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Assessment of genetic diversity and relationships among some grape varieties using ISSR markers

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Abstract

As a result of large-scale introduction, the origin and authenticity of many grape varieties is unclear and the subject of some controversy. This has led to confusion regarding their correct identification. Molecular markers have proved to be useful to analyze the genetic relationships as well as diversity between different grape varieties. In the present study, 34 grape varieties have been characterized using Inter Simple Sequence Repeat (ISSR) markers. Out of 93 ISSR primers screened initially, 11 showed good polymorphism. Total 174 bands were obtained, out of which 145 were polymorphic. The pair wise similarity indices were calculated from the band data. Cluster analysis of the varieties resulted in the formation of two main clusters, one belonging to *Vitis vinifera* and other to *V. labrusca*. Varieties belonging to *V. vinifera* appeared more diverse and formed distinct sub-clusters based on their colour, flavour and seeds. Out of 34 varieties screened, 10 varieties with green/yellow berries were grouped together in one subcluster and 15 with red/black berries in the other. Three varieties with green/ yellow berries, Italia, Queen of Vineyard and Thompson seedless were grouped with the varieties with red/black berries. The cluster of labrusca varieties showed homogeneity and had five varieties except Dakh, which belongs to vinifera. Concord separates initially from all other varieties. Incidentally, Concord is a pure selection from *V. labrusca*, while other varieties like Bangalore Blue, Black Muscat, Catawba and Muzzafar Nagar in labrusca group, may be the hybrids of *V. abrusca* x *V. vinifera*. The current study thus revealed that genetic relationships among grape cultivars could be assessed using ISSR markers.

Key words: Diversity, genetic relationships, ISSR markers, grape varieties

Introduction

Grape is one of the most important and oldest fruit crop throughout the world. In India, more than 90% of grape produce is utilized for table purpose and a small quantity for raisins, juice and wine making. The long history of viticulture, vegetative propagation of cultivars and the reliance on ampelography pose difficulties in accurate cultivar identification. Most of the commercially cultivated grape cultivars are introductions from exotic sources and the genetic relationships among them are not clear which is important for planning breeding programme and conservation of germplasm.

The use of DNA markers has been proposed as an objective and viable alternative for ampelography (Thomas *et al.*, 1993). There are number of reports involving use of DNA markers for studying genetic relationships, fingerprinting of clones and cultivars as well as parentage studies (Bowers *et al.,* 1993, 1996; Moreno *et al.,* 1995; Cervera *et al.*, 1998; Sefc *et al.,* 1998). Particularly, PCR based DNA markers, provide powerful tools for genetic analysis because of their simplicity and ease of handling. Markers generated by Inter Simple Sequence Repeat amplification (ISSR; Zietkiewicz et al., 1994) have been shown to be useful for detecting polymorphisms and overcome many technical limitations of RFLP and RAPD analyses. ISSR analyses have been applied to grapes earlier mainly for detecting intravarietal differences (Moreno *et al.,* 1998) and distinguishing cultivars (Herrera *et al.,* 2002). In the present work; we have used ISSR markers to characterize seeded grape varieties from India.

Materials and methods

Plant material: A total of 32 seeded grape varieties were analysed in the present study. Two seedless varieties, Thompson Seedless and Flame Seedless were also included in the analysis as standard varieties. All these were obtained from the germplasm maintained at National Research Centre for grapes, Pune. List of varieties is given in Table-1

Table 1. List of varieties analysed in present study

	Green / Yellow berries		Red / Black berries
1	Anab-e-Shahi	18	Black Muscat
2	Angur Kalan	19	Black Prince
3	Banqui Abyad	20	Catawba
4	Cheema Sahebi	21	Coarna Regia
5	Chenin Blanc	22	Concord
6	Gold	23	Convent Large Black
7	Itatia	24	Dakh
8	Jaos Beli	25	Diamond Jubilee
9	Muller Thergau	26	Muscat Hamburg
10	Palomino	27	Muzzafar Nagar
11	Queen of Vineyard	28	Red Globe
12	Sahebi Ali	29	Red Muscat
13	Sundekhani	30	Shiraz
14	Walthom Cross	31	Spin Sahebi
	Red / Black berries		**Standards**
15	Bangalore Blue	32	Thompson Seedless
16	Bharat Prince	33	Flame Seedless
17	Black Champa	34	Gulabi

DNA isolation: DNA was extracted from young, fully expanded leaves by modified CTAB method (Lodhi *et al.,* 1994). The isolated DNA was processed, quantified and used for PCR reactions.

Table 2. List of ISSR primers used and polymorphic bands

S. No.	Primer	Sequence	Number of bands	Polymorphic bands	Polymorphism (%)
1	807	AGA GAG AGA GAG AGA GT	11	7	64.1
2	827	ACA CAC ACA CAC ACA CG	14	13	93
3	855	ACA CAC ACA CAC ACA CYT	13	7	54
4	856	ACA CAC ACA CAC ACA CYA	23	9	39
5	857	ACA CAC ACA CAC ACA CYG	16	16	100
6	859	TGT GTG TGT GTG TGT GRC	6	6	100
7	860	TGT GTG TGT GTG TGT GRA	16	15	94
8	888	BDB CAC ACA CAC ACA CA	19	14	73.7
9	889	DBD ACA CAC ACA CAC AC	19	17	89.5
10	890	VHV GTG TGT GTG TGT GT	23	23	100
11	891	HVH TGT GTG TGT GTG TG	24	18	75
		Total	174	145	83.3

Y=C/T; R=A/G; B=C/G/T; D=A/G/T; H=A/C/T; V=A/C/G

ISSR amplifications and Gel electrophoresis: ISSR amplifications were carried out using Primer set#9; obtained from University of British Columbia, Vancouver, Canada in 25µl reaction volume. Reaction mixture contained 1X PCR buffer containing 1.5mM $MgCl_2$, 12.5ng of genomic DNA, 0.5 U Taq DNA Polymerase, 0.1mM of each dNTP, 0.04 mM spermidine, 2% formamide and 0.3 µM of primer. The thermal cycling was performed in PTC 200 Thermal Cycler (MJ Research Inc, USA) following the protocol of Nagaoka and Ogihara (1997). The PCR reaction was performed at least three times for each primer to ensure reproducibility. The amplification products were separated on 1.5% agarose gels. The gels were stained with ethidium bromide and visualized on a UV transilluminator.

Data Analysis: Bands in the amplification profiles were recorded as present (1) and absent (0). Based on the band data, the similarity matrix was calculated using Dice coefficient and the cluster analysis was carried out using SAHN module in NTSYS pc 2.1 software.

Results and discussion

Total 93 ISSR primers from set #9 were screened initially for polymorphism and 11 were finally selected based on the basis of clear scorable band pattern. The list of the primers used and polymorphic bands recorded is given in Table 2. The amplification profile obtained with primer UBC 857 is shown in Fig. 1.

Total 174 bands were obtained with the 11 selected primers; out of which 145 were polymorphic. The bands ranged from 70 bp to 1.4 kb in size. Primer 891 showed the maximum number of bands. The number of polymorphic bands ranged from 6 (UBC 859) to 23 (UBC 890). All these primers contained dinucleotide repeats. Primers with $(AC)_n$ repeats were maximum in number (5/11) followed by $(TG)_n$ (3/11). In general, percentage polymorphism obtained by primers containing $(AC)_n$ repeats was lower (75%) as compared to those with $(TG)_n$ (89.66%). All the bands obtained with primers 857, 859 and 890 were polymorphic. Few genotype specific bands were observed with primers 856 and 860 in variety Black Prince and primer 889 in varieties Concord and Diamond Jubilee.

Based on the band data, similarity matrix was generated using Dice coefficient and cluster analysis was carried out. The dendrogram generated by UPGMA algorithm using NTSYS pc 2.1 software is shown in Fig. 2. The similarity coefficient ranged from 0.48 to 0.89. Two major clusters were observed; one consisting of *V. labrusca* and its derivatives and the other of varieties from *V. vinifera*. Variety Concord separated initially from all others and showed least similarity. Cluster I consisted of mainly *V. labrusca* or *labrusca* x *vinifera* hybrids except Dakh. The cluster II consisted mainly of *V. vinifera* varieties. In this cluster, Black Prince and Red Muscat separated initially from other vinifera varieties. Several subgroups based on their colour, flavour and seeds were observed in this cluster indicating the diverse nature of these varieties. A distinct sub cluster of 10 varieties with green / yellow berries could be distinguished. Similarly, 15 varieties with (red / black) berries were grouped together. Three varieties, Italia, Queen of Vineyard and Thompson Seedless having yellowish green berries were also grouped with these varieties. Varieties Anab-e-Shahi, Angur Kalan and Cheema Sahebi, all high yielding and hard seeded were grouped together, while three varieties in the other subgroup namely Gold, Muller Thergau and Sundekhani, have soft seeds and muscat flavour. Four flavoured varieties; Italia, Bharat Prince, Muscat Hamburg and Gulabi were grouped together in the same group.

The cluster of labrusca varieties showed homogeneity and had five varieties including Dakh, which however, belongs to vinifera. Varieties Catawba and Dakh showed maximum similarity of 89%. Such a close relationship and the grouping of Dakh in *labrusca* group is surprising since Dakh belongs to *V. vinifera* (Chadha and

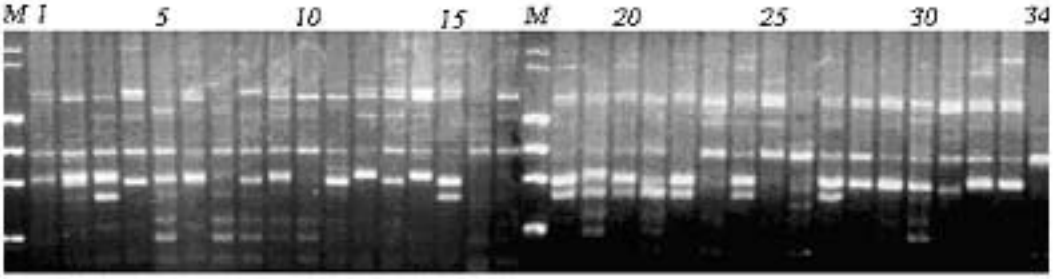

Fig. 1. Amplification profile obtained with UBC primer 857. M: Molecular weight marker, PhiX174DNA/ HaeIII digest. 1-34 DNA samples as listed in Table 1

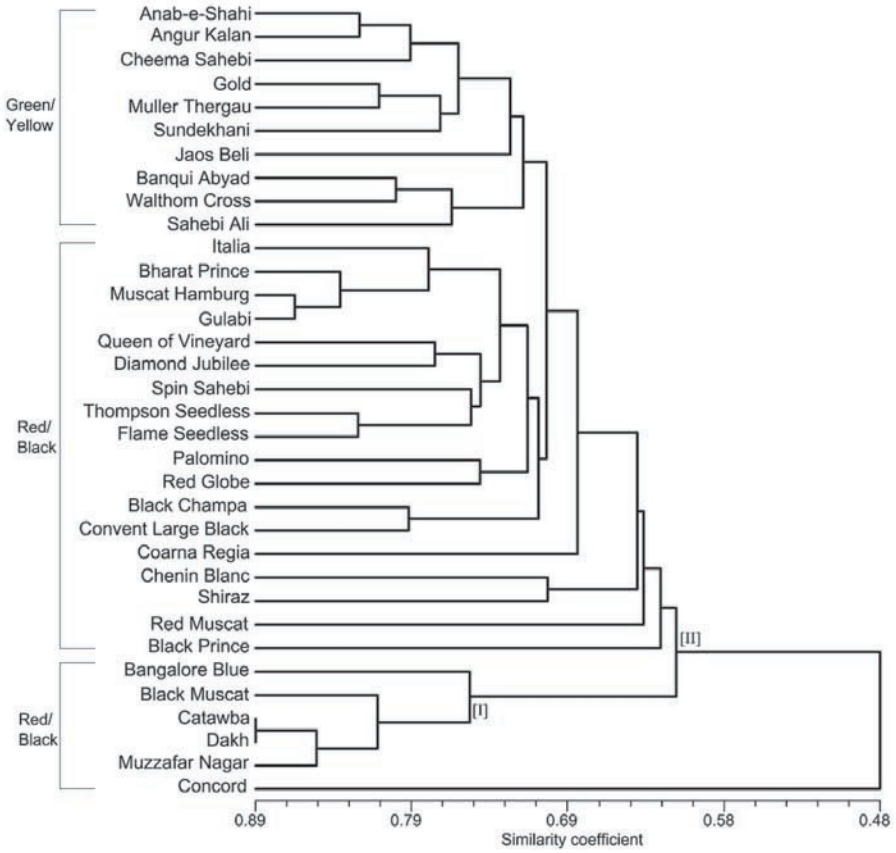

Fig. 2. Dendrogram based on ISSR band data and UPGMA algorithm showing relationships among the grape varieties [I]: *Vitis labrusca* cluster [II]: *Vitis vinifera* cluster

Randhawa, 1974). Therefore, it appears to be a case of mistaken identity. The separation of Concord from *V. labrusca* group is also unexpected since it is one of the oldest *labrusca* cultivar. According to Hedrick (1938), Concord has all the morphological characters of the American species (*V. labrusca*), while, several other workers have reported this variety to be a hybrid between *V. labrusca* and *V. vinifera* (Chadha and Randhawa, 1974). In our earlier analysis using RAPD markers also it had grouped along with other *labrusca* varieties (Tamhankar *et al.*, 2001). The analysis of multiple samples collected from different sources will be useful to confirm these results. Although ISSR markers are efficient and reliable for the assessment of genetic relationships among grape varieties, application of other marker systems like microsatellites (STMS) and AFLP is necessary for resolving the controversial grouping of some varieties.

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