International Journal of Genetic Engineering and Biotechnology.
ISSN 0974 3073 Volume 5, Number 2 (2014), pp. 179-184

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Valepotriates Content Variation and Genetic Diversity in High Value Medicinal Herb, *Valeriana jatamansi*

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Abstract

Valeriana jatamansi is an important medicinal herb from Caprifoliaceae family found in the Himalayan region at an altitude of 1500-3600 m. It is known for its medicinal compounds Velerenic acid and Valepotriates. It is recommended for the treatment of insomnia, stimulates the central nervous system, and reduces spasms and anxiety. The aim of the present study was to study genetic diversity in twelve accessions of Valeriana jatamansi varying in Valepotriates content collected from different locations in Himachal Pradesh. Quantification of Valepotriates was done using RP-HPLC. Valepotriates content varied from 1.2%-1.9%. We utilized DNA-based molecular marker technique i.e. simple sequence repeats (SSRs) designed from whole genome transcriptomes of Picrorhiza kurroa to study polymorphism. 220 SSR primers including mono-, di-, tri-, tetra-, penta-, and hexa-nucleotide repeat motifs were tested on genomic DNA of Valeriana jatamansi genotypes, out of which 14 showed polymorphisms. The highest percentage of polymorphic primers were mononucleotide repeats (42.9%) followed by di- (21.4%), tri- and hexa- (14.3%) and tetra- (7.14%) nucleotides. This is the first report on polymorphism study in Valeriana jatamansi using microsatellite markers. This study would be helpful in the development of DNA diagnostics for the authentication of plant material and detection of adulterant plant species.

Keywords:- SSR; polymorphism; markers; genetic diversity

1. Introduction

Valeriana jatamansi is a perennial herb from the *Caprifoliaceae* family and is commonly known as 'Tagar'. It grows wild in temperate Himalayan region an altitude of 1500-3600 m asl (above to sea level). 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. The species is phytochemically well investigated (Mathela et al., 2005; Singh et al., 2006, 2010). The medicinal properties of this plant is due to the presence of Valerenic acid and Valepotriates. The genetic and chemical diversity of *Valeriana jatamansi* has been studied using ISSR and AFLP markers (Rajkumar et al., 2011; Sundaresan et al., 2012). However, no reports are available on DNA fingerprinting of *Valeriana jatamansi* strains by 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).The objective of this study was to study genetic diversity and the extent of polymorphism using microsatellites in twelve strains of *Valeriana jatamansi* collected from different locations in North-Western Himalayan region varying in valepotriates content. The genetic diversity study using SSR markers is being reported for the first time in this medicinal plant.

2. Material and Methods

2.1 Plant material

Twelve genotypes of *Valeriana jatamansi* varying in valepotriates content from 1.2%-1.9% were obtained from Himalayan Forest Research Institute (HFRI), Shimla, Himachal Pradesh.

2.2 Isolation of genomic DNA and PCR amplification

Genomic DNA of 12 genotypes of *V. jatamansi* was isolated using the CTAB method described by Murray and Thompson (1980) with few modifications. Fresh young leaves (100–150 mg) were ground in liquid nitrogen using pestle and mortar. 700µl of prewarmed extraction buffer (100 mM Tris-HCl, pH 8.0; 1.4 M NaCl; 20 mM EDTA; 2% CTAB; 1% PVP; β-mercaptoethanol) was added. The samples were incubated for 60 min at 60 °C in a water bath, protein was removed by extraction with chloroform-isoamyl alcohol (24:1) mixture and DNA was precipitated with chilled isopropanol at -20°C for 1 hour. DNA pellet was washed with 70% ethanol twice and dissolved in 100 µl of autoclaved distilled water. Stock solutions of DNA were stored at -20 °C.

The quality of DNA was checked on 0.8% agarose gel. Quantification of DNA was done in a NanoDrop spectrophotometer and concentration of each DNA sample was adjusted to 100ng/µl.

The SSRs including mono-, di-, tri-, tetra-, penta-, and hexa- nucleotide repeat motifswere identified from *P. kurroa* transcriptome and primers were designed from the region flanking SSR motifs by using an online primer design tool Primer3 (v. 0.4.0) (http://bioinfo.ut.ee/primer3-0.4.0/primer3/). The target amplicon sizes were set at 100-500 bp with optimal annealing temperature of 52°C and optimal primer length of 20 bp. The primers were amplified on genomic DNA of different genotypes of *Valeriana jatamansi* using Polymerase Chain Reaction (PCR). PCR reactions were performed in 12.5µl volume and each PCR reaction consisted of 100ng genomic DNA, 0.5µm of each primer, 200µm dNTPs, and 0.5 units *Taq* polymerase. PCR amplification programs included 94°C for 5 minutes, 35 cycles of 94°C for 45 sec, annealing temperature (47–55C) for 45 sec, 72°C for 2 minutes, and a final extension of 7 minutes at 72°C. 12µl of each PCR product was mixed with 2µl of 6X loading dye (0.25% bromophenol blue, 0.25% xylene cyanol, and 30% glycerol in MilliQ water) and subjected to agarose gel electrophoresis using 2.5% metaphor agarose gel prepared in 1X TAE buffer. The gel was run at a constant voltage of 100 volts for 1.5 to 2 h, stained with ethidium bromide, and analyzed using the gel documentation system AlphaImager EP (Alpha Innotech Corp., USA).

2.3 Polymorphism and statistical analysis

Polymorphism analysis was done by comparing SSR fragments according to their respective sizes on agarose gel by comparing with DNA ladder. Amplicon bands obtained from SSR amplification were scored as 1 or 0 for the presence or absence of amplicons respectively. Binary data matrix was generated from scoring of bands. Popgene (version 1.31) was used to measure variability at each locus: Overall gene frequency, observed number of alleles (na), effective number of alleles (ne), Nei's gene diversity (h) and Shannon's Information index (I).

3. Results And Discussion

3.1 SSR amplification and polymorphism analysis

220 SSR primer pairs were tested for amplification and polymorphism analysis in *Valeriana jatamansi*. Out of 220 primers,203 showed amplification. 14 SSR markers were found to be polymorphic in different genotypes. The highest percentage of polymorphic primers were mononucleotide repeats (42.9%) followed by di- (21.4%), tri- and hexa- (14.3%) and tetra- (7.14%) nucleotides.**Table 1** shows the list of polymorphic SSR markers along with their repeat motif, primer sequence and annealing temperature. A representative polymorphic gel picture is shown in *Figure 1*.

Table 1. Polymorphic SSR markers

S. No.	SSR primer	Repeat Unit	Nucleotide Sequence	Annealing temperature (°C)
1	PKSTS M5	(A)14	5'-ACAAAGACAACAGACGCTCT-3' 5'-GATTCGCAAGAATTGAGAAG-3'	52
2	PKSTS M11	(T)14	5'-GGGACTCTCTTCCTGTTTCT-3' 5'-AAGCTTTCAACCACAAGAAA-3'	51
3	PKSTS H3	(GGTGAA)3	5'-CAAAGTCAACAAGAAGGGAG-3' 5'-CTTGCTGATCTTCTTAGCGT-3'	52
4	PKSTS H5	(CGGGAA)8	5'-AAGGTCTTCTTTACGCTCCT-3' 5'-CTATCTCTTTCCCGTACCCT-3'	53
5	PKR 25 M15	(A)12	5'-ATGACCTGATCAATTCGAAC-3' 5'-CCTCCCCTACAGTATCATCA-3'	51
6	PKR 25 M4	(A)12	5'-CCAACTATGTCAGGAAAAGG-3' 5'-CGCGATTGATTTCTAAGATC-3'	50
7	PKR 25 M12	(C)12	5'-AGGAGGAGCTTAATTTCGTT-3' 5'-GAACTTGGTGTGAGGAAAAC-3'	51
8	PKR 25 M14	(T)12	5'-CACCCACTTTCTGAGATCAT-3' 5'-CCTTTGAAAACATTGTAGCC-3'	49
9	PKR 25 D1	(TA)8	5'-ACTGTGGCCTAGTTGAAGAA-3' 5'-ATGAAACGTCATCTCGAATC-3'	52
10	PKR 25 D5	(AG)7	5'-AAAAGGTCTCCTTCATCCTC-3' 5'-TCTTTTGGTGGTCTTATGCT-3'	52
11	PKR 25 D6	(AT)10	5'-CAGCCAAGAAAGATCTATCG-3' 5'-CCAGGTGATAAATCCACAGT-3'	51
12	PKR 25 TT2	(GTTT)3	5'-TTTCTGGTGGGTATCCTTAA-3' 5'-TCGACTATATCCGCATTTTT-3'	52
13	PKS25T9	(AAG)5	5'-AAGAGGAAGGTGAAAAGACC-3' 5'-GGCACATTCTTGAGATCTGT-3'	51
14	PKS25T13	(TCT)5	5'-ATGCTGTTGGATTTTTACGT-3' 5'-ATCTTGAAAGCCTTCAATCA-3'	52

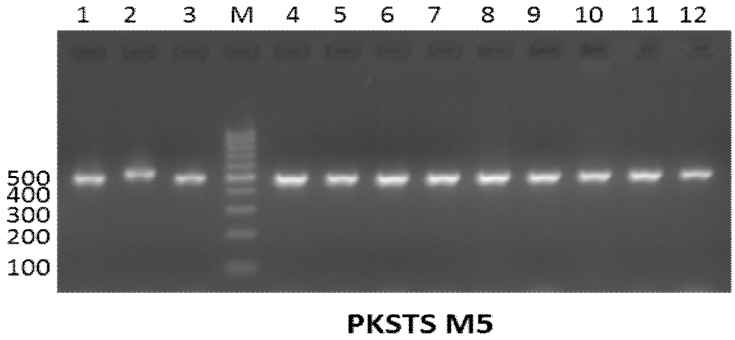

Fig. 1: SSR marker PKSTS M5 showing polymorphism in 12 genotypes (1-12) of *Valeriana jatamansi*. M is 100bp DNA ladder.

3.2 Genetic diversity analysis

Genetic diversity analysis was done using Popgene. Among all genotypes Nei's genetic diversity (h) ranged from 0.28 to 0.48 with a mean value of 0.40 (St. Dev. 0.069). Shannon's information index (I) ranged from 0.45 to 0.68 with mean value of 0.59 (St. Dev. 0.076). The observed number of alleles (na) was 2.0 whereas effective number of alleles (ne) ranged from 1.38 to 1.94 with mean value of 1.69 (St. Dev. 0.185). Summary of genetic variation statistics for all loci is given in **Table 2**. Overall low genetic diversity was observed in twelve genotypes of *Valeriana jatamansi*. The study indicates that *Valeriana jatamansi* populations in the North-Western Himalayan region are not genetically highly diverse as low levels of both polymorphism and genetic diversity was observed; although a large number of populations need to be investigated for significant assessment of genetic diversity.

Table 2. Summary of genetic variation statistics for polymorphic loci

Locus	**Sample size**	**ne**	**h**	**I**
PKS25-T9	12	1.9459	0.4861	0.6792
PKR 25-D5	12	1.6000	0.3750	0.5623
PKSTS-M5	12	1.3846	0.2778	0.4506
PKS25-T13	12	1.9459	0.4861	0.6792
PKSTS-H3	12	1.8000	0.4444	0.6365
PKR 25-TT2	12	1.8000	0.4444	0.6365
PKSTS-M11	12	1.6000	0.3750	0.5623
PKR 25-D6	12	1.6000	0.3750	0.5623
PKR 25-M4	12	1.8000	0.4444	0.6365
PKR 25-D1	12	1.8000	0.4444	0.6365
PKR 25-M14	12	1.6000	0.3750	0.5623
PKSTS-H5	12	1.8000	0.4444	0.6365
PKR 25-M12	12	1.3846	0.2778	0.4506
PKR 25-M15	12	1.6000	0.3750	0.5623
Mean	**12**	**1.6901**	**0.4018**	**0.5895**
St. Dev.		**0.1848**	**0.0689**	**0.0756**

4. Conclusion

Assessment of genetic diversity is an essential component in germplasm characterization and conservation. Further association mapping can be done to correlate genetic diversity with valepotriates content to find out the genetic regions linked to high valepotriates content. Microsatellite markers associated with high valepotriates content would be helpful in the development of DNA diagnostics for the authentication of quality plant material as well as planning a genetic improvement strategy.

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