ISSN 2394 - 7837

ARTICLE

Tissue culture studies in an important medicinal plant *Bacopa monnieri* Linn.

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Received 04/03/2018 Accepted 15/04/2018

Abstract

A protocol was developed for the in vitro propagation of *Bacopa monnieri*. For nodal segment culture MS medium with different concentrations of BAP (0.1 -2 mg/l) was used and BAP 1 mg/l was found to be the best concentration with the development of 30 shoots after 40 days. Leaf and stem explants produced white compact callus in presence of 2, 4-D (0.1 mg/l) in combination with BAP (0.5 mg/l)) and 2, 4-D (0.1 mg/l and 0.5 mg/l) in combination with kinetin (0.5 mg/l). Green callus was observed in medium with equal concentrations of BAP and 2, 4-D (0.5 mg/l). Callus regeneration was observed 0.5 mg/l BAP containing medium.

Keywords: 2, 4-D, BAP, Kinetin, In vitro propagation

Introduction

Mankind has been dependent on plants for food, flavours, medicinal and many other uses since ancient times. The use of herbal medicines is growing in developed countries and about 40% of compounds used in pharmaceutical industry are directly or indirectly derived from plants because the chemical synthesis of such compounds is either not possible or economically not viable. Therefore a large number of medicinal plant species are under threat of extinction because of their over exploitation. In vitro culture techniques are increasingly exploited for clonal propagation and conservation of valuable germplasm threatened for extinction. Micropropagation offers a great potential for large scale multiplication of such useful species and subsequent exploitation.

Micropropagation offers a good regular supply of medicinal plants, using minimum space and time (Prakash and Van Staden, 2007). Several medicinal plants have been micrpropagated till date. Eclipta alba (Husain and Anis, 2006), Cardiospermum helicacabum (Shekhawat et al, 2012), Bacopa monniera (Tiwari KN and Singh J, 2010), Adhathoda vasica (Abhyankar G and Reddy VD, 2007) and Guava (Amin and Jaiswal, 1987).

Bacopa monniera, also referred to as *Bacopa monnieri*, Herpestis monniera, water hyssop, and "Brahmi," is a member of the Scrophulariaceae family. It is a small, creeping herb with numerous branches, small oblong leaves, and light purple flowers. In India and the tropics it grows naturally in wet soil, shallow water, and marshes. The herb can be found at elevations from sea level to altitudes of 4,400 feet, and is easily cultivated if adequate water is available. It has been used in the Ayurvedic system of medicine for centuries. Traditionally, it was used as a brain tonic to enhance memory development, learning, and concentration. It provides relief to patients with anxiety or epileptic disorders, used in India and Pakistan as a cardiac tonic, digestive aid, and to improve respiratory function in cases of bronchoconstriction. Bacopa's antioxidant properties may offer protection from free radical damage in cardiovascular disease and certain types of cancer. Bacopa is used as a cardiotonic and also the extract of this species increased the thyroid hormone. It also has a protective effect against certain drugs and their negative side effects. The constituents responsible for Bacopa's cognitive effects are bacosides A and B.

The main objectives of this work were in vitro multiplication, callus induction and callus regeneration of B. monnieri in order to provide healthy planting materials and thus building a solid foundation for future pharmacological and biochemical studies.

Materials and Methods

Bacopa monnieri Linn. Plants (Fig.1) were grown in the garden of Department of Botany, Mahatma Gandhi College, Thiruvananthapuram. Nodes, leaves and internodes from young parts were used as explants in the present study.

All glasswares used were thoroughly washed with water, autoclaved and dried in an oven. Inoculations were done in a horizontal laminar air flow cabinet. Scalpels, surgical blades and forceps were autoclaved and their distal ends were immersed in 95% alcohol and alcohol flamed at periodic intervals during inoculations.

MS (Murashige and Skoog, 1962) medium was used for

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all the experiments. Sucrose 3% was used as carbon source. The pH of the medium was adjusted between 5.6 and 5.8 and gelled with 0.8% agar. This medium was sterilised by autoclaving at 120°C for 20min. The cultures were grown at 26±2°C, under an 12:12hr light:dark regime (3000lux).

The fresh sprouts from mature plants were thoroughly washed with tap water and treated with 0.5% solution of teepol for 15min. Then the explants were kept under running tap water for one hour. After that they were disinfected with 0.1% mercuric chloride for 5 min. and washed 4-5 times with sterile distilled water to remove mercuric chloride completely before inoculation.

For nodal segment culture MS medium with different concentrations of BAP (0.1-2mg/l) was used. Intermodal segments and leaves were used as explants for callus induction. MS medium with 2, 4-D (0.1-1.5mg/l) with BAP /Kinetin (0.5mg/l) were used for callus induction. The callus obtained were subcultured to medium BAP (0.1-2mg/l).

Shoots were rooted in MS basal medium and transferred to autoclaved vermiculite in plastic cups and watering was done. After three weeks of hardening the plantlets were transplanted to pots and then to field.

Results

Explants after proper sterilisation were inoculated in different media for inducing various in vitro responses.

Nodal Segment Cultures

Nodal segments were cultured in MS basal medium supplemented with different concentrations of BAP (0.1-2mg/l). The explants showed visible sprouting after one week of inoculation. After 14 days 8-10 shoot buds were produced in medium BAP 1mg/l. A maximum of 30 shoots appeared after 40 days in 1mg/l BAP (Fig. 2) which also showed adventitious roots at their lower ends. The nodal segment cultures showed varied responses in different concentrations of BAP. In lower (0.1mg/l) and higher concentrations (1.5mg/l) of BAP number of shoots were reduced.

Indirect Organogenesis

Callus induction from leaf and intermodal explants was observed one week after inoculation. The appearance of callus was first observed at the cut surface but later extended to the entire surface of the explant.

Leaf and stem explants produced white compact callus in presence of 2,4-D (0.1mg/l) in combination with BAP (0.5mg/l) (Fig. 3) and 2,4-D (0.1mg/l and 0.5mg/l) in combination with kinetin (0.5 mg/l). Green callus was observed in medium with equal concentrations of BAP and 2,4-D (0.5mg/l) (Fig.4). Keeping the concentration of BAP and kinetin constant (0.5mg/l), the concentration of 2,4-D was varied at an increasing rate (0.1-1.5mg/l). Increasing concentrations of 2,4-D produced brown compact callus and became necrotic eventually.

Callus Regeneration

Morphogenetic calli were transferred to regeneration medium containing BAP (0.1-2mg/l). White and green calli showed sprouting of shoot buds after 30 days (Fig. 5). A maximum of 10 shoots were initiated in 0.5 mg/l BAP containing medium. Only 3-4 shoot buds were initiated in medium containing 1.5mg/l BAP and in higher concentrations initiation of shoot buds were inhibited.

Rooting, Hardening and Transplanting

The plantlets with roots were transferred to plastic cups with sterile vermiculite and covered with plastic bag with holes to maintain humidity. They were watered at regular intervals and transferred to pots and were kept in green house and all the plants survived.

Discussion

Bacopa monnieri is one of the important medicinal plants which has gained considerable importance in ayurvedic medicine due to its use as vitalising tonics and other similar preparations.

Nodal segment culture

Multiple shoots were initiated through depression or elimination of apical dominance when the apical meristem was injured or surgically separated from axillary buds. Nodal segments cultured in MS medium fortified with BAP (0.1-2mg/l) produced multiple shoots. Shoot multiplication in the presence of BAP has been reported in different species. A range of cytokinins were tested (6-benzylaminio purine, kinetin, thidiazuron and 2-isopentenyl adenine) for multiple shoot induction in Baccopa. Of the four cytokinins tested TDZ (6.8μM) and BAP (8.9μM) proved superior to other treatments (Tiwari KN and Singh J, 2010). In general, BAP was more effective than other cytokinins. The stimulating effect of BAP on multiple shoot formation has been reported for several medicinal plant species (Wang et al, 2004).

Indirect Organogenesis

In the present study, higher concentration of 2,4-D was found to suppress morphogenesis. In Baccopa callus developed from leaf explants on MS medium supplemented with 2,4-D (0.5mg/l) and BAP (0.5mg/l) gave best results. The callus produced was and green with fast growth. Similar results was observed in Eclipta alba shown by Zafar and Sagar (1999). Some researchers observed similar results on 2, 4-D and BAP supplemented media in Tridax procumbens L. (Wani et al, 2010) and Gardenia latifolia Ait. (Lakshmi and Reddy, 2012).

Skoog and Miller were the first to report that the ratio of auxin and cytokinin determined the type and extent of organogenesis in plant cell cultures. Considerable variability exists among genera, species and even cultivars in the type and amount of auxin and cytokinin required for shoot induction. The cytokinins are generally added to culture to stimulate cell division, shoot formation, axillary shoot pro-

liferation and to inhibit root formation.

To induce shoot, the callus was grown in fresh MS medium containing different concentrations of cytokinin. The most suitable hormones concentration to induce explant regeneration was 0.5mg/l BAP where maximum number of 10 shoot buds were formed from callus within 6-7 weeks. Cytokinins are reported to promote chloroplast development and chlorophyll synthesis, by enhancing the formation of one or more proteins to which chlorophylls bind and become stabilized (Salisbury and Ross, 1986). Whipkey et al (1992) reported organogenesis from *Artemisia annua* callus using BA alone. In *Casuarina cunninghamiana* Miq. BAP and NAA induced shoot differentiation from callus (Jiang et al, 2012). Similarly in *Saussurea obvallata*, callus differentiation was obtained on BA and NAA (Dhar and Joshi, 2005). Many authors report that cytokinin is required in optimal quantity for shoot proliferation in many genotypes but inclusion of a low concentration of auxin along

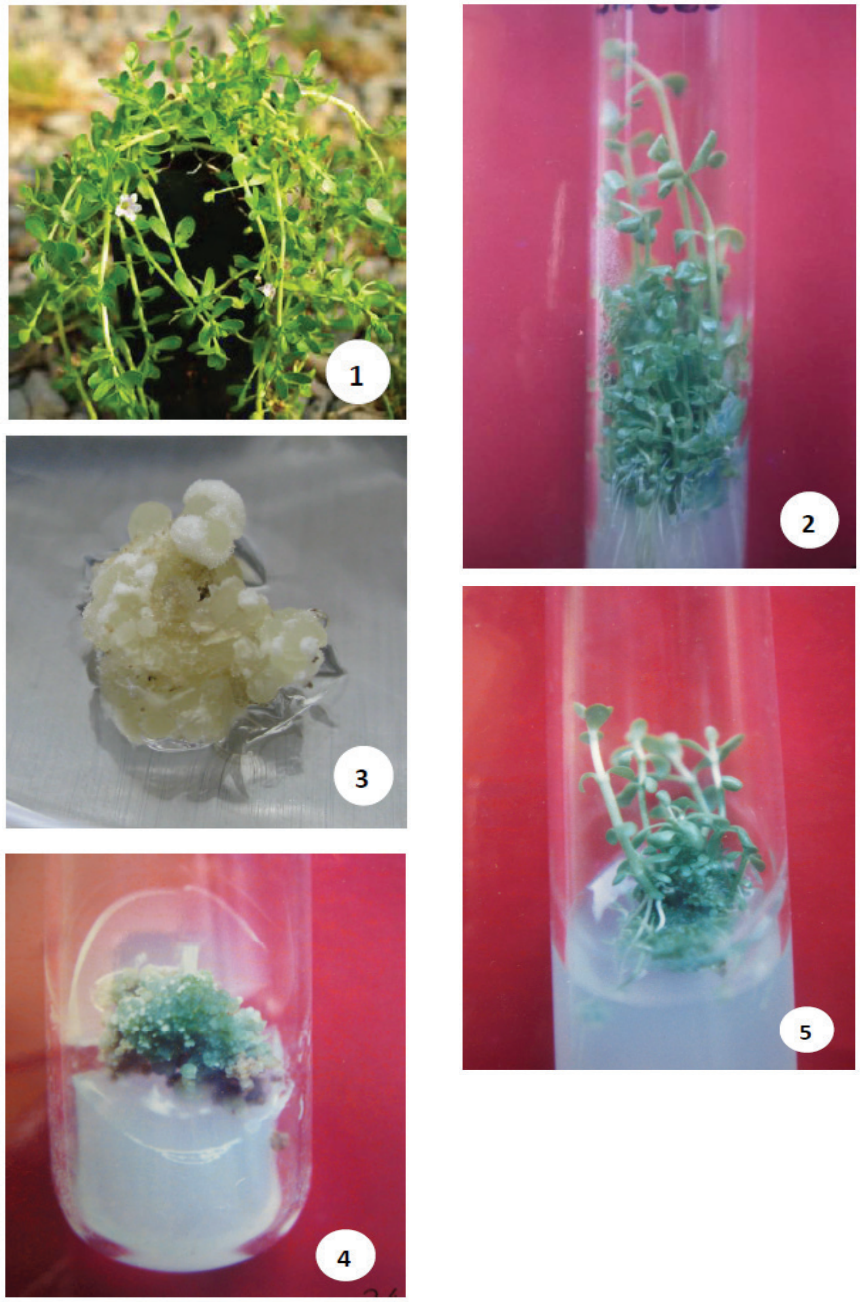

Figure 1. Fig.1. Habit of *Bacopa monnieri*.
Figure 2. High rate of shooting from nodes in BAP 1mg/l containing MS medium.
Figure 3. White compact callus in MS medium with BAP (0.5mg/l) and 2,4-D (0.1mg/l).
Figure 4.Green callus in MS medium fortified with BAP (0.5mg/l) and 2,4-D (0.5mg/l).
Figure 5. Development of maximum of 10 shoots from callus in BAP 0.5mg/l containing MS medium.

with cytokinin increases the rate of shoot multiplication.

Conclusion

The major results are summarized below of the present study are:

1. Nodal segments cultured on MS medium containing BAP (0.1-1.5mg/l) produced multiple shoots. Medium containing 1mg/l BAP induced a maximum of 30 shoots.
2. Callus was initiated from leaf and intermodal explants in MS medium with 2,4-D (0.1-1.5mg/l) and BAP /Kinetin (0.5 mg/l). Leaf and stem explants produced white compact callus in presence of 2,4-D (0.1mg/l) in combination with BAP (0.5mg/l) and 2,4-D (0.1mg/l and 0.5mg/l) in combination with kinetin (0.5mg/l). Green callus was observed in medium with equal concentrations of BAP and 2,4-D (0.5mg/l).
3. Morphogenetic white and green calli showed sprouting of shoot buds after 30 days in regeneration medium containing BAP (0.1-2mg/l).

Acknowledgement

The authors are grateful to the Principal, Mahatma Gandhi college for providing the facilities for conducting the above work.

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