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EFFECT OF DIFFERENT PLANT GROWTH REGULATORS ON *IN-VITRO* PROPAGATION OF *BARLERIA PRIONITIS* L. – A THREATENED MEDICINAL PLANT.

Corresponding Author

SHABIR A. LONE

Molecular Biology and Seed Technology Laboratory, Govt. Motilal Vigyan Mahavidyalaya

Co Authors

A.S. YADAV[1], YOGESH BADKHANE[1], AJIT K. SHARMA[1], SAJAD HUSSAIN BAKHSHI[2] AND D.K. RAGHUWANSHI[1].

[1]Molecular Biology and Seed Technology Laboratory, Govt. Motilal Vigyan Mahavidyalaya
[2]Department of Bioscience Barkatullah Vishwavidhyalaya Bhopal

ABSTRACT

Barleria prionitis L. is a threatened medicinal plant belongs to Acanthaceae family. No protocol has been developed yet for plant regeneration of this important medicinal plant. The objective of this study was to develop an efficient in vitro propagation protocol for *Barleria prionitis L*. Significantly more shoots (10 shoots per nodal explants) were induced on a medium containing 0.4mg/l thidiazuron (TDZ) and 1.5mg/l 6-benzylaminopurine (BA) than any other treatment. Sub-culturing regenerated maximum rate of shoot multiplication on a medium with 5.0 mg/l 6-benzylaminopurine (BA) with 2.0 mg/l ά-naphthaleneacetic acid (NAA). *In-vitro* produced shoots exhibited good rooting response in half strength MS medium supplemented with different concentrations of auxins. After 40 days of hardening of plantlets almost 99% of rooted plants could be successfully transferred and acclimatized *ex-vitro* under poly-house conditions, followed by their establishment in the field.

KEYWORDS

Tissue culture; conservation strategies; *Barleria prionitis L*; Thidiazuron.

INTRODUCTION

Barleria prionitis L. is an erect bushy shrub of family Acanthacae. The height is about 1.5 metres. The leaves are narrowly to broadly elliptic lanceolate, entire, and appraised hairy beneath. The flowers are yellow, in axillary, spikate clusters. The flowering season is September to March. In Ayurveda the leaves and the tender branches are used for treatment of toothache, strengthening of gums, whooping cough and premature ejeculation. Whole-plant extracts of porcupine flower contain iridoid glycosides, barlerin, and verbascoside, which have shown potent activity against respiratory syncytial virus *in vitro* and may account for the plant's use in treating fever and several respiratory diseases in herbal medicine[2].

The plant leaves and roots are used for a variety of purposes in traditional Indian medicine. For example, the leaves are used to promote healing of wounds and to relieve joint pains and toothache[15]. Because of its antiseptic properties, extracts of the plant are incorporated into herbal cosmetics and hair products to promote skin and scalp health[16,17,19]. The flavonoids extracted from callus culture of *Barleria prionitis* L. showed maximum antibacterial activity against S. *aureus*[4]. In 2006 survey cum study on Threat Assessment For Prioritized Medicinal Plant Species Of Madhya Pradesh by Madhya Pradesh Biodiversity Board through Foundation for Revitalization of Local Health Traditions (FRLHT) listed *Barleria prionitis* L. under vulnerable species of Central Eco-Region[20].

During literature review no report on micro-propagation of *Barleria prionitis* L. has been found. Hence, with two main objectives, the current study was undertaken: firstly to explore the possibility of developing an efficient culture medium for multiplication of *Barleria prionitis* L and secondly to assess the efficacy of TDZ, a growth regulating compound in inducing regeneration of *Barleria prionitis* L. along with other traditionally used growth hormones. There are many reports showing that the application of thidiazuron (TDZ) results in a better shoot regeneration capacity in comparison with other cytokinins[1,22]. The list of plant species exhibiting increased morphogenesis in the sole presence of TDZ has continued to increase over the years, facilitating the improvement of tissue culture technology[14]. In this study, an efficient procedure for in vitro multiplication of *Barleria prionitis* L which may facilitate conservation efforts of this threatened medicinal plant is being reported.

MATERIALS AND METHODS

Explants of *Barleria prionitis* L were collected from healthy plants growing in the Botanical Garden of Govt. Motilal Vigyan Mahavidyalaya, Bhopal (M.P.). Axillary, apical segments and nodal segments were used a explants. The explants were surface sterilized with 0.1% mercuric chloride solution for 2 minutes. The disinfectant was removed by several successive washes with sterile distilled water. The cut surfaces exhibiting mercuric chloride damage were aseptically trimmed with a sharp and sterile surgical blade.

MS basal medium[12] supplemented with 3% Sucrose and 1% Agar (Bacteriological Himedia, India) was used in the study. The medium was supplemented with various concentrations of BAP (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg l^{-1}), Kn (0.1, 0.2, 0.4, 0.5 and 0.8 mg l^{-1}), IBA (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg l^{-}

^{1}), IAA (0.1, 0.2, 0.4, 0.5 and 0.8 mg l^{-1}), NAA (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg l^{-1}), TDZ (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg l^{-1}) and different combinations of TDZ (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg l^{-1}) with BAP (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg l^{-1}) and different combinations of IAA (0.1, 0.2, 0.4, 0.5 and 0.8 mg l^{-1}), with NAA (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg l^{-1}) depending upon the objective of the experiment.

The pH of the media was adjusted to 5.8 prior to the addition of gelling agent. The media was autoclaved at 121^{0}C for 15 minutes. Cultures were incubated at 25 $\pm$ 2^{0}C under 16 hours of photoperiod from cool white fluorescent tube giving 1000lux at culture level. Observations were made at regular intervals of 10 days.

STATISTICAL DESIGN

5 replicates for each treatment were tested for shooting medium and 5 replicates for each treatment were tested for rooting medium. Data from each experimental stage were analyzed separately and mean and standard error of each experimental stage were calculated.

RESULTS AND DISCUSSION

SHOOT INDUCTION AND PROLIFERATION

Explants of *Barleria prionitis L*. were inoculated in MS medium supplemented with different concentration of Cytokinins and there effect on shoot regeneration was summarized. The effect of BAP on shoot regeneration was found to be highest in the concentration of (2.0 mg l^{-1}) and recorded 30 % of explants produced shoots and maximum number of shoots per explant was recorded 6 with shoot length varying between 3-4 cm. In this medicinal plant shoot regeneration was also found in the sole presence of auxin a-naphthaleneacetic acid (NAA) which is found in rare cases. The effect of NAA on shoot regeneration was found to be highest in the concentration of (0.8 mg l^{-1}) and recorder 15 % of explants producing shoots and maximum number of shoots per explant was recorded (4) with shoot length varying between 1-2 cm. The combined effect of BAP and NAA was found to be highest in the concentration of BAP (2.0 mg l^{-1}) and NAA (0.5 mg l^{-1}) and recorded 75% of explants producing shoots and maximum number of shoots per explant was 8 with shoot length varying between 4-5 cm (Fig 1. A).

The effect of TDZ on shoot regeneration was found to be highest in the concentration of TDZ (0.4 mg l^{-1}) with BAP (1.5 mg $l^{-1)}$ and recorded 90% of explants producing shoots and number of shoots per explant was recorded (10) with shoot length varying between 4-5 cm (Fig.1 B). Above this concentration the percentage of explant producing shoots, number of shoots per explant and height of shoots were decreased. (Table1).

Sub-culturing regenerated maximum rate of shoot multiplication on a medium with 5.0 mg/l 6-benzylaminopurine (BA) with 2.0 mg/l ά-naphthaleneacetic acid (NAA).

It was found that TDZ addition in the medium enhances the shoot proliferation, thus acted as a potent growth regulator for shoot organogenesis in *Barleria prionitis* L., also reported earlier in other plants viz; *C. maritima* Linn.[3], *Glycine max* (L.) Merr.[7], *Hypericum perforatum* cv 'Anthos[13], *Rosa damascene*[9] and *Artemisia judaica* L.[11]

Table 1

Effect of growth regulators on shooting pattern during in-vitro culture of Barleria prionitis L. on MS media.

Growth regulators [mg l^{-1}]			Percentage of explants producing shoots	Number of shoots per explant Mean* ± SE	Height of shoots (in cm) Mean ± SE
BAP	NAA	TDZ			
1.5	0	0	20	3.6 ± 0.33	2.36 ± 0.06
2.0	0	0	**30**	**5.3 ± 0.32**	**3.10 ± 0.05**
2.5	0	0	18	6.0 ± 0.0	2.73 ± 0.05
0	0.4	0	10	1.6 ± 0.33	1.36 ± 0.06
0	0.5	0	10	2.3 ± 0.32	1.36 ± 0.03
0	**0.8**	**0**	**15**	**3.3 ± 0.74**	**1.50 ± 0.03**
1.5	0.4	0	70	6.3 ± 0.33	3.06 ± 0.02
2.0	**0.5**	0	**75**	**7.6 ± 0.33**	**4.43 ± 0.12**
2.5	0.8	0	75	7.5 ± 0.76	4.06 ± 0.06
1.5	**0**	**0.4**	**90**	**10.0 ± 0.32**	**4.50 ± 0.03**
2.0	0	0.5	60	9.3 ± 0.34	4.36 ± 0.03
2.5	0	0.6	55	9.0 ± 0.34	4.34±0.07

* *Mean and standard error of 5 replicates each.*

Table2

Effect of growth regulators on rooting pattern of Barleria prionitis L.during in-vitro culture (1/2 strength MS media)

Growth regul [mg l^{-1}]		Percentage of shoots producing roots	Number of roots per shoot Mean* ± SE	Days for emergence of roots	Root length (in cm) Mean ± SE
IAA	IBA				
0.3	0	80-90	1.4 ± 0.25	19	1.18 ± 0.06
0.4	0	90.0	1.4 ± 0.27	18	1.46 ± 0.24
0.5	**0**	**100.0**	**1.6 ± 0.20**	**17**	**1.54 ± 0.02**
0	0.3	90.0	1.8 ± 0.20	18	1.26 ± 0.06
0	**0.4**	**100.0**	**2.3 ± 0.25**	**16**	**1.16 ± 0.02**
0	0.5	80-90	1.4 ± 0.24	18-19	1.06 ± 0.07
0.3	0.3	90-100	1.4 ± 0.20	17-18	1.44 ± 0.198
0.4	0.4	90-100	1.2 ± 0.19	17	1.46 ± 0.03
0.5	**0.5**	**100**	**1.6 ±0.20**	**18-20**	**1.88 ± 0.06**

* *Mean and standard error of 5 replicates each.*

ROOT INDUCTION AND ACCLIMATIZATION

Different auxins in different concentrations and combinations were used for root induction in *Barleria prionitis L*. The effect of IAA on root induction was found to be highest in the concentration of IAA (0. 5mg l^{-1}) half strength MS media. 100% of shoots produced roots and maximum number of roots per shoot was recorded 2.0 with root length varying between 1-2 cm and it took 17 days for

emergence of roots. The effect of IBA on root induction was found to be highest in the concentration of IBA (0.4 mg l^{-1}) half strength MS media. 100% of shoots producing roots and maximum number of roots per shoot was recorded (3.0) with root length varying between 1-2 cm and it took 16 days for emergence of roots (Fig.1. C). The combined effect of IAA and IBA was found to be highest in the concentration of IAA (0.5 mg l^{-1}) and IBA (0.5 mg l^{-1}) and recorded 100% of shoots producing roots and maximum number of roots per shoot being 2.0 with root length varying between 1-2 cm and it took 18-20 days for emergence of roots. In this study, the rooting response of microcuttings on an IBA containing medium was better than that in IAA containing medium. Being of a stable nature, IBA is the preferred auxin for adventitious root initiation in many species e.g. *Syzygium cuminii*[21]. In addition, Hutchinson (1981) and Litz, and Jaiswal[10] found IBA to be a superior auxin compared with IAA or NAA for the *in vitro* rooting of apple shoots.

During hardening, the plantlets were irrigated with one fourth strength of MS basal medium (without sugar and vitamins) for one week. This helped the plantlets to recover the shock resulting from a change of environment. Earlier, Kar and Sen[8] reported maintenance of plantlets in half strength of MS medium, prior to their transfer to the soil. Humidity was maintained by covering it with rigid plastic cover and frequently spraying of water. Similar process of maintaining humidity was practiced for hardening of banana[6] and *Alpinia*[18]. The hardened plants were transferred to the pots after 45 days of good growth (Fig. 1.D). Almost 99% of plantlets survived in pots. All hardened plants were successfully established in field, where the plants appeared morphologically uniform with normal leaf form, shape and growth pattern. Thus the protocol described here could be of considerable commercial importance for large scale propagation of *Barleria prionitis* L. an important and threatened medicinal plant.

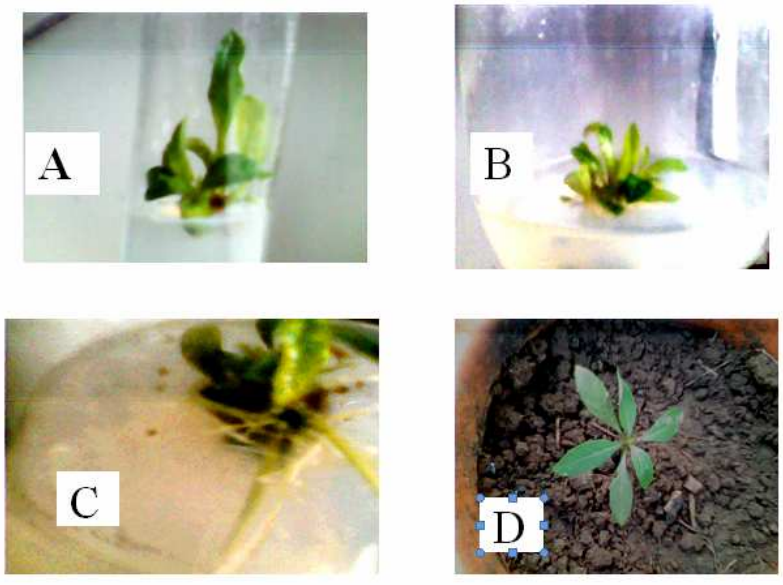

Fig.1

(A-D) Stages of in-vito propagation in Barleria prionitis L.

A. Shoot induction in MS medium supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA.
B. Multiple shoot induction MS medium supplemented with 1.5 mg/l BAP and 0.4 mg/l TDZ.
C. Root induction in MS medium suplemnted with IBA (0.4 mg l^{-1}) half strength MS
D. Hardened plant in pot containing potting mixture of soil, sand and farmyard manure after 5 weeks of transfer.

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