Indian J. Genet. Plant Breed., (2022) 82(1): 81-88
doi: 10.31742/IJGPB.82.1.11

ISSN: 0975-6906

www.isgpg.org

RESEARCH ARTICLE

Optimization of aerial node mediated emergence and field performance in lower altitude of a higher altitude specific endangered medicinal plant, *Valeriana jatamansi* Jones

Sidhartha Shankar Sharma[1] and Hossain Ali Mondal[1]*

Abstract

A study was undertaken to develop a convenient, low cost, and an efficient technique for rapid propagation from aerial node of *Valenaria jatamansi* Jones directly in artificial soil formulations. The performance of aerial node mediated emergence was also evaluated in higher altitude (HA) as well as lower altitude (LA) to monitor the above and below ground growth parameters in a year in addition to active marker component analysis. The combination of supplemented artificial soil (SAS) having NPK, PSB and *Tricoderma asperellum* along with critical environment that initiated the high emergence as well as growth benefit at early time point (49 days). The two-node containing aerial cutting (wt.=0.580 ± 0.156g; emergence=87.383 ± 2.483%) showed maximum emergence as compared to one node containing aerial cutting (avg.=0.165 ± 0.016g; avg. emergence=63.883 ± 2.77). Concomitantly, injection of 2 µL of 6-Benzylaminopurine (6-BA; 0.25 mg.lt^{-1}) into an aerial node also enhanced early time point growth benefits like significantly higher number of root initiation. The 4-month-old plant produced around 10 additional aerial nodes that could be explored for next round planting material production and original plant will be directly transplanted into main field with more than 90% survival rate without additional acclimatization and hardening steps. Therefore, a continuous and exponential rate of mass clonal propagation could be achieved from one-time requirement of aerial nodes. The 235 days exposure in lower altitude environment produced a significantly higher number of leaves, larger petiole length, smaller leaf length and breadth as compared to natural habitat grown plant in higher altitude. The above ground mass and root production were significantly higher in LA environment as compared to HA environment, but not in total rhizome production with variation in marker active component. The present study developed for the first time a new plant emergence from aerial node in SAS. Hence, it was breakthrough that aerial node produced Quality Planting Material (QPM) directly. Hence, it will be easy to bypass the costly process like tissue culture for clonal propagation of *V. jatamansi*, an endangered medicinal plant.

Keywords: Bud emergence, clonal propagation, higher altitude, lower altitude, micro node, supplemented artificial soil, *Valeriana jatamansi*

Introduction

The genus *Valeriana* belonging to the family *Valerianaceae* is a natural habitat in northern temperate regions of the Himalayan area comprising of nearly 250 species distributed across the countries, namely, Afghanistan, Southwest China, India, Nepal, Bhutan, and Myanmar at an altitude of 1000 to 3000m amsl (Jugran et al. 2013; Polunin and Stainton 1987). *V. jatamansi* (Syn. *V. walichii*, Indian Valerian/Sugandhbala in Hindi) is a natural tetraploid (2n=32) with high morphological variation recorded in northern temperate regions of the Himalayan. Different cytotypes (2n=16, 32 and 64) are found with a diploid species (2n=16) has recently been also reported (Rani et al. 2015). It is reproduced through sexual (seeds) and asexual (rhizome) means (Kaur et al. 1999; Khajuria et al. 2011). However, the genetic variation and very low initial growth among population were the demerit. The vegetative mean was another potent way to achieve the Quality Planting

Department of Genetics and Plant Breeding, Uttar Banga Krishi Viswavidyalaya, Cooch Behar-736 165, West Bengal, India
[1]School of Crop Improvement, College of Post Graduate Studies in Agricultural Sciences (under Central Agricultural University, Imphal, Manipur), Umiam 793103, Meghalaya, India
Corresponding Author: Hossain Ali Mondal, School of Crop Improvement, College of Post Graduate Studies in Agricultural Sciences (under Central Agricultural University, Imphal, Manipur), Umiam 793103, Meghalaya, India, E-Mail: hossainalimondal@gmail.com; ORCiD id- http://Orcid.org/0000-0001-5181-0300 (HAM)

How to cite this article: Sharma S. S., Mondal H. A. 2022. Optimization of aerial node mediated emergence and field performance in lower altitude of a higher altitude specific endangered medicinal plant, *Valeriana jatamansi* Jones. Indian J. Genet., **82**(1): 81-88.
Source of support: NMPB, Ministry of Ayush, India
Conflict of interest: None.
Received: March 2021 **Revised:** Nov. 2021 **Accepted:** Dec. 2022

© ISGPB 2022. This article is Published by the Indian Society of Genetics & Plant Breeding, NASC Complex, IARI P.O., Pusa Campus, New Delhi 110 012; www.isgpb.org

Material (QPM) production but sacrificed rhizome and adventitious root. The active ingredient enriched rhizome and/or adventitious root of *V. jatamansi* are of high medicinal value (Mishra 2004; Singh et al. 2006; Bhatt et al. 2012; Jugaran et al. 2018). Valerenic acid and valepotriates, two marker medicinal compounds are isolated from the rhizome of *V. jatamansi* (Singh et al. 2006). The presence of valerenic acid and valerinone in *V. jatamansi* was a source of drug 'valerian', ranked eighth among the top selling herbal supplements (Blumenthal 2001). The global acceptance of complementary and alternative medicine was the major ground for the increasing demand of the species. *V. jatamansi* was one among the 178 traded medicinal plants, which was recorded in high volume i.e., over 100 mt/year (Sharma et al. 2008).

Demand for *V. jatamansi*'s raw materials in the international trade made this plant into an endangered species due to over and unscientific exploitation (Sher et al. 2011). Therefore, the immediate rescue and conservation required a low cost and convenient mass-propagation. The propagation of plants and trees through tissue culture method using different ex-plant has long been practiced for conservation of plants including endangered species and for enhancing selection efficiency of desirable traits at cellular level (Huynh et al. 2017; Heidary et al. 2018; Pandey et al. 2019). Most scientific conservation efforts were channelized into tissue culture for achieving an efficient propagation technique (Murashige and Skoog 1962). Several studies since then have focused on clonal propagation of *V. jatamansi* via hormone supplemented Murashige and Skoog (MS) media-based tissue culture method.

New agricultural technologies such as soilless and aeroponic cultivation systems have also been developed for enhancing yield and quality biomass production, root harvest and secondary metabolite production in *V. jatamansi* (Pratap et al. 2020) to meet the demand of the pharmaceutical industries. Gautam et al. (2021) have also standardized the propagation techniques for large –scale quality plant material production of essential metabolites for aromatic and pharmaceutical industry based on *V. jatamansi* produce. However, they advocated the use of different concentrations of hormonal use to enhance uniformity in quality and yield, biomass. Kaur et al. (1999) developed protocols for an efficient regeneration of *V. jatamansi* from MS based tissue culture through somatic embryogenesis (Chen et al. 2014) and organogenesis considering apical and axillary buds (Kaur et al. 1999), nodal segments e.g., in *Moringa oleifera* Lam. (Chand et al. 2019), nodal portion at the base (Pandey et al. 2019) and shoot-apices, rhizome and leaf explants (Das et al. 2013). The response of callus formation has been however, varied as per the medium supplemented by plant growth regulators.

Singh 1 et al. (2015) reported plant hormone supplemented MS media using nodal part as explant for developing plantlets in *Valeriana wallichii* DC. Usually, a successful callus formation from leaf explant in plant hormone supplemented MS media and its rooting in media takes 60-80 days and almost one-year required for complete acclimatization for field transfer. For one QPM required 1 year and 75 days for ready of field transplantation. Therefore, a study was undertaken to develop a convenient, low cost, and efficient technique over previously reported methods exploring aerial node which was not considered for conventional propagation. Moreover, the performance of aerial node mediated emergence was evaluated in higher altitude (HA) as well as lower altitude (LA) to monitor the above and below ground growth parameter in a year in addition to active marker component analysis.

Materials and methods

The species, *V. jatamansi* was chosen as the experimental plant. The one and two node containing aerial nodal part harvested from side branch were used for new emergence. The experiment was conducted in the laboratory situated in the foot hill region, known as Terai zone located in West Bengal, India. The specific location was at 28°19'N latitude and 89°23'E longitude and at an altitude of 43m amsl. The experiment was performed at plant growth room (PGR) conditioned with 22°C, 70% relative humidity, 6000 LUX lights intensity for 14 hours per day, open room (OR) and shade house (SH).

Different soil formulations

Artificial soil (AS) was formulated with perlite, peat moss and vermiculite (1:1:1). Perlite was reported for maintaining aeration to ensure an excellent air/water balance which impact on better root growth including better uptake of nutrients in more effective manner. Peat moss retained moisture for better plant growth which also saves irrigation frequency. Moreover, the release of water and nutrients to the right proportions for optimum plant growth of plant and reduce the application of manuring. Vermiculite was reported to improve soil porosity as well as act as a medium for water and nutrient exchange. The water soluble NPK (20:20:20) @1g per liter of water, PSB and *T. asperellum* power @5g (2.5g+2.5g each) per 100 mL were used for irrigation in artificial soil for 2 kg. Both were used only once.

Artificial Soil (AS) was composed of Perlite, peat moss and vermiculite in 1:1:1 ratio. Supplemented artificial soil (SAS) was formulated with the further adding of NPK, PSB and *Tricoderma*. Farm yard manure (FYM) supplemented soil (FSS) in 1:1 ratio was also prepared. Used and autoclaved supplemented artificial soil (UASAS) was also used to evaluate the remaining merit without additional supplementation of NPK, PSB and *Tricoderma*.

Controlled environment for emergence from aerial node

The initial environmental incubation is very crucial for new plant emergence. The transparent box with tight lid was

used for maintaining the humidity. The pot was filled with SAS and kept in transparent box which was again incubated in the environment of 3000 LUX light intensity, 14 h light condition per day, 70% of relative humidity in PGR. At least, 14 days was followed for initial humid in the transparent box for assuring the emerging of new plant. The same process was followed in OR and SH experiment in the respective experimental conditions. An amount of 2 µL of 6-BA (0.25 mg.lt^{-1}) was injected onto the upper side of the micro nodal explant having two nodes. The injected aerial nodal explant was incubated in 100% humid condition for 14 days, 3000 LUX, and 14 Hours light per day under the transparent plastic box.

After emergence from aerial nodal explant, the plant was incubated in 6000 LUX of light intensity, 14-hour light condition per day, 70% of relative humidity in lower altitude in PGR. Very interestingly, profuse branching was observed but not realized in high altitude region in the field condition. This characteristic profuse branching was crucial for micro node harvest from the side branches for the next round new plant emergence from nodal parts.

Growth characteristics on LA and HA

Aerial two nodes mediated emergence was grown in SAS in PGR (2-SAS-PGR) for 120 days and transplanted to LA and HA plot. Each field was shaded with 50% shade mesh. The plant was sown in valley in Kalimpong, Darjeeling (1,250 m or 4,101 ft) and Pundibari, Cooch Behar (43 m) as HA and LA plot, respectively.

The plants were harvested from the lower and high altitudes plots after exposure of 235 days. The growth specific parameters *viz.,* total shoot length, total number of branches, total number of nodes, largest petiole diameter at middle position, total number of leaves, largest petiole length, and largest leaf breadth were recorded. After careful uprooting, the whole plant was considered for recording the total above ground weight excluding roots and rhizomes, total rhizome weight and total root weight from both LA and HA plots after exposure of 235 days.

Valerenic acid estimation in root and rhizome

The marker active component, valerene acid was extracted from root and rhizome according to protocol described by Gao and Björk (2000). An amount of 1600 mg fan drying for 15 days root and rhizome sample were homogenized into fine powder in mortar and pestle using liquid nitrogen. The fine powder was added into 10 mL methanol (Rankem HPLC grade) and incubated for 24 hours at 30°C. The sample was ultrasonicated for 20 minutes in Citizon Ultrasonic cleaner, 220V/50Hz, Power 50W, Frequency 40kHz. The sonicated sample was filtrated through Whatman No. 1 filter paper and volume was made to 10 mL by adding additional methanol. Before loading into HPLC, the sample was further filtrated through 0.2 µm pore size syringe filter to 100 µL. The filtered volume of 60µL was injected into HPLC in the mobile phase of acetonitrile and 0.1% ortho phosphoric acid at 50:50 ratio with a detector of 220 nm wave length according to the described protocol (Gao and Björk 2000). The HPLC details was as follows: Binary pump Waters Milford, MA, USA 515 HPLC, with pump A at the top and pump B at bottom, maximum pressure 600 psi, maximum flow 1-mL per minute, during purging 5 mL per minute. Symmetry column of C18, 5µm, 4.6×250 mm and a software Empower 3 were used for detecting the peak of valerenic acid. Column temperature was maintained at 35°C. Before running the sample, standard of three doses of valerenic acid (analytical standard; 51964-1MG, CAS- 3569-10-6; Sigma-Aldrich) was also run for standard curve preparation. Flow rate was maintained as 1-mL per minute with run time 30 minutes and valerenic acid was detected at 8 minutes; 60 uL of 0.125 mg/mL concentrated valerenic acid was run and area of peak was estimated through a software, Empower 3.

Statistics analysis

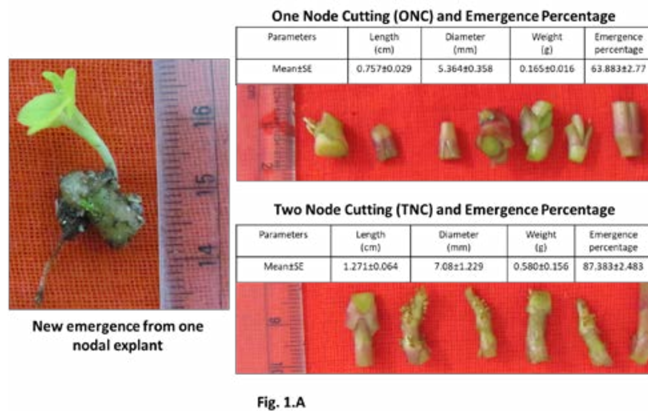
One-way ANOVA and Tukey's HSD Calculator was used for calculation (https://www.icalcu.com/stat/anova-tukey-hsd-calculator.html) to calculate p values at 0.05% level of significance to see any significant difference. The MedCalc statistical software (https://www.medcalc.org/calc/comparison_of_means.php) was also explored to calculate the difference between the observed means in two independent samples.

Result and discussion

Indiscriminate exploitation of *V. jatamansi* from its natural habitat put this plant into an endangered category (Sher et al. 2011). It is of utmost importance to conserve this species for its high pharmaceutical value in Indian medicine. The results obtained on successful emergence of aerial nodal cuttings are presented here below. The emergence from one node and two nodes showed a significant variation in different formulations of soil under controlled environment. The information generated may be prerequisite for commercial clonal propagation.

Emergence from nodal explants

The aerial nodal cutting having one node and two nodes from *V. jatamansi* were grown in environment conditioned with 22°C, 6000 LUX light intensity, 14h light condition per day, 70% of relative humidity were considered for the experiment (Fig. 1A and 1B). The one node cutting (ONC) had characterised as the length of 0.757 ± 0.029 cm, and 5.364 ± 0.358 mm in diameter. Importantly, the mean fresh weigh was 0.165 ± 0.016g which was considered for direct plant emergence from SAS, a formulated artificial soil. The two-node cutting (TNC) had the length of 1.271 ± 0.064 cm, and 7.08 ± 1.229 mm in diameter. The mean fresh weight was 0.580 ± 0.156 g. It has been reported



One Node Cutting (ONC) and Emergence Percentage

Parameters	Length (cm)	Diameter (mm)	Weight (g)	Emergence percentage
Mean±SE	0.757±0.029	5.364±0.358	0.165±0.016	63.883±2.77

Two Node Cutting (TNC) and Emergence Percentage

Parameters	Length (cm)	Diameter (mm)	Weight (g)	Emergence percentage
Mean±SE	1.271±0.064	7.08±1.229	0.580±0.156	87.383±2.483

Fig. 1A. New emergence from aerial node in artificial soil (AS) supplemented additionally with NPK, PSB and *Tricoderma*. The new plant emergence from ONC (0.165 ± 0.016 mg) was recorded to be 63.88 ± 2.77% and 87.38 ± 2.48% from two node cutting (0.580 ± 0.156 mg)

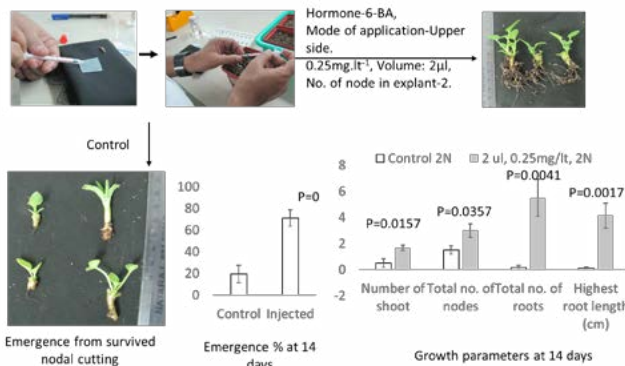


Fig. 1B. Hormone injected aerial node enhanced an emergence. 2 µL of 6-BA (0.25mg.lt^{-1}) injection in TNC in AS showed 70.83 ± 7.68% survival as compared to 19.44 ± 7.98 emergence recorded in AS

earlier that the same kind of explant was explored in MS media for new emergence (Singh et al. 2015). The aerial two nodes showed 87.38 ± 2.48% emergence in SAS with initial incubation period for 14 days in 3000 LUX of light intensity, 14h light condition per day, 100% of relative humidity at 22°C (Fig. 1.A). The same challenge was overcome by hormone supplemented MS media in tissue culture as reported by Singh et al. (2015) and Purohit et al. (2015). AS without supplementation showed 19.44 ± 7.98% new plant emergence (data not shown) from aerial two nodes.

The results on hormone injection of a small quantity of 2 µL of 6-BA (0.25 mg.lt^{-1}) in aerial two node (Fig. 1.B) and incubation in AS for 14 days with 22°C, 3000 LUX light intensity for 14 hours per day with 100% relative humidity showed an enhanced new plant emergence percentage. The growth characteristic of 14-days old emergent was compared from control. The number of shoots, total no. of nodes production, total number of root production, and highest root length were recorded significantly higher

Fig. 2. A and B. Growth comparison among aerial node emergence from SAS, FSS and UASAS at 49 days. A: The picture depicted the growth in SAS, FSS, and UASAS in controlled environment at 49 days and, B: Plant height from ONC and TNC in SAS, FSS and UASAS

in hormone injected two nodal explants as compared to control. Hormone injection also enhanced an emergence percentage from 19.44 ± 7.98 to 70.83 ± 7.68 % (Fig. 1.B). Chand et al. (2019) had also supplemented the basal medium with different hormones in variable quantity and recorded enhanced number of embryos in the nodal segment.

Environmental effect on growth in different soil formulations under controlled conditions

The results on growth benefits of environmental effect showed a significant variation (Fig. 2A). The height was considered at 49 days for evaluating the growth benefit in SAS over FSS and UASAS. The plant height was significantly lower in UASAS in comparison to SAS. The similar results were also observed in TNC. Autoclaving the supplemented artificial soil destroyed soil merits, which was evidenced in SAS. The FSS was recognised as good as SAS. Conclusively, TNC in SAS was the best for achieving highest height at 49 days (Table 1).

A total number of leaves produced at 49 days in all soil formulations in controlled environment were not significant within ONC. The significant difference was recorded between ONC and TNC. The highest number of leaves produced in SAS (Table 2.A). The maximum number of node production was recorded in SAS but not comparable to FSS and UASAS. Therefore, an aerial two-node produced highest number of node production the SAS as compared to FSS and UASAS (Table 2.B).

Plant growth characteristic in OR and SH conditions

All three formulated soils were explored in open room (OR) condition having temperature of 26-30°C and 14 h of light to see the bud break mediated emergence and subsequent growth characteristics. The result showed that only survival

Plant Growth Room	Height in cm	Mean	SE
SAS	ONC	0.7	0.110
	TNC	**1.25**	0.116
FSS	ONC	0.387	0.063
	TNC	0.763	0.111
UASAS	ONC	0.6	0.039
	TNC	0.740	0.093

P Value

		SAS ONC	SAS TNC	FSS ONC	FSS TNC	UASAS ONC	UASAS TNC
SAS	ONC		0.0105			0.0105	
	TNC			0	0.0314	0.0269	0.0137

Table 1. Growth comparison among aerial node emergence from SAS, FSS and UASAS at 49 days. The analysis depicted that aerial two node was experienced early time growth benefit in SAS over FSS and UASAS.

	No. of leaves	Mean	SE
SAS	ONC	10.625	2.692
	TNC	**23.625**	4.200
FSS	ONC	8.444	0.915
	TNC	16.25	3.249
UASAS	ONC	8	1.715
	TNC	22.7	1.468

P Value

		SAS ONC	SAS TNC	FSS ONC	FSS TNC	UASAS ONC	UASAS TNC
SAS	TNC	0.0123		0.0016		0.004	0.0354

	No. of node	Mean (No. of nodes)	SE
SAS	ONC	1.5	0.327
	TNC	**4.75**	0.491
FSS	ONC	2	0.457
	TNC	2.25	0.491
UASAS	ONC	1.833	0.500
	TNC	1.625	0.168

P Value

		SAS ONC	SAS TNC	FSS ONC	FSS TNC	UASAS ONC	UASAS TNC
SAS	TNC	0		0.0003	0.0014	0.0005	0.000[illegible]

Table 2. A and B. Number of leaves and nodes developed from ONC and TNC grown in SAS, FSS and UASAS in controlled environment. A: The result depicted that TNC in SAS was the best for achieving highest number of leaves among ONC and TNC grown in FSS and UASAS and, B: The result depicted that TNC in SAS showed maximum number of nodes production.

was significantly higher in 1-PGR-SAS (1 node containing explant cultured on SAS in plant growth room) as compared to 1-FSS and 2-UASAS grown in open room. Rest of the characters, *viz.,* no. of leaves and no. of nodes developed were non-significant. But OR grown plant was very less vigorous in nature (data not shown).

The shade house was also considered using three formulated soils for bud break-based emergence and subsequent growth parameters. The growth parameters from SH were comparable with the plant growth room grown plants. The result showed that height in 1-PGR-SAS was significantly higher as compared to 1-SH-FSS and 1-SH-UASAS (data nor shown). But no. of leaves and no. of nodes developed had no difference within 1-node mediated emergence in SH among three soil formulations. But within 2-nodes cutting, 2-PGR-SAS showed significantly higher survival percentage of no. of leaves and no. of nodes as compared to 2-SH-SAS, 2-SH-FSS and 2-SH-UASAS (data not shown).

Survival percentage under field conditions

The nodal emergence at 120 days from PGR in SAS was considered for transplanting into different field conditions. The four months old plants grown on SAS from plant growth room were transplanted and recorded survival percentage in different field conditions. The minimum survival percentage was 91 ± 5.56% and maximum was 95 ± 5.00%. However, the survival data are non-significant to each other depicting those 120 days old plant without any additional hardening

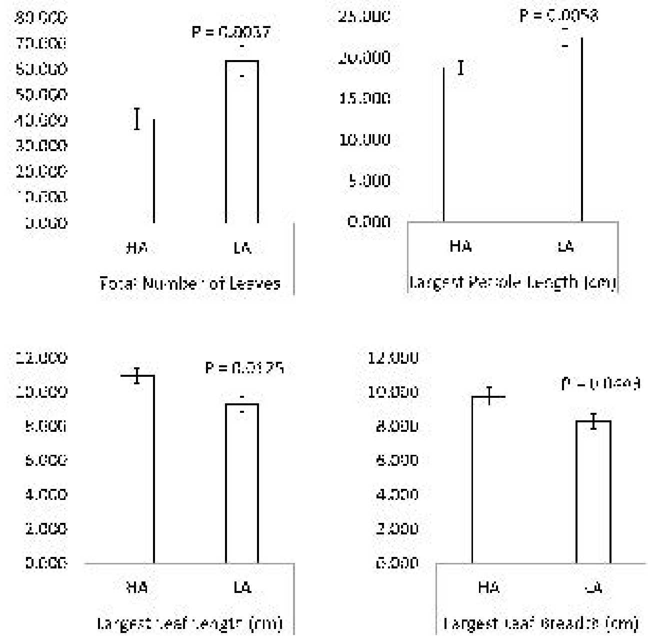


Fig. 3. Above ground growth specific probes on LA and HA grown *V. jatamansi*. The result depicted that 235 days exposed *V. jatamansi* in LA showed significant variation in 'total number of leaves', and 'largest petiole length'. LA exposed *V. atamans* showed significant 'largest leaf length', (p=0.0125) and 'largest leaf breadth' (p=0.0443) but characteristically, 'length/breadth ratio of largest leaf' was insignificant (p=0.891).

and acclimatization showed high performance in field standing.

Above ground characters differentiation in LA and HA

The result showed that 235 days exposure of *V. jatamansi* in HA and LA did not show any significant variation in total shoot length, total number of branches, total number of nodes, and largest petiole diameter at middle position (data not shown). But 235 days exposed *V. jatamansi* in LA showed significant variation in total number of leaves (p=0.0037), and largest petiole length (p=0.0058) (Fig. 3). Moreover, LA exposed *V. jatamansi* reduced significantly the length (p=0.0125) and breadth (p=0.0443) in the 'largest leaf' (Fig. 3). Characteristically, length/breadth ratio of the largest leaf remained insignificant (p=0.891) in LA. In summary, the leaf area was reduced in LA exposed plant as compared to HA exposed plant and leaf become smaller but number of leaves was significantly increased in LA exposed plant (p=0.0037). Interestingly, the LA exposed leaf produced lengthy petiole (p=0.0058) (Fig. 3).

Yield differentiation in LA and HA conditions

The plants of *V. jatamansi* exposed for 235 days in higher altitude and lower altitude were harvested carefully and recorded the yield related parameters like total above ground weight excluding roots and rhizomes (g), total rhizome weight (g) and total root weight (g). Interestingly,

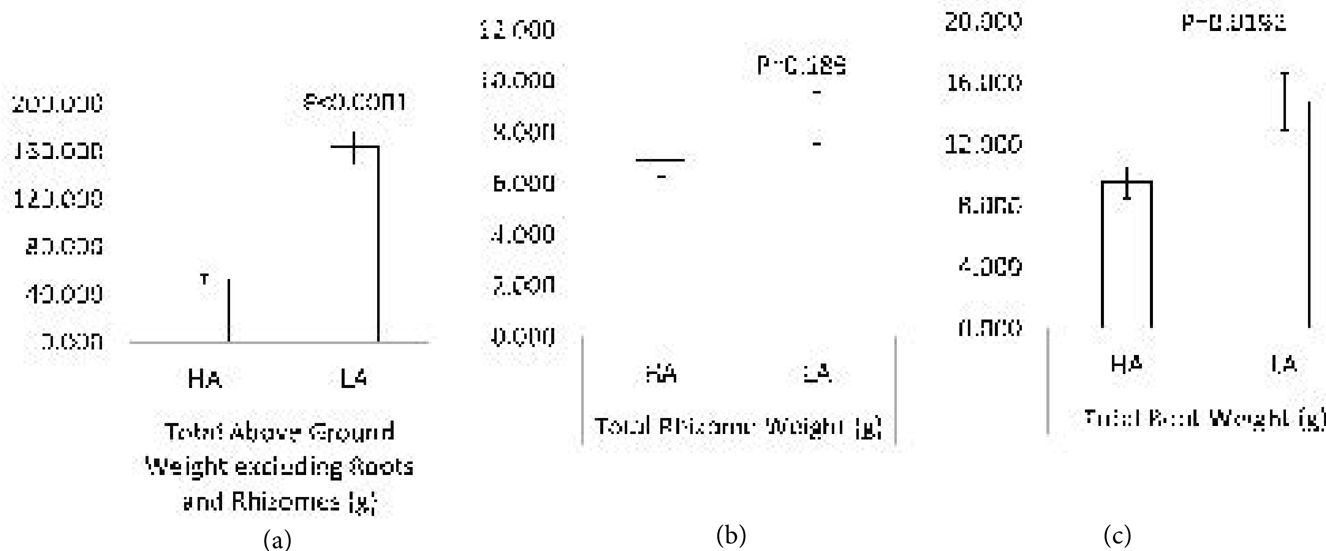


Fig. 4. A, B and C. Yield performance both in HA and LA. A: The result revealed that 235 days exposure in LA produced significantly (p <0.0001) higher above ground mass excluding root and rhizome as compared to HA. B: Very interestingly, total rhizome weight was remained comparable (p=0.189) in both HA and LA environments and, C: The adventitious root production was significantly higher (p=0.0192) in LA grown plant as compared to HA grown plant

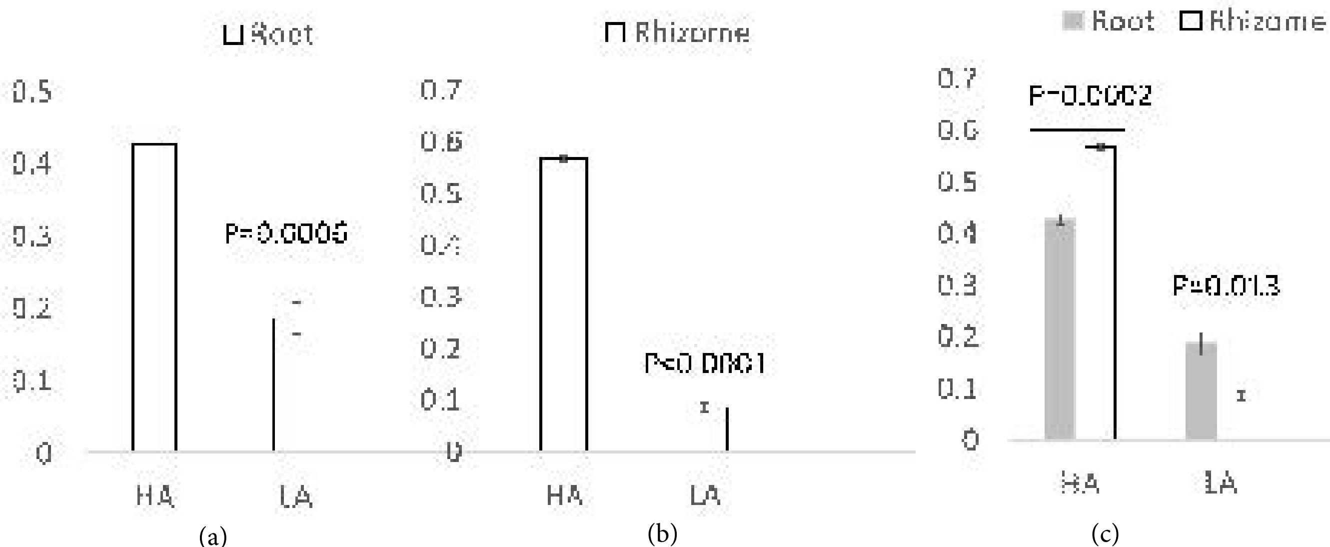


Fig. 5. A, B and C. Marker active component monitoring in root and rhizome harvested from HA and LA plot. A: The marker active component in root. B: The marker active component in rhizome and, C: The rhizome was more sensitive in marker active component as compared to root.

235 days LA exposed *V. jatamansi* produced significantly (p <0.0001) higher total above ground weight excluding roots and rhizomes (g) as compared to HA exposed plants (Fig. 4.A). The above ground mass from 235 days LA exposure was recorded to be 163.385 ± 13.896g as compared to 53.022 ± 4.339g realized in HA exposed plant. However, total rhizome weight remained comparable (p=0.189) (Fig. 4.B) in both HA and LA exposed plant (6.918 ± 0.679g in HA vs. 8.558 ± 1.018g in LA). Interestingly, the adventitious root production was significantly (p=0.0192) higher (Fig. 4.C) in LA grown plant (14.778 ± 1.871g) as compared to HA grown plant (9.494 ± 1.064g). In conclusion, LA exposure accumulated more mass in the above ground as well as root but not in rhizome.

Active component analysis in root and rhizome

The result showed that room dried HA root contained 0.426 ± 0.009% valerenic acid. Due to exposure of 235 days in LA, the active component was estimated to 0.186 ± 0.022% on dry weight basis (Fig. 5A). Therefore, significant marker active component was reduced (p=0.0006). The dried rhizome contained 0.567 ± 0.005% valerenic acid on dry weight basis when rhizome was collected from HA (Fig. 5B). Due to exposure of 235 days in LA, the active

component in rhizome was estimated to 0.085 ± 0.008% on dry weight basis (Fig. 5B). Therefore, significant marker active component was reduced (p=0.0001). In conclusion, exposure of LA environment significantly reduced marker active component. Root showed 2.28 times less marker active component in LA as compared to HA (Fig. 5A). The rhizome also showed 6.64 time less marker active component as compared to HA grown rhizome. Hence, rhizome is more sensitive to LA environment (Fig. 5B). The HA grown rhizome was significantly (p=0.0002) enriched with marker active component as compared to HA grown root. Interestingly, the LA grown root was significantly (p=0.013) enriched with marker active component as compared to LA grown rhizome (Fig. 5C). This indicated that LA environment was more sensitive to marker active component in rhizome as compared to root.

To the best of our knowledge, the aerial node was explored directly for new plant emergence without exploring hormone supplemented MS media which consumed huge time for new transplantable plant. The planting material production with field performance to both LA and HA was also completed within one year with special reference to the marker active component analysis. In India, *V. jatamansi* was recognized in the list of 178 medicinal plants with high volume trade/consumption about 123 MT (Ved and Goraya 2008). The high demand of the rhizome and root provoked the local people to uproot the plant from natural habitat in an uncontrolled way and categorized in the endangered category in India (CAMP 2003; Samant et al. 1998). Therefore, very simple, potent, and low-cost technology was the most relevant issue for achieving mass clonal propagation. A number of research articles were already published focusing on propagation exploring hormone-supplemented MS media (Singh et al. 2015; Purohit et al. 2015). The bud break mediated emergence in hormone-supplemented MS media was the initial step for successful tissue culture. The present protocol directly regenerated new emergence from aerial nodal explant in AS (Fig. 2.A). The present study recorded 87.38 ± 2.48% bud break mediated emergence in SAS (Fig. 1.A). A number of experiments optimized of supplementation in water soluble NPK, PSB and *Tricoderma* (Fig. 1B). Whereas the same aerial nodal explant in control having only AS without any supplementation of NPK, PSB and *Tricoderma asperellum* and initial environment recorded 19.44 ± 7.98% emergence (data not shown). The emerged plant produced profuse branching in the environment of 6000 LUX of light intensity, 14-hour light condition per day, 70% of Relative Humidity at 120 days in SAS previously supplemented with NPK, PSB and *Tricoderma* (data not shown). The node from branching will be explored for next round clonal propagation without destroying mother plant which was recognized as QPM for the field transfer without additional requirement of hardening and acclimatization (data not shown). Among all soil formulation, SAS was the best and the environment of 6000 LUX of light intensity, 14-hour light condition per day, 70% of Relative Humidity was the optimum for enhanced profuse branching having highest number of node production (data not shown). This invention could potentially produce QPM in continuous and exponential rate (data not shown).

The study concluded that *V. jatamansi* was naturally found in the ecology of higher altitudes. However, lower altitude exposed plant showed more number leaves, shorter leaf size, and shorter leaf length and breadth ratio. More number of leaves might contribute more mass in the above ground as compared to higher altitude exposed plant. Interestingly, the rhizome yield was insignificant due to lower altitude exposure, but the root production was higher. Finally, lower altitude exposure significantly reduced active component both in root and rhizome but rhizome was more sensitive to environment in term of marker active component.

Authors' contribution

Conceptualization of research (SSS, HAM); Designing of the experiments (HAM); Contribution of experimental materials (SSS, HAM); Execution of field/lab experiments and data collection (HAM, SSS); Analysis of data and interpretation (HAM); Preparation of manuscript (HAM).

Acknowledgement

The author Hossain Ali Mondal acknowledge sincerely to NMPB, Ministry of AYUSH for the financial support (Grand Number-Z.18017/187/CSS/R&D/WB-1/2016-17-NMPB-IVA, Dated: 05.08.2016). The author also acknowledges sincerely to Central Instrumental Facility (CIC), UBKV for accessing in HPLC. The communicating author (HAM) acknowledged sincerely to Pratik Saha, Bablu Paul, Albina Gurung and Moumita Mallick for back up service and assisting in scientific experiment.

References

Bhatt I. D., Dauthal P., Rawat S., Gaira K. S., Jugran A., Rawal R. S. and Dhar U. 2012. Characterization of essential oil composition, phenolic content, and antioxidant properties in wild and planted individuals of *Valeriana jatamansi* Jones. Sci. Horticulturae, **136**: 61-68. https://doi.org/10.1016/j.scienta.2011.12.032

Blumenthal M. 2001. Herb sales down 15 percent sales in mainstream market. Herbal Gram, **59**: 69.

CAMP. 2003. Conservation assessment and management prioritization for the medicinal plants of Himachal Pradesh, Jammu & Kashmir and Uttarakhand. In: Proceedings of the workshop held at Shimla, Hosted by Foundation for Revitalisation of local Health Traditions (FRLHT), Bangalore, India.

Chand S., Pandey A. and Verma O. 2019. *In vitro* regeneration of *Moringa oleifera* Lam.: A medicinal tree of family Moringaceae. Indian J. Genet. Plant Breed., **79**(3): 606-613.

DOI: 10.31742/IJGPB.79.3.10

Chen et al. 2014. Shoot organogenesis and somatic embryogenesis from leaf explants of *Valerianajatamansi* Jones. Scientia Horticulturae, **165**: 392–397.

Chevallier A. 1999. The encyclopedia of medicinal plants. Dorling Kindersley London (seen at http://www.diet-and-healthnet/neuropathy/valerian.html).

Das J., Mao A.A. and Handique P.J. 2013. Callus-mediated organogenesis and effect of growth regulators on production of different valepotriates in Indian valerian (*Valeriana jatamansi* Jones.). Acta Physiol. Plant, **35**: 55–63.

Gao X. and Björk L. 2000. Valerenic acid derivatives and valepotriates among individuals, varieties and species of *Valeriana*. Fitoterapia, **71**(1): 19-24.

Gautam R., Kumar A., Kumar R., Chauhan R., Singh S., Kumar M., Kumar D., Kumar Ashok and Singh S. 2021. Clonal propagation of *Valenaria jatamansi* retains the essential oil profile of mother plan: An approach toward generating homogenous grade of essential oil for industrial use. Front. Plant Sci., **14**(12): 738247.

Heidari P., Etminan A., Azizinezhad Reza and Khosroshahli M. 2018. In vitro-examination of genetic parameters and estimation of seedling physiological traits under drought and normal conditions in durum wheat. Indian J. Genet. Plant Breed., **78**(2): 217-227. DOI: 10.5958/ 0975-6906.2018.00028.7.

Huynh H. N., Lal S. K., Singh S. K., Talukdar A. and Vinod. 2017. Screening of soybean [*Glycine max* (L.) Merrill] genotypes for somatic embryogenesis and plant regeneration potential. Indian J. Genet. Plant Breed., **77**(3): 387-393. DOI: 10.5958/0975-6906.2017.00052.9.

Kaur R., Sood M., Chander S., Mahajan R., Kumar V. and Sharma D.R. 1999. *In-vitro* propagation of *Valeriana jatamansi*. Plant Cell Tiss. Org. Cult., **59**: 227–229.

Khajuria A., Verma S. and Sharma P. 2011. Stylar movement in *Valeriana wallichii* DC. A contrivance for reproductive assurance and species survival. Curr. Sci., **100**: 1143–1144.

Jugran A.K., Rawat S., Bhatt I.D. and Rawal R.S. 2018. *Valeriana jatamansi*: An herbaceous plant with multiple medicinal uses. Phytotherapy Res., **2019**: 1–22. https://doi.org/10.1002/ptr.6245

Jugran A.K., Bhatt I. D., Rawal R. S., Nandi S. K. and Pande, V. 2013. Patterns of morphological and genetic diversity of *Valeriana jatamansi* Jones in different habitats and altitudinal range of West Himalaya, India. Flora, **208**: 13-21. https://doi.org/10.1016/j.flora.2012.12.003

Mishra L.C. 2004. Scientific Basis for Ayurvedic Therapies. CRC Press, NewYork, USA.

Murashige T., Skoog, F. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant, **15**: 474-497, doi: 10.1111/j.1399-3054.1962.tb08052.x.

Pandey A., Verma O. and Chand S. 2019. In vitro propagation of *Boerhaavia diffusa* L.: An important medicinal plant of family Nyctagimaceae. Indian J. Genet. Plant Breed., **79**(1): 89-95. DOI: https://doi.org/10.31742/ IJGPB.79.1.12.

Polunin O. and Stainton A. 1987. Concise flowers of the Himalaya. London: Oxford University Press.

Prakash V. 1999. Indian *Valerianceae*: A Monograph on Medicinally Important Family. Scientific Publishers, Jodhpur, India, pp. 70.

Pratap M., Kumar P. Kumar A., Joshi R., Kumar D. and Warghat A.R. 2020. Effect of elicitors on morpho-physiological performance and metabolites enrichment in *Valeriana jatamansi* cultivated under aeroponic conditions. Front. Plant Sci., https://doi.org/10.3389/fpls.2020.01263

Purohit S., Rawat V., Jugran A.K., Singh R.V., Bhatt I.D. and Nandi S.K. 2015. Micropropagation and genetic fidelity analysis in *Valeriana jatamansi* Jones. J. Appl. Res. Med. Aromatic Plants, **2**. pp 15-20.

Rajkumar S., Singh S.K., Nag A. and Ahuja P.S. 2011. Genetic structure of Indian valerian (*Valeriana jatamansi*) populations in western Himalaya revealed by AFLP. Biochem. Genet., **49**: 674-681.

Rani S., Sharma T.R., Kapila R. and Chahota R.K. 1970. Identification of new cytotypes of *Valeriana jatamansi* Jones, (Valerianaceae) from North-Western Himalayan region of India. Comp. Cytogenet., **9**(4): 499-512. DOI:10.3897/CompCytogen.v9i4.8875.

Sharma A., Shanker C., Tyagi L. K., Singh M. and ChV R. 2008. Herbal medicine for market potential in India: An overview. Acad. J. Plant Sci., **1**: 26-36.

Sher H., Elyemeni M., Khan A.R. and Sabir A. 2011. Assessment of local management practices on the population ecology of some medicinal plants in the coniferous forest of Northern parts of Pakistan. Saudi. J. Biol. Sci., **18**: 141-149.

Singh N., Gupta A. P., Singh B. and Kaul V. K. 2006. Quantification of valerenic acid in Valeriana jatamansi and *Valeriana officinalis* by HPTLC. Chromatographia, **63:** 209-213. DOI: https://doi.org/10.1365/s10337-005-0713-6

Singh S., Purohit V.K., Prasas P. and Nautiyal A.R. 2015. Micropropagation of Valeriana wallichii DC. (Indian Valerian) through nodes. Indian J. Biotechnol., **14**. pp127-130.

Ved D.K., Goraya G.S. 2008. Demand and Supply of Medicinal Plants in India. Bishen Singh Mahendra Pal Singh, Dehradun.

Violon C., Van C.N. and Vercruysse A. 1983. Valepotriate content in different in-vitro cultures of *Valerianaceae*. Pharmacy World and Science, **5:** 205-209.