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Exploring Regenerative Mechanisms: Comparative Analysis of Callus Induction and Shoot Regeneration in *Valeriana jatamansi* Jones through *In-vitro* and *In-vivo* Cultivation

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Authors' contributions

This work was carried out in collaboration among all authors. Author SP and BP conceived and designed the experiments; analyzed and interpreted the data; wrote the paper. Author DS and DJ interpreted the data; help to wrote the paper. All authors read and approved the final manuscript.

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ABSTRACT

Valeriana jatamansi Jones, commonly known as Tagar, belongs to the Caprifoliaceae family and is valued for its medicinal properties. This research aims to assess the impact of specific plant growth regulators on different explants obtained from natural and in-vitro cultivated plants. Leaves and nodal explants from both sources were utilized, treated with 2,4-D, NAA, and BAP, and monitored

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for 30 days. Results indicate widespread callus formation among various explant types. Particularly intriguing is the robust response of in-vitro cultivated explants, in contrast to those from naturally grown plants. Nodal explants exhibited a remarkable tendency for producing multiple shoots, especially in a medium enriched with 20 µM BAP, highlighting BAP's efficacy in promoting shoot regeneration in controlled settings. This study underscores the potential of tissue culture for establishing effective protocols to conserve this medicinal plant, offering insights into sustainable and responsible utilization for informed conservation strategies.

Keywords: Medicinal plant; 6-benzylaminopurine; multiplication; callus; conservation.

1. INTRODUCTION

Valeriana jatamansi Jones, a vital medicinal herb in the Caprifoliaceae family, faces critical endangerment according to the IUCN list [1]. Known also as *V. wallichii*, it flourishes at 1000 to 3000 meters above sea level in the Himalayas [2,3,4,5] (Bhatt et al., 2013), often in wet zones, woodlands, and stream edges. Its rhizome has been a staple in Ayurvedic and Unani medicine for ages, used for obesity, epilepsy, snakebite, and mental health [2]. Additionally, this plant produces natural sedatives called valepotriates [6,7,8]. Urgent conservation measures are essential to safeguarding this plant's role in traditional and modern medicine due to its unique qualities and significance.

The unauthorized and unscientific collection of wild plants, often with valuable medicinal uses, has significantly diminished their natural habitats, resulting in the classification of species like this one as "endangered" in India [9,10]. It becomes crucial for us to ensure the preservation of such species while using them sustainably. Traditionally, Tagar is grown from seeds, which have a slow germination process and prolonged dormancy. Another method involves root suckers, but this approach has limitations based on population size [11]. Considering this, a promising approach might be large-scale multiplication using in vitro techniques, which has shown success for various Himalayan medicinal plants [12,13,14,15]. This method is recognized as a potent solution for cultivating challenging-to-propagate species, particularly those that are endangered or rare, serving both commercial production and conservation goals [16]. This avenue holds significant promise for safeguarding the future of such valuable plant species.

2. MATERIALS AND METHODS

The purpose of this research was to ascertain how various explant types from *V. jantamansi* plants grown in vivo and in vitro responded to various PGRs for tissue culture-based regeneration.

2.1 Medium and Culture Conditions for Tissue Culture

Different amounts of auxin and cytokinin were added to Murashige and Skoog's (MS, 1962) medium, which had been gelled with 8% agar. Auxins similar to cytokinin, 2, 4-D, and NAA similar to BAP were utilized at concentrations of 5, 10, 15, and 20 µM and autoclaved at 121°C and 15 lb (pressure). Subsequently, the media were kept with a photoperiod of 12 hours and a light intensity of 2000 lux from the cool fluorescent light and the pH was adjusted to 5.8 with the help of a digital pH meter. Cultures underwent continuous incubation at 22 ± 4 °C, illuminated consistently for 24 hours.

2.2 Steps of Micropropagation

A. Collection of explants

For this study, we sourced natural *V. jatamansi* plants from Khirsu villages in the Pauri Garhwal district of Uttarakhand, India (30.1736184 N, 78.8714654 E, 1744 masl) (https://earth.google.com/web/search/Khirsu,+Uttarakhand/). In vitro plants were obtained from HAPPRC, HNBGU Srinagar Garhwal, Uttarakhand (30.2190407 N, 78.7906375 E, 656 m asl). This meticulous collection process laid the foundation for our research.

Preparation and sterilization of explants

The method of preparation and sterilization of explants was used as per Razdan (2000). The sterilization process was done with the following important steps:

Rinsing: The explant was rinsed thoroughly with tap water to remove the large dust particles, and then the selected explants were washed with

double distilled water. After that, the explants were washed with a surfactant like Tween-20 or Triton-X for the removal of contamination.
B. 2. Bavistin: 0.1% to 1% Bavistin is used as a fungicide for 30 minutes.

Ethanol: 70% ethanol is used for a short period of time of 30 sec to 1minutes.

Sodium Hypochlorite (NaClO): NaClO is a commercial bleach generally available with 4% active chlorine content. 2-5% NaClO can be used for 5-10 minutes for sterilization, as per the condition of the explant.

Mercuric Chloride ($HgCl_2$): 0.1% solution of mercuric chloride can be used for 2-5 min.

2.3 Surface Sterilization of Plant Material

The following procedures were used throughout the sterilization process: The Collected explant was washed thoroughly in tap water, then washed 4 to 5 times with distilled water (dw), rinsed in a few drops of Tween-20 for 10 to 15 minutes, and then rewashed 4 to 5 times with dw. The explant was then kept in 0.2% Bavistin for 30 minutes, treated with NaClO (2%) for 5 minutes, placed in a laminar airflow chamber, and rinsed several times (3 to 4 times) with autoclaved distilled water. The explant was then kept in 0.1% HgCl2 for 3 minutes and then rinsed 3 to 4 times with autoclaved distilled water. After that, it was subjected to an additional treatment with 70% alcohol for 30 seconds, followed by a final washing with distilled water (3–4 times).

2.4 Micro-propagation of *V. jatamansi*

After surface sterilization of the explants, cut the explants to a size of about 1-2 cm by scalpel and inoculate them in MS media containing different concentrations of cytokinin and auxin for inducing callus and shoot regeneration. To achieve micro-propagation by in vitro methods, the shoot of a sterilized plant is dissected with the help of a fine scalpel and forceps on a sterilized Petri plate under laminar airflow.

3. RESULTS AND DISCUSSION

This study investigated the impact of various plant growth regulators (PGRs) on distinct explants from both naturally cultivated and tissue culture-based *V. jatamansi* plants. Nodes (runner nodes), petioles, and leaves of naturally grown and in-vitro grown plants were used as explant types for the study. The effect of 2, 4-D, NAA, and BAP on different explants was evaluated. The response was documented after 30 days of inoculation.

3.1 Effect of 2, 4-D

Nodes, petioles, and leaves of naturally grown and *in vitro* grown plants of *Valeriana* were inoculated in MS media supplemented with 5 µM, 10 µM and 15 µM 2,4-D. Saxena et al. [17] documented the organogenesis of callus cultures made from *Psoralea corylifolia* plant's stems, leaves, and petioles. In almost all explant types, callus formation was induced, the magnitude of which varied with the concentration of 2,4-D and the explant type [18] also reported callus formation in leaf explants using 2,4-D). Root induction also varied with the concentration of 2,4-D and the explants type. The observations made have been illustrated in Figs. 1 and 2. It was observed that explants of *In vitro* origin were exclusively responsive in terms of callus development in comparison to naturally grown plants. Results obtained indicate that lower and higher concentrations of 2,4-D i.e., 5 µM and 15 µM 2,4-D had a lower response than 10 µM 2,4-D in the case of nodes of naturally grown plants, indicating the latter as the optimum concentration for callusing. Nodes from *in vitro* origin showed minimum callusing accompanying root formation in 10 µM 2, 4-D while 5, 10, and 15µM 2,4- D induced maximum callusing without root regeneration. 10µM 2, 4-D was found sufficient to produce callus from the petiole of naturally grown plants in the presence of rooting. 5 and 15µM 2,4-D had also produced callus without roots in the latter but 10µM 2,4-D remained at optimum concentration. In the case of petioles of in-vitro grown plants 5, 10, and 15µM 2,4-D were found optimum to generate callus (Fig. 5). It was noticed that root regeneration was reduced when 2,4-D was increased since only 5 and 10 µM 2,4-D induced rooting. Simultaneous rooting with callusing was observed in every leaf explant from nature as well as *in vitro* grown plants (Fig. 1). Moderate callusing was observed in leaves of naturally grown plants in all concentrations of 2,4-D whereas leaves of in-vitro grown plants showed a good callusing response. The data were illustrated in Figs. 1 and 2.

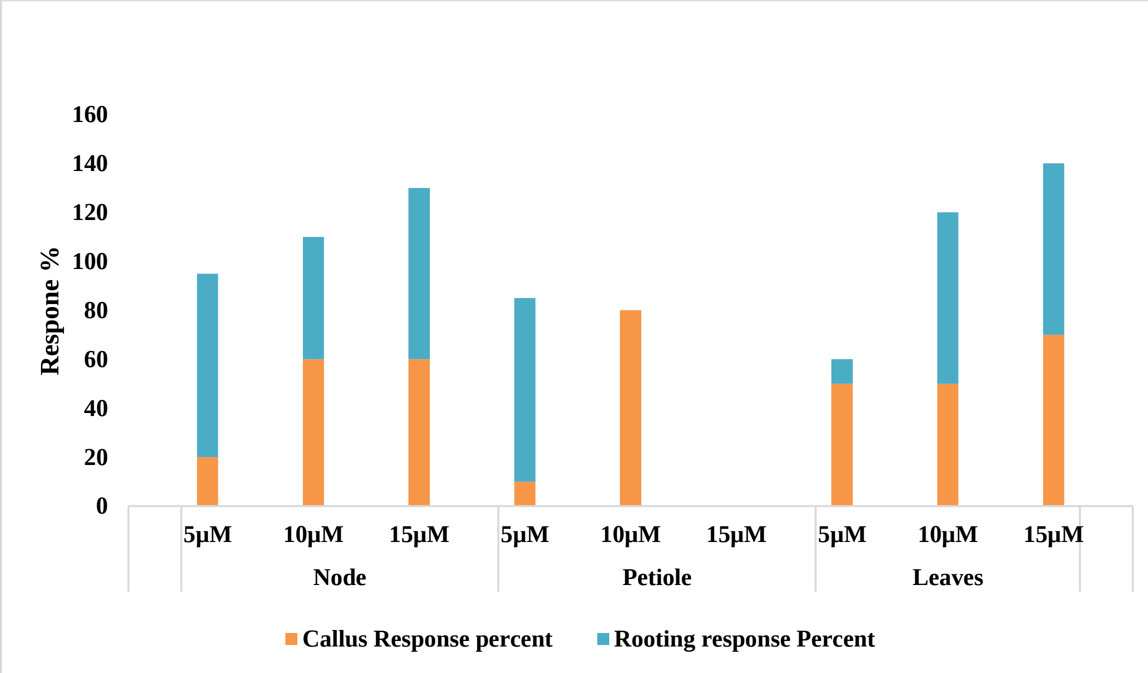

Fig. 1. Effect of different concentrations of 2, 4-D on explant of in vivo plant

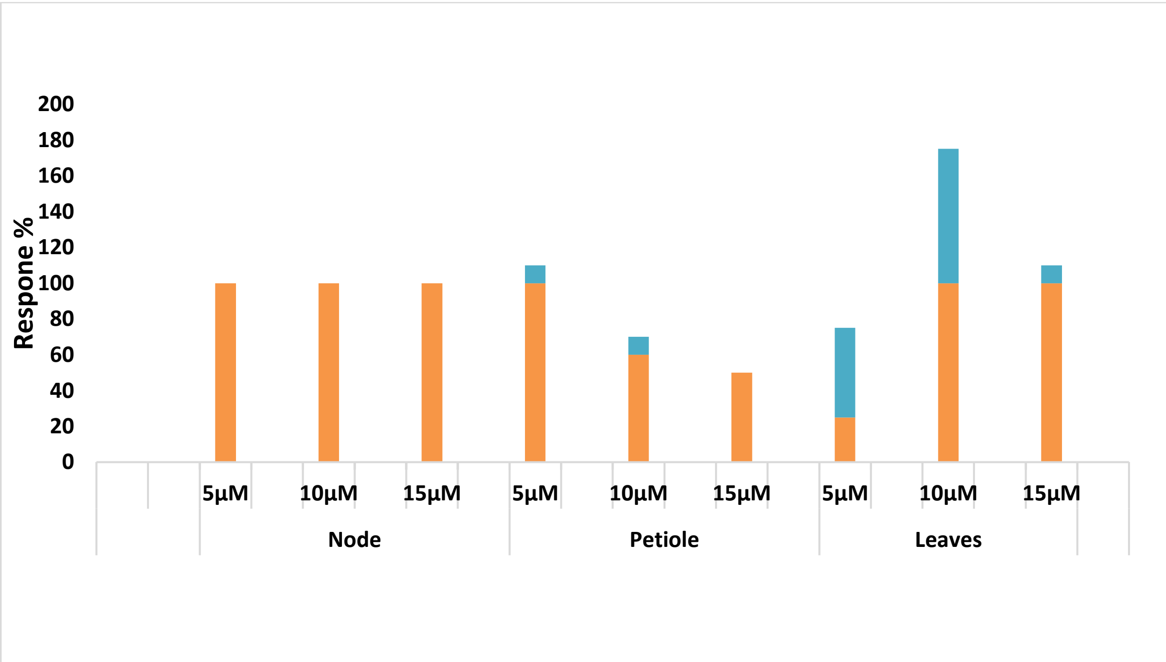

Fig. 2. Effect of different concentrations of 2, 4-D on explant of in vitro plant

3.2 Effect of NAA

The effect of NAA on different explant types was also evaluated, like 2,4-D. 5–15 µM concentrations of NAA were used to evaluate the response of different explant types of naturally grown and in-vitro grown plants (Fig. 3). Again, explants from in-vitro produced plants showed more capacity for differentiation than explant from plants that were in vivo. Nodes of in-vitro grown plants showed good callusing in comparison to nodes of in vivo plants without root induction in all concentrations of 2, 4-D used. 10 µM NAA induced optimum callusing in comparison to 5 and 15 µM NAA, Which were quite low, and no response was noted respectively in petioles of in vivo plants. Nodal explants from naturally grown plants showed moderate callus along with good rooting in all treatments evaluated, whereas nodes from in-vitro grown plants showed well. In the case of petioles of in vitro origin, 5µM NAA induced maximum callus with the presence of a low level of rooting in comparison to 10 and 15 µM NAA. Callus regeneration from petioles of *Valeriana wallichii* using different concentrations of NAA was also reported by Mathur, [19,20]. Again in the leaves of both types of plants, simultaneous callus with rooting was observed in all the treatments of NAA used. Leaves of naturally grown plants in 15µM NAA and of in vitro grown plants in 10 and 15µM NAA showed maximum callus induction in comparison to other concentrations. The data illustrated on Figs. 3 and 4.

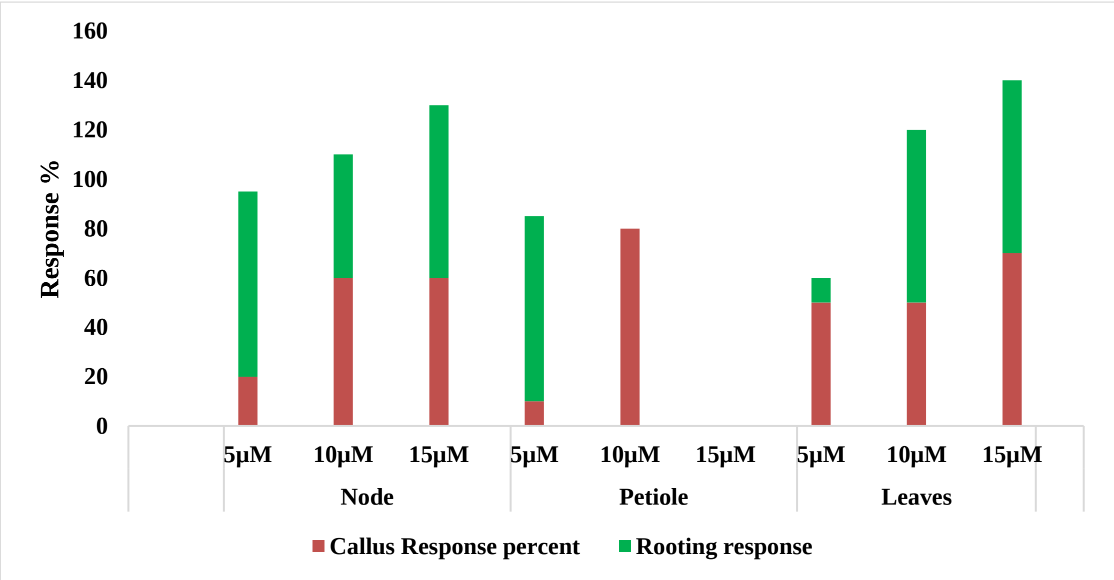

Fig. 3. Role of several concentrations of NAA on different explant types of in-vivo grown plants

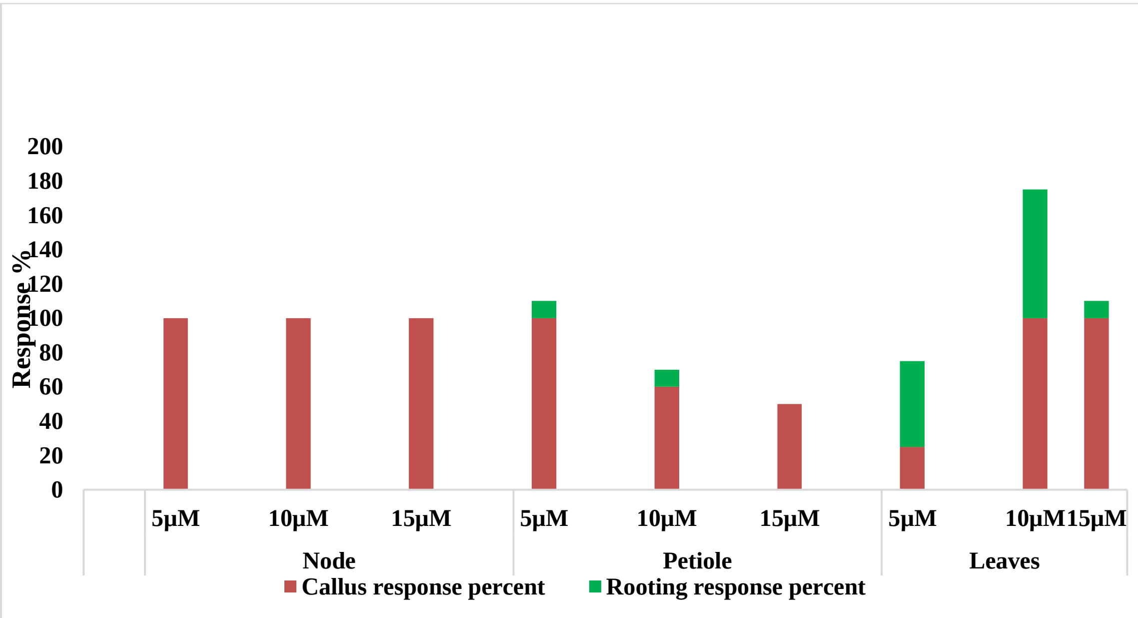

Fig. 4. Role of several concentrations of NAA on different explant types of in-vitro grown plants

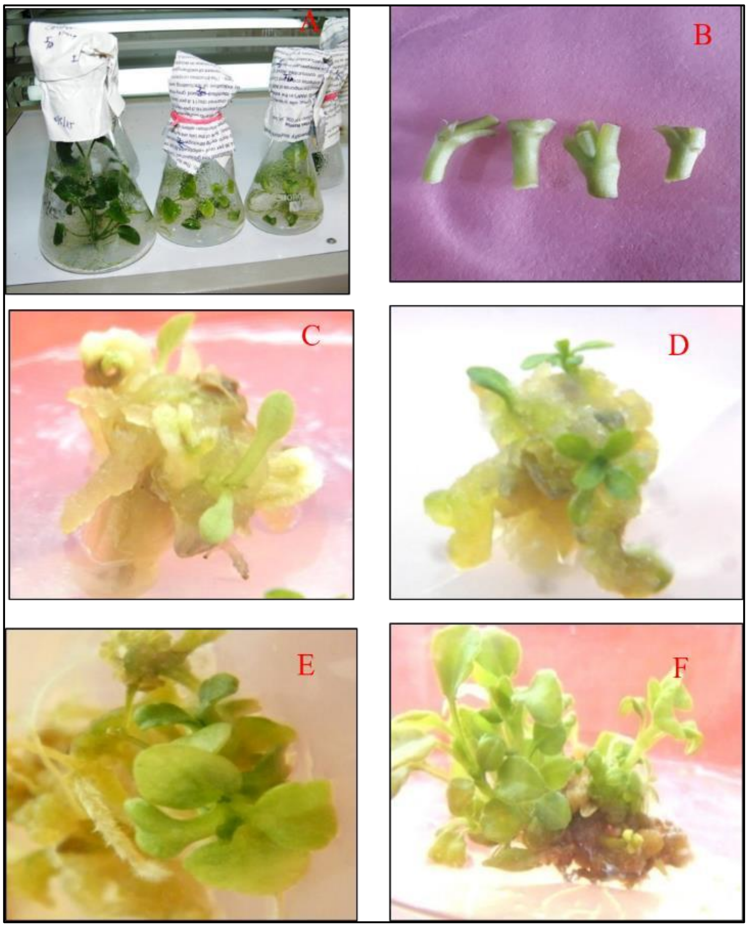

Fig. 5. *In vitro* propagation *V. jatamansi* (A) in vitro grown plant (B) Nodal explant (C) callus induction (D, E) shooting initiation (F) Multiple shoot regeneration

3.3 Effect of BAP on Callus

Callus obtained from explants of in-vitro grown plants was transferred to media containing 5- 20 µM BAP. It was observed that after 30 days of culture, only the callus obtained from nodal explants was able to regenerate shoots from it while the callus obtained from petioles and leaves showed no response. Shoot multiplication was recorded only in media containing 20µM BAP where an average of 20 shoots per callus clump were recorded (Fig. 5 F) Efficiency shoot multiplication using BAP has been developed for several medicinal plant's species such as *Ocimum basilicum* [21], and *Hippophae rehmnoides* [22,23]. Only two shoots per callus clump were found in the remaining treatments that were already present in the callus clump before shifting to BAP from 2, 4-D. These results showed that only BAP has a different physiological response in calluses originating from different sources [24-28].

4. CONCLUSION

This concluded that in almost all explants types, callus was induced. The magnitude of which varied with the concentration of PGRs and the explant type. Root induction also varied with different concentrations of PGRs and the explant type. The higher callus proliferation increases the rate of plant multiplication. These methods will help increase the propagation rate. Callus regenerated from nodes induces multiple shootings in media containing a high concentration of BAP while explants such as petiole and leaves fail to generate shooting responses in tissue culture medium. The findings of this study indicate that tissue culture of *V. jatamansi* can be used to develop efficient protocols for the conservation of this important medicinal plant species. These results can be used to aid in the conservation and sustainable utilization of this species.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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