Indian Journal of Science and Technology Vol. 3 No. 7 (July 2010) ISSN: 0974- 6846

In vitro propagation of *Bacopa monnieri* L. - a multipurpose medicinal plant

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Abstract

We report the micropropagation of *Bacopa monnieri* L. a medicinal plant from Shervarayan Hills, Salem, Tamil Nadu, India. Effect of auxins (IAA, NAA & 2, 4-D) and cytokinins (BA, KIN & TDZ) on shoot induction and IAA and TDZ on root induction were assessed. Callus induction on Murashige and Skoog's medium (MS) supplemented with NAA, 2,4-D and TDZ at various combinations/concentrations were also investigated. The auxiliary buds formed from both leaf and nodal explants on hormone free MS medium within 9 d. Growth hormones at different combinations brought out remarkable variations in shoot, root and callus induction. Rooted plantlets were transferred to 10 different soil mixtures with various ratio/combinations of biological and natural fertilizers such as humus rich soil alone (control), soil with vesicular arbuscular mycorrhizae (VAM) (10:1 & 10:2) soil with vermicompost (4:1 & 4:2) soil with farmyard manure (4:1 & 4:2) soil with flyash (1:1 & 1:2) and soil with agropeat (4:1 & 4:2) in sterile polythene bags. When the plantlets were transplanted to the actual field, this pre-adaptation increased their survival as much as 80-90% (observed for 45 days).

Keywords: *Bacopa monnieri* L., growth hormones, auxiliary shoot proliferation, transplantation.

Introduction

WHO estimated that 80% of the population of developing countries rely on traditional medicines, mostly plant drugs, for their primary health care needs. The developed nations are also looking for eco-friendly treatment of various diseases through plant based source. In addition, many valuable herbal drugs have been discovered by knowing that particular plant was used by the ancient folk healers for the treatment of some kind of ailment (Ekka & Dixit, 2007). Technology Information forecasting and assessment council (TIFAC) has recommended 45 medicinal plant species of which 7 plants were recommended specifically for immediate attention during this decade, they are as follows: *Aloe vera* (Ghrita Kumari), *Bacopa monnieri* L. (Brahmi), *Centella asiatica* (Mandukparni, Gotukola), *Rawolfia serpenrtina* (Sarpagandha), *Catharanthus roseus* (Periwinkle), *Taxus bacatal T. wallichiana* (Himalayan Yew) and *Artemisia annua*. Traditionally, *Bacopa monnieri* L. is used as a brain tonic to enhance memory development, learning and concentration and to provide relief to patients with anxiety or epileptic disorders. The plant has also been used in India and Pakistan as a cardiac tonic. Recent research has been focused primarily on *Bacopa*'s cognitive-enhancing effects. Especially, memory, learning and concentration and results support the traditional ayurvedic claims. Research on anxiety, epilepsy, bronchitis and asthma, irritable bowel syndrome and gastric ulcers also supports the ayurvedic uses of *Bacopa* (Shakoor *et al*., 1994).

Plant tissue culture is the process of small pieces of living tissues (explants) isolated from a plant and grown aseptically for indefinite periods on a semi defined or defined nutrient medium (Ignacimuthu, 1997). It is considered in wide sense which comprises the various culture methods of plant organs, tissues which facilitates experimental approach with a large objective of developmental biology and crop modification. It provides new possibilities for *in vitro* propagation and manipulation of plants and also recognized as an efficient tool for rapid clonal propagation (Negrutiu *et al*., 1984). Murashige and Skoog's medium is commonly used for plant tissue culture studies (Murashige & Skoog's, 1962). Hence, the present study is justifiably planned to propagate the valuable medicinal plant *Bacopa monnieri* L. in *in vitro* condition with various combinations/ concentrations of plant growth regulators, and transplant the plants from laboratory in to field condition.

Materials and methods

Collection of plant material

Bacopa monnieri L. plant was collected from Shervarayan Hills, Western Ghats of India at Salem district of Tamil Nadu. The collected plant parts were kept under green house condition for further study.

Murashige & Skoog's medium

Murashige and Skoog's medium was used for the cultivation of *B. monnieri* L. at *in vitro* condition. The MS

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medium was prepared by adding required amounts of stock solutions and final volume was made up with distilled water, the composition of the MS medium was given in Table 1. The pH of the medium was adjusted to 5.8 using 1 N NaOH/KCl. About 50 ml of the medium was poured into sterile culture bottles. The culture bottles with MS medium was autoclaved at 121°C for 20 min. at 15 lbs pressure and transferred to the media storage room where they were kept under aseptic condition for further experimental study (Murashige & Skoog, 1962).

Table 1. Different compositions of MS medium for shoot & root induction

Essential elements	Conc. in stock solution (mg/l)	Conc. in medium (mg/l)
Macro elements		
NH_4NO_3	33000	1650
KNO_3	38000	1900
$CaCl_2.2H_2O$	8800	440
$MgSO_4.7H_2O$	7400	370
KH_2PO_4	3400	170
Micro elements		
KI	166	0.83
H_3BO_3	1240	6.2
$MnSO_4.4H_2O$	4460	22.3
$ZnSO_4.7H_2O$	1720	8.6
$Na_2MoO_4.2H_2O$	50	0.25
$CuSO_4.5H_2O$	5	0.025
$CoCl_2.6H_2O$	5	0.025
Iron source		
$FeSO_4.7H_2O$	5560	27.8
$Na_2EDTA.2H_2O$	7460	37.3
Organic supplement		
Myoinositol	22000	100
Nicotinic acid	100	0.5
Pyridoxine - HCL	100	0.5
Thiamine - HCl	100	0.5
Glycine	400	2.0
Carbon source		
Sucrose	Added as solid	30000

Selection of explants & sterilization

The explants were first washed with running tap water for 30 min. to remove the soil particles and other extraneous fine particles. The explants parts such as nodal segment, stem, leaf and root were cut from the healthy plant of *B. monnieri* L. and washed with tap water for 5-10 times, and they were soaked in 0.2-0.5% bavistin and 0.03% streptomycin aqueous solution for 10 min. It was gently washed twice in sterile double distilled water. The explants were immersed in aqueous solutions of savlon (1.5% v/v chlorohexidine gluconate solution & 3% w/v cetrimide) for 10 min. Then the explants were washed twice thoroughly with sterile double distilled water. After this treatment, the explants were surface sterilized with 0.01% $HgCl_2$ aqueous solution for 1 min. and rinsed thoroughly with sterile double distilled water (Tiwari *et al.,* 2001).

Initiation of cultures

There is a high risk of contamination of the MS medium at the time of transfer of the explants into the culture medium. Therefore, surface sterilized explants were transferred aseptically to sterile glass plate. Then undesirable and dead portions of both basal and the top portion of the explants were removed. The nodal explants were placed in an erect position in the culture bottle containing MS medium with the help of sterile forceps. Then lid was closed carefully and sealed with Klin film. The same procedure was used for all the explants. The culture bottles were kept in the growth room at 25±2°C, with a photoperiod of 16 h daylight and 8 h night breaks under the cool white fluorescent light (Anilkumar & Sajeevan, 2005).

Establishment of cultures

The explants with bud proliferation cultures were transferred to culture tubes containing fresh MS medium. After 21-25 d of incubation the initiated plants were taken out from the culture bottle and transferred into fresh semi-solid MS media. Then the bottles were kept in culture room at 25±2°C for 8-16 h of day and night under the low temperature with white fluorescent light (Murashige & Skoog, 1962). After experimental days, the full matured culture was obtained and they were further subcultured in MS medium supplemented with different plant growth hormones at different concentrations for regeneration of shoots.

Auxiliary shoot proliferation

The stem explants were inoculated on MS medium supplemented with different plant growth regulators at different concentrations and multiplication of shoots were carried out by repeated sub-culturing in MS medium. Multiple shoots and cluster were transferred from the culture bottle to a sterile glass plate and the debris parts were removed. These nodal segments were transferred to the multiplication media with 0.5 mg/l IAA/0.5 mg/l KIN as growth regulators. These culture bottles were incubated at 25±2°C. These steps were repeated at every 25-30 d intervals and shoot induction rate was observed (Ray *et al*., 2005).

Effect of plant growth regulators on shoot induction

The MS medium was supplemented with the plant growth regulators like auxin (IAA & NAA) and cytokinin (BA, KIN & TDZ) at 42 different combinations. The combinations and their concentrations were mentioned in Table 2. The explants were inoculated at appropriate condition in culture bottles, and the shoot induction rate was observed, triplicates were used for each treatment (Yadav & Padmaja, 2005; Anilkumar & Sajeevan, 2005).

Rooting of the shoots

The surface sterilized explants were cut into small pieces. For root induction, MS basal medium was supplemented with IAA 0.2 mg/l and TDZ 0.04 mg/l and the initial pH of the medium was adjusted to 5.8. The explants were implanted aseptically on the culture media.

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All the cultures were maintained in culture room at 25±2°C for 8-16 h of day and night break under the white fluorescent light (Mahendran & Sampath, 2005). After incubation the root induction rate was observed. Triplicates were maintained in each treatment.

Effect of growth hormones on root induction

MS basal medium was supplemented with different concentrations of auxin (IAA) 0.1- 0.5 mg/l and TDZ 0.01-0.06 mg/l and pH of the medium was adjusted to 5.8. The explants were implanted on the culture media. All the cultures were maintained at culture room at 25±2°C for 8-16 h of day and night break under the cool white fluorescent light and the root induction rate was observed (Thorat *et al.*, 2006).

Callus induction

Leaf explants were taken from established cultures of *B. monnieri* L. for callus induction. The MS basal medium was supplemented with 0.5 mg/l NAA and 0.25 mg/l TDZ. After inoculation with established culture, the culture bottles were sealed properly, labeled and the triplicates were maintained. Then they were transferred to the incubation room and kept in appropriate condition. After two weeks, the callus induction rate was recorded (Nagaraja *et al.,* 2003).

Effect of plant growth regulators on callus induction

The plant growth regulators such as auxin and cytokinin were supplemented into MS medium at 25 different combinations (Table 4) and callus induction rate was observed (Anilkumar *et al.,* 2005).

Ex vitro rooting of B. monnieri L.

Plantlets were taken from the culture bottles with the help of forceps and washed thoroughly with water to remove any remaining of the medium. The plantlets were treated with 0.1% bavistin in order to protect from the fungal infection. The plantlets were separated into single shoots by cutting their bases gently with the help of scalpels. The plantlets were carefully planted into the pots containing soil, and adequate water was poured and the growth of the plant was recorded.

Transplantation with soil mixture

The rooted plantlets were taken out from the culture bottles and washed with water to remove the excess medium. The plantlets were kept under mist chamber for 10-15 d. Then, it was transferred to 10 different soil mixtures with various ratio/combinations such as humus rich soil alone as a control, soil with vesicular arbuscular mycorrhizae (VAM) (10:1 & 10:2), vermicompost (4:1 & 4:2), farmyard manure (4:1 & 4:2), flyash (1:1 & 1:2) and agropeat (4:1 & 4:2) in sterile polythene bags. The small holes were made in the polythene bag for proper air circulation and removal of excess water. After 3 weeks

Table 2. Different growth regulators for shooting response of B. monnieri L.

Growth regulator	Conc. (mg/l)	Shoot explants		Leaves explants	
		Shoot formation (%)	Shoot length (cm)	Shoot formation (%)	Shoot length (cm)
IAA	0.1	25	0.64±1.12	10	0.40±0.17
	0.2	25	0.70±00	22	0.43±0.09
	0.3	32	0.83±0.4	32	0.54±0.18
	0.4	50	1.36±00	50	1.01±0.01
	0.5	70	2.76±0.03	70	1.28±0.89
NAA	0.1	30	0.89±0.19	20	0.69±0.13
	0.2	50	1.56±0.11	40	0.98±0.9
	0.3	70	2.86±1.12	60	1.01±1.12
	0.4	90	3.06±0.18	90	1.92±0.98
	0.5	95	3.17±0.12	95	1.98±0.16
KIN	0.1	50	2.01±0.13	25	1.96±0.02
	0.2	50	2.21±0.02	50	1.98±0.89
	0.3	60	2.92±0.18	75	2.01±00
	0.4	75	3.02±00	90	2.61±0.08
	0.5	95	4.09±0.02	95	3.00±0.61
TDZ	0.05	50	3.01±0.89	50	1.01±00
	0.10	65	3.68±0.61	60	1.38±0.11
	0.25	80	4.52±0.89	75	1.96±0.86
	0.50	90	4.81±0.11	90	2.01±0.92
	0.75	95	5.01±0.89	95	2.22±0.61
	1.0	95	5.86±0.12	95	2.30±0.18
BA	0.25	55	3.41±0.11	30	0.98±0.28
	0.50	65	4.01±0.84	50	1.12±0.19
	0.75	80	4.83±0.13	50	1.89±0.12
	1.0	90	5.03±0.9	70	2.01±0.22
	1.25	95	5.83±0.13	90	2.81±0.10
	1.5	95	6.36±0.9	95	2.96±0.33
BA/KIN/ NAA	0.5/0.5/0.1	50	2.38±0.28	20	1.18±0.62
	0.5/0.5/0.2	65	2.81±0.99	20	1.28±0.22
	0.5/0.5/0.3	70	2.89±0.11	40	1.61±0.98
	0.5/0.5/0.4	80	3.01±0.01	60	1.80±0.88
	0.5/0.5/0.5	95	4.89±0.01	75	1.98±0.10
	1.0/0.1/0.1	60	0.99±0.86	30	0.51±0.03
	1.0/0.2/0.2	75	4.98±0.01	50	0.69±0.21
	1.0/0.3/0.3	90	5.98±0.22	85	0.98±0.19
	1.0/0.4/0.4	95	6.38±0.99	95	1.79±0.67
	1.0/0.5/0.5	95	0.88±0.11	95	1.83±0.28
	0.5/0.1/0.5	30	1.63±0.41	40	0.72±0.55
	0.5/0.2/0.5	50	1.92±0.77	50	0.98±0.69
	0.5/0.3/0.5	50	2.01±0.89	65	1.28±0.83
	0.5/0.4/0.5	60	2.76±0.33	75	1.82±0.27
	0.5/0.5/0.5	90	3.81±0.99	90	2.01±0.08

IAA = Indole acetic acid; NAA = α-Naphthalene acetic acid; KIN = kinetin; TDZ = thiodizuron; BA = Benzyl adenine; 2-4-dichlorophenoxyacetic acid

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Table 3. Effect of growth regulators on in vitro rooting of B. monnieri L.

Growth regulators	Conc. (mg/l)	Average no. of roots	Root length (cm)
TDZ	0.01	5.80±0.96	1.60±0.51
	0.02	6.40±0.96	2.10±1.37
	0.03	6.80±0.96	3.00±1.15
	0.04	7.00±0.96	3.80±1.12
	0.05	8.00±0.96	4.01±0.96
	0.06	8.60±0.96	4.89±0.64
IAA	0.1	1.18±0.26	2.11±0.58
	0.2	2.36±0.26	3.15±0.77
	0.3	4.12±0.26	6.21±0.89
	0.4	5.01±0.26	6.99±0.22
	0.5	6.81±0.26	7.31±0.89

IAA = Indole acetic acid; TDZ = thiodizuron

the growth patterns of the plants were observed and recorded (Das *et al*., 2005).

Transplantation to cultivation field

The rooted plantlets from the polythene bags were transferred to various cultivation field soil types including red soil, clay soil and sandy soil and water was poured at regular intervals for the growth of the plants. While transfer, the polythene bags were cut using sterile blade for better rooting into the soil. After 45 d, the plants from various soil types were uprooted, washed and the shoot and root length were measured using meter scale.

Results and discussion

Plant tissue culture techniques for ornamental as well as herbaceous plants have been well established. *In vitro* propagation technique is a powerful tool for plant germplasm conservation hence tissue culture is the only rapid process for the mass propagation of plants. The ability to generate plants directly for explants is fundamental to clonal multiplication of elite germplasm *via* micropropagation (Ignacimuthu, 1997). Plant biotechnology is considered in a wide sense which comprises the various culture methods of plant organs and explants to facilitate experimental approaches with a large objective of developmental biology in grain legumes for crop modification (Ramawat, 2003). In the present study, to raise stock culture, nodal explants were taken from the field growing wild plants. The auxiliary bud was found initiated from both leaf and nodal explants on hormone free MS medium within 9 d. Shoot buds of *B. monnieri* L. were also initiated on the MS basal medium supplemented with 0.5 mg/l IAA and 0.5 mg/l KIN within 25-30 d from the nodal explants. Correspondingly, Thejavathi *et al.* (2001) has also been used shoot tip and nodal explants for the micropropagation studies of *B. monnieri* L. Most of the other research studies for other medicinal plant species have shown the use of cytokinin alone or in combination with other different concentrations for plant culture initiation. For eg. in *Paederia foetida* and *Centella asiatica* multiple shoots were obtained in MS medium supplemented with BAP 1.0 mg/l (Singh *et al.,* 1999) and *Rauwolfia serpentina* on MS medium supplemented with benzyladenine and NAA (Sehrawat *et al*., 2001).

Table 4. Effect of growth regulators on callus formation of B. monnieri L.

Growth regulator	Conc. (mg/l)	Callus formation (%)
NAA	0.1	20
	0.2	50
	0.3	72
	0.4	85
	0.5	90
2,4-D	0.1	50
	0.2	50
	0.3	80
	0.4	85
	0.5	90
TDZ	0.05	10
	0.10	20
	0.15	50
	0.20	60
	0.25	90
2,4-D/ TDZ	0.5/0.05	50
	0.5/0.10	50
	0.5/0.15	90
	0.5/0.20	75
	0.5/0.25	75
TDZ/NAA	0.05/0.1	25
	0.05/0.2	40
	0.05/0.3	50
	0.05/0.4	90
	0.05/0.5	90

NAA= α-Naphthalene acetic acid; TDZ= thiodizuron; 2-4-dichlorophenoxyacetic acid

Effect of growth regulators on induction of shoot buds

The present study was investigated the effect of various plant growth regulators (PGR) on shoot induction in 42 different combinations of which better results was observed in MS medium containing the following combinations of PGRs such as 0.5 mg/l NAA, 1 mg/l TDZ, 1.5 mg/l BA, 0.5 mg/l IAA and 0.5 mg/l BA + 0.5 mg/l KIN + 0.5 mg/l NAA. Remarkably, the MS medium containing 1 mg/l BA + 0.4 mg/l KIN + 0.4 mg/l NAA was showed excellent shoot formation. In this combination, the shooting response was observed as 100% and maximum shoot length was recorded as 6.38±0.99 cm after 3 weeks. Similarly from leaf explants, excellent shoot formation was observed in 0.5 mg/l KIN containing MS medium and maximum shoot length was recorded as 3.00±0.61 cm when compared with other growth regulators combinations (Table 2). The moderate level of shoot formation was observed with the other growth regulators. Similar type of work has been reported by Kameri *et al.* (2005) for *Wedelia chinensis.* MS media containing different concentrations and combinations of growth regulators were found to promote multiple shoots from both nodal and shoot tip explants. Multiple shoots start arising from nodes after 18-22 d of inoculation. Growth regulator combinations of BAP (2 mg/l) + IBA (0.5 mg/l) was producing maximum number of shoots (5.2±1.55) and longer shoot length (5.2±1.12 cm) from nodal segments. Thus, it has been reported that the plant growth regulators greatly influence the shoot induction but it is varied depends upon the selected plant

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species used.

Effect of growth regulators on root induction

The plant growth regulators not only control the shoot bud formation but also influence the root and callus induction. In accordance with this, the effect of IAA and TDZ on root induction was carried out. Among them, maximum number of roots (8.60±0.96) and root length (7.31± 0.89 cm) of the plants were noted in the MS medium containing 0.06 mg/l of TDZ and 0.5 mg/l of IAA respectively. The maximum number roots (6.81±0.26) were produced when the medium supplemented with IAA similarly maximum root length (4.89±0.64) contributed by TDZ at 0.06 mg/l on MS medium. The other concentrations of TDZ and IAA could be response moderate to minimum level (Table 3). Ahmed *et al.* (2007) has also been reported that the maximum root induction (97.66%) was observed in MS medium fortified with 0.1 mg/1 of IAA. The root induction of sweetener plant (*Stevia rebaudiana* Bertoni.) gradually decreased with increasing concentration of auxin except 0.1, 0.2, 0.5 mg/l IAA and 0.1 mg/l IBA. The rooting was not obtained on auxin omitted medium.

Effect of growth regulators on callus induction

Production of callus and its subsequent regeneration are the prime steps in crop plant to be manipulated by biotechnological means and to exploit somaclonal variation (Monirul Islam *et al.,* 2005). In the present study remarkable callus induction rate was observed with leaves were used as explants and the appearance of *B. monnieri* L. callus was globular and pale yellow in colour. The explants were enlarged within 12-14 d of inoculation; however callus formation was started after 20-25 d. Rapid callus growth (90%) response was observed in the MS medium with 0.5 mg/l NAA, 0.5 mg/l 2,4-D and 0.25 mg/l TDZ individually and 2,4-D 0.5 mg/l with TDZ 0.15 mg/l, TDZ 0.05 + NAA 0.4 mg/l and TDZ 0.05 + NAA 0.5 mg/l in combinations of growth regulators. Minimum callus formation rate (10%) was noted in the MS medium containing 0.05 mg/l of TDZ. The effect of growth regulators on callus formation was presented in Table 4. Tiwari *et al.* (1998) has also been reported greatest callus formation observed from nodal explants of *Bacopa* cultured on MS medium containing 0.5 mg/l 2,4-D. Hence, the present and previous findings revealed that the callus induction was greatly influenced by growth hormones namely auxin or cytokinin in the nutrient media depending on the source of explants. Among the different factors influencing callus induction and regeneration, genotype of the plants used, incubation condition and composition of nutrient media are the major factors that decide the *in vitro* raised cultures.

Transplantation of B. monnieri L.

The successfulness of the *in vitro* micropropagation is achieved when the plant survives in the field soil without support of growth hormones and synthetic media by transplantation technique. *B. monnieri* L. rooted plantlets from culture bottle were transferred to polythene bags containing eleven different sterile soil mixtures. Among them, maximum shoot length (10.78 ± 0.36 cm) and root length (7.89 ± 0.14 cm) were observed with plant grown in soil + VAM (10:2) and soil with vermicompost (4:1) respectively. The percentage of survival was recorded as 80-90% in the transplantation. Minimum shoot length (6.01±0.09 cm) and root length (4.29±0.12 cm) were recorded with plant grown in soil + flyash (1:2) and soil + agropeat (4:1) respectively. The other soil mixtures induced moderate to minimum shoot and root growth of plants (Table 5). Thus, it has been reported that the growth of plants in the soils was controlled by various plant growth promoting substances like organic compounds. Previous workers carried out similar transplantation studies at *in vitro* conditions using various plants such as *Terminalia arjuna* Roxb. (Thomas *et al*., 2003); *Withania somnifera* Dunal. (Sivanesan & Murugesan, 2005); *Morus indica* L. (Kavyashree *et al.,* 2005); *Hyptis suaveolens* Poit. (Jain & Chaturvedi, 2005) and *Musa acuminata* Colla (Anilkumar & Sajeevan, 2005).

Table 5. Growth response of plant at different soil mixtures

Soil mixtures	Conc. (%)	Shoot length (cm)	Root length (cm)
Soil alone	NA	7.2±0.16	6.01±1.23
Soil + VAM	10:1	10.49±0.11	7.59±1.01
Soil + VAM	10:2	10.78±0.36	7.63±0.18
Soil + Vermicompost	4:1	9.26±0.26	7.89±0.14
Soil + Vermicompost	4:2	10.46±0.27	7.38±1.28
Soil + Farmyard manure	4:1	7.92±0.02	6.79±1.12
Soil + Farmyard manure	4:2	6.56±0.46	5.19±0.98
Soil + Flyash	1:1	6.21±0.10	4.92±1.01
Soil + Flyash	1:2	6.01±0.09	4.89±0.83
Soil + Agropeat	4:1	7.38±0.02	4.29±0.12
Soil + Agropeat	4:2	8.39±0.31	7.12±1.43

NA = Not applicable; VAM = Vesicular arbuscular mycorrhiza

Transplantation of B. monnieri L. to soil

In cultivated field soil, the plant was grown well after 45 d. Remarkably, the maximum shoot and root length was recorded as 9.4±0.21 and 8.5±1.12 cm in clay soil and clay + red soils respectively, followed by red soil and clay with red soil combination, whereas the minimum growth rate of shoot length (2.5±0.07 cm) and root length (1.3±0.02 cm) were observed when it was grown in loam + sand soil combination. The percentage of the survival of the plant was 90% (Table 6). Thus, it is clearly revealed that the growth of the plant not only influenced by nature and texture of the soil, but also the physico-chemical parameters of the soil greatly affected the growth and biochemical processes of the plants.

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Conclusion

In the present study, a fruitful protocol was set up for *B. monnieri* through multiple shoot induction. This protocol can be exploited for commercial propagation and conservation of potential endangered medicinal plant resources.

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