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ISSR-PCR for assessment of genetic relationships among grape varieties cultivated in India

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Summary

Genetic relationships among 43 varieties cultivated in India were characterized using ISSR PCR. Out of total 139 reproducible fragments generated by 13 informative primers, 96 were polymorphic. The similarity coefficient ranged from 0.65 to 0.96. Cluster analysis resulted in the formation of two main clusters, consisting mainly of *Vitis labrusca* and *V. vinifera* types. One single variety belonging to *V. rotundifolia* James grouped with *V. labrusca* types but separated initially from them. Two varieties, Lake Emerald and Muscat, were completely outgrouped. Varieties belonging to *V. vinifera* appeared to be more diverse and separated further into many subgroups. In contrast, *V. labrusca* types were homogeneous. The results showed that ISSR is an efficient and reliable marker system for genetic analysis of grape cultivars.

K e y w o r d s : Genetic relationships, grapes, ISSR, *Vitis*.

Introduction

More than 400 accessions including grape varieties, *Vitis* spp. and their wild relatives are preserved at the Agharkar Research Institute Farm at Hol (Baramati). Most of the commercially cultivated grapes in India were introduced initially from major grape growing countries and hence interrelationships between them are not very clear. Such knowledge on genetic relationships and correct identification of varieties is not only important for evolutionary studies but also for plant breeding and germplasm preservation. Often the same variety is known by different names and this can lead to a confusion of nomenclature (Chadha and Randhawa 1974, Chadha and Shikhamany 1999). The difficulties to accurately identify cultivars are due to the vegetative propagation of cultivars and the reliance on ampelography. The use of molecular markers for grapevine identification has been shown to be an objective and viable alternative or supplement to ampelography (Thomas *et al*. 1993). Initially the markers based on hybridization such as RFLPs were applied (Bourquin *et al*. 1993, Bowers *et al.* 1993, Gogorcena *et al.* 1993, Bowers and Meredith 1996). PCR-based DNA markers provide powerful tools for genetic analysis mainly because of their simplicity and ease of handling. The use of microsatellite (STMS) markers has been propagated mainly for fingerprinting of clones and cultivars as well as parental studies (Sefc *et al*. 2001), while multilocus marker systems like RAPD, AFLPs have been used for analysis of genetic relationships (Gogorcena *et al.* 1993, Grando *et al.* 1995, This *et al.* 1997, Cervera *et al*. 1998, Ye *et al.* 1998, Vidal *et al*. 1999, Tamhankar *et al*. 2001). Inter simple sequence repeat amplification (ISSR) (Zietkiewicz *et al*. 1994) is a simple, quick and reliable technique used in various species for detecting polymorphism and genetic mapping (http://www.biosci.ohio.state.edu/~awolfe/ISSR/ISSR.html). ISSR analyses are easier than SSR as there is no need of prior sequence information. ISSR analyses have been applied to grapes for detecting intravarietal differences (Moreno *et al.* 1998) but also for distinguishing cultivars (Herrera *et al.* 2002). In the present work, we have employed this technique to characterize 43 grape varieties. To our knowledge, this is the first report of application of ISSR markers for assessing genetic relationships of grape varieties grown in India.

Material and Methods

P l a n t m a t e r i a l : Leaf blade tissue from 43 grape varieties was collected from vines cultivated at the Institute farm within the AICRP-STF programme. Out of these, 41 are seeded varieties. Two seedless varieties, namely Thompson Seedless and Flame Seedless have been included in the analysis as standard varieties (Tab. 1).

D N A a m p l i f i c a t i o n a n d g e l e l e c t r o p h o r e s i s : DNA was extracted from young, fully expanded leaves by modified CTAB method (Lodhi *et al.* 1994). The PCR reaction was performed in a 25 ml volume containing the following components: 1x PCR buffer containing 1.5 mM $MgCl_2$, 1 unit of Taq DNA polymerase, 0.1 mM of each dNTP, 0.4 mM of spermidine, 2 % formamide, 0.3 µM of a single primer and 15 ng of genomic DNA. Amplifications were carried out in PTC 200 (M. J. Research Inc., USA) thermal cycler as described by Nagaoka and Ogihara (1997). The PCR reaction was performed at least three times for each primer to ensure reproducibility.

The reaction products were separated on 1.5 % agarose gel electrophoresis in TAE buffer and photographed on a UV Transilluminator.

D a t a a n a l y s i s : Bands in the gel profiles were recorded as present (1) and absent (0). The similarity matrix

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Table 1

List of grape varieties used for ISSR analysis

V. vinifera		*V. labrusca*
Almeria	Hussaini Black Kabuli	Banglore Blue
Amber Queen	Jawahar	Buckland Sweet Water
Anab-e-shahi	Kali Sahebi	Catawba
Bain Shirai	Karachi	Concord
Banglore Purple	Motia	Goethe
Bhokari	Muscat	Isabella
Black Champa	Muscat White	Khalili
Black Damascus	Pale Green	Large White
Black Monukka	Pandhari Sahebi	Lake Emerald
Cheema Sahebi	Phakdi	Malaga
Convent Large Black	President	Oval White
Convent Large White	Red Prince	*V. rotundifolia*
Country Banglore	Ribier	James
Foster Seedling	Rubired	Standard varieties
Gulabi		Thompson Seedless
		Flame Seedless

was calculated using Dice coefficient. The dendrogram based on UPGMA (unweighted pair group with arithmetic mean) algorithm was generated using SAHN module in NTSYS pc 2.1 package.

Results and Discussion

Assessment of ISSR primers for polymorphism: Ninety-three primers from set no. 9 (University of British Columbia, Vancouver, Canada) were screened for polymorphism on a small subset of samples. Based on the initial screening, 13 primers giving clear band pattern were selected for further analysis. The details of primers are given in Tab. 2. One hundred and thirty-nine bands were obtained from selected primers, out of which 96 were polymorphic (Tab. 2). The amplification profile generated by primer UBC-889 for 43 grape varieties is shown in Fig. 1. The size of the amplification products ranged from 300 to 1,400 bp. The number of polymorphic bands varied from 3(UBC-856) to 12(UBC-891). The average number of polymorphic bands per primer was 7.3. The relative polymorphism was lowest for primer 856 (42.8 %) and highest for primer 850 (84.6 %). Since these primers contain simple sequence repeat motifs with a 1-3 base anchor in their sequence, the primers were analyzed for their sequences. All the selected primers contained dinucleotide repeats. This agrees well with the reported prevalence of dinucleotide repeats in plants (WANG *et al.* 1994) and earlier report for grapes (MORENO *et al.* 1998). Among these, primers with $(AC)_n$ repeats were maximum (5/13). There were differences in resolving polymorphism. The primers containing $(GT)_n$ repeats and $(CA)_n$ repeats were most polymorphic with 76.9 and 77.3 %, respectively. However, relative polymorphism obtained with $(AC)_n$ primers was the lowest (61.7 %). The same is true for the average number of bands per primer and the number of polymorphic bands per primer. Although, the average number of bands per primer was maximum for $(TG)_n$ containing primers (13.5 bands per primer); the number of polymorphic bands per primer were higher for those containing $(GT)_n$. Nucleotides at the 5' and 3' end of the primer played an important role in detecting polymorphism. Nine primers had anchors at the 3' end, 4 at the 5' end. The primers containing single base anchors at the 3' end were least polymorphic (61.1 %) while those with three bases anchor at the 5' end were most polymorphic.

Table 2

Selected ISSR primers used for generating amplification profiles

Primer	Repeat	Total no. of bands	Polymorphic bands	Polymorphism %
807	5'$(AG)_n$T 3'	6	4	66.7
827	5'$(AC)_n$T 3'	12	7	58.3
841	5'$(GA)_n$YC 3'	11	8	72.7
848	5'$(CA)_n$RG 3'	11	8	72.7
850	5'$(GT)_n$YC 3'	13	11	84.6
855	5'$(AC)_n$YT 3'	8	5	62.5
856	5'$(AC)_n$YA 3'	7	3	42.8
857	5'$(AC)_n$YG 3'	9	7	77.7
859	5'$(TG)_n$RC 3'	11	6	54.5
888	5'BDB$(CA)_n$ 3'	11	9	81.8
889	5'DBD$(AC)_n$ 3'	11	7	63.6
890	5'VHV$(GT)_n$ 3'	13	9	69.2
891	5'HVH$(TG)_n$ 3'	16	12	75.0
	Total bands	139	96	69.0

D = A/G/T, H = A/C/T, V = A/C/G, Y = C/T and R = A/G

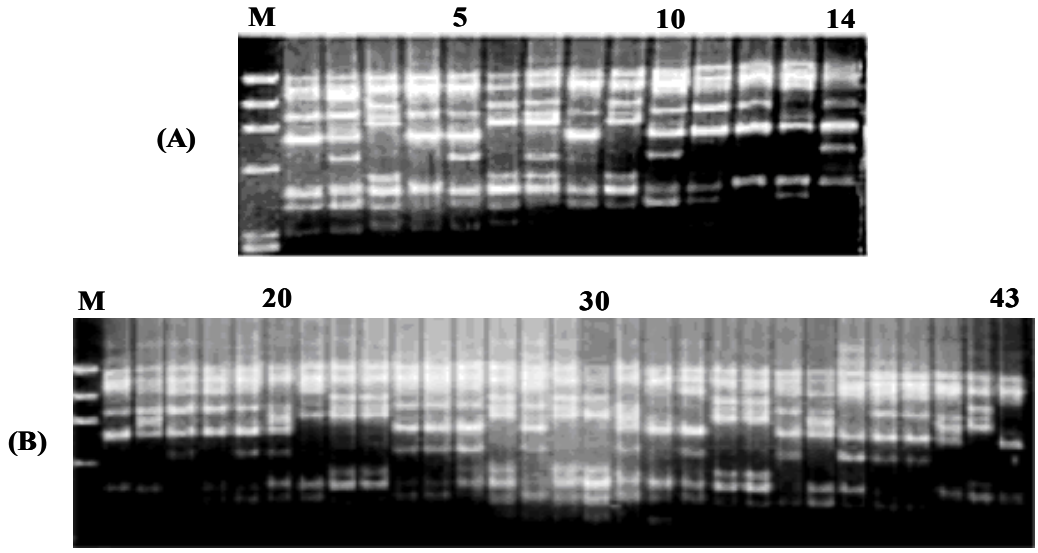

Fig. 1: Amplification profiles of grape varieties generated by ISSR primer 889. **A**: **M** = FX174 DNA / *Hae*III digest; 1 = BainShirai, 2 = Bhokari, 3 = Buckland Sweet Water, 4 = CheemaSahebi, 5 = Foster Seedling, 6 = Khalili, 7 = Lake Emerald, 8 = Motia, 9 = Oval White, 10 = Pale Green, 11 = Pandhari Sahebi, 12 = Phakdi, 13 = Thompson Seedless, 14-Gulabi. **B**: M = FX174 DNA / *Hae*III digest; 15 = Almeria, 16 = Amber Queen, 17 = Anab-e-shahi, 18 = Banglore Purple, 19 = Black Champa, 20 = Black Damascus, 21 = Black Monukka, 22 = Catawba, 23 = Concord, 24 = Country Banglore, 25 = Convent Large Black, 26 = Convent Large White, 27 = Goethe, 28 = Hussaini Black Kabuli, 29 = Isabella, 30 = James, 31 = Jawahar, 32 = Kali Sahebi, 33 = Karachi, 34 = Large White, 35 = Malaga, 36 = Muscat, 37 = Muscat White, 38 = President, 39 = Red Prince, 40 = Ribier, 41 = Rubired, 42 = Banglore Blue, 43 = Flame Seedless.

The percentage of polymorphism revealed in the present analysis is much higher than that reported by Moreno *et al*. (1998). This may be because ISSR markers were used by these authors for characterizing the intravarietal differences while in the present paper different grape varieties have been analyzed. Secondly, preselection of primers for the ability to generate clear and polymorphic band pattern may also have led to higher polymorphism. Genotype specific bands were observed only for cv. Lake Emerald with primers 827, 856 and 888. These variety specific markers could be further utilized for the identification of this particular variety.

Genetic relationships as derived from cluster analysis: Based on the band data, the similarity matrix was calculated using Dice coefficient. The dendrogram generated using Dice coefficient and UPGMA algorithm is shown in Fig. 2. The similarity coefficient ranged from 0.65 to 0.96. Two major clusters were observed, one consisting of *V. labrusca* and its derivatives and the other consisting of varieties from *V. vinifera*. Cluster I consisted of 11 varieties out of which 10 are *V. labrusca* or *V. labrusca* x *V. vinifera* hybrids. James, a variety from *V. rotundifolia,* was also grouped in the *V. labrusca* cluster, but separated initially from them. The second cluster consisted mainly of *V. vinifera* varieties. However, several subgroups were observed in this cluster. Two varieties, Lake Emerald and Muscat, were completely outgrouped and separated initially from all the varieties.

The grouping of the varieties as observed in the present analysis was compared with the available morphological and parentage data. In the cluster of *V. vinifera* varieties, distinct subgroups based on berry colour could be distinguished. The only exceptions were Kali Sahebi, although reddish purple, grouped in subgroup with yellow-green berries while Motia, Foster Seedling and Thompson Seedless with green-yellow berries were grouped with the black/purple berries. Cv. Cheema Sahebi was grouped along with Phakdi in the same subgroup indicating a close relationship between the two. Actually, Cheema Sahebi is a natural seedling selection from an open pollinated progeny of Pandhari Sahebi. Since Pandhari Sahebi is a partially male sterile variety, the possibility that Cheema Sahebi is a result of open pollination from Phakdi cannot be ruled out (Phadnis *et al.* 1968). The close grouping of Cheema Sahebi and Phakdi in the dendrogram sharing 86 % similarity is in accordance with this. Country Banglore and Convent Large Black showed maximum similarity, as high as 96 %, indicating that they are probably synonyms. In the key prepared on the basis of fruit characters, these two varieties fall into the same subgroup as well (Chadha and Randhawa 1974).

Banglore Blue is a *V. vinifera* and *V. labrusca* hybrid extensively used for juice and wine making. It is resistant to many diseases and hence used in breeding programmes. Berries are dark purple with pulp having foxy flavour while Banglore Purple is a *V. vinifera* grape having bluish black berries and a musky flavoured pulp. In some earlier reports, Banglore Purple was considered to be synonymous with Banglore Blue (Phadnis 1965, Gandhi 1960). However, the vegetative and fruit characters of the two varieties are entirely different from one another (Chadha and Randhawa 1974). In the present analysis marked differences were observed in the groupings. Banglore Purple was grouped with *V. vinifera* varieties, while Banglore Blue was in the *V. labrusca* cluster. Similar differences in their grouping were also observed in our earlier analysis using RAPD markers (Tamhankar *et al*. 2001). The present analysis thus reiterates the genetic distinctness of the two varieties.

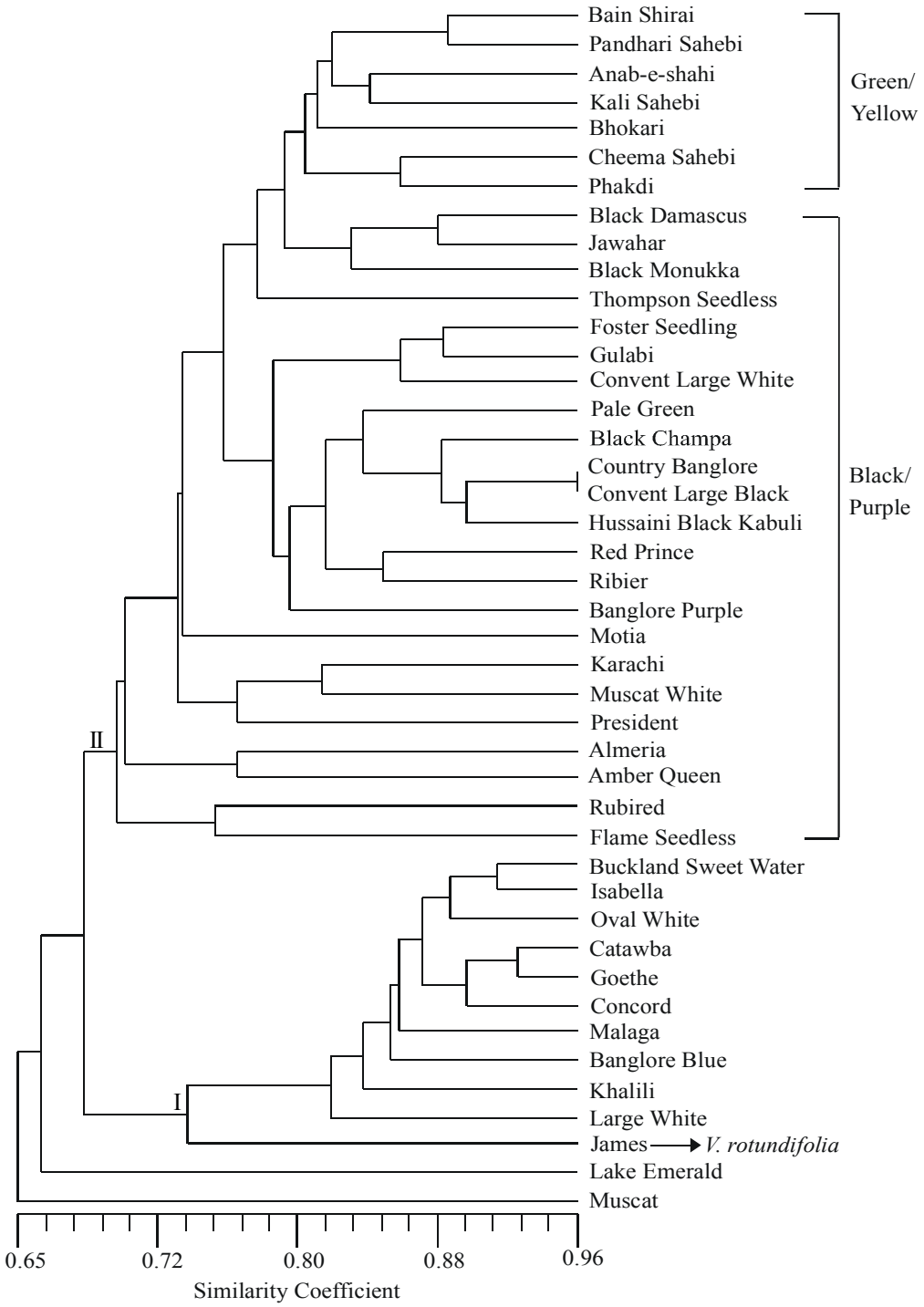

Fig. 2: Genetic relationships among 43 grape varieties based on ISSR band data. The dendrogram was obtained by Dice coefficient and UPGMA analysis using NTSYS pc 2.1 software. Cluster I: *V. labrusca*; Cluster II: *V. vinifera.*

Cvs Muscat and Lake Emerald showed the lowest similarity values with other types and separated initially from all other varieties. This was rather unexpected, because Muscat belongs to *V. vinifera*, originally introduced from USSR (CHADHA and RANDHAWA 1974), while Lake Emerald is a hybrid bunch grape variety with characters of *V. labrusca* and *V. vinifera* (CHADHA and RANDHAWA 1974). Thus, these varieties appear to be misnomers. Analysis of multiple samples from other sources is necessary for further authentification of these varieties.

In contrast to *V. vinifera,* varieties of *V. labrusca* showed homogeneity. James, a reported *rotundifolia* grape variety (VAILE 1939), also showed close affinity with the *V. labrusca* group sharing 75 % similarity. The grouping of James with *V. labrusca* cultivars was also observed earlier by RAPD analysis (TAMHANKAR *et al*. 2001). This is also in accordance with the earlier classification of grape varieties based on fruit characters, where it is grouped in the same subgroup along with a few *V. labrusca* cultivars like Banglore Blue, Catawba, Concord and Large White (CHADHA and RANDHAWA

1974) In spite of the high similarity among the *V. labrusca* varieties, all the varieties could be distinguished from each other. Six varieties from this group were also analyzed earlier using RAPD markers (Tamhankar *et al*. 2001); however, it was not possible to differentiate between them even after using 19 different RAPD primers. Thus, the use of ISSR markers seems to be more effective. Also, a smaller number of ISSR markers (13) were required to detect the differences between the varieties.

Thus, the ISSR markers appear to be reliable and efficient for the assessment of genetic relationships among grape varieties. However, other marker systems like microsatellites and AFLPs should be applied to substantiate the unexpected grouping of some varieties.

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