DEVELOPMENT OF TISSUE CULTURE CONDITIONS AND MOLECULAR CHARACTERIZATION OF *VALERIANA JATAMANSI*

Project report submitted in partial fulfillment of the requirement for the degree of Bachelor of Technology In Biotechnology

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DECLARATION

I hereby declare that the work presented in this report entitled "Development of Tissue Culture conditions and molecular characterization of *Valeriana jatamansi.*" in partial fulfilment of the requirements for the award of the degree of Bachelor of Technology in Biotechnology submitted in the Department of Biotechnology/Bioinformatics Jaypee University of Information Technology Wakanaghat, Solan-173234, Himachal Pradesh is an authentic record of my own work carried out over a period from August 2015 to June 2016 under the supervision of Dr Hemant Sood Assistant Professor(Senior Grade), Department of Biotechnology/Bioinformatics.

The matter embodied in the report has not been submitted for the award of any other degree or diploma.

Medhavi Behl

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CERTIFICATE

This is to certify that the work entitlied “Development of Tissue Culture conditions and molecular characterization of *Valeriana jatamansi.*” pursued by Vasudha Verma 121566 and Medhavi Behl 121554 in partial fulfillment for the award of degree of Bachelor of Technology in Biotechnology from Jaypee University Of Information Technology, Waknaghat has been carried out under my supervision. This work has not been submitted partially or wholly to any other university or institute for the award of any degree or appreciation.

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Place: - Waknaghat

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SUMMARY

*V. jatamansi*is a perennial herb from the Caprifoliaceae family, commonly known as 'Tagar' or 'Indian valerian', The species is found growing on moist slopes in shrubberies and open slopes between 1500 and 4000 m. It is known for its medicinal compounds Valerenic acid, Valepotriates and essential oils.It is a well known medicinal plant used for insomnia, hysteria, excitability, mild sedative action for nervous tension, stress and anxiety, encourages ulcer and wound healing, high blood pressure and intestinal colic.

In order to see the growing demand in market different accessions, genotypes containing different concentrations of valepotriates are of high significance. So in present study we optimized concentrations of different growth hormones with different combinations to see the effect on metabolites biosynthesis and accumulation.

We also characterized the variants by testing 25 primers for valepotriates synthesis. We found one accession (Vj-7) which gave good content of valepotriates quite at par with field grown. So these could provide an alternate resource to the herbal and pharmaceutical industry.

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Valeriana jatamansi

CHAPTER - 1

1.1. Introduction

*V. jatamansi*is a perennial herb from the Caprifoliaceae family, commonly known as 'Tagar' or 'Indian valerian', The species is found growing on moist slopes in the Himalayas and Khasi hills in shrubberies and open slopes between 1500 and 4000 m elevation from Pakistan to Southwest China, Burma, and South-East Asia (Sunil Kumar Singh,*et.al*2015). The plant also grows well in different agroclimatic regions of India. The species is characterized as rhizomatous, occasionally stoloniferous, perennial herb with erect, unbranched flowering stem revealing considerable morphological variability. It is dioecious, polygamous or occasionally polygamomonoecious species (Prakash, 1999). The word 'Valeriana' is the first met with in writings of the ninth and tenth century. The plant has been valued for centuries in Ayurvedic in Indian, Unani in ancient Greek and Arab, and in ancient Egypt and Rome for its medicinal values.

It is a well known medicinal plant used for insomnia, hysteria, excitability, mild sedative action for nervous tension (Houghton, 1999), stress and anxiety, encourages ulcer and wound healing, high blood pressure and intestinal colic(Mathela*et al.*, 2005; Singh *et al.*, 2010). The active principle of this plant besides having antibacterial and antiprozal activity can be taken as a remedy for snake bite as well as scorpion sting. The active ingredient – valerian, derived from its rhizomes is used for the treatment of various diseases (Prakash, 1999). The roots and rhizomes of this species are also used for the preparation of phytomedicines with mild sedative action (Houghton,1999).

Even though *V. jatamansi* has high medicinal value but is threatened medicinal plant of Himalayan region. In study (Dhiman Mukherjee et.al 2014), ecology, phenology and seed germination of this species domesticated at different altitudes, viz., 1290 m, 1550 m, 1800 m and 2000 m in district Darjeeling, West Bengal, India, were observed. The studies have revealed that *V. jatamansi* was a glabrous and more or less pubescent herb, nearly 15 – 45 cm tall herb. Optimum survival of V. jatamansi across 1290 to 2000 m indicated their adaptability at a wider range of altitudinal zones. However, low seed germination percentage of jatamansi above 1800 m restricted their cultivation possibilities up to low altitudinal zones only. Further, flowering and fruiting periods in

most of the species varied greatly amongst the domestication sites. It is known for its medicinal compounds Valerenic acid, Valepotriates and essential oils. The roots and rhizomes of this species are also used for the preparation of phytomedicines with mild sedative action (Houghton,1999).

Himalayan region is well known for high diversity of native, endemic, rare and endangered medicinal plants which are valued worldwide due to their unique active compounds of therapeutic use. The species is being labeled as critically endangered due to over-exploitation of rhizomes for its medicinal value habi-tat degradation and other biotic interferences in its distribution ranges (Polunin and Stainton, 1987). Like many other non-timber forest products (NTFPs), this critically endangered plant is taken as forest gift and hence there is neither any control system in its harvest nor its domestication. Locally it is being used for medicinal purpose especially for headache and eye trouble (Mukherjee, 2009). The species witnessed a tremendous decline in its population size. It tells the tale of biotic interferences, which have brought it to the brink of extinction. If left as such and exploited at the same rate, in near future, the species will disappear forever. Thus, convention on international trade on endangered species notified V. jatamansi in its schedule for conservation and additionally, it has been listed an endangered species on the list of National Medicinal Plant Board, New Delhi, India (www.nmpb.nic.in). Despite the fact, a very few medicinal plants have been adopted under commercial level farming. Among important parameters, study of phonological behaviour of any wild plant species, which is being targeted for cultivation, is a pre-requisite exercise; it is helpful in developing and standardizing agro-techniques of targeted species. Identification of phonological stages is very crucial (Sanz Cortes et al., 2002).

Morphology in V. jatamansi is of great interest for its impressive diversity of forms, mainly resulting in adaptation to wide range of ecological conditions, concerning both vegetative and reproductive forms. This diversity has been well studied from the inflorescence structural perspective, with different forms and levels of complexity by (Weberling, 1989 and Hidalgo, 2004). The unique character of family is the pattern of four different stamen numbers in a series wherein mainly four are found in Patrinia and

Nardostachys, three in Plectritis,
Valerianella and Valeriana, two in Fedia while in Centranthus have only one stamen (Donoghue, 2003). Valerianaceae exhibits a considerable diversity in flower and fruit morphology (Erickson, 1989). This implicitly assumes particularly habitat diversification as well as prevailing climatic conditions have prominent effect on plant morphological characters and seed germination behavior, and its progeny in response to altered environmental conditions. The species jatamansi inhabit diverse habitats of Darjeeling - Sikkim Himalaya, so it was thought worthwhile to undertake detailed ecology and enable the species to survive in these varied habitats. Growing awareness of the importance of plant diversity and rapid decline of these valuable plants, have given an unprecedented impetus for their monitoring and conservation (Victor, 2010). The relative response of the V. jatamansi for sprouting duration and sprouting percentage was in most cases different both between and among locations.

The studies have reported that the methanol extract of roots of *V.jatamansi* possesses remarkable antioxidant activity as compared to its essential oil. Thus root extract of V. jatamansi can prove beneficial in food and pharmaceutical industry (SakshimaThusoo*et al* , 2014). More recent reports have established its antioxidant potential (Kalim*et al.*, 2010), which can largely be attributed to the presence of polyphenols and their free radicals scavenging properties and known protective effects against certain chronic and degenerative diseases (Meyer *et al.*,1998; Record *et al.*, 2001).

The quantification of Valerenic Acid in field grown *V. jatamansi* by HPTLC (N. Singh *et.al* 2006) has been reported. However, no reports are available on quantification of Valerenic Acid (Valepotriates) in tissue cultured grown *V.jatamansi* by HPLC.

The genetic and chemical diversity of *V. jatamansi*has been studied using ISSR and AFLP markers (Rajkumar*et al.*, 2011; Sundaresan*et al.*, 2012). However, no reports are available on using of SSR markers.SSRs or microsatellites are short nucleotide repeats of 1-8 bp which vary in length in different individuals.

Variation in number of repeats gives the length polymorphism which is identified by

amplifying the primers designed for the sequences flanking the SSRs. The number of times the unit is repeated in a given microsatellite can be highly variable, a characteristic that makes them useful as genetic markers. The primers designed for one species can also be utilized for another species. The use of SSR markers has many advantages over other markers and length-polymorphisms can be easily detected on high resolution gels. Microsatellites allow the identification of many alleles at a single locus, co dominant, little DNA is required and the analysis can be semi-automated and performed without the need of radioactivity (Gianfranceschi*et al.*, 1998, Guilford *et al.*, 1997).

Somaclonal variation is the variation seen in plants that have been produced by plant tissue culture. How a single plant genotype can result in a variety of phenotypic outcomes under the same in vitro culture conditions is still far from being completely understood. Several bases for somaclonal variation have been proposed, which include changes in chromosome number (Mujib et al. 2007; Leva et al. 2012), point mutations (D'Amato 1985; Ngezahayo et al. 2007), somatic crossing over and sister chromatid exchange (Duncan 1997; Bairu et al. 2011), chromosome breakage and rearrangement (Czene and Harms-Ringdahl1995; Alvarez et al. 2010), somatic gene rearrangement, DNA amplification (Karp 1995; Tiwari et al. 2013), changes in organelle DNA (Cassells and Curry 2001; Bartoszewski et al. 2007), DNA methylation (Guo et al. 2007; Linacero et al. 2011), epigenetic variation (Kaeppler et al. 2000; Guo et al. 2006; Smulders and de Klerk 2011), histone modifications and RNA interference (Miguel and Marum2011), segregation of pre-existing chimeral tissue (Brar and Jain 1998; Vázquez2001; Ravindra et al. 2012; Nwauzoma and Jaja2013) and insertion or excision of transposable elements (Gupta 1998; Sato et al. 2011b). In particular, transposable elements are one of the causes of genetic rearrangements in in vitro culture (Hirochika et al. 1996; Sato et al. 2011a). In in-vitro plants the amount of secondary metabolites produced should be same through out, the quality of the plant is needed to be maintained. So to check out the presence of variation in tissue culture plants we go for SSR studies.

1.2. Objective

1. To develop tissue culture conditions for somaclonal varients of *Valeriana jatamansi.*

2. To carry out the molecular screening of somaclonalvarientsfor Valepotriates production in tissue cultured and field grown plants.

CHAPTER- 2

2.1. Review of Literature

*V. jatamansi*or Indian Valerian of the family Caprifoliaceae is an important medicinal plant used in several indigenous systems of medicine practiced in India. This species has been listed as critically endangered species in Western Himalayas (Kaul&Handa, 2000) and is therefore of considerable interest for various studies which centreon the following aspects.

- Study was attained to develop in-*vitro* regeneration protocol for *V. jatamansi* using nodal explants on Murashige and Skoog (MS) basal medium supplemented with various combinations of PGRs. The study has practical implications as it will be helpful to meet out industrial as well as domestic demand (Purohit et al. 2015).

- A callus-mediated shoot regeneration system was developed for the large-scale production of *V. jatamansi* Jones. Effect of Murashige and Skoog (MS) medium supplemented with different concentrations of (2,4-D), (NAA) and (IBA) on callus induction and production of valepotriates accumulation. (Das et al. 2013).

- The volatile constituents of *V.jatamansi* Jones and *V.hardwickii* Wall. (Valerianaceae) collected from the Khasi Hills of north-east India were analyzed by GC and GC/MS. (Das et al. 2011).

- To explore the diversity in the essential oil yield and composition of *V. jatamansi*

- jones (syn. *V. wallichii* DC) growing wild in Uttarakhand (Western Himalaya). (Verma et al. 2011).

- A simple, rapid, cost-effective and accurate high performance thin layer chromatographic method has been developed for quantification of valerenic acid in *V. jatamansi* and *Valerianaofficinalis*. *(*Singh et al. 2006).

- To investigate the effect of magnesium and calcium as abiotic elicitors on *valerianaofficinalis* hairy root for scale-up production of valerinic acid (Mohammad Reza DiniTorkamani et.al 2014).

- HPTLC was done for the quantification of Valerenic Acid in *V. jatamansi* and Valerianaofficinalis (N. Singh *et.al* 2006).

- The study to check the seasonal variation of bioactive components in *V. jatamansi* from Himachal Pradesh (Bikramsingh et.al 2010).

- Characterization of essential oil composition, phenolic content, and antioxidant properties in wild and planted individuals of *V. jatamansi* Jones (Indra D. Bhat et.al 2012)

- Quantification of Valerenic Acid in *V. jatamansi* and *Valerianaofficinalis* by HPTLC (N. Singh et.al 2006)

- Impact of Geographic Range on Genetic and Chemical Diversity of Indian Valerian (*V. jatamansi*) from Northwestern Himalaya(V. Sundaresan et.al 2012)

- Assessment of Genetic Diversity in *V. jatamansi* Jones. Germplasm Using RAPD Marker (Amit Kumar et.al 2012)

- Genetic Structure of Indian Valerian (*V. jatamansi*) Populations in Western Himalaya Revealed by AFLP (Subramani Rajkumar et.al 2011)

- Genetic and Biochemical Diversity among *V. jatamansi* Populations from Himachal Pradesh (Sunil Kumar Singhet.al 2015)
- Valepotriates Content Variation and Genetic Diversity in High Value Medicinal Herb, *Valeriana jatamansi* (Vikas Singh et.al 2014)

2.2. Taxonomy:

Valeriana jatamansi

Local Name:	Sugandhawal (Nep)
Family:	VALERIANACEAE
Genus:	*Valeriana*
Species:	*jatamansi*
IUCN Category:	N/A
CITES Appendix:	N/A
Altitude (meter):	1500 to 3300
Habitat:	N/A
Habit:	Herb

2.3. Geographical distribution:

Distribution in Protected Area

- Kanchenjunga Conservation Area
- Khangchendzonga Biosphere Reserve

- Nepal side of KBR and BRS adjoining KCA
- Singhalila National Park
- Toorsa Strict Nature Reserve
- Toorsa-JigmeDorji

Conservation of organism is important for identification so if we cannot conserve organisms that we cannot identify and our understanding the consequences of environmental change and degradation are compromised fatally, if we cannot recognize and describe the interacting components of natural ecosystems (Mace 2004). The familial status of Valerianaceae is debatable in light of recent studies (Bell and Donoghue, 2005). Judd *et al.*,(1994) on the basis of morphological and anatomical similarities merged it in Caprifoliaceae.

Recent report shows that Valerianaceae comprises of 350 species distributed throughout the World (except Australia and New Zealand), mostly at high elevations and with many species in alpine zones (Backlund and Moritz, 1998). Bell (2004) labeled Valerianaceae as a natural group of 350 species of cosmopolitan distribution comprising of 13 genera with 200 species chiefly confined to temperate regions. *V. jatamansi*Jones (Syn. *Valerianawallichi)* popularly known as Indian Valerian (English), Mushkibala (Hindi), Suganthdhawal or Tagara (Sanskrit), is distributed in all the temperate regions except Australia.Benet,1987).

Several species of Valeriana have also been reported from Andean Chile, Brazil, South Africa and Sub-tropical Asia. About 12 species of genus Valeriana have been reported from India (Rao*et al.*,1977). Out of these, *V. jatamansi*strainshasbeen reported to be widely distributed in temperate Himalayas at an altitude ranging from 1500m in Khasi Hills to 3600m in Jammu and Kashmir, Himachal Pradesh and Bhutan (Kritikar and Basu, 1975).

Mukerjee (1953) while assessing distribution of *Valerianaofficinalis is* reported it to be growing only in restricted sites of North Kashmir at an altitude of 2400 m to 2700m. However, *V. jatamansi*according to Chauhan and Khosla (1988) is sporadically found in whole NorthWestern Himalaya range.Polunin and Stainton, (1987) reported the

distribution of *V. jatamansi*from Afghanistan to South west China and Burma. The herb was seen to grow at an altitude of 1500 m to 2600m (a.s.l).

2.4. Species morphology

V. jatamansi is a perennial herb with pubescent stem, radical leaves, several long petiolatedcordate-ovate, cauline few or much smaller entire or pinnate, fruits hairy or nearly glabrous. Root stocks thick, horizontal with thick descending fibers; stem 6-18cm, radical leaves often 1-3cm in diameter, deeply cordate, usually acute toothed. Cymes corymboselypanicled; bracts small oblong or linear persistent, calyx limb in flower obscure, unrolling in fruit into 5-15 plumose bristles united at base equal or sub-gibbous, flowers white, stamens 3, ovary 3celled, 1 ovuled, stigma shortly 2-3 fid or sub-entire, fruits oblong lanceolate, compressed crowned by persistent pappus calyx (Hooker, 1881).

2.5. Phenology

Robertson, (1924) proposed that a natural group of flowering plant has a definite position. It begins at a given time period, attains peak at a given point and retards until all of its members are out of bloom, showing complete senescence and complete seasonal growth. Their seasons do not coincide but they often overlap. Flowering phenology is an important life history trait because the timing of reproduction and the schedule of reproductive expenditures across time can strongly influence individual fitness (Primack, 1985; and Rathcke and Lacey, 1985; Fenner, 1998) and hence flowering phenology is of fundamental interest for understanding of species interaction and community functions. Thus phenology in general and reproductive phenology in particular is a critical and important trait of a plant because it determines the growth, developmental pattern and number of reproductive isolations or speciation over time (Bronstein et al., 1990).

2.6. Pollen production and pollen ovule ratio

Pollen-ovule ratio reflects pollination efficiency, i.e., the likelihood of a pollen grain reaching a stigma (Cruden, 1977). P/O ratio is also used to examine how breeding system, sexual system, pollen vectors and dispersal units influence pollen grain number (Cruden,

2000). He also reported that there is substantial decrease in P/O ratio from xenogamy to facultative xenogamy to autogamy suggesting that there is a cost associated with changes in sexual system. The P/O ratio of the wind pollinated plants are substantially higher than those of animal pollinated plants. The P/O ratio of the plants whose pollen is dispersed in tetrads, polyads or polliniaare substantially lower than those of species whose pollen is dispersed as monads (Cruden, 2000). The P/O' s of plants that provide only pollen as reward are higher than those that provide nectar as a reward. In general more efficient the transfer of pollen, the lower the P/O ratio should be. Thus it logically depicts that cleistogamous flowers will have lower P/O's than xenogamous flowers i.e., P/O's are correlated with the breeding systems (Cruden, 2000).Layton and Ganders (1984) analyzed the pollen ovule ratio in two species of family Valerianaceae and reported that wind pollinated species (Plectritisbrachystemon) produce more pollen grains than insect pollinated (Plectritiscongesta). The latter had fewer ovules per flower than the former. He concluded that differences in pollen-ovule ratio may possibly reflect the differences in pollination efficiency.

2.7. Breeding system

Plants cannot choose their mates; nevertheless they have diverse methods by which genetic structure of their populations and the patterns of their evolution is influenced by their mating patterns. Plant breeding systems are under genetic control and can themselves be selected for. They are fluid and respond to selection pressures in an infinite variety of subtle and interrelated ways because breeding systems are genetically controlled and affect genotype structure(Richards, 1986). Thus breeding system is the purposeful manipulation of plant species in order to create desired genotypes and phenotypes for specific purposes.

2.8. Pollination system

One of the most exquisite features of many flowering plants is their interaction with different species of pollinating insects (Olesen and Warncke, 1989). Out crossing plants mate only with the assistance of pollen vectors so that the abundance and efficiency of

vectors determine mating success (Burd, 1994; Larson and Barret, 2000). In recent years pollination failure in small, isolated population has been identified as a potential threat to the long term persistence of declining plant species (Rathcke and Jules, 1993). The size and density of a plant population may affect interactions with pollinators and pollen transfer in several ways. First, increase in size and density of a population increases pollinator visits to plants, as well as the amount of pollen received per flower, (Powell and Powell, 1987). Second, pollinator foraging behavior may change the size and density of the population (Zimmerman, 1981; Goulson, 2000), thereby affecting the composition of pollen deposited. If the overall visitation rate decreases with decreasing population size or density, then this may increase standing crop of nectar and pollen in individual flowers. This in turn may induce pollinators to visit more flowers per plant (Pyke, 1978; Harder, 1990). This depicts that relative abundance of different pollinator species may vary with plant population size (Sowing, 1989) which can influence both the quality and composition of pollen deposited.Leppik, (1953)

2.9. Seed Biology

In most angiosperms, flowering date varies among plants within a population, and such variation may result in differences in reproductive output among plants like fruit: flower ratio (Dieringer, 1991; Kelly and Levin, 2000), seed: ovule ratio (Widden, 1991), number of seeds produced by a plant (Schmitt, 1983; Kelly and Levin, 2000) and number of seeds per fruit produced by a plant (Widden, 1991). The reproductive strategies among plants may evolve from fitness tradeoff between seed dispersal and seedling establishment. Large seeds of small crops generally are not dispersed as compared to small seeds of large crops, but large seeds contain more reserves for seedling establishment (Preciak, 2002). However, variation in seed size vs. number can reflect a compromise between seed dispersal (i.e., movement of seeds away from parent plants) and seedling establishment including seed germination and seedling emergence, growth and survival (Sollabanks, 1992). Seeds play an important role in the development of civilization by supplying food, feed and natural products and traditional medicines, thus acquiring knowledge of seed biology has been a priority for most cultures (Jaimie et al., 2005).Weins, (1984) on the basis of studies on seed productivity reported that seed ovule ratio of out crossing

perennials tends to be lower than selfing species. However, Argen and Willson, (1991) have reported that in gynodioecious species, seed production in hermaphrodite plants was lower than in female plants.

2.10. In vivo/In vitro studies

Propagation is the practice of rapidly multiplying stock plant material to produce large number of progeny plants. It is used to multiply novel plants such as those that have been genetically modified or bred through conventional plant breeding methods. It is also used to provide a sufficient number of plantlets which do not produce seeds or do not respond well to vegetative reproduction (Horn, 1992).Kaur et al. (1999) through in vitro propagations established productive method for rapid and mass multiplication of Valerianajatamansi through induction of shoot proliferation from shoot buds. The optimum results were obtained by using solid media supplemented with benzyl adenine alone or in combination with the indole acetic acid or naphthalene acetic acid. Further, culturing on media supplemented with BA and IAA or NAA facilitated the shoot formation which produced roots on the same .medium within 3-4 weeks. The survival of progenials was hundred percent.

Luissa et al. (2002) developed a practical method for the multiplication of Valerianaglechomifolia. They also assessed the valtrate synthesis in the plantlet. Doing their study the auxiliary buds and shoot tips were cultured in 0.3µ Murrashige and Skoog basal medium supplemented either with 0.4mg BAP or without plant growth regulators. The cultured segment grew on both media producing roots after 3-4 weeks. Singh et al. (2005) assessed the impact of transplantation time on the growth and yield of Valerianajatamansi. The maximum plant yield in terms of height, aerial biomass, underground biomass, rhizome yield, and root yield was obtained after 9 months of transplantation. The assessment of impact of habitat conditions depicted highest values for plant height, biomass, underground biomass, rhizome yield and root when plantation was carried out on ridges.

2.11. Medicinal properties

Chopra *et al.*,(1956) reported that rhizome and roots of *Valerianajatamansi*contain an essential oil-valepotriate, which has antibacterial and antiprozoal activity. The isolation of valepotriates and determination of its medicinal properties resulted in wide spread use of this compound as a sedative in west Germany under the trade name "valmane" (Thies1966). The valmane comprises of standarized mixture of valepotriates containing valtrate (15%), didrovaltrate (80%) and acevaltrate (5%). Although some clinical testing of these alkaloids were carried out earlier also, but the first report on their medicinal properties was published by Von Eickstedt and Rehman, (1969).

Bounthanh*et al.*,(1981) conducted tests on the cytotoxity of some valepotriates for their use as potential anti-tumor agents. Pande*et al.*,(1994) reported roots of *Valerianajatamansi*are acrid and bitter with a flavor. These are used as carminative, laxative, antiperiodic, hypnotic and aphrodisiac. The roots are also used for curing diseases of blood, burning sensation, leprosy, cholera, skin disease throat troubles and ulcers. Further the roots increase the lusture of eyes, promote growth and blackness of hair and also useful in the treatment of cough, chest pain and kidney troubles. Prakash and Mahrotra, (1994) reported that *Valerianajatamansi*rhizome and roots have been used in the treatment of hysteria, epilepsy, asthma. However, Gupta *et al.*, (1996) assessed that the drug Valerian extracted from roots of Valerianais being used immemorial for curing different diseases.

Table 2.1.: Summary of pathophysiological conditions treated with *V. jatamansi*

Sedative effects	Antispasmodic	Gastrointestinal
Emotional Stress	Muscular pain	Digestive disturbances
Insomnia	Menstrual cramps	Stomach cramps
Nervousness	Bronchial spasms	Diarrhea
Anxiety	Coughs	Bloating
Nervous heart conditions		Colic
Children's anorexia		
Restlessness		
Trembling		

2.12. Effects

Neurological Effects

Overall, in vivo studies showed that valerian and its constituents produced sedative, anxiolytic, and antidepressant effects. In vitro studies suggest that the effects of valerian and its components may occur through modulation of a variety of neurotransmitter systems including γ-aminobutyric acid, adenosine, and serotonin systems. Valerian components and extracts may specifically bind to receptors and modulate neurotransmission.

Anti-inflammatory Effects

The ethyl acetate extract of V. officinalis inhibited NF-κB activity in HeLa cells, and transforming growth factor beta 1 expression was downregulated by V. officinalias var. latifolia in dietary-induced hypercholesterolemia in male rats. Polysaccharides isolated from V. officinalis exhibited mitogenic and co-mitogenic activity in rat thymocytes. Comparatively, a short-term in vivo study showed that research grade valerian had no effect on natural killer cell activity in rats.

Effect on Enzymes

Components of valerian and valerian extracts modulate a variety of enzyme activities (e.g., inhibited glucuronidase activity and stimulated glutamic acid decarboxylate). There are conflicting studies on the effects of valerian on cytochrome P450 activities.

2.13. Activities

Endocrine Activity

A petroleum ether extract of V. officinalis (root) exhibited binding affinity for estrogen receptors α and β in MCF-7 cells. Both petroleum ether and dichloromethane extracts induced luciferase activity in an estrogen receptor α positive cell line.

Antifungal-Activity

Jatamansi essential oil demonstrated fungi static activity against Aspergillusflavus, Aspergillusniger and FusariumoxysporumMucorfragilis, Rhizopusstolonifer and this oil was found to be fungi static of fungicidal to one or the molds, depending upon the concentration.

Hepatoprotective Activity

The roots extract of jatamansi also possess the hepatoprotective activities and it has been proved by several studies. Pre-treatments of rats with 800 mg/kg body wt of the 50% ethanolic extract of jatamansi DC demonstrated significant hepatoprotective activity

against thioacetamide induced hepatotoxicity. Marked reduction in raised levels of serum transaminase and alkaline phosphatase was observed. Pre treatment of the animals with the extract further resulted in an increase in survival in rats intoxicated with LD90 dose of the hepatotoxic drug.

CNS Activity

Valeranone prolonged barbiturate anesthesia, impaired rotarod performance, inhibited electroshock convulsions, and Potentiated the hypothermic effects. Limited results from behaviour- ral tests revealed that an extract from jatamansi exhibited significant antidepressant activity.Studied the effect of acute and sub chronic administration of alcoholic extract of the roots of jatamansi DC on nor epinephrine (NE), dopamine (DA), serotonin (5-HT), 5- hydroxyindoleacetic acid (5-HIAA), gamma-amino butyric acid (GABA), and taurine on male albino Wistar rats. The acute oral administration of the extract did not change the level of NE and DA but resulted in a significant increase in the level of 5-HT and 5-HIAA. A significant increase in the level of GABA and taurine was observed in the drug-treated groups when compared to the controls. A 15-day treatment resulted in a significant increase in the levels of NE, DA, 5-HT, 5-HIAA, and GABA.

Anticonvulsant Activity

Rao VS et al. studied ethanol extract of the roots of jatamansi DC was studied for its anticonvulsant activity and neurotoxicity, alone and in combination with phenytoin in rats. The results demonstrated a significant increase in the seizure threshold by jatamansi DC root extract against maximal electroshock seizure (MES) model as indicated by a decrease in the extension/flexion ratio.

However, the extract was ineffective against pentylene- tetrazole-induced seizures. Further, pre-treatment of rats with phenytoin at a dose of 12.5, 25, 50 and 75 mg/kg in combination with 50 mg/kg of jatamansi DC root extract resulted in a significant increase in the protective index (PI) of phenytoin from 3.63 to 13.18.

Neuroprotective Activity

Salim S et al pre treatment with an alcoholic extract of jatamansi DC dosed at 250 mg/kg of for 15 days protected rats against focal ischemia caused by middle cerebral arteryocclusion. The protective effect may be associated with improving glutathione content, inhibiting lipid peroxidation, and activity on the Na+/K+ ATP aseand catalase enzyme systems.

Antiparkinson's Activity

Parkinson's disease is a most common neurodegenerative diseases, and oxidative stress has been evidenced to play a vital role in its causation. It was evaluated that the ethanolic extract can slow the neuronal injury in caused by parkinson's rats.

Ahmad M et al. treated with 200, 400, and 600 mg/kg of jatamansi DC roots for 3 weeks in rats. Antiparkinsonism activity was studied on 6-OHDA (12 µg in 0.01% in ascorbic acid-saline) induced Parkinsonism. Three weeks after the 6- OHDA injection, the rats were tested for neurobehavioural activity and quantification of catechol amines, antioxidants, dopaminergic D2 receptor binding and tyrosine hydroxylase expression were also estimated. The increase in drug-induced rotations and deficits in locomotor activity and muscular coordination due to 6-OHDA injections were significantly and dose-dependently restored by jatamansi DC.

Tranquilizing Activities

German R et al investigated sesquiterpenevaleranone (Yatamanson) isolated from jatamansi DC rhizomes for tranquilizers activity in rodents and significantly the prolongation of barbiturate hypnosis, the impairment of rotarod performance, as regards the hypotensive property was demonstrated .

Antioxidant Activity

The antiperoxidative property of jatamansi was investigated as an iron-induced lipid peroxidation model in rat liver, quantified by thiobarbituric acid reactive substance (TBARS) content. They have observed in their study that the extract provide protection against lipid peroxidation. In other study an aqueous root extract of jatamansi was

investigated for its antioxidant and anticataleptic effects on haloperoidal-induced catalepsy rat model of the disease by measuring various behavioural and biochemical parameters.

Antidiabetic activity

The extract of jatamasni has been shown to a significant hypoglycemic activity. It decreases glucose level significantly in diabetic and non-diabetic rats as compared to respective controls. The present study was carried out to evaluate the antidiabetic activity of N. jatamansiethanolic extract in alloxan induced diabetic rats for 7 days. The ethanolic extract at high dose (1200 mg/kg) exhibited significant antihyperglycemic activity in diabetic rats. The results showed that it has significant antihyperglycemic effect in experimental model of diabetes mellitus.

Others activity

Animal studies done on jatamansone have reported anti estrogenic activity, moreover, jatamansone have reported antiarrhythmic and antihypertensive activity, anti asthmatic activity ,nematicidal activity and antibacterial activity.

2.14. Simple sequence repeats (SSR):

Simple sequence repeat (SSR) markers are repeats of shortnucleotide sequences, usually equal to or less than six bases in length, that vary in number (Rafalski*et al.*, 1996). SSR markers have become quite useful in various aspects of molecular genetic studies in the past decade, including assessment ofgenetic diversity (Amsellem*et al.*, 2001; Ashley *et al.*, 2003),fingerprinting (Rongwen*et al.*, 1995), ecological-genetic studies (Li *et al.*, 2000), marker-assisted selection (Fazio *et al.*, 2003), gene flow characterization (Aldrich and Hamrick, 1998; Chase *et al.*, 1996), and genetic linkage mapping (Akkaya*et al.*, 1995; Broun and Tanksley, 1996). They are desirable because they are often codominant, highly reproducible, frequent in most eukaryotes, and reveal high allelic diversity (Mohan *et al.*, 1997). SSR markers are amplified using the PCR, thus allowing for the rapid generation of data from a relatively small amount of plant tissue. However, SSR markers

are also expensive to generate and can be very laborious in certain species. Because of these expense and labor issues, many researchers have attempted to use SSR primers developed from one species for studies on related species and genera.

2.15. Chemical Constituents:

Following are the major compounds of *V. jatamansi*:-

β Vatirenene

β Patchoulene

Dehydroaromadendrene

β Gurjunene

Patchoulic alcohol

β Guaiene

Alpha Muurolene

Following listed compounds are also found in *V.jatamansi*:-

Maaliol

Seychellene

calarene/ß-gurjunene

α-santalene

bornyl acetate,

α-guaiene

α-bulnesene/δ-guaiene

7-epi-α-selinene

kessane

spathulenol

viridiflorol

α-patchoulene

ß-patchoulene

CHAPTER-3
MATERIAL AND METHODS

3.1. Plant Material

Shoots regenerated from callus were taken from the plant tissue culture chamber of Department of Biotechnology/Bioinforamtics, Jaypee University of Information Technology, Waknaghat, Solan.

3.2. Material Required

Beakers (1000, 500, and 100 ml), graduated cylinders (1000, 500, and 100 ml), conical flasks, reagent bottles, micropipettes (1000 µl), distilled water, spatulas, weighing balance, tissue, pH meter,aluminium foil, autoclave machine, and chemicals needed: stock of solutions macronutrient, micronutrient, vitamins, plant growth regulators such as IBA (Indole-3-butyric acid, Kinetin, BAP (6-benzylaminopurine), sucrose, agar,NaOH and HCl to adjust pH.

3.3. Media Preparation

Modifications of MS (Murashige and Skoog, 1962) media supplemented with different concentrations and combinations of IBA (indole-3-butyric acid), KN (kinetin) and BAP (6-benzylaminopurine) were prepared. pH was adjusted to 5.7 using 0.1N HCl and 0.1N NaOH. 30g/L sucrose was added.Media was solidified by adding 9 g as a gelling agent. The media were autoclaved at 121°C and 15 lb/in2 pressures for 15-20 min in 150mL Erlenmeyer flasks by dispensing 40 mL molten media in each flask.

Table 3.1. MS Media supplemented with different concentration of growth hormones.

	IBA(mg/L)	KN(mg/L)	BAP(mg/L)
MS-1	0	0	0
MS-2	1	0	1
MS-3	1	3	0
MS-4	3	1	0
MS-5	0	1	3
MS-6	1	0	0
MS-7	3	1	0
MS-8	0	1	0
MS-9	1	0	3
MS-10	3	0	1

Sub-culturing was performed under sterilized conditions in Laminar Air Floor. Shoots from previously cultured plants of *V. jatamansi* were cut and transferred to new media supplemented with various concentrations of growth hormones.

The cultures were incubated at 25±1°C in plant tissue culture chamber with 70% relative humidity under 16h photoperiod provided by cool fluorescent light (3000 lux). For the development of variants we subcultured the regenerated microshoots from callus 25 times on the defined media.

3.4. Quantification of Valepotriates by HPLC

Preparation of extracts

The 9 subcultured plants were taken for HPLC analysis. The extracts were prepared using liquid nitrogen. The dried and powdered roots of *V. jatamansi* were extracted successively three times with 70: 30 (v/v) ethanol: water and then filtered. The filtrates were combined and dried over rotavapour. The concentration of amount 100 mg was

reconstituted with 2ml of degassed mobile phase 0.1% phosphoric acid: water (6:4) and then samples were sonicated for 2-3 min and subjected to HPLC. The standard preparation involved 1mg of standard velerenic acid in 5 ml of HPLC graded methanol.(Vikas et.al 2014).

High Pressure Liquid Chromatography

Samples of *V. jatamansi*were processed for characterization of Valepotriates. The dried and powdered extracts of *V. jatamansi* were extracted successively three times with 70:30(v/v) ethanol:water and then filtered. The filtrates were combined and dried over rotavapour. The concentration amount of 100mg was reconstituted with 2ml of degassed mobile phase 0.1% phosphoric acid:water (6:4) and then samples were sonicated for 2-3 min and subjected to HPLC. The standard preparation involved 1mg of standard valerenic acid in 5 ml of HPLC graded methanol.

3.5. Genomic DNA Extraction Using CTAB:

Materials

Microfuge tubes

Mortar and Pestle

Liquid Nitrogen

Extraction buffer

60ºC water bath

70 % Ethanol (ice cold)

CTAB Extraction Buffer

Chloroform: Isoamyl Alcohol (24:1)

Isopropanol (chilled)

Autoclaved Water

Agarose

Ethidium Bromide

1xTAE solution

Agarose gel electrophoresis system

Table 3.2. Extraction Buffer (100ml)

1% CTAB	1gm
50 mMTrisHcl	5ml of 1M
50mM EDTA	10ml of 0.5M
700Mm Nacl	70ml of 1 M
Water	Raise volume to 99ml and autoclave
1% β-Mercaptoethanol (added after autoclaving)	1ml

TAE Buffer

50X Stock solution of TAE was prepared by adding the following:

Table 3.3: Composition of TAE Buffer

Tris base	24.2gm
Glacial acetic acid	5.71ml
EDTA(0.5M, pH 8.0)	10ml
Distilled water	Make up the vol. to 100ml

3.6 DNA Isolation

DNA of field grown as well as tissue cultred plants of *V. jatamansi*was isolated using CTAB method.

- Leaves of the samples were ground in liquid nitrogen.
- Ground samples were transferred to a microfuge tube.
- 700µL pre warmed extraction buffer was added to the vials.
- The vials were incubated at 60ºC for 1hour in a water bath.
- 700µL chloro:isoamylalcohol (24:1) was added and mixed.
- Vials were centrifuged for 10 minutes at 10,000 rpm.
- Supernatant was transferred to fresh vials.
- 500µL chilled isopropanaol was added and mixed well.
- This was stored at -20ºC for 1hour.
- The vials were again centrifuged at 10,000 rpm for 10minutes.
- Supernatant was discarded.
- Washing was done using 200µL 70% ethanol and centrifuged at 10,000rpm for 1 minute.
- Ethanol was drained and the above step was repeated to wash again.
- The pellet was air dried and dissolved in 50µL autoclaved water.
- Stored DNA at -4ºC
- Checked the DNA quality and contamination on 0.8% of agarose gel.

3.7. DNA quality confirmation

- Prepared 1 % solution of agarose by melting 1g of agarose in 100 mL of 0.5x TAE buffer in a microwave for approximately 2 min. Allowed it to cool for a couple of minutes.
- Added 2.5µl of ethidium bromide.
- Gel was cast using a supplied tray and comb. Allowed the gel to set for a minimum of 20min at room temperature on a flat surface.

- Following was loaded into separate wells
- 10 µL 1kb ladder
- 5 µL sample + 2 µL 6x Loading Buffer
- Gel was run for 30 min at 100 V
- Gel was analysed using a Gel Doc System.
- Confirmed DNA quality. Presence of a highly resolved high molecular weight band indicates good quality DNA, presence of a smeared band indicates DNA degradation.

Table 3.4.Polymorphic SSR Markers and Sequences

S. No.	SSR primer	Repeat Unit	Nucleotide Sequence	No. of alleles	Annealing temp. (°C)
1	PKSTS M5	(A)14	5'-ACAAAGACAACAGACGCTCT-3' 5'-GATTCGCAAGAATTGAGAAG-3'	2	52
2	PKSTS M11	(T)14	5'-GGGACTCTCTTCCTGTTTCT-3' 5'-AAGCTTTCAACCACAAGAAA-3'	3	51
3	PKSTS H3	(GGTGAA)3	5'-CAAAGTCAACAAGAAGGGAG-3' 5'-CTTGCTGATCTTCTTAGCGT-3'	3	52
4	PKSTS H5	(CGGGAA)8	5'-AAGGTCTTCTTTACGCTCCT-3' 5'-CTATCTCTTTCCCGTACCCT-3'	3	53
5	PKR 25 M15	(A)12	5'-ATGACCTGATCAATTCGAAC-3' 5'-CCTCCCCTACAGTATCATCA-3'	2	51
6	PKR 25 M4	(A)12	5'-CCAACTATGTCAGGAAAAGG-3' 5'-CGCGATTGATTTCTAAGATC-3'	2	50
7	PKR 25 M12	(C)12	5'-AGGAGGAGCTTAATTTCGTT-3' 5'-GAACTTGGTGTGAGGAAAAC-3'	2	51

8	PKR 25 M14	(T)12	5’-CACCCACTTTCTGAGATCAT-3’ 5’-CCTTTGAAAACATTGTAGCC-3’	2	49
9	PKR 25 D1	(TA)8	5’-ACTGTGGCCTAGTTGAAGAA-3’ 5’-ATGAAACGTCATCTCGAATC-3’	5	52
10	PKR 25 D5	(AG)7	5’-AAAAGGTCTCCTTCATCCTC-3’ 5’-TCTTTTGGTGGTCTTATGCT-3’	2	52
11	PKR 25 D6	(AT)10	5’-CAGCCAAGAAAGATCTATCG-3’ 5’-CCAGGTGATAAATCCACAGT-3’	3	51
12	PKR 25TT2	(GTTT)3	5’-TTTCTGGTGGGTATCCTTAA-3’ 5’-TCGACTATATCCGCATTTTT-3’	3	52
13	PKS25T9	(AAG)5	5’-AAGAGGAAGGTGAAAAGACC-3’ 5’-GGCACATTCTTGAGATCTGT-3’	3	51
14	PKS25T13	(TCT)5	5’-ATGCTGTTGGATTTTTACGT-3’ 5’-ATCTTGAAAGCCTTCAATCA-3’	2	52
15	PKSTST1		5’-GGACCTTCTGGGCTAAATAT-3’ 5’-AAATGGTGTTCCATTGGTAG-3’		48
16	PKSTST9		5’-CTTTGCTCTCCAGTTCAATC-3’ 5’-TAGATGATTTGCAGACGATG-3’		48
17	PKSTSD15		5’-ATCTGTAACCCTCTGCACTG-3’ 5’-CCCTTTTGGATATTTTCACA-3’		49
18	PKSTST6		5’-AGGTGCTCCTGCTAATAATG-3’ 5’-CGAAAACGGTATAGGTGAAG-3’		50
19	PKSTSD8		5’-CCCAAGGGAAGATCTAAATT-3’ 5’-CCAAGGTCTTTGAACTTCAC-3’		48
20	PKSTSD9		5’-TCTTCTCCTTCATCTTCAACA-3’ 5’-AGAAGGGAGTTTGAGGGTT-3’		53
21	PKSTSD10		5’-CAACTTTCACGACCGTTAAT-3’ 5’-GAAGCTCTTGCAGAAAAAGA-3’		54

22	PKR25H5	(CGGGAA)8	5’-AAGGTCTTCTTTACGCTCCT-3’ 5’-CTATCTCTTTCCCGTACCCT-3’		52
23	PKSTST7		5’-GCCTACAGCAGTATTTCCAG-3’ 5’-CACAACAACAGCAGGTTCTA-3’		49
24	PKSTST13		5’-ATGCTGTTGGATTTTTACGT-3’ 5’-ATCTTGAAAGCCTTCAATCA-3’		51
25	PKSTST14		5’-AAAGAGTGACGAAGGTGCT-3’ 5’-CTCTTGCTGCTACTGTTTCC-3’		50

Primers were prepared by taking 5µL stock primer and 45µL distilled autoclaved water.

3.8. Polymerase Chain Reaction

- PCR was performed on five different DNA samples of Valerianajatamnsi; three were field grown samples and two were tissue cultured.
- To perform several parallel reactions, master mix was prepared containing autoclaved distilled water, PCR buffer, dNTPs, primers and Taq DNA polymerase in a single tube.
- Tube was gently vortexed.
- Template DNA solution (1µL) was added into five separate PCR vials.
- 14µL master mix was added to each PCR vial.
- Samples were vortexed in order to collect all drops from walls of tube.
- PCR reaction was started using Veriti 96 well thermocycler (Applied Biosystems).
- Products were resolved using 1X TAE, 2.5% agarose gel.

Table 3.5. PCR Cycle

S.No.	Stage	Step	Temperature	Time
1.	STAGE 1*1 CYCLE		94° C	4mins
2.	STAGE 2*30 CYCLES	STEP 1	94° C	30 sec
		STEP 2	52° C	1 min
		STEP 3	72° C	1min
3.	STAGE 3*1 CYCLE		72° C	7mins
4.	STAGE 4		4° C	∞

Table 3.6. PCR Master Mix

Materials	1x	6x (MasterMix)
Autoclaved Water	11.05µL	66.3µL
10x PCR Buffer	1.5µL	9µL
dNTPs	0.3µL	1.8µL
Taq DNA Polymerase	0.15µL	0.9µL
Primers	0.5µL(each)	3µL(each)
Genomic DNA	1µL	
Total	15µL	84µL

3.9. Agarose Gel Electrophoresis of PCR Products

Agarose gel was prepared by dissolving 2.5grams agarose in 100ml 1xTAE buffer. 5µL EtBr(ethidium bromide) was added. 10µL PCR product was mixed with 2µL loading dye was loaded on agarose gel and electrophoresed at 100V for 30 minutes. After electrophoresis, the gel was visualized and photographed in Gel Documentation system (BioRad).

CHAPTER 4
RESULTS AND DISCUSSIONS

4.1. Regeneration and multiplication of somaclonal variants

The in-vitro grown shoots regenerate from callus were subcultured on MS-media supplements with growth hormones IBA and KN in above mentioned concentrations, so we subcultured the shoots for 25 times on those respective media for developing variants having different concentrations of Valepotrites. Though we did not notice distinct morphological varitions but content varitions were there (table4.2)

Out of tested 10 different media combinations, MS medium supplemented with KN:IBA in the ratio 3:1 was found to be the best for the micropropagation of

V. jatamansi with maximum number of shoots (32) under in vitro conditions(Fig4.1-4.4). Sood and Chauhan,(2009) have also shown MSmedia supplemented with KN:IBA in the ration 3:1 as the best media for the micropropagation of Picrorhizakurroa plants under invitro conditions.

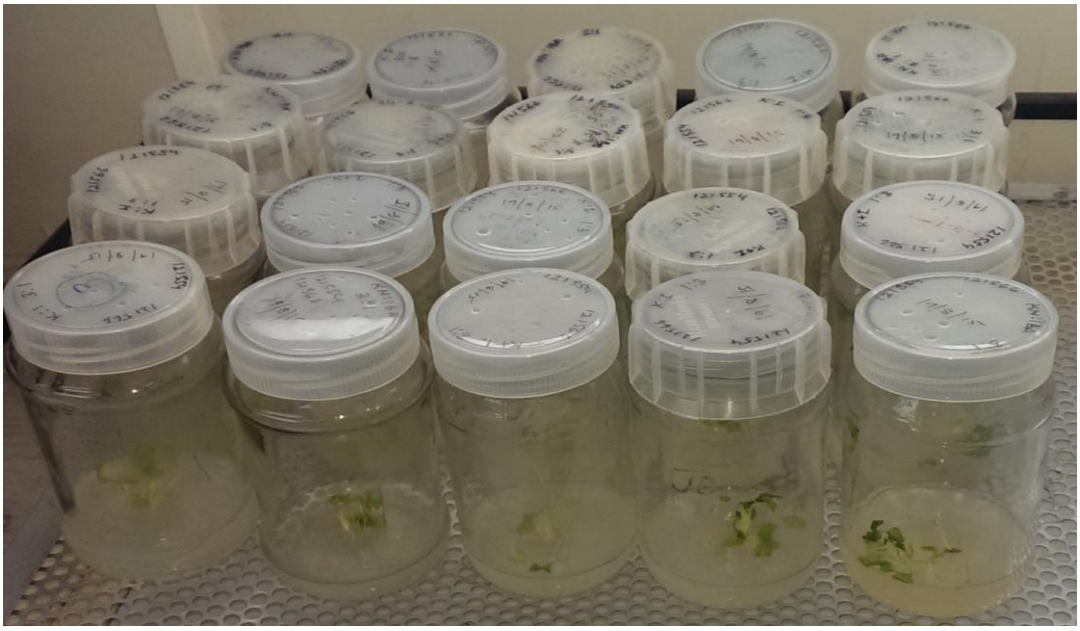


Fig 4.1. Incubation of somaclonalvariants of V. jatamansi on MS Media

Fig 4.2.Subcultured plants of *V. jatamansi*showing growth

Fig 4.3. V.j 3 grown after 25 subcultures

Fig 4.4 V.j 7 grown after 25 subcultures

Table 4.1. Effect of Ms media on number of shoots with different growth hormone combination in somaclonal variants of V. jatamansi

MS Media	Somaclonal variants	IBA:KN:BAP	Number of Shoots
MS-1	Vj-1	1:2:0	23
MS-2	Vj-2	1:0:1	24
MS-3	Vj-3	3:1:0	30
MS-4	Vj-4	3:1:0	27
MS-5	Vj-5	0:1:3	25
MS-6	Vj-6	1:0:0	24
MS-7	Vj-7	1:3:0	32
MS-8	Vj-8	0:1:0	25
MS-9	Vj-9	1:0:3	26
MS-10	Vj-10	3:0:1	25

Maximum numbers of shoots were observed in the MS Media having growth hormone concentration in the ratio IBA: KN: BAP (1:3:0)on which VJ 7somaclonal variant gave max. of 32 shoots after 25thsublutring . The leaf size was observed to be larger in this combination as compared to the other combinations but VJ 3also gave 30 number of shoots on Ms media IBA: KN: BAP(3:1:0) of growth hormones.So these two variants were further utilized for molecular screening for already designed primers for high content of valepotriates.

4.2.Quantification of valepotriates

Field grown shoots and *in-vitro* grown shoots of somaclonal variants of *V. jatamansi*were estimated for Valepotriate content .Out of in ten variantsVJ-3 and VJ-7 gave highest content (0.8 and 0.9%) as compared to the field grown 1.4% which were grown under the ten different combinations of IBA, Kinetin and BAP. (Table 4.2)

Table 4.2. HPLC forValepotriate content in different samples of *V. jatamansi*

S. No.	Sample Name	Valepotriates %
1	VJ-1	0.01
2	VJ-2	0.1
3	VJ-3	0.8
4	VJ-4	0.1
5	VJ-5	0.3
6	VJ-6	0.09
7	VJ-7	0.9
8	VJ-8	0.2
9	VJ-9	0.3
10	Vj-10	0.4
11	Vj-11 field grown	1.4

4.3. DNA isolation using CTAB method

DNA of shoots from field grown Vj-11 and somaclonal variants Vj-1, Vj-2, Vj-3 and Vj-7 of *V. jatamansi* was successfully extractedusing CTAB method.

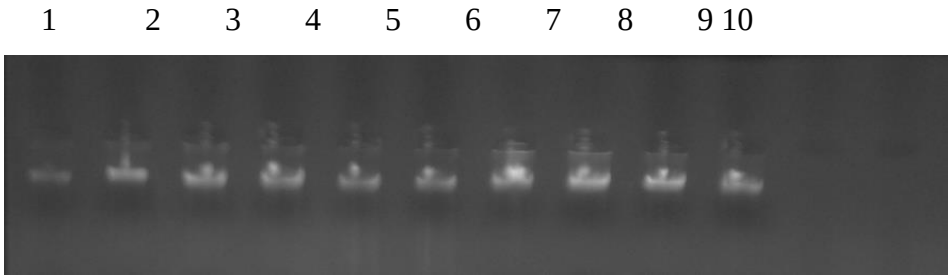


Fig 4.5. Gel Electrophoresis of isolated DNA samples

Wells indicating:

1-2: Field grown samples

3-6: Tissue culturedVj-3 samples

7-9: Tissue culturedVj-7 samples

10µL isolated DNA samples mixed with 2µL gel loading dye were loaded on 0.8% agarose gel and electrophoresed in 1xTAE buffer at 100V for 30minutes. The Gel was visualized using Gel documentation system (BioRad).

4.4. Polymorphism using SSR Markers

Out of tested 25 primer pairs, PKR25D5, PKS25T13 and PKR25M12 showed polymorphism between field grown and tissue cultured plants. Approximately 300bp band and 500bp band was shown by PKR25D5 and PKS25T13 respectively Polymorphism was found among tissue culture grown plants regenerated from same callus and in field grown *V.jatamansi*. Among the tissue from somaclonal variants of Vj-7 and field grown Vj-11 the polymorphism indicates that somaclonal variant shares same set of information for valepotraite production as lies with field grown one. So it could be used as best substitute for field grown variant.

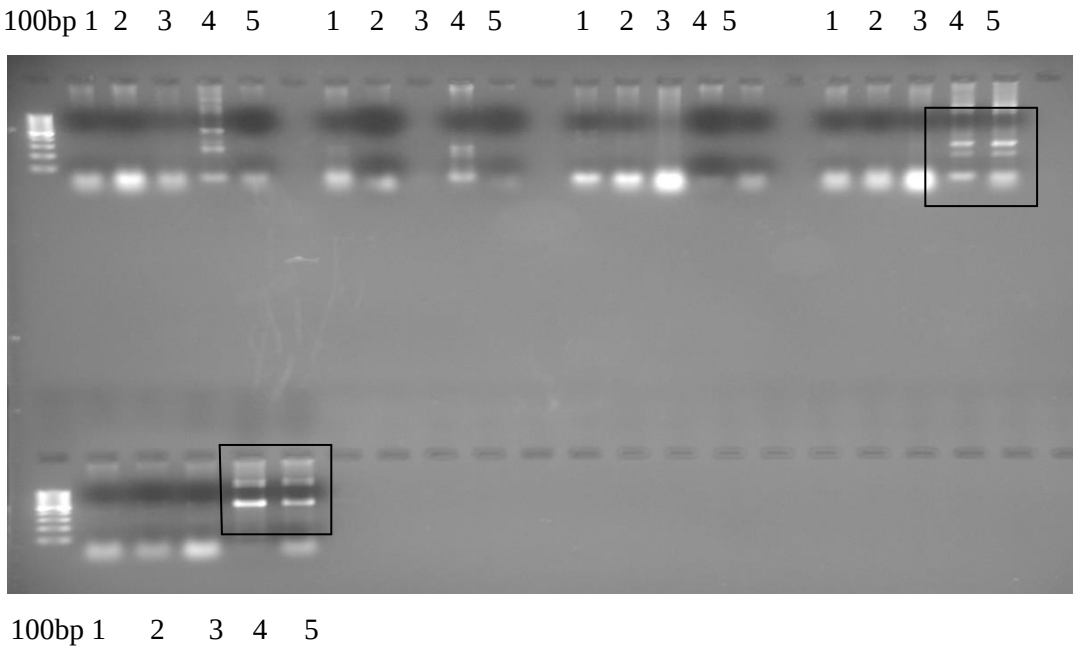


Fig 4.6. Gel electrophoresis showing polymorphism in DNA samples

PCR gel run using PKSTSH5, PKR25M4, PKR25D1, PKR25D5, PKS25T13primers.

Wells indicating:

1– Vj-1Tissue culture

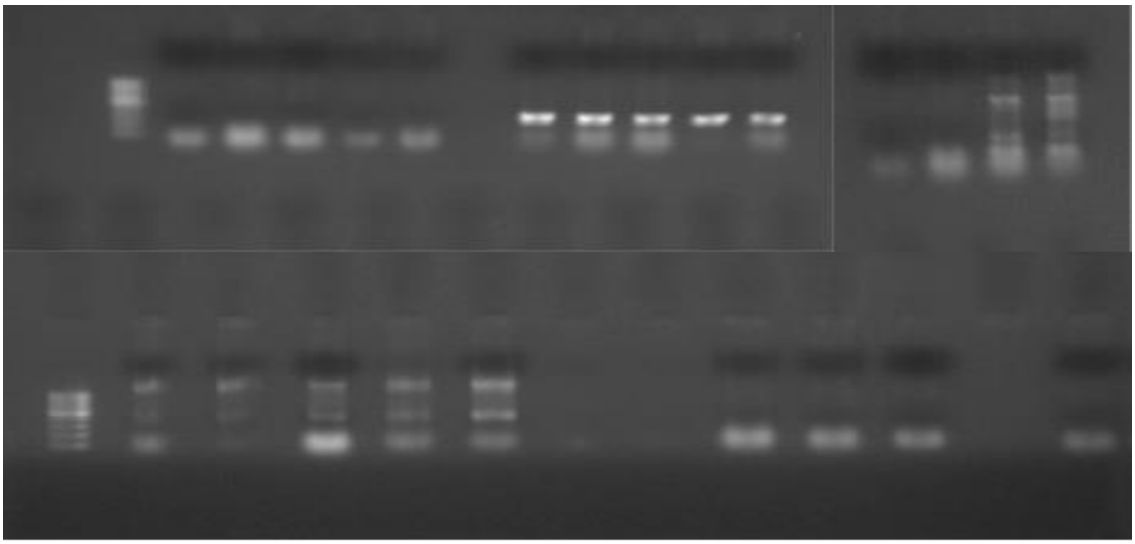
2 – Vj-2 Tissue culture

3 – Vj-3 Tissue culture

4 – Vj-7 Tissue culture

5– Vj-11 Field grown

L 1 2 3 4 5 1 2 3 4 5 1 2 3 4 5



L 1 2 3 4 5 1 2 3 4 5

Fig 4.7. Gel electrophoresis showing polymorphism in field grown plants

PKSTSM11, PKR25M15, PKR25M12,PKS25T9,PKR25D6

Wells indicating:

1– Vj-1Tissue culture

2 – Vj-2 Tissue culture

3 – Vj-3 Tissue culture

4 – Vj-7 Tissue culture

5– Vj-11 Field grown

CHAPTER 5

CONCLUSION

This study shows the utilization of developed tissue culture conditions and molecular characterization of *Valerianajatamansi*. The shoots were regenerated from callus, taken from plant tissue culture chamber of Department of Biotechnology/Bioinforamtics, Jaypee University of Information Technology, Waknaghat, Solan. We tested 10 combinations of MS-media out of which Kinetin and IBA in ratio of 3:1 showed maximum growth. On continuous subculturing of *in vitro* grown shoots of *V. jatamansi*we found variation in valepotriate content in VJ-3 and VJ-7 to be 0.8% and 0.9% respectively, out of ten different genotypes grown under ten different combinations of IBA, Kinetin and BAP.

The rhizome and roots of *Valerianajatamansi*contain an essential oil-valepotriate, which has antibacterial and antiprozoal activity. The isolation of valepotriates and determination of its medicinal properties resulted in wide spread use of this compound as a sedative and in the treatment of hysteria, epilepsy, asthma. The major compounds of *V. jatamansi* are β Vatirenene, β Patchoulene, Dehydroaromadendrene,,β Gurjunene, Patchoulic alcohol, β Guaiene, Alpha Muurolene. The need to grow some variant lines having good content of valepotraites so in future those could be used as alternative raw material for herbal industry and products for aroma therapy. Therefore in this study we found one variant Vj-7 which showed good content of valepotraites which was at par with the field grown sample. We found that three primers PKR25D5, PKR25M12, PKR25T13 showed polymorphism for Vj-7 and field grown as compared to other Vj-1, Vj-2 and Vj-3 somaclonal variant whereas other primers didn't. These studies could be further validated by carrying out expression analysis for their specific characters.

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