Indian Res. J. Genet. & Biotech, 8(3) : 202-209 (Aug. 2016)

Molecular Characterization of Different Grape Varieties by Using RAPD and ISSR Marker

Shinde A. S.[1], Mahajan S. R[2], Adlinge P. M. Hembade V. L[3] and Khandale T. M. [4]

Lokmangal Agricultural Biotechnology College, Wadala, Solapur, Maharashtra- 413 222

(Received : May, 2016 : Revised : June, 2016 : Accepted : July 2016)

Abstract

Six genotypes of grape were characterized by using eight RAPD and five ISSR primers. The RAPD studies revealed 71.42% average polymorphism across six genotypes of grape with ten mean numbers of polymorphic bands and polymorphism ranged from 33 to 100%. The dendrogram constructed using pooled RAPD loci data clearly showed two clusters. The highest genetic similarity (0.52) was found between sarita and nanasaheb while lowest genetic similarity (0.22) found between manikchaman and sharad cultivars. The mean similarity index was found to be 0.55 among all six grape cultivars. The ISSR analyses with five primers produced 117bands with23 average numbers of bands per primer and the polymorphism ranged from 42 to 100%. The average percent polymorphism was 69.23%. Cluster analysis based on ISSR data clearly grouped all six varieties in two main clusters. In the ISSR analysis thompson seedless and sarita were shown to be more genetically similar while sharad seedless and nanasaheb purple seedless were shown to be distant varieties. The findings of RAPD and ISSR studies with different varieties of grapes will be helpful for grape improvement. The present study also indicates RAPD as well as ISSR markers are reliable for molecular diversity analysis in grape.

Key words: ISSR, RAPD, Molecular Characterization.

Introduction

Grape (Vitis vinifera) belongs to family Vitaceae, and genus Vitis (Ren et al., 2000). Vitisis the only genus with economic importance in the world with a long history of domestication being first reported in Occidental Europe in the Tertiary-Quaternary transition. The tendency to cultivate this crop increases worldwide due to consumption diversity, high income, low production expenses and resistance against drought, particularly in dry cultivated varieties (Lowe et al., 2006). It is useful in preparation of raisin, juice, and wine (Winkler et al., 1974). It is a diploid plant and can be easily crossed and selfed. It has a small genome of approximately 500Mbp, equivalent to four times the genome size of Arabidopsis, i.e. 125Mb and has a number of unique features including a novel shoot architecture and non-climacteric fleshy fruit produced from a perennial deciduous woody vine. The fruit also has unique secondary metabolism producing color pigments, tannins, flavor and aroma compounds. Considering grape as one of the most important agricultural products in the world, it is necessary to have a breeding plan to improve its fruits and leaves.

Corresponding Author's E-mail : amolshinde0110@gmail.com
Published by Indian Society of Genetics, Biotechnology Research and Development,
5, E Biotech Bhawan, Nikhil Estate, Mugalia Road, Shastripuram. Sikandra, Agra 282007
Online management by www.isgbrd.co.in, www.irigbt.co.in

Genetic diversity is of great importance in biology sciences such as ecology, evolution biology, taxonomy and genetics. Nowadays, enormous markers with different technical diversity have been identified. So far, morphological, biochemical and molecular markers have been used to distinguish relationship of different grapes varieties. Choosing a certain marker for assigning the DNA polymorphism is always depends on the case of study, special use of markering system, available laboratory equipments and facilities. The desirable features of molecular markers are high polymorphism, codominant inheritance, and frequent occurrence, even distribution throughout the genome, neutral behavior, easy access, easy and fast assay, low cost, high throughput, high reproducibility and transferability between laboratories, populations and/or species. Genetic polymorphism is classically defined as the simultaneous occurrence of a trait in the same population of two or more discontinuous variants or genotypes. Welsh and McClelland (1991) developed a new PCR-based genetic assay namely randomly amplified polymorphic DNA (RAPD) along with these many other markers like Simple Sequence Repeat (SSR) (Tautz, 1989), Inter Simple Sequence Repeat (ISSR) (Zietkiewicz et al., 1994), Amplified Fragment length Polymorphism (AFLP) and Restriction Fragment Length Polymorphism (RFLP) (Botstein et al., 1980) has been existed. Among these RAPD is technically simple, robust and useful to characterize the different plant genotypes and are require PCR amplification (Saiki et al., 1985, 1988) of random genomic DNA segment with single primer of arbitrary sequence and it has no need of prior information of the DNA sequence and feasibility of automation. The Inter Simple Sequence Repeat (ISSR) is a PCR based marker in which DNA fragments located between adjacent, oppositely oriented microsatellites are amplified by Polymerase Chain Reaction (PCR) using primers that are anchored at 5' or 3' end of a repeat region and extend into the flanking region. The ISSR marker is very repeatable and produces a high polymorphism in most systems and does not require information on genome sequence. The use of such techniques for germplasm characterization facilitates the conservation and utilization of plant genetic resources (Kapteyn and Simon, 2002). The paper deals with the molecular diversity between different varieties of grapes hence it can be used in grape breeding program as well as used for character specific study.

Materials and Methods

Plant Material and DNA Isolation : The six different genotypes of grape viz, Thompson seedless, Sarita, Sonaka,Nanasaheb purple seedless, Sharad seedless, Manikchaman were collected from different grape growing region of Maharashtra and used for DNA isolation. The DNA isolated from six varieties of grapes with the help of CTAB (Cetyltrimethylethyl Ammonium Bromide) method of Doyle and Doyle (1990) and SDS method of Dellaporta et al (1983). The quantification of DNA was carried out at 260nm in spectrophotometer and stock DNA was diluted to make final solution of 20ng/µl for PCR analysis.

RAPD Assay : For assessment of molecular diversity among six genotypes of grape through RAPD followed the method given by Mathews et al. (2007) with minor modifications. The genomic DNA extracted from each genotype was subjected to polymerase chain reaction using 21 random decamer primers of RAPD. Among 21 primers eight were selected for further analysis. Amplification was carried out in a 200 µl thin walled PCR tube containing a 25 µl reaction mix (1X Taq buffer, 15.5µl sterile DDH_2O, 2.5mM MgCl2, 0.25mM dNTP, 10pmol Primer, 1.5U Taq DNA Polymerase, 30 ng DNA). Amplification reactions were carried out for 40 cycles. Each cycle comprised of 1 min at 45ºC, 1 min at 36 ºC and 2 min at 72 ºC. Amplified product were separated on 1.8% agarose gel, stained with ethidium bromide and photographed under UV light.

ISSR Assay : The ISSRs analysis was done following the procedure given by Herrera et al., 2002. Twelve universal primers were used to screen all six genotypes. Among 12 primers five were used for further analysis. The PCR reactions consisted of 1X Taq buffer, 16.5µl sterile DDH_2O, 2.5mM MgCl2, 0.4mM dNTP, 10pmol Primer, 1.5U Taq DNA Polymerase and 50ng DNA for 40 cycles. Cyclic condition were consisted of 94ºC for 1min, 55-65 ºC (depends on melting temperature) for 1:30 min and 72 ºC for 2min. Amplified product were separated on 2% agarose gel.

Data Analysis : For computer analysis molecular data was scored on the basis of the presence or absence of the PCR products. It was designated as '1' if a product was present in a genotype and if absent; it was designated as '0'. The data generated by RAPD and ISSR loci were analyzed with the software NTSYSpc version 2.02 (Rohlf 1994).

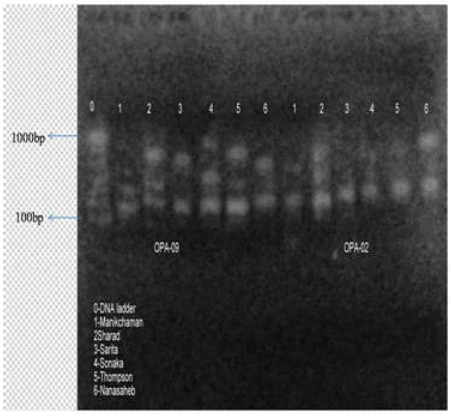
Results and Discussion

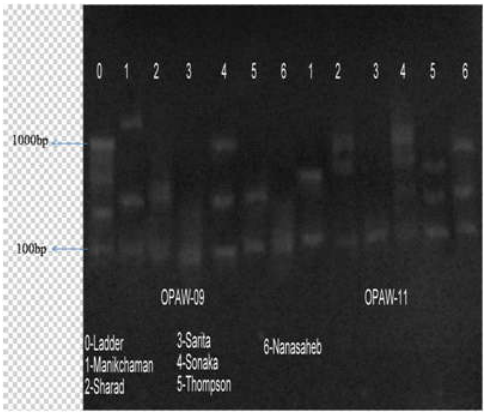
RAPD Markers : Total six genotypes of grape were analyzed by using 21 RAPD primers. Among 21 primers eight primers were selected for final analysis. A total of 109 bands 80 were

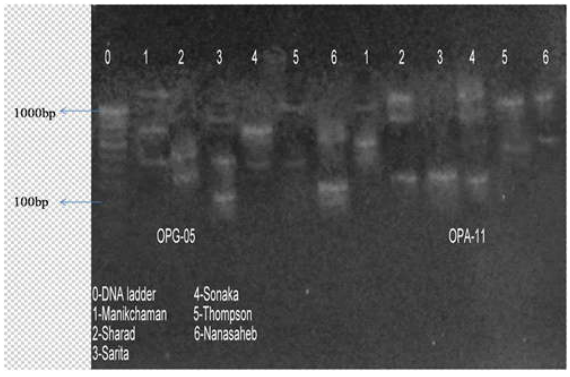
polymorphic and given 71.42% average polymorphic percentage. The same results were found by Singh et al., (2011) analyzed genetic diversity in grape and they reported that OPG-09, OPX-15, OPG-05, OPA-02 and OPA-9given 100% polymorphism. Aras et al., (2005) showed primer OPA-11 and OPA-09 given the polymorphism percentage ranged from 41.1% - 65.2% in different grape varieties. The number of DNA fragment varied from 8 to 20 and mean number of polymorphic bands per primer among six grape genotypes was 13.6 and the size of PCR amplified DNA fragment ranged from 91 to 452bp (Table no 1).The highest polymorphism (100%) was exhibited by primers OPA 11, OPAW 9, OPAW 11 (plate -1) and the PIC value varied from 0.52 to 0.81 (Table no 1).

Table 1: Results of RAPD analysis in six genotypes of grape

Sr No	Primer Name	Primer sequence	LMWB(bp)	HMWB(bp)	TB	PB	Percent polymorphism	PIC value
1	OPA-09	GGGTAACGCC	227.98	1024.5	14	8	57.14	0.71
2	OPA-02	TGCCGAGCTG	102.9	804.01	9	3	33.33	0.52
3	OPG-05	CTGAGACGGA	218.0	982.47	14	9	64.28	0.76
4	OPA-11	CAATCGCCGT	132.17	1098.4	14	14	100	0.82
5	OPAW-09	ACTGGGTCGG	91.83	1047.6	12	6	50	0.67
6	OPAW-11	CTGCCACGAG	153.17	1136.4	8	8	100	0.78
7	OPG-09	CTGACGTCAC	224.41	728.4	20	20	100	0.81
8	OPX-15	CAGACAAGCC	452.71	1228	18	12	66.66	0.74
Total					**109**	**80**		
Average					**13.6**	**10**	**71.42**	**0.72**







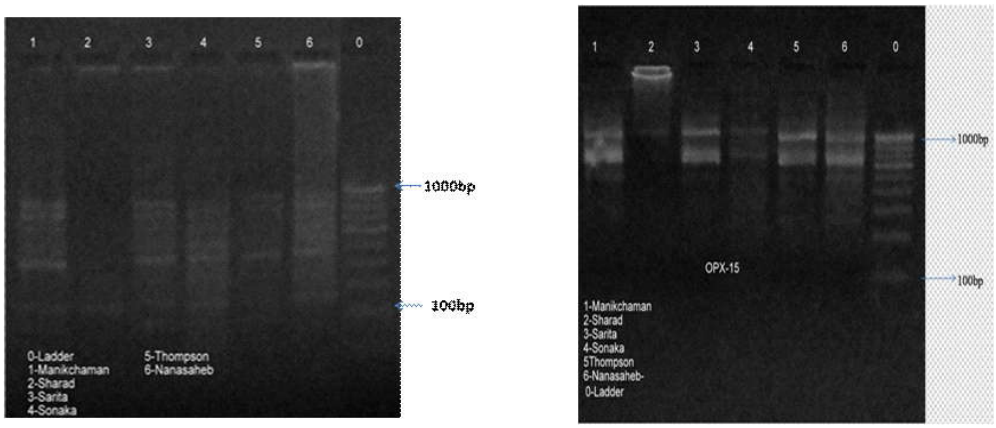
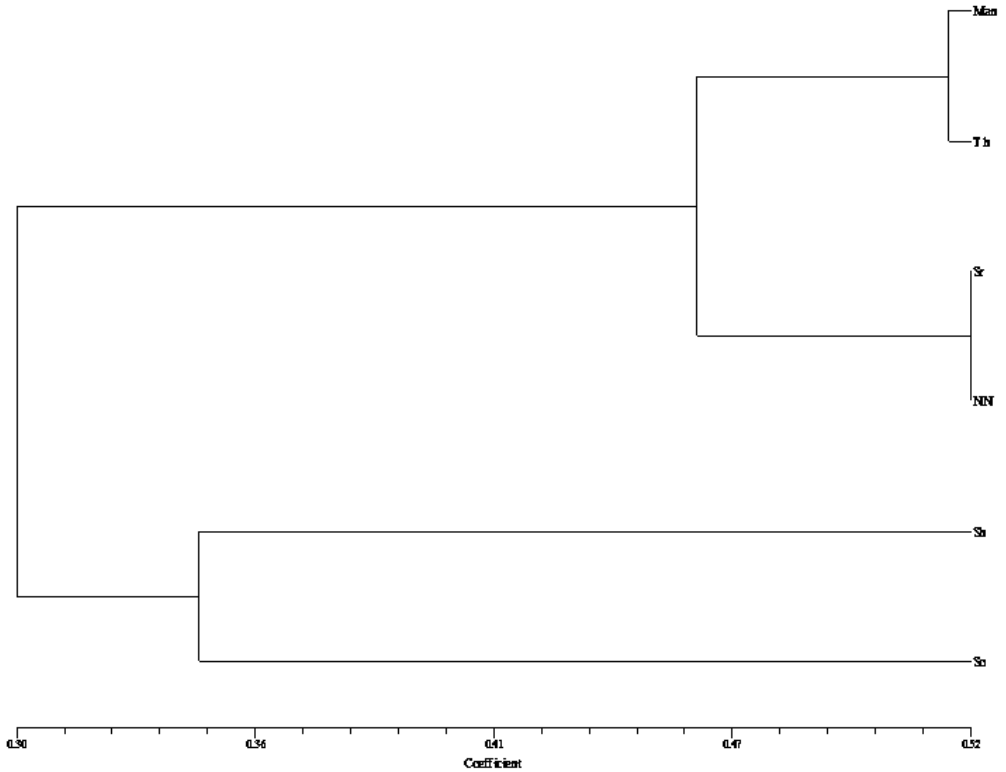


Plate 1 Banding pattern produced by all eight RAPD primers

Genetic Diversity

On the basis of data generated by all eight RAPD primers there wereconstructed dendrogram which grouped the six grape genotypes into two clusters i.e cluster A and cluster B. Cluster A were comprised total four varieties viz, Manikchaman, Thompson, Sarita and Nanasaheb with average similarity coefficient 0.45 while cluster B comprised remaining two varieties viz, Sharad and Sonaka which shown 0.34 similarity coefficient. The highest similarity coefficient revealed by the RAPD's analysis was 0.52 among Sarita, Nanasaheb, followed by Thompson, Manikchaman (0.51). On the other hand, the lowest genetic similarity (0.22) was found between Manikchaman and Sharad cultivars (Table 2). The mean similarity Index was found to be 0.55 among all grape cultivars.



(Man- Manikchaman, Th- Thompson, Sr- Sarita, NN- Nanasaheb purple seedless, So- Sonaka, Sh- Sharad)

Fig. 1 Dendrogram showing clustering of grape genotypes constructed using UPGMA based on Jaccard's similarity coefficient obtained from RAPD primers.

Table 2: Jaccard's similarity coefficient for different grape genotypes based on RAPD data analysis

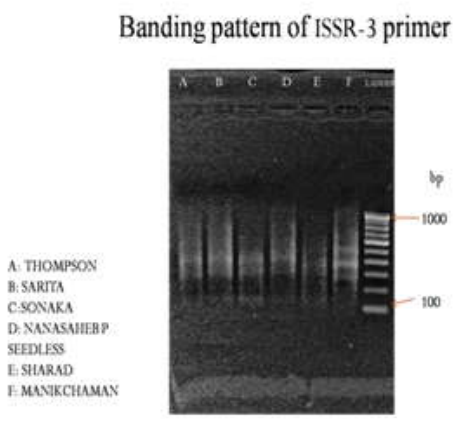
Varieties	Manikchaman	Sharad	Sarita	Sonaka	Thompson	Nanasaheb
Manikchaman	1.00					
Sharad	0.2258	1.00				
Sarita	0.5000	0.3636	1.00			
Sonaka	0.3666	0.3461	0.3750	1.00		
Thompson	0.5185	0.2068	0.4347	0.2666	1.00	
Nanasaheb	0.4285	0.3076	0.5238	0.3214	0.4800	1.00

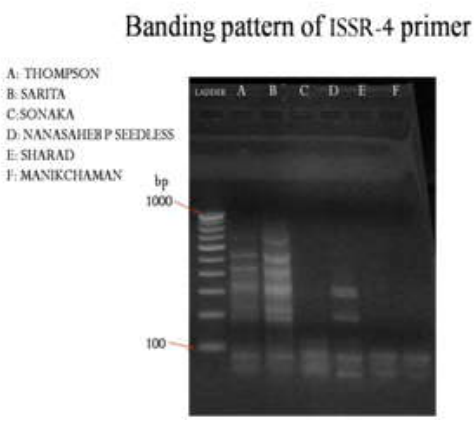
ISSR Markers

Twelve ISSRs were amplified to analyze the genetic variation among six genotypes of grape at molecular level and out of twelve; five were selected on the basis of their ability to produce polymorphic, unambiguous and clear bands for further analysis. Five selected different primers of ISSRs were given total 117 bands ranged from 66 to 670bp and the total numbers of bands were varying from 12 to 29 in six genotypes of grape (plate no 2). Of 117 bands 81 were polymorphism and showed 69.23 average polymorphic percent across six genotypes. The percent polymorphism was ranged from 42 to 100 percent while highest and lowest percent polymorphism found in ISSR-11 and ISSR-9 respectively. The PIC value varied from 0.63 to 0.86 with a mean 0.78 (table 3).The average number of bands per primer was23.4.The highest number of bands (26) found in ISSR-4 and ISSR-6 and high PIC (0.86) value was found in ISSR-6.

Table 3: Results of RAPD analysis in six genotypes of grape

Sr No	Primer Code	Sequence	LMWB (Size in bp)	HMWB (Size in bp)	TB	PB	Percent polymorphism %	PIC Value
1.	ISSR-3	$(GA)_8YC$	129	434	12	6	50.00	0.63
2.	ISSR-4	$(GA)_8A$	66	659	29	17	58.62	0.86
3.	ISSR-6	$(CA)_8RG$	122	633	29	23	79.31	0.84
4.	ISSR-9	$HVH(TG)_7$	77	527	21	9	42.85	0.76
5.	ISSR-11	$(GTG)_4AC$	131	670	26	26	100	0.83
Total					**117**	**81**		
Average					**23.4**	**16.1**	**69.23**	**0.78**





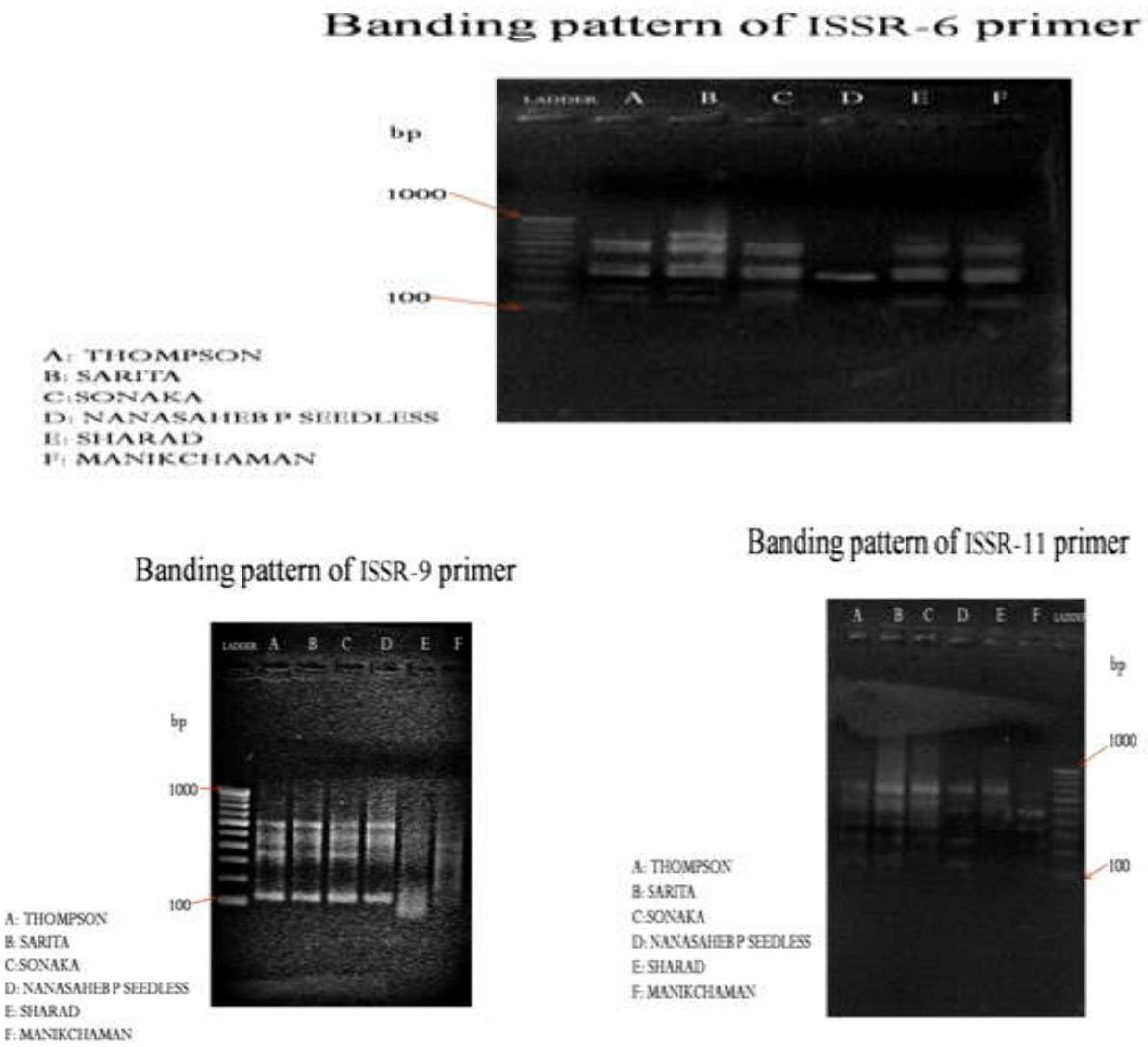
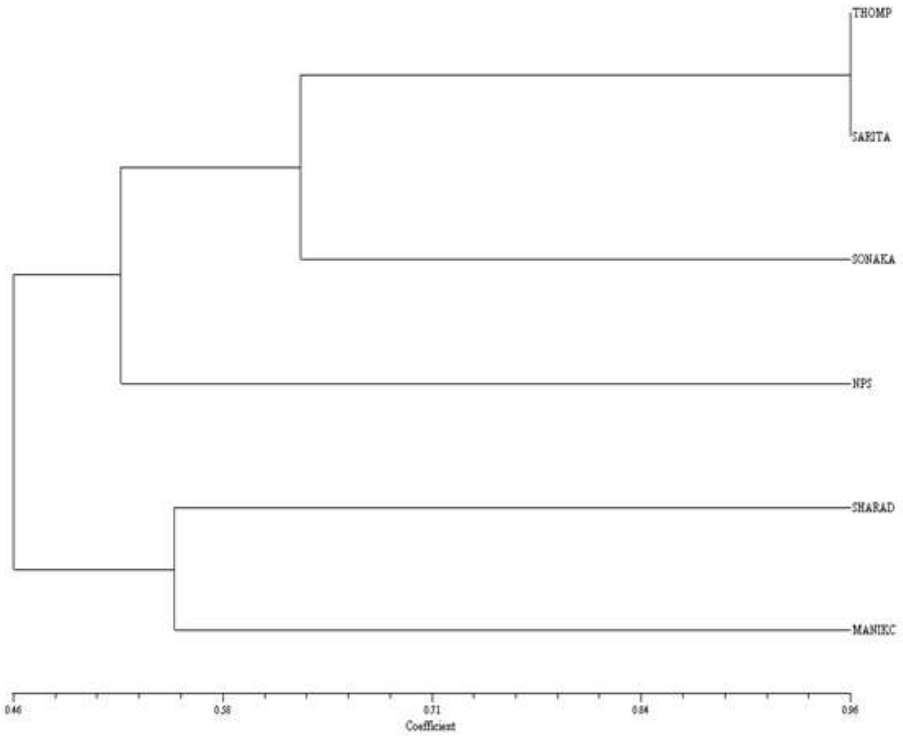


Plate 2 Banding pattern produced by all five ISSR primers

Genetic Diversity

The dendrogram based on the UPGMA clustering method showed two major clusters i.e cluster A and cluster B. Cluster A were grouped total four varieties i.e Thompson seedless, Sarita, Sonaka seedless, Nanasaheb purple seedless while remaining two varieties were grouped in cluster B i.eSharad seedless and Manikchaman (fig 2). The highest genetic similarity (0.96) found between Thompson seedless and Sarita and lowest genetic similarity (0.33) found between Nanasaheb purple seedless and Sharad table no 4. The average genetic similarity present in all six genotypes was 0.66.



(Man- Manikchaman, Th- Thompson, Sr- Sarita, NPS- Nanasaheb purple seedless, So- Sonaka, Sh- Sharad)

Fig. 1 Dendrogram showing clustering of grape genotypes constructed using UPGMA based on Jaccard's similarity coefficient obtained from ISSR primers.

Table 2: Jaccard's similarity coefficient for different grape genotypes based on ISSR data analysis

Varieties	Thompson	Sarita	Sonaka	Nanasaheb	Sharad	Manikchaman
Thompson	1.0000					
Sarita	0.9642	1.0000				
Sonaka	0.6428	0.6206	1.0000			
Nanasaheb	0.5555	0.5357	0.4782	1.0000		
Sharad	0.4285	0.4137	0.5238	0.3333	1.0000	
Manikchaman	0.5000	0.4827	0.6190	0.3636	0.5555	1.0000

Conclusion

The result of RAPD was indicating that among all eight screened RAPD primers three (OPA-11, OPAW-09 and OPG-09) were very informative because they were giving 100% polymorphism. The dendrogram constructed on the basis of data created by eight screened RAPD primer showed highest similarity in-between Nanasahe purpal seedless and Sarita. Similarly the result of ISSR was indicating that ISSR-11 ans ISSR-06 were very informative as compared to all other screened ISSR primers because both primers showed 100% and 79% polymorphism across six grape varieties and the dendrogrm constructed by five ISSR primer showed highest similarity between sarita and Thompson grape variety.

References

Botstein, D, White, R.L., Skolnik, M. and Davis, R.W., 1980. Construction of a linkage map in man using restriction fragment length polymorphism. Hum Genet. 32: 314-31.

Dellaporta, S. L., Wood, J. and Hicks, J. B., 1983. A plant DNA minipreparation: version II. Plant Mol Biol Rep.1: 19-21.

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

Herrera, R., Cares, V., Wilkinson, M.J. and Caligari, P. D., 2002. Characterization of genetic variation between Vitis vinifera cultivars from central Chile using RAPD and Inter simple sequence repeat markers. Euphytica.124:139 145.

Lowe and Davey M. R., 2001. Relationships in pineapple by random amplified polymorphic DNA (RAPD) analysis. Plant Breedidng, 120: 265-267.

Mathews, M. D., Srinivasachary, Sujatha, R., JeVrey L. B., Mike, D., Gale and Katrien M. D., 2007. The genetic map of Finger millet Eleusinecoracana. Theor Appl Genet., 114:321-332.

Ren, Z., Lamikanra, O. and Lu, J., 2000. Identification of RAPD marker closely linked to the fruit color in Muscadine grapes (Vitis rotundifolia). Acta Hort. 528: 263-266.

Rohlf, F. J., 1994. NTSYS-PC Numerical taxonomy and multivarate analysis system verson 2.02. Stat Unversity of New York, Stonybrook, NEWYORK, U.S.A.

Saiki, R. K., Gefland, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K.B., Erlich, H. A., 1988. Primer directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science. 239: 487-489.

Saiki, R.K., Scharf, S., Faloona, F., Mullis, K.B., Horn, G.T., Erlich, H.A., Arnheim, N., 1985. Enzymatic amplification of betaglobin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science. 230: 1350–4.

Tautz, D. ,1989. Hyper variability of simple sequences as general source of polymorphic DNA marker. Nucleic Acid Research, 17, 6463-6471.

Welsh, J. and McClelland, M., 1991. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res.18: 7213-7218.

Winkler, A. J., Cook, J. A., Kliewer, W. M., and Lider, L. A., 1974. General Viticulture. Univ. of California Press, Berkeley.

Zietkiewicz, E., Rafalski, A. and Labuda, D., 1994. Genome fingerprinting by simple sequence repeat (SSR) - anchored polymerase chain reaction amplification. Genomics. 20:176-183.