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In vitro Mass Propagation from Shoot Tip Explants of *Vernonia cinerea* (L.) Less. - An Antioxidant, Anti-inflammatory Medicinal Plant

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Abstract

Plantlets were regenerated from the shoot tip explants of *Vernonia cinerea* (L.) Less. in MS supplimented with BA and Kn. Maximum number of shoots in BA (13.32 mg/l) and roots in IBA (7.38 mg/l) developed. The rooted plantlets were successfully established in the field.

Introduction

Fatty oil, β-amyrin acetate, β-amyrin benzoate, β-sitosterol, stigmosterol, α-spinnasterol (+)-lirioresinol B, stigmasterol, stigmasterol-3-O-β-D-glucoside, 4-sulfo-benzocyclobutene compounds inducing NGF activity are extracted from *Vernonia cinerea* (L.) Less. (Asteraceae) (Zhu et al. 2008). It also contains medicinal properties for eczema, ringworm, elephantiasis, conjunctivitis, diarrhea, leucoderma, dysiria, skin diseases, leprosy, fevers, anti-cancer, anti-oxidant and anti-inflammatory (Kumar and Kuttan 2009). Nowadays, tissue culture techniques are actively employed biotechnological methods to improve the metabolites and medicinal plants in a large scale. Here, to develop an effective *in vitro* method for plant regeneration of *V. cinerea* and the propagated plants were successfully established in field conditions.

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Materials and Methods

Shoot tips of *Vernonia cinerea* (L.) Less. were collected from field grown plants (Fig. 1a). The explants were washed with 70% ethanol, 0.1% $HgCl_2$ (w/v) for 3 min and rinsed with sterile distilled water for three to four times. Explants were cultured on MS containing 3.0% sucrose (w/v) and BA (2.22 to 22.20 μM/l) and Kn (2.32 to 23.20 μM/l) for multiplication of plants with optimum pH (5.8 ± 1), light intensity (85 $\mu mol/m^2/s^1$), 16 h. The regenerated shoots were kept into the rooting medium with IBA (2.46 to 14 μM/l). Plantlets were transferred to plastic cups containing sterile soil, sand, vermiculite (1 : 1 : 1), and then transferred to field. All the treatments were statistically analyzed by DMRT) (Gomez and Gomez 1976).

Results and Discussion

Table 1 shows the successful results. Shoot tips were cultured on MS supplemented with different concentrations of BA (2.22 to 22.20 μM/l) and Kn (2.32 to 23.20 μM/l). Higher number of multiple shoot induction was observed in 30 days and significantly higher in BA than Kn (Fig. 1b, c). The maximum number of multiple shoots (148.2/shoot tip) was obtained in MS with BA 13.32 μM/l (Table 1; Fig. 1d, e). Similar results were reported in *Emblica offcinalis* (Verma and Kant 1996) and *Withania sominifera* (Deka et al. 1999).

Table 1. Effect of Kn and BA on multiple shoot induction from shoot tip explants of *Vernonia cinerea* (L.) Less.

Plant growth regulators (μM/l)	Regenerating shoots (%)	No. of shoots/ explants	Shoot length (cm)
Kn			
2.32	33.8 ± 3.7^{f}	39.0 ± 3.6^{f}	0.8 ± 0.2^{f}
4.64	40.3 ± 2.5^{e}	69.0 ± 3.6^{de}	2.6 ± 0.5^{de}
9.28	48.0 ± 2.6^{c}	83.0 ± 7.2^{b}	4.0 ± 1.0^{c}
13.92	69.0 ± 1.0^{a}	131.6 ± 10.4^{a}	6.5 ± 0.5^{a}
18.56	62.6 ± 2.5^{b}	81.0 ± 3.6^{bc}	4.6 ± 0.5^{b}
23.20	46.6 ± 1.5^{cd}	71.3 ± 3.2^{d}	3.0 ± 1.0^{d}
BA			
2.22	38.1 ± 0.7^{f}	46.6 ± 1.5^{f}	1.6 ± 0.5^{f}
4.44	46.3 ± 1.5^{e}	70.0 ± 1.0^{e}	3.3 ± 0.5^{de}
8.88	57.3 ± 2.5^{bc}	101.6 ± 7.6^{b}	5.6 ± 0.5^{c}
13.32	73.0 ± 2.6^{a}	148.3 ± 2.8^{a}	9.0 ± 1.0^{a}
17.76	59.6 ± 2.0^{b}	91.6 ± 5.6^{c}	6.3 ± 0.5^{b}
22.20	52.0 ± 2.0^{d}	88.3 ± 7.6^{cd}	4.0 ± 1.0^{d}

Values are mean of 30 replicates per treatment and repeated thrice. Values with the same superscript are not significantly different at 5% probability according to DMRT.

Patnaik and Chand (1996) reported BA was superior for multiple shoot induction than other cytokinins in shoot tip explants. Beena et al. (2003) reported that the Kn did not support the proliferation of multiple shoots in *Ceropegia candelabrum*, but the Kn also favoured for high rate of multiple shoot induction.

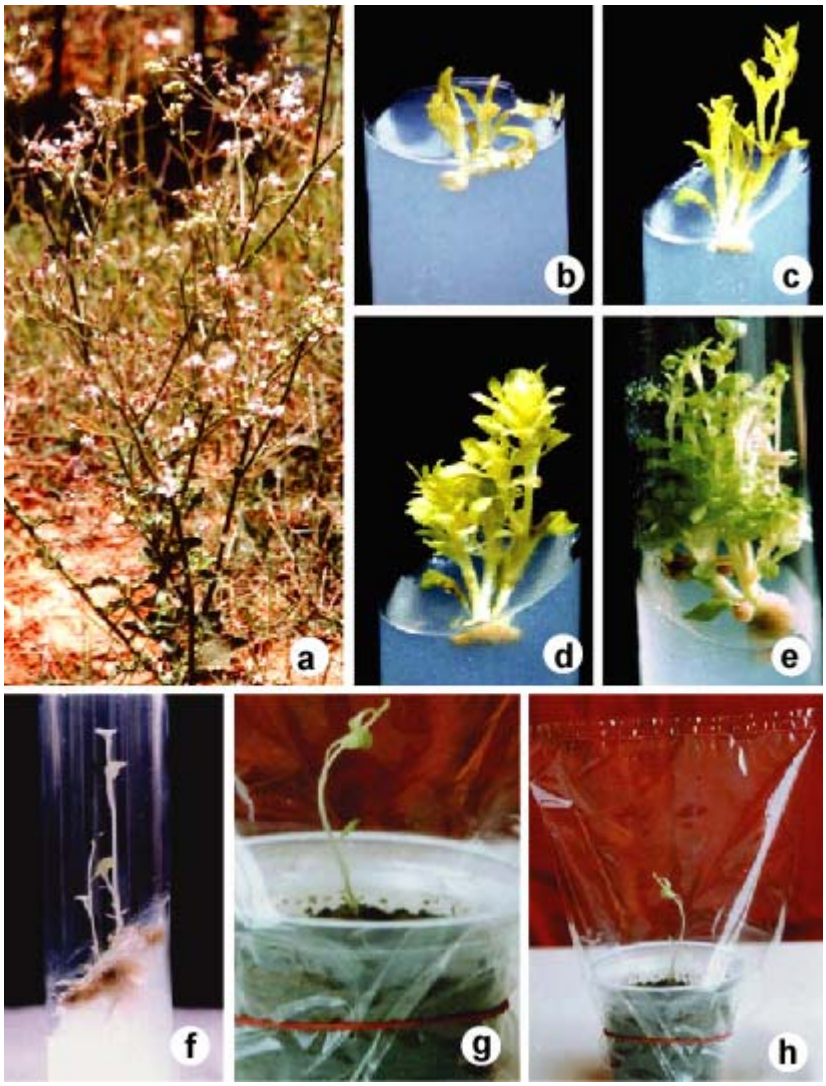

Fig. 1. a. Habitat, b-c. Multiple shoot initiation, d-e. Multiple shoots, f. Rooting and g, h. Hardening.

The well developed shoots were transferred for root induction in medium containing IBA (2.46 to 14.76 μM/l) and IAA (2.85 to 17.13 μM/l) at 25 days. Maximum number of roots per shoot was observed in IBA 7.38 μM/l (Table 2; Fig. 1f), whereas, the higher level of IBA showed low frequency root induction.

Patnaik and Chand (1996) suggested that the best rooting medium contained IBA. Well-developed rooted plantlets were transferred to plastic cups and covered with polythene and maintained in tissue culture conditions (Fig. 1g, h). Finally, the developed plantlets were kept in greenhouse, and then transferred to field. The survival rate was 55%.

Table 2. Effect of IAA and IBA on root induction from shoot tips derived plantlets of *Vernonia cinerea* (L.) Less.

Plant growth regulators (μM/l)	Root formation (%)	Average No. of roots/shoot
IAA		
2.85	32.0 ± 4.89^{d}	1.6 ± 0.47^{e}
5.71	54.0 ± 1.63^{b}	2.9 ± 0.34^{c}
8.56	62.3 ± 2.05^{a}	6.0 ± 0.81^{a}
11.42	50.0 ± 1.23^{bc}	4.3 ± 0.47^{b}
17.13	30.0 ± 4.89^{cd}	2.3 ± 0.37^{cd}
IBA		
2.46		
4.92	32.0 ± 4.89^{d}	1.6 ± 0.47^{e}
7.38	56.6 ± 2.62^{bc}	3.3 ± 0.27^{c}
9.84	70.6 ± 4.08^{a}	8.0 ± 0.81^{a}
14.76	62.3 ± 2.05^{b}	6.6 ± 1.24^{b}
	30.0 ± 4.89^{de}	3.0 ± 0.81^{cd}

Values are mean of 30 replicates per treatment and repeated thrice. Values with the same superscript are not significantly different at 5% probability according to DMRT.

In conclusion, the micropropagation protocol was established from shoot tip explants of *V. cinerea.* The rehabilitation micropropagation development total process was completed in 60 days. This efficient micropropagation protocol will be useful to conservation, and in the improvement of *V. cinerea* using genetic transformation.

References

Beena MR, Martin KP, Kirti PB and **Hariharan M** (2003) Rapid *in vitro* propagation of medicinally important *Ceropegia candelabrum*. Plant Cell Tiss. Org. Cult. **72:** 285-289.

Deka AC, Kalita ML and **Baruah A** (1999) Micropropagation of a potent herbal medicinal plant, *Withania sominiferea*. Environ. Ecology. **17**: 596.

Gomez KA and **Gomez AA (1976)** Statistical procedures for agricultural research with emphasis on rice. International Rice Research Institute. Los Banos, Philippines, p. 264.

Kumar P and **Kuttan G (2009)** *Vernonia cinerea* (L.) Less. scavenges free radicals and regulates nitric oxide and proinflammatory cytokines profile in carrgeenan induced paw edema model. Immunopharma. Immnotoxicol. **31:** 94-102.

Patnaik S and **Chand PK** (1996*) In vitro* propagation of the medicinal herbs *Ocimum americanum* L.Syh. *Ocimum sims* (Hoary basil) and *Ocimum sanctum* L. (Holy basil). Plant Cell Reports **15:** 846-850.

Verma B and **Kant U** (1996) Micropropagation of *Embilica officinale* Gaertz through mature nodal explant. J. Phytal. Res. **9**: 107-109.

Zhu HX, Tang YP, Pan LM and **Min ZD (2008)** Studies on bioactive constituents of whole herbs of *Vernonia cinerea*. Zhongguo Zhongyao Zazhi. **33:** 1986-1988.