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Interaction Effect of Auxin and Cytokinin on *in Vitro* Shoot Regeneration and Rooting of Endangered Medicinal Plant *Valeriana jatamansi* Jones through Tissue Culture

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Abstract

Micropropagation of *Valeriana jatamansi* Jones by using small segments of rhizome on full strength MS medium having various concentrations and combinations of auxin Naphthaleneacetic acid (NAA) and cytokinin Benzylaminopurine (BAP) was conducted. The highest mean shoot length (3.71 cm) was achieved when media was fortified with BAP 2 mg/L in combination with NAA 1 mg/L. The highest mean leaf number *i.e.* 6.00 was observed when BAP was used alone at 2 mg/L. Average root length (0.77 cm) was recorded when BAP 1.5 mg/L along with NAA 0.5 mg/L was used. Maximum mean root numbers 2.57 were obtained when BAP and NAA were used at equal concentrations *i.e.* 1.5 mg/L. Observed data demonstrated that BAP up to 1 mg/L, 1.5 mg/L and 2 mg/L promotes shoot length, leaf number and leaf growth when used along with NAA at 0 mg/L, 0.5 mg/L and 1 mg/L. However lower quantities of both NAA (0 mg/L, 0.5 mg/L) and BAP (1 mg/L and 1.5 mg/L) produced significantly higher root length of *Valeriana jatamansi* Jones but the higher concentrations of plant growth hormones BAP (2 mg/L, 3 mg/L) and NAA (1 mg/L, 1.5 mg/L) were found unfavorable for increase in root length but the root number increases at higher concentration of NAA (1 mg/L and 1.5 mg/L).

Keywords

Valeriana jatamansi, Medicinal Plant, Tissue Culture, NAA, BAP

1. Introduction

Plants are used throughout the world for various health care requirements and

for the formation of medicine on large scale. Medicinal plants are used as a source of bioactive secondary compounds by pharmaceutical industries [1]. *Valeriana jatamansi* is a species of family Valerianaceae belonging to the genus *Valeriana*. Common name of *Valeriana jatamansi* is Mushkbala, sugandhbala in Hindi and Tagar in Sanskrit [2] [3]. *Valeriana jatamansi* Jones has tremendous medicinal importance, used for treating diseases such as leprosy [4], nerve diseases, epilepsy and hysteria, cholera, scorpion and snakebite, asthma and neurosis [5] [6] [7] and aromatherapy [8]. The roots and rhizomes of *Valeriana jatamansi* contain valepotriates [4] which have cytotoxic and anticancer activity. Cytotoxicity of valepotriates has potential antitumor properties which minimize tumor size after 24 hours [6].

Valeriana jatamansi Jones is endangered [9] and high valued medicinal herb generally utilized for its rhizomes and roots. It is proliferated through seeds but because of poor seed viability, slow germination and prolonged dormancy of the species and over-harvesting from its natural environment it is necessary to develop a practical and reproducible method for production and rapid multiplication of *Valeriana jatamansi* Jones over large scale by using tissue culture techniques. Tissue culture techniques are applied for forestry, horticulture as well as for the conservation and recovery of rare and endangered herbs.

Zamini *et al.* [10] organized a method for callus initiation of *Valeriana officinalis* by using NAA and 2,4-D. Excellent induction of callus (95.83%) resulted with KIN 1 mg/L along with 2,4-D 1.5 mg/L. Roots were induced on the same media. Regeneration through tissue culture by using leaf of *Valeriana jatamansi* as explants and induction of adventitious shoots along with somatic embryos was done by Chen *et al.* [11]. Current research work is based on the evaluation of the effect of plant growth regulators 6-benzyl amino purine (BAP) and α-naphthalene acetic acid (NAA) in culture medium for the successful *in vitro* shoot and root development of *Valeriana jatamansi* through rhizome cuttings.

The aim of present study was to evaluate the effect of different concentrations and combinations of 6-benzyl amino purine (BAP) and α-naphthalene acetic acid (NAA) in culture medium for the successful *in vitro* shoot and root regeneration of *Valeriana jatamansi* through tissue culture technique, successful hardening and transplantation of rooted plants in the soil and to optimize Lab Protocol for successful *in vitro* regeneration of endangered medicinal herb *Valeriana jatamansi.*

2. Materials and Methods

2.1. Plant Material Collection

The present study was conducted in the tissue culture laboratory at Hazara Agriculture Research Station Abbottabad. *Valeriana jatamansi* was collected from its natural habitat of Nathiagali hills Abbottabad during the month of July. Plant was identified by using plant identification key "Flora of Pakistan" [12].

2.2. Media Preparation

Murashige and Skoog [13] basal medium was prepared to contain 1 mg/L Ca-pantothenate, 100 mg/L myoinositol, 0.25 mg/L Gibberellic acid (GA3) with 30 g/L sucrose as a carbohydrate source and 8 g/L agar as a solidifying agent. Plant growth hormones *i.e.* 6-benzyl amino purine (BAP) and α-naphthalene acetic acid (NAA) were added for shoot growth and rooting. pH was adjusted to 5.8 with 1N HCl or 1N NaOH before autoclaving at 121˚C and 15 psi pressure for 15 minutes. There were 16 different combinations of BAP and NAA concentrations as experimental treatments each replicated 10 times.

2.3. Inoculation and Surface Sterilization of Explants

Valeriana rhizome cuttings were washed under running tap water for 30 minutes to remove any traces of dirt. Then washed with mild detergent containing 2% (v/v) solution of Tween 20 for 15 minutes and were again washed with sterilized distilled water thoroughly. Small segments of rhizome were cut and surface sterilized in a solution of 0.1% mercuric chloride for 1 min and washed with sterilized distilled water for three times then treated with 0.1% (w/v) Streptomycine sulphate (bactericide) solution for 15 minutes followed by three times washing with sterilized distilled water. Finally the rhizome segments were treated with 0.3% (w/v) Carbendazim (fungicide) solution for 10 minutes under laminar flow cabinet and washed thrice with sterilized distilled water. After sterilization, rhizome segments were inoculated aseptically @ one segment in each test tube.

2.4. Incubation in Growth Chamber

Cultured test tubes were incubated at 20˚C ± 2˚C in 16 h light and 8 h dark periods in the growth chamber. Data were collected on growth parameters *i.e.* shoot and root emergence, shoot length, root length, root number and leave number.

2.5. Statistical Analysis

The experiment was carried out in Complete Randomized Design (CRD) and the recorded data were subjected to analysis of variance (ANOVA). Least significance difference test (LSD) was used for comparing means at 5% probability level. The Computer software Statistix 8.1 was used to analyze the data.

3. Results

3.1. Shoot Emergence

All the treatment combinations of cytokinin and auxin (6-benzyl amino purine (BAP) and α-naphthalene acetic acid (NAA) showed shoot emergence after 7 days of culturing (**Table 1**) (**Figure 1**). The highest mean shoot emergence *i.e.* 85.7% was obtained in treatments T9 and T11 with BAP 2 mg/L alone and along with NAA 1 mg/L, respectively (**Table 1**) followed by 71.4% when media was fortified with BAP 1 mg/L along with 0.5 mg/L and 1 mg/L NAA, with BAP 1.5 mg/L

Table 1. Effects of 6-benzyl amino purine (BAP) and *α*-naphthalene acetic acid (NAA) at different concentrations on shoot emergence percentage of *Valeriana jatamansi* Jones.

Treatments	Plant growth regulators concentration (mg/L) media		Shoot Emergence %	
	BAP	NAA	After first week of culture	After second week of culture
T1	1	0	57.1%	100%
T2		0.5	71.4%	100%
T3		1	71.4%	100%
T4		1.5	24.2%	42.8%
T5	1.5	0	71.4%	100%
T6		0.5	71.4%	85.7%
T7		1	57.1%	71.4%
T8		1.5	28.5%	28.5%
T9	2	0	85.7%	100%
T10		0.5	71.4%	100%
T11		1	85.7%	100%
T12		1.5	42.8%	57.1%
T13	3	0	28.5%	42.8%
T14		0.5	14.2%	42.8%
T15		1	14.2%	57.1%
T16		1.5	14.2%	28.5%

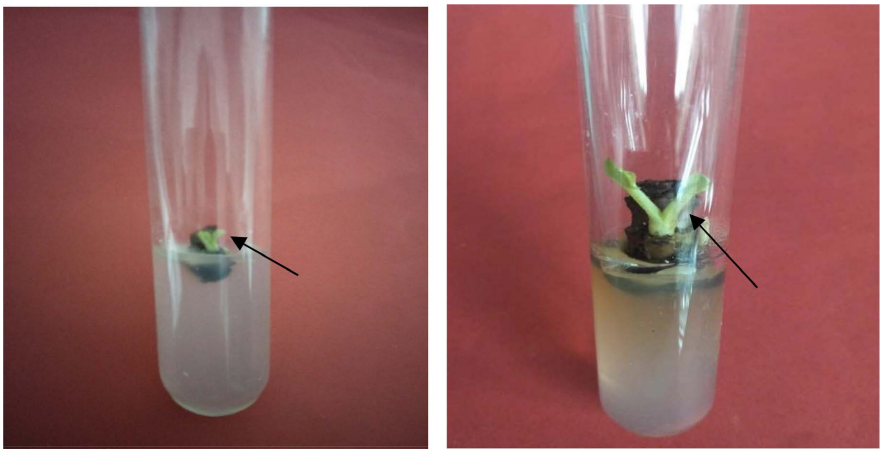

Figure 1. Rizome segemnts of *Valeriana jatamansi* Jones showing shoot emergence after 1st week of culture at BAP 1 mg/L + NAA 0 mg/L (left) and BAP 1 mg/L + NAA 0.5 mg/L (right).

alone (T5) and in combination with 0.5 mg/L NAA (T6), in treatment T10 at BAP 2 mg/L along with NAA 0.5 mg/L (Table 1, Figure 1). When BAP and NAA were used at equal concentration *i.e.* 1.5 mg/L shoot emergence was re-

duced to 28.5%. The lowest shoot emergence (14.2%) resulted at higher concentration of BAP (3 mg/L) along with all the three quantities (0.5 mg/L, 1 mg/L, and 1.5 mg/L) of NAA (Table 1).

After 2nd week of culture 100% emergence was observed at BAP 1 mg/L, 1.5 mg/L and 2 mg/L alone in treatments T1, T5 and T9, and with NAA 0.5 mg/L and 1 mg/L in treatments T2, T3, T10 and T11 (Table 1) followed by 85.7% shoot emergence at BAP 1.5 mg/L along with NAA 0.5 mg/L. The least shoot emergence 28.5% was recorded in treatments T8 with equal concentration of both BAP and NAA *i.e.* 1.5 mg/L and in T16 at BAP 3 mg/L along with NAA 1.5 mg/L (Table 1).

3.2. Shoot Length

After two weeks of inoculation, the highest mean shoot length (2.11 cm) was observed in treatment T11 with BAP 2 mg/L along with NAA 1 mg/L followed by 2.05 cm and 1.97 cm at media fortified with BAP 2 mg/L along with NAA 0.5 mg/L and 0 mg/L respectively (Table 2, Figure 2). It was observed that BAP at 1.5

Table 2. Influence of different concentrations and combinations of auxin and cytokinin on shoot length and number of leaves of *Valeriana jatamansi* Jones after two weeks of culture.

Treatments	Plant growth regulators concentrations (mg/L)		Mean Shoot length (cm)	Mean Number of leaves
	BAP	NAA		
T1	1	0	1.57^{a}	2.00^{abcd}
T2		0.5	1.68^{a}	2.28^{abc}
T3		1	1.74^{a}	1.71^{bcde}
T4		1.5	0.31^{b}	0.85^{def}
T5	1.5	0	1.78^{a}	1.71^{bcde}
T6		0.5	1.84^{a}	1.71^{bcde}
T7		1	1.94^{a}	2.57^{ab}
T8		1.5	0.34^{b}	0.85^{def}
T9	2	0	1.97^{a}	2.00^{abcd}
T10		0.5	2.05^{a}	2.00^{abcd}
T11		1	2.11^{a}	3.14^{a}
T12		1.5	0.38^{b}	1.14^{cdef}
T13	3	0	0.41^{b}	1.14^{cdef}
T14		0.5	0.35^{b}	1.14^{cdef}
T15		1	0.27^{b}	0.57^{ef}
T16		1.5	0.18^{b}	0.28^{f}

Mean values followed by same letters in a column are not significantly different at $P \leq 0.05$.

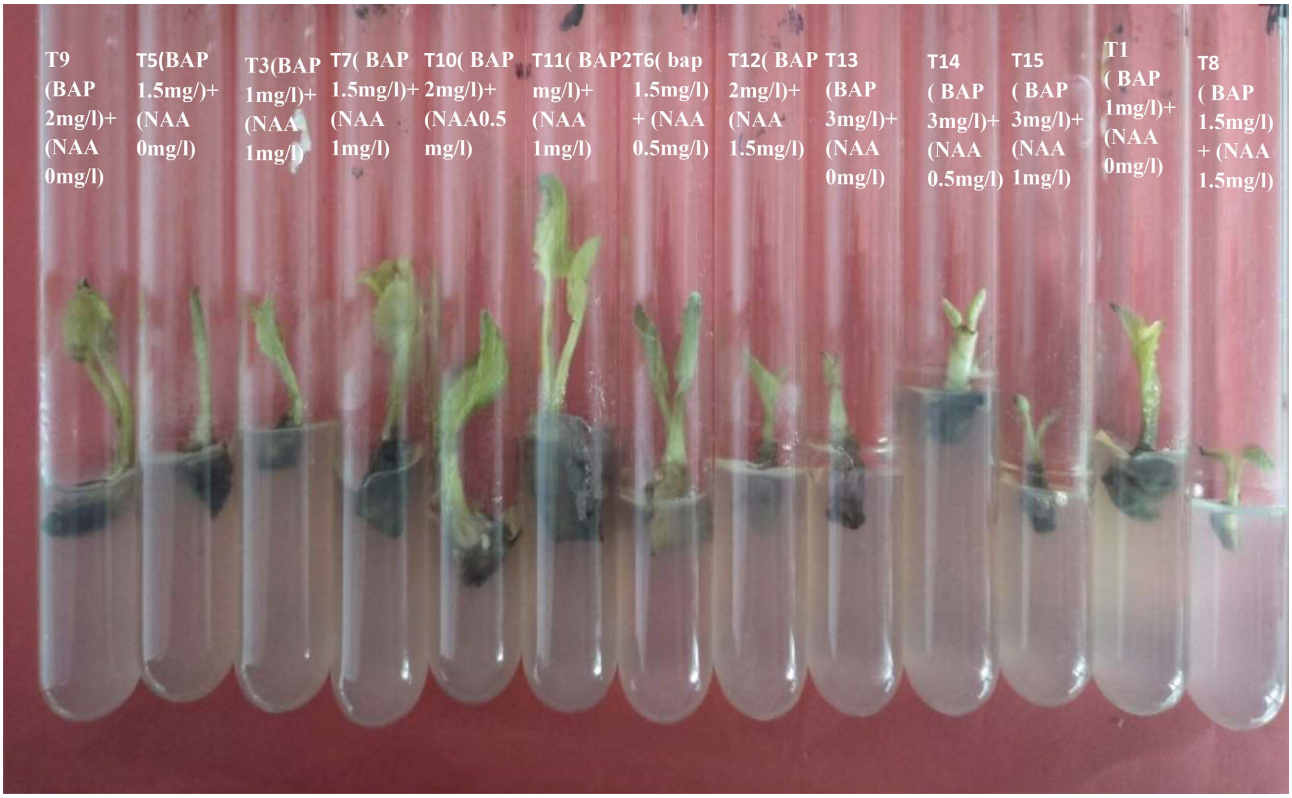

Figure 2. Shoot growth and mean number of leaves of *Valeriana jatamansi* Jones at different combinations and concentrations of BAP and NAA after two weeks of culture.

mg/L and 2 mg/L increases shoot length when used along with NAA at 0.5 mg/L and 1 mg/L (T6, T7, T10, T11) respectively (Table 2). But at 3 mg/L the shoot growth significantly decreased in combination with all the 4 concentrations of NAA. The highest amount of NAA *i.e.* 1.5 mg/L also decreased the shoots length. The lowest shoot length (0.18 cm) was recorded in treatment T16 *i.e.* highest amount of both NAA and BAP (1.5 mg/L; 3 mg/L) respectively (Table 2).

After four weeks of culturing a positive response in shoot growth was observed (Figure 3) and significantly highest shoot length *i.e.* 3.44 cm was recorded when BAP 2 mg/L was used along with NAA 1 mg/L followed by 2.84 cm at BAP 2 mg/L along with NAA 0.5 mg/L, but NAA at higher concentration 1.5 mg/L decreased the shoot growth to (0.51 cm) (Table 3). The data demonstrated that BAP alone at three different concentrations (1 mg/L, 1.5 mg/L and 2 mg/L) has a positive impact on shoot length and shoot growth enhanced but at 3 mg/L it showed negative effect on shoot growth and shoot length decreased to 0.57cm (Table 3). Further, in combination, BAP at all the concentrations with maximum amount of NAA (1.5 mg/L) decreased the shoot length (Table 3).

Statistical analysis of Data recorded after 6th week of culturing revealed the same trend in shoot growth *i.e.* 3.71 cm when BAP at 2 mg/L was added in MS media along with NAA 1 mg/L followed by treatment T10 and T9 (Table 4). But at higher concentration of NAA *i.e.* 1.5 mg/L the shoot length decreased to 0.62 cm. of NAA (0 mg/L, 0.5 mg/L, 1 mg/L and 1.5 mg/L).

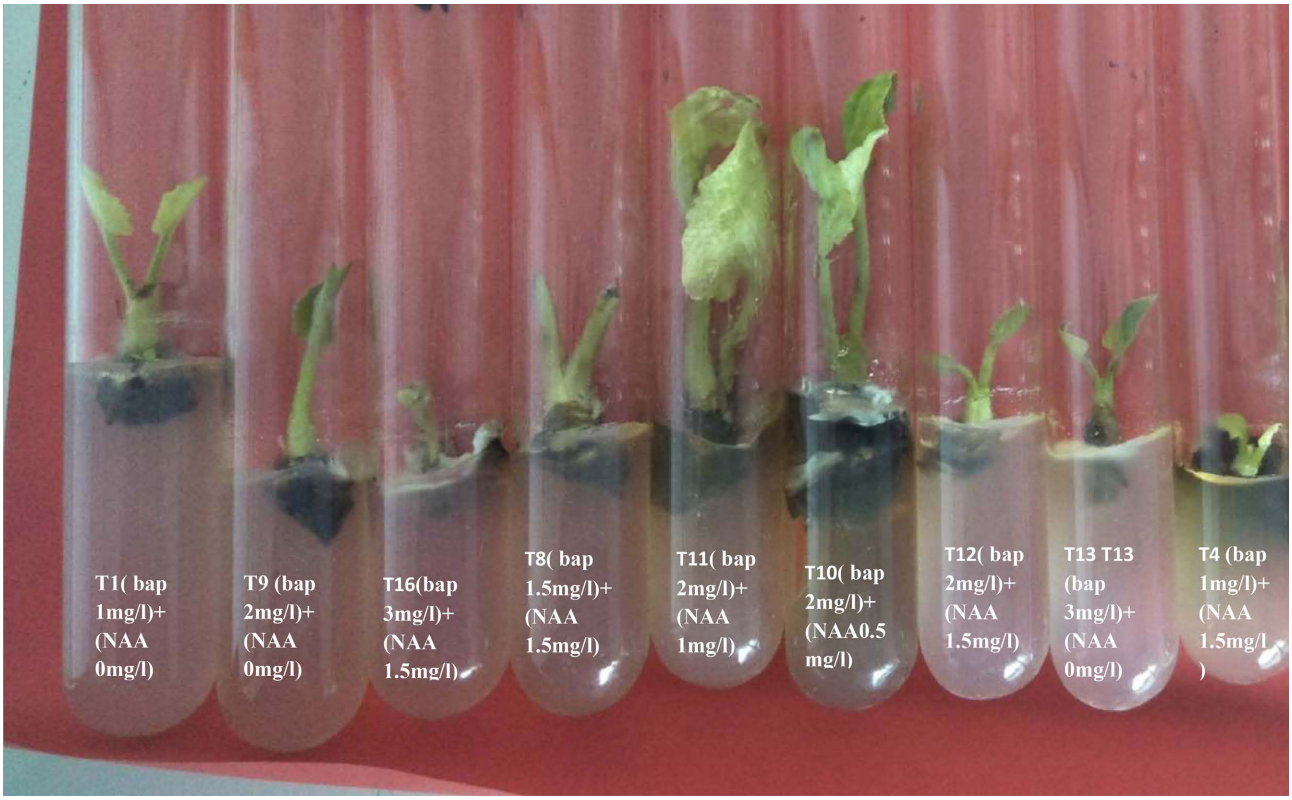

Figure 3. Treatments showing shoot growth of *Valeriana jatamansi* Jones at different concentrations of BAP and NAA after four weeks of culture.

3.3. Number of Leaves

After two weeks of culturing significantly the highest mean leaf number (3.14) was obtained with 6-Benzylaminopurine 2 mg/L in combination with *α*-naphthalene acetic acid 1 mg/L (Table 2) followed by (2.57) when media was supplemented with BAP 1.5 mg/L along with NAA 1 mg/L and (2.28) at BAP 1 mg/L with NAA 0.5 mg/L respectively (Table 2). The least mean leaf number *i.e.* 0.57 and 0.28 was observed in T15 and T16 *i.e.* at 3 mg/L of BAP in combination with and NAA 1 mg/L and 1.5 mg/L, respectively (Table 2).

Statistical analysis of Data recorded after four weeks of culturing revealed that the highest mean leaf number *i.e.* 4.00 was observed in treatment T11 at BAP 2 mg/L along with NAA 1 mg/L followed by 3.42 at BAP 2 mg/L alone (TT9) or together with least amount of NAA (0.5 mg/L) (T10) (Table 3; Figure 3). It was observed that in treatments T4, T8, T12, T16 in which NAA at highest concentration 1.5 mg/L in combination with all the four concentrations of BAP caused a decrease in mean leaf number (Table 3).

After six weeks of culturing excellent response was resulted in leaf number and significantly highest leaf number *i.e.* (6.00) was observed in treatment T9 when BAP was used alone at 2 mg/L (Table 4). Data showed that BAP alone up to concentration 2 mg/L has positive effect on mean leaf number and it increased significantly but in combination with NAA the mean leaf number has been decreased (Table 4).

Table 3. Effects of different concentrations and combinations of auxin and cytokinin on shoot length and number of leaves of *Valeriana jatamansi* Jones after four weeks of culture.

Treatments	Plant growth regulators concentrations (mg/L)		Mean Shoot Length (cm)	Mean Number of Leaves
	BAP	NAA		
T1	1	0	1.97^{c}	3.14^{abc}
T2		0.5	2.17^{bc}	2.57^{abcde}
T3		1	2.21^{bc}	2.57^{abcde}
T4		1.5	0.44^{d}	1.42^{cde}
T5	1.5	0	2.35^{bc}	2.85^{abcd}
T6		0.5	2.47^{bc}	2.57^{abcde}
T7		1	2.58^{bc}	2.57^{abcde}
T8		1.5	0.47^{d}	1.14^{de}
T9	2	0	2.78^{ab}	3.42^{ab}
T10		0.5	2.84^{ab}	3.42^{ab}
T11		1	3.44^{a}	4.00^{a}
T12		1.5	0.51^{d}	1.14^{de}
T13	3	0	0.57^{d}	1.71^{bcde}
T14		0.5	0.48^{d}	1.71^{bcde}
T15		1	0.38^{d}	1.42^{cde}
T16		1.5	0.28^{d}	0.85^{e}

Mean values followed by same letters in a column are not significantly different $P \leq 0.05$.

Table 4. Impact of various concentrations and combinations of auxin and cytokinin on shoot length and number of leaves of *Valeriana jatamansi* Jones after six weeks of culture.

Treatments	Plant growth regulators (PGRS) concentrations (mg/L)		Mean Shoot Length (cm)	Mean Number of Leaves
	BAP	NAA		
T1	1	0	2.72^{a}	5.71^{a}
T2		0.5	2.88^{a}	2.85^{cd}
T3		1	3.04^{a}	2.85^{cd}
T4		1.5	0.54^{b}	2.00^{cd}
T5	1.5	0	2.97^{a}	5.14^{a}
T6		0.5	3.07^{a}	2.57^{cd}
T7		1	3.17^{a}	2.57^{cd}
T8		1.5	0.58^{b}	1.71^{cd}

Continued

T9	2	0	3.57[a]	6.00[a]
T10		0.5	3.67[a]	4.85[ab]
T11		1	3.71[a]	3.14[bc]
T12		1.5	0.62[b]	1.14[d]
T13	3	0	0.72[b]	2.28[cd]
T14		0.5	0.64[b]	2.28[cd]
T15		1	0.52[b]	2.00[cd]
T16		1.5	0.41[b]	1.71[cd]

Mean values followed by same letters in a column are not significantly different $P \leq 0.05$.

3.4. Root Emergence

For *in vitro* multiplication of *Valeriana jatamansi* Jones all the treatment combinations of plant growth regulators (BAP and NAA) showed rooting after 5th week of culturing (Figure 4). Maximum mean root emergence *i.e.* 85.7% was resulted in T4, T7 and T8 with BAP 1 mg/L and 1.5 mg/L together with NAA 1 mg/L and 1.5 mg/L (Table 5). Least root emergence 28.5% was resulted at BAP 3 mg/L alone (Table 5). It was revealed from the data that higher concentrations of NAA had positive effect on rooting percentage (Table 5).

After 6th week of culture rooting % enhanced to 100% at BAP 1.5 mg/L together with NAA 0.5 mg/L, 1 mg/L and 1.5 mg/L respectively (Table 5). BAP at 1 mg/L also resulted in 100% root emergence when used together with NAA 1.5 mg/L (Table 5). It was observed that BAP concentration up to 1.5 mg/L has a positive effect on rooting either alone or with all the four concentrations of NAA, but when BAP amount was increased it created a negative effect and the root emergence percentage decreased (Table 5).

3.5. Root Length

Root growth recorded after 7 weeks of culturing on MS media fortified with BAP and NAA showed that the highest mean root length (0.57 cm) was observed with BAP 1.5 mg/L in combination with naphthalene acetic acid 0.5 mg/L (T6) (Table 6) followed by 0.50 cm when BAP 1 mg/L was used in combination with NAA 0.5 mg/L (T2) (Figure 5). Equal concentration of BAP and NAA *i.e.* 1 mg/L caused root length decreased to 0.40 cm (Table 6). When BAP concentration was increased to 2 mg/L and 3 mg/L, the root length decreased.

Statistical analysis of data recorded after 8 weeks of culture revealed same trend in root growth *i.e.* 0.77cm with BAP 1.5 mg/L together with NAA 0.5 mg/L followed by 0.74cm at BAP 1 mg/L along with NAA 0.5 (Table 6). The highest concentration of both hormones in interaction caused root length to decrease considerably.

Figure 4. Rhizome cuttings of *Valeriana jatamansi* Jones showing rooting.

Table 5. Effects of different treatment combinations of NAA and BAP on root emergence percentage of *Valeriana jatamansi* Jones.

Treatments	Plant growth regulators (PGRS) concentrations (mg/L) in media		% Root Emergence	
	BAP	NAA	After five weeks of culture	After Six weeks of culture
T1	1	0	57.1%	71.4%
T2		0.5	71.4%	85.7%
T3		1	71.4%	85.7%
T4		1.5	85.7%	100%
T5	1.5	0	71.4%	85.7%
T6		0.5	71.4%	100%
T7		1	85.7%	100%
T8		1.5	85.7%	100%
T9	2	0	42.8%	57.1%
T10		0.5	57.1%	71.4%
T11		1	57.1%	71.4%
T12		1.5	71.4%	85.7%
T13	3	0	28.5%	42.8%
T14		0.5	28.5%	57.1%
T15		1	42.8%	57.1%
T16		1.5	57.1%	57.1%

Figure 5. Effect of various treatments on root growth of *Valeriana jatamansi* after 7 weeks of culturing.

3.6. Root Number

After seven weeks of culturing BAP and NAA in combination at equal concentration (1.5 mg/L) significantly increased mean root number (2.42) in T8 followed by (2.0) in T7 when media was fortified with BAP 1.5 mg/L along with NAA 1 mg/L (**Table 6**). After eight weeks of culturing a significant increase in root number (2.57) was observed when BAP and NAA were used at equal concentration *i.e.* 1.5 mg/L (T8) (**Table 7**) followed by (2.28) when media was fortified with BAP 1.5 mg/L together with NAA 1 mg/L. Least root number (0.85) was resulted in treatment T1 and T13 where no NAA was used (**Table 7**).

The rooted plantlets were transplanted in the Greenhouse after 60 days of culturing and were acclimatized for about 5 days (**Figure 6** and **Figure 7**). After about 10 days of transplantation the plantlets showed successful growth and started flowering in the month of May (**Figure 8**).

4. Discussion

Micropropagation has been reported in different species of genus *Valeriana*. For shoot and root induction from the rhizome cuttings of *Valeriana jatamansi*

Table 6. Interaction effect of various treatments of BAP and NAA on Root length and number of roots of *Valeriana jatamansi* Jones after 7 weeks of culturing.

Treatments	Plant growth regulators concentrations (mg/L)		Average Root length (cm)	Mean number of Roots
	BAP	NAA		
T1	1	0	0.42^{abcd}	0.57^{c}
T2		0.5	0.50^{ab}	1.00^{bc}
T3		1	0.40^{abcd}	1.28^{abc}
T4		1.5	0.37^{abcd}	1.57^{abc}
T5	1.5	0	0.48^{abc}	1.00^{bc}
T6		0.5	0.57^{a}	1.85^{ab}
T7		1	0.41^{abcd}	2.00^{ab}
T8		1.5	0.35^{abcd}	2.42^{a}
T9	2	0	0.28^{abcd}	0.57^{c}
T10		0.5	0.25^{abcd}	1.00^{bc}
T11		1	0.24^{abcd}	1.57^{abc}
T12		1.5	0.22^{abcd}	1.71^{abc}
T13	3	0	0.20^{bcd}	0.57^{c}
T14		0.5	0.17^{bcd}	0.85^{bc}
T15		1	0.12^{cd}	0.85^{bc}
T16		1.5	0.10^{d}	1.00^{bc}

Mean values followed by same letters in a column are not significantly different at $P \leq 0.05$.

Table 7. Effect of different concentrations and combinations of auxin and cytokinin on Root length and number of roots of *Valeriana jatamansi* Jones after 8 weeks of culturing.

Treatments	Plant growth regulators concentrations (mg/L)		Average Root length (cm)	Mean number of Roots
	BAP	NAA		
T1	1	0	0.71^{ab}	0.85^{d}
T2		0.5	0.74^{a}	1.14^{cd}
T3		1	0.70^{ab}	1.57^{abcd}
T4		1.5	0.60^{ab}	1.71^{abcd}
T5	1.5	0	0.72^{a}	1.42^{bcd}
T6		0.5	0.77^{a}	2.14^{abc}
T7		1	0.67^{ab}	2.28^{ab}
T8		1.5	0.65^{ab}	2.57^{a}

Continued

T9	2	0	0.62^{ab}	1.00^{d}
T10		0.5	0.61^{ab}	1.28^{bcd}
T11		1	0.50^{ab}	1.71^{abcd}
T12		1.5	0.45^{ab}	1.85^{abcd}
T13	3	0	0.44^{ab}	0.85^{d}
T14		0.5	0.34^{ab}	1.00^{d}
T15		1	0.18^{b}	1.00^{d}
T16		1.5	0.16^{b}	1.28^{bcd}

Mean values followed by same letters in a column are not significantly different at $P \leq 0.05$.

Figure 6. Transplantation of rooted plants of *Valeriana jatamansi* Jones in greenhouse after 60 days of culture.

Figure 7. Transplanted plantlets of *Valeriana jatamansi* Jones showing growth after acclimatization in greenhouse

Figure 8. *Valeriana jatamansi* Jones plant developed through tissue culture started flowering in the greenhouse.

Jones the present research was conducted with different concentrations of BAP and NAA alone or together.

Regarding shoot emergence, the results revealed that lower concentrations of both 6-benzyl amino purine (BAP) and α-naphthalene acetic acid (NAA) significantly have positive effect on shoot emergence (**Table 1**). These findings are in consistent with the results of Zamini *et al.* [10] who reported that the excellent regeneration (82.5%), shoot length (5.96 cm) and shoot number (5.87) of *Valeriana officinalis* was resulted at lower level of BAP 0.5 mg/L along with IBA 0.5 mg/L. Excellent shoot regeneration percentage (60.00%) was also reported by Zebarjadi *et al.* [14] with 0.5 mg/L NAA + 2 mg/L BAP in *Valeriana officinalis*.

BAP is a cytokinin, which accelerates or initiates cell growth. Our results revealed that BAP at lower concentration produced significantly higher shoot length but its highest concentration (3 mg/L) inhibits shoot development. Whenever the amount of cytokinin (BAP) was enhanced or reduced below the optimum level it caused detrimental effect on shoot induction. BAP is a synthetic cytokinin that in combination with auxins elicits plant growth and development responses. Cytokinin along with lower concentration of auxin-induced more shoots [15]

It was observed that rhizome cuttings used to produce shoots showed good results and the growth increased with time at various concentrations and combinations of BAP (1 mg/L, 1.5 mg/L and 2 mg/L) and NAA (0.5 mg/L and 1 mg/L). Similarly, Martin [16] reported that BAP 2.0 mg/L and 0.5 mg/L IBA was best for axillary bud propagation of *Holostemma ada-kodien Schult* and eight shoots per node were induced. Moreover, Gailite *et al.* [17] stated that shoot propagation of *Saussurea esthonica* occurred best at the lower amount of 6-benzyl

amino purine (0.5 to 1 mg/L). Iriondo *et al.* [18] reported that multiple buds induction and leaves development of *Coronopus navasii* (Brassicaceae) were resulted in MS modified medium with BAP l mg/L along with NAA 0.1 mg/L.

Regarding root emergence the findings of the current study revealed that BAP up to 2 mg/L alone and along with all concentrations of NAA (0 mg/L, 0.5 mg/L, 1 mg/L and 1.5 mg/L) significantly showed positive effect on root emergence (**Table 5**). The process of root formation is influenced by different internal and external factors [19]. Our findings are in favor with the observations of Ekhteraei *et al.* [20] who stated that NAA was more effective for the root initiation in *Valeriana Officinalis*. Kristiansen *et al.* [21] reported that BAP (cytokinins) at high concentration cause negative effect on root formation.

In our study lower levels of both BAP (1 mg/L and 1.5 mg/L) and NAA (0 mg/L, 0.5 mg/L) produced significantly higher root length of *Valeriana jatamansi* Jones but the higher concentrations of plant growth hormones BAP (2 mg/L, 3 mg/L) and NAA (1 mg/L, 1.5 mg/L) inhibit root elongation. Increased quantities of both BAP and NAA was found unfavorable for root length but the root number increases at higher concentration of NAA (1 mg/L and 1.5 mg/L) (**Table 7**). Hossain and Urbi [22] studied that NAA stimulate root initiation. It also induces growth of roots as well as formation of adventitious root. As native auxin is transferred down towards the roots, it stimulates roots growth. However, higher concentrations of auxin prevent root elongation and instead increase adventitious root formation. Secondary root formation was inhibited when the root tip was removed. Auxin stimulates wall loosing factors such as elastin to stimulate cell elongation to loosen cell walls. The effect becomes stronger in the presence of gibberellins. Cell division is also stimulated by auxin in the presence of cytokinins. The present study revealed that BAP and NAA not only induced shoots but also induced roots from the rhizome cuttings of *Valeriana jatamansi* Jones. According to the analyzed data lower concentration of BAP and NAA had significant effect on root formation.

Our observations are in favor with the findings of Seyyedyousefi *et al.* [23] who reported that NAA was found suitable for root induction and the lowest root length (1.30 cm) of *Alstroemeria* was obtained at BAP 2.50 mg/L along with NAA 0.5 mg/L. Kaur *et al.* [4] reported that NAA at various concentrations affect rooting. NAA at 0.75 mg/L and 3% sucrose was found most suitable for root induction of *Valeriana jatamansi*. They also observed that root length (28.3 ± 0.1) and highest root number (13.5 ± 0.6) was resulted at NAA 0.75 mg/L alone. Ozcan *et al.* [24] observed that at low auxin levels long roots were developed and short roots at higher concentration of auxin.

Nikolelis *et al.* [25] stated that NAA is rooting agent and used for vegetative propagation of various plants. In the present report we observed that higher concentration of NAA (1 and 1.5 mg/L) along with BAP @ 1 mg/L and 1.5 mg/L increases root number (**Table 7**). Yan *et al.* [26] demonstrated that adventitious rooting in *Hemarthria compressa* was significantly increased by the lower con-

centration of NAA. Moreover, Hossain and Urbi [22] stated that NAA at higher concentration promotes number of adventitious rooting per cutting.

5. Conclusion

It was concluded from the present study that BAP up to 2 mg/L in combination with NAA 1 mg/L produced significantly higher shoot length. The highest leaf number was obtained at BAP 2 mg/L alone. Lower levels of both BAP (1 mg/L and 1.5 mg/L) and NAA (0 mg/L, 0.5 mg/L) produced significantly higher root length of *Valeriana jatamansi* Jones. When the concentration of NAA increased up to 1.5 mg/L root number was increased.

6. Recommendations

As *Valeriana jatamansi* Jones is a high valued medicinal herb and due to its extreme industrial importance for secondary metabolites, the *in vitro* propagation techniques can be used for more research work on metabolic pathways and transgenic approaches for the production of quality material of *Valeriana jatamansi* Jones. It is further recommended to use other parts of the plant like leaf and apical portion for regeneration and multiplication to conserve the species instead of destroying the whole plant. Further research can also be recommended for using other Plant growth regulators instead of or along with (BAP) and (NAA) at different concentrations for successful tissue culture of *Valeriana jatamansi* Jones.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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