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In vitro clonal propagation of *Achyranthes aspera* L. and *Achyranthes bidentata* Blume using nodal explants

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ABSTRACT

Objective: To develop the reproducible *in vitro* propagation protocols for the medicinally important plants *viz.*, *Achyranthes aspera* (*A. aspera*) L. and *Achyranthes bidentata* (*A. bidentata*) Blume using nodal segments as explants. **Methods:** Young shoots of *A. aspera* and *A. bidentata* were harvested and washed with running tap water and treated with 0.1% bavistin and rinsed twice with distilled water. Then the explants were surface sterilized with 0.1% (w/v) $HgCl_2$ solutions for 1 min. After rinsing with sterile distilled water for 3-4 times, nodal segments were cut into smaller segments (1 cm) and used as the explants. The explants were placed horizontally as well as vertically on solid basal Murashige and Skoog (MS) medium supplemented with 3% sucrose, 0.6% (w/v) agar (Hi-Media, Mumbai) and different concentration and combination of 6-benzyl amino purine (BAP), kinetin (Kin), naphthalene acetic acid (NAA) and indole acetic acid (IAA) for direct regeneration. **Results:** Adventitious proliferation was obtained from *A. aspera* and *A. bidentata* nodal segments inoculated on MS basal medium with 3% sucrose and augmented with BAP and Kin with varied frequency. MS medium augmented with 3.0 mg/L of BAP showed the highest percentage (93.60±0.71) of shootlets formation for *A. aspera* and (94.70±0.53) percentages for *A. bidentata*. Maximum number of shoots/explants (10.60±0.36) for *A. aspera* and (9.50±0.56) for *A. bidentata* was observed in MS medium fortified with 5.0 mg/L of BAP. For *A. aspera*, maximum mean length (5.50±0.34) of shootlets was obtained in MS medium augmented with 3.0 mg/L of Kin and for *A. bidentata* (5.40±0.61) was observed in the very same concentration. The highest percentage, maximum number of rootlets/shootlet and mean length of rootlets were observed in 1/2 MS medium supplemented with 1.0 mg/L of IBA. Seventy percentages of plants were successfully established in polycups. Sixty eight percentages of plants were well established in the green house condition. Sixty five percentages of plants were established in the field. **Conclusions:** The results have shown that use of nodal buds is an alternative reproducible and dependable method for clonal propagation of *A. aspera* and *A. bidentata*. The high rate of direct shoot-root multiplication and their high rate of post-hardening survival indicate that this protocol can be easily adopted for commercial large scale cultivation.

1. Introduction

Achyranthes aspera (*A. aspera*) L. and *Achyranthes bidentata* (*A. bidentata*) Blume belong to the family Amaranthaceae and are found to possess lots of medicinal properties. *A. aspera* is a small herb found all over India possessing valuable medicinal properties. It is useful in cough, bronchitis, rheumatism, malarial fever, dysentery, asthma, renal and cardiac dropsy, hypertension and diabetes mellitus[1]. *A. aspera* can stimulate the immunity, enhance the antigen clearance, potentiate antibody production, elevate thyroid hormone levels, decrease hepatic lipid peroxidation and also possesses spermicidal, chemopreventive, anti-inflammatory, anti-arthritic and hypoglycaemic activities[2-4]. In Chinese traditional medicine, the hot water extract of the plant has been used as an antiarthritic to alleviate arthritic pain. *In vitro* and *in vivo* studies of the root extract have found the spermicidal activity[1]. The dried leaf powder mixed with honey is useful

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in the early stages of asthma[1]. Oleanolic acid is one of the constituents of *A. aspera* L. *A. bidentata* Blume extract can promote neuronal growth, protect hippocampal neurons against toxicity, and also has anti-stress and anti-apoptosis activities[5-8]. Both the plants are found to be a source of many secondary metabolites[9].

Successful development of regeneration of shoots is a prerequisite for clonal propagation and for genetic transformation. A growing demand for *A. aspera* L. and *A. bidentata* Blume in pharmaceutical industry has resulted in a serious reduction as a consequence of unforeseen climatic conditions, which affect the production capacity and deforestation. It is observed that phenolic compounds from medicinal plants are sensitive to various environmental factors. The major constraint in conventional propagation through seeds is the high mortality of seedlings in early stage and there is always the possibility of losing the mother plant during this process. *In vitro* propagation methods offer highly efficient tools for medicinal plants useful for pharmaceutical industry[9]. Plant tissue culture techniques offer a powerful tool for mass multiplication of medicinally important plant species[9-20]. There has been progress in tissue culture studies in many Amaranthaceae members such as *Alternanthera sessilis*, *Amaranthus* spp, *Amaranthus paniculatus*[20,21]. Preliminary work on *in vitro* callus production of *A. aspera* was reported by Kayani *et al*[21]. But there is no report on direct regeneration of *A. aspera* and *A. bidentata*i using nodal segments. Therefore, the present investigation was aimed to elucidate the protocol for *in vitro* regeneration through nodal segments of *A. aspera* and *A. bidentata* for better exploitation.

2. Materials and methods

Plants of *A. aspera* L. and *A. bidentata* Blume were collected from Kollimalai, Salem, Tamil Nadu, India and were established in the green house and herbal garden. Young shoots were harvested and washed with running tap water and treated with 0.1% bavistin and rinsed twice with distilled water. Then the explants were surface sterilized with 0.1% (w/v) $HgCl_2$ solutions for 1 min. After rinsing with sterile distilled water for 3-4 times, nodal segments were cut into smaller segments (1 cm) and used as the explants. The explants were placed horizontally as well as vertically on solid basal Murashige and Skoog (MS)[22] medium supplemented with 3% sucrose, 0.6% (w/v) agar (Hi-Media, Mumbai) and different concentration and combination of 6-benzyl amino purine (BAP), kinetin (Kin), naphthalene acetic acid (NAA) and indole acetic acid (IAA) for direct regeneration. For rooting, the *in vitro* raised shootlets were sub-cultured on 1/2 strength MS medium supplemented with various concentrations and combinations of auxins. The pH of the medium was adjusted to 5.8 before autoclaving at 121 ℃ for 15 min. The cultures were incubated at (25±2) ℃ under cool fluorescent light (2 000 lux 12 h/day photoperiod). For acclimatization, the plantlets with well developed roots (5 cm) were removed from culture tubes and washed in running tap water to remove the remnants of agar. Then the plantlets were planted separately in polycup (1-10 cm in diameter) filled with potting mixture river sand, garden soil and farm yard manure (1:1:1). Plants were in mist chamber with relative humidity of 70% and were irrigated with 10 × liquid MS medium (5 mL) at 8 h intervals for 2 weeks and establishment rate was recorded and tabulated.

3. Results

The frequency of explants survival and regeneration of explants varied with concentration of $HgCl_2$ and sterilization time. 0.1% $HgCl_2$ for 3 min showed the highest percentage (95%) of survival rate of explants. 0.1% $HgCl_2$ for 2 min showed the highest percentage (90%) of microbial contamination. It was observed that when the explants were sterilized with 0.1% $HgCl_2$ for 5 min, 100% of microbes are free from explants but the mortality rate of explants was high up to 95% to 100%. Proliferation of multiple shoots was observed with high frequency from nodal segments and shoot tips within five days of inoculation. These explants were capable of directly developing multiple shoots on MS medium containing different concentrations and combinations of cytokinin (BAP and Kin). MS medium augmented with 3.0 mg/L of BAP showed the highest percentage (93.60±0.71) of shootlets formation for *A. aspera* and (94.70±0.53) percentage for *A. bidentata*. Maximum number of shoots/explants (10.60±0.36) for *A. aspera* and (9.50±0.56) for *A. bidentata* was observed in MS medium fortified with 5.0 mg/L of BAP (Table 1 and Figure 1A-1E). For *A. aspera*, maximum mean length (5.50 ±0.34) of shootlets was obtained in MS medium augmented with 3.0 mg/L of Kin and for *A. bidentata* (5.40±0.61) was observed in the very same concentration (Table 1). The *in vitro* raised shootlets were sub-cultured on 1/2 strength MS medium augmented with various concentrations of IBA. On the 17th day, the *in vitro* raised shootlets produced *in vitro* rootlets without any callus proliferation (Figure 1F-1H). The highest percentage, maximum number of rootlets/ shootlet and mean length of rootlets were observed in 1/2 MS medium supplemented with 1.0 mg/L of IBA (Table 2). After 10 days, *in vitro* raised plantlets were hardened in polycups containing a mixture of sterile garden soil: sand (3:1), covered with polypropylene bags and irrigated with 10 × diluted MS liquid medium. The plants were kept in a culture room for 15 days. Seventy percentages of plants were successfully established in polycups (Figure 1I, 1J). After 15 days, the polycups hardened plants were transferred to pots and kept in green house. Sixty eight percentages of plants were well established in the green house condition.

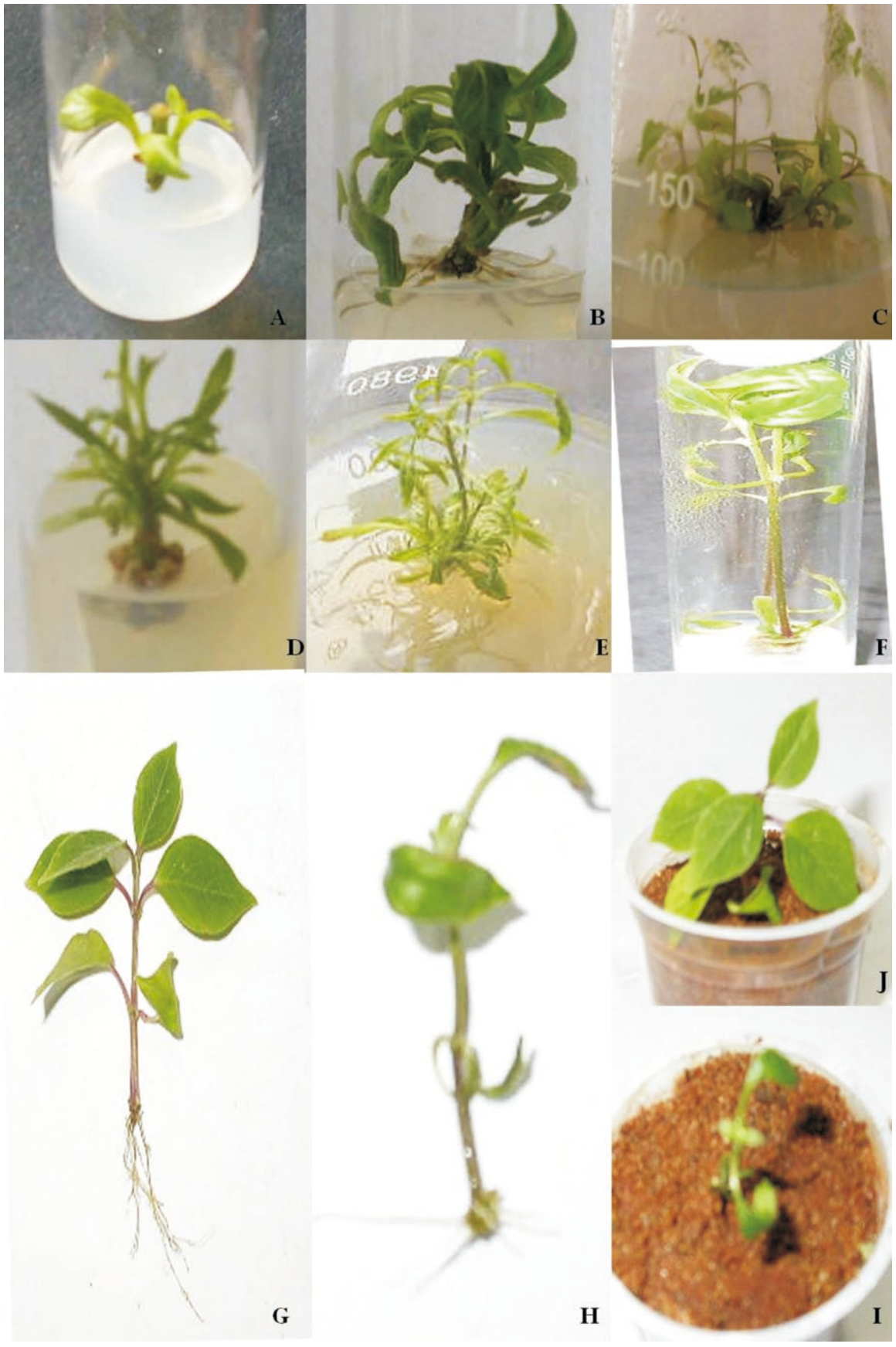

Figure 1. *In vitro* propagation of *A. aspera* and *A. bidentata* Blume.
A: *A. aspera* at the shoot initial stage–after five days; B: *A. aspera* at the stage of multiple shoot formation on MS+BAP (3.0 mg/L); C: *A. aspera* at the stage of multiple shoot formation on MS+BAP (5.0 mg/L); D: *A. bidentata* at the stage of multiple shoot formation on MS+Kin (3.0 mg/L); E: *A. bidentata* at the stage of multiple shootlet formation on MS+BAP (3.0 mg/L); F: *In vitro* raised plantlet of *A. aspera*; G: *In vitro* raised plantlet of *A. bidentata*; H: *In vitro* raised plantlet of *A. aspera*; I: Hardened plant of *A. aspera* in the polycups; J: Hardened plant of *A. bidentata* in the polycups.

Table 1
Effect of cytokinin on multiple shoot induction from nodal explants of *A. aspera* and *A. bidentata* (Mean±SE).

Cytokinin conc. (mg/L)	Percentage of shoot initiation		Number of shoots/explants		Length of shoot (cm)	
	A. aspera	*A. bidentata*	*A. aspera*	*A. bidentata*	*A. aspera*	*A. bidentata*
BAP (0.0)	58.30±0.82	53.30±0.46	1.30±0.42	1.80±0.35	2.10±0.81	2.30±0.82
BAP (1.0)	80.30±0.76	83.80±0.61	2.40±0.35	2.70±0.63	4.90±0.46	5.30±0.67
BAP (2.0)	89.40±0.63	91.70±0.62	3.40±0.63	4.40±0.52	4.20±0.73	4.80±0.64
BAP (3.0)	93.60±0.71	94.70±0.53	3.90±0.34	4.60±0.37	3.70±0.52	4.20±0.68
BAP (4.0)	92.40±0.68	84.40±0.47	8.40±0.63	6.70±0.51	3.00±0.28	3.80±0.46
BAP (5.0)	79.40±0.61	79.70±0.52	10.60±0.36	9.50±0.56	2.70±0.42	3.10±0.53
Kin (1.0)	75.40±0.34	61.70±0.26	2.10±0.32	2.10±0.54	5.10±0.34	4.90±0.62
Kin (2.0)	82.40±0.49	76.40±0.42	2.40±0.62	2.70±0.45	4.90±0.43	5.10±0.36
Kin (3.0)	74.40±0.26	73.40±0.72	3.40±0.72	2.40±0.55	5.50±0.34	5.40±0.61
Kin (4.0)	69.80±0.46	68.60±0.64	3.10±0.56	2.40±0.32	5.40±0.53	5.20±0.42
Kin (5.0)	61.70±0.37	51.90±0.73	2.40±0.34	1.90±0.46	5.20±0.38	4.70±0.43

Table 2
Effect of indole 3 butyric acid (IBA) on root formation on *in vitro* raised shootlets of *A. aspera* and *A. bidentata* (Mean±SE).

IBA (mg/L)	Percentage of root formation		Number of rootlets/shootlets		Length of rootlets (cm)	
	A. aspera	*A. bidentata*	*A. aspera*	*A. bidentata*	*A. aspera*	*A. bidentata*
0.0	1.30±0.32	1.50±0.56	1.10±0.75	2.10±0.46	2.30±0.36	2.40±0.46
0.5	56.30±0.48	54.60±0.73	6.40±0.42	6.80±0.49	4.10±0.53	4.50±0.48
1.0	73.50±0.63	74.70±0.72	8.80±0.72	9.40±0.76	4.80±0.38	5.20±0.46
1.5	64.80±0.56	66.70±0.92	4.70±0.64	5.80±0.63	3.50±0.42	4.10±0.36
2.0	53.70±0.43	51.40±0.82	3.80±0.56	2.40±0.68	2.90±0.42	3.20±0.43

4. Discussion

High period of exposure with $HgCl_2$ leads the browning of explants and death. Our results were in agreement with the observations of Johnson *et al*[25], Wesely *et al*[26] and Johnson *et al*[27-30]. They reported that surface sterilization exceeding 5 min was lethal to explants whereas, the present study showed that above 3 min of surface sterilization was lethal to nodal segments and shoot tip of *A. aspera* and *A. bidentata*. MS medium supplemented with various concentration of BAP and Kin stimulated the shootlets formation with varied percentage. Our results were in agreement with the observations of Kant, Sri Rama Murthy and Johnson *et al*[23-25,31-34], Wesely et al[35,36]. The present study was directly coinciding with previous observations of Johnson *et al*[25] in *Rhinacanthus nasutus*, Gadidasu *et al*[37] in *Streblus asper*, Loc and Kiet[38] in *Solanum hainanense*, Gutiérrez *et al*[39] in *Bauhinia cheilantha*, Saha *et al*[40] in *Coleus forskohlii*, Subramanian *et al*[41] in *Vicoa indica* and Robinson *et al*[42] in *Costus speciosus*. They got maximum number of rootlets on 1/2 MS medium augmented with IBA (1.0 mg/L). In the present investigation we also observed the root induction in the very same medium.

Here, the results have shown that use of nodal buds is an alternative reproducible and dependable method for clonal propagation of *A. aspera* and *A. bidentata*. The high rate of direct shoot-root multiplication and their high rate of post-hardening survival indicate that this protocol could be easily adopted for commercial large scale cultivation.

Conflict of interest statement

We declare that we have no conflict of interest.

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