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A Review on *In Vitro* Propagation and Bacoside Production in *Bacopa monnieri*

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ABSTRACT

Bacopa monnieri (Brahmi) is an important medicinal plant used and it has several pharmacological properties which include memory and learning enhancer, immunomodulator, adaptogen, cerebral activator, etc. This plant is over exploited due to the preference of its natural drugs over synthetic which compelled us for its conservation. The aim of this review article is to summarize the in vitro propagation studies, conservation methods, commercial production of bacoside from Bacopa monnieri and its dosage and side effects.

Keywords: *Bacopa monnieri,* Bacoside*, In vitro* propagation, Conservation, Side effects

INTRODUCTION

Bacopa monnieri is an important medicinal plant which rejuvenates memory and intellect. It has been used by Ayurveda in India for almost 3000 years [1]. It is belongs to 'Scrophulariaceae' family and a small perennial herb which grows in wetland and muddy shores habitat. The plant has a soft stem which is approximately 10-30 cm long, 1-2 mm in thickness. According to National Medicinal Plants Board (NMPB) it is also known as *Gratiola monnieria* (L.)*, Moniera cuneifoli, Herpestis monnieri* (L.), *Moniera cuneate* and locally known as brahmi, pan brahmi, water hyssop, jalbuti, jalnim, etc. Genus Bacopa includes more than 100 species of aquatic herbs. It is found in India, Nepal, Vietnam, Sri Lanka, China, Taiwan, Hawaii, Florida and some other Southern states of USA. In the list of important medicinal plants *Bacopa monnieri* is placed in second position. Whole plant of *Bacopa monnieri* is medicinally useful [2]. According to National Medicinal Plants Board the estimated market demand for *Bacopa monnieri* is around 1,000 tonnes per year. *Bacopa monnieri* also helps in repairing connective tissues and skin and smoothing out cellulite. It possesses different pharmacological activities due to presence of several compounds which include alkaloids, saponins, glycosides, flavonoids and stigmasterols. Saponins include hersaponin, monnierin, Bacoside A and Bacoside B [3]. Bacoside A is the major component that is responsible for the memory enhancement effect. Bacoside A differs from Bacoside B in optical rotation where Bacoside A is levorotatory and Bacoside B is dextrorotatory. The present review gives the detail study of *in vitro* propagation studies done in *Bacopa monnieri* and its conservation process. Further it also discusses the commercial production of bacosides and dosages and side effects.

In vitro propagation

For conservation of *B. monnieri* tissue culture is one of the alternative methods. In short period of time clonal propagation through tissue culture can be achieved. Different *in vitro* propagation studies show that *B. monnieri* can be propagated through different parts of the plant which includes nodes, internodes, leaves, axillary bud and callus shown in Table 1. It was seen that generally Murashige and Skoog's media used in most cases along with the growth hormones or elicitors or both for plant propagation [37-40].

Table 1: *In vitro* propagation of *B. monnieri* using different explants

Explants	Media	Growth hormone	Shooting/Rooting/ Callusing	References
Node	MS	BAP (1.0 mg/l)+IAA (0.5 mg/l)	Shooting	Dixit and Thakur [4]
Node	MS	BA (0.2 mg/l)	Shooting	Sharma et al. [5]
Node	MS	BA (0.2 mg/l)	Rooting	
Node	MS	BAP (3.0 mg/l)	Shooting	Shashikanta and Nibedita [6]
Leaf	MS	BAP (0.2 mg/l)+IAA (0.2 mg/l)	Shooting	Uma et al. [7]
Shoot tips, leaves, nodal and internodes	MS	BAP (2 mg/l)+Kinetin (1 mg/l)+ IAA (1 mg/l)	Shooting	Ayyappadas and Renugadevi [8]
		2,4-D (0.5 mg/l)	Callus induction	
Nodal and leaf	MS	2,4-D (2 mg/l)	Callus induction	Talukdar [9]
Nodal	MS	Kinetin (2 mg/l)	Shoot regeneration	Umesh [10]
Leaf		BAP (1 mg/l)+IAA (0.5 mg/l)	Callus induction	
Leaf	MS	-	Shoot initiation	Koul et al. [11]
	B5			
Leaf, nodal and intermodal	MS	BAP (2.0 mg/l)+NAA (0.5 mg/l)	Callus induction	Priya [12]
Nodal	MS	BAP (1.5 mg/l)	Shoot multiplication	Pandey et al. [13]
		IBA (1.0 mg/l)	Rooting	
Nodal	MS	BAP (0.5 mg/l)	Shoot induction	Kumari et al. [14]
		IBA (0.2 mg/l)	Rooting	
Nodal	MS	BAP (1.5 mg/l)+IAA (0.5 mg/l)	Shoot multiplication	Kapil and Sharma [15]
		IAA (1.0 mg/l)	Rooting	
Nodal	MS	BAP (1.0 mg/l)	Shoot induction	Vollala et al. [16]
		TDZ (1.0 mg/l)		
		2ip (4.92 mg/l)		
Nodal	MS	IAA (0.5 mg/l)	Axillary shoot proliferation	Mohanta and Sahoo [17]
		NAA (0.5 mg/l)		
		BAP (0.5 mg/l)+2,4 D (1 mg/l)	Callus Initiation	
		BAP (1.0 mg/l)+IAA(1 mg/l)	Callus Regeneration	
Leaf	B5	2,4-D (0.5 ml/l)	Callus induction	Jain et al. [18]
Nodal and Internodal	MS	BAP (1.0 mg/l)+NAA (0.5 mg/l)	Shoot initiation	Jain et al. [19]
Nodal	MS	BAP (1.0 mg/l) IBA (1.0 mg/l)	Shoots multiplication	Kaur et al. [20]
			Rooting	
Axillary buds	MS	BAP (4.44 µM) and NAA (0.53 µM)	Shoot regeneration	Sharath, et al. [21]
Leaf	MS	BAP (0.5 mg/l)+NAA (1.0, 2.0 mg/l), 2,4D (2.0 mg/l)+BAP (0.5, 1.0 mg/l)	Callus induction	Singh [22]
Nodal	MS	BAP (2.5 mg/l)+IAA (0.01 mg/l)	Shooting	Jain et al. [23]
Nodal	MS	BAP (1.5 mg/l)	Shoot induction	Pandiyan and Selvaraj [24]
		IBA (1.0 mg/l)+NAA (0.5 mg/l)	Rooting	
Nodal	MS	BAP (3 mg/l)	Shoot regeneration	Gurnani et al. [25]
		BAP (1 mg/l)+IAA (3 mg/l)	Rooting	
Auxiliary nodes and internodes	MS	IAA (0.5 mg/l)+Kn (0.5 mg/l)	Auxiliary shoot proliferation	Tanner et al. [26]
Leaves		NAA (0.5 mg/l)+TDZ (0.25 mg/l)	Callus induction	
Nodal, Internodal and Leaf	MS	BAP (4.4 µM)	Shoot regenration	Yusuf et al. [27]
Leaf	MS	2,4-D (0.5 mg/l)	Callus induction	Showkat et al. [28]

Leaf	MS	BAP (5 µM)	Shoots regeneration and multiplication	Parale et al. [29]
		NAA (5 µM)+2,4-D (1 µM)	Callus induction	
Leaf and node	MS	BAP (1 mg/l)+Kn (0.5 mg/l)+NAA (0.5 mg/l)	Shoot regeneration	Khan et al. [30]
		IAA (0.5 mg/l)	Rooting	
		BAP (1 mg/l)+2,4D (0.5 mg/l)	Callus induction	
Leaf	MS	BAP (6 µM)	Shoot regeneration	Pathak et al. [31]
		IBA (2 µM)	Rooting	
Nodal	MS	BAP (0.2 mg/l)	Shoot multiplication	Sharma et al. [32]
		IBA (0.15 mg/l)	Rooting	
Young shoot	MS	IBA (0.5 mg/l)	Rooting	Kumari et al. [33]
Internodal	MS	BAP (1.0 mg/l)+Kn (0.5 mg/l)	Shooting	Banerjee and Shrivastava [34]
Axillary nodes, young leaves and internodes	MS	BAP (1.1 µM)+IAA (0.2 µM)	Shoot regeneration	Yogesh et al. [35]
Leaf and nodal segments.	MS	BAP and NAA	Shoot regeneration	Mohapatra and Rath [36]
	B5	BAP and NAA		

Cryopreservation

In vitro shoot tips of *B. monnieri* was developed through Cryopreservation. Shoot tips (about 1 mm in length) were taken from 4 to 24 week old proliferating shoot cultures were pre-cultured on MS medium supplemented with various cryoprotectants before dehydration with PVS2 at 0°C. After rapid rewarming at 45°C the dehydrated shoot tips were directly immersed in liquid nitrogen, then they were quickly washed with 1.2 M sucrose solution and plated on solidified shoot culture medium [41-45]. By vitrification the shoot tips were successfully cryopreserved when they were pre-cultured on medium supplemented with 5% DMSO for two days before dehydration [46-51]. The survival rate and shoot regeneration rate of cryopreserved shoot tips is significantly improved by pre-culturing the shoot tips with sucrose at 25°C [52-56]. Regenerated plantlets were successfully transferred to soil [57,58]. A method was developed for slow growing, medium-term conservation of *B. monneiri* by using mineral oil. Nodes of *B. monnieri* were conserved using mineral oil for 24 months. Single node explants were inoculated in MS medium which contains 0.2 mg/l BA and were covered with mineral oil. There is significant enhancement of subculture duration from 6 to 24 months was observed on the above medium. Regeneration of plant from conserved culture was established in soil. Results show that mineral oil can be used for medium-term conservation of *B. monnieri* germplasm without any adverse genetical and biochemical effects [37].

Production of bacosides

In natural circumstances, the production of secondary metabolites is in fewer quantities so there is a requirement of large scale production. Secondary metabolite of *B. monnieri* from leaf, nodes and axillary buds was produced by using cheap alternative media [38]. In the same study MS medium used with 0.5 mg/l Indole Butyric Acid (IBA) and 3 mg/l Kinetin. For cost minimization, 70% strength MS medium with 20 g/l Jaggery, 10 g/l Sucrose and 5 g/l Agar were used [44-47]. Result shows that there were maximum multiple shoot regeneration from leaf up to number of shoots per explants- 7.25 ± 0.96, shoot length- 11.30 ± 0.26 cm, nodes number per shoots- 11.50 ± 0.58. Different compositions of 6-benzyl amino purine (BAP) were used for comparison instead of Kinetin and it was better than Kinetin [50-56]. Also, rooting response against NAA and IBA were compared and it was found that NAA gave better result than IBA and by this there was a 40% reduction in medium cost and 25% reduction in production cost [38]. The elevated production of saponins in short duration was measured using Gamboreg's media with different combination of hormones [18]. Callus were formed and after 20 days viable callus were transferred to modified MS liquid medium which was supplemented with 1 mg/l naphthalene acetic acid, 0.5 mg/l kinetin, 1 g/l casein hydrolysate and 30 g/l sucrose [49]. After 40 days cell mass in suspension along with field grown natural plant system and plant parts were dried separately in dark for 4 days at 37°C. Total methanolic extraction of saponin was performed. Then spectrophotometric technique and TLC (Thin layer Chromatography) were performed and TLC

plate exhibiting different bands of saponins obtained from Root system, Shoot system, Callus and Total plant system of *B. monnieri* [19]. Elicitors also help in the production of the secondary metabolites. It enhances the synthesis of secondary metabolites by the plants. [39] Investigated the effect of abiotic stress (salinity and drought) on proline and protein content in *in vitro* shoot culture for a period of 5, 15 and 30 days and showed an increase in proline but decease in protein content with an increase in both mannitol and NaCl concentration as well with culture age [42]. The effect of 150 mg/l chitosan and 2 mg/ml yeast extract in whole plant cultures has showed an increased pseudojujubogenin glycoside production (40.83 ± 2.24 mg/g DW and 40.05 ± 2.37 mg/g DW, respectively) after 7 days. This production was 6 times higher than that of control cultures [40]. In a study when shoot cultures were co-cultivated with a novel symbiotic fungus *Piriformospora indica*, it showed an enhancement in the plant growth, biomass, bacoside contant, antioxidant activity and nuclear hypertrophy. Reported enhanced bacoside A production (4.4 mg/g) in *in vitro* shoot cultures treated for one week with 50 µM methyl jasmonate [59,60]. Increase in shoot growth, antioxidant defense and bacoside A content under the influence of NaCl has been reported [59] and recently, also observed enhanced bacoside A and bacoside I content in *B. monnieri* shoots when treated with a concentration of 10 µM Cadmium after a culture period of one week. In a study it was reported that rhizospheric microbes, i.e., *Bacillus megaterium, Glomus intraradices, Trichoderma harzianum* and their combinations used for the increase production of bacoside content and the production was 1.40-fold higher than control [42]. Effect of different abiotic elicitor's, i.e., jasmonic acid, copper sulphate and salicyclic acid at varying concentrations increases the biomass and bacoside production. Shoot cultures treated with 45 mg/l of $CuSO_4$ showed highest content of bacoside, i.e., 8.73 mg/g dry weight which was approximately 1.42-fold higher than in control cultures [40].

Dosage, side effects and toxicity

5-10 g of non-standardized powder of *B. monnieri*, 8-16 ml of infusion and 30 ml of Brahmi syrup are recommended for daily doses. For adults, 5-12 ml per day dosages of a 1:2 fluid extract and two and half to ml per day for children ages 6-12. For standardized to 20% Bacosides A and B, *Bacopa monnieri* extract the dosage is 200-400 mg daily for adults (in divided doses), and for children, 100-200 mg/day [43,48]. Therapeutic doses of *B. monnieri* are not associated with any known side effects. In a study it has been seen that LD50 of aqueous extracts of *B. monnieri* when administered to rats orally was 5 g/kg and the alcohol extract was 17 g/kg. None of the extract shows gross behavioural change at these concentrations [61]. In double-blind, placebo controlled clinical trial of healthy male volunteer's safety of pharmacological doses of isolated bacosides over a four-week period was investigated. Daily concentrated bacosides given in form of single (20-30 mg) and multiple (100-200 mg) dose, and doses were well tolerated and without showing any adverse effects [62,63].

CONCLUSION

Bacopa monnieri is an important medicinal plant and contains various phytocompounds like alkaloids, saponins, glycosides, flavonoids, etc. Due to the presence of various phytocompounds it possesses various pharmacological activities which make this plant important for the various studies. The present review summarizes the *in-vitro* propagation studies which showed that the plant has maximum growth in MS media with different concentration of auxins and cytokinins. Most commonly used cytokinin was BAP in the concentration range of 0.2-3mg/l for the shooting and for rooting and callusing 2,4-D, IAA and NAA in the concentration range of 0.2-2mg/l). Micropropagation technique can be used for the conservation of this plant as due to the medicinal importance of this plant it is overexploited.

Saponin is one of the major phytocompound presents in this plant and it is responsible for the treatment of several diseases due to this reason there is a requirement of alternative techniques for a large amount of saponin production. Cell suspension culture containing different types of elicitors and plant growth hormones enhance the production of the saponin up to 1.4 times. In future, various concentrations of elicitors and growth hormones can be utilized for the enhanced production of saponin to meet the commercial requirement.

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