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Direct organogenesis and somatic embryogenesis in *Beloperone plumbaginifolia* (Jacq.) Nees

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Beloperone plumbaginifolia (Jacq.) Nees is a small branched medicinal shrub. *In vitro* studies were conducted, employing explants from node, internode, petiole, shoot bud and leaf lamina. Murashige and Skoog (MS) medium fortified with 6.66 μ*M* BA enabled the proliferation of axillary and apical buds. MS medium supplemented with 5.37 μ*M* NAA was better for callogenesis from nodal and internodal explants, while a combination of IBA (2.46 μ*M*) and 2,4-D (4.52 μ*M*) was good for leaf lamina explant. MS medium with 5.37 μ*M* NAA and 2.22 μ*M* BA was found superior for shoot induction from nodal explants. Half-strength MS medium with 5.37 μ*M* NAA induced adventitious roots and 85% plantlets survived when transferred in the field conditions.

Keywords: *Beloperone plumbaginifolia*, callogenesis, organogenesis, somatic embryogenesis

Introduction

Beloperone plumbaginifolia (Jacq.) Nees of Acanthaceae is a branched small shrub, which is being used by tribals in Kerala as an antidote for snakebite. In Brazil, its native place, it is grown as an ornamental[1]. Natural propagation of this plant is hampered by low rate of germination due to limited seed viability. However, its rapid multiplication is a prerequisite to meet the pharmaceutical requirements and to prevent it from becoming endangered[2]. A perusal of literature revealed that no studies have so far been conducted for its *in vitro* multiplication. Therefore, the objective of the present study was to establish a protocol for direct shoot multiplication and callogenesis of *B. plumbaginifolia.*

Materials and Methods

Explants were collected from plants growing in the Botanical Garden, University of Calicut, Kerala and washed in dilute solution of Teepol (5% v/v) for 5 min, followed by thorough wash with double distilled water. Explants were surface sterilized with 0.1% $HgCl_2$ for 13-15 min and repeatedly washed with autoclaved distilled water to remove traces of $HgCl_2$. Surface sterilized explants were blot-dried and cultured on Murashige and Skoog (MS) basal medium[3] fortified with various concentrations and combinations of auxins and cytokinins. Sucrose (3%) was used as the carbon source and agar (0.8%) as the gelling agent. *p*H of the medium was adjusted to 5.8 before autoclaving at 1.05 kg cm^{-2} for 20 min. The cultures were incubated in 16:8 h light and dark periods under fluorescent light (1600 lux) in the culture room.

Experiments were conducted at least in triplicate and the data are presented with standard deviation. The responses of explants cultured on different media were recorded weekly. Fully developed plantlets were transferred to plastic pots containing fertile sand and soil (1:1), covered with polythene bags for hardening. Hardened plants were transplanted to earthen pots.

Results

Direct Organogenesis

Nodal, internodal and shoot bud explants were cultured on MS medium fortified with different growth regulators individually or in combinations (Table 1). MS medium with 6.66 μ*M* BA was found most effective for the development of axillary and apical buds (Fig. 1A). MS with Kn (4.65 μ*M*) also induced proliferation of axillary and apical buds, but the effect was inferior to that of BA. Synergistic action of IBA (7.36 μ*M*) and BA (2.22 μ*M*) or NAA (5.37 μ*M*) and BA (2.22 μ*M*) (Fig. 1B) was also better for axillary bud development.

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Abbreviations: BA, 6-benzyladenine; 2,4-D, 2,4-dichloro-phenoxyacetic acid; IBA, indole-3-butyric acid; Kn (Kinetin), 6-furfurylaminopurine; NAA, ∝-naphthalene acetic acid; MS, Murashige and Skoog medium.

Table 1—Direct organogenesis from nodal and shoot tip explants of *B. plumbaginifolia* cultured on MS media with various growth regulators

Growth regulators (µ*M*)				% response			
BA	Kn	IBA	NAA	Node	(±SD)	Shoot tip	(±SD)
4.44	–	–	–	70	(±4.50)	70	(±1.15
6.66	–	–	–	90	(±1.52)	80	(±2.00)
8.88	–	–	–	60	(±2.00)	60	(±1.73)
11.10	–	–	–	40	(±1.15)	30	(±0.57)
–	2.32	–	–	50	(±1.00)	30	(±1.52)
–	4.65	–	–	80	(±2.08)	60	(±1.52)
–	6.97	–	–	60	(±1.15)	40	(±1.00)
2.22	4.65	–	–	60	(±1.52)	40	(±1.00)
4.44	2.32	–	–	50	(±1.00)	50	(±1.52)
2.22	–	7.36	–	70	(±3.21)	60	(±2.08)
2.22	–	–	5.37	60	(±1.52)	80	(±0.57)

Callogenesis

Petiolar, nodal, internodal and leaf lamina explants were cultured on MS medium with different growth supplements. Callus showed variations in colour and texture, based on the type of growth regulators used. MS with NAA (5.37 µ*M*) was better for inducing callus from node and internodes (Fig. 1C; Table 2) after 4 wk. MS fortified with IBA (2.46 µ*M*) and 2,4-D (4.52 µ*M*) was optimal for callogenesis from leaf lamina (Table 2). Synergistic action of NAA (2.69 µ*M*) and 2,4-D (6.78 µ*M*) also induced callogenesis from node and internodes; similar observation was also made in case of MS fortified with 2,4-D (6.78 µ*M*) and BA (2.22 µ*M*). However, MS with 2,4-D (4.52 µ*M*) and IBA (4.90 µ*M*) induced embryogenic calli from leaf lamina within 4 wk of incubation (Figs 1D & E).

***In Vitro* Rooting**

In *in vitro* derived shoots, falf-strength (½) MS medium fortified with 5.37 µ*M* NAA gave the best results for profuse rhizogenesis (Fig. 1F; Table 3). MS medium with IBA (4.90 µ*M*) was also better in inducing rhizogenesis.

Transfer to Field Conditions

When rooted plantlets were transferred to poly ethylene bags for hardening, 85% of them survived. These hardened plants were then transplanted to earthen pots and shifted in the garden, where they established well (Fig. 1G).

Fig. 1 (A-G)—Different stages of direct organogenesis and somatic embryogenesis in *B. plumbaginifolia* on MS medium with various growth regulators: A. Proliferation of shoot tip on 6.66 µ*M* BA; B. Proliferation of axillary bud on 5.73 µ*M* NAA + 2.22 µ*M* BA; C. Callogenesis from distal end of internode on 5.73 µ*M* NAA; D & E. Embryogenic calli from leaf lamina on 4.52 µ*M* 2,4-D + 4.90 µ*M* IBA; F. *In vitro* rhizogenesis on ½ MS with 5.37 µ*M* NAA; & E. *In vitro* derived plants in the field.

Discussion

The *in vitro* propagation of *Beloperone plumbaginifolia,* an important ethnomedicinal plant, is reported here for the first time. *In vitro* propagation through axillary bud proliferation has been the safest and faithful strategy to maintain genetic integrity of the developing progenies[4]. Among Acanthacean members, direct organogenesis has been achieved with *Justicia gendarussa*[5], *Crossandra infundibuliformis*[6] and *Asystasia dalzelliana*[7]. In the present study, it was observed that MS medium with 6.66 µ*M* BA was the most efficient treatment for axillary bud multiplication of *B. plumbaginifolia*. Superior effect of BA for axillary bud multiplication

Table 2—Callus induction from different explants of *B. plumbaginifolia* on MS medium with various growth regulators

Growth regulators (μ*M*)				% response					
NAA	IBA	2,4-D	BA	Node	(±SD)	Internode	(±SD)	Leaf	(±SD)
2.69	–	–	–	50	(±2.00)	50	± 1.52	40	(±2.51)
5.37	–	–	–	70	(±2.51)	90	± 0.57	50	(±3.05)
8.06	–	–	–	60	(±3.05)	60	± 3.51	40	(±1.52)
–	7.36	–	–	60	(±4.05)	70	± 2.64	50	(±1.52)
–	9.80	–	–	50	(±1.15)	50	± 2.08	40	(±1.52)
2.69	–	6.78	–	70	(±2.30)	80	± 1.52	50	(±4.00)
–	–	4.52	–	40	(±1.52)	60	± 2.08	20	(±2.64)
–	–	6.78	–	60	(±2.51)	70	± 3.51	40	(±4.16)
–	–	9.05	–	40	(±4.16)	60	± 1.52	30	(±2.00)
–	2.46	4.52	–	60	(±3.6)	70	± 3.6	90	(±2.08)
–	9.8	9.05	–	50	(±2.00)	60	± 2.51	70	(±0.57)
–	12.26	4.52	–	40	(±0.57)	50	± 2.64	50	(±2.51)
–	–	6.78	2.22	70	(±1.52)	80	± 4.5	60	(±0.57)
–	–	9.05	2.22	40	(±2.08)	60	± 2.3	40	(±1.00)

Table 3—*In vitro* rooting of *B. plumbaginifolia* on MS media with various growth regulators

Growth regulators (μ*M*)		% response			
NAA	IBA	½MS	(±SD)	MS	(±SD)
2.69	–	80	(±2.00)	70	(±1.52)
5.37	–	90	(±1.15)	80	(±0.57)
8.06	–	70	(±2.51)	60	(±1.15)
–	2.46	60	(±2.08)	50	(±1.15)
–	4.90	70	(±2.00)	60	(±2.51)
–	7.36	60	(±3.78)	40	(±2.00)

has been reported among various medicinal plants of Acanthaceae, like *Crossandra unduleafolia*[8] and *C. infundibuliformis* var. Danica[9]. As in the present study, the reduction of shoot regeneration potential by the synergy of BA and an auxin compared to a medium with cytokinin alone was also demonstrated in *Mentha piperita*[10]. On the contrary, a combination of BA and auxin had shown better response in *Curcuma amada*[11]. The shoots developed by BA over its optimal level (8.88 μ*M*) showed stunted growth. A similar phenomenon has also been reported in *Crossandra infundibuliformis*[4] and *Orthosiphon* sp.[12].

Callus developed on all types of explants of *B. plumbaginifolia*, except petiole, on MS medium supplemented with suitable growth regulators. However, the best results were obtained with 5.37 μ*M* NAA. Similar results were also reported in case of *Andrographis paniculata* and *Tinospora crispa*[13]. Cytokinin in combination with auxins was found effective in callogenesis and similar effect was noticed in *Lavendula viridis*[14] and *Andrographis paniculata*[15]. Interestingly, explants cultured on MS medium in combination with 2,4-D and IBA developed embryogenic calli.

For root induction, ½ MS basal medium with 5.37 μ*M* NAA was found superior. Higher efficacy of NAA over other auxins has been demonstrated in many medicinal plants, like *Pinellia ternata*[16], *Calendula officianalis*[17] and *Aloe vera* var. Chinensis[18].

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