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In vitro Shoot Multiplication of *Vernonia cinerea* (L.) Less. – an important medicinal herb

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A rapid and efficient protocol was developed for inducing direct organogenesis using shoot tip explants of Vernonia cinerea L. Shoot tip explants were cultured on MS medium supplemented with different concentrations of BAP (2.22 to 22.20 µM/l) and KN (2.42 to 23.20 µM/l) for direct shoot induction. The frequency of multiple shoot induction and proliferation increased with increasing concentration of BAP (13.32 µM/l) and KN (13.92 µM/l) at optimal level. The high frequency of multiple shoot proliferation was observed on MS medium containing 13.32 µM/l BAP and 13.92 µM/l KN. The regenerated shoots were successfully rooted on MS medium supplemented with IBA 7.38 µM/l, after sequential hardening, survival rate was 90 %.

Key works: *Vernonia cinerea*, Shoot tip, Direct organogenesis

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Introduction

Vernonia cinerea (L.) Less is an important medicinal herbs, belongs to the family Asteraceae, commonly called as iron weed. The principal constituents of V. cinerea is β-amyrin acetate, β -amyrin benzoate, lupeol and its acetate, β -sitosterol, stigmasterol, a-spinasterol and Kcl. The whole plants are useful in anthelminitic, depurative, diuretic, lithontriptic, anodyne, anti-inflammatory, sudorific, stomachic, alexeteric, antibacterial, antifungal, antiviral, antiperiodic and alexipharmic. The roots are useful in skin diseases, leprosy, strangury, renal, versical calculi, intermittent fevers and vitiated conditions of Vata. The leaves are useful in humid, herpes, eczema, ring worm, guinea worms and elephantiasis. The flowers are useful in

conjuctivities, vitiated conditions of Vata and fevers. The seeds are useful in leucoderma and dysiria. The plant possesses anticancerous activities and good for cancerous mal formations. It is alternative and purifies blood, piles and semen (Pullaih 2002, Prajapathi *et al*.2003). Plant cell and tissue culture have become major tools in the study of an increasing number of fundamental and a applied programs in plant science. Tissue culture techniques are being used globally for the ex situ conservation of plants. The endeavor is to adopt that method to multiply the medicinal herbs and monitor their secondary metabolites. Conservation of endangered medicinal plants has also been achieved through cell cultures with significance (Rao *et al*.1996). Medicinal Plant biotechnology is a new field, which comprises multiplication by micro propagation, creation of soma clonal variants, genetic improvement using recombinant DNA techniques and bioproduction of useful secondary metabolites of pharmaceutical significance. Medicinal plants are of great interest to the researchers in the field of biotechnology as most of the drug industries depend, in part, on plants for the production of pharmaceutical compounds (Chand *et al*. 1997). Reports of in vitro Plant regeneration from tissues of medicinal plants are available (Hiraoka and Oyanagi 1988, Sharma *et al.* 1991, Lu *et al.* 1995, Sahoo and Chand 1998, Gururaj et al. 2004, Karuppusasmy and Pullaiah 2007, Jawahar *et al.* 2008) However, there is no report on in vitro regeneration of *Vernonia cinerea* (L.) Less. The Present study was to establish an efficient protocol for high frequency of plant regeneration from shoot tips of *Vernonia cinerea* (L) Less.

Materials and methods

Field grown young healthy plants were used as source of explants. Shoot tips were selected as explants for direct regeneration. The explants were washed in running tap water followed by treatment with surfactant, Tween 20 (5 % v/v) for 10 min. The explants were further treated with 70% ethanol for 10-15 sec and rinsed in double distilled water (3-4 times), followed by 0.1 % (w/v) HgCl2 for 2-3 min. Finally, the explants were washed in sterile distilled water for 3-5 times and inoculated on MS medium supplemented with various concentrations of growth regulators. Sucrose (20 g) and 0.8 %agar were used. After adjusting the pH (5.8), the medium was autoclaved at 1210C for 15minutes. All the cultures were incubated at 25 ± 20C under 16 : 8 light and dark: All the treatments were repeated at least there times with 30 replicates and data were subjected to statistical analysis.

Results and Discussion

Vernonia cinerea (L.) Less plant was efficiently regenerated from shoot tips. Explants were capable of directly developing multiple shoots on MS basal medium supplemented with various concentrations of cytokinins. Multiple shoot initiation from shoot tip explants was observed after seven days of inoculation (Fig.1a) and multiple shoot proliferation was obtained with in 30-35 days without subculture (fig.1 b). The morphogenic responses of shoot tip to various growth regulators such as BAP and KN are presented in Table 1. Between two cytokinin tested, multiple shoot proliferation was observed to be the best on MS medium supplemented with BAP. The highest number of shoots (73.0 / explant) was observed in the medium containing 13.32 µM/l BAP followed by KN 13.92 µM/l with 69.0 shoots per explant. The elongation of shoots and nodes were achieved on the same medium. The capacity of multiple shoot bud initiation and proliferation from shoot tips of *Vernonia cinerea* (L.) depended on hormonal content of the culture medium. There was good shoot bud initiation and proliferation response only in the presence of cytokinin and absence in the basal medium. Similar observation was made by Pattnaik and Chand (1996) in *Ocimum* species. The potential for shoot multiplication in Vernonia cinerea appears to be high in the presence of cytokinin alone in the culture medium. The stimulatory effect of singular supplement of BAP on bud burst and multiple shoot formation is similar to that reported in other medicinal plant species by Verma and Kant (1996) in *Emblica officianale,* Sahoo and Chand (1998) in *Vitex negundo*, Deka *et al.* (1999) in *Withania sominifera*, and Ravipaul *et al.*(2008) in *Sphaeranthus amaranthoides*. Sharon and Marie (2000) reported that the shoot tip explants were preferred over meristem to produce large number of genetically identical clones in *Bixa ovellana* L. in the medium containing BAP and KN alone. Pawar *et al.* (2002) reported that BAP and KN individually and combination induced a higher frequency of adventitious shoots form a single explants of *Solanum xanthocarpum*. This result was similar to that recorded in the present study. Regenerated shoots (3cm and above in length) were excised and placed on MS medium with various concentrations of IAA (2.85 to 17.13 µM/l) and IBA (2.46 to 14.76 µM/l) for root induction. Optimal rooting and growth of micro roots were observed without intervening callus 5-10 days after transfer. The percentage of root formation and the number of roots per shoot significantly varied depending on concentrations of IAA and IBA (Table 2). The higher frequency of rooting (70 %) with highest root numbers (8.0 ± 0.8) was obtained in medium

containing 7.38 µM/l IBA followed by 8.56 µM/l IAA (Fig. 1c). However, there was no significant difference between the effect of IBA and IAA. Similar results were reported by Jawahar *et al.* (2008) in *Cardiospermum helicacabum*. Sunichan *et al.* (1998) reported that IBA was effective for root induction in Sterculia urens. Quarishi and Mishra (1998) obtained rooting response in IAA. Pattnaik and Chand (1996) obtained best rooting on the medium containing IBA. In most of the medicinal Plant species IBA and IAA are considered as the most effective growth regulators for the induction of roots. Wakhlu and Sharma (1999) reported that the medium containing IBA produced maximum number of adventitious roots in *Heracleum candicans*. The rooted plants (Fig.1d) were first transferred to plastic cups having vermiculite and garden soil (3:1). The plastic cups were covered with polythene pack and kept for a week in a culture room at 25 ± 20C under 16 h photoperiod. After a week, these were transferred to the green house and then to the field. From our experimental data, it is evident that BAP and KN induced higher frequency of multiple shoot initiation and proliferation, and IBA induced a higher frequency of rooting in shoot tip explants of *Vernonia cinerea* (L.) Less. In conclusion, this direct organogenesis system in suitable for conservation of germplasm of this important multipurpose medicinal plant.

Plate - 1

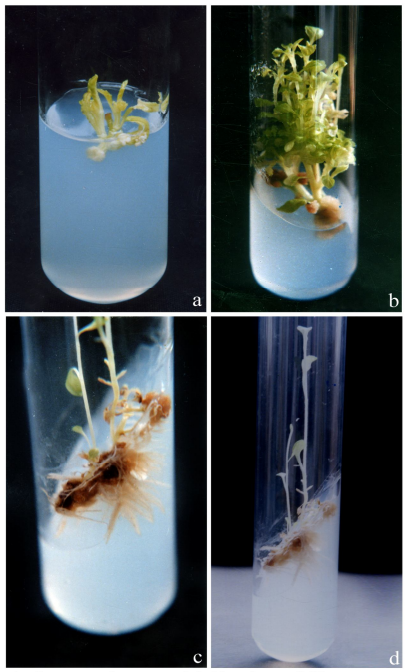

Fig. 1. *In vitro* shoot multiplication of *Vernonia cinerea*
(a) Multiple shoot initiation. (b) Multiple shoot proliferation.
(c) Root induction. (d) Well developed plantlet.

Table 1. Effect of different concentration of cytokinins for *in vitro* shoot multiplication from shoot tip explant of *Vernonia cinerea* (L.) Less.

Growth regulators (µM/l)	% of response	No. of shoots per explant	Average length of soot (cm)
BAP			
2.22	35	38.1 ± 0.7^{k}	1.6 ± 0.5^{ij}
4.44	46	46.3 ± 1.5^{hi}	3.3 ± 0.5^{g}
8.88	58	57.3 ± 2.5^{e}	5.6 ± 0.5^{d}
13.32	80	73.0 ± 2.6^{a}	9.0 ± 1.0^{a}
17.76	60	59.6 ± 2.0^{d}	6.3 ± 0.5^{bc}
22.20	52	52.0 ± 2.0^{f}	4.0 ± 1.0^{f}
KN			
2.32	34	33.8 ± 3.7^{l}	0.8 ± 0.2^{k}
4.64	40	40.3 ± 2.5^{j}	2.6 ± 0.5^{i}
9.28	49	48.0 ± 2.6^{g}	4.0 ± 1.0^{f}
13.92	70	69.0 ± 1.0^{b}	6.5 ± 0.5^{b}
18.56	63	62.6 ± 2.5^{c}	4.6 ± 0.5^{e}
23.20	47	46.6 ± 2.5^{h}	3.0 ± 1.0^{gh}

Each value represents the mean $\pm$ SD of 30 replicates and each experiment was repeated three times. Values with the same superscript are not significantly different at the 0.05% probability level according to DMRT.

Table 2. Effect of different concentration of auxins on root induction in regenerated plantlets.

Growth regulators (µM/l)	% of response	Average number of roots per shoot
IAA		
2.85	32	1.6 ± 0.4^{hi}
5.71	54	2.3 ± 0.4^{h}
8.56	62	6.0 ± 0.8^{c}
11.42	52	4.3 ± 0.4^{d}
17.13	30	2.3 ± 0.4^{h}
IBA		
2.46	32	2.6 ± 0.5^{fg}
4.92	56	3.3 ± 0.4^{e}
7.38	70	8.0 ± 0.8^{a}
9.84	60	6.6 ± 1.2^{b}
14.76	30	3.0 ± 0.8^{ef}

Each value represents the mean $\pm$ SD of 30 replicates and each experiment was repeated three times. Values with the same superscript are not significantly different at the 0.05% probability level according to DMRT.

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