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Microsatellite Markers and Genetic Diversity Analysis in Grape (*Vitis vinifera* L)

Venkata Rao
Associate Professor of Fruit Science, College of Horticulture, Bengaluru, University of Horticultural Sciences, Bagalkot, Karnataka, India

Abstract *Grape (Vitis vinifera L), commonly cultivated in India, shows a wide range of ripening periods and fruit quality and is an unexploited resource for breeding programs. The main purpose of this study was to fingerprint these accessions and to construct a molecular database including the cultivars commonly grown in India. A total of 42 genotypes were analyzed using seven microsatellite simple sequence repeat (SSR) markers and their main morphological and agronomic characteristics were com-pared. 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 dis-similarity matrix showed that a maximum of 110 units was obtained between the genotypes "Convent Large Black" and "Arka Hans," while a minimum dissimilarity if 37 units were obtained between the genotypes "Anab-e-Shahi" and "Dilkhush." In the dendrogram the microsatellite segregated the genotypes into two clusters (A and B) at 476 units with two subclusters each. The sub-clusters grouped genotypes predominantly as A1 with seeded fruits, A2 with seedless fruits, B1 with pigmented and seedless fruits, and B2 with colorless and seeded fruits. The use of seven polymorphicmicrosatellite 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 diversity analysis and subsequently in crop improvement programs.*
Keywords: Microsatellites Markers, Genetic diversity, Vitis vinifera L.

I. INTRODUCTION

Grape cultivars (*Vitis vinifera* L) have a long history of domestication. The world's vineyards occupy about 8.7 million hectares. More than 9600 grape cultivars exist around the world and almost 16,000 prime names appear in the *Vitis* International Variety Catalogue. Some of these are not easily distinguishable by morphology and many cultivars appear to be synonyms, having been distributed around the world and acquiring new names in the process . Moreover, the wide distribution and long history of cultivation have led to the develop-ment of numerous cultivars with many synonyms, resulting in complexity among germplasm collections Grape in India are reported to have been introduced in 620 BC. and commercial cultivation was started in the beginning of the 20th century. Presently, grapes are suc-cessfully grown in India over an area of 60,000 ha with a production of approximately 1.67 million MT (Anonymous, 2005), primarily for use as fresh fruit. Grape breeding had mainly relied on selection among naturally occur-ring 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 eco-geographical conditions. 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 charac-terize the commercially important germplasm available in India.

Morphological characterization has been attempted among several grape cultivars for identification purposes. However, superiority of molecu-lar markers over morphological characterization in grape cultivars is well established. The usefulness of molecular testing for grapevine identification is widely accepted. In the grape germplasm, characterization of endangered cultivar, parentage analysis, identification of the clones (Ye *et al.*, 1998), analysis of genetic diversity (Narayanaswamy *et al.*, 2009), and molecular mapping (Doligez *et al.*, 2006) is been carried out. Molecular markers like RFLP, RAPD, microsatellite or SSR and AFLP are successfully used to characterize grape germplasm. In this study, a combination of morphological and micro-satellite amplification with appropriate statistical analysis has enabled us to identify the relationships among 42 genotypes.

II. MATERIALS AND METHODS

A. Plant Materials

Plant material from 42 grape genotypes was collected from Indian Institute of Horticultural Research, Bangalore. Approximately, 50 g of recently matured leaves (15–20 days old) were collected, washed using distilled water, wiped with 70% (v/v) ethanol, then air dried prior to storage in sealed plastic bags at 4^0C.

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B. Morphological Data

Morphological characterization of each genotype with respect to their vegetative and reproductive characters was done in triplicate plants according to IPGRI (International Plant Genetic Resources Institute, Rome) descriptors for grape (Anonymous, 1995). The dendrogram was constructed based on the descriptors value for all genotypes using SPSS for windows, version 11.5.0.

C. DNA Isolation

DNA was extracted from the stored leaves of grapevine using a cetyl trime-thyl ammonium bromide (CTAB) method (Simon *et al*., 2007). 2 g of leaf sample were powdered in liquid nitrogen to extract the DNA. The powder was mixed with 10 ml extraction buffer, preheated to 65^0C, containing 100 mM Tris-HCl, pH 8.0, 20 mM EDTA, 1.4 M NaCl, 3% (w/v) CTAB, 2% polyvinylpyrrolidone and 1% (v/v) ¾β-mercaptoethanol, then incubated at 65^0C for 1 h. The mixture was cooled to room temperature, 10 ml cold 24:1 (v/v) chloroform:isoamylalcohol was added, and the contents were mixed well. After centrifugation at 7500 rpm for 12 min at 4^0C, the superna-tant was transferred to a fresh tube and the chloroform:isoamylalcohol step was repeated until a clear supernatant was obtained. 5 M NaCl was added to the supernatant [0.5 (v/v)] and mixed gently, followed by addition of 1 volume of cold isopropanol to precipitate the DNA. The mixture was incubated overnight at 4^0C, then centrifuged at 6500 rpm for 5 min. The resulting pellet was washed with 70% (v/v) ethanol, air-dried, and dissolved in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). 2 µg RNase (bovine pancreatic ribonuclease; Bangalore Genei, Bangalore, India) was added to each sample which was incubated for 3 h at 37^0C, mixed with an equal volume of phenol, and centrifuged at 7500 rpm for 12 min at room temperature. This step was followed by washing with an equal volume of 1:1 (v/v) phenol:chloroform, then with chloroform alone. The DNA was precipitated overnight at 4^0C with 0.5 (v/v) 5 M NaCl and 1 volume of cold isopropanol. The resulting pellet obtained after centrifugation was dissolved in TE buffer, analyzed in an agarose gel and quantified using a spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

D. Microsatellite Analyses

A total of seven SSR primers characterized in previous studies were used. The primers were VVMD 14, VVMD 25, VVMD 27, VVMD 28, VVMD 31, VVMD 36, and VMC 7f2. 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 mM KCl, 10 mM Tris-HCl, pH 9.0, 0.05% (v/v) NP40, and 0.05% (v/v) Triton X-100), 1.5 mMof 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 in Bowers *et al*., 1996 and 1999b; Pellerone *et al*., 2001; 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.

III. RESULTS AND DISCUSSION

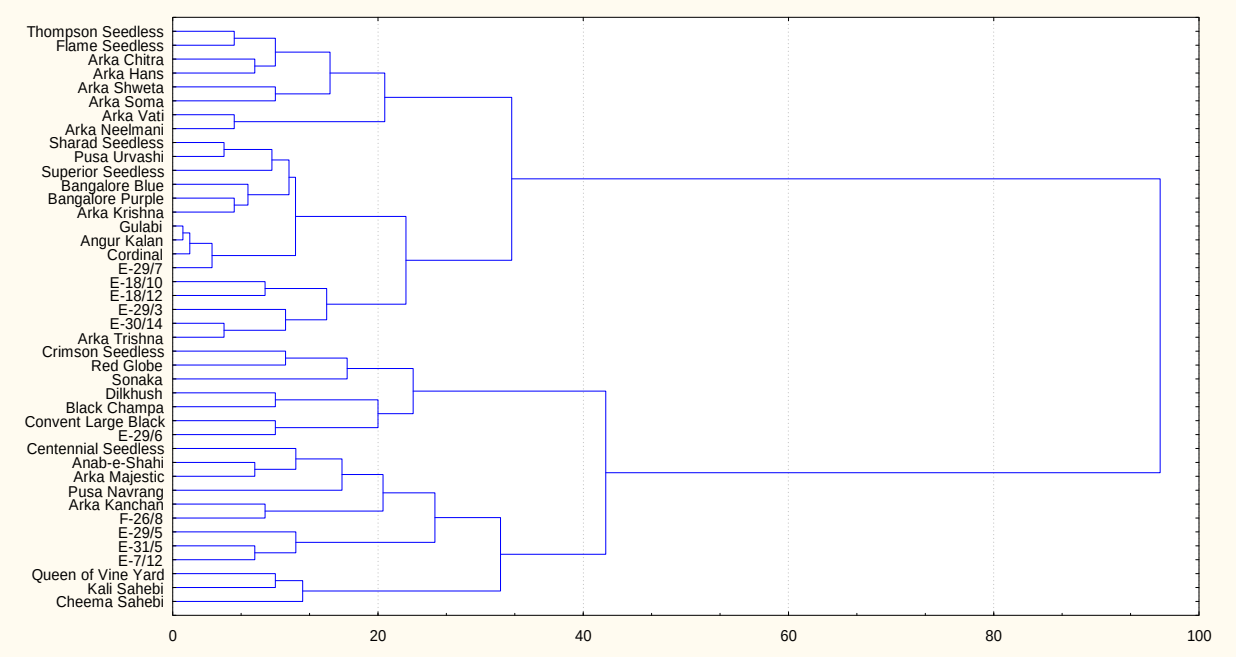
The objective of this study was to estimate the extent of the genetic diversity among Indian grapevine lines using SSR markers. Information on genetic diversity in crop plant species is important for efficient utilization of plant genetic resources. The traditional method of identifying species by morpho-logical characters is now accompanied by DNA profiling that is more reliable on proteins, largely because of several limitations of their morphological data (Nayak *et al*., 2003). Therefore, when investigating organisms with a high tendency for morphological differentiation, studies considering both molecular and morphological characters are highly relevant (Bartish *et al*., 2000).

A constructed dendrogram based on 27 morphological characters clus-tered the grape genotypes into two major clusters (I and II) at 96 units (Fig. 1). Cluster I consisted of 23 genotypes grouped into two subclusters (A and B) linked at a distance of 33 units. Subcluster A 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 com-pact and elongated bunches and pale- and red-colored berries, respectively. The genotypes “Arka Chitra,” “Arka Hans,” “Arka Shweta,” and “Arka Soma” showed seeded berries with greenish yellow color, but varied with their bunch characters. The genotypes “Arka Vathi” and “Arka Neelamani” 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.

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Group 1 clustered 10 genotypes predominantly characterized by large bunches and dark berry types. The genotype "Angur Kalan" showed golden yellow berries with pinkish tinge, genotype "Sharad Seedless" evidenced a crispy pulp, and genotype E-29/7 had spherical berries. Group II consisted of 5 genotypes clustering at 15 units. All genotypes of the group showed medium to large bunches with dark colored berries. The genotypes E-18/12 and "Arka Trishna" were distinctly characterized by cylindrical-bunch and oval-shaped berries, respectively.

Cluster II consisted of 19 genotypes forming into two subclusters (C and D) at 43 linkage distance in the dendrogram. Subcluster C was comprised of 7 genotypes at 23 distances. All the genotypes under this subcluster showed colored berries and were seeded, except the genotypes "Sonaka"and "Dilkhush," which had a pale greenish color, and genotypes "Crimson Seedless" and E-29/6 with a seedless nature. The genotypes "Crimson Seed-less" and "Red Globe" clustered together, respectively showing light-red and wine-red colored berries. Genotypes "Black Champa" and "Convent Large Black" both had spherical berries. The subcluster D with 12 genotypes was clustered at 32 units. The genotypes were predominantly characterized by greenish golden yellow color and seeded berries with the exception of genotypes "Arka Majestic," "Pusa Navrang," and "Arka Kanchan" which evidenced a dark color. The remaining genotypes "Centennial Seedless," "Arka Kanchan," and E-29/5 produced seeded berries. The genotypes "Pusa Navrang" and "Kali Sahebi" were characterized by berries with reddish pulp, while genotypes "Queen of Vine Yards," "Kali Sahebi," and "Cheema Sahebi" were clustered together and characterized by obovate-shaped berries.



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 (Martin *et al*., 2003). The most informative locus was VVMD 28 (13 alleles per locus) and the least informative one was VVMD-14 (4) (Table 1). 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. More-over, 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 (Wagner *et al*., 2006). 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 (Bandelj *et al*., 2004; Khadari *et al*., 2003). Hence, a choice criterion should be used to avoid loci displaying null alleles being included.

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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

Pairwise comparisons were made between all genotypes included in this study and the average dissimilarity values were calculated based on microsatellite data. A maximum dissimilarity of 110 units was obtained between the genotypes “Convent Large Black” and “Arka Hans” where the former genotype was characterized by spherical bluish black seeded berries and the later genotype with colorless seeded berries; while a minimum dis-similarity of 37 units were obtained between the genotypes “Anab-e-Shahi” and “Dilkhush,” where both the genotypes were characterized by pale-greenish colored seeded berries. The dendrogram presented demonstrates clearly the ability of microsat-ellites to detect a large amount of genetic variation in genetically closely related genotypes of grapevine and to identify groups with different levels of genetic distance. The markers segregated the genotypes into two major clusters (I and II) at 476 linkage distance (Fig. 2). Major cluster I consisted of 30 genotypes grouped into two subclusters (A and B) at 223 linkage dis-tance. Subcluster A with 17 genotypes was grouped into two groups (A1 and A2) with 12 and 5 genotypes, respectively. The genotypes “Anab-e-Shahi” and “Dilkhush” were closely linked in group A1 by 31 linkage distance with both the genotypes showing greenish seeded berries. The genotypes “Pusa Urvasi” and “Cordinal” were closely linked at 39 units and grouped with “Pusa Navrang” at 59 units where “Cordinal” and “Pusa Navrang” were producing pigmented berries. The genotypes “Centenal Seedless” and “Superior Seedless” were linked together at 35 units, both with colorless berries. These genotypes were grouped with “Shrad Seedless” (pigmented berries) at 52 units and linked with cultivars “Sonaka” and “Gulabi” at 73 units. The genotypes “Thompson Seedless” and “Flame Seedless” were grouped together at 45 units and differentiated with colorless and red berries, respectively. The five genotypes of group A2 were linked at 71 units, of which “Black Champa” and “Red Globe” were closely linked at 41 units with both pigmented and seeded berries. The “Convert Large Black” (pigmented and seeded berries) was grouped with “Queen of Vineyards” (golden yellow colored and seeded berries) at 56 units. The genotype “Crimson Seedless” (red colored berries) stood separate and linked to the group at 71 units.

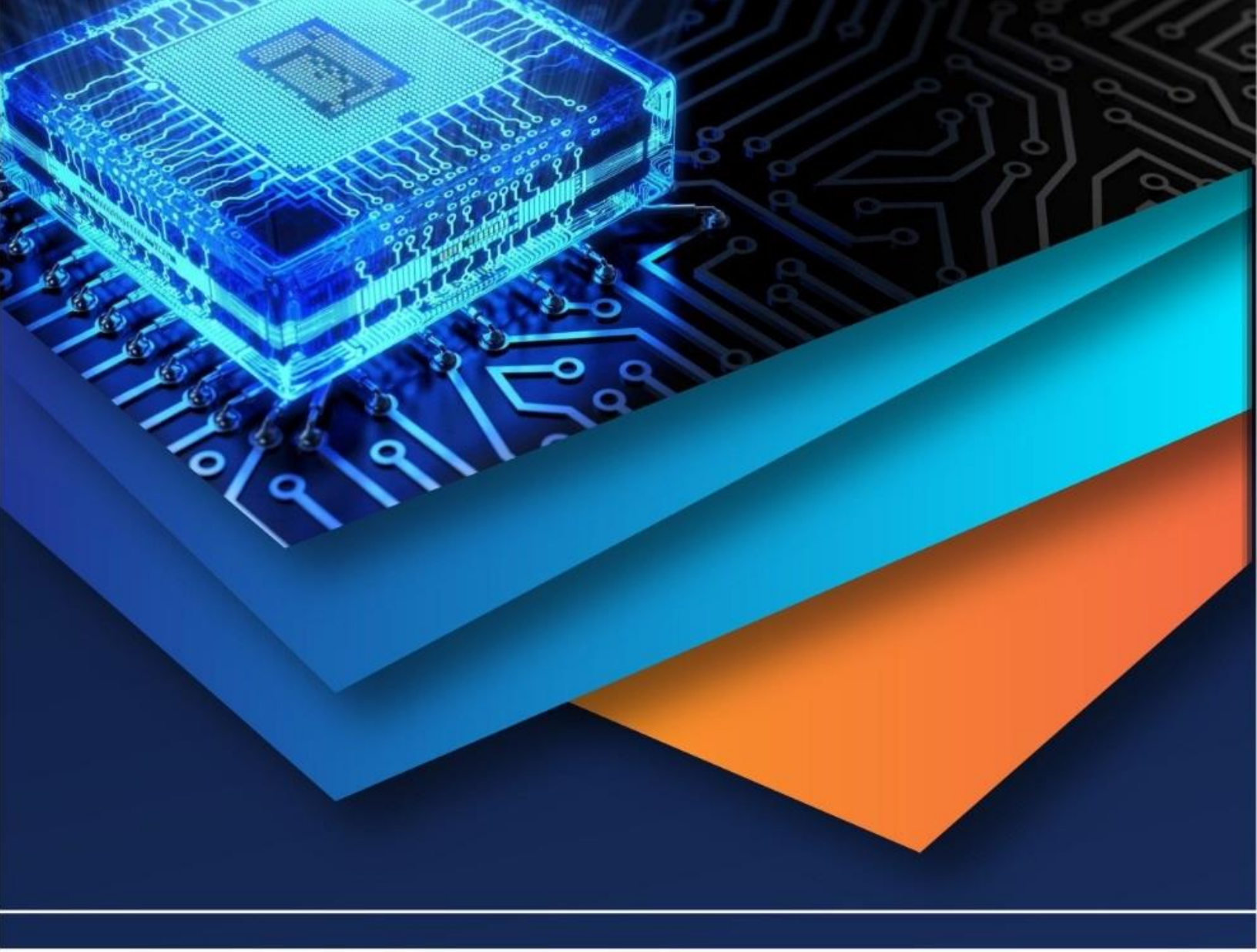
The subcluster B consisted of 13 genotypes segregated into two groups (B1 and B2) at 145 units in the dendrogram. The group B1 with eight geno-types was clustered at 81 units, of which “Arka Neelamani” and “Arka Shweta” were closely linked at 31 units—both were seedless. The large bunches with colored genotypes, namely, “Angur Kalan” (pinkish) and “Bangalore Blue” (purple) were linked at 33 units. “Bangalore Purple” (purple) and “Kali Sahebi” (reddish pulp) were linked at 63 units. The geno-types “Cheema Sahebi” and “Arkavathi” both with pyramidal shaped and tightly packed bunches were clustered together at 45 units. The group B2 consisted of five genotypes linked at 65 units and divided into two minor clusters. The first minor cluster consists of three colorless and seedless gen-otypes E-29/5, E-31/5, and E-29/7, which are linked at 55 units. The second minor cluster with two genotypes, E-29/6 and E-7/12, is linked at 52 units and both genotypes showed medium-sized bunches and seedless berries. The characteristic feature of cluster I predominantly shows subcluster A with more seeded genotypes and subcluster B with seedless genotypes.

IV. SUMMERY

In summary, 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 (Narayanaswamy *et al*., 2009). The clustering of the genotypes was predominantly based on the pigment and seed charac-ters in fruits. These groupings can be used in selecting diverse parents in breeding improved cultivars and in maintaining variation in the germplasm. Evaluation of genetic diversity among germplasm, particularly of crops, is crucial in utilizing genetic potential to improve traits needed for adaptation to various stress conditions (Amer *et al*., 2001). To our knowledge, this study is the first attempt in using molecular markers for assessing genetic diversity in Indian grape vine germplasm, and the outcome of this work could be use-ful for future characterization and exploitation of that germplasm. Understanding the spatial organization of genetic diversity within the plant populations is of critical importance for the development of strategies designed to preserve genetic variation. It has been shown that species with limited gene flow (i.e., with restricted seed and pollen movement) have considerably more among-population variation for total amount of genetic diversity . Since the *ex situ* collection cannot exceed a limited number of accessions, it is difficult to preserve the evolutionary potential of the species hence, the future of this fruit depends on the selection of high quality cultivars. Thus, conservation strategies among grape vine cultivars should be developed with the morphological characters in mind. Taken together, the microsatellites markers can be used for establishing relationships between related species, but their efficiency depends on the amount of variability obtained within the cultivars.

REFERENCES

[1] Amer, I.M.B., A. Borner, and M.S. Roder. (2001). Detection of genetic diversity in Libyan wheat genotypes using wheat microsatellite markers. Genet. Resour. Crop Ev. 48:579–585.
[2] Anonymous. (1995). IPGRI, Descriptors for grapevine (Vitis vinifera L.). International Plant Genetic Resources Institute, Rome.
[3] Anonymous. 2005. Statistical Databases—Agricultural Data. http://faostat.fao.org/ site/567/default.aspx#ancor
[4] Bandelj, D., J. Jakse, and B. Javornik. (2004). Assessment of genetic variability of olive varieties by microsatellite and AFLP markers. Euphytica 136:93–102
[5] Bartish, I.V., K. Rumpunen, and H. Nybom. (2000). Combined analyses of RAPDs, cpDNA and morphology demonstrate spontaneous hybridization in the plant genus Chaenomeles. Heredity 85:383–392.
[6] Bourquín, J.C., L. Otten, and B. Walter. (1993). Restriction fragment length poly-morphism and molecular taxonomy in Vitis vinifera. Theor. Appl. Genet. 87: 157–162
[7] Doligez, A., A.F. Adam-Blondon, G. Cipriani, G. Di Gaspero, V. Laucou, D. Merdinoglu, C.P. Meredith,
[8] Narayanaswamy Papanna, Venkata Rao, Srinivasmurthy and Luke Simon (2009). Microsatellite-Based Genetic Diversity Assessment in Grape (Vitis vinifera L) Germplasm and its Relationship with Agronomic Traits. International Journal of Fruit Science. 9:92–105, 2009
[9] Pellerone, F.I., K.J. Edwards, and M.R. Thomas. (2001). Grapevine microsatellite repeat: Isolation, characterization and use of genotyping grape germplasm from Southern Italy. Vitis 40:79–186.
[10] Wagner, A. P., S. Creel, and S.T. Kalinowski. (2006). Estimating relatedness and rela-tionships using microsatellite loci with null alleles. Heredity 97:336–345.



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