International Journal of Aquatic Science
ISSN: 2008-8019
Vol 12, Issue 03, 2021

Role of micropropagation for propagating medicinal plant Bacopamonnieri L

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Abstract: The role of micro propagation for propagating medicinal plant Bacopa monnieri L through MS medium was investigated.To perform the experiments soil beds were prepared for planting the plant materials and after six months of plantation the internodes of the B. monnieri L were collected from the beds as explants for the micro propagation under aseptic condition.

Sterilised explants of B. monnieriL were making ready to designed and perform the experiments of a) Invitro propagation under aseptic condition b) Effect of growth regulators on micro propagation.

To perform the experiments culture media were prepared and to study the effect of BAP and Kinetin a series of experiments were performed.

In the first experiments the results showed that after 15 days of inoculation direct organogenesis occur in BASAL medium. The induction of callus formation both from leaf and internodes does not occur. The formation of lateral shoot, length of lateral shoot and number of roots were less.

From the second experiments it was found that after 15 days of inoculation direct organogenesis occur in the BAP. The induction of callus formation both from leaf and internodes occur.

From the third experiments it is found that after 15 days of inoculation direct organogenesis occur in the BAP+KINETIN. The induction of callus formation both from leaf and internodes occur. The formation of lateral shoot, length of lateral shoot and number of roots are less than BAP treatment.

From the experiments it was found that internodes and leaf segments cultured on MS media supplemented with plant hormones like BAP, Kinetin induces organogenesis from callus and subsequent rooting. Maximum number of lateral shoot development observed in MS media supplemented with plant hormone BAP at a concentration of 0.4mg/L and minimum number of lateral shoot found on BASAL medium.

Callus formation from the basal cut end of the internodes and leaf segments of explants were recorded in all concentration of BAP and Kinetin. However, callus induction from basal cut end of internodes and leaf segments was not observed even after 12 days of experiments treated with BASAL medium.

From the present study it was also observed that shoot bud induction occurred in all plant hormone treatments and also observed that callus induction from basal cut end suppress the induction of growth and development of lateral shoot from upper portion.

Induction of root formation was also recorded from the present investigation in almost all treatments. The highest number of root induction was recorded with the treatment of BAP at a concentration of 0.4mg/L.

From the present investigation it was also found that all plant hormones induce the growth of shoot length. Longest shoot is recorded on BAP with formation of maximum number of node per shoot.

Keywords: Bacopamonnieri, Organogenesis, BASAL, BAP, Kinetin.

1. INTRODUCTION

Indian forests are abode of medicinal plants. Medicinal plants are used as a source of medicine in the form of isolates extractives or a lead compounds for synthetic optimization.
According to WHO (1989) in a medicinal plant contain one or more medicinal substances that can be used for therapeutic uses or contain substances that can be used as precursor for chemotherapeutic uses.
According to Islam (1997), micro propagation or in vitro culture technique refers as the in vitro cultivation of plant seeds or various parts of plant organs, embryos, tissues, single cell and protoplast. The cultivation process is carried out in a nutrient medium under aseptic conditions. The first concept of micro propagation was developed by German Botanist Gottlieb (1902).According to Debnath et.al. (2006) and Benniamin et.al. (2004) micro propagation is the tool for production of high quality plant.
Plant tissue culture is one of the most rapidly growing areas of Bio- technology because of its high potential to developed improved crops and ornamental plants. Development of tissue culture has helped to produce several pathogen free plants, besides the synthesis of many biologically important compounds.
In micro propagation system the highly mature and differentiated cells can retain the ability of change to meristematic state and differentiate into a whole plant.
In India a lots of work have been done in micro propagation .Tiwari et.al.(1998), had done shoot regeneration and somatic embryogenesis from different explants of B. monnieri L
In 1999, Rajanul and Shrivastav studied shoot regeneration on Bacopamonnieri L through tissue culture. They induced adventitious shoots from leaf and stem explants of Bacopamonnieri L. through MS medium, supplemented with Benzyl adenine and kinetin.
According to Binita et.al. (2005), Bacopamonnieri L is a rapid, efficient and cost effective micro ricus. and it is an valuable Indian medicinal plant(Neelam et.al.,2016)
AlijandroSalvio et.al. (2005), worked on the Bacopamonnieri L in vitro polypolidization,which is very much commercially important in Argentina.
Tiwari et.al. (2005), worked in vitro propagation of BacopamonnieriL.In their investigation they used a range of cytokinins for multiple shoot induction from node, internodes and leaf explants.
Lie et.el. (2006), studied on plant regeneration via somatic embryogenesis of Blymussbiricus. In this experiment MS medium was supplemented with 5.0mg/L 2,4-D and 0.05mg/L Kinetin in the dark at 26Degree celcious, the callus were produced.
Mahapatra and Rath(2006), worked on in vitro study of BacopamonnieriL, an important medicinal plant with reference to its biochemical variation. In the experiment they found that inBacopamonnieri L. plants micro propagation was achieved on MS medium and B5 medium with BAP and NAA using leaf explants and nodal segment.
Debnath et.al.(2006), worked micro propagation as a tool for production of high quality of plant mainly based on medicinal value.

By keeping above observation an attempt has been made to study micro propagation as a method for propagating plants in mass and also grow plants in large numbers in invitro condition and also aseptic and controlled environment. In a traditional stem cutting method only two or three new plants can be produce. But under micro propagation techniques by taking square centimetre of meristematic cell can produce thousands of new plants. In this project medicinal plant Bacopamonnieri L. plant has been selected to produce disease free medicinal plant through micro propagation technique, so that a large number of plants can be produced in a short period and can also accelerated growth compare to plants produced by traditional methods.

2. MATERIALS METHODS

Bacopamonnieri L. commonly known as " Brahmi", is a perennial creeping herb, belongs to family scorphulariaceae, whose habitat includes wetlands and muddy shores. The leaves of this plant are succulent and relatively thick.
To perform the experiments soil beds are to be prepared for planting the plant material B. monnieriL plants. After five months the internodes of the B. monnieri L plants are to be collected from the beds as explants for the micropropagation under aseptic condition. After collection explants are sterilised, then explants were washed in running water and treated with Teepol. Then materials were washed several times with distil water.
The present investigation is designed with the following parameters:
a) Invitro propagation Bacopamonnieri L plant under aseptic condition.
b) Effect of plant growth regulators on micro propagation and their growth.

Plant materials and Sterilization of explants:
A bed is prepared for cultivation of medicinal plants Bacopamonnieri L plants. After four months of cultivation plant materials in the form of internodes and leaf portion are taken as explants for the experiments and sterilized with 70% alcohol and vaporized on sterile hood of laminar flow.The process is repeated several times and finally the plant explants are ready for inoculation in the nutrient medium.

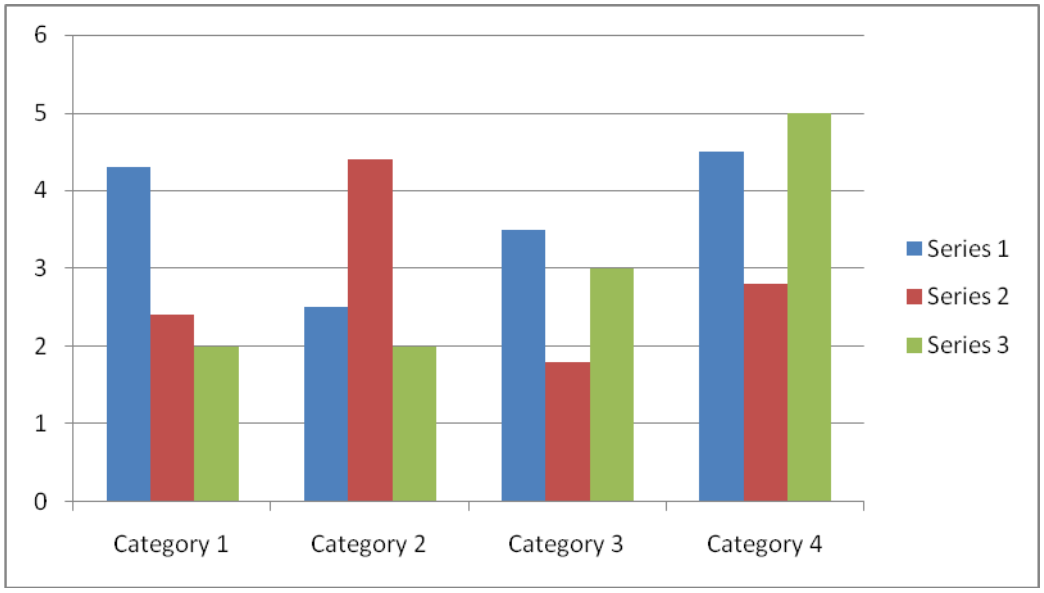
Inoculation of explants:
At the time of inoculation the explants are taken on a pair of sterilized Petridis. After that with the help of a pair of flame sterilized forceps the plant explants are transferred within the media supplemented with
i) Different concentration of BAP
ii) Different concentration BAP+ Kinetin
iii) one replica without hormone
In the present study three replications are prepared for each treatment and each treatment is repeated thrice and each observation is taken for 15 days.

3. EXPERIMENTAL RESULTS

Experiment No. 1: Effect of BASAL on the *in vitro* growth of shoot, root and callus formation *in Bacopamonneri* L. plants for 15 days inoculation**.**

Sl. No.	**BASAL WITHOUT**	**No. Of lateral**	**Length of lateral**	**No. Of node/multiple**	**No. Of root**	**% OF Callusing**

	Plant growth regulator	shoot	shoot(mm)	shoot.	explants	
1	BASAL	28	2	3	18	-
2	BASAL	35	3	4	22	-
3	BASAL	33	2.8	2	20	-
4	BASAL	25	1.9	3	17	-



From the results it was observed that during the first experiments which was considered after 15 Days of inoculation direct organogenesis occurred in BASAL medium. The induction of callus formation both from leaf and internodes does not occur. The formation of lateral shoot, length of lateral shoot and numbers of roots are less number.

Experiment No. 2: Effect of plant growth hormone BAP on the *in vitro* growth of shoot, root and callus formation *in Bacopamonneri* L. plants for 15 days inoculation.

Sl. No.	Plant growth regulator BAP	No. Of lateral shoot	Length of lateral shoot(mm)	No. Of node/multiple shoot.	No. Of root explants	% OF Callusing
1	BAP(0.2)	50	5	3	23	100
2	BAP(0.3)	56	6	4	25	100
3	BAP(0.4)	57	5.5	2	24	100
4	BAP(0.5)	45	4	3	22	100

From the results it was observed that during the first experiments which was considered after 15 Days of inoculation direct organogenesis occurred in the BAP. The induction of callus formation both from leaf and internodes occurred.

Experiment No. 3: Effect of plant growth hormone BAP and Kinetin on the *in vitro* growth of shoot, root and callus formation *in Bacopamonneri* L. plants for 15 days inoculation.

Sl. No.	Plant growth regulator BAP+Kinetin mg/litre	No. Of lateral shoot	Length of lateral shoot(mm)	No. Of node/multiple shoot.	No. Of root explants	% OF Callusing
1	BAP+ Kinetin(0.2)	41	4	2	20	100
2	BAP+ kinetin(0.3)	42	6	4	21	100
3	BAP+ Kinetin(0.4)	40	5	3	22	100
4	BAP+ Kinetin(0.5)	39	3	2	20	100

From the results it was observed that during the first experiments which was considered after 15 Days of inoculation direct organogenesis occurred in the BAP+KINTIN. The induction of callus formation both from leaf and internodes occurred. The formation of lateral shoot, length of lateral shoot and numbers of roots are less than BAP treatment.

From the above observation it was found that internodes and leaf segments cultured on MS media supplemented with plant hormones like BAP, Kinetin induces organogenesis from callus and subsequent rooting. Maximum number of lateral shoot development was found on MS media supplemented with plant hormone BAP at a concentration of 0.4mg/L and minimum number of lateral shoot was recorded on BASAL medium.

Callus formation from the basal cut end of the internodes and leaf segments of explants was recorded in all concentration of BAP and Kinetin. However, callus induction from basal cut end of internodes and leaf segments was not observed even after 12 days of experiments treated with BASAL medium.

From the present study it was also observed that shoot bud induction occurred in all plant hormone treatments. In the present study it was observed that callus induction from basal cut end suppress the induction of growth and development of lateral shoot from upper portion.

Induction of root formation was also recorded from the present investigation in almost all treatments. The highest number of root induction was recorded with the treatment of BAP at a concentration of 0.4mg/L.

From the present investigation it was also found that all plant hormones induce the growth of shoot length. Longest shoot was recorded on BAP with formation of maximum number of node per shoot.

4. DISCUSSION

Importance of medicinal plants is both locally and globally. Therefore, medicinal plants need conservation for their existence and future availability. In-vitro cultivation of medicinal plants provides constant supply from in-vitro cultivated sources. In 1981 Arora and Bhajwani has been successfully developed in-vitro multiplication protocol for the conservation endangered plant. The success of in-vitro culture is largely depend on explants choice, medium composition and control of physical environment (Thorpe and Patel, 1984)

Keeping in mind the importance micro propagation, in the present investigation an attempt has been made to study the effect of growth hormones BAP, BAP+Kinetin on shoot bud proliferation from intermodal and leaf segment of BacopamonnierL.plants.

Status of materials i.e. Juvenile and Adult and position of materials(original) in the plant reflects the endogenous level of hormone which have an important effects on the formation of cell division and both organ and embryo formation. Thus the physiological state of the explants depends on the nature of the level of endogenous hormone. In 1957, Skoog and Miller suggested that concentration of growth hormone had a critical role in culture medium for morphogenesis.

Benniamin et.al.(2004) and Hoqueet.el.(1999) reported the role of BAP in breaking of bud in medicinal plants Crataeva manga . Mahapatra and Rath(2005), reported the role of BAP in regeneration of explants of Bacopamonnieri L plants from leaf and nodal explants at higher percentage.

In micro propagation, organogenesis is a process of differentiation where plant organs root, shoot and buds are formed. Organogenesis is an outcome of the process of differentiation of cells.

Regeneration of rooting as a result of in vitro micro propagation from micro shoot consider as an important technique. Generally roots are induced by presence of plant hormones namely auxin in the medium. In the present investigation it was observed that micro shoot bearing callus from the basal portion cultured on MS medium, supplemented with hormone BAP initiate rooting from internodes and leafy portion of Bacopamonnieri L plants.

From the present study it was observed that call genesis occur from the intermodal and leaf segments of Bacopamonnieri L plants cultured on cytokines and auxin enriched MS medium.

According to Marks and Seinpson(1994) call genesis is due to action of accumulated auxin at the basal cut ends which stimulates cell proliferate especially in the presence of cytokine. After 15 days from callus induction shoot bud induction was recorded on MS medium supplemented with BAP. Maximum number shoot bud different ion was recorded on 0.5 mg/litre BAP.

According to Singh and Tiwari(2002), shoot bud regeneration from the explants of Bacopamonnieri L. plants was induced by cutting internodes and leaf explants on MS medium supplemented with an antibiotic or fungicide Bavistin. Bavistin showed kinetin like activity. At optimum adventitious shoot bud induction occurred at 300mg/lit bavistin from internodes explants. In present investigation the MS media is also supplemented with various concentrations of cytokines and Auxin for regeneration of bud and roots. Because Auxins, Kinetins are the important plant hormones. They stimulate root, shoot and internodes growth. The in vitro regenerated shoots were elongated and rooted before transferred to the field with 85% survival.

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