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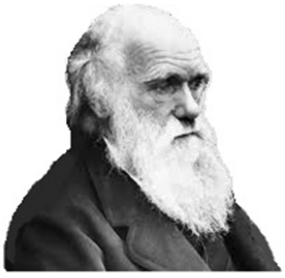

World News of Natural Sciences

An International Scientific Journal

WNOFNS 27 (2019) 141-152 EISSN 2543-5426

In Vitro Micropropagation of *Vernonia cinerea* (L.) Less. - An important medicinal herb

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ABSTRACT

In vitro micropropagation was achieved from Nodal and leaf explants of *Vernonia cinerea* L. on MS medium supplemented with different concentrations of BAP (1.0 to 3.0 mg/l), GA_3 (1.0 to 5.0 mg/l) and KN (0.5 mg/l) for direct shoot induction. Maximum numbers of shoots were obtained from nodal and leaf explants at BAP (2.5 mg/l) GA_3 (2.0 mg/l) and KN (0.5 mg/l) respectively. The multiple shoots elongation and their development were better on MS medium supplemented with 1.5 mg/l BAP and 3.0 mg/l GA_3 for both the explants. The regenerated shoots were successfully rooted on MS medium supplemented with IBA 2.0 mg/1, after sequential hardening, survival rate was 91%. The *in vitro* raised plantlets were acclimatized and established successfully in the field.

***Keywords*:** Micropropagation, Nodal and leaf explants, BAP, KN and IBA, *Vernonia cinerea*

1. INTRODUCTION

Vernonia cinerea (Family Asteracea) also called little iron weed is a perennial grass with erect stem seen in the mainland of China, Fujian, Guangdong, Guangxi, Jiangxi, Hunan, Sichuan, Yunnan and Vietnam, Myanmar, India, Bangladesh, Srilanka, Malay island, the Philippines, Australia, Africa, New Zealand, Asia and other places [1-3]. The herbal medicine is widely practiced throughout the world from time immemorable.

(Received 20 October 2019; Accepted 07 November 2019; Date of Publication 08 November 2019)

These medicines are safe and environment friendly. Nearly 80% of the world population depends upon traditional system of health care. The indigenous traditional knowledge of medicinal plants of various ethnic communities, where it has been transmitted orally for centuries is fast disappearing from the face of the earth due to the advent of modern technology and transformation of traditional culture [4]. 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 [5].

It also contains medicinal properties for eczema, ringworm, elephantiasis, conjunctivitis, diarrhea, leucoderma, dysiria, skin diseases, leprosy, fevers, anti-cancer, anti- oxidant and anti-inflammatory. The present paper describes a simple protocol for the rapid and large scale propagation of *V. cinerea in vitro* through shoot proliferation from cotyledonary nodes derived from seedlings [6] here, to develop an effective *in vitro* method for plant regeneration of *V. cinerea* and the propagated plants were successfully established in field conditions.

2. MATERIALS AND METHODS

2. 1. Plant material and surface sterilization

Young branches of *Vernonia cinerea* (L.) Less. Were collected from kaveri river Trichy district, Tamil Nadu. The branches were covered in polythin bag and brought to the laboratory within 2 hrs and cultured after sterilization. The young leaves were collected and washed with 1% (v/v) Teepol solution for 10 min followed by washing in running tap water for 5 min. The explants were then surface-disinfected by immersion for 7 min in 0.1% (w/v) aqueous mercuric chloride solution. After three rinses in sterile distilled water, the explants were excised into 1.5 cm x 4 mm in diameter and cultured on shoot induction medium.

2. 2. Shoot regeneration

In a laminar air flow cabinet, sterilized Nodal and leaf explants (about 1.0 cm in length) were inoculated in culture tubes (22 × 150 mm) containing 25 ml of sterile MS [7] medium, 3% (w/v) sucrose 8 g/l agar and pH adjusted to 5.8. Explants were maintained in a growth room in the dark at a temperature of 25 ºC. After 35 d, callus induction was evaluated, and callus fresh weight and dry weight was determined. The basal medium was supplemented with different growth regulators in different concentrations and combinations. In the present study, two types of media were employed on the basis of growth regulator used.

The first category was callus initiation and induction medium and the second include shoot differentiating medium. The former medium was fortified with various concentrations of (BAP 3.0 mg/l) alone or in combination with 2.0 mg/l (GA_3). The latter consisted of different concentrations of N6-benzylaminopurine (BAP 1.0–5.0 mg/l) or GA_3 (1.0–3.0 mg/l) alone or in combination with (KIN 0.5–2.5 mg/l), (NAA 0.5-2.5 mg/l). The callus was periodically sub-cultured on MS medium supplemented with 2.0 mg/l GA_3. For shoot regeneration from callus, 50 mg calli were transferred to each culture tube. Data on percentage of calli forming shoots and mean shoot number and length of differentiated shoots were recorded after 35 days of culture. MS medium lacking growth regulators served as the control.

2. 3. Shoot elongation, rooting and field transfer

The shoots below 2.5 cm in length were excised and sub-cultured on MS medium supplemented with 3.0 mg/l BAP for shoot elongation. The shoots having approximately 3.0 cm in length were harvested from the shoot elongation medium and cultured on half strength MS medium supplemented with various concentrations of indole-3-butyric acid (IBA; 0.5–2.5 mg/l) or NAA (0.5–3.0 mg/l)for root induction. Data were recorded for percent rooting, root number and length after 45 d of transfer on rooting medium. Plantlets with well developed roots were removed from culture tubes, washed well to remove the remnants of agar from roots and transplanted to plastic cups (6 cm diameter) containing garden soil and sand (1:1). The plantlets were placed in glasshouse set at 24 ±2 ºC, 85–95% relative humidity and irradiance (60 mol $m^{-2}s^{-1}$) provided by cool white fluorescent tubes. Plants were irrigated with half-strength MS salt solution for 3 weeks and thereafter with water. After 45 days the plants were transferred to larger pots and kept under shade in a net house for another 2 weeks before transferring outside under full sun to develop into mature plants.

2. 4. Culture conditions

The pH was adjusted to 5.8 with 1.0 N HCl or 1.0 N NaOH before autoclaving the medium at 1.06 kg cm^{-2} and 121 ºC for 20 min. The cultures were maintained in a culture room with a 16h/8h light/dark photoperiod at 23 ±2 ºC unless otherwise mentioned. Light was supplied at intensity of 80 mol $m^{-2}S^{-1}$ supplied by two Philips TL 40W cool-white fluorescent lamps. Each treatment consisted of 20 tubes and all experiments repeated 3 times. The data were presented as mean and its standard deviation (mean ± SD).

3. RESULT AND DISCUSSION

3. 1. Multiple Shoot Induction from Nodal and leaf Explants

Table 1 shows the successful results. Nodal and leaf explants were cultured on MS supplemented with different concentrations of KIN, GA_3 and BAP in combination with GA_3 and KIN were tested. In all the tested combination of plant growth regulators induced multiple shoots induction was noticed from the Nodal and leaf explants. Among the different treatments medium comprising of MS salt B5 vitamins BAP (2.5 mg/l), GA_3 (2.0 mg/l) and KIN 0.5 mg/l, nodal explants (Plate-I; Fig. 1) and leaf explants (Plate-I; Fig. 5&6) showed best result response for multiple shoot induction the nodal and leaf explants in this concentration large number of the nodal and leaf were observed from single (94.3%) Individual treatment of cytokinins and combination treatment of cytokinins along with small amount of auxins showed low percentage of multiple shoots induction when compared with above concentration.

When compared with BAP and KIN. BAP showed superior percentage of response for multiple shoots induction. Hence for combination treatment studies along with auxins BAP was selected in combination treatments supplementation of KIN (0.5 mg/l) along with BAP (1.0 mg/l) showed low response when compared with BAP and IAA treatment. Similar observation was made by Pattnaik and Chand, 1996 [8] 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 [9] in *Emblica officianale*, *Vitex*

negundo [10], *Withania sominifera* [11], and [12-13] *Sphaeranthus amaranthoides*. A 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 [14].

3. 2. Shoot elongation

The multiple shoots were transferred to shoot elongation medium, medium comparing with MS salts B5vitamines and different concentration of GA_3 and BAP. The highest percentage of shoot elongation 3.0 mg/l GA_3 and 1.5 mg/l BAP (98.8 %) for nodal and leaf explants (Table: 2: Plate-I; Fig. 2 & 7). The elongation of nodal and leaf were achieved on the same medium. The capacity of multiple shoot bud initiation and proliferation from nodal and leaf 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 reports of *in vitro* conditions of different genera of Asteraceae have been described [15-16].

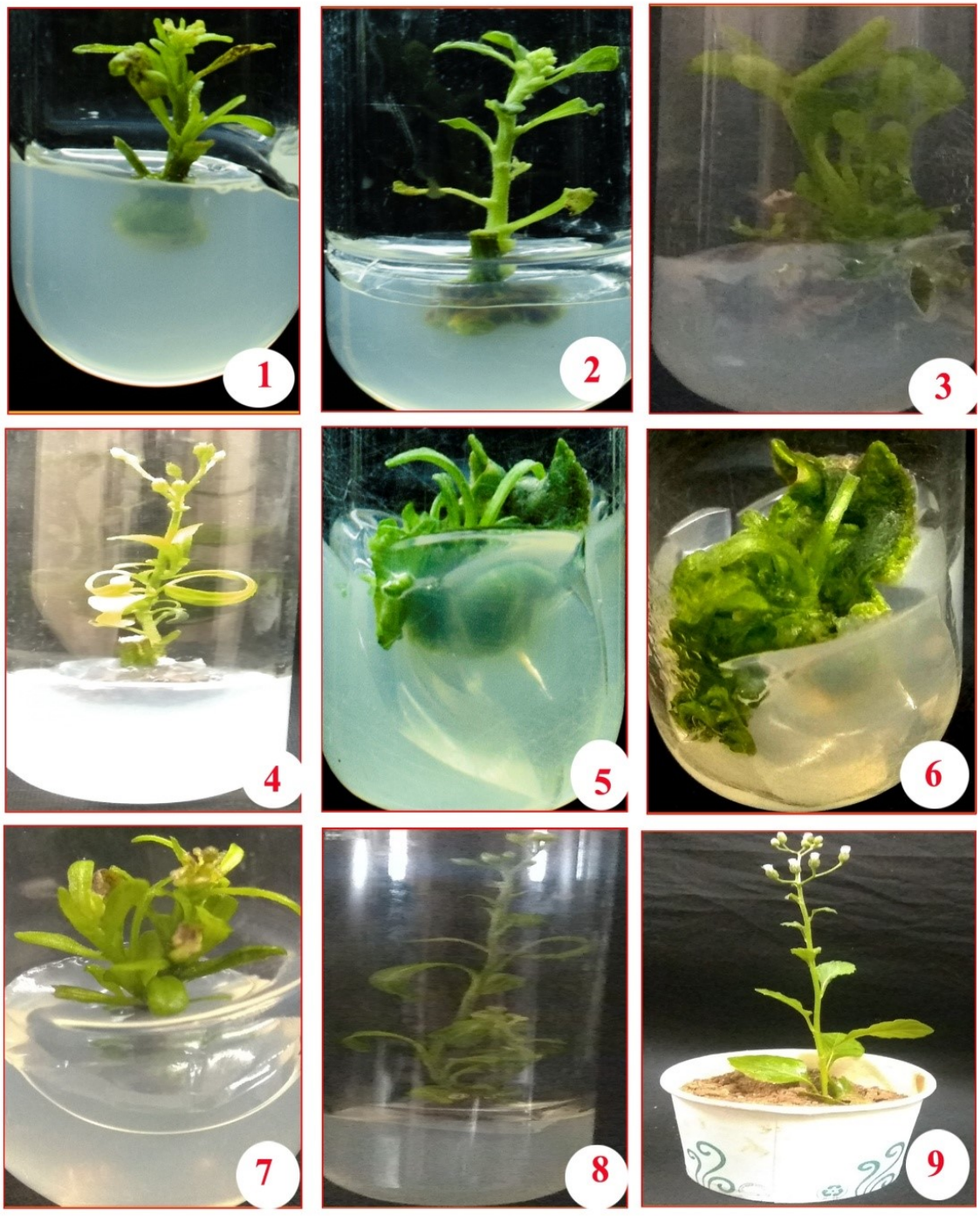
3. 3. Rooting and Hardened

The root induction studies the elongation multiple shoots from the shoot clusters were individually separated and were kept for rooting in MS medium supplemented with various auxins. Initially for root induction supplementation of all the auxins tested different concentration of IAA, IBA and NAA the high frequency of root induction form leaf explants IBA 2.0 mg/l (91 %) (Table 3: Plate-I; Fig. 8) However, there was no significant difference between the effect of IBA and IAA. Similar results were reported by [17] Jawahar *et al*., 2008 in *Cardiospermum helicacabum*.

Reported that IBA was effective for root induction in *Sterculia urens*. [18-19] obtained rooting response in IAA. The 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 [20] reported that the medium containing IBA produced maximum number of adventitious roots in *Heracleum candicans*. The rooted plants (Plate-I; Fig. 9) 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 ±2 ºC 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.)

The shoots with root were transferred to plastic pots for hardening survival of about 91 % of *in vitro* derived plants was observed after 1 month. The fully regenerated plantlets under green house condition were transferred to field for further development. In conclusion, the micropropagation protocol was established from nodal and leaf explants of *V. cinerea*. The rehabilitation micropropagation development total process was completed in 75 days. This efficient micropropagation protocol will be useful to conservation, and in the improvement of *V. cinerea* using genetic transformation [21-54].

Plate-I



Nodal explants

1. Shoot induction
2. Shoot elongation
3. Multiple shoot induction
4. Flower induction

Leaf explants

5 & 6 Multiple Shoot induction
7. Shoot elongation
8. Root induction
9. Hardening

Table 1. Multiple Shoot induction response from Nodal and leaf Explants of *Vernonia cinerea* L.

PGR (mg/L)			% of response	Nodal and leaf Explants	
				No of shoot bud / Explants Mean ± SD.	Shoots length Mean ± SD.
BAP					
1.0			41.2	2.7 ± 0.86	1.3±0.26
1.5			53.3	3.4 ± 0.13	3.2±0.30
2.0			71.4	4.5 ± 0.55	3.4±0.24
2.5			90.7	5.3 ± 0.44	4.5±0.41
3.0			64.4	4.6 ± 0.24	2.6±0.31
GA_3					
1.0			62.4	4.1 ± 0.32	1.2±0.42
2.0			76.0	5.2 ± 0.43	2.4±0.65
3.0			75.6	6.3 ± 0.24	3.4±0.44
4.0			46.8	3.4 ± 0.55	1.1±0.17
5.0			45.5	4.5 ± 0.33	1.5±0.35
BAP	**GA_3**	**KIN**			
2.5	1.0	0.5	76.5	4.3±0.43	1.1±0.22
2.5	1.5	0.5	86.2	5.2±0.37	1.2±0.44
2.5	**2.0**	**0.5**	**94.3**	**6.4±0.53**	**2.3±0.89**
2.5	2.5	0.5	81.4	4.1±0.32	0.4±0.46
2.5	3.0	0.5	76.6	4.5±0.34	1.8±0.32

Observations were made up to 35 days of inoculation. Values represent mean ± standard deviation of 20 replicates per treatment and each experiment was repeated thrice for percentage of explants response.

Table 2. Shoot elongation responses of multiple shoots raised from nodal and leaf Explants of *Vernonia cinerea* L.

PGR (mg/l)		Nodal and leaf Explants		
		% response	No. of shoots per Explants Mean ± SD.	Shoot height (cm) Mean ± SD.
GA3	**BAP**			
1.0	0.5	76.4	3.1±1.32	2.8±2.12
2.0	1.0	85.9	3.7±1.81	2.6±2.28
3.0	**1.5**	**98.8**	**4.0±1.36**	**3.4±2.56**
4.0	2.0	77.5	2.5±1.33	2.3±2.17
5.0	2.5	74.7	2.2±1.24	1.9±2.19

Observations were made up to 68 days of inoculation. Values represent mean ± standard deviation of 20 replicates per treatment and each experiment was repeated thrice for percentage of explants response.

Table 3. Effect of different forms and concentrations of auxins on rooting in *Vernonia cinerea* L.

PGR (mg/l)	% Response	Leaf Explants	
		Mean root number Mean ± SD	Mean root length (cm) Mean ± SD
IBA			
0.5	45.1	2.3±1.15	2.3±1.12
1.0	58.2	3.2±1.18	2.8±1.24
1.5	63.4	3.5±1.04	3.6±1.23
2.0	**91.0**	**5.7±1.17**	**5.5±1.28**
2.5	84.5	3.9±1.14	4.4±1.21

NAA			
0.2	47.8	3.9±0.15	2.4±0.18
0.4	59.9	2.4±1.12	1.4±0.13
0.6	75.4	3.5±0.22	2.1±0.24
0.8	81.3	4.3±1.25	3.7±0.34
1.0	72.5	3.6±1.17	2.4±0.13
IAA			
0.2	40.4	1.3±0.16	1.3±0.74
0.4	48.0	2.3±0.14	1.6±0.35
1.0	67.4	2.6±0.41	1.8±0.53
1.5	79.3	3.8±1.37	2.8±1.55
2.0	64.9	2.4±0.94	2.3±1.47

Observations were made up to 85 days of inoculation. Values represent mean ± standard deviation of 20 replicates per treatment and each experiment was repeated thrice for percentage of explants response.

4. CONCLUSIONS

The present study describes an efficient *in vitro* plant regeneration protocol for micropropagation of *Vernonia cinerea* L. Promising plant regeneration from nodal and leaf explants was influenced markedly by combinations of BAP, GA_3 and Kinetin. All the *in vitro* regenerated shoot were rooted successfully for direct methods from *Vernonia cinerea* L. This study aims to develop a standard protocol to initiate multiple shoot culture at a standardized media and hormonal concentration of plant that maybe beneficial for *in vitro* large scale propagation of the plant this could reduce the time, energy, labor and cost of production of plantlets. The protocol can be avail in drug research, genetic transformation, adventitious root cultures for *in vitro* secondary metabolic production and commercial cultivation of *V. cinerea* L. Moreover this study could concentrate on rapid multiplication and germplasm conversation of *V. cinerea* L. with bioreactor technology and optimizing transformation conditions for genetic improvements.

Acknowledgement

The authors are thankful to the management of National College (Autonomous), Tiruchirappalli, Tamil Nadu, India, for providing the necessary facilities.

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