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Regeneration of Plantlets from Leaf Derived Callus of *Aerva lanata*, Juss: A Medicinal Plant

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ABSTRACT

Plantlets regeneration through callus has been achieved in medicinal plant *Aerva lanata* of Amaranthaceae. Callus obtained by culturing young leaf discs on Murashige and Skoog's (MS) medium with 1.0 mg L^{-1} 2, 4.D (dichlorophenoxyacetic acid) was subcultured on MS medium containing 2.0 mg L^{-1} 6, Benzyl Amino Purine (BAP), produced 30-40 multiple shoots. Rooting was achieved on half or full strength MS medium with 0.5-0.1 mg L^{-1} Naphthalene Acetic Acid (NAA) in 14 days. Regenerated plantlets were acclimatized and transferred to soil were showed normal morphological characteristics. Survival of transplants was 90-95% under green house condition.

Key words: *Aerva lanata,* callus, multiple shoots, regeneration, rooting

INTRODUCTION

Aerva lanata. Juss. of Amaranthaceae is perennial erect or prostrate herb with medicinal and ornamental uses, common weed in south India, distributed throughout India, Ceylon, tropical Africa, Java and Philippines etc. Its leaves and roots are used in folk medicine, mainly for diuretic, Lithiasis (Soundararajan *et al*., 2006), Anthelmintic, headache, cough and also vermifuge for children (Nadakarni, 1976), Antimicrobial activity and cytotoxicity (Chowdhury *et al*., 2002). Since the plant is a weed, most of the farmers destroying the plant without knowing the medicinal value and folk doctors intensively collected the plants growing naturally in the field. This has resulted in gradual eradication of the plant. Weeds have been a part of civilization and many ancient documents speak of humans battling weeds in the crops they grow (Prakash *et al*., 2012). Knowledge of management, proper identification and ecological status of the Weeds are important to point out the useful weeds (Nasir and Sikander, 2006; Adebayo and Uyi, 2010). Tissue culture has been investigated as an alternative tool for rapid progression of this plant species, thus reducing the risk of its extinction. *In vitro* technique has proven as a potential technology for clonal propagation and conservation of medicinal plant species; micropropagation of *Hemidesmus indicus* (Siddique and Bari, 2006) and *Hibiscus sabdariffa* (Gomez-Leyva *et al*., 2008) has been reported.

MATERIALS AND METHODS

Aerva lanata collected from the Bangalore University campus, Bangalore and maintained as stock plant in green house (Fig. 1a). Juvenile leaves were collected from young branches of mature

plant were washed thoroughly with 5% Labolean solution under running tap water for 15 min. The leaves were disinfected with aqueous mercuric chloride ($HgCl_2$) solution (0.1 w/v) for 5-7 min and then washed thoroughly with sterile distilled water. Leaf discs were inoculated on MS medium containing 3% sucrose, 0.8% agar and fortified with 1.0 mg L^{-1} 2, 4.D (dichlorophenoxyacetic acid) for callus induction. Initially the discs were cultured in test tubes. After 15 days, proliferating callus were transferred to 100 mL conical flasks to obtain more callus. 2.0 mg L^{-1} BAP was used for shoot bud differentiation. Shoot buds were harvested and transferred on the half or full strength MS medium with 0.5-1.0 mg L^{-1} NAA for rooting. The pH of the media was adjusted to 5.8 before adding agar and closed with non absorbent cotton. Media were autoclaved at 121°C (C) for 15 min. All cultures were incubated under white cool florescent light (1000 Lux) at 25±2°C with 16 h photo period. Callus cultures were maintained by subcultures, every 30 days on fresh medium. The experiment was repeated and 12 explants per treatment were cultured. Rooted plants were removed from the culture media, washed thoroughly with distilled water and transferred to small pots containing vermiculate, yard manure and soil (1:1:1) for hardening and kept in the growth chamber. After 14 days they were transferred to green house.

RESULTS AND DISCUSSION

The effect of NAA, 2, 4.D, BAP and KN (kinetin) at 0.1 to 2.0 mg L^{-1} were tested on MS Medium for callus initiation. Among the concentration and combinations of hormones used, 2, 4.D showed better callus initiation and growth. Friable, greenish and white callus was obtained from leaf discs cultured on MS +1 mg L^{-1} 2, 4.D, within 2 weeks. Similarly regeneration of multiple shoots were achieved by several workers from leaf explants in *Terminalia arjuna* (Arumugam and Gopinath, 2011); *Ananas comosus* (Amin *et al*., 2005) and *Memecylon edule* (Elavazhagan and Arunachalam, 2010). Callus color varied from white, light brown and light orange (Fig. 1b and c). All the leaf explants responded well with 2, 4.D in the media ranging from 0.5 to 3.0 mg L^{-1}. Initially the growth of the callus was observed to be slow but when subcultured on the same media and growth hormone, accelerated the growth rate. Approximately 50 mg of the induced calluses, broken in to tiny pieces were subcultured on MS medium containing 01 mg L^{-1} BAP either alone or combination with IAA, NAA and KN (0.1-2.0 mg L^{-1}). The BAP alone promotes shoot organogenesis when the MS medium is fortified with (Table 1). The combination of BAP and NAA promotes both shoot and root formation but size and number of roots are not satisfactory. Increase in BAP concentration did not increase the number of shoots. Elongated multiple shoots (30 to 40) were obtained within three weeks of subculture (Fig. 1d). NAA induced direct root organogenesis at the concentration of 0.5-1.0 mg L^{-1} (Table 2).

Table 1: Effect of BAP on induction of multiple shoots in *Aerva lanata* callus

BAP	No. of multiple shoots per 50 mg callus	Callus shows multiple shoots (%)
0.0	0	0
0.3	2	45
0.5	10	60
0.8	15	90
1.0	40	95
1.3	40	95
1.5	40	95
1.3	30	95
1.8	33	95
2.0	29	95

Values are Mean of the two experiments with 12 explants each

Fig. 1(a-f): (a) *In vivo Aerva lanata* plant, (b) Rooting and callus formation on NAA + 2,4-D, (c) Callus on 2,4-D, (d) Multiple shoots obtained after 30 days of culture on MS medium containing 2 mg L^{-1} BAP (e) and (f) Rooting on MS+0.5 mg L^{-1} NAA

Table 2: Effect of NAA on root induction of multiple shoots in *Aerva lanata* callus

MS medium+NAA	Shoots rooted (%)	Mean No. of roots $shoot^{-1}$
0.0	0	0
0.1	40.4±0.5	2.3±0.4
0.2	45.4±0.5	2.3±0.4
0.4	80.2±0.8	5.2±0.7
0.5	92.8±0.8	7.3±0.4
0.8	83.4±0.6	5.3±0.6
1.0	83.4±0.6	5.3±0.6
1.5	67.4±0.6	3.6±0.7

Values are Mean±SEM (standard error of the mean) of the two experiments with 12 explants each

Better rooting of micro shoots was obtained in the presence of NAA 0.5 mg L^{-1} containing half or full strength MS media in 14 days (Fig. 1e, f). The rooted plants were removed from the cultures, washed thoroughly with water and transferred to pots for 2-3 weeks for hardening in the green house. The regenerated plantlets showed normal morphological characteristics.

Survival percentage was 90 to 95 shows that the tissue culture technique can be used for large scale production in limited time and space.

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