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Effect of phytohormones on micropropagation and phytochemical studies of *Aerva lanata* (Linn.) Juss.ex Schult-A seasonal and vulnerable plant

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ABSTRACT

Aerva lanata (Linn.) Juss. ex Schult (Family-Amaranthaceae) is an medicinally important plant known for its prolonged hypertensive and hypoglycemic activity. This study is focused on standardization of in vitro propagation and phytochemical studies of A. lanata. Direct organogenesis of shoots from nodal segments was achieved by culturing on MS medium supplemented with different concentrations and combinations of growth regulators BAP (0.5–2.5 mg/l), IAA (0.5–2.5 mg/l) and KIN (0.2–1.0 mg/l). The maximum number of multiple shoots per explants was obtained in the combination BAP and (1.5 mg/l) and KIN (0.6 mg/l). Half and full strength of MS medium with different concentrations of auxins such as IAA and IBA, were used for in vitro root formation. IAA (1.5 mg/l) with half strength MS medium supported the greatest increase in the number of average roots. Hardening of regenerated plants was achieved with on 1:2:1 ratio of sterilized garden soil, red soil, compost. In the current investigation, 70% of A. lanata plantlets survived transfer to ex situ conditions.

Keywords: *Aerva lanata*, MS medium, PGR, *In vitro* regeneration, Phytochemical.

INTRODUCTION

Aerva lanata L. a medicinally important plant commonly called as Polpala belongs to the family Amaranthaceae which is recognized as repellants to many pests and birds. This plant is native to Indomalaya, Afrotropic and Australia, can be found in open forests on mountain slopes, on waste and disturbed ground, deserted cultivation and coastal scrub and at altitudes from sea level to 900 meters (3000 ft) [1]. *Aerva lanata* is prostrate to decumbent, sometimes erect herb, 30-60 cm in height, wooly, tomentose throughout; leaves simple, alternate, short petioled, densely tomentose, usually smaller in the flowering branches; flowers very small, sessile, bisexual, greenish or hairy white, often clustered in spikes, perianth calycine membranous, five free filaments of the five stamens connate at the base with alternating linear staminodes; fruit greenish, roundish, compressed urticle and seeds kidney shaped with shining black coriaceous testa [2].

Several medicinally active components of *Aerva lanata* have been identified including alkaloids, flavonoids, tannins, phenols and saponins, which boost the immune system and increase the amount of phagocytic cells [3, 4]. In traditional medicine, this plant is being widely used for the treatment of anti-helmintic, anti-diabetic, anti-hepatotoxic, expectorant and in treatment of lithiasis [5]. Recent pharmacological investigation of the leaf extract of this plant revealed immunomodulatory and antitumor activity [6]. The flowers are used in dysentery, diarrhea and bronchitis [7]. The seeds find use in rheumatism and bronchitis treatments. The leaves are used as anti-malarial, in fever, in hepatitis, and to expel stones from kidney and also as an antidote for scorpion sting, spermaorrhoea, urinary

troubles and as an antirheumatic [8]. The roots are used in headache, scabies, and cough, as demulcent, diuretic, to cure diarrhea, jaundice, cholera, and dysentery and in snake bite [9]. The herb *A. lanata* L. is seen soon after the onset of monsoon and disappears by the beginning of January. Dormancy and seasonal appearance in plants are a temporary suspension of visible growth of structures containing a meristem and also an important mechanism ensuring a seasonal synchronization of plant growth and also contributes to the control of architecture [10].

Emphasized that medicinal plants continue to be an important source of life saving drugs for humankind. Micropropagation and eco-restoration of medicinal plants in fact support the in-situ conservation activities, facilitate population enhancement of species where natural propagation is hindered due to destructive harvesting and reproductive barriers. Therefore, the present study reports the *in vitro* response of *A. lanata* explants, their reproducibility and reliable techniques for shoot multiplication within short period of time [11].

A. lanata has a narrow germplasm base and continuously exploited for its varied advantages and medicinal properties. To combat the problem, conventional propagation methods were employed but the plant took very long time for its development with a low rate of fruit set and poor seed germination. Moreover, this plant is susceptible to fungi, bacteria and viruses which further reduce the yield. In the case herbs land degradation, transformation or clearing would wipe out the whole population [12].

In vitro shoot multiplication by direct organogenesis of *A. lanata* was not yet attempted and this study would be the first of its kind to report. Due to unprecedented genetic erosion, disappearance of species, their ecosystem and conservation of natural resources *in vitro* regeneration and phytoconstituents of plantlets is mandatory. Medicinal plants continues to be an important source of lifesaving drugs for humankind. Therefore, the present study reports the *in vitro* response of *A. lanata* explants, their reproducibility and reliable techniques for shoot multiplication within a short period of time.

MATERIALS AND METHODS

Source of explants

For the present study, medicinally and economically important plant *A. lanata* was selected. The plant was collected from wildly growing plant population of *A. lanata* in and around the botanical garden of "Save Life Research Center" in Thanjavur, Tamil Nadu, India.

Selection of explants

Fresh nodal (35 days old) explants were taken from the plant and used for inoculation for direct organogenesis respectively.

Sterilization of explants

The explants were washed thoroughly in running tap water for 30 minutes to remove the soil or sand particles in the surface of the explants and placed in detergent solution (Teepol, 5% (v/v) for 10 minutes. Then the explants were washed in running tap water until the removal of last traces of detergent solution. After that the explants were washed thoroughly with double distilled water. Then the explants were transferred in front of laminar air flow. Explants were then treated with 70% ethanol for 20 seconds and washed with sterile distilled water for 4 times. Thereafter, the explants were immersed in 0.1% (w/v) mercuric chloride for 2–3 minutes. Finally, they were rinsed with several changes of sterile distilled water.

Preparation of media

. The surface sterilized explants were placed vertically into 25×150 mm culture tubes containing 15 ml of MS media containing 30% (w/v) sucrose and 0.6% (w/v) agar and supplemented with plant growth factors. The pH of the media was adjusted with 0.1 N sodium hydroxide and 0.1 N hydrochloric acid prior to autoclaving at 15 lbs pressure at 120°C for 15 minutes [13].

Culture conditions

The explants were transferred aseptically on MS media supplemented along with growth regulators (BAP, IAA, IBA, KIN). The cultures were kept under 16/8 hours, light/dark (3000 Lux) photo period at 25 ± 2°C. Twenty culture tubes with explants were assigned to each treatment. The results were observed and regulated at regular time intervals.

Shoot induction
After 2 weeks incubation, tissue growth was observed in the culture tubes with different concentrations and combinations of growth regulators: BAP (0.5–2.5 mg/l), IAA (0.5–2.5 mg/l) and KIN (0.2–1.0 mg/l). The shoot induction was assessed after 15 days in culture by counting the proliferated shoots which attained the length of 4.0 cm and above. The subsequent sub culture was made only on the medium which showed maximum shoot growth.

Shoot multiplication
Proliferated shoots of 4.0 cm and above were divided into small cultures of 2–3 shoots. They were sub cultured on MS medium containing growth regulators: BAP (0.5–2.5 mg/l) and KIN (0.2–2.5 mg/l). The cultures were incubated under the same conditions as cultures were initiated. After two weeks, shoots which are longer than 6.0 cm were counted and transferred to rooting medium.

Root induction from shoots and hardening
In vitro shoots developed through organogenesis (6.0 cm and above in length) were excised from the culture tubes and sub cultured into medium containing two different concentration of MS salts (half and full strength) supplemented with IAA and IBA (0.5–2.0 mg/l). The cultures were kept under 16 hours light/day photoperiod. The rooted plantlet were removed from the culture tubes and washed in running tap water. The number of shoots that formed roots in each plantlets were counted and they were transplanted in to plastic pots containing a mixture of sterilized and unsterilized garden soil, red soil and compost (1:2:1). The pots were covered with transparent plastic bags to maintain humidity under 16 hours light/ day photo period (2000 Lux) at 25 ± 2°C. The plants were watered using MS nutrient solution or ordinary water as and when required. Fifty percent of the plantlets were maintained under AC room after covering with plastic bags. The remaining plantlets were placed under shade with a short exposure (2–3 hours) to sunlight every day. After 15 days, the plantlets were transferred to pot containing 2: 1: l of the soil mixtures (garden soil, red soil, compost) and placed in green house. The plants were transplanted to field. The plants growing in field were observed for their growth and survival after 40 days.

Phytochemical screening of *Aerva lanata* plant extracts
The phytochemical components of the *Aerva lanata* plant extracts were screened by using the methods of [14, 15]. The components analysed were alkaloids, volatile oils, fatty acids, emodins, flavonoids, triterpenoids, antharacene glycosides, tannins, phenolics and sponins.

Seperation of the compounds
The compounds present in the *Aerva lanata* plant extracts were qualitatively analysed by using thin layer chromatography which is commercially available, TLC aluminium sheets with silica gel $60F_{354}$ were used. The isolation and seperation of monoterpenes and sesquiterpenes was done by using the procedure of [14, 16].

Statistical analysis
The percentage of response, callus formation, number of shoots, shoot length, number of roots, root length and antimicrobial activity indicated by zone of growth inhibition were monitored as growth parameters. Data of three independent experiments represented by 10 and 3 replicates from each experiment were subjected to statistical analysis (mean ± SE), according to New Duncan's Multiple Range Test [17].

RESULTS AND DISCUSSION

Shoot multiplication
For direct organogenesis, nodal segments were inoculated on MS basal medium containing different plant growth regulators (Figure 1). After 2 weeks of inoculation of the nodal segments on MS medium growth was observed in the culture tubes with different concentrations and combination of growth regulators like BAP (0.5–2.5 mg/l), IAA (0.5–2.5 mg/l) and KIN (0.2–1.0 mg/l) (Table 1). The shoot induction was assessed after 15 days in culture by counting the maximum proliferated shoots which attained the length of 4.0 cm and above which was obtained in the combination of BAP (1.5 mg/l) and KIN (0.6 mg/l). Thus the subsequent sub culture was made on the medium BAP and KIN which showed maximum shoot growth. The explants responded well to different concentrations and combination of plant growth regulators (Table 2). Maximum number of multiple shoots was obtained in the combination BAP 1.5 mg/l and KIN 1.5 mg/l with a length of about 8 cm using shoot tips as explants (Figure 1 B). The average time for shoot bud elongation was 28 to 30 days.

Table 1. Effect of BAP, IAA and KIN in shoot induction on MS media from nodal explants after 4 weeks of culture

S. No.	Growth regulators (mg/l)			% of response	Average length of shoots/explant (cm) (mean ± SE)
	BAP	IAA	KIN		
1	0.5	–	–	60	1.1±0.2 c
2	1.0	–	–	65	1.4±0.2 c
3	**1.5**	–	–	**70**	**2.0±0.3 b**
4	2.0	–	–	60	2.5±0.3 bc
5	2.5	–	–	50	1.7±0.2 c
1	0.5	0.5	–	40	1.0±0.2 c
2	1.0	1.0	–	50	1.4±0.2 c
3	**1.5**	**1.5**	–	**60**	**2.1±0.3 bc**
4	2.0	2.0	–	50	2.4±0.3 bc
5	2.5	2.5	–	50	1.8±0.3 b
1	0.5	–	0.2	60	2.5±0.3 bc
2	1.0	–	0.4	65	3.1±0.4 b
3	**1.5**	–	**0.6**	**90**	**4.0±0.5 a**
4	2.0	–	0.8	80	3.5±0.4 ab
5	2.5	–	1.0	75	3.0±0.4 b

Each value represents the mean ±standard error (SE) of ten replicates per treatment in three repeated experiments. Means within a row followed by the same letters are not significant at P = 0.05 according to DMRT.

In the present investigation, MS media gave better response through morphogenesis investigation of *A. lanata*. Similar to our work MS media was found effective in shoot regeneration from nodal explants, apical buds and leaf callus in other plant species (*Asclepias curassaavica, Lycopersicon esculentum, Withania somnifera*) [18-20]. In contradictory to our results L_2 media was found better compared to MS media in the morphogenesis investigation of *A. lanata* done by [12]. Related to our results for shoot induction and multiplication growth regulators have been used both individually and in combination. Nodal explants produced more number of multiple shoots in MS medium with BAP and KIN in other plants (*Eclipta alba, Arachis hypogaea, Carthamus tinctorius and Artemisia vulgaris*) [21-24].

Table 2. Effect of BAP, KIN in shoot multiplication on MS media from shoot explants after 4 weeks of culture

S. No.	Growth Regulators (mg/l)		% of response	Average no. of multiple shoots/explant (cm) (mean ± SE)
	BAP	KIN		
1	0.5	0.2	40	4±0.2 c
2	1.0	0.4	50	4±0.2 c
3	1.5	0.6	60	5±0.2 c
4	**2.0**	**0.8**	**70**	**7±0.3 bc**
5	2.5	1.0	60	6±0.3 bc
1	0.5	0.5	60	10±0.4 b
2	1.0	1.0	60	11±0.4 b
3	**1.5**	**1.5**	**90**	**13±0.5 a**
4	2.0	2.0	80	12±0.5 a
5	2.5	2.5	70	11±0.4 b

Each value represents the mean ±standard error (SE) of ten replicates per treatment in three repeated experiments. Means within a row followed by the same letters are not significant at P = 0.05 according to DMRT.

Root induction

Regenerated shoots did not form root spontaneously. For this reason, elongated shoots (6–8 cm) were excised and cultured on root induction medium to stimulate root development. In the present experiment, half and full strength of MS medium with different concentrations of auxins such as, IAA and IBA, were used for *in vitro* root formation in *A. lanata*. Irrespective of plant growth regulator supplements all cultured shoot showed rooting response and showed positive response towards healthy root development (Figure 1 C). Results of these observations are presented in the (Table 3). In case of IAA thin long roots were initiated from the cut ends at the base of shoots. While in case of IBA, though similar type of roots were formed in all concentrations *Aerva lanata* L. a medicinally important plant commonly called as Polpala belongs to the family Amaranthaceae which is recognized as repellants to many pests and birds. This plant is native to Indomalaya, Afrotropic and Australia, can be found in open forests on mountain slopes, on waste and disturbed ground, deserted cultivation and coastal scrub and at altitudes from sea level to 900 meters (3000 ft), but the root growth in IBA supplemented media was slow. Among the four concentrations tested, 1.5 mg/l IAA with half MS medium showed best result with increased number of average roots (6.5). Days required

for root initiation varied to the different types of media. The minimum days (6-7) required for root initiation when the explant treated with half MS medium supplemented with 1.5 mg/l IAA, well develop roots were observed in 16-20 days.

Table 3. Effect of combination of IAA and IBA on root induction

S. No.	MS basal media	Growth regulators (mg/ml)		% of response	Average number of roots (mean ± SE)
		IAA	IBA		
1	Half-Strength	0.5	–	50	4.1±0.3 bc
2		1.0	–	60	4.8±0.3 bc
3		**1.5**	–	**90**	**6.5±0.5 a**
4		2.0	–	80	6.0±0.5 a
5		–	0.5	40	5.2±0.4 ab
6		–	1.0	50	4.7±0.3 bc
7		–	1.5	60	3.5±0.2 c
8		–	2.0	50	3.1±0.2 c
1	Full-Strength	0.5	–	40	3.7±0.2 c
2		1.0	–	50	4.2±0.3 bc
3		**1.5**	–	**70**	**5.0±0.4 ab**
4		2.0	–	60	5.5±0.5 ab
5		–	0.5	30	5.1±0.4 ab
6		–	1.0	40	4.4±0.3 bc
7		–	1.5	50	4.0±0.3 bc
8		–	2.0	40	3.5±0.2 c

Each value represents the mean ±standard error (SE) of ten replicates per treatment in three repeated experiments. Means within a row followed by the same letters are not significant at P = 0.05 according to DMRT.

Both the auxins (IBA and IAA) used in this study induced rooting of *in vitro* raised shoots. In the present study IAA was found to be more potent auxin, in terms of root production compared to IBA. According to [25] several reports deal with the better performance of IAA versus IBA during the rooting process in bamboo. This was attributed to parameters such as stability, transport or metabolism. In contradictory to our results IBA was found to be the best rooting plant growth regulator in other plants (papaya, *Psidium guajava* L. and *Cichorium intybus* L.,) as reported by [26-29].

Establishment and performance of plantlets in natural environment

Healthy and fully developed rooted plantlets were successfully transplanted into plastic pots containing a mixture of sterilized and unsterilized garden soil, red soil and compost (1:2:1). Roots formed in IAA showed better survivability in the soil than those induced with IBA. The rooted plantlets were transplanted into the plastic cups, containing sterile soil, sand and compost (1:2:1). Following transplantation survival rate of the regenerated plantlets were found to be 70%. Transplanted plantlet is shown in (Figure 1 D). The survived plantlets were transferred to the field. Success of *in vitro* response of these explants is strongly influenced by the source of the explants, media supplementation, growth regulators and controlled environment. Methodology standardised in the present study for complete plant regeneration of this *A. lanata* can be applied to produce plants throughout the year irrespective of season. Similarly [30] observed a remarkable influence of the season on tissue culture response of barley (*Hordeum vulgare*) for cultivars Salome and Golden Promise.

Phytochemical screening of *A. lanata* plant extracts:

The preliminary phytochemical screening of the *A. lanata* plant extracts using different solvents was reported (Table 4). All the four organic solvents such as ethanol, chloroform, acetone, benzene showed positive result for the presence of volatile oils and fatty acids which were absent in the water extract. In the water extract, flavonoids and tannins were present which were absent in the organic solvent extracts [31-35].

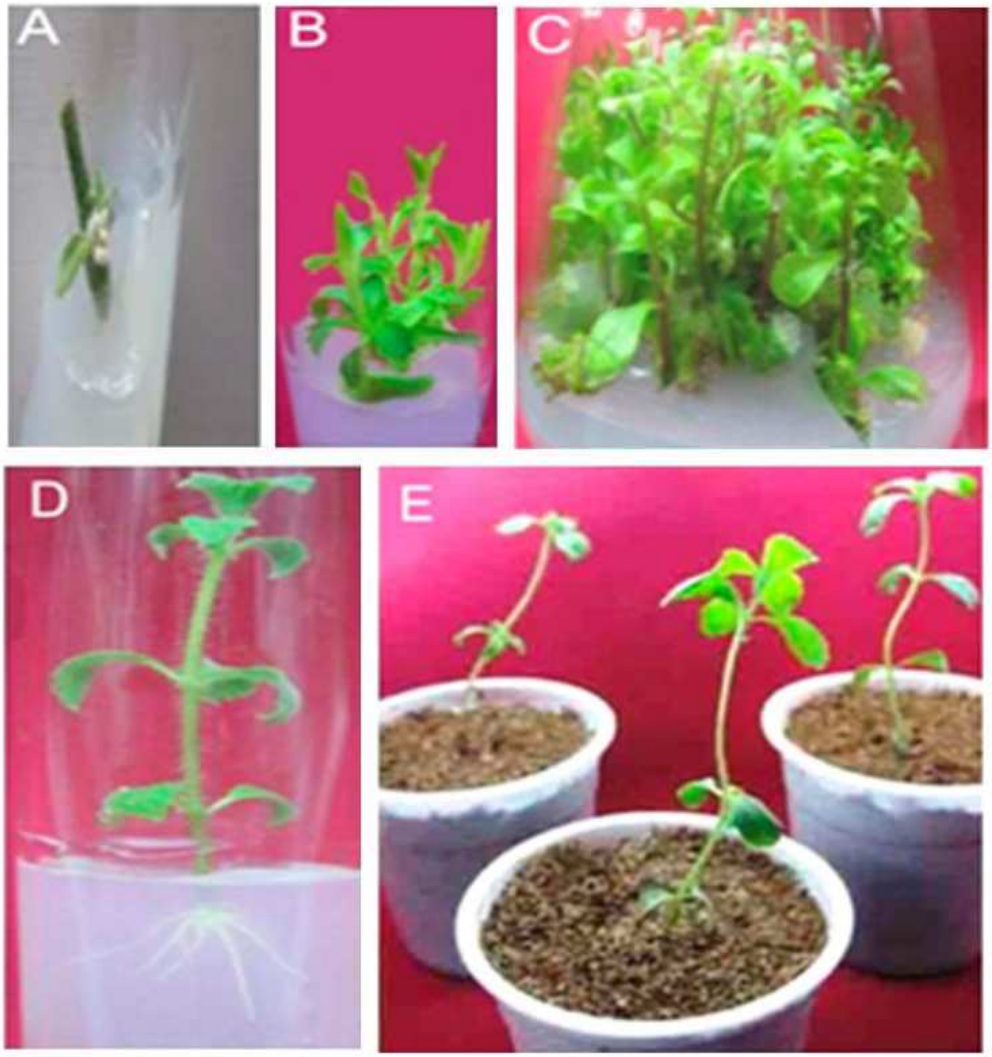

Figure 1. Direct regeneration of Aerva lanta from nodal explant: A: Inoculation of nodal explant B: Shoot mulitplication in 15 days C: Shoot mulitplication after 30 days D: Root initiation after 35 days E: Hardened plantlet in form cup

Table 4. Preliminary phytochemical analysis of *A. lanata* with different solvents

Sl. No	Name of the compound	B	C	A	E	W
1	Alkaloids	-	-	-	+	+
2	Volatile oils	+	+	+	+	-
3	Fattyacids	+	+	+	+	-
4	Emodins	-	-	-	-	-
5	Flavonoids	-	-	-	-	+
6	Triterpenoids	-	-	+	-	-
7	Antharacene glycosides	-	-	-	-	-
8	Tannins	-	-	-	-	+
9	Phenolics	-	-	-	-	-
10	Saponins	+	+	-	+	+

+ Present, - Absent, A-Acetone, B-Benzene, C-Chloroform, E-Ethanol, W-Water.

CONCLUSION

Aerva lanata L. a medicinally important plant that belongs to the family Amaranthaceae. Direct organogenesis of shoots from nodal segments was achieved by culturing on MS medium supplemented with different concentrations and combinations of growth regulators BAP, IAA and KIN. The maximum number of multiple shoots per explants was obtained in the combination BAP and (1.5 mg/l) and KIN (0.6 mg/l). Half and full strength of MS medium with different concentrations of auxins such as IAA and IBA, were used for *in vitro* root formation. IAA (1.5 mg/l) with half strength MS medium supported the greatest increase in the number of average roots. In the present investigation, MS media gave better response through morphogenesis investigation of *A. lanata*. The preliminary phytochemical screening of the *A. lanata* plant extracts using different organic solvents. All the four organic solvents such as ethanol, chloroform, acetone, benzene showed positive result in *A. lanata* plant extracts.

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