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ROLE OF SSR MARKERS IN CHARACTERIZATION OF GRAPE (*VITIS VINIFERA* L) GENOTYPES AND HYBRIDS

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ABSTRACT

The aim of this study was to fingerprint and characterize the grapegenotypes and hybrids to construct a molecular database including the cultivars commonly grown in India.A total of 42 genotypes including eighteen hybrids and seven parents were analyzed using seven microsatellite simple sequence repeat (SSR) markers and their main morphological and agronomic characteristics were compared. The dissimilarity matrix showed that a maximum of 110 units was obtained between the genotypes "Convent Large Black" and "Arka Hans," while a minimum dissimilarity of 37 units were obtained between the genotypes "Anab-e-Shahi" and "Dilkhush."The hybrid nature of all the progenies used was marked by VVMD-32. In VVMD-32, unique banding pattern was observed in Black Champa (male parent) in Group A. In which six bands were prominent, out of which first and last band were absent in Thompson Seedless (female parent). While in case of primer VVS-29, three prominent bands were observed in male parents. A total of 45 alleles in all 42 genotypes were obtained with 7 primers with an average number of 6.4 alleles per locus. The use of seven polymorphic microsatellite markers and the level of genetic variability detected within Indian grapevine germplasm suggested that this is a reliable, efficient, and effective marker system that can be used for molecular characterization and subsequently in crop improvement programs.

INTRODUCTION

Grape(*Vitisvinifera*L) cultivation is one of the most remunerative farming enterprises in India. Famous Indian medicine scholars, Sasruta and Charaka in their medical treatises entitled '*SasrutaSamhita'* and '*CharakaSamhita',* respectively, written during 1356-1220 BC, mentioned the medicinal properties of grapes. Kautilya in his *'Arthashastra'* written in the fourth century BC mentioned the type of land suitable for grape cultivation. Native spp. resembling *Vitislanata* and *Vitispalmata* grow wild in the northwestern Himalayan foothills.

Commercial cultivation was started in the beginning of the 20th century. Presently, grapes are successfully grown in India over an area of 1, 10,000 ha with a production of approximately 1.73 million MT (Anon. 2013), primarily for use as fresh fruit. Grape breeding had mainly relied on selection among naturally occurring spontaneous crosses for ages and to a lesser extent, due to conventional breeding during the last century. The varieties currently available are the results of a selection process by human and ecogeographical conditions (Bisson, 1995). Information on genetic diversity among plant species is important for efficient utilization of genetic resources. The existence of close genetic relationships among cultivars grown in the same region or under similar climatic influence could lead to dilution of genetic

resources. Hence, studies on grape have been carried out to characterize the commercially important germplasm available in India. Microsatellite technology has been extensively used in grapevine biology and genetics. The number of microsatellite loci available has greatly increased in the last few years largely through the establishment of the International Vitis Microsatellite Consortium, leading to the discovery of more than 350 new loci. Microsatellite markers, being abundant, multiallelic and highly polymorphic, provide an efficient and accurate means of detecting genetic polymorphism. Most importantly, their codominant nature makes them the markers of choice for population genetic analysis to assess genetic organization in germplasm collections.

Microsatellites have been used to determine parent-progeny relationships in grape (Narayanaswamy*et al*. (2009), to develop a database of DNA profiles for use in cultivar identification and as markers for mapping genetic linkage The present study evaluates the hybrids for their parentage analysis to confirm the hybrid nature using polymorphisms revealed by twelve microsatellite loci.

MATERIALS AND METHODS

Plant Materials

Plant material from 42 grape genotypes was collected from Department of Horticulture, University of Agricultural Sciences, Bangalore and Indian Institute of Horticultural Research, Bangalore. Approximately, 50 g of recently matured leaves (15–20 d old) were collected, washed using distilled water, wiped with 70% (v/v) ethanol, then air dried prior to storage in sealed plastic bags at 4°C.

SSR Analysis

A total of twelve SSR primers characterized in previous studies were used. The primers were VVS1, VVS2, VVS29, VVMD 7, VVMD 14, VVMD 25, VVMD 27, VVMD28, VVMD31, VVMD32, VVMD 36 and VMC7b1 (Bowers *et al*., 1996 and 1999b).These microsatellites were selected as they were the same core set in the screening programme used to access the target grapevine collections. Primer pairs were synthesized from MWG Biotech, Bangalore, India based on their published gene sequence. PCR was performed in 96-well plates in MJ Research PTC100 thermocyclers (Bio-Rad Laboratories, Bangalore, India).

PCR reactions were carried out in 25 µl reactions containing 50 ng of DNA, 5 pmoles of each primer, 10x of *Taq*polymerase buffer (50 mMKCl, 10 mMTris-HCl, pH 9.0, 0.05% (v/v) NP40, and 0.05% (v/v) Triton X-100), 1.5 mM of MgCl2, 0.5 mM of dNTPs (Finzymes Pvt. Ltd., India), and 1 U of *Taq*polymerase (Sigma-Aldrich Pvt. Ltd., India). The final volume was adjusted with sterile distilled water. The PCR amplifications were carried out with respect to the protocols for primer sets published (Bowers et al., 1996 and 1999b and Thomas and Scott, 1993). Amplification was confirmed with agarose gels, and alleles were separated by running on 6% polyacrilamide denaturing gels and electrophoresed in 1 × TBE at 55 W for 2 h. The amplified products were visualized with silver staining previously described.

Statistical Analysis

Amplified fragments from each SSR primer set were scored manually for their presence (1) or absence (0). The profiles of 25 accessions of grapevine using 12 primer pairs were assembled for statistical analysis. The sizes of the fragments were estimated using 50 bp standard DNA markers (Bangalore GeneiPvt. Ltd., India), coelectrophoresized with the amplified products. A genetic dissimilarity matrix was developed using Euclidean Distances, which estimates all pairwise differences in the amplification products (Sokal and Sneath, 1973). A cluster analysis was based on Ward's method using a minimum variance algorithm.

RESULTS AND DISCUSSION

The objective of this study was to confirm the parentage of the hybrids developed. To detect hybridity there must be polymorphism between the parents. The polymorphic bands which are present in male parent should be present in all the hybrids and should not be present in female parent.

Molecular characterization

A constructed dendrogram based on 27 morphological characters clustered the grape genotypes into two

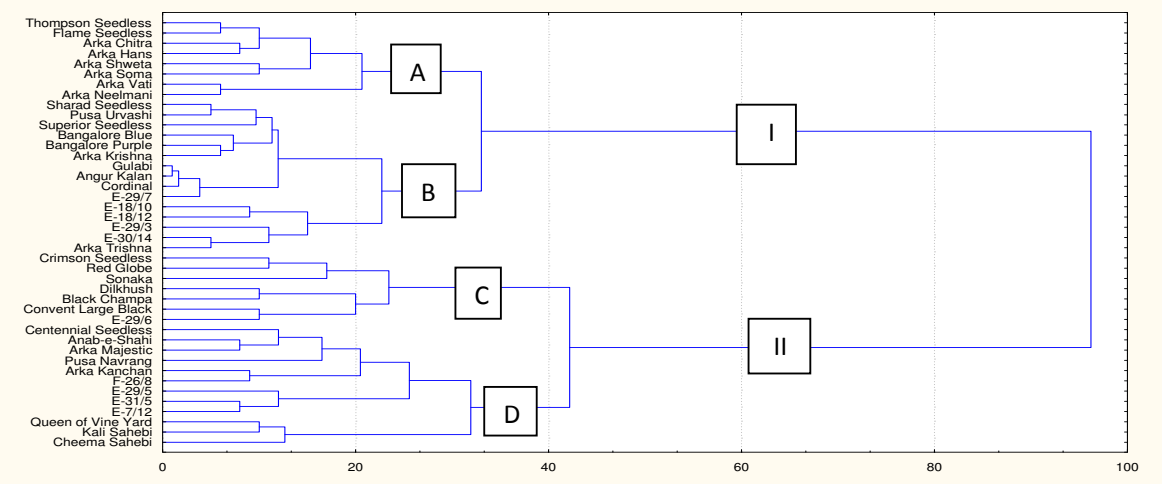


Figure 1:Dendrogram showing the clustering patterns of 42 Indian grapevine genotypes based on morphological characters.

Cluster I consisted of 23 genotypes grouped into two subclusters (A and B) linked at a distance of 33 units. SubclusterA clustered at a distance of 21 units and consisted of 8 genotypes. The seedless genotypes "Thompson Seedless" and "Flame Seedless" were clustered together, but both differed with compact and elongated bunches and pale and red colored berries, respectively. The genotypes "ArkaChitra," "Arka Hans," "ArkaShweta," and "Arka Soma" showed seeded berries with greenish yellow color, but varied with their bunch characters. The genotypes "ArkaVathi" and "ArkaNeelamani" clustered together showed large and elongated bunches, but contained green and black colored berries respectively. Subcluster B consisted of 15 genotypes and clustered into two groups at 23 linkage distances. Group 1 clustered 10 genotypes predominantly characterized by large bunches and dark berry types.

The genetic variability of this germplasm (Table 1) was evaluated on the basis of the number of alleles (mean: 6.4), gene diversity (GD: 0.71), observed heterozigosity (Ho: 0.849), and probability of coincidence (PC: 0.13). These data indicated the presence of a lower genetic variability in the Indian grapevine germplasm, comparable to the variability found in the Algeria and Mediterranean basin and similar to Spanish grape germplasm. The most informative locus was VVMD 28 (13 alleles per locus) and the least informative one was VVMD-14 (4) (Table 1).

Table 1: Descriptive statistics and Genetic Diversity of Indian Grape genotypes at Twelve Microsatellite Loci

Loci	Allele Number	Allele size range (bp)	Expected Hetezygosity (He)	Observed Heterozygosity (Ho)	Probability of Identity (PI)	Probability of null alleles (r)	Genetic diversity (GD)	Discrimination power (d)
VVS 1	6	180-230	7.93	7.90	0.09	-0.017	0.61	0.79
VVS 2	7	140-210	8.12	8.33	0.13	-0.035	0.67	0.82
VVS 29	4	150-175	8.35	8.14	0.16	-0.130	0.74	0.80
VVMD 7	4	140-170	8.16	8.83	0.06	-0.036	0.41	0.86
VVMD 14	4	140-165	7.69	7.65	0.08	0.011	0.53	0.47
VVMD 25	5	135-165	7.81	7.93	0.10	0.064	0.60	0.61
VVMD 27	7	135-175	8.40	8.16	0.13	-0.026	0.64	0.73
VVMD 28	13	140-200	8.66	9.21	0.15	-0.045	0.85	0.81
VVMD 31	6	130-165	8.10	9.47	0.11	-0.016	0.79	0.84
VVMD 32	9	130-205	8.20	8.16	0.19	-0.024	0.77	0.89
VVMD 36	5	180-205	8.56	8.94	0.16	-0.019	0.78	0.86
VMC 7b1	5	150-205	8.64	8.09	0.20	-0.015	0.80	0.84
Mean	6.3	146-189	8.10	8.20	0.13	-0.021	0.68	0.77

The most informative primers VVMD 28 are VVMD 32 were good candidates to be used for paternity testing due to the high direct count heterozygosity, high number of alleles, and even distribution of allelic frequencies. Moreover, in the populations studied, the observed heterozygosity was very sim-ilar to the expected heterozygosity at each nuclear SSR locus, suggesting no excess of homozygosity in populations. Since the analysis did not display null alleles hence, the marker should be suitable for a genetic population study among wild relatives and parentage studies. Such an absence of null alleles is probably due to the choice of nuclear SSR loci which have revealed very low deviation in the observed heterozygosity from the expected heterozygosity. Hence, a choice criterion should be used to avoid loci displaying null alleles being included.

Cluster analysis of hybrids

A total of15 primers were used for the analysis out of which 12 primers were polymorphic. Linkage among 18 hybrids and their parents as revealed by a dendorgram is presented in Fig.1. The cluster analysis grouped the parents and hybrids into two major groups. Group I is divided into group I (a), I (b) and I (c). Under group I (a) Arkavati, E-31/5 and E-29/7 come under one cluster to which Arkavati and E-29/5 are related at a linkage distance of 54 and 55, respectively. In the same group E-29/6 and E-7/12 formed a single sub cluster and are closely related at a linkage distance of 51. Groups I (b) comprises of 4 genotypes. Among them ArkaNeelamani and ArkaShweta are closely related at linkage distance of 34. AngurKalan and Bangalore Blue Share much similarly and are related at a linkage distance of 40.

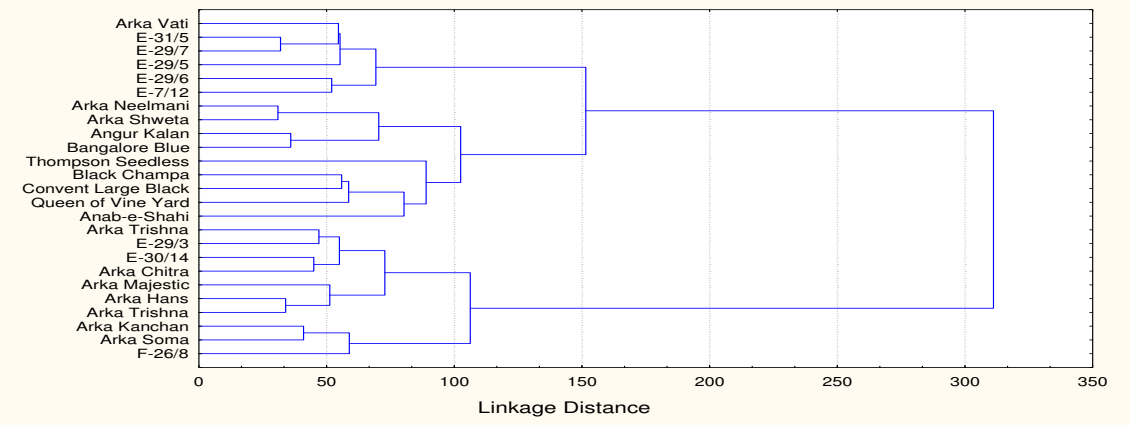
Group I (c) comprised of 5 genotypes, among them Black Champa and Convent Large Black are closely related and formed a single sub cluster at a linkage distance of 58, whereas, Thompson Seedless, Queen of Vine Yard and Anab-e-Shahi separated from this group and formed individual clusters.

Group II comprises of 10 hybrids and is divided into group I (a), I (b) and I (c), under group II (a), ArkaTrishna and E-29/3 are closely related at a linkage distance of 45 whereas E-30/14 and ArkaChitra formed a separate sub cluster at a linkage distance of 42. Group II (b) comprises 3 hybrids among them Arka Hans and ArkaTrishna are closely related at a linkage distance of 45 whereas E-30/14 and ArkaChitra formed a separate sub cluster at a linkage distance of 42 (Table 1).

Hybridity confirmation

Eighteen hybrids from different parent combinations were tested for their hybridity. Out of 18 hybrids 9 were from cross between Black Champa x Thompson Seedless, two each from cross between Anab-e-Shahi x Queen of Vine Yard, AngurKalan x Black Champa. One each from a cross between Anab-e-Shahi x Thompson Seedless, Anab-e-Shahi x Convent Large Black, AngurKalan x Anab-e-Shahi, Bangalore Blue x Anab-e-Shahi and Bangalore Blue x Convent Large Black.

Fifteen primers were used for analysis to confirm the hybridity and the 7 primers giving clear and reproducible bands were chosen for PCR analysis of samples. The banding patterns of the parents and progenies were compared to test the hybridity of the progenies used. Out of the seven primers used individually for amplification of samples, three primers VVMD-32, VVS-2 and VVS-29 gave the amplification patterns which reveal the hybrid nature of the progenies. In case of primer VVMD-32 (Group A), unique banding pattern was observed in Black Champa (male parent) in which 6 bands were prominent, out of which first and last band were absent in Thompson Seedless (female parent). Whereas the first band was present in all the hybrids except 9^{th} and the last band was absent in 8^{th} hybrid. In group B, 5^{th} band was present in male parent and the hybrids but was absent in female parent. In group C and D, the last band was prominent among the male parent and hybrids. In case of groups E and F, 4^{th} and 5^{th} bands were prominent in male parent and hybrids. This primer has not proved the hybridity of progenies of group G and H.



grapevine

Conclusion

Thus, this study, using microsatellite markers on Indian grape vine genotypes, showed considerable genetic diversity existing among the population. This is most likely due to different conditions under which the populations are grown and conserved. It can be concluded that, all the progenies under investigation were confirmed to be hybrids. Similar kind of observations were reported by Narayanaswamy*et al*. (2009) by using SSR markers.

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