Received: 09 Aug 2021 | Revised: 17 Mar 2022 | Accepted: 18 Mar 2022

Electronic Journal of Plant Breeding

Research Article

Genetic diversity analysis of selected mulberry accessions using microsatellite markers

Suraksha Chanotra[1*], Romesh Kumar Salgotra[2], Ramesh Kumar Bali[1] and Kamlesh Bali[1]

[1]Division of Sericulture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu Chatha- 18009, India.
[2]School of Biotechnology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu Chatha- 18009, India.
***E-Mail**: chanotrasuraksha@gmail.com

Abstract
The current study was carried out with a set of 44 mulberry accessions in order to evaluate the degree of distinctiveness and relatedness among the selected germplasm accessions. A total of 40 microsatellite markers were selected on the basis of earlier studies, out of which 26 primers yielded a total of 182 polymorphic bands. The PIC (polymorphism information content) values was above 0.5. Based on clusters obtained from unweighted pair group method with arithmetic mean, all the genotypes were grouped into three major cluster namely cluster *viz*., A, B and C having sub-sub-clusters comprising of genetically different accessions. Cluster analysis displayed relatively high degree of genetic variation among the genotypes belonging to neighbouring clusters. Grouping of genotypes was further confirmed by principal component analysis which revealed similar diversification among the studied genotypes. Therefore, the current investigation can be utilized in creating unique genetic profile of mulberry genotypes.

Keywords: Mulberry, fingerprinting, microsatellite, dendrogram, diversity

INTRODUCTION

Mulberry represents one of the most diversified groups of plants belonging to genus *Morus,* family moraceae, division magnoliophyta, class magnoliopsida and order urticales (Arab *et al.,* 2017; Rohela *et al.,* 2020; Gull *et al.,* 2021, Sathyanarayana and Sangannavar., 2021). Most of the cultivated varieties of mulberry are diploid (2n=28) and a few are polyploids (Venkatesh, 2014). Genetic identification or fingerprinting of mulberry germplasm resources is essential for the effective utilization of mulberry genotypes as parental material in breeding programmes in order to make best possible parental combinations for region and season specific genotypes or hybrids. Conventionally, morpho-physiological characterization of mulberry played significant role in phenotypic specification and differentiation (Banerjee *et al.,* 2011; Chanotra *et al.,* 2019; Rahman *et al.,* 2020).

Many investigations were carried out in mulberry genome characterization aided with molecular markers. Simple Sequence Repeats (SSR) (Aggarwal and Udaykumar, 2004; Zhao *et al.,*2005) and Inter- Simple Sequence Repeats (ISSR) (Awasthi *et al*.,2004; Vijayan *et al.,* 2006 a-b; Zhao *et al.,*2007 and Rohela *et al.,* 2018) have been demonstrated as most preferred markers for diversity analysis in mulberry. They were widely used to generate molecular profile and genetic fidelity studies of mulberry genotypes to analyze the genetic relatedness

among germplasm accessions. In view of the advantages concerned with molecular identification of mulberry over morphological identification, the present investigation was carried out with SSR markers for elucidating the genetic diversity among different mulberry cultivars so as to create genetic profile of the germplasm stock for conducting future breeding programmes.

MATERIALS AND METHODS

The current experiment was conducted at Division of Sericulture, Udheywalla in collaboration with School of Biotechnology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu - Jammu, Chatha-India (SKUAST- Jammu). Forty four mulberry genotypes available in mulberry germplasm bank Udheywala campus, SKUAST- Jammu were selected in order to know the extent of genetic relatedness or diversity among them (**Table 1**).

Young leaf samples were harvested randomly from selected genotypes (approximately 5-7g of fresh weight) for genomic DNA isolation using CTAB (Cetyltrimethyl ammonium bromide) method of Doyle and Doyle (1990) with slight modifications. Leaf lamina (500 mg) was finely grounded to powder form with the help of pre-chilled mortar and pestle by adding sufficient amount of liquid nitrogen. To the grounded powder, 1 ml of extraction buffer (pre warmed) was added and incubated in water bath at 65ºC for 35 minutes with occasional stirring. To the mixture, 1:1 of phenol: chloroform was added in equal volume. The samples were subjected to centrifugation at 10,000 rotations per minute (rpm) for 15 minutes. The supernatant layer was transferred to afresh tube and mixed well with Phenol: chloroform: isoamyl alcohol in the ratio of 25:24:1(P:C:I) followed by centrifugation. To the supernatant, 24:1 of chloroform: isoamylalcohol was added and centrifuged. To this, 0.6 ml of Iso-propanol (chilled) was added to the tubes and stored at 4ºC for 1-2 hours. Centrifugation was carriedunder conditions of 4ºC at 10,000 rpm for 10 minutes. The supernatant was discarded and pellet was rinsed with 0.01 M ammonium acetate (200 µl-300 µl) to remove contamination. A volume of 0.6 ml of Iso-propanol was added for precipitation and the content was again subjected to centrifugation at 5,000 rpm for 10 minutes. The white pellet thus collected was again rinsed with 70 per cent ethanol and air dried at room temperature. Genomic DNA thus obtained was dissolved by adding TE buffer (200 µl) and stored at 4ºC. For further purification, 300 µl of RNase (10mg/ml) was added to the DNA sample and incubated for 1 hour in a water bath at 37ºC. The isolated DNA was treated with P:C:I (25:24:1), mixed by tilting and centrifugation was carried out at 10,000 rpm for 10 minutes. The supernatant was then collected in a fresh tube, and 0.6 ml of chilled Iso-propanol was added and centrifuged. The resultant DNA pellet thus obtained was once again rinsed with 70 per cent ethanol and kept for drying for approximately 30 minutes. Finally the dried DNA pellet was dissolved with the addition of 125 µl of TE buffer (Tris-cl, EDTA) and stored at -20ºC.

The concentration of DNA samples was estimated by agarose gel electrophoresis. Presence of fairly pure DNA was detected and thus subjected to amplification bypolymerase chain reaction (PCR) with 40 microsatellite markers (**Table 2**).Clustering of the chosen genotypes was performed through UPGMA analysis based on scoring of data as 1 for presence of band and 0 as absent for generating binomial data matrix (Sneath and Sokal, 1973) and dendrogram based on Jaccard's Similarity Cofficient were generated by using NTSYS program version 2.0 software. Analysis of various parameters including major allele frequency, gene diversity, heterozygosity, polymorphism percentage (PP%) and polymorphic information content (PIC) were derived with the help of R-software.

RESULTS AND DISCUSSION

The capability of SSR primers to discriminate genetic diversity among different genotypes of mulberry was assessed based on characters like major allele frequency, gene diversity, heterozygosity, polymorphism percentage (PP%) and polymorphic information content (PIC) as presented in **Table 3**. Molecular study yielded 182 highly polymorphic bands with 26 primers for forty four mulberry genotypes with selected primers (**Fig.1**). Results presented higher values for major allele frequency of 0.558 for primer M2 at band size of 190 bp, high heterozygosity of 0.870 for primer Mul3SSR105 at 240 bp. The average value for major allele frequency was recorded as 0.351 and primer M2 presented the highest value as 0.558 at 190-210 bp. The highest value for genetic diversity was recorded as 0.909 for primer M6 at 310 bp and highest range of heterozygosity was recorded as 0.870 for primer Mul3SSR105 at 240 bp. The results depicted presence of high polymorphism percentage and PIC value above 0.5, revealing the great amount of genetic variation among forty four different genotypes of mulberry. Hence, the genotypes could be believed to have different genetic makeup with considerable variation in their expression. Similar studies have been reported earlier by Ramesh *et al.* (2004) and Wani *et al.* (2010) utilized similar microsatellite markers for characterization of mulberry genotypes and reported presence of genetic divergence among different mulberry accessions. Kala *et al.* (2016) utilized nineteen mulberry genotypes from the same germplasm bank and conducted assessment of divergence on the basis of morphological and molecular analysis. The results of the study showed close similarity with the present investigation as they too mentioned the mulberry accessions to exhibit wide range of divergence. In addition to this, Wang *et al.* (2017) also recorded presence of genetic diversity in *Morus* spp. with the help of SSR and ISSR markers.

The dendrogram analysis for 44 mulberry genotypes resulted into three major clusters (A, B and C) validating the presence of genetic divergence between them (**Fig. 2**). Cluster-A comprised of twenty one (21) genotypes namely; BC-259, Tr-10, C-763, Asayuki,

Table 1. Pedigree record of studied mulberry genotypes

S.No.	Name of genotype	Donor Name	Nature of breeding	Genetic Nature
1	Asayuki	CSR & TI, Mysore	Cross Pollinated Hybrid	Exotic
2	Enshutukasuka	CSR & TI, Mysore	Collection	Exotic
3	Fukushima	CSR & TI, Berhampore	Collection	Exotic
4	Goshyerami	CSR & TI, Mysore	Selection	Exotic
5	Ichinose	RSRS, Kodathi	Cross Pollinated Selection	Exotic
6	Kairyoroso	CSR & TI, Mysore	Cross Pollinated Hybrid	Exotic
7	Kamabori	CSR & TI, Mysore	Cross Pollinated Hybrid	Exotic
8	Kokuso-20	CSR & TI, Mysore	Mutation	Exotic
9	Kokuso-27	CSR & TI, Mysore	Cross Pollinated Hybrid	Exotic
10	Limencina	CSR & TI, Mysore	Collection	Exotic
11	Miuraso	CSR & TI, Mysore	Collection	Exotic
12	Rokokyoso	RSRS, Kodathi	Clonal Selection	Exotic
13	Shimanouchi	CSR & TI, Mysore	Cross Pollinated Hybrid	Exotic
14	BC-259	CSR & TI, Berhampore	Back Cross Selection	Indigenous
15	Bhrem C-776	CSR & TI, Pampore	Cross Pollinated Selection	Indigenous
16	Behrampur	CSR & TI, Berhampore	Clonal Selection	Indigenous
17	C-763	CSR & TI, Mysore	Cross Pollinated Hybrid	Indigenous
18	Chakmajra	DOS, J&K Govt.	Natural Selection	Indigenous
19	Chinese white	CSR & TI, Mysore	Collection	Indigenous
20	Dhar local	DOS, J&K Govt.	Open Pollinated Hybrid	Indigenous
21	Kanva-2	CSR & TI, Mysore	Cross Pollinated Hybrid	Indigenous
22	KNG	CSR & TI, Mysore	Clonal Selection	Indigenous
23	LF-1	CSR & TI, Mysore	Clonal Selection	Indigenous
24	LF-2	CSR & TI, Mysore	Clonal Selection	Indigenous
25	NS-1	SKUAST-Jammu.	Open Pollinated	Indigenous
26	NS-2	SKUAST-Jammu.	Open Pollinated	Indigenous
27	NS-3	SKUAST-Jammu.	Open Pollinated	Indigenous
28	S-1	CSR & TI, Mysore	Clonal Selection	Indigenous
29	S-30	CSR & TI, Mysore	Mutation	Indigenous
30	S-36	CSR & TI, Mysore	Mutation	Indigenous
31	S-41	CSR & TI, Mysore	Mutation	Indigenous
32	S-54	CSR & TI, Mysore	Mutation	Indigenous
33	S-146	RSRS, Kodathi	Open Pollinated Selection	Indigenous
34	S-799	CSR & TI, Mysore	Open Pollinated Hybrid	Indigenous
35	S-1531	CSR & TI, Mysore	Open Pollinated Selection	Indigenous
36	S-1608	CSR & TI, Berhampore	Open Pollinated Hybrid	Indigenous
37	S-1635	CSR & TI, Berhampore	OPH Selection	Indigenous
38	S-1708	CSR & TI, Berhampore	Open Pollinated Selection	Indigenous
39	Sujanpur	DOS, J&K Govt.	Open Pollinated Collection	Indigenous
40	Tr-1	CSR & TI, Berhampore	Colchiploid	Indigenous
41	Tr-4	RSRS, Kodathi	Polyploid	Indigenous
42	Tr-8	RSRS, Kodathi	Polyploid	Indigenous
43	Tr-10	RSRS, Kodathi	Polyploid	Indigenous
44	V-1	CSR & TI, Mysore	Cross Pollinated Hybrid	Indigenous

Table 2. List of microsatellite primers (SSR) used for diversity analysis

S.No.	Primer	Sequence	Amplicon size (bp)
1	Mul3SSR4 F Mul3SSR4 R	GGAGCAGTCAATCTCTTG CTGGGGTTCAAACTAAGCTC	314
2	Mul3SSR9 F Mul3SSR9 R	GACCAGCCATGAGCCTAC GGTTCACAACCACAATCTCC	365
3	Mul3SSR16 F Mul3SSR16 R	CTAGTAGCAGATCACCAC CGGTCTCTCCCTAATCC	207
4	Mul3SSR17 F Mul3SSR17 R	GTCTTGCACTAGGAGAGG CTCACAGGAGAACACCACC	345
5	Mul3SSR19 F Mul3SSR19 R	CCAAGTCCTCCTCCAG GTTTTGTGACTTGCCG	170
6	Mul3SSR20 F Mul3SSR20 R	CTAGCAGATCGTGGCATTG CTCCGCCCAAAATATCACAC	252
7	Mul3SSR50 F Mul3SSR50 R	CTAGCAGATCCACCAAACC GTTGTTGTACTCTCGCACG	161
8	Mul3SSR53 F Mul3SSR53 R	CAGCTATGACCATGATTACGCC GGACCCTTGATGGCATTG	124
9	Mul3SSR65 F Mul3SSR65 R	CTGGAGTACAAGAACCGCAAC GCCCTCCACCATTGAACTAAG	210
10	Mul3SSR70 F Mul3SSR70 R	GAAGAGGGGAGAGGGAGAGA CAACCAGGATCCAAATAGAAGC	170
11	Mul3SSR71 F Mul3SSR71 R	GGATACTACCTGTTTGGTTGCTG ATTCCCTCCTCAACGAC	360
12	Mul3SSR74 F Mul3SSR74 R	CCCATTGAGGGTTTTGTGAG ATGTGAGCTCGGGATTTGAC	400
13	Mul3SSR80 F Mul3SSR80 R	GAGCCGTTTGATTTCCGTC CAACGGTCGGTGAAAAAGC	140
14	Mul3SSR91 F Mul3SSR91 R	CATGAACCGTTGGATCACAG ATCCCAGATCCCAAATACCC	227
15	Mul3SSR93 F Mul3SSR93 R	CAGCCAATGCACTTTTAACG GTGGAGCTTCTGTTGAGC	340
16	Mul3SSR94 F Mul3SSR94 R	CCCTCATGTGTTCCATCTACC CAGAATCACAGCCGAGGAAG	195
17	Mul3SSR95 F Mul3SSR95 R	GATCATCGTGCCAATAAGCC TAAGAGCTGAGAGGGGAAGC	209
18	Mul3SSR97 F Mul3SSR97 R	TCCACCACTGAACCAAATC ATTAGGGTTGTGACGACGAC	292
19	Mul3SSR102F Mul3SSR102R	TTGGTTGCTGAGAAATGCAG TTGTCGATGGAAAACACGAC	225
20	Mul3SSR103F Mul3SSR103R	GGTCAGATCAGTTTCGTTGC GTAAGAGCTGAGAGGGGAAG	235
21	Mul3SSR105F Mul3SSR105R	GCAGAATCCCAAGTTAATGCC CCTCATAGAGTACAGGAACCG	240
22	Mul3SSR114F Mul3SSR114R	GCAACTCTGCCTTGTTTTC TGGTGCCTTAGACCAGAC	102
23	Mul3SSR116F Mul3SSR116R	CCAAGGAAGGTGAAATCC CATGAACCGTTGGATCACAG	277
24	Mul3SSR122F Mul3SSR122R	GGTGATGGGCTTTTGATG GTTGGATCTGAGGAGGGTC	219
25	Mul3SSR131F Mul3SSR131R	ACTGTGCTTCGTGGAGTTG GAGAGCTTCGAGAGGGAGG	300
26	Mul3SSR187F Mul3SSR187R	GGACATTTCACAACCCTG AACTGCAAGTTGGCACAG	324

27	Mul3SSR197F Mul3SSR197R	GGTGAAAGTTCGTGTGAGTCC CAGCAACTAGAGTGACTTTG	180
28	Mul3SSR203F Mul3SSR203R	GACCGTAGGAGAGAGTGC GGATACCCGCTAAACCCAC	440
29	Mul3SSR229F Mul3SSR229R	CCTTATAGCCGATTTTGCAGGC GAAATTCCGACTCCATGGTC	240
30	Mul3SSR230F Mul3SSR230R	CGGGTGAGCTGGTTTGTTTC CAGCCCCACAATCCCTACT	380
31	SS05	F:TCCAGCAAAGATGTGACAAAAGTT R:TTGCCTTCCCGATTATGCTG	350
32	SS02	F:GCTTCGATCAATCTAGCTTCCC R:GCAAACTACGCCACCCCG	355
33	SS04	F:CGAGGGAGGGATGAGGAGC R:CACATTCATCCACCCTCCTATA	190
34	SS17	F:TACAGGGCTCGGGCAAATG R:TGATCCGAAGCTTGGGGTCT	220
35	SS06	F:ACTCAAAATGAAGGAAAAGGAATTATAC R:TTTACTTAAATCCCAGCCACA	180
36	SS19	F:TTCTGTCGTGTCCTCCGTCAA R:TGAGAACATACACTAATAGGTGAAAAC	300
37	SS09	F:AGAACCCTTCCGCCCTATG R:CCTTGGCGTAGGCAAAGTTG	200
38	SS18	F:TCTTCGCCCGTTGTTCGC R:AGCAATTTTCTTCAACTCACCTTCT	180
39	M2	F:CGTGGGCTTAGGCTGAGTAGAGG R:CACCACCACTACTTCTCTTCTTCCAG	190
40	M6	F:TCCTTAGGTTTTTGGGGTCTGTTTACAT R:CCTCATTCTCCTTTCACTTATTGTTG	310

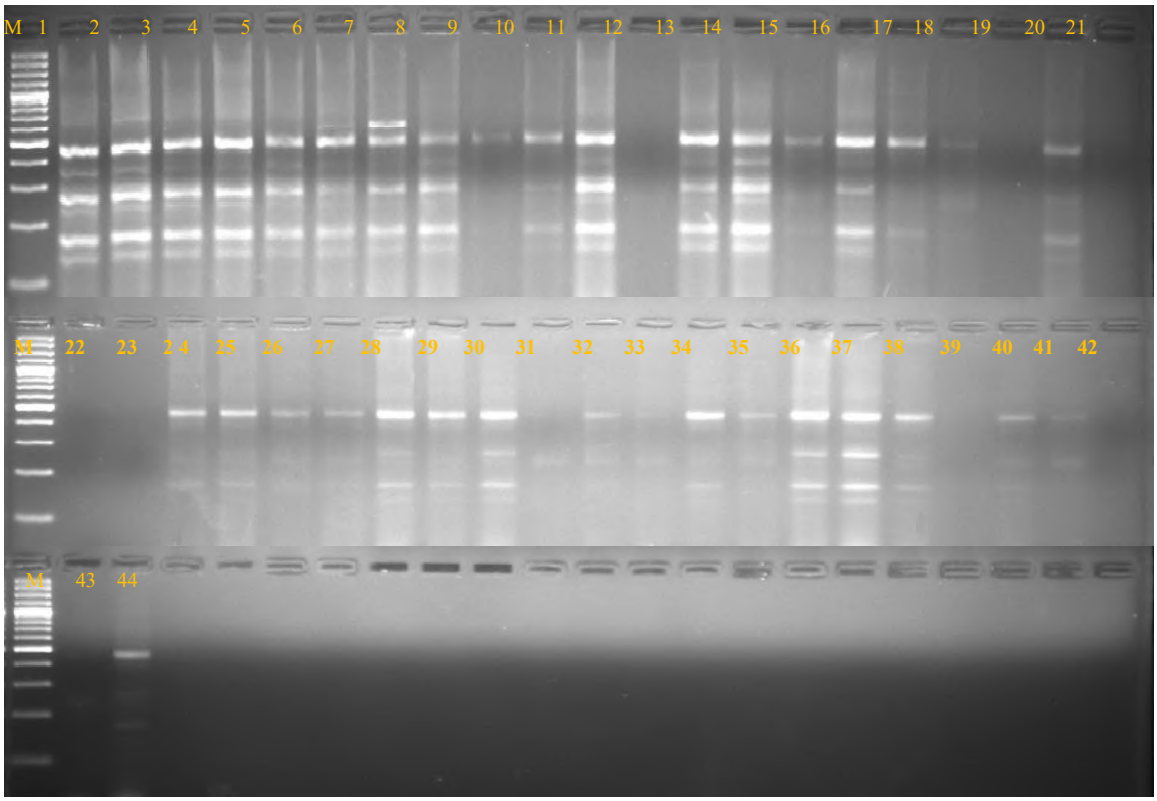

Fig. 1. PCR amplification obtained with primer Mul3SSR9: M; molecular ladder, 1-44; mulberry genotypes

Table 3. Major allelic frequency, gene diversity, heterozygosity, polymorphism percentage and PIC content for the studied primers

S.No.	Primer	Band size (bp)	Major allele frequency	Gene diversity	Heterozygosity	PP	PIC
1.	Mul3SSR9	365	0.289	0.850	0.137	50.00	0.836
2.	Mul3SSR19	170	0.173	0.850	0.057	33.30	0.832
3.	Mul3SSR65	210	0.428	0.752	0.125	60.00	0.726
4.	Mul3SSR70	170	0.277	0.827	0.034	100.00	0.805
5.	Mul3SSR74	400	0.266	0.773	0.036	100.00	0.737
6.	Mul3SSR80	140	0.314	0.813	0.159	14.20	0.790
7.	Mul3SSR91	227	0.454	0.578	0.045	33.30	0.486
8.	Mul3SSR93	340	0.423	0.742	0.108	20.00	0.710
9.	Mul3SSR94	195	0.500	0.648	0.066	33.30	0.592
10.	Mlrl3SSR97	292	0.256	0.820	0.850	14.20	0.796
11.	Mul3SSR102	225	0.400	0.700	0.026	33.30	0.645
12.	Mul3SSR103	235	0.318	0.801	0.580	40.00	0.775
13.	Mul3SSR105	240	0.268	0.855	0.870	25.00	0.840
14.	Mul3SSR114	102	0.285	0.836	0.022	100.00	0.818
15.	Mul3SSR122	219	0.342	0.788	0.186	16.60	0.759
16.	Mul3SSR131	300	0.500	0.580	0.770	33.30	0.493
17.	Mul3SSR197	180	0.548	0.626	0.237	25.00	0.580
18.	Mul3SSR203	440	0.428	0.704	0.037	66.60	0.657
19.	Mul3SSR229	240	0.333	0.753	0.062	25.00	0.711
20.	Mul3SSR230	380	0.291	0.815	0.079	60.00	0.792
21.	SS05	350	0.294	0.807	0.023	16.60	0.781
22.	SS02	355	0.447	0.720	0.023	40.00	0.684
23.	SS19	300	0.333	0.780	0.072	20.00	0.748
24.	SS18	180	0.275	0.78	0.228	20.00	0.744
25.	M2	190	0.558	0.634	0.264	20.00	0.600
26.	M6	310	0.136	0.909	0.107	36.30	0.901
	Mean	-	-	**0.759**	**0.200**	**39.84**	**0.724**

Kanava-2, S-799, Sujanpur, Fukushima, Kamabori, Chakmajra, Kairyoroso, Behrampur, S-1, Goshyoerami, Kokuso-20, Enshutukasuka, Rokokyoso, Kokuso-27, Shimanouchi, V-1 and S-146. Cluster-B comprised of twelve (12) genotypes namely; Tr-4, Tr-8, LF-1, LF-2, S-36, S-30, Miuraso, Dal-local, Bhrem C-776, S-1708, S-1608 and S-1635. Cluster-C comprised of eleven (11) genotypes namely; NS-2, NS-1, KNG, Chinese-white, Ichinose, S-1531, S-41, S-54, T-1, NS-3 and Limencina. Cluster analysis revealed high level of genetic variation among the genotypes belonging to neighbouring groups showing distinctiveness among the genotypes under different clusters. The degree of divergence between genotypes under different groups could be attributed to the expression of their genetic constitution and its interaction towards their native places. Similar reports on genetic variation in *Morus* spp. due to the extent of genetic expression and its interaction with surrounding environment had been presented earlier by different workers such as; Rao *et al*. (2011), Chikkaswamy *et al.* (2012 and 2014), Sharma *et al*. (2015) Wani *et al*. (2015) and Sheet *et al.* (2018).

Grouping of 44 mulberry genotypes as revealed by UPGMA analysis was further confirmed by Principal component analysis (PCA) and results of studied accessions thus obtained were represented in 2D and 3D plot (**Fig. 3and 4**). PCA revealed similar results with relation to similarity matrix as depicted by UPGMA dendrogram. PCA grouped 44 accessions into three major groups as Group-I, II and III, each containing genotypes with high degree of relatedness within the group and high divergence between the groups. PCA observations revealed similar genetic differentiation as reported earlier by Zhao *et al.* (2006). Further genotypes belonging to Cluster-A and B combined to form Group-I

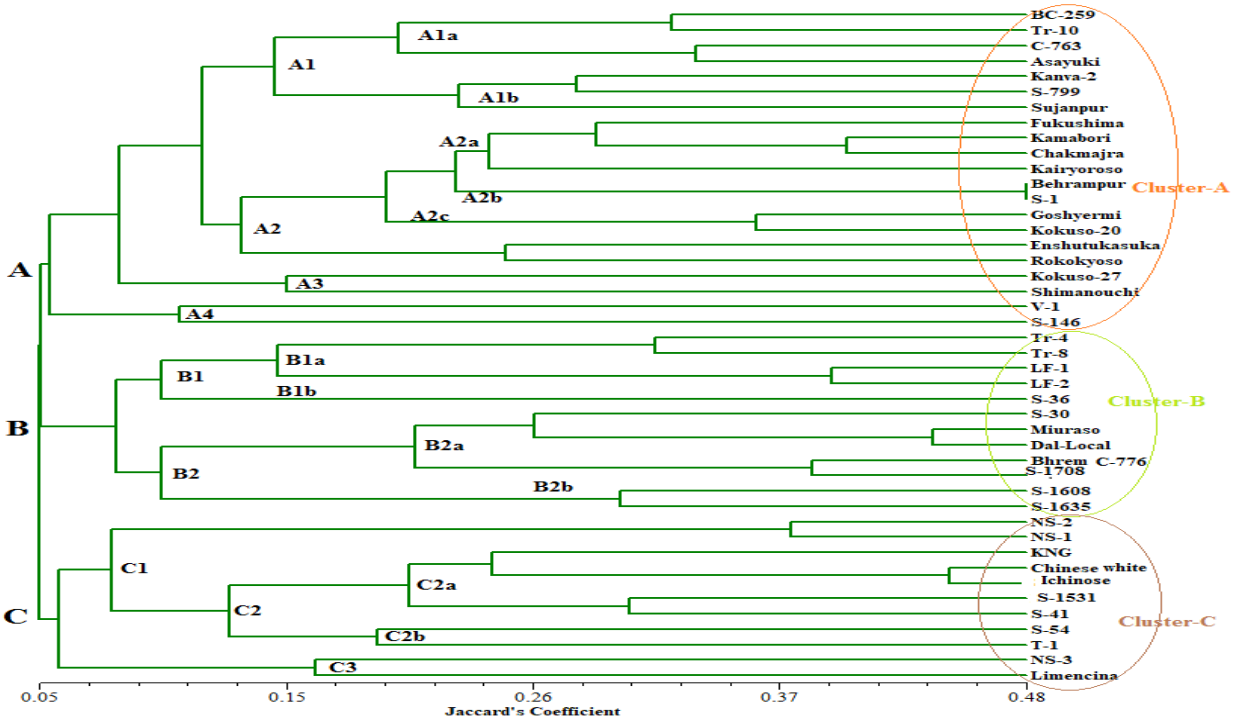

Fig. 2. Cluster dendrogram of 44 mulberry genotypes based on Jaccard's Cofficient

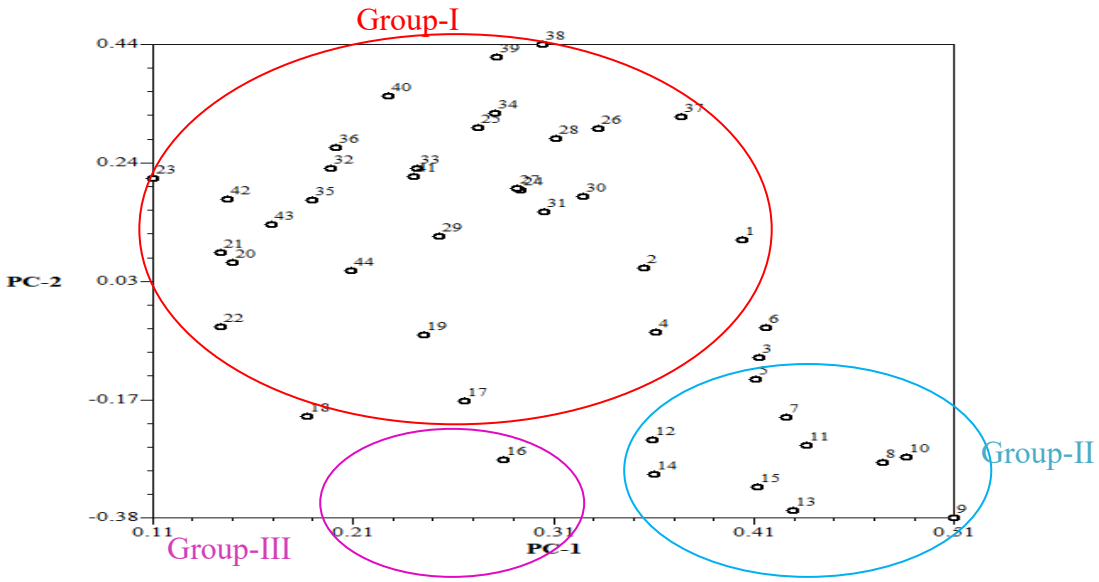

Fig. 3. Centroids of 44 mulberry genotypes according to the 2-D PCA based on similarity matrix

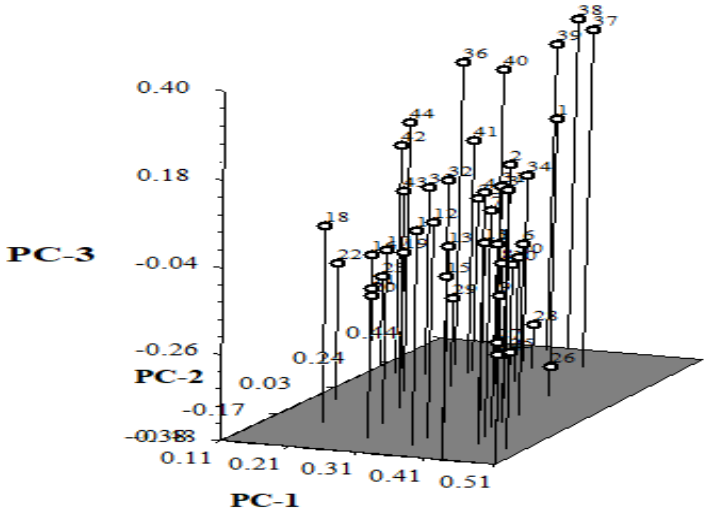

Fig. 4. Centroids of 44 mulberry genotypes according to the 3-DPCA based on similarity matrix

under PCA. Such results had earlier been advocated by Wang *et al.* (2017), where they described combining of genotypes of Group-III and Group-IV to form Cluster-V. Similar reports on PCA was also demonstrated by Boeing *et al.* (2014) and Das and Mandal, (2018) for describing the divergence among mulberry collections.

Estimation of genetic variability among germplasm collections is useful tool which facilitates efficient management and utilization of available resources. As such, it is prudent to have the genetic characterization of mulberry by using microsatellite markers to elucidate the genetic diversity and to eliminate duplicates accessions through DNA fingerprinting so as to create genetic profile of the available germplasm accessions. Molecular analysis of 44 mulberry accessions available at mulberry germplasm bank of SKUAST-Jammu comprising of mulberry genotypes commonly cultivated in temperate and tropical areas of the country with the help of SSR marker yielded 182 highly polymorphic bands with 26 primers. Results showed higher values for major allele frequency of 0.558 for primer M2 at band size of 190 bp and high heterozygosity of 0.870 for primer Mul3SSR105 at 240 bp. Whereas, value for highest genetic diversity was recorded as 0.909 for primer M6 at 310 bp. High polymorphism percentage and PIC value above 0.5, revealing the great amount of genetic variation among forty four different genotypes of mulberry was recorded in case of molecular analysis. Further the genotypes were subjected to clustering based on Jaccard's similarity cofficient which categorised 44 genotypes into three main cluster with genetically different accessions. The current investigation thus provides a platform for selection of diverse parental stock for mulberry breeding programmes aimed at improvement of the mulberry genetic stock.

REFERENCES

Aggarwal, R. K., Udaykumar, D., Hendre, P. S., Sarkar, A. and Singh, L. I. 2004. Isolation and characterization of six novel microsatellite markers for mulberry (*Morus indica*). *Molecular Ecology Notes,***4**: 477-479. [Cross Ref]

Arab, J. R., Nirmale, D. and Jain, B. B. 2017. Evaluation of anthelmintic activity of *Morus alba* stem. *European Journal of Biomedical and Pharmaceutical Sciences*, **4** (05): 303-307.

Awasthi, A. K., Nagaraja, G. M., Naik, G. V., Kanginakudru, S., Thangavelu, K. and Nagaraju, J. 2004. Genetic diversity and relationships in mulberry (genus *Morus*) as revealed by RAPD and ISSR marker assays. *BMC Genetics,* **5**(1): 1-9. [Cross Ref]

Banerjee, R., Roychoudhury, S., Sau,H., Das, B. H., Saha, A. K., Saratchandra, B. and Bajpai, A. K. 2011. Phenotypic divergence in mulberry (*Morus* spp.) germplasm collections. *Journal of Crop Improvement,***25**(5): 459-471. [Cross Ref]

Boeing, J. S., Barizao, E.O., Silva, B.C., Montanher, P, F., Almeida, V.C. and Visentainer, J. V. 2014. Evaluation of solvent effect on the extraction of phenolic compounds and antioxidant capacities from the berries: application of principal component analysis. *Chemistry Central Journal*: **4(**8):48. [Cross Ref]

Chanotra, S., Bali, R.K. and Bali, K. 2019. Morpho-physiological characterization of mulberry genotypes (*Morus* spp.) for inheritance in future

breeding programme. *Journal of Pharmacognosy and Phytochemistry,***8**(1): 2695-2701. [Cross Ref]

Chikkaswamy, B. K. and Parananik, C. R. 2014. An investigation of RAPD and ISSR molecular marker studies in mulberry varieties. *International journal of current microbiology and applied sciences,* **3**(6): 986-995.

Chikkaswamy, B. K., Paramanik, R. C., Debnath, A. and Sadana, S. M. 2012. Evaluation of genetic diversity in mulberry varieties using molecular markers. *Nature and Science,***10**(6): 45-60.

Das, D. and Mandal, P. 2018. A comparative study of foliar micro–morphology of six genotypes of *Morusalba* L. from Darjeeling district, West Bengal, INDIA. *Plant Archives,***18**(2): 1471-1478.

Doyle, J. J. and Doyle, J. L. 1990. Isolation of plant DNA from fresh tissue. *Focus,***12**: 13-15. [Cross Ref]

Gull, A., Ramegowda, G. K. and Illahi, I. 2021. Genetic appraisal of frost damage in mulberry germplasm accessions in temperate climate of Jammu and Kashmir, India. *Electronic Journal of Plant Breeding.* **12**(1): 104-108. [Cross Ref]

Kala. P., Zargar, S.M., Bali, R.K., Gupta, N., Salgotra, R.K. and Koul, A. 2016. Assessment of genetic diversity in mulberry using morphological and molecular markers. *Electronic Journal of Plant Breeding*, **7**(1): 94-103. [Cross Ref]

Rahman, M.S. and Islam, S.M.S. 2020.Genetic diversity analysis based on morphological characters in mulberry (*Morus*spp.). *J. Bio-Sci,***28**: 111-119. [Cross Ref]

Ramesh, Aggarwal, D., Udaykumar, D., Hendre, P. S., Sarkar, A. and Singh, L. I. 2004. Isolation and characterization of six novel microsatellite markers for mulberry *(Morus*spp.). *Molecular Ecology Notes*, **4**:477-479. [Cross Ref]

Rao, A.A., Vijayan, K., Krubakaran, M., Borpujari, M.M. and Kamble, C. K. 2011. Genetic diversity in mulberry (*Morus* spp.) revealed by isozyme markers. *The Journal of Horticultural Science and Biotechnology,***86**(3): 291-297. [Cross Ref]

Rohela, G.K, Jogam, P., Mir, M.Y., Shabnam, A.A., Shukla, P., Abbagani, S., Azra, N.K. 2020. Indirect regeneration and genetic fidelity analysis of acclimated plantlets through SCoT and ISSR markers in *Morusalba* L. cv. Chinese white: *Biotech Rep,* **25**: 1-12. [Cross Ref]

Rohela, G.K., Jogam, P., Shabnam, A.A., Shukla, P., Abbagani, S., Ghosh, M.K. 2018. *In vitro* regeneration and assessment of genetic fidelity of acclimated plantlets by using ISSR markers in PPR-1 (*Morus* sp.): An economically important plant. *SciHorti,* **241**: 313–321. [Cross Ref]

Sathyanarayana, K. and Sangannavar, P.A. 2021. Genotype x Environment interaction and stability analysis for leaf yield of mulberry (*Morus alba* L.) under alkali affected soils. *Electronic Journal of Plant Breeding*, **12**(2): 435-443. [Cross Ref]

Sharma, A., Krisha, V., Kour, P. and Reyal, R. 2015. Characterization and screening of various mulberry varieties through morpho-biochemical characteristics. *Journal of global bioscience,***4**(1): 1186-1192.

Sheet, S., Ghosh, K., Acharya, S., Kim, K. P. and Lee, Y. S. 2018. Estimating genetic conformism of Korean mulberry cultivars using Random Amplified Polymorphic DNA and Inter-Simple Sequence Repeat profiling. *Plants*, **7**(21): 01-10. [Cross Ref]

Sneath, P. H.A. and Sokal, R. R. 1973. Numerical Taxonomy: The Principles and Practice of Numerical Taxonomy.W. H. Freeman and Co., San Francisco, CA, USA. P. 513.

Venkatesh, K. H. 2014. Studies on micromorphology and karyotype analysis of three mulberry genotypes (*Morus*spp.). *American Journal of Phytomedicine and Clinical Therapeutics,* **3**(02) 192-198.

Vijayan, K, Srivatsava, P. P., Nair, C. V., Awasthi, A. K., Tikader, A., Sreenivasa, B. and Urs, S. R. 2006a. Molecular characterization and identification of markers associated with yield traits in mulberry using ISSR markers. *Plant Breeding,* **125**: 298-301. [Cross Ref]

Vijayan, K., Tikader, A., Kar, P. K., Srivastava, P. P., Awasthi, A. K., Thangavelu, K. and Saratchandra, B. 2006b. Assessment of genetic relationships between wild and cultivated mulberry (*Morus*) species using PCR based markers. *Genetic Resources and Crop Evolution,* **53**(5): 873-882. [Cross Ref]

Wang, Z., Zhang, Y., Dai, F., Luo, G., Xiao, G. and Tang, C. 2017. Genetic diversity among mulberry genotypes from seven countries. *Physiology and Molecular Biology of Plants,***23**(2): 421-427. [Cross Ref]

Wani, S. A., Bhat, M. A., Malik, G. A. and Mir, M. R. 2015. Molecular characterization of mulberry (*Morus* spp.) genotypes by Inter-simple Sequence Repeat markers. *Research Journal of Biotechnology,***10**(4):53-61.

Wani, S. A., Bhat, M. A., Malik, G. N., Kamilli, A. S., Mir, M. R., Bhat, S. A., Wani, N., Razvi, M. S., Sabina, A. and Bhat, A. K. 2010. Molecular markers and their role in mulberry improvement. *International Journal of Current Research*, **4**: 022-024.

Zhao, W. G., Zhou, Z. H., Miao, X. X., Wang, S. B., Zhang, L., Pan, Y. and Huang, Y. P. 2006. Genetic relatedness among cultivated and wild mulberry as revealed by inter-simple sequence repeat (ISSR) analysis in China. *Canadian Journal of Plant Science*, **86**:251-257. [Cross Ref]

Zhao, W. G., Zhou, Z. H., Miao, X. X., Zhang, Y., Wang, S. B., Huang, J. H., Xiang, H., Pan ,Y. L. and Huang, Y. P. 2007. A comparison of genetic variation among wild and cultivated *Morus* species (Moraceae*: Morus*) as revealed by ISSR and SSR markers. *Biodiversity and Conservation,* **6**:275– 290. [Cross Ref]

Zhao, W., Miao, X., Jia, S., Pan, Y. and Huang, Y. 2005. Isolation and characterization of microsatellite loci from the mulberry, *Morus* L. *Plant Science,* **168**: 519-525. [Cross Ref]