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In vitro propagation of *Aerva lanata* (L.) Juss. ex Schult. through organogenesis

Alok Ranjan Sahu, Sobha Chandra Rath and Jogeswar Panigrahi*

Plant Biotechnology Laboratory, School of Life Sciences, Sambalpur University, Sambalpur 768 019, India

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In vitro plantlet regeneration in *Aerva lanata* (L.) has been achieved from nodal segments cultured on MS medium supplemented with growth regulators. Nodal explants from healthy field grown plants were cultured on MS medium fortified with various concentrations of BA (N^6-benzyladenine), KN (kinetin) and NAA (α-napthalene acetic acid) either alone or in combinations. The combination of BA and KN (3.0 mg/L each) was the best for multiple shoot induction through direct organogenesis. While addition of NAA (1.0 or 1.5 mg/L) to the optimal combination of BA and KN in the medium was the best and produced the maximum (10.66±1.15) multiple shoots *via* callus mediated organogenesis. Well-developed shoots (>3cm) were successfully rooted on half strength MS medium containing IBA (indole butyric acid; 0.5 mg/L). The regenerated plantlets were successfully established in soil with survival rate of 72±4%. The protocol described is simple, rapid and efficient for *in vitro* propagation of *A. lanata* from nodal explants.

Key words: *in vitro*, organogenesis, nodal explant, medicinal herb

Introduction

Aerva lanata (L.) Juss. ex Schult., a medicinal herb belongs to the family Amaranthaceae. This herb is known as 'Chhaya' in Hindi, 'Bhadram' in Sanskrit, 'Pulai' in Telugu and 'Lopang Ark' in Oriya. It is a branched, woody, prostrate, perennial herb and growing wild in the hot regions of India, predominantly in the habitat of Odisha, Andhra Pradesh, Karnataka and Tamilnadu. The herb possesses anti-inflammatory, diuretic[1], antimicrobial[2], anticancer[3], nephroprotective[4], antinociceptive[5], antidiabetic[6-8], antihelimintic[9], antifertility[10] activities. The plant is also useful in lithiasis, cough, sore throat, wounds and pediatric diarhea[9,11-14]. *A. lanata* is one of the 10 auspicious herbs that constitute the group "Dasapushpam" – ten sacred flowers[15] for tradition and culture. The extracts of *A. lanata* is endowed with flavonoids, alkaloids, triterpenes, steroids, polysaccharides, tannins and saponins[15,16]. The root extract contains aervin, methylaervin and aervoside[17-19], which possibly contribute to its diverse uses in folklore medicines. Secondary metabolites including β-carboline, β-sitosterol and vanillic acid were also isolated from the leaves[19,20]. Hence, the whole plant and plant parts including roots and leaves has been used in several herbal preparations including Bhadraveradi Kashyam, Dasmolam Kashayam, Nishakathakadi Kashyayam (Ashoka pharmaceuticals, Kerala; www.asoka.co.in), Mahasiddhartha oil (www.aparmita.lv) for curing of several ailments. Further, the raw plant extract was rich in antioxidants, hence used as tonic by rural poor during pregnancy, and even during natal care stage[11].

The requirement of this medicinal herb is presently met from the natural populations. However, extensive utilization of this plant poses a potential threat for its existence. *In vitro* culture techniques including organogenesis and somatic embryogenesis offer a viable tool for clonal multiplication and conservation of this medicinal herbs[21]. Commensurate with this, the intervention of *in vitro* culture for accelerating clonal multiplication of this important herb and their conservation are warranted in the right earnest. Therefore, the present study has been initiated to standardize a simple protocol for *in vitro* propagation of this important medicinal herb *A. lanata*.

Materials and Methods

Plant Materials and Preparation of Explants

Seeds of *A. lanata* (L.) were collected from western part of Odisha during May, 2010 and the plants were maintained in the experimental garden of Biotechnology Unit, School of Life Sciences, Sambalpur University, Odisha. Healthy shoots were washed with 5% (v/v) teepol for 2 min and then surface sterilized by quick dip in 70% alcohol for 45 sec, followed by 2-3 min of soaking in 0.05% (w/v) $HgCl_2$ (E-Merck, India). The sterilized shoots were washed three times with sterilized

*Author for correspondence:
Mobile: +91-9437130857; Fax: +91-663-2430158
E-mail: drjpanigrahi@gmail.com; drjogeswar_ panigrahi@ymail.com

double distilled water. The 2nd to 5th apical node (5-10 mm length) segments were used as explants.

Culture Medium and Conditions

The explants were cultured on Murashige and Skoog's (MS)[22] medium supplemented with 100 mg/L mesoinositol (Hi-Media, India) and 3% (w/v) sucrose (Hi-Media, India). The medium was augmented with different concentrations and combinations of N^6-benzyladenine (BA), kinetin (KN), α-napthalene acetic acid (NAA) and indole butyric acid (IBA) (Hi-Media, India). The *p*H of the medium was adjusted to 5.8 prior to gelling it with 0.8% agar (bacteriological grade, Hi-Media, India). All the culture vessels containing media were autoclaved at 121°C and 1.05 kg/cm^2 for 20 min. One explant was implanted in each tube and cultures were incubated at 25±2°C with 60-70% relative humidity under 16 h photoperiod of 35 µE m^{-2} s^{-1} irradiance, provided by cool white fluorescent tubes (Philips, India).

Rooting and Acclimatization

Elongated shoots (>3 cms long) with 5-6 leaves were excised and transferred to ½ strength MS medium containing different concentrations of NAA and indole butyric acid (IBA). Cultures were incubated as described previously. Plantlets with well-developed roots were transferred to plastic cup containing autoclaved sand and soil (1:1). The regenerated plantlets were hardened by covering them with a thin perforated transparent polythene bag to maintain humidity. Plantlets were watered with 1/10th strength MS salts solution and maintained in the culture condition. After a wk the plantlets were shifted to the experimental garden under shade, and then to day light.

Statistical Analysis

Data were recorded based on explant response, number of shoot buds per explant, shoot length and number of roots per elongated shoot. Each phytohormone treatment consisted of 5 replicated tubes and was repeated thrice. Data were statistically analyzed using a completely randomized block design and means were compared at $p \leq 0.05$ level of significance using Duncan's multiple range test[23]. For this SPSS V 16.0 software used with parameters, one way ANOVA and homogeneity of variance.

Results and Discussion

Direct Organogenesis

Surface sterilized nodal explants were cultured on MS media supplemented with various concentrations and combinations of cytokinins (BA & KN) and an auxin (NAA) (Table 1). The cultured nodal explants enlarged

Table 1—Effect of BA, KN and combination of BA, KN and NAA on development of multiple shoots from nodal explants of *A. lanata* after 10 wk of culture

Media code	MS medium with plant growth regulators (mg/L)			*Explant response (%)	**No. of shoots per explant (mean±SD)	**Shoot length in cm (mean±SD)
	BA	KN	NAA			
M_1		Control		66.67	0.33 ± 0.57^{a}	0.9 ± 0.1^{a}
M_2	1.0	-	-	73.33	0.66 ± 0.57^{a}	1.12 ± 0.11^{b}
M_3	2.0	-	-	80.0	1.33 ± 0.57^{abc}	1.14 ± 0.09^{b}
M_4	3.0	-	-	86.67	2.33 ± 0.57^{cd}	1.5 ± 0.1^{c}
M_5	4.0	-	-	80.0	4.0 ± 0.57^{de}	2.28 ± 0.11^{e}
M_6	5.0	-	-	93.33	3.66 ± 0.57^{cde}	2.26 ± 0.11^{e}
M_7	-	1.0	-	80.0	1.33 ± 0.57^{ab}	1.12 ± 0.06^{b}
M_8	-	2.0	-	93.33	1.67 ± 0.57^{ab}	1.24 ± 0.06^{b}
M_9	-	3.0	-	86.67	2.33 ± 0.57^{bc}	1.56 ± 0.06^{c}
M_{10}	-	4.0	-	100.0	2.66 ± 0.57^{bcd}	1.82 ± 0.08^{d}
M_{11}	-	5.0	-	100.0	2.33 ± 0.57^{bc}	1.86 ± 0.13^{d}
M_{12}	1.0	1.0	-	80.0	2.66 ± 0.57^{bcd}	1.96 ± 0.06^{d}
M_{13}	2.0	2.0	-	86.67	4.66 ± 1.15^{e}	2.42 ± 0.08^{e}
M_{14}	3.0	3.0	-	86.67	13.33 ± 0.58^{i}	3.1 ± 0.1^{f}
M_{15}	4.0	4.0	-	86.67	8.66 ± 0.58^{g}	3.04 ± 0.15^{f}
M_{16}	3.0	3.0	0.5	80.0	5.66 ± 0.57^{ef}	3.08 ± 0.11^{f}
M_{17}	3.0	3.0	1.0	80.0	10.66 ± 1.15^{h}	4.16 ± 0.11^{h}
M_{18}	3.0	3.0	1.5	86.67	10.66 ± 1.15^{h}	4.46 ± 0.09^{i}
M_{19}	3.0	3.0	2.0	93.33	7.0 ± 1.0^{f}	3.36 ± 0.16^{g}
M_{20}	3.0	3.0	2.5	93.33	4.66 ± 1.15^{e}	3.14 ± 0.13^{f}

* Percentage response was calculated by combining three replication data simultaneously.

**Means within a column having the same letter were not statistically significant (p=0.05) according to Duncan's multiple range test (SPSS V 16.0).

and produced protuberances from the cut ends within 7 to 10 d of culture initiation (Fig. 1a) on MS medium supplemented with BA and KN. Initially, these protuberances were induced at the both the cut ends of nodal segments, subsequently developed on the entire surface of the explant as well as from the abaxial surface, which were in contact with the medium. By the end of 6th wk, these structures develop into shoot buds without intervening callus phase and among these 66±2% of them were flanked by green leaves (Fig. 1b). At the end of 10th wk, well developed shoots (Fig. 1c) were obtained on the same medium. Maximum number of healthy shoots per explant (13.33± 0.58) was obtained on MS medium supplemented with BA (3.0 mg/L) and KN (3.0 mg/L). The comparative evaluation of data revealed that BA was more effective for direct organogenesis in *A. lanata* in comparison to KN (Table 1). The combined effect of BA and KN showed higher efficiency of multiple shoot induction than individual effect of both the cytokinins.

Fig. 1 (a-g)—Plant regeneration in *A. lanata*: (a) Protuberances at the cut end of nodal explants; (b) Induction of multiple shoots after 6th wk on MS medium+BA (3.0 mg/L)+KN (3.0 mg/L); (c) Well-developed multiple shoots after 10th wk; (d) Pigmented, hard, compact calli developed after 4th wk on MS medium+BA (3.0 mg/L)+KN (3.0 mg/L)+NAA (1.0 mg/L); (e) Shoot bud regeneration from the callus after 8th wk; (f) Shoot showing initiation of roots on ½ strength MS medium+IBA (0.5 mg/L); & (g) Regenerated plantlets in plastic cups containing sterilized sand and soil mixture.

Similar kind of synergistic effect was observed in the *in vitro* culture of cotyledonary explants of *Feronia limonia*[24] and *Lagenaria siceraria*[25,26]. This synergistic effect of BA and KN on regeneration potential of nodal explants of *A. lanata* might be due to less ethylene production induced by simultaneous addition of cytokinins to the medium, and this led to accelerated cell differentiation and ensured higher regeneration[25,26].

Callus Mediated Organogenesis and Elongation of Shoots

Cytokinins promote cell division but they require exogenously added auxin to resume cell division[27], whereas cytokinins modulate auxin induced organogenesis *via* regulation of the efflux dependent intracellular auxin distribution[28]. In addition to tissue sensitivity[29], plant hormone content and auxin to cytokinin ratio appear to modulate the cell division, cell elongation and plant regeneration[30]. In the present study, addition of NAA (0.5 to 2.5 mg/L) to MS media containing optimum concentration of BA (3.0 mg/L) and KN (3.0 mg/L) induced profused pigmented callus development on the surface of the explant by the end of 4th wk (Fig.1 d). After the end of 8th wk, the calli were enriched with multiple shoot initials (Fig. 1e). The number of shoots per explant was the maximum (10.66±1.15) on MS medium supplemented with BA (3.0 mg/L)+KN (3.0 mg/L) +NAA (1.0 or 1.5 mg/L) at the end of 10th wk (Table 1). Addition of auxin to a cytokinin enriched MS media promoted callus mediated multiple shoot bud induction from nodal explants in several species including *Asteracantha longifolia*[31,32], *Stevia rebaudiana*[33] and *Morinda citrifolia*[34]. At the end of 10th wk the length of shoot were also measured, and the data showed that addition of NAA along with BA and KN to the medium also have better effect on elongation of shoots (Table 1; Fig. 2). This led to the

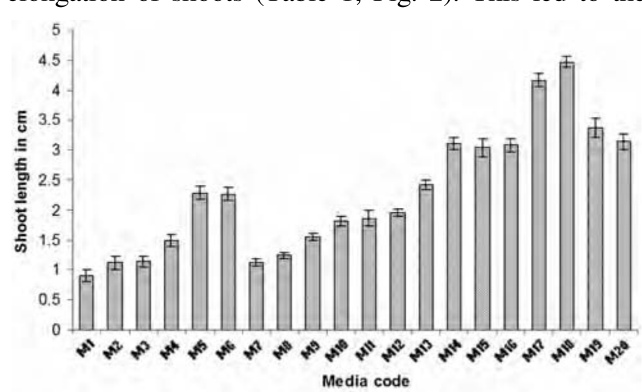

Fig 2—Effect of plant growth regulators (BA, KN & NAA) supplemented to MS medium at various combinations (M_1-M_{20} as per Table 1) on elongation of shoots in *A. lanata*.

Table 2—Effect of auxins on *in vitro* rooting of shoots of *A. lanata* (L) after 3 wk of culture initiation

Media code	½ strength MS medium with plant growth regulator (mg/L)		Rooting frequency (%)	No. of roots per shoots (mean±SD)*	Remarks
	IBA	NAA			
R_1	0.0	0.0	-	0.0 ± 00^{a}	Sproutings but no root developed
R_2	0.1	-	86.67	1.0 ± 0.45^{b}	-
R_3	0.2	-	80.0	1.8 ± 0.45^{cd}	-
R_4	0.5	-	80.0	3.6 ± 0.55^{c}	-
R_5	1.0	-	80.0	2.0 ± 0.71^{cd}	-
R_6	-	0.1	-	0.0 ± 00^{a}	Callus development
R_7	-	0.2	53.33	1.0 ± 0.0^{b}	-
R_8	-	0.5	-	0.0 ± 00^{a}	Sproutings but no root developed
R_9	-	1.0	-	0.0 ± 00^{a}	Sproutings but no root developed

*Means having the same letter were not statistically significant (p=0.05) according to Duncan's multiple range test (SPSS V 16.0).

conclusion that callus mediated organogenesis in *A. lanata* require critical amount of cytokinin and auxin in addition to macro/micro-nutrient composition of media. Combined cytokinins (BA & KN) stimulate the development of meristems, and further enrichment with low dose of auxin (NAA) promotes shoot proliferations as well as elongation in *A. lanata*.

Rooting and Acclimatization

For root induction, healthy individual shoots (>3cms long) were separated and transferred to ½ strength MS medium containing either IBA (0.1, 0.2, 0.5 & 1.0 mg/L) or NAA (0.1, 0.2, 0.5 & 1.0 mg/L). Root induction was observed on IBA (0.1, 0.2, 0.5 & 1.0 mg/L) or 0.2 mg/L NAA (Fig. 1f). However, the average number of roots per shoot and rooting frequency was optimum on ½ strength MS medium containing 0.5 mg/L IBA (Table 2). After 3 wk, the rooted plants were transferred into plastic cups containing autoclaved sand and soil mixture (2:1) and were maintained in the culture room for 2 wk (Fig. 1g). Further, the pots were transferred to shade and later the plantlets were transferred into soil. The survival rate of field transferred plantlets was 72±4%.

The protocol described in the present communication could be useful for *in vitro* propagation of the *A. lanata*, a herb with several medicinal value. Further, this approach can be used for mass multiplication of *A. lanata* in short span of time to cater the need of herbal industries and in the *ex-situ* conservation of this species.

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