Mining of Genetic diversity of *Lavatera cachemiriana* native to Kashmir Valley

Mudasir A Mir[1*], Amrina Shafi[2], Insha Zahoor[3]

[1]*Centre for Plant Biotechnology, Division of Biotechnology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, 191121, India.*

[2]*Division of Biotechnology, CSIR-Institute of Himalayan Bioresource Technology, Palampur, Himachal Pradesh, 170761, India.*

[3]*Department of Biotechnology, School of Biological Sciences, University of Kashmir, Srinagar, Jammu and Kashmir, 190006, India.*

[*]**Corresponding author details**:
Email: mudasirbt@gmail.com

ABSTRACT

Lavatera cachemiriana is an important endemic medicinal plant of Kashmir Himalayas, the conservation and sustainable utilization are important areas to safeguard it for the future generations. The study of Genetic diversity proves to be useful tool to devise strategies for tackling extinction of any species. Therefore, In the current research work, genetic diversity of L.cachemiriana was undertaken using microsatellite based ISSR markers. The results indicated statistically significant ($p<0.05$) percentage of polymorphism revealed by markers as 7.50%, 57.14%, 100%, and 100% by ISSR-1, ISSR-2, ISSR-8 and ISSR-9 primers respectively. Nei-Li's similarity index ranged between 0.716 to 0.945. In conclusion, the ISSR based genetic evaluation has revealed lower but significant levels of genetic diversity among collected accessions.

Key words: *Genetic characterization, polymorphism, ISSR, molecular diversity, and monormorphic*

1.INTRODUCTION

Exploration of genetic diversity levels and molecular characterization are emerging as new ways to understand the variation in the genetic makeup of a species. It helps to devise the for conservation, sustainable utilization of any species (Sundaram and Purwar 2011). PCR based molecular markers helps to evaluate genetic diversity and among which Inter Simple Sequence Repeat (ISSR) are ideal ones for study of genetic diversity. These markers are simple, mostly dominant and occasionally co-dominant, highly polymorphic molecular markers, designed from SSR motifs which shows reproducible results and does not require target DNA sequence information (Chikkaswamy and Prasad, 2012).

Lavatera cachemiriana Cambess (Malvaceae) being an important ethno-medicinally important plant of Kashmir Himalayas which is being exploited to such an extent that its distribution is restricted and is considered as an endangered species as per IUCN status (Dar *et al*., 2002; Kaul, M.K. 1997). It is imperative to devise the strategies for its conservation, germplasm collection and sunsequently sustainable utilization. Therefore, in the current work, we have evaluated genetic diversity of different accessions of *Lavatera cachemiriana* native to Kashmir.

2. MATERIALS AND METHODS

Sample Collection

Leaf samples of *Lavatera cachemiriana* Cambess were collected different geographical locations of Kashmir Himalaya, India. The samples were authenticated by a taxonomist and voucher specimen numbers were assigned by Centre for Biodiversity and Taxonomy, University of Kashmir herbarium (KASH).

DNA extraction

DNA was extracted using CTAB method (Doyle and Doyle, 1987; Chikkaswamyand Prasad, 2012).

DNA Quantification and purity check

DNA was quantified using spectrophotometeric approach (Chemito Technologies (Ahmed *et al*., 2013). The ratios of 260nm/280nm was determined and DNA was run on 0.8% (w/v) agarose gel for ~45 minutes. The gel was later on visualized using Gel Documentation system (Vilber Lourmat, Germany) and DNA was kept at -20°C.

Polymerase chain reaction amplification (PCR amplification)

The PCR amplification of target DNA was carried out initially using 8 synthesized ISSR primers and only 4 primers were selected because they gave clear, reproducible and scorable bands. PCR amplification was performed in a final volume of 20µl reaction using 20ng template DNA, the contents of mastermix were added as per manual instructions (Thermo Fischer scientific, USA).

Molecular data collection and analysis:

Markers characteristics were determined such as total number of bands (TB), number of polymorphic bands (PB), number of monomorphic bands (MB), and percentage of polymorphic bands (PPB). Further, polymorphic information content (PIC), effective multiplex ratio (EMR), marker index (MI), resolving power (RP) were calculated (Varshney *et al*., 2007). The pairwise genetic similarity matrices was created using MVSP 3.1 software (Kovach, 2007) to generate by Nei-Li's coefficients (Nei and Li, 1979).

3. STATISTICAL ANALYSIS

All the measurements were carried out in triplicates (mean ± SD). Graph Pad Prism 5.01 (Graph Pad Software, San Diego, CA, USA) was used for analysis.

4. RESULTS AND DISCUSSION

Four out of eight ISSR primers produced clear, scorable and reproducible bands. The primers which didn't produce any proper amplification were as ISSR169,ISSR170, ISSR171 & ISSR172. A total of 35 amplicons/loci were generated in the range of 7-12 with an average of 8.75 bands per primer. The size of amplified bands ranged between 100-15, 00 bp. The highest number of total bands produced by ISSR-8 were 12 and lower number of bands were generated by ISSR-2 primers. Both ISSR-1 and ISSR-9 have produced same number of bands i.e. 8. Furthermore, out of total 35 amplicons, 31 were found to be polymorphic and only 4 were found as monomorphic with an average percentage of polymorphism as 86.16% (Table-1) ISSR profile from various representative gels (Fig.1). A statistically significant ($p<0.05$) percentage of polymorphism was reported as 87.50%, 57.14%, 100%, and 100% by ISSR-1, ISSR-2, ISSR-8 and ISSR-9 primers respectively. The stronger polymorphic percentage of showed by these primers could make them potential markers for identification of genetic diversity in *Lavatera cachemiriana*.

PIC, MI, RP, EMR were calculated and results are presented in Table 1. The highest resolving power (RP) among ISSR primers was showed by ISSR-8 (4.0), followed by ISSR-1 (3.20), then ISSR-9 (2.0) and ISSR-2 (1.60). An average value of RP per primer was found as 2.7. Marker index (MI) data has showed that highest value was contributed by ISSR-1 (1.84) and rest of the primers showed values as 0.64, 1.07 and 0.50 for ISSR-2, ISSR-8 and ISSR-8 respectively. PIC value of each primer was calculated using average PIC value of each loci and it was found that ISSR-1 has shared highest value as 0.30, followed by ISSR-8 (0.21). The effective multiplex ratio (EMR) of each primer was calculated and the highest EMR was showed by ISSR-1 (6.12), followed by (5.0), ISSR-2 (3.54) and ISSR-9 (2.80).

Nei-Li's similarity index of L. *cachemiriana* ranged between 0.716 to 0.945 with an average value as 0.866 (Table 2). The genetic closeness of all five accessions were analysed via UPGMA algorithm i.e. Unweighted Pair-Group Method of Arithmetic average cluster analysis and dendrogram was created based on genetic distance obtained from ISSR markers (Fig.2). The molecular dendrogram has revealed two clusters which joined to form a major cluster at 0.865 level of similarity. The cluster 2 is composed of two accessions (DACH and AHB) which

joined to form a single cluster at 0.938 level of similarity and cluster 1 is again composed of two accessions (TANG and PAMP) which joined at 0.945 level of similarity. The clustering report and dendrogram has indicated that accession of GUL did not form any cluster and showed similarity level of 0.716 with other two clusters.

The results reflected existence of significant but limited genetic differences among collected samples. The presence of smaller levels of molecular diversity within *Lavatera cachemiriana* species has been also indicated by an earlier study on nursery cultivars in the United Kingdom using RAPD markers (Grant and Miller, 2001). The ISSR markers identified in the current work can be used for further studies which could help to devise strategies to safeguard this plant and use it sustainably. The percentage of polymorphism (PPB) data has revealed that ISSR primers showed more percentage of average polymorphic bands I.e. 86.16% as compared to 24.30% by RAPD primers. This plant species has tremendous potential to act as a commercial medicinal plant and its conservation is possible through in-depth genetic studies which would pave way for its use sustainably. The evaluation of genetic diversity of any endangered plant species is vital to i decipher variation at DNA level. As these measures could further help to devise strategies for their sustainable survival.

5. ACKNOWLEDGMENTS

The authors would like to acknowledge the Centre for Biodiversity and Taxonomy, University of Kashmir herbarium (KASH) for identification of collected samples.

6. REFERENCES

Dar,G.H., Bhagat, R.C. and Khan, M.A. 2002. Biodiversity of Kashmir Himalaya. Valley book house, Srinagar Kashmir, J&K.

Sundaram, S. and Purwar, S. 2011. Assessment of genetic diversity among fenugreek (*Trigonellafoenum-graecum* L.), using RAPD molecular markers. *J Med Plants Res.*5, 1543–1548.

Chikkaswamy, B.K. and Prasad, M.P. 2012. Evaluation of genetic diversity and relationships in mulberry varieties using RAPD and ISSR molecular markers. *Int J Mol Bio*. 3, 3.

Kaul, M.K. 1997. Medicinal Plants of Kashmir and Ladhak. Indus PublisihningCo.,New Delhi, 144.

Doyle, J.J., Doyle, J.L., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem bull.*19, 11-15.

Ahmed, N., Nawaz, S., Iqbal, A., Mubin, M., Butt, A., Lightfoot, D. A., Maekawa, M. 2013. Extraction of High-quality Intact DNA from Okra Leaves Despite Their High. *Bioscience Methods. 4.*

Varshney, R. K., Chabane, K., Hendre, P. S., Aggarwal, R. K., Graner, A., 2007. Comparative assessment of EST-SSR, EST-SNP and AFLP markers for evaluation of genetic diversity and conservation of genetic resources using wild, cultivated and elite barleys. *Plant Science*. 6, 638-649.

Kovach, W. L., 2007. MVSP 3.1: Multivariate Statistical Package.

Nei M., Li, W., 1979. *Proc.Natl.Acad.Sci.USA.* 74: 5267-5273.

Grant, M.L. and Miller, D.M. 2001. Genetic and Morphological Analysis of the Origin and Identity of Perennial Lavatera (Malvaceae) Cultivars. *J.Amer.Soc.Hort.Sci.*125, 593–598.

Tables and Figures

Table 1. ISSR Primer characteristics used genetic analysis of *Lavatera cachemiriana.*

Primer name	Primer Sequence	TB	MB	PB	PPB (%)	RP	MI	PIC	EMR
ISSR-1	CACACACACACAGG	8	1	7.00	87.50	3.20	1.84	0.30	6.12
ISSR-2	CTCTCTCTCTCTAC	7	3	4.00	57.14	1.60	0.64	0.18	3.54
ISSR-8	GAGAGAGAGAGAGG	12	0	12.00	100.00	4.00	1.07	0.21	5.00
ISSR-9	GTGTGTGTGTGTGG	8	0	8.00	100.00	2.00	0.50	0.18	2.80

Note: TB- total number of bands, MB- monomorphic bands, PB- polymorphic bands, PPB-percentage of polymorphism, RP-resolving power, MI-marker index, PIC-Polymorphicinformation content, EMR-effective multiplex ratio.

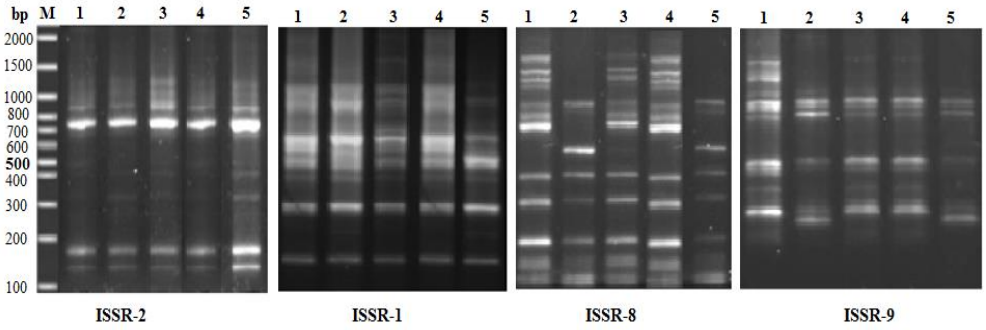

Fig 1. Selected ISSR gel profiles of *Lavatera cachemiriana*

Table 2. Nei-Li's similarity matrix among accessions of *Lavatera cachemiriana* based on ISSR data.

	AHB	PAMP	DACH	GUL	TANG
AHB	1.00				
PAMP	0.83	1.00			
DACH	0.94	0.86	1.00		
GUL	0.76	0.64	0.79	1.00	
TANG	0.85	0.95	0.92	0.68	1.00

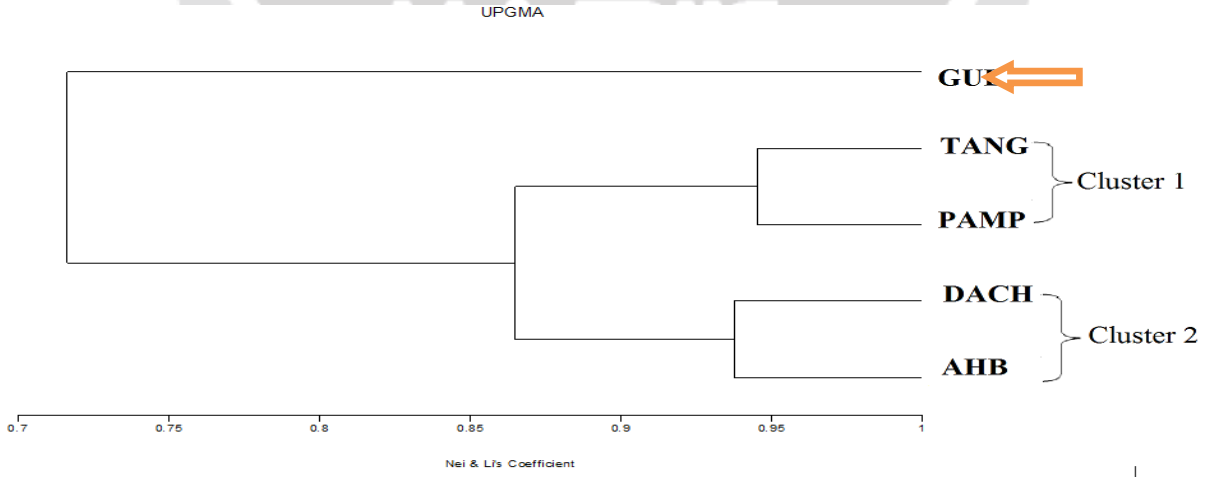

Fig.2. Dendrogram illustrating genetic relationship among different samples of *Lavatera cachemiriana* using ISSR UPGMA clustering method