In vitro culture studies of an important medicinal herb, *Vernonia cinerea* (L.) Less (Asteraceae) under varying hormonal concentrations and effect of Indole 3-Acetic Acid (IAA) on subculture media

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ABSTRACT: Development of an efficient *in vitro* propagation method for the important medicinal herb *Vernonia cinerea* has been successfully established. Multiple shoots were obtained from the stem explants of *Vernonia cinerea* on MS media supplemented with varying concentrations of plant growth hormones (2, 4-D, Kinetin, IAA and BAP). Maximum number of shoots was observed in Medium D (2 mg/l IAA and 2mg/l BAP). It was observed that BAP in combination with IAA was more effective for shoot proliferation than varying concentrations of 2, 4-D and kinetin. Differentiated shoots were subcultured and maximum number of roots was obtained in the medium A (2 mg/l IAA and 1 mg/l BAP). From this study, it is revealed that too much concentration of IAA or BAP do not cause any significant changes in the *in vitro* propagation. The rooted plantlets were successfully transferred to the green house with 90% survivability.

Keywords: *Vernonia cinerea*, stem explants, IAA, BAP

1. INTRODUCTION

Vernonia cinerea (L.) Less (Asteraceae), one of the members of "Dasapushpa" is reported to have several therapeutic uses. The plant has been used in the treatment of fever, cold, headache, infection, inflammation, malaria, fever, worms, pain, diuresis, cancer, abortion, various gastro-intestinal disorders, filariasis, haematological disorders, eczema, bleeding, leprosy, scabies, leucoderma, asthma, bronchitis, psoriasis and other chronic skin diseases (Kirtikar and Basu, 1993; Khare, 2007; Alagesaboopathi, 1994; Sasidharan *et al*. 2010; Jeyapardha *et al*. 2011). The plant has been scientifically reported to have antitumour, antidiabetic, renoprotective, anticancer, antioxidant, anticholineesterase, antiviral, antimicrobial, antidiarrhoel, antimalarial, antihyperglycemic, analgesic, anti-inflammatory, antipyretic, antiyeast, antibacterial and sedative-hypnotic activities (Sangeetha and Venkatarathinakumar, 2011; Rizvi *et al*. 2011, Yadava and Raj, 2013; Haque *et al*. 2012; Rao and Rao, 1998; Ganesh *et al*. 2011, Chea *et al*. 2006, Choudhary *et al*. 2013; Mazumder *et al*. 2003; Iwaleva *et al*. 2003; Gupta *et al*. 2003 a, 2003 b; Latha *et al*. 2005, Sathyanathan *et al*. 2012).

Since all medicinal plants are not cultivated those living in wild habitats are under threat due to overexploitation for their byproducts. The diversity of such medicinal plants is found to be decreasing due to anthropogenic and environmental activities and their higher demand in pharmaceutical applications. Therefore, the present investigation was to establish an efficient protocol for the propagation of an important medicinal herb, *Vernonia cinerea* (L.) Less and the study also aimed to evaluate the effect of IAA on subculture media.

2. MATERIALS AND METHODS

Plant material, explant preparation and sterilization

The explant material was collected from the plant *Vernonia cinerea* grown in the field. Explants used in the present study were stem. The explants were washed in running tap water followed by treatment with teepol solution. To remove teepol remains, the explants were washed with double

distilled water for three times. Then the explants were washed in 70% ethanol for one minute and then rinsed with double distilled water for three times. Then the explants were sterilized in 0.1% $HgCl_2$ for one minute. Finally the explants were washed in double distilled water for three times.

Culture medium and conditions

The explants were inoculated asceptically on MS medium supplemented with varying concentrations of plant growth hormones (Table 1). pH of all the media were adjusted to 5.7 and 8 g/l agar were added. About 15 ml of the medium was dispensed in each culture tubes and then autoclaved at 121 ^{0}C for 15 minutes. The media were left to cool in a slanting position in a culture rack. Sterilized explants of suitable size (5 mm) were placed on the medium by pressing it gently for providing the maximum contact with the medium. The water particles on the explant were blotted out using filter paper. Before and after inoculation, the rim of the culture tubes was flamed to avoid the microbial attack and they were plugged tightly. Keep the culture tubes in dark for 48 hours. After that the culture tubes were transferred to the incubation room and incubated at 25 ^{0}C to 27 ^{0}C. Light (1000 lux) was given for 8 hours per day.

Table 1: Showing Culture Media Used and Growth Regulators

Culture medium used	Plant growth regulators mg/l	
A	2,4-D	2
	Kinetin	1
B	IAA	1
	BAP	2
C	IAA	1
	BAP	4
D	IAA	2
	BAP	2

Subculturing

Differentiated shoots were subcultured on MS medium containing plant growth regulators; BAP was kept constant with varying concentrations of IAA (Table 2). After subculturing, it was kept in incubation room at a temperature of 25 ^{0}C. Results were observed at regular intervals, data were collected and photographs were taken.

Table 2: Showing growth regulators and subculture media used

Plant growth regulators	Subculture medium used		
	A	B	C
IAA mg/l	2	4	6
BAP mg/l	1	1	1

Hardening and Acclimatization

The rooted plants were taken out from the culture tubes and washed to remove the excess of agar medium and were transplanted to a plastic pot containing potting mixture. Plants were kept at 90% humidity with low light intensity. The sterilized potting mixture should not be too wet and water drops should not form on the plantlets. The humidity was gradually decreased to the ambient level after 7-15 days and the light intensity was increased. The plants were finally exposed to green house conditions.

3. RESULTS

For establishing an efficient *in vitro* propagation of *Vernonia cinerea* (L.) Less from stem explants, the nodal stem segments were incubated on MS medium supplemented with varying concentrations of auxin and cytokinin. Observations were taken at regular intervals of 4th day after inoculation and the results are shown in the Table 3. The inoculum was green in colour at the time of inoculation. But later it is noticed that the intensity of green colour of the explants decreased throughout the days and develop pale yellow colour. On 16th day the explants showed no further colour change. After 20 days, the colour of the explants slightly turned greenish.

Among the four culture media used, medium D supplemented with 2 mg/l IAA and 2 mg/l BAP was found to be best suited for shoot proliferation and also showed maximum number of shoots (Table 4; Fig.1; Fig.3 a). The result showed that BAP in combination with IAA was more effective

for shoot proliferation than varying concentrations of 2, 4-D and kinetin. However, Medium C showed the response only after the 20th day of inoculation and later it shows stunted growth. In medium A and C, only small light green coloured callus mass was formed and also the enlargement and growth of explant was very slow.

Table 3: *In vitro* callus response of *Vernonia cinerea* (L.) Less stem explants in MS basal media with varying concentration of auxin and cytokinin

Culture medium	Explants response at various time intervals				
	4th day	8th day	12th day	16th day	20th day
A	0	0	0	+	++
B	0	0	+	++	+++
C	0	0	0	0	+
D	0	+	++	+++	++++

0 : No growth
\+ : Callus initiation
++ : Moderate growth
+++ : Initiation of shoot
++++ : Vigorous growth of shoots

Table 4: Showing number of shoots in different medium

Culture medium	Number of shoots
A	0
B	5
C	0
D	8

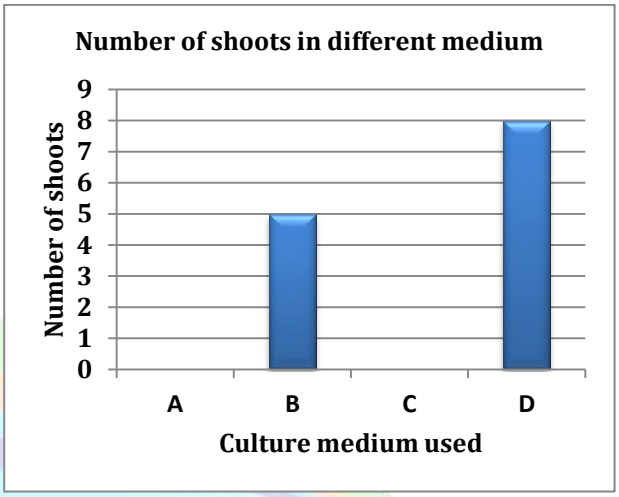


Figure 1: Showing Number of shoots in different medium

Effect of IAA on subculture media

Differentiated shoots were obtained from the medium B and medium D were subcultured on MS medium containing different concentrations of IAA with fixed levels of BAP (1 mg/l). Among the three subculture media used, medium A seems to be the best for root induction and also showed the maximum number of root proliferation (Table 6; Fig.2). The shoot inoculated in the medium A supplemented with 2 mg/l IAA and 1 mg/l BAP started swelling on 8th day and enlarged on 16th day and vigorous growth was observed on 20th day of inoculation (Table 5; Fig.3 b). Further increase in the concentration of IAA resulted in the retardation of growth of roots in the medium B and C.

Table 6: *In vitro* root formation of *Vernonia cinerea* (L.) Less in subcultured shoot explants in MS basal media with varying concentration of IAA and BAP

Subculture medium used	Shoot response at various time intervals				
	4th day	8th day	12th day	16th day	20th day
A	0	+	++	+++	++++
B	0	0	+	++	+++
C	0	0	0	+	++

0 : No growth
\+ : Small swelling
++ : Enlarged mass

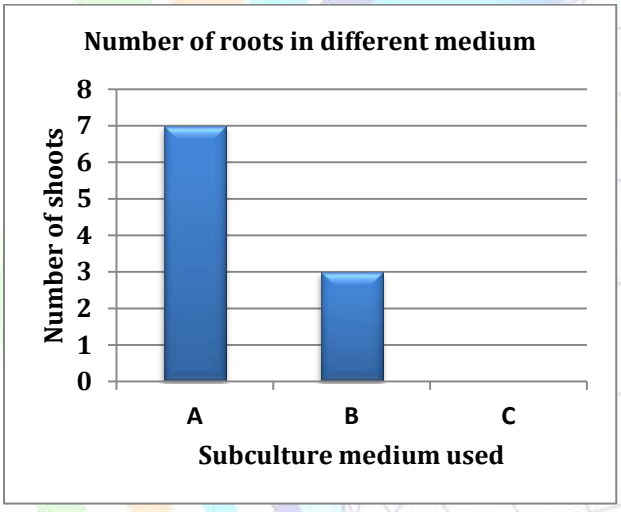
+++ : Moderate with initiation of root

++++ : Vigorous growth of roots

Table 6: Showing number of roots in different medium

Subculture medium	Number of roots
A	7
B	3
C	0

Figure 2: Showing Number of shoots in different medium



Hardening and Acclimatization

The rooted plant in the test tube was transferred to plastic pot containing sterilised potting mixture. During the first five days the colour of the plant was pale green and gradually it became dark green (Fig.3 c).

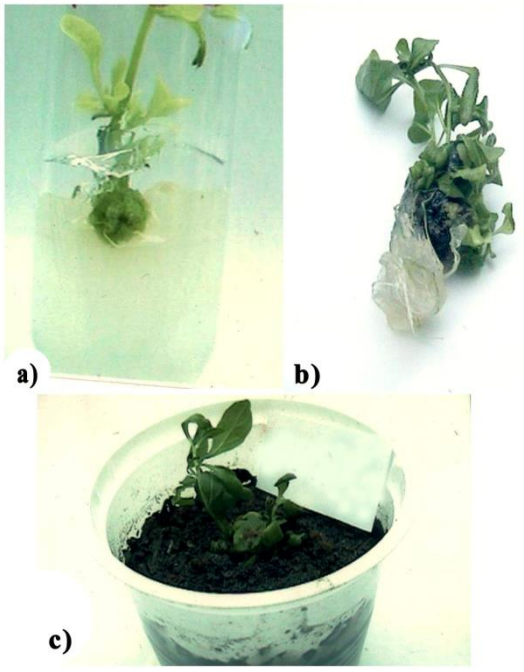


Figure 3.a) Shoot formation in Medium D supplemented with 2 mg/l IAA and 2 mg/l BAP; **b)** Subculture: Root proliferation in Medium A supplemented with 2 mg/l IAA and 1 mg/l BAP; **c)** Acclimatised plants of one month old

4. DISCUSSION

The present study elucidates a protocol for developing an efficient and reproducible *in vitro* propagation and acclimatization methodology of an important medicinal herb, *Vernonia cinerea.*

Various studies on the effects of auxins and cytokinins on shoot and root initiation of *Vernonia* species have been reported. A viable protocol was developed for direct and indirect shoot regeneration of *Vernonia cinerea* by using leaf and stem as explants on MS medium supplemented with different combinations of NAA, IAA and BAP (Maheshwari and Kumar, 2006). Khalafalla *et al*. (2007) reported that maximum number of multiple shoots was observed on the medium containing BAP in combination with NAA from the nodal segments of *Vernonia amygdalina*. Multiple shoots were induced *in vitro* on MS medium containing BAP alone or in combination with NAA and Kinetin alone or in combination with 2, 4-D and the regenerated shoots were rooted with MS medium supplemented with 2 mg/l NAA. Seetharam *et al*. (2007) observed that maximum number of shoot obtained *in vitro* from leaf and

nodal segments of *vernonia cinerea*, shoot multiplication and their development was better on MS medium supplemented with BAP (2 mg/l) and NAA (1.5 mg/l) and rhizogenesis was observed on the half strength MS medium containing IAA (1.5 mg/l). Vincente *et al*. (2009) carried out in vitro multiplication and acclimatization of *Vernonia condensata* by using axillary buds as explants on MS medium containing different concentrations of BAP (0-5 mg/l). Maximum number of shoots was observed in BA (13.32 mg/l) and roots in IBA (7.38 mg/l) by culturing of shoot tip as explants of *Vernonia cinerea*, in another study (Maharajan *et al*. 2010). Another study by Sunder and Jawahar (2011), established a rapid and efficient protocol was developed via shoot tip explants of *Vernonia cinerea* on MS medium containing different concentrations of 13.32 µM/l BAP and 13.92 µM/l KN and the regenerated shoots were rooted on MS medium supplemented with IBA (7.38 µM/l).

The present study showed that BAP in combination with IAA was more effective for shoot proliferation than varying concentrations of 2, 4-D and kinetin. It is because of the growth regulator 2, 4-D having the capacity to inhibit cell proliferation (Milia *et al*. 1996). For calli proliferation, an appropriate balance between IAA and BAP plant growth regulators is essential (Ram *et al.* 2014) and it is revealed that too much concentration of IAA or BAP do not cause any significant changes in the *in vitro* propagation. It is evident from the results that *Vernonia cinerea* can be easily propagated *in vitro* via stem explants. The procedure makes it possible to get rooted plantlets that are ready to be transplanted into the field within one month. The plant after achieving self-sustained growth will use their own photosynthetic apparatus and this may be the reason behind the active growth of the plant and production of more leaves. The vigorous growth of the plant may be due to the high degree of carbon dioxide fixation *in vivo*.

5. CONCLUSION

The present investigation reports a protocol for efficient *in vitro* propagation of *Vernonia cinerea* via stem explants and successful acclimatization of the plants with 90% survivability. The result of the study exposed that *in vitro* techniques can play an important role in the clonal propagation and also in the production of secondary metabolites of high medicinal values.

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