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Establishment of *In Vitro* Cultures and Genetic Fidelity Analysis in *Valeriana jatamansi*-a High Value Endangered Medicinal Herb

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ABSTRACT

The present study was conducted in the Department of Biotechnology, College of Horticulture and Forestry, Neri, Hamirpur, Himachal Pradesh, India during 2018–2020 for 18 months to develop an efficient, rapid and reproducible protocol for *in vitro* establishment along with analysing antioxidant activity. In the present study, surface sterilization protocol was standardized using Sodium hypochlorite and mercuric chloride as sterilant. Maximum direct shoot induction frequency from nodal explant was achieved on Murashige and Skoog medium enriched with BAP (5.0 mg l^{-1}), TDZ (3.0 mg l^{-1}) and NAA (1.0 mg l^{-1}) for 18 days. Microshoots inoculated on MS media supplemented with 4.0 mg l^{-1} BAP, 2.0 mg l^{-1} TDZ, 1.0 mg l^{-1} NAA and 0.5 mg l^{-1} GA_3) showed maximum frequency of shoot multiplication with average shoot length (4.36±0.08 cm) and average shoot number (14.00±0.57). A 75.00±1.15% rooting with significantly high mean root number (8.00±0.57) and root length was achieved in full strength MS medium, supplemented with same concentration *i.e.* 4.0 mg l^{-1} BAP, 2.0 mg l^{-1} TDZ, 1.0 mg l^{-1} NAA and 0.5 mg l^{-1} GA_3) combination. Maximum plantlets survive after 1 year of acclimatization. RAPD and ISSR markers were used to confirmed genetic stability of *in vitro* raised plants by showing 100% monomorphism. High multiplication rate associated with genetic stability ensure the efficacy of the present *in vitro* clonal propagation protocol of this important medicinal plant.

KEYWORDS: Micropropagation, molecular marker, phytohormones, shoot induction

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1. INTRODUCTION

Valeriana jatamansi is an important medicinal plant belongs to the family Caprifoliaceae (Jugran et al., 2020). *Valeriana jatamansi* grows abundantly from Kashmir to Bhutan at an altitude of 1200 to 4000 m, its rhizome is thick and horizontal, whereas descending roots are fibrous (Houghton, 1999; Cornara et al., 2020). In Himachal Pradesh it grows profusely in Bharmour division of Chamba, Kanda area of Karsog and Chansil of Rohru forest division (Purohit and vasu, 2005). *V. jatamansi* is locally threatened endangered species of Himachal Pradesh.

It is used for various medicinal purposes in the Indian, British, and Chinese Pharmacopoeia. It has been reported as a psychopharmacological agent and a natural source of valepotriates. It possesses antibacterial, anticancer, anticoagulant, antifungal, anti-inflammatory, antioxidative, antiprotozoal, hepatoprotective, and neuroprotective activities. Therefore, the demand for this rare plant is increasing day by day in pharmaceutical as well as perfumery industries that leads to dwindling of herb's availability in the forest (Nandhini et al., 2018. However, increasing human pressures and herbal market, coupled with climatechange, have decimated the population and distribution of medicinal plants for the last couple of decades (Schickhoff et al., 2015; Lenoir and Svenning, 2015). Therefore, increasing demand and 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. With increasing threats due to unsustainable harvesting from the natural habitat as well as climate change, it is necessary to understand the distribution, production, use and trade of medicinal plants used in a scientific way, which will help management, and sustainable production in the future (Charmakar et al., 2021). Conventionally, *V. jatamansi* is propagated through seeds, however, in nature seeds germinate slowly and remain dormant for a long time. Alternatively, root sucker can be used but low population size hampers the process. Therefore, *in vitro* methods for large scale multiplication would be a viable option, and has been reported for several other Himalayan medicinal herbs.

Plant tissue culture techniques are economically a powerful and most promising tool for plant propagation of this important medicinal plant. (Kumar et al., 2001). In a plant tissue culture any cells, tissues or organs can be used for on specially formulated on nutrient rich media. Under aseptic and right conditions, an entire plant can be regenerated from a single cell. This technique is able to produce a disease-free, high quality planting material and rapid production of genetically uniform plants throughout the year. 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 micropropagation (Chen et al., 2014; Purohit et al., 2015). Micro-propagation protocol is severely hindered due to the incidence of somaclonal variations (Kumar et al., 2011; Pandey et al., 2020). All the variations which are widely documented with increased passages of subcultures, genotype, growth regulator concentration etc. So, it is necessary to monitor somaclonal variability within the micro propagated plants, it is necessary to monitor and assess the genetic constitution and stability of *in vitro* raised plants (Lattoo et al., 2006), which in turns help us to analyze the variation present between mother plant and *in vitro* raised plant. Thus, for the analysis of genetic fidelity of *in vitro* raised plants molecular techniques are valuable tool (Bhattacharya et al., 2017).

2. MATERIALS AND METHODS

The present investigation was carried out in the Department of Biotechnology, College of Horticulture and Forestry, Neri, Hamirpur at 31°.6 N longitude and 76°.5 E Latitude) Himachal Pradesh during 2018–20 for 18 months.

2.1. *In vitro* regeneration studies of *valeriana jatamansi*

Different explants viz. leaves, nodal segments, rhizomes and shoot buds were excised from field grown plants of *Valeriana jatamansi* and used for culturing. In case of *Valeriana jatamansi* explants were surface sterilized with various chemical sterilant such as: Sodium hypochlorite (3–12%) and mercuric chloride (0.5–1.5%). The explants *viz.* leaf, nodal segment, rhizome and shoot buds were washed with tap water followed with tween-20 (detergent) in a beaker under running tap water covered with muslin cloth for about 20 minutes. Then, under laminar air flow these explants were treated with 3.0% (w/v) carbendazim (fungicide) followed by rinsing twice with autoclaved distilled water (ADW). Then explants were transfers into separate flask giving treatment of various concentration and combination of sterilant (sodium hypochlorite or mercuric chloride) for different durations. Finally, they were rinsed thrice with ADW prior to inoculation.

2.2. *Cultural conditions and preparation during inoculation*

Maintenance of constant environmental conditions throughout incubation is important. Before inoculation process, all the required materials were properly sterilized and kept in laminar air flow. Throughout experiment, growth chamber was kept at aseptic conditions and cultures (explants of *Valeriana jatamansi*) were maintained at 25±2°C with 60–70% relative humidity in a growth chamber with 16 h photoperiod using white fluorescent light (1500–3000

Lux). Aseptic condition controlled by proper sterilization and immediately discarding the contaminated flasks from growth chamber.

2.3. Direct or indirect shoot regeneration

The sterilized explants viz. leaf, nodal segment, rhizome and shoot buds were aseptically inoculated on sterilized MS medium supplemented with different concentrations and combinations of phytohormones such as NAA, BAP, TDZ and kinetin (Indirect shoot induction) and (Direct shoot induction). Continuous sub culturing was done at 15–20 days interval for avoiding browning of the media.

2.4. Rooting of in vitro raised shoots

For root induction, regenerated shoots from 4–5 weeks old cultures were transferred to MS medium containing different concentration of auxins (IAA, IBA and NAA) either alone or in combination with Cytokinins (BAP, TDZ and Kinetin) and gibberellic acid (GA_3) for root induction. Healthy shoots with well-developed roots were then hardened.

2.5. Hardening of in vitro raised plantlets

In vitro raised plantlets with well-developed shoots and roots were taken out carefully from the culture medium to avoid any damage to root system, washed thoroughly in running tap water to remove excess agar and traces of sugar, and rinsed with distilled water. The plantlets thus obtained were subsequently transplanted into earthen pots containing a mixture of soil: sand: FYM (1:1:1), soil: sand: vermicompost (1:1:1), soil: sand: peat (1:1:1) and covered with transparent polythene bags for proper aeration and maintained under controlled conditions (23±2°C and 70% humidity).

2.7. Assessment of genetic stability of in vitro raised plants using RAPD and ISSR markers

Molecular marker are powerful tools to estimate genetic variability among mother plant and *in vitro* plant, since they are low price, abundant and unaffected by the environment. Using PCR technology, several markers have been made available for the evaluation of genetic stability. In the present study, the RAPD and ISSR marker system were used to assess the genetic stability of *Valeriana jatamansi* developed through tissue culture. The DNA based marker system offers a more reliable and neutral alternative to the environment to detect genetic polymorphisms useful for genotype identification.

2.8. Statistical analysis

The data were analyzed using one-way and two-way analysis of variance (ANOVA). The statistical analysis was carried out by using MS-Excel and OPSTAT (Cochran and Cox, 1963 and Gomez and Gomez, 1984).

3. RESULTS AND DISCUSSION

3.1. Surface sterilization

Surface sterilization is the first and necessary step in starting and establishment of aseptic cultures. The excised explants *viz.* leaf and nodal segment of *V. jatamansi* plants were subjected to an initial treatment with running tap water followed with tween-20 (detergent) to remove debris, then with distilled water twicely. The explants were treated with different concentration and combination of sterilant (sodium hypochlorite, mercuric chloride and sodium hypochlorite plus mercuric chloride) with varying exposure of time.

Among various sterilization treatments, treatment T_7 mercuric chloride alone was found to be the best with survival rate. But in combination of the both sterilant (mercuric chloride and sodium hypochlorite) survival rate of explants (leaf and nodal segment) was more (Table 1).

3.2. Shoot bud induction from callus

Callus derived from leaf explant of *V. jatamansi* were transferred to MS medium supplemented with various concentration and combinations of auxins (NAA, IAA) and cytokinin (BAP, TDZ, Kn) for shoot induction. Among all tested PGRs, BAP and TDZ were best for shoot induction than combination with other plant growth regulators *viz.* Kn, IAA and NAA. Shoots were initiated in 17 days on MS10 medium supplemented with 4.0 mg l^{-1} BAP±1.0 mg l^{-1} TDZ±0.5 mg l^{-1} NAA. Shoot induction took longer time (37 days) on MS3 medium with 3.0 mg l^{-1} BAP±1.0 mg l^{-1} IAA. It might be due to synergistic effect of two cytokinin's (BAP and TDZ) that lead to shoot induction from callus (Chen et al., 2014).

All these studies revealed that high concentration of BAP and low concentration of auxin was responsible for shoot induction from callus irrespective of explant. High cytokinin to auxin ratio promotes shoot regeneration whereas, high auxin to cytokinin ratio promotes root induction which indicate that auxin and cytokinin have cross link during organogenesis (Skoog and Miller, 1957). High cytokinin/ auxin ratio promote shoot regeneration since cytokinin activates the expression of WUS (WUSCHEL) gene which induced shoot regeneration from callus and auxin upregulate the expression of cytokinin receptor gene AKH4 which is required for WUS activation. Thus, auxin and cytokinin both play significant role in initiating shoot induction from callus (Gordon et al., 2007) (Table 2). Maximum percent of shoot multiplication (80.66±0.57%) was achieved on MS7 medium supplemented with 4.0 mg l^{-1} BAP+2.0 mg l^{-1} TDZ±1.0 mg l^{-1} NAA±0.5 mg l^{-1} GA_3 while minimum (53.33±0.88) on MS2 medium fortified with 4.0 mg l^{-1} BAP±1.0 mg l^{-1} NAA (Table 3).

Table 1: Effect of different sterilant varying exposure of time on percentage survival of leaf and nodal segment explants of *V. jatamansi* after 15 days of inoculation

Treatments	Sterilant	Percentage of un-contaminated cultures		Percentage of contaminated cultures	
		Leaves	Nodal segment	Leaves	Nodal segment
T_0	Control (No sterilant)	0	0	100	100
T_1	Sodium hypochlorite (3%) 15 min	29.66±0.88 (32.98±0.55)	23.66±0.33 (29.09±0.22)	70.33±0.88 (56.98±0.55)	76.33±0.88 (60.86±0.22)
T_2	Sodium hypochlorite (6%) 15 min	36.66±0.88 (37.24±0.52)	27.00±0.57 (31.29±0.37)	63.33±0.88 (52.71±0.52)	73.00±0.57 (58.67±0.37)
T_3	Sodium hypochlorite (9%) 15 min	45.00±0.57 (42.11±0.33)	35.33±0.33 (36.45±0.19)	55.00±0.57 (47.85±0.33)	64.66±0.33 (53.50±0.19)
T_4	Sodium hypochlorite (12%) 15 min	58.33±0.33 (49.77±0.19)	41.00±1.52 (39.79±0.89)	41.66±0.33 (40.18± 0.19)	59.00±1.52 (50.17±0.89)
T_5	$HgCl_2$ (0.5%) 1 min	60.66±0.88 (51.14±0.51)	44.66±0.33 (41.92±0.19)	39.33±0.88 (38.82±0.51)	55.33±0.33 (48.04±0.19)
T_6	$HgCl_2$ (1.0%) 1 min	67.33±0.33 (55.14±0.20)	56.00±0.57 (48.42±0.33)	32.66±0.33 (34.84± 0.20)	44.00±0.57 (41.53± 0.33)
T_7	$HgCl_2$ (1.5%) 1 min	73.66±0.88 (59.10±0.57)	67.33±0.88 (55.12±0.54)	26.33±0.88 (30.85±0.57)	32.66±0.88 (34.84±0.54)
T_8	Sodium hypochlorite (9%) 15 min.+$HgCl_2$ (1.0%) 1 min	76.66±0.88 (61.09±0.59)	73.66±0.88 (59.10±0.57)	23.33±0.88 (28.86±0.59)	26.33±0.88 (30.85±0.57)
T_9	Sodium hypochlorite (12%) 15 min $HgCl_2$ (1.0%) 1 min	80.33±0.33 (63.65±0.24)	77.00±0.57 (61.32±0.39)	19.66±0.33 (26.31±0.24)	23.00±0.57 (28.64±0.39)
SEm±		-	-	-	-
CD (p=0.05)		2.13 (1.33)	2.281 (1.39)	2.13 (1.33)	2.281 (1.39)

Figures in parenthesis are angular transformed values; Values are Mean±Standard error

3.3. Rooting in in vitro raised shoots

After shoot multiplication, micro shoots were inoculated on MS medium fortified with different concentration and combinations of plant growth regulators *viz.* IBA, IAA, NAA, TDZ, BAP to initiate root induction. Maximum root induction (77.33±0.88%) was observed on MS10 medium supplemented with 4.0 mg l^{-1} BAP±2.0 mg l^{-1} TDZ±1.0 mg l^{-1} NAA±0.5 mg l^{-1} GA_3 within 17 days while, minimum root induction (56.33±0.33%) was observed on MS5 media with BAP (4.0 mg l^{-1}) ±NAA (1.0 mg l^{-1}) in 23 days. Maximum number of roots (8.00±0.57) with maximum root length (9.30±0.05 cm) was recorded in MS10 media fortified with 4.0 mg l^{-1} BAP±2.0 mg l^{-1} TDZ±1.0 mg l^{-1} NAA±0.5 mg l^{-1} GA3, while MS5 media with BAP (4.0 mg l^{-1}) ±NAA (1.0 mg l^{-1}) showed minimum number of roots (3.33±0.33) with 4.30±0.05 cm root length. Auxin along with cytokinin substantially improved root induction response (Skoog and Miller, 1957). Similarly, Purohit et al., 2015) also found that MS media enriched with 1.5 µM BAP+0.5 µM NAA±0.1 µM GA_3 gave 100% root response in *V. jatamansi* with 27.5±1.98 average root number with 50.00±1.35 cm root length (Table 4).

3.4. Hardening of in vitro raised plantlets

For the growth and survival of *in vitro* raised plantlets a similar environment that exists in nature is likely to be most suitable after the transfer (Zeng et al., 2011). For hardening and establishment of plantlets under the natural conditions, the well rooted plantlets were taken out from culture flask to avoid any damage to root system and gently washed under running water to remove traces of agar and rinse with distilled water followed with Bavistin treatment to avoid fungal contamination. The plantlet thus obtained were transferred to the earthen pots containing different sterilized substrates Sand: Soil: Vermicompost (1:1:1), Sand: Soil: FYM (1:1:1) and Sand: Peat: Soil (1:1:1). Covered earthen pots with polythene bag and holes were made for maintaining proper humidity and aeration and kept in green house (23±2°C and 80% humidity). Micro-plants were hardened in earthen pots filled with Sand: Soil: Vermicompost (1:1:1) showed highest survival rate 76% and

Table 2: Effect of different concentrations and combinations of cytokinin's (BAP, TDZ and Kn) and auxin (NAA) on shoot induction from callus of leaf explants of *Valeriana jatamansi* (Mean±SE)

Treatment	Plant growth regulators (mg l^{-1})	Percent shoot induction	Number of days to initiate shoot	Number of shoots
MS_1	Control	0.00	0.00	0.00
MS_2	BAP+NAA (3.0+1.0)	44.00±3.05 (41.52±1.76)	33.66±0.88 (5.88±0.07)	4 .00±0.57 (2.22±0.13)
MS_3	BAP+IAA(3.0+1.0)	34.66±1.76 (36.04±1.05)	37.66±0.88 (6.21±0.07)	3 .00±0.57 (1.98±0.14)
MS_4	BAP+NAA (6.0+1.0)	55.66±1.20 (48.23±0.69)	25.66±2.18 (5.15±0.20)	6.00±0.57 (2.64±0.10)
MS_5	BAP+NAA (9.0+1.0)	64.00±2.08 (53.12±1.23)	22.66±0.33 (4.86±0.03)	9.00±1.52 (3.14±0.23)
MS_6	TDZ+NAA (3.0+1.0)	66.33±066 (54.51±0.40)	23.33±0.33 (4.93±0.03)	7.00±0.57 (2.82±0.10)
MS_7	TDZ+IAA (3.0+1.0)	58.33±2.33 (49.78±1.35)	26.00±0.57 (5.19±0.05)	6.33±0.33 (2.70±0.06)
MS_8	TDZ+NAA (5.0+1.0)	72.00±0.57 (58.03±0.36)	20.66±0.88 (4.65±0.09)	12.33±1.20 (3.64±0.16)
MS_9	BAP+TDZ+NAA (2.0+1.0+0.2)	69.66±0.88 (56.56±0.54)	19.33±0.88 (4.50±0.09)	11.66±0.33 (3.55±0.04)
MS_{10}	BAP+TDZ+NAA (4.0+1.0+0.5)	74.33±0.66 (59.54±0.43)	17.33±0.88 (4.27±0.10)	15±0.57 (3.99±0.07)
SEm±		-	-	-
CD (p=0.05)		5.056 (2.970)	3.031 (0.297)	2.376 (0.393)

Figures in parenthesis are angular transformed values; Values are Mean±Standard error

Table 3: Effect of different concentrations and combinations of cytokinin's (BAP and TDZ), auxin (NAA) and gibberellic acid (GA_3) on shoot multiplication of *in vitro* raised micro shoots of *Valeriana jatamansi* from leaf and nodal segment explants (Mean±SE)

Treatment	Plant growth regulators (mg l^{-1})	Percent no. of shoots	Average length cm	Average no. of shoots
MS_1	Control	0.00	0.00	0.00
MS_2	BAP+NAA (4+1)	53.33±0.88 (46.89±0.50)	2.30± 0.05 (1.81±0.01)	5.00±0.57 (2.44±0.11)
MS_3	BAP+NAA (6+1)	60.33±1.85 (50.95±1.09)	2.56± 0.17 (1.88±0.04)	7.00± 0.57 (2.82±0.10)
MS_4	TDZ+NAA (3+1)	67.00±1.15 (54.92±0.70)	3.00±0.05 (2.00±0.01)	8.00±0.57 (2.99±0.09)
MS_5	TDZ+NAA (5.0+1.0)	72.33±0.88 (58.24±0.56)	3.56±0.12 (2.13±0.02)	9.33±0.88 (3.20±0.13)
MS_6	BAP+TDZ+NAA+GA_3 (3.0+1.0+0.5+0.2)	78.00±0.57 (62.00±0.39)	4.20±0.15 (2.28±0.03)	11.66±0.88 (3.55±0.12)
MS_7	BAP+TDZ+NAA+GA_3 (4.0+2.0+1.0+0.5)	80.66±0.57 (63.41±0.41)	4.36±0.08 (2.31±0.01)	14.00±0.57 (3.87±0.07)
SEm±		-	-	-
CD (p=0.05)		3.36 (2.04)	0.36 (0.08)	2.16 (0.34)

lowest survival rate 21% was observed in Sand: FYM: Soil (1:1:1). However, 90% survival rate was reported on Sand: Vermicompost: Perlite (1:1:1) in *V. jatamansi* (Purohit et al., 2015).

3.5. Assessment of genetic stability of in vitro raised plantlets

3.5.1. Analysis of genetic stability by RAPD

RAPD markers used for studying genetic variation in tissue culture raised plants. In the present study 10 RAPD primers used to assess the genetic similarity between *in vitro* and mother plants of *V. jatamansi.* Out of 10 RAPD primer, 2 primers OPA 11 and OPA 13 showed monomorphic banding pattern (Figure 6). Similar to our studies there are reports in *Valeriana offcinallis* (Ghaderi and Jafari, 2014), *V. jatamansi* (Purohit et al., 2015) and *Amomum subulatum* plant (Purohit et al., 2017) where RAPD markers have been implied for confirming clonal fidelity of micro propagated plants (Figure 1).

3.5.2. Analysis of genetic stability by ISSR

ISSR markers used for studying genetic variation in tissue culture raised plants. In the present investigation 10 ISSR

Table 4: Effect of different concentrations and combinations of cytokinin's (BAP and TDZ), auxin NAA, IBA and IAA) and gibberellic acid (GA_3) on root induction of *in vitro* raised shoots of *Valeriana jatamansi* (Mean±SE)

Treatment	Plant growth regulators (mg l^{-1})	Percent root regeneration	Days taken for rooting	Average root length (cm)	Number of roots
MS_1	Control	0.00	0.00	0.00	0.00
MS_2	NAA	0.00	0.00	0.00	0.00
MS_3	IAA	0.00	0.00	0.00	0.00
MS_4	IBA	0.00	0.00	0.00	0.00
MS_5	BAP+NAA (4+1)	56.33±0.33 (48.61±0.19)	25.00±0.57 (5.09 ±0.05)	4.30±0.05 (2.30±0.01)	3.33±0.33 (2.07±0.07)
MS_6	BAP+NAA (6+1)	58.66±2.02 (49.97±1.17)	22.00±0.88 (4.82±0.09)	4.93±0.88 (2.43± 0.01)	4.00±0.57 (2.22±0.13)
MS_7	TDZ+NAA (3+1)	65.66±1.20 (54.11±0.72)	23.00±1.15 (4.89 ±0.11)	5.46±0.08 (2.54± 0.01)	5.00±0.57 (2.44±0.11)
MS_8	TDZ+ NAA (5.0+1.0)	68.00±1.20 (55.94±0.74)	22.33±0.882 (4.78±0.21)	6.00±0.15 (2.64±0.02)	7.00±1.73 (2.79±0.31)
MS_9	BAP+TDZ+NAA+GA_3 (3.0+1.0+0.5+ 0.2)	73.66±0.88 (59.10±0.57)	17.66±1.20 (4.31±0.13)	8.63± 0.08 (3.10±0.01)	7.66±0.33 (2.94±0.05)
MS_{10}	BAP+TDZ+NAA+GA_3 (4.0+2.0+1.0+0.5)	77.33±0.88 (61.55±0.60)	16.33±1.45 (4.15±0.17)	9.30±0.05 (3.20±0.00)	8.00±0.57 (2.99±0.09)
SEm±		-	-	-	-
CD (p=0.05)		3.74 (2.27)	4.08 (0.44)	0.29 (0.05)	2.61 (0.48)

Figures in parenthesis are angular transformed values; Values are Mean±Standard error

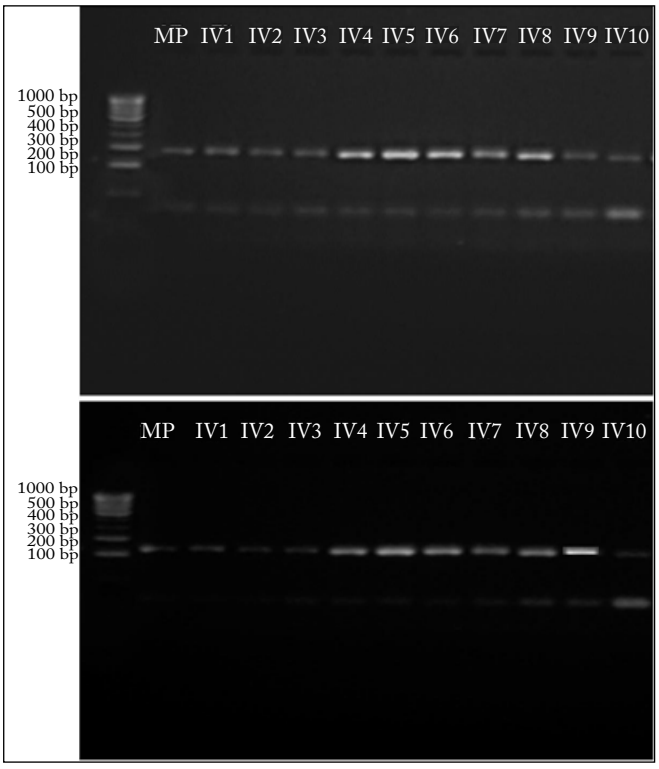

Figure 1: RAPD pattern of mother plants and *in vitro* raised plants of *Valerina jatamansi* by OPA 11 and OPA 13

primers used to study the genetic similarity between *in vitro* and mother plants of *V. jatamansi.* Out of 10 ISSR primer, 3 primers HB 8, HB10 and HB13 showed monomorphic banding pattern. Many investigators have also reported genetic stability of several micro propagated crops *viz. Morus* spp. (Rohela et al., 2018), *Clerodendrum thomsoniae* (Kar et al., 2019) and *Helicteres isora* (Kumar et al., 2020). Therefore, it is concluded that micro propagated plants were genetically identical to that of the mother plant and no variation was induced during *in vitro* regeneration. RAPD and ISSR primer showed 100% similarity between mother plant and *in vitro* raised plant.

4. CONCLUSION

The current work is focused on *in vitro* regeneration investigations of *Valeriana jatamansi*. Various explants were utilised for culture, as well as various sterilisation procedures. Shoot regeneration was promoted both directly and indirectly, and rooting was accomplished utilising several plant growth regulators. Plantlets produced in vitro were successfully toughened and transplanted to soil. Using RAPD and ISSR markers, genetic stability analysis verified that the micropropagated plants were genetically similar to the mother plant, with no induced changes.

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