Vitis **40** (3), 157–161 (2001)

Assessment of the genetic diversity of some important grape genotypes in India using RAPD markers

SHUBHADA A. TAMHANKAR, S. G. PATIL and V. S. RAO

Genetics Group, Agharkar Research Institute, Agarkar Road, Pune, India

Summary

Genetic relationships in a set of important grape genotypes in India, comprising cultivated varieties, rootstocks and wild species were analysed using RAPD markers. A total of 250 bands were obtained by 19 informative primers, most of which could clearly distinguish between the wild and cultivated genotypes. Wild species and rootstocks showed a maximum number of bands (205) and the highest polymorphism (94 %), followed by cultivars belonging to *Vitis vinifera* (165 bands, 90 %), while cultivars from *V. labrusca* showed only 75 bands of which almost all were monomorphic. Cluster analysis resulted in the formation of three main clusters. Wild species and rootstocks separated early from cultivated genotypes. Cultivated types formed two separate clusters one consisting mainly of the *labrusca* and the other of the *vinifera* types. In the cluster of *vinifera* genotypes, seeded and seedless varieties were further separated into different subgroups. High bootstrap values at most of the nodes supported the stability of the dendrogram. The grouping of most varieties agreed well with previous reports based on morphological characters as well as parentage, emphasizing the suitability of RAPD analysis for such studies. The present report is the first attempt to determine the genetic relationships in important grape genotypes in India using molecular markers.

Key words: genetic diversity, grapes, RAPD, *Vitis*, India.

Introduction

Commercial grape cultivation in India is one of the most remunerative fruit crops, which is mainly confined to table grapes. More than 80 % are cultivated under the tropical conditions of the Southern peninsular region, while viticulture in North India is under subtropical conditions. Due to the diversity of climate, different cultivars are planted in different regions (CHADHA and SHIKHAMANY 1999). Although some wild grapes are known, most commercially cultivated grapes were introduced; very often the interrelationships between these genotypes are not known. Such knowledge is important for germplasm conservation, evolutionary aspects as well as for breeding objectives. The present work was therefore undertaken to assess genetic relatedness of different genotypes of *Vitis* spp. mainly important in India.

Due to their potential to yield unlimited numbers of polymorphic markers, various DNA-based markers are increasingly used in diversity analyses. However, among all these marker types, RAPD technique requires only a minute quantity of DNA, requires no prior sequence information and is simple and capable of detecting high levels of genetic variation, therefore, it has been widely used in assessments of genetic diversity of grape as well as other taxa. Thus, we used RAPD markers for analyzing the relationships between important grape genotypes. Most of the important *Vitis vinifera* varieties presently under cultivation were selected for analysis. In contrast to *V. vinifera*, some of the non-*vinifera* sources of germplasm are resistant to insects, diseases and environmental stress (REISCH and PRATT 1996). Rootstock development is also entirely dependent on non-*vinifera* germplasm; therefore, these genotypes were included in the analysis. Some of the promising hybrids developed at our institute were also selected (PATIL *et al.* 1996). The present paper describes the results of RAPD analysis of 43 grape genotypes belonging to 9 *Vitis* spp. To the best of our knowledge, this is the first report of application of molecular markers for assessing the genetic relationships of grape genotypes in India.

Material and Methods

Genotypes: A total of 43 genotypes, belonging to 9 different species of *Vitis* were used in the present study (Tab. 1). These genotypes could be broadly classified into two categories: 1: cultivated, comprising mainly genotypes belonging to *V. vinifera* and *V. labrusca*, 2: rootstocks and wild species. Leaf material of these genotypes was obtained from the grape germplasm collection, maintained at the institute or from commercial farms.

DNA extraction: DNA extraction was carried out from young partially expanded at inserted one or two nodes below the shoot tips, following the protocol of LODHI *et al.* (1994). DNA samples were quantified by agarose gel electrophoresis by comparison with standard dilutions of lambda DNA.

PCR amplification: PCR amplification was carried out in a Perkin Elmer DNA thermal cycler 480 following the protocol of WILLIAMS *et al.* (1990). Random primers from kits A,B,C,E,F,G,H,J,K,O,P,U,V and Y (Operon Technologies, Alameda, USA) were used. Amplified products were separated on either 1.5 or 2 % agarose gels and visualized by ethidium bromide staining.

Scoring and data analysis: The bands were scored from the photographs of gel profiles. Only bands

Correspondence to: Dr. S. A. TAMHANKAR, Genetics Group, Agharkar Research Institute, Agarkar Road, Pune 411 004, India. Fax: +91-20-5651542. E-mail: prakash@aripune.ernet.in

Table 1

List of genotypes analysed in the present study

Vitis spp.	*Vitis labrusca*
Vitis berlandieri	Bangalore blue
Vitis candicans	Bangalore purple
Vitis champini	Catawba
Vitis longii	Concord
Vitis palmata	Isabella
Vitis parviflora	Khalili
	Large white
Vitis vinifera	
Seeded	*Vitis rotundifolia*
Anab-e-shahi	James
Bhokri	
Cheema sahebi	*Vitis champinii*
Gulabi	Champanel
Kali sahebi	Dogridge
Pandhri sahebi	Degrasset
Ruby red	
	Rootstocks
Seedless	*Solonis* x Othello
Anab-e-shahi	*Solonis* x *Riparia*
mutant 302	*Riparia* x *Rupestris*
Flame seedless	
Kishmish beli	Hybrids
Kishmish chorni	H-27 (Diamond jubilee x Rubired)
Manik chaman	H-56 (Cheema sahebi x
Perlette	Tas-a-ganesh)
Pusa seedless	H-144 (Cheema sahebi x Catawba)
Sentenal seedless	H-324 (Gulabi x James)
Sharad seedless	H-516 (Catawba x
Sonaka	Beauty seedless)
Tas-a-ganesh	

that were observed in three separate amplifications were scored. For each genotype, the presence of a band (1) or its absence (0) was entered in the RAPDISTANCE computer program (ARMSTRONG *et al.* 1994). These data were used for calculation of pairwise genetic distances using Jaccard, Dice and simple matching coefficients. The distance matrix was then used for cluster analysis using the unweighed pair-group method, arithmetic average (UPGMA) algorithm. The dendrogram was generated using NEIGHBOR and DRAWGRAM programs in the PHYLIP version 3.2 package (FELSENSTEIN 1993). The stability of the dendrogram was evaluated by the bootstrap approach using Winboot program (YAP and NELSON 1996). Based on band data, distribution of each band within the sampled genotype was calculated which was further used to calculate resolving power of each primer (PREVOST and WILKINSON 1999).

Results

Screening of the primers for polymorphism: The primers were screened initially using a sample set of two wild species and two cultivated varieties. Out of 256 primers tested, only 57 primers could distinguish between the cultivated genotypes. Finally, 19 primers that yielded clear and scorable patterns were selected for amplifying DNAs from all genotypes.

A total of 250 bands was amplified using the selected 19 primers. The results obtained for individual primers are listed in Tab. 2. The size of amplification products ranged from 300 to 3000 bp. Almost all bands were polymorphic and most of the primers could clearly distinguish between wild and cultivated genotypes. In group-wise comparison, wild species and rootstocks showed a maximum number of bands (205), 94 % of which were polymorphic, followed by *V. vinifera* varieties with 165 bands and more than 90 % polymorphism. Varieties belonging to the *V. labrusca* group showed only 75 bands and almost all bands except $OPC13_{1000}$ were monomorphic. The primers also differed in their informativeness (Tab. 2). Five primers OPA 02, OPG 06, OPH 03, OPK 11 and OPK 19 generated most informative band profiles and were able to distinguish more than 30 genotypes. A strong positive correlation was obtained between the number of genotypes identified and resolving power (r = 0.7672). However, no primer could differentiate between all 43 genotypes.

Table 2

Polymorphism obtained with selected informative primers

Primer	Total no. of bands	No. of polymorphic bands	No. of genotypes identified	Resolving power
OPA 02	22	22	32	8.965
OPA 09	10	10	13	1.966
OPA 11	11	11	27	3.602
OPC 13	09	09	19	3.011
OPG 06	15	15	30	6.606
OPH 03	18	18	31	5.619
OPH 04	10	09	23	4.454
OPH 07	08	08	12	2.408
OPK 11	14	14	33	6.375
OPK 17	14	14	20	5.465
OPK 19	21	21	30	7.552
OPO 05	09	09	25	5.323
OPO 13	09	09	20	3.505
OPP 02	10	10	17	4.545
OPP 17	14	14	22	5.347
OPU 03	12	12	20	3.977
OPV 12	16	16	23	5.017
OPV 19	15	15	22	5.647
OPY 07	13	13	28	3.863

Genotype and group specific bands: Detailed analysis of RAPD profiles revealed a number of genotype-specific products. A total of 45 bands were unique and present only in one genotype. The majority of these bands were specific to wild and rootstock species. Fifteen products were specific to *V. vinifera* genotypes. The pres-

ence of some of the group-specific products, particularly in the *V. labrusca* group, was also noted.

Genetic relationships: Genetic distance matrices generated by Jaccard, Dice and simple matching coefficients showed a very high correlation (r >0.98). UPGMA analysis of distance matrices resulted in overall similar dendrograms with 3 main clusters. The dendrogram generated with Jaccard coefficient is shown in the Figure. Cluster-I consisting of 10 genotypes, mainly wild species and rootstocks, separates early from cultivated genotypes. Cultivated types are further subdivided into two separate clusters consisting of mainly *labrusca* (cluster-II) and *vinifera* genotypes (cluster-III). In cluster-II out of 11 genotypes, 6 belong to *V. labrusca*. In cluster III which consists of genotypes belonging to *V. vinifera*, two cultivars, Perlette and Centennial seedless, separate out initially. At second bifurcation, a group of 7 genotypes separates out. Interestingly, all varieties in this group (except Cheema sahebi) are seedless. The other branch further divides into different subgroups.

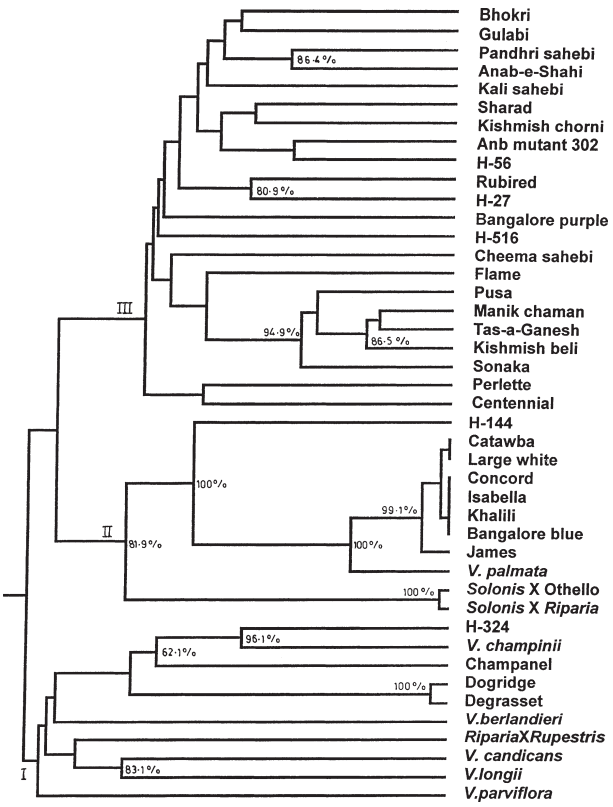

Figure: Dendrogram showing relationships among 43 important grape genotypes in India obtained by Jaccard coefficient and UPGMA analysis. Three main clusters are shown as I, II and III. Percentage values at forks indicate the number of times the group consisting of the genotypes at the right occurred among the trees in the bootstrap method. Some of the nodes with high values are shown.

The stability of the dendrogram was also analysed by the bootstrap method, which provides confidence for the different nodes. The consensus tree showed the three main clusters as observed earlier. High bootstrap values (more than 50 %) were observed at about 26 out of 41 nodes in the consensus tree. Some of the nodes at which high values were observed are shown in the Figure. The confidence values were especially high for cluster-II.

Discussion

RAPD, a suitable approach to detect variability in the Indian grape germplasm: We have used RAPD markers to analyse genetic diversity in a set of 43 grape genotypes. Some serious doubts regarding the reproducibility of the RAPD bands have been raised in earlier reports with grape (Büscher *et al.* 1993, Xu *et al.* 1995). However, subsequently, it has been shown that if proper care is taken the technique can be applied to study genetic relationships. In a detailed study on the stability of RAPD markers in grape, the most important factor affecting stability was found to be the origin of *Taq* DNA polymerase (This *et al.* 1997). Lower reproducibility, especially for the larger fragments and weak bands, has also been reported (Vidal *et al.* 1999 b). It was, therefore, suggested that for studies of genetic relationships, conclusions should be based on large number of bands and the exclusion of large and weak bands could give a reasonable level of reproducibility. Considering all these points, in our study, care was taken to avoid any variation due to these factors. Only bands that were observed in three independent amplifications were scored and faint bands and the high molecular weight bands >2 kb were not scored. The number of RAPD bands scored is also important to obtain accurate estimates of genetic relationships. In a recent report in *V. vinifera,* it was observed that the clusterings in the dendrogram were completely rearranged if the number of scored bands was 100-150. The estimation of genetic distances improved with the progressive increase of the number of bands (Fanizza *et al.* 1999). We have scored 250 bands which are notably higher than those suggested earlier (Guirao *et al.* 1995); this is in accordance with recent studies with grapes (This *et al.* 1997, Vidal *et al*. 1999 a, b).

The present study revealed three main clusters in a set of Indian grape germplasm. The value of more than 50 % observed at most of the nodes in the bootstrap analysis also confirmed the stability of the main groups in the dendrogram. Thus our study reiterates the usefulness of RAPD analyses in assessing the genetic relationships in grape germplasm. Although there are several reports on diversity analysis of grapes using RAPD approach, most of them have analysed either cultivars (Jean-Jaques *et al.* 1993, Ye *et al.* 1998, Vidal *et al.* 1999 a, b) or rootstocks (This *et al.* 1997) separately. Up to now there is only one report (Grando *et.al.* 1995), in which both wild and cultivated genotypes of *V. vinifera* have been analysed together. Our report thus differs from others in that we have analysed a total of 43 genotypes belonging to nine different *Vitis* species at the same time using RAPD approach.

Genetic diversity in the grapes grown in India: The percentage of polymorphism (99 %) reported in the present study is much higher than values reported earlier (Vidal *et al*. 1999 a, b). This may be due to the fact that very diverse germplasm comprising wild and

cultivated genotypes belonging to 9 different species was analysed together. A higher degree of polymorphism (90 %) was also found within *V. vinifera* cultivars. Most varieties were introduced from different grape growing countries, *e.g.* USA, Australia and Russia; this may have increased diversity. Secondly, the preselection of primers based on their ability to distinguish between cultivars also may have led to reveal higher polymorphism among cultivars. In contrast, the *V. labrusca* group of cultivars, introduced mainly from USA, appeared to be a highly homogeneous group.

In our analysis several genotype-specific amplification products were also observed. Although most of them were present in wild species, about 15 bands were specific to *V. vinifera* cultivars. These variety-specific markers will be useful for varietal identification and there is a need to identify such markers for many more varieties. Furthermore, these bands seem to be useful in defining the genetic relationships more clearly (data not shown). This observation is in accordance with earlier reports (Bourqin *et al.* 1993; Vidal *et al.* 1999 b).

Groupings in relation to parentage: When the groupings obtained in the study were compared with the parentage of the cultivars, some interesting findings were revealed. Varieties Tas-a-Ganesh, Manik chaman and Sonaka are bud sport selections from Thompson Seedless, while Pusa seedless is a selection from unknown germplasm having characteristics very similar to Thompson Seedless. Clustering of all these varieties in the same cluster and also in the same subgroup suggested very close genetic homology. Similarly, cv. Kishmish chorni and its bud sport Sharad seedless are also present in the same subgroup. Another interesting finding was that seeded and seedless varieties formed separate subgroups within the cluster of *V. vinifera* genotypes.

Within the *V. labrusca* group two genotypes, Bangalore blue and Bangalore purple, are *V. vinifera* x *V. labrusca* hybrids considered to be synonymous (Chadha and Randhawa 1974). However, the vegetative and fruit characters of the two varieties are quite different from each other. Our study clearly demonstrated that both varieties are also quite distinct genetically. While Bangalore blue was grouped with other *V. labrusca* cultivars the profiles obtained for Bangalore purple were more close to *V. vinifera* rather than *V. labrusca* types resulting in clustering of Bangalore purple with seeded types of *V. vinifera*.

In the group of wild species and rootstocks, two varieties of *V.champinii*, Dogridge and Degrasset, and its only cultivated type Champanel, which is a cross between *V. labrusca* and *V. champinii*, are all clustered in the same subgroup.

Out of the 5 hybrids developed at our institute, H-27 and H-144 were grouped along with one of the parents. H-27, which is a hybrid between Diamond jubilee and Rubired, was present in the same subgroup with Rubired. H-144, a hybrid between Cheema sahebi and Catawba is grouped in the cluster of Catawba. Thus the clustering of most of the varieties in our study agreed well with previous studies and parentage.

Relevance for grape breeding: This is a first report on the genetic variability of grape germplasm used in India. Based on the viticultural management, grape-growing in India can be classified according to the climatic regions which are distinctly different. Moreover breeding objectives as well as the traits for which improvement is needed differ region-wise. Therefore, the knowledge on the genetic interrelationships among cultivated and wild genotypes obtained by such studies will be very useful for planning future breeding programs. Keeping with the changing trends in Indian viticulture more seedless varieties as well as varieties for wine making need to be analysed in the future.

Acknowledgement

Authors thank Dr. Vidya Gupta, Division of Biochemical Sciences, National Chemical Laboratory, Pune, for critical reading of the manuscript.

References

Armstrong, J.; Gibbs, A.; Peakall, R.; Weiller, G.; 1994: 'The RAPDistance Package'. http://life.anu.edu.au/molecular/software/rapd.htm

Bourquin, J. C.; Sonko, A.; Otten, L.; Walter, B.; 1993: Restriction fragment length polymorphism and molecular taxonomy in *Vitis vinifera* L. Theor. Appl. Genet. **87**, 431-438.

Büscher, N.; Zyprian, E.; Blaich, R.; 1993: Identification of grapevine cultivars by DNA analyses: Pitfalls of random amplified polymorphic DNA techniques using 10mer primers. Vitis **32**, 187-188.

Chadha, K. L.; Randhawa, G. S.; 1974: Grape Varieties in India - Description and Classification, ICAR Technical Bulletin u.c.o. 48, Indian Council for Agricultural Research, New Delhi.

- -; Shikhamany, S. D.; 1999: The Grape: Improvement, Production and Post-harvest Management. Malhotra Publishing House, New Delhi.

Fanizza, G.; Colonna, G.; Resta, P.; Ferrera, G.; 1999: The effect of number of RAPD markers on the evaluation of genotypic distances in *Vitis vinifera*. Euphytica **107**, 45-50.

Felsenstein, J.; 1993: PHYLIP - phylogenic inference package (Version 3.2). Cladistics **5**, 164-166.

Grando, M. S.; De Micheli, L.; Biasetto, L.; Scienza, A.; 1995: RAPD markers in wild and cultivated *Vitis vinifera.* Vitis **34**, 37-39.

Guirao, P.; Moya, A., Cenis, J. L.; 1995: Optimal use of random amplified polymorphic DNA in estimating the genetic relationship of four major *Meloidogyne* spp. Phytopathology **85**, 547-551.

Jean-Jaques, I.; Defontaine, A.; Hallet, J. N.; 1993: Characterization of *Vitis vinifera* cultivars by random amplified polymorphic DNA markers. Vitis **32**, 189-190.

Lodhi, M. A.; Ye, G. N.; Weeden, W. F.; Reisch, B. I.; 1994: A simple and efficient method for DNA extraction from grapevine cultivars and *Vitis* species. Plant Mol. Biol. Reptr. **12**, 6-12.

Patil, S. G.; Honrao, B. K.; Karkamkar, S. P.; 1996: New promising grape hybrids. Drakshavritta Souvenir **16** (7), 79-82.

Prevost, A.; Wilkinson M. J.; 1999: A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. Theor. Appl. Genet. **98,** 107-112

Reisch, B. I.; Pratt, C.; 1996: Grapes. In: J. Janick, J. N. Moore (Eds.): Fruit Breeding, Vol. II, 297-369. John Wiley & Sons.

This, P.; Cuisset, C.; Boursiquot, J. M.; 1997: Development of stable RAPD markers for the identification of grapevine rootstocks and the analysis of genetic relationships. Am. J. Enol. Vitic. **48**, 492-501.

Vidal, J. R.; Coarer, M.; Defontaine, A.; 1999 a: Genetic relationships among grapevine varieties grown in different French and Spanish regions based on RAPD markers. Euphytica **109**, 161-172.

- -; Moreno, S.; Gogorcena, Y.; Masa, A.; Ortiz, J. M.; 1999 b: On the genetic relationships and origins of six grape cultivars of Galicia (Spain) using RAPD markers. Am. J. Enol. Vitic. **50**, 69-75.

Williams, J. G. K.; Kubelik, A. R.; Livak, K. J.; Rafalsky, J. A.; Tingey, S. V.; 1990: DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl. Acids Res. **18**, 6531-6535.

Xu, H.; Wilson, D. J.; Arulsekar, S.; Bakalinsky, A. T.; 1995: Sequence-specific polymerase chain reaction markers derived from randomly amplified polymorphic DNA markers for fingerprinting grape (*Vitis*) rootstocks. J. Am. Soc. Hort. Sci. **120**, 714-720.

Yap, I.; Nelson, R.J.; 1996: WinBoot: A program for performing bootstrap analysis of binary data to determine the confidence limits of UPGMA-based dendrograms. IRRI Discussion paper Series No. 14, International Rice Research Institute, Manila, Philippines

Ye, G. N.; Soylemezoglu, G.; Weeden, N. F.; Lamboy, W. F.; Pool, R. M.; Reisch, B. I.; 1998: Analysis of the relationship between grapevine cultivars, sports and clones via DNA fingerprinting. Vitis **37**, 33-38.

Received February 19, 2001