (Himedia, India) each containing 50 mL of MS agar (0.8 %) medium supplemented with same cytokinin concentration where they have come from [16]. After 2 months of culture, the explants subcultured for every 2 months up to ten subcultures. The explants were examined and number of shoots per explant, fresh weight of shoot cluster along with explant was recorded for each subcultures. The first 2 months culture consider as parental culture (0^{th} subculture). Shoot cluster along with original explant were collected and oven dried at 60 ^{0}C for 1 day and dry weight was recorded.

Extraction and analysis of bacoside A

Extraction and High performance liquid chromatography (HPLC) analysis of bacoside A were carried out by following the method of Murthy et al. [17] with some modifications. Thirty milligram of powdered plant material were extracted in 25 mL of 70 % methanol by heat refluxing for 45 minutes and filtered through 0.45 µm membrane filters. The bacoside fractions were analyzed using Shimadzu HPLC system equipped with Phenomenex C18, 5 µm (4.6x250 mm) column, LC10AT VP lamps, SCL-10AVP system controller, SIL-10 AD VP auto injector, SPD-M10 AVP photodiode array detector. The mobile phase was a mixture of acetonitrile and water (60:40, v/v) at flow rate of 1 mL/min and column temperature was maintained at 30 ^{o}C. The detection wavelength was set at 205 nm. The injection volume was 20 µL. The chromatography system was equilibrated by the mobile phase. The standard bacoside A was purchased from Chromadex (Laguna Hill, CA, USA). The standard bacoside A chromatogram was used to quantify the concentrations of bacoside A in *Bacopa monnieri* extracts.

Statistical analysis

The experiment was conducted with a minimum of 12 replicates and the experiment was repeated three times. The data was analyzed statistically using SPSS ver. 17.0 (SPSS Inc. Chicago, USA). The significance of differences among means was carried out using Duncan's multiple range test (DMRT) at P = 0.05. The results are expressed as the means ± SE of three experiments.

RESULTS AND DISCUSSION

In the present study, we have tested the effect of subculture on adventitious shoot regeneration, biomass and bacoside A accumulation from leaf explants of *B. monnieri*. Our present study showed that highest number of shoots (79.00 shoots per explant) was obtained in 6^{th} subculture against the parental culture (0^{th} subculture) in which 64.35 shoots per explants was obtained (Table 1). There was gradual increase in the shoot number from parental culture and attained optimum on 6^{th} subculture, thereafter decrease in the shoot number. Similar

results were obtained in *Potentilla fruticosa* and banana, in which shoot multiplication was at its maximum at the beginning of the experiment and decline eventually with increase in the number of subcultures [11, 18]. In contrast to these results, in cherry and plum significant decrease in shoot multiplication was observed after the first subculture [19].

Table 1:Effect of subcultures on adventitious shoot regeneration and biomass accumulation from leaf explants of Bacopa monnieri cultured for 2 months on MS medium supplemented with 2 mg/L Kin and 2 % sucrose at pH 5.8*.

No. of subculture	Mean No. Shoots per explant	Mean fresh weight (g)	Mean dry weight (g)
0	64.350±2.864c	1.131±0.070f	0.078±0.002f
1	64.400±1.528c	1.137±0.034f	0.080±0.001f
2	69.500±2.797c	1.516±0.011e	0.117±0.002e
3	70.000±3.511bc	2.360±0.063b	0.133±0.004d
4	71.660±2.410abc	2.410±0.073b	0.136±0.005d
5	78.200±2.318ab	2.632±0.059a	0.159±0.005bc
6	79.000±4.041a	2.800±0.063a	0.190±0.004a
7	72.000±2.000abc	2.344±0.069bc	0.164±0.002bc
8	70.750±1.520abc	2.390±0.090b	0.166±0.002b
9	69.160±2.199c	2.158±0.074c	0.153±0.003c
10	66.250±2.529c	1.842±0.050d	0.128±0.003d

*Data were collected after two months of culture. Values represent the mean ± SE. Mean values following the same letter within columns are not significantly different, according to Duncan's multiple range (p = 0.05) test

Optimum biomass (fresh weight = 2.800 g and dry weight = 0.190 g) was observed in 6th subculture (Table 1), it was significantly higher (fresh weight = 2.475 times and dry weight = 2.435 times) when compared to the parental culture (0th subculture). After the 6th subculture there was decline in the biomass accumulation. In *Melastoma malabathricum* biomass of calluses continued to increase until 10th subculture, after that random check in the growth [20]. Production of *Artemisia annua* callus peaked at the 20th subculture thereafter decreased in the callus growth [14]. Bacoside A production was also highest in the 6th subculture (13.750 mg/g DW) when compared to the parental culture (0th subculture), there was a steady increase in the bacoside A content up to 6th subculture and thereafter decrease in the accumulation of bacoside A content (Figure 1). In contrast to these results1st subculture produced highest content of asiaticoside in *Centella asiatica*, but parental culture and 2nd subculture showed lower content of asiaticoside [21].

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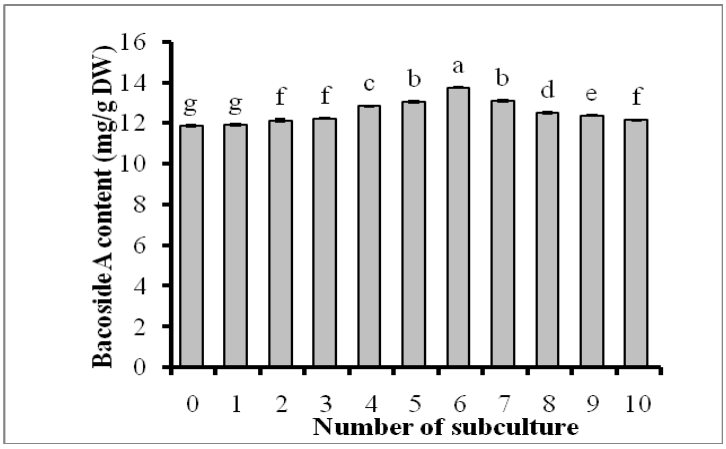

Figure 1: Effect of subcultures on bacoside A content accumulation from leaf explants of Bacopa monnieri cultured for 2 months on MS medium supplemented with 2 mg/L Kin and 2 % sucrose at pH 5.8*

*Bars represent the standard errors, mean values following the same letter are not significantly different, according to Duncan's multiple range (p = 0.05) test

CONCLUSIONS

The present study on adventitious shoot cultures of *Bacopa monnieri* has shown that both the biomass and bacoside A was influenced by subculture. The highest number of adventitious shoots, fresh weight, dry weight and the production of bacoside A content was recorded on 6th subculture. The above results are useful for the large scale cultivation of *Bacopa monnieri* adventitious shoots for the production of bacoside A.

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