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In vitro Direct Rhizogenesis of an Endangered Medicinal Herb: *Valeriana jatamansi* Jones

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A B S T R A C T

V. jatamansi Jones is an important medicinal wild herb of North Eastern Himalayan Region belonging to family Valerianaceae. The herb is widely used in treatment of leprosy, epilepsy, hysteria and asthma and also in making perfumed powder. Due to over-exploitation of roots and rhizomes, *V. jatamansi* is included in endangered species as per National Medicinal Plant Board, New Delhi, India. The harvest of roots and rhizomes causes destruction of whole plant at its natural habitat. An alternative and efficient protocol was developed for *in vitro* root mass multiplication from leaf explant in order to promote *in situ* conservation of the herb. The leaf explant sterilized with 5% (v/v) Tween-20 (5 min), 0.1% Bavistin (5 min), 0.1% (w/v) $HgCl_2$ (1 min) and 70% ethanol (1 min) and exhibited 80% survival after 7 days of implantation on MS medium supplemented with 2 mg/L each of BAP and NAA. However, 90 % of leaf explant in same medium induced roots exhibiting maximum root number (70) and maximum root length (5 cm.).

Introduction

Uttarakhand, part of Indian North western Himalaya represents a mountainous region that encompasses agroclimatic conditions ranging from tropical to alpine and, thus, possesses a rich biodiversity. *Valeriana jatamansi* Jones is a medicinal herb of genus Valeriana and family Valerianaceae also called as Indian Valerian or Tagar-Ganthoda. *V. jatamansi* is a forest perennial herb, 0.5-2 ft tall, with velvet-hairy to hairy appearance. Rhizomes are elongate with fibrous root system. Stems are 3-6 in number on rhizome and covered with heart shaped leaves. Flowering occurs during March-May months. Snow-white flowers are borne in flat-topped clusters on top of the stems (Raina and Srivastava, 1992). The genus Valeriana with about 200 species is distributed in Asian continent from Afghanistan to SW China. It spans throughout the Himalayas in India. The

North Indian state Uttarakhand shows its distribution in five districts *i.e.*, Ginoti in Uttarkashi, Kanda in Bageshwar, Niglat in Nainital, Dunagiri in Almora and Mussorrie in Dehradun (Raina and Negi, 2015) at altitudes of 1500-3600m asl. *V. jatamansi* is well known in traditional Indian medicine**.** The Indian Valerian has long been used in Ayurveda (Charak Samhita and Susruta) and Unani systems of medicine, which describe its use in obesity, skin disease, insanity, epilepsy and snake poisoning. The paste of roots mashed in water is applied on forehead to alleviate the pain. Both the crude drugs from roots/ rhizomes and Valerian derived phytomedicines are used as mild sedatives in pharmaceutical industry. The phytomedicinal activity of Valerian compounds is largely attributed to the presence of valepotriates (Kinnula and Crapo, 2004).

The over-exploitation of *V. jatamansi* roots and rhizomes for medicinal usages and the biotic interferences in its distribution range have caused habitat degradation thus creating nearly extinct condition of the herb. Thus, convention on international trade of endangered species notified *V. jatamansi* in its schedule for conservation and additionally, it has been enlisted an endangered species in the list of National Medicinal Plant Board, New Delhi, India (Nawchoo *et al.*, 2012).

Hence, it is of immediate concern that different conservation measures and strategies be adopted to stop further depletion of the herb from its natural habitat**.** Conventionally the herb is propagated through seeds. Despite being the common method of its propagation, it is not an attractive practice, since the seeds germinate slowly and remain dormant for long time. Therefore, establishment of an efficient protocol and propagation system is necessary for large-scale production and plantation of *V. jatamansi* which would also help in developing new varieties with high levels of important compounds via biotechnology (Kaur *et al.*, 1999).

In vitro propagation of rare and threatened plants is generally undertaken to enhance the biomass and conserve the germplasm especially when population numbers are low in the wild. *In vitro* propagation or micropropagation is a viable alternative for species which are difficult to regenerate by conventional methods; where populations have decreased due to over exploitation by destructive harvesting and can effectively be used to meet the growing demand for clonally uniform elite plants. *In vitro* propagation can provide an alternate source of plants and alleviate pressures on wild populations (Bapat *et al.*, 2008).

Materials and Methods

Plant material

Plant material of *V. jatamansi* was collected from medicinal plant block of Uttarakhand University of Horticulture and Forestry (UUHF), Bharsar situated at 1720m asl in Distt Tehri, Uttarakhand. For regular use the plant material was potted in autoclaved soil and maintained in shade and dark period of 12 hrs at 10-12ºC in laboratory.

In vitro establishment

The 4 explants (leaf, petiole, nodal segment and shoot buds) were excised from three-month-old plants. These explants were first washed with tap water to remove debris and then with distilled water having 0.2 % Tween-20. The explants were surface sterilized with 0.1% bavistin containing 0.2 % Tween- 20 for 6 min followed by 70% ethanol for 1 min and 0.1% mercuric chloride for 1 min and washing was done with 2-3 times, 2 times and 5-6 times of sterilized water in that order. Four explants per bottle were aseptically inoculated

on sterilized MS medium (Murashige and Skoog, 1962) containing 3% (w/v) sucrose, 10mg/L mesoinositol and different concentrations of BAP and NAA. The pH of medium was adjusted to 5.8, followed by addition of 0.6% (w/v) agar and autoclaved at 15 psi for 20 min at 121°C. The culture bottles were kept in growth chamber at 21±1 ºC, ~ 70% relative humidity and 16 hr photoperiods provided by 40W fluorescent tubes having a photon flux density of approximately 21,500 lux. Data obtained during the investigation of present study, were analyzed using analysis of variance (ANOVA) technique "STPR".

Direct *in vitro* rhizogenesis

Various concentrations of BAP and NAA in MS medium were used for direct root induction from leaf explant (Table 1). Subculturing for mass multiplication of roots was done at similar culture growth condition after every 25 days in the same medium supplemented with BAP and NAA.

Results and Discussion

In vitro establishment

Different explants such as leaf, petiole, nodal segment and shoot bud were used for *in vitro* root development. The maximum survival rate 80% was observed for leaf explant after 7 days of implantation amongst all the explants. However, 90% of leaf explant was success for root induction in MS medium supplemented with 2 mg/L each of BAP and NAA.

Direct *in vitro* rhizogenesis

Direct rhizognesis was observed from leaf explant after 20 days of implantation in the MS medium supplemented with 2 mg/L of each BAP and NAA. Profuse rooting was observed after 90 days of implantation (Fig. 3). Growth response of the explant, average root number (Fig. 1) and average root length (Fig. 2) per explant were recorded from 20 to 90 days of implantation. The analyzed data showed that maximum number of roots and the highest root length were observed in MS medium supplemented with 2mg/L each of BAP and NAA and the growth increased along with time amongst all the combinations of BAP and NAA (Table 1 and 2). Earlier leaf induced callus developed into adventitious shoots from *V. jatamansi* Jones leaf explant grown on MS medium supplemented with different concentrations and combinations of phytohormones such as 2,4-D, NAA, IBA and BAP (Chen *et al.*, 2014). There had been no reports on direct root regeneration (rhizogenesis). Our unique study focused towards standardizing an efficient protocol for *in vitro* root mass multiplication rather than whole plant regeneration from miniature explant without disturbing whole plant; a step towards conserving the herb in its natural habitat (Table 3).

Table.1 Various concentrations of BAP and NAA used in MS medium for root induction

BAP / NAA	0 ppm	1 ppm	2 ppm
0 ppm	0/0	0/1	0/2
1 ppm	1/0	1/1	1/2
2 ppm	2/0	2/1	**2/2**

Table.2 Effects of various concentrations of BAP and NAA with time interval on root no. of *V. jatamansi*

Treatments (NAA:BAP) mg/L	20 days	40 days	60 days	90 days
1 : 0	0.000±0.000^{bC}	1.333±0.333^{cB}	2.333±0.333^{eB}	4.000±0.000^{faA}
2 : 0	0.000±0.000^{bB}	0.333±0.333^{cB}	1.667±0.882^{eA}	2.333±1.202^{gA}
0 : 1	1.000±0.000^{bBC}	1.667±0.333^{bB}	2.667±0.333^{eB}	4.333±0.333^{fA}
1 : 1	1.667±0.333^{aC}	2.667±0.333^{bC}	6.667±0.333^{cB}	9.667±0.333^{eA}
2 : 1	1.000±0.000^{bD}	3.000±0.000^{bC}	6.333±0.333^{cdB}	11.333±0.667^{dA}
0 : 2	1.000±0.000^{bC}	1.667±0.333^{bC}	7.667±0.333^{cB}	14.333±0.667^{cA}
1 : 2	1.000±0.000^{bC}	1.667±0.333^{bC}	10.667±0.333^{bB}	18.333±0.333^{bA}
2 : 2	1.667±0.333^{aD}	10.000±0.000^{aC}	21.333±0.667^{aB}	**69.667±0.333^{aA}**
CD at 5%	**0.4162020**	**0.5885985**	**1.177197**	
CV	**10.35608**			

Small letter denotes significant within column and capital letter significant within row

Table.3 Effects of various concentrations of BAP and NAA with time interval on root length of *V. jatamansi*

Treatments (NAA:BAP) mg/L	20 days	40 days	60 days	90 days
1 : 0	0.000±0.000^{abBC}	0.100±0.000^{dB}	0.233±0.033^{eB}	0.500±0.000^{eA}
2 : 0	0.000±0.000^{abC}	0.033±0.033^{dC}	0.167±0.088^{eB}	0.333±0.167^{fA}
0 : 1	0.100±0.000^{aD}	0.467±0.033^{bC}	0.967±0.033^{cB}	1.533±0.033^{cA}
1 : 1	0.100±0.000^{aD}	0.200±0.000^{cC}	0.567±0.067^{dB}	1.000±0.000^{dA}
2 : 1	0.100±0.000^{aD}	0.300±0.000^{cC}	0.500±0.000^{dB}	1.500±0.000^{cA}
0 : 2	0.100±0.000^{aD}	0.500±0.000^{bC}	0.967±0.033^{cB}	1.400±0.100^{cA}
1 : 2	0.167±0.033^{aD}	0.933±0.067^{aC}	1.700±0.100^{bB}	2.933±0.067^{bA}
2 : 2	0.200±0.000^{aD}	1.000±0.000^{aC}	2.933±0.067^{aB}	**4.933±0.067^{aA}**
CD at 5%	**0.5164933**	**0.7304318**	**0.1460864**	
CV	**10.82834**			

Small letter denotes significant within column and capital letter significant within row

Fig.1 Root number in leaf explants of *V. jatamansi* Jones. Observation was recorded after 20 days interval upto 90 days. The values shown are mean values of three replication (*i.e.*, mean±SEm)

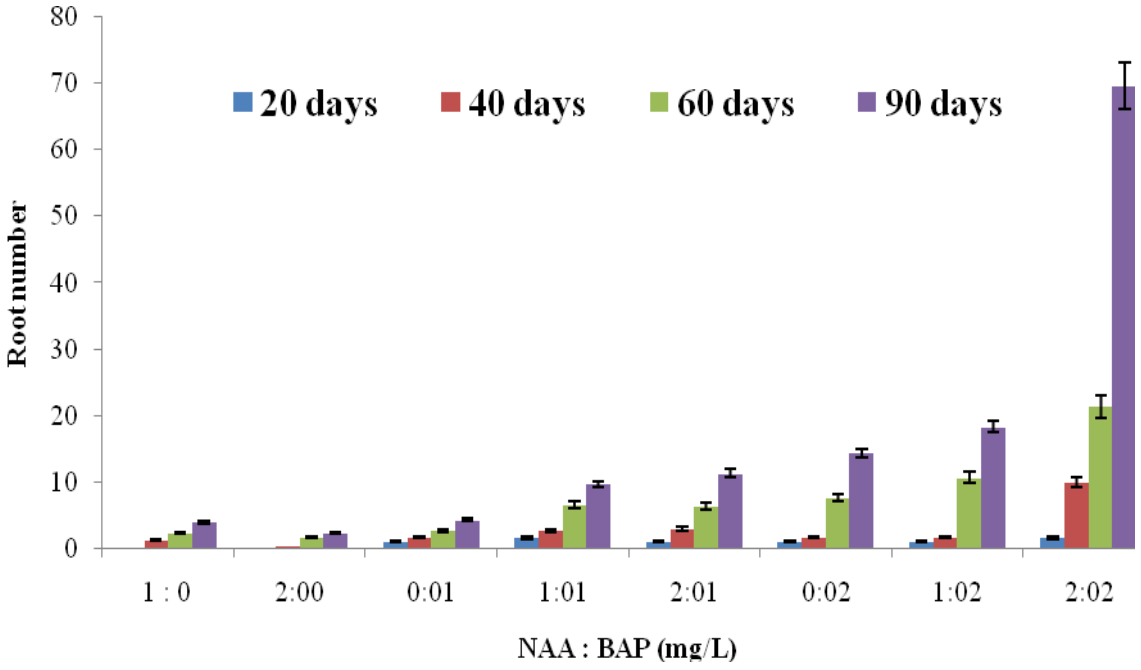


Fig.2 Root length in leaf explants of *V. jatamansi* Jones. Observation was recorded after 20 days interval upto 90 days. The values shown are mean values of three replication (*i.e.*, mean±SEm)

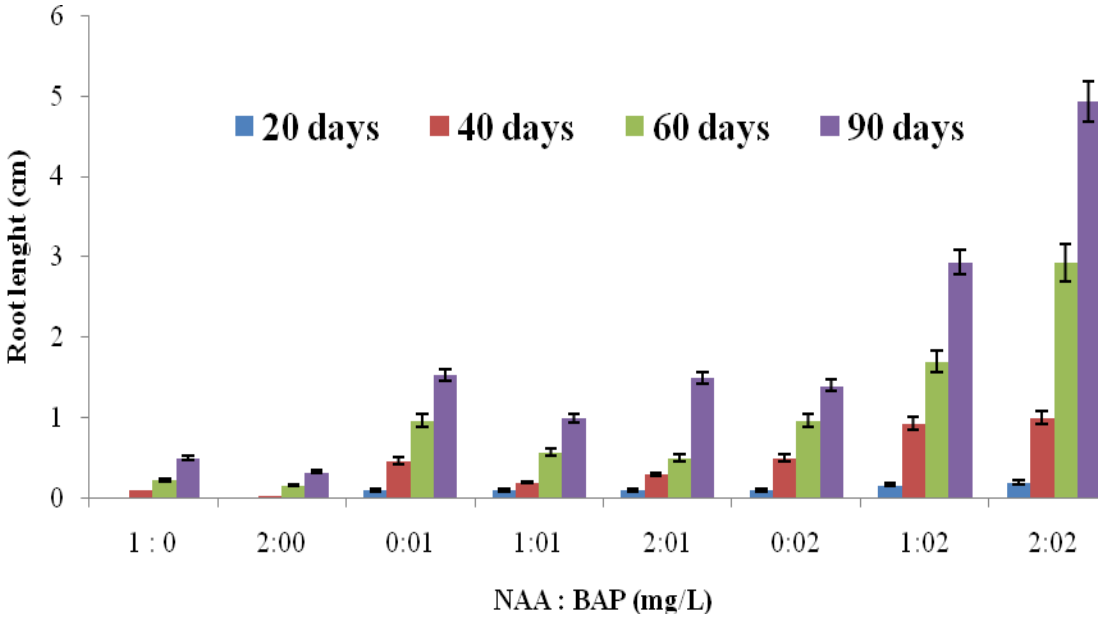
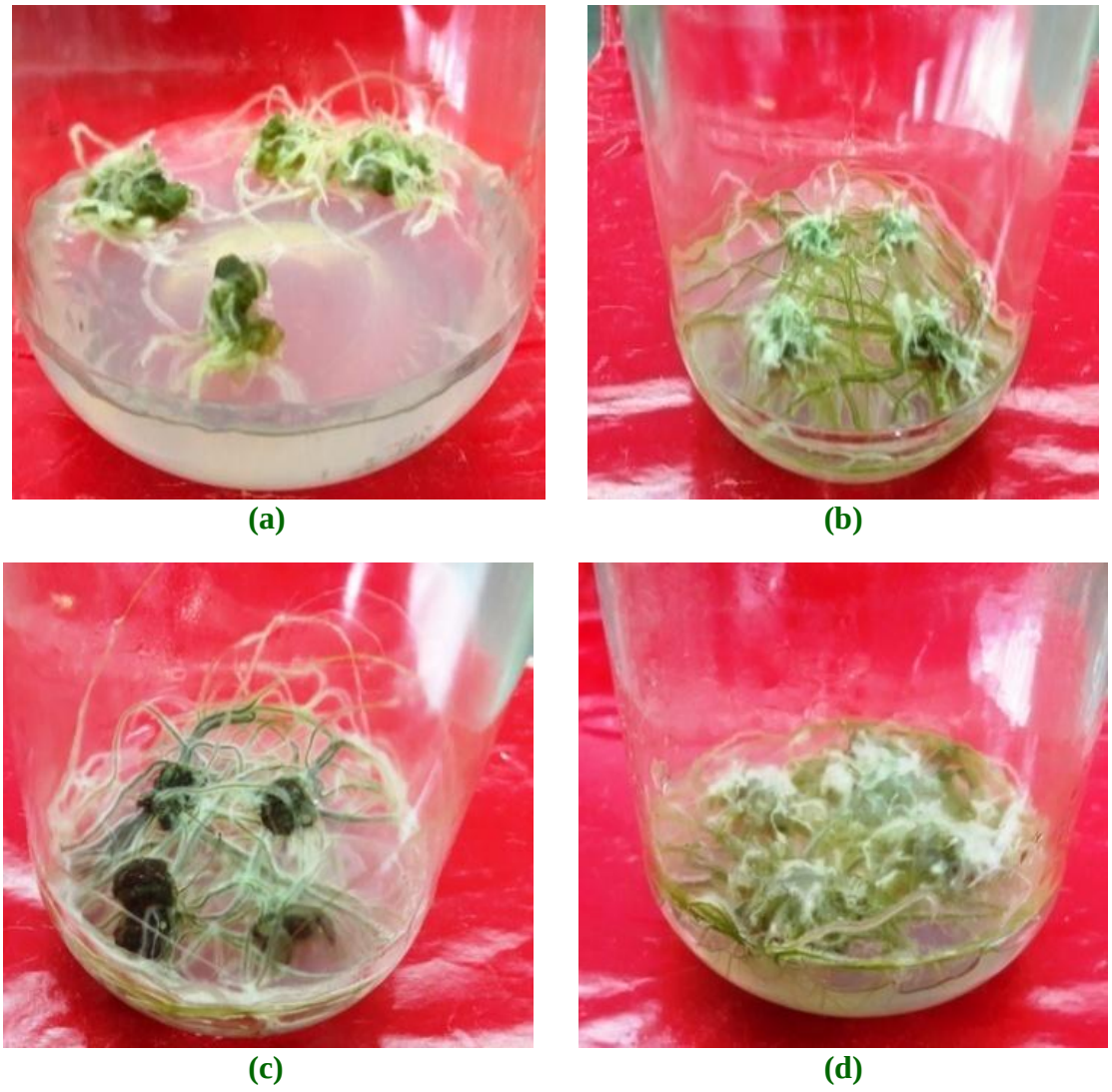


Fig.3 *In vitro* direct rhizogenesis from leaf explants of *V. jatamansi* at (a) 20 days (b) 40 days (c) 60 days (d) 90 days, in MS medium supplemented with BAP and NAA



In vitro propagation using miniature explant is a suitable method to prevent destruction of whole plant and enabling *in situ* conservation of *V. jatamansi*, an endangered species. The *in vitro* technique using miniature explant is applicable to mass multiplication of roots in a short span of time. Due to medicinal value of this herb, its root production is extremely important. The protocol has been standardized and found successful to enhance large-scale root mass production. The results obtained demonstrated feasibility of applying leaf culture technique for enhancing *in vitro* root mass multiplication of *V. jatamansi* in MS medium supplemented with specific concentration of BAP and NAA.

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