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IN VITRO SHOOT PROLIFERATION IN *BARLERIA PRIONITIS* LINN. FROM NODAL EXPLANTS

DEEPA AND ASHA GOSWAMI
Department of Botany, Govt.
Dungar College, Bikaner – 334 001

An efficient protocol for *in vitro* shoot proliferation of *Barleria prionitis* has been developed by using cotyledonary node as explant. MS medium supplemented various concentrations of BAP (ranging from 0.2 mg/l to 2mg/l) with all possible combinations of NAA and IAA separately in four different concentrations (0.1, 0.2, 0.5 and 1.0 mg/l) were used for selecting best suitable BAP-NAA and BAP-IAA combinations. Percentage of shoot proliferation increased from 0.2 to 0.5 mg/l BAP and reached maximum (76% and 72%) at 1.0 mg/l BAP with 0.1 mg/l NAA and IAA, respectively. Higher concentrations of BAP (1.5 to 2.0 mg/l) as well as of auxins (0.2, 0.5 and 1.0 mg/l) suppressed shooting and induced callus formation.

Keywords : *Barleria prionitis*, cotyledonary node, growth hormones, shoot proliferation.

Barleria prionitis Linn. (commonly called as 'Vajradanti') is a herb common in India. It is a xerophytic plant of family Acanthaceae and is native of Tropical East Africa and Asia (Burkill *et al.* 1985). It is found in many parts of the world, like USA, Australia, Indonesia, Malaysia, Philippines, Naharu. It is widely cultivated as an ornamental and as a hedge. In Ayurveda, the leaves and the tender branches of this plant are used for treatment of tooth ache, strengthening of gums, whooping cough, premature ejaculation, leucoderma, blood-complaints, and skin inflammations, etc. It is considered as good appetizer. The juice of the leaf is used in cataract and fever. Conventionally, it is propagated by seeds and stem cuttings, which is a slow process. In recent years, tissue culture techniques are increasingly being used for rapid multiplications of several economically and medicinally important plants, but there has been no report on in vitro multiplication of *B. prionitis*. In the present investigation, in vitro shoots of *B. prionitis* were developed using cotyledonary nodes as explants on different media.

MATERIAL AND METHODS

Healthy, top shoot cuttings were collected in polythene bags from plants growing in natural conditions and brought to laboratories. After excision nodal parts of these shoots were washed under running tap water thoroughly, then with 1% savlon and again with tap water. These nodal segments were further surface sterilized with 0.1% $HgCl_2$ for 5 minutes and washed 4 to 5 times with sterilized distilled water. Now some of the nodal segments were split longitudinally aseptically under the laminar flow. Intact as well as split nodal segments were used as explants for regeneration on freshly prepared and sterilized MS medium (Murashige and Skoog 1962). The medium was supplemented with various concentrations and combinations of BAP with IAA or NAA (at pH 5.8±0.2). Cultures were incubated under 16 hrs light cycle with 3000 lux intensity at 25°C ± 2°C and 60% relative humidity.

Besides intact and split nodal segments, cotyledonary nodal segments of sterilized germinating seeds were also used as explants for regeneration. For this, seeds were washed and sterilized in same manner as nodal segments and incubated in sterilized test tubes containing paper bridges in water, for germination. Cotyledonary nodes of these germinating seeds were aseptically transferred as explants on MS medium with various combinations of cytokinins (BAP) and auxins (IAA and NAA separately). Experiment was carried out in five replicates and mean values were calculated.

RESULTS AND DISCUSSION

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In present studies intact nodal segments, split nodal segments and cotyledonary nodes were used as explants for initiation of multiple shooting. Various concentrations of BAP (ranging from 0.2 mg/l to 3 mg/l) with all possible combinations of NAA and IAA separately in four different concentrations (0.1, 0.2, 0.5 and 1.0 mg/l) were used for selecting best suitable BAP-NAA and BAP-IAA combinations. Complete nodal segments and split nodal segments failed to induce proliferation on any combination of BAP+NAA and BAP +IAA. Best response was seen with cotyledonary nodal explants. The relative amount and ratios of BAP with NAA or IAA present in the medium remarkably influenced proliferation of shoots. Axillary shoot formation was observed in all concentrations of BAP (0.2 to 2.90 mg/l) with NAA and IAA separately ranging from 0.1 mg/l to 0.5 mg/l, NAA and IAA in 1.0 mg/l concentration did not give positive response with any concentration of BAP. 0.2 mg/l BAP showed shoot proliferation only with 0.1 and 0.2 mg/l NAA as well as IAA with slight variation.

Percentage of shoot proliferation increased from 0.2 to 0.5 mg/l BAP and reached maximum (76% and 72%) at 1.0 mg/l BAP with 0.1 mg/l NAA and IAA, respectively.

With further higher concentrations of BAP (1.5 to 2.0 mg/l), percentage of shooting started decreasing and proceeded towards callusing.

Maximum number of shoots per culture (12.6 and 12.2) as well as maximum length (4.5cm and 4.3cm) of shoots were also achieved on 1.0mg/l BAP + 0.1 mg/l NAA and IAA respectively.

Although NAA and IAA in combination with BAP gave nearly same results, still results of NAA were comparatively better than IAA, as shown in table.

It was concluded that shoot proliferation depends upon the balance of cytokinins and auxins and maximum variation in BAP and auxin concentrations is best suitable combination for getting maximum percentage of proliferation, number of shoots as well as average length of shoots as given in table.

Present study showed the importance of growth hormones in regeneration process to achieve the maximum number of shoots of *B. prionitis in vitro*, which can later be rooted, acclimatized and transferred to natural conditions.

Micropropagation of various plant species, including many medicinal plants, has been reported (Murashige 1978). Plant regeneration from shoot and stem meristems has yielded encouraging results in medicinal plants like *Catharanthus roseus, Cinchona ledgeriana* and *Digitalis* spp., *Rehmannia glutinosa, Rauvolfia serpentina, Isoplexis cananriensis* (Perez-Bermudez *et al.* 2002).

It has been observed that cytokinin is required in optional quantity for shoot proliferation in many genotypes but inclusion of low concentrations of auxins along with cytokinin triggers the rate of shoot proliferation.

There are many reports showing the micropropagation of various plants as *Camellia sinensis* (Phukan and Mitra 1984), *Helianthus annuus* (Nataraja and Ganapathi 1989), *Prosopis juliflora* (Nandwani and Ramawat 1991), *Terminalia chebula* (Shyamkumar *et al.* 2004), *Cuculigo orchioides* (Hiren *et al.* 2004) *Leucaena leucocephala* (Saafi and Borthakur 2002), *Emblica officinalis* (Goyal and Bhadauria 2008), *Withania somnifera* (Anjali *et al.* 2000, Sivanesan 2007), *Cajanus cajan* (Vijaykumari *et al.* 2001), *Rauvolfia tetraphylla* (Faisal *et al.* 2005), *Capsicum annuum* (Venkataiah *et al.* 2003) and *Adhatoda vasica* (Abhyankar and Reddy 2007).

This protocol adds new information of successful and rapid technique that can be used for *in vitro* shoot proliferation of *B. prionitis*.

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Table 1. Regeneration of shoots from cotyledonary nodal segments in *B. prionitis*

Growth Regulators (mg/l)	% of explant shooting proliferation		No. of total shoots per culture		Average length of the longest shoots (cm)	
BAP+NAA/IAA	BAP+NAA	BAP+IAA	BAP+NAA	BAP+IAA	BAP+NAA	BAP+IAA
0.2+ 0.1	55	56	3.5±0.21	3.7±0.33	3.2±0.37	2.9±0.32
+ 0.2	25	27	1.2±0.20	1.2±0.14	1.0±0.25	1.0±0.12
+0.5	-	-	-	-	-	-
+1.0	-	-	-	-	-	-
0.5+0.1	68	64	8.8±0.19	7.9±0.34	3.3±0.26	3.0±0.36
+0.2	60	56	4.6±0.34	5.1±0.53	2.3±0.19	2.6±0.29
+0.5	48	42	2.1±0.28	2.4±0.36	1.1±0.12	1.0±0.21
+1.0	-	-	-	-	-	-
1.0+0.1	76	72	12.6±0.17	12.2±0.39	4.5±0.38	4.3±0.17
+0.2	58	53	9.7±0.43	9.7±0.21	3.6±0.27	3.1±0.45
+0.5	21	20	2.9±0.29	2.9±0.29	2.1±0.63	2.0±0.22
+1.0	-	-	-	-	-	-
1.5+0.1	46	43	3.9±0.32	3.6±0.18	2.8±0.44	2.0±0.27
+0.2	37	37	2.8±0.28	2.5±0.41	1.6±0.23	1.5±0.36
+0.5	19	18	1.1±0.13	0.9±0.15	0.6±0.11	0.7±0.18
+1.0	-	-	-	-	-	-
2.0+0.1	35	36	2.3±0.21	2.0±0.37	1.9±0.32	1.6±0.32
+0.2	23	21	1.6±0.31	1.5±0.19	1.7±0.12	1.3±0.17
+0.5	11	10	0.8±0.16	1.2±0.21	0.5±0.29	0.5±0.13
+1.0	-	-	-	-	-	-

Values are mean of five replicates ± SD

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