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PROFILLING OF GRAPE VARIETIES USING OIV DESCRIPTORS AND MOLECULAR MARKERS

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ABSTRACT
Sixteen grape varieties were characterized based on twenty-seven ampelographic traits and five microsatellite markers as per OIV descriptors at ICAR-National Research Centre for Grapes, Pune (INDIA). Variety, Champanel and Manjari Naveen displayed loose bunch compactness.White Sweet and Christmas Rose recorded with firm berry attachment with pedicel. Using principle component analysis, first three component described 50% variation. Highly significant differences were exhibited by all quantitative traits. White Sweet variety exceled in terms of traits like bunch weight (510.00 ± 17.93g),berry length (24.57 ± 0.60mm) and berry diameter (21.53 ± 0.95mm).Maximum bunch length (21.64 ± 0.62mm),10 berry weight (45.04 ± 1.14gm) and total soluble solids (23.77 ± 1.03°B) was recorded in Crimson Seedless, Maroo Seedless and Centennial Seedless respectively. Among sixteen varieties, seven were seeded (Champanel, White Sweet, Angoor Kalan, Spin Sahebi, JaosBelyi, Black Round and Christmas Rose). Five microsatellite markers had shown 55% of allelic diversity among the varieties based on 24 alleles generated. Marker VrZag79 had displayed maximum allelic diversity (75.78%).Total three clusters were generated in the dendrogram, where Black Round and Centennial Seedless were classified individually in two separate clusters.These information will be useful in selection of parents in breeding programme and profile generated will provide the identity to the variety.

INTRODUCTION

Grape scenario in India presents total of 1,10,000 ha area under grape cultivation with 1,737 metric ton per hectare annual production (National Horticultural Board, 2014).As returns from the grape is a much higher, it is reckoned to be one of the important fruit crops in India.Major belts for grape cultivation in India are: Western Maharashtra followed by Tamil Nadu, Karnataka and Andhra Pradesh.More than 80% of the grapes are freshly consumed in India followed by raisin making. Grape is a temperate crop, howerver it is grown in tropical conditions of India under various climatic challenges. Expression of traits varies with exteme climatic conditions. Traits such as fruitfulness, bunch and berry traits, total soluble solids, pH influenced by the environtal conditions. Identification of variety with its best expressed traits will aid in parental selection in improvement programme. Traditionally, ampelographic characterization was the only mean for discrimination varieties and these were influenced by the environment(This *et al.*, 2004). Use of molecular marker is a powerful tool to overcome such limitations (Merkouropoulos *et al.*, 2015 and Oliveira *et al.*, 2006). Therefore, sixteen varieties with some traits of interest were discriminated based on morphological and molecular markers. Profile generated thorugh this study will be useful for deciding the better parental combinations in grape improvement programme by identifying the traits contributing in variation and also provide the identity to the variety.

MATERIALS AND METHODS

Plant material

Grape varieties; Arkavati, Marro Seedless, Champanel, White Sweet, Pusa Seedless, Beauty Seedless, Crimson Seedless, Centennial Seedless, Thompson Seedless, Angoor Kalan, Spin Sahebi, JaosBelyi, Manjari Naveen, Black Round, Christmas Rose and Sonaka formed material for the experiment.

Ampelographic observations

The experiment was conducted during the year 2013 and 2014 at ICAR-National Research Centre for Grapes. International Organisation of Vine and Wine (OIV) is a reference organisation for the entire vitivinicultural field.Sixteen grape varieties were differentiated based on OIV descriptors (OIV, 2009). Visual observations were recorded for ampelographic traits. At harvest, bunch and berry traits were asessed. Juice-sugar, acidity and pH were measured using FOSS instrument. All observations were made at the stage specified by OIV. ANOVA was carried out to detect the significant differences. Tukey's test was performed to compare the variable among the varieties. Principle component analysis (PCA) was done using data on morphological parameters.GLM procedure of SAS version 9.3 were used to perform all calculations.

Moleular analysis

DNA isolation

The DNA was isolated from the leaf sample withDNeasy® Plant Mini Kit (Qiagen, Hilden, Germany) as described by the manufacturer.

PCR ans SSR analysis

PCR for microsatellite loci was carried out in 10 μl of standard

reaction.PCR reaction mixure comprised of containing10 çg DNA, 1x PCR buffer, 2.5 mM $MgCl_2$, 100 μM each dNTP, 0.5 μM each of forward and reverse primer and 0.5 U Taq Polymerase and volume make up to 10μl using double distilled water. Fivemicrosatellite markers(OIV, 2009; Upadhyay *et al.*, 2010 and This, Jung A Fau - Boccacci *et al.*, 2004)*viz*.VVMD7, VVMD27, VrZAG62 and VrZAG79 and VVII52were used for characterization (Table 1). GeneAmp PCR system 9700 (AppliedBiosystems, USA) was used to perform PCR.The thermal cycle regime for polymerase chain reaction was composed of following steps (Upadhyay, *et al.,* 2010 with some mofication): 5 minutes at 94ºC followed by 35 cycles of denaturation (1 minute at 94ºC), annealing (1 minute at 50-57ºC depends on primer), 2 minutes at 72ºC and final extension at 72º C for 10 minutes. Agarose gel (1.5%) stained with ethidium bromide was used for product separation.Data was analyzed using JMP genomics software. Dendrograme was generated using morphological and molecular data through SAS9.3 version.

RESULTS

Majority of grape quality traits are quantitative in nature and influence by the environment. Therefore sixteen varieties with some trait of interest were taken into consideration to identify the traits contributing indiversity using principle component analysis. Also five microsatellite markers were used for generating genetic profiles of the varieties.

Evaluation of descriptors

Principal component analysis was carried out using program SAS 9.3 (Table 2a). Nine principal components were brought out in declining series of their importance, presenting 90.00% of the total variance of the varieties studied. First three components have shown more than 10% contribution in variability. The factors contributing in principle component 1 and their relative contribution in principle component 2 and 3 can be easily depicted from the Fig. 1, 2 and 3. Major contributing variables in the first principle component were (table 2b): berry attachment (26.47%), bunch weight (35.05%), bunch length (29.93%), berry diameter (34.71%) and total soluble solids or TSS (25.06%). Alba *et al.*, 2014reported the the veins trait and leaf area contributed 43.2% variation in twenty-six grape genotypes. Similar ampelographic method of characterization was used by Boselli *et al.*, 2000 and Gargin *et al.*, 2011.

Ampelographic characterization

Total 27 morphological descriptors were considered for discrimination of sixteen grape varieties.

Mature leaves of most of the varieties had pentagonal leaf blade shape and with three leaf lobes. Based on OIV descriptors particularly for leaf and shoot characters, Rusjan *et al.*, 2015 carried out phenotyping of old varieties Refošk and Teran to reveal their denomination and origin.Among the varieties studied, more than three inflorescences per shoot were observed in White Sweet, Beauty Seedless and Black Round. Except Champanel, Ruby Red, Centennial Seedless and Manjari Naveen; no anthocyanin colouration of mid vein was observed in the varieties studied and more than three numbers of consecutive tendrils were recorded in general. Configuration of teeth was either straight or concave from both sides except in grape variety Christmas Rose. In general, shoot attitude was erect or semi-erect in habit. Bunch shape was observed as cylindrical and conical in most of the varieties. Colour of mesocarp was not pigmented in eleven varieties. But Maroo Seedless, Beauty Seedless and Black Round had bluish-black; Champanel with purple-black; Ruby Red with reddish-black and Christmas Red with Red colourmesocarp. Champanel and Manjari Naveen had loose bunch compactness. Berry skin colour ranged from green yellow to blue black. Total twelve varieties observed with berry uniformity within the cluster. White Sweet and Christmas Rose had a firm berry attachment with the pedicel. Globose berry shape was recorded in twelve varieties. Seediness ranged from seedless to complete seed formation.Alonso *et al.*, 2007 characterized two clones of Albarino based on cluster and berry characters such as cluster weight, length, width, number of berries per cluster, pedicel length, berry length, width and weight.

In this study, all quantitative traits have shown highly significant differences (table 4). Higher variability was contributed by bunch and berry traits (Fig. 1, 2 and 3). Bunch weight ranged from 129.45 $\pm$ 3.58 g in Black Round to 510.00 $\pm$ 17.93 g in

Table 1: Details of microsatellite markers used for characterization

Microsatellite markers	Primer sequence	Annealing temperature (ºC)	Allele size(bp)
VVMD 7	F:AGAGTTGCGGAGAACAGGATR:CGAACCTTCACACGCTTGAT	52ºC	235-255bp
VVMD 27	F:GTACCAGATCTGAATACATCCGTAAGTR:ACGCCTATAGAGCAAACGTTGT	56ºC	173-192bp
VrZag 62	F:GGTGAAATGGGCACCGAACACACGCR:CCATGTCTCTCCTCAGCTTCTCAGC	50.5ºC	185-203bp
VrZag 79	F:AGATTGTGGAGGAGGGAACAAACCGR:TGCCCCCATTTTCAAACTCCCTTCC	50.5ºC	238-260bp
VVII 52	F:AGATTTGACGAAAAAGGGTR:CTTGATCTTTAGTTGCAGTCTG	57ºC	94-104bp

Table 2a : Evaluation of morphological descriptors and variability contribution from them

Principal components														
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Variation contribution (%)														21.56
15.37	13.79	9.75	8.45	7.35		5.53 4.68	3.54	2.89	2.18	1.79	1.65	1.01	0.04	
Related OIV descriptors														
OIV 006	OIV 016	OIV 051	OIV 067	OIV 068	OIV 070	OIV 076	OIV 079	OIV 151	OIV 153	OIV 202	OIV 204	OIV 208	OIV 220	OIV 221
OIV 222	OIV 223	OIV 225	OIV 231	OIV 240	OIV 241	OIV 243	OIV 502	OIV 503	OIV 505	OIV 506	OIV 508			

Table 2b: Contribution of individual variable in the principle components

Eigenvectors Parameters	Prin1	Prin2	Prin3	Prin4	Prin5	Prin6
Bladeshape	-0.032	0.064	-0.062	-0.01	0.02	-0.011
Lobenumbers	0.002	-0.072	-0.023	0.05	-0.065	0.134
Inflorescencespershoot	0.053	-0.203	-0.088	0.003	0.195	0.478
Upperbladecolour	0.111	0.071	0.12	0.045	0.033	-0.226
Leafanthocyanin	0.009	-0.024	-0.03	0.031	-0.148	0.112
Consecutivetendrils	-0.035	0.023	-0.049	-0.02	-0.1	-0.014
Sexualorgan	-0.028	0.018	0.092	-0.016	0.183	0.187
Teethshape	-0.01	0.024	0.078	-0.079	0.144	0.118
Shootattitude	0.044	0.344	0.071	0.204	-0.177	0.066
Degreeofopening	0.083	-0.094	-0.094	0.334	0.391	-0.457
Bunchshape	0.031	0.024	-0.057	-0.106	-0.076	0.047
Berrymesocarpcolour	0.769	0.117	0.257	0.059	-0.047	0.009
Bunchcompactness	0.01	-0.138	0.112	-0.002	0.302	0.019
Berryskincolour	0.39	-0.025	0.111	0.192	-0.125	0.031
Berryuniformity	-0.029	-0.018	0.027	0.04	0.01	-0.086
Berryattachment	-0.034	0.131	0.051	0.043	0.108	-0.025
Berryshape	0.156	0.131	0.324	-0.587	0.414	0.048
Seedformation	0.058	0.148	-0.149	0.012	0.082	0.146
Bunchweight	-0.1885	0.127	0.187	-0.058	0.01	0.201
Bunchlength	-0.178	0.123	0.389	-0.184	-0.033	-0.478
Berry length	-0.152	0.402	0.277	0.258	-0.104	0.112
Berry diameter	-0.185	0.409	0.188	0.054	-0.103	0.14
Berry weight	0.08	0.045	0.102	0.408	0.21	0.162
Totalacid	0.108	0.043	-0.214	-0.077	-0.299	-0.229
Ph	0	0	0	0	0	0
TSS	-0.192	0.029	0.064	0.362	0.38	-0.072
Seedwt	0.126	0.597	-0.596	-0.143	0.277	-0.057

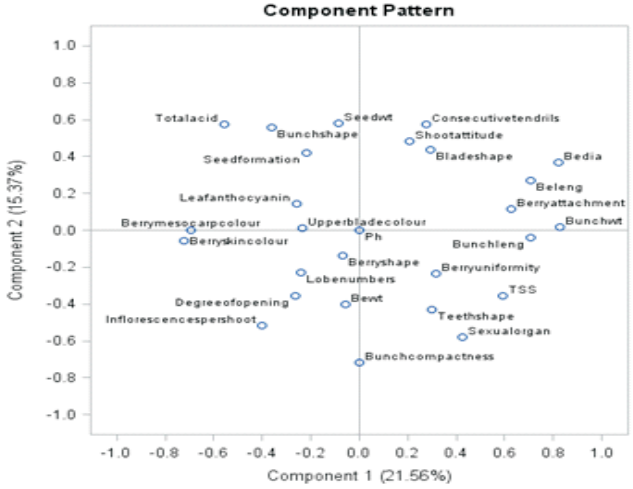

Figure 1: Variability contribution by morphological traits in PCA1 and PCA2

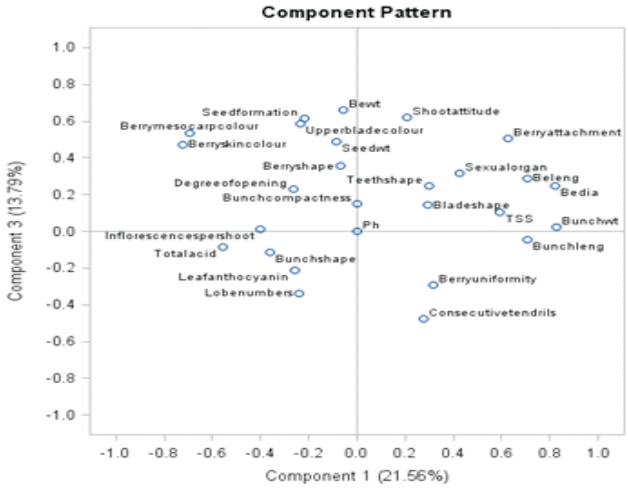

Figure 2: Variability contribution by morphological traits in PCA2 and PCA3

White Sweet. Longest bunch length was recorded in Crimson Seedless and Thompson Seedless. More than 20 mm berry length was observed in the varieties; White Sweet, Maroo Seedless, Christmas Rose, Centennial Seedless, Spin Sahebi, Manjari Naveen and Sonaka. Maroo Seedless, White Sweet, Centennial Seedless, Spin Sahebi and Christmas Rose had bolder berry size (>20 mm). Ten Berry weight ranged from 6.20±0.10 g in Black Round to 45.04±1.14 gm in Maroo Seedless followed by White Sweet (42.29±1.56 g). Wider range was recorded for the acidity content. Most of the varieties had medium acidity content (between 5 to 8 g/l tartaric acid content) except 4.40±0.17 g/l in White Sweet (lower acidity) and 13.30±0.5 g/l in Champanel (higher acidity). Among the varieties, pH ranged between 3 – 4. Arkavati, Centennial Seedless, Spin Sahebi, Black Round and Sonaka had more than 20 ºBrix. Varieties were grouped into seeded, rudimentary seeds and seedless.

Microsatellite analysis

Overall, more than 55% of allelic diversity was recorded. Total 24 alleles were generated. Among the markers, maximum number of alleles (6) and diversity (75.78%) was observed with marker VrZag79. Further VVMD7 and VVMD 27 rated

Table 3: Ampelographic traits of sixteen grape varieties

Sr.No	Variety	Shape of leaf blade	No. lobes	Inflo-rescence-nces per shoot	Color of upper side of blade	Anthocya-nin colouration of leaf	No. of consec-utive tendrils	Flower sexual organ	Shape of teeth
1	Arkavati	Pentagonal	3	1 to 2	Green	Absent	> 3	Male & Female fully developed	Both side straight
2	Marro Seedless	Pentagonal	3	1	Green with bronze spot	Absent	>3	Female with straight stamens	Both side concave
3	Champanel	Pentagonal	5	1 to 2	Copper	Upto 1st bifurcation	>3	Male & Female fully developed	Both side concave
4	White Sweet	Pentagonal	3	3 & more	Green with bronze spot	Absent	>3	Male & Female fully developed	Both side straight
5	Pusa seedless	Pentagonal	5	2 to 3	Green	Absent	>3	Female with straight stamens	Both side straight
6	Beauty Seedless	Pentagonal	3	3 & more	Copper	Absent	2	Male & Female fully developed	Both side straight
7	Crimson Seedless	Circular	5	1 to 2	Green with bronze spot	Absent	>3	Male & Female fully developed	Both side straight
8	Centennial Seedless	Pentagonal	5	1	Copper	Upto 1st bifurcation	1	Male & Female fully developed	Both side straight
9	Thompson Seedless	Pentagonal	3	1 to 2	Copper	Absent	3	Female with straight stamens	Both side straight
10	Angoor Kalan	Circular	3	2 to 3	Copper	Absent	3	Female with descending stamens	Both side concave
11	Spin Sahebi	Circular	3	1 to 2	Green with bronze spot	Absent	>3	Male & Female fully developed	Both side straight
12	JaosBelyi	Circular	3	1 to 2	Green	Absent	>3	Female with straight stamens	Both side concave
13	Manjri Naveen	Pentagonal	7	1	Green	Upto 1st bifurcation	>3	Male & Female fully developed	Both side straight
14	Black Round	Pentagonal	5	3 & more.	Copper	Absent	2	Male & Female fully developed	Both side straight
15	Christmas Rose	Pentagonal	3	1	Green with bronze spot	Absent	>3	Male & Female fully developed	Both side convex
16	Sonaka	Pentagonal	3	1 to 2	Green with bronze spot	Absent	>3	Female with straight stamens	Both side concave

Table 3: Continue..

Sr. No	Shoot attitude habit	Degree of leaf opening	Bunch shape	Anthocyanin coloration of berry mesocarp	Berry compact-ness	Berry skin colour -mity	Berry uniform pedicel	Berry attach-ment with	Berry shape	Seed form-ation
1	Erect	Moderately open	Long cylindrical	Green	Compact	Green yellow	Uniform	Loose	Globose	Rudimentary
2	Horizontal	Narrowly open	Winged conical	Bluish black	Medium	Blue black	Uniform	Medium	Globose	Rudimentary
3	Semi-erect	Moderately open	Conical	Purple black	Loose	Black red violet	Not uniform	Loose	Globose	Well developed
4	Semi-erect	Narrowly open	Winged cylindrical	Green	Medium	Green yellow	Not uniform	Firm	Globose	Well developed
5	Erect	Moderately open	Winged conical	Green	Medium	Green yellow	Uniform	Medium	Short elliptical	Seedless
6	Erect	Narrowly open	Conical	Bluish black	Compact	Black red violet	Not uniform	Loose	Oblate	Rudimentary
7	Semi-erect	Very wide open	Cylindrical	Green	Medium	Rose red	Uniform	Medium	Globose	Seedless
8	Semi-erect	Narrowly open	Cylindrical	Green	Medium	Green yellow	Uniform	Medium	Long elliptical	Rudimentary
9	Semi-erect	Narrowly open	Globular	Green	Compact	Green yellow	Uniform	Medium	Globose	Rudimentary
10	Erect	Narrowly open	Winged conical	Green	Medium	Rose	Uniform	Medium	Globose	Well developed
11	Horizontal	Narrowly open	Globular	Green	Medium	Green yellow	Not uniform	Medium	Short elliptical	Well developed
12	Semi-erect	Narrowly open	Conical	Green	Medium	Green yellow	Uniform	Medium	Globose	Well developed
13	Erect	Narrowly open	Winged conical	Green	Loose	Green yellow	Uniform	Loose	Globose	Rudimentary
14	Semi-erect	Lobes overlapping	Cylindrical	Bluish black	Compact	Blue black	Uniform	Medium	Globose	Well developed
15	Semi-erect	Moderately open	Winged conical	Red	Medium	Rose red	Uniform	Very firm	Obovte	Well developed
16	Erect	Lobes overlapping	Cylindrical	Green	Medium	Green yellow	Uniform	Medium	Globose	Seedless

Table 4: Analysis of sixteen grape varieties based on quantitative traits

Sr. no.	Variety	Bunch Weight (g)	Bunch Length (cm)	Berry Length (mm)	Berry Diameter (mm)	10 Berry Weight (g)	Acidity (g/l)	pH	Total Soluble Solids(°B)	100 Seed Weight (mg)
1	Arkavati	206.67 ± 3.93^{g}	16.10 ± 0.69^{de}	17.08 ± 0.19^{e}	16.17 ± 0.60^{e}	16.24 ± 0.57^{g}	6.67 ± 0.00^{de}	3.44 ± 0.01^{cdef}	21.97 ± 0.55^{b}	0 ± 0.00^{f}
2	Marro Seedless	240.33 ± 7.80^{f}	16.79 ± 16.79^{cd}	24.40 ± 0.55^{a}	21.68 ± 0.53^{a}	45.04 ± 1.14^{a}	6.70 ± 0.04^{de}	3.71 ± 0.12^{abcd}	18.63 ± 0.24^{cd}	0 ± 0.00^{f}
3	Champanel	130.01 ± 2.58^{h}	9.35 ± 9.35^{g}	13.98 ± 0.15^{f}	12.80 ± 0.32^{f}	20.87 ± 0.43^{f}	13.30 ± 0.5^{a}	3.24 ± 0.10^{f}	14.77 ± 0.53e	41.67 ± 1.01^{d}
4	White Sweet	510.00 ± 17.93^{a}	14.45 ± 0.10^{f}	24.57 ± 0.60^{a}	21.53 ± 0.95^{ab}	42.29 ± 1.56^{b}	4.40 ± 0.17^{j}	3.77 ± 0.03^{ab}	19.63 ± 0.19^{c}	25.40 ± 0.11^{e}
5	Pusa Seedless	200.00 ± 5.59^{g}	15.18 ± 0.12^{ef}	13.40 ± 0.57^{f}	12.32 ± 0.34^{fg}	17.27 ± 0.73^{g}	7.40 ± 0.32^{c}	3.57 ± 0.12^{abcde}	19.70 ± 0.34^{c}	0 ± 0.00^{f}
6	Beauty Seedless	225.67 ± 7.32^{fg}	16.89 ± 0.62^{cd}	12.84 ± 0.28^{f}	10.96 ± 0.36^{g}	11.11 ± 0.05^{i}	6.00 ± 0.21^{fghi}	3.78 ± 0.08^{ab}	13.43 ± 0.07^{e}	0 ± 0.00^{f}
7	Crimson Seedless	430.67 ± 1.16^{b}	21.64 ± 0.62^{a}	19.83 ± 0.68^{c}	19.01 ± 0.74^{cd}	26.13 ± 0.33^{e}	5.67 ± 0.16^{i}	3.50 ± 0.09^{bcdef}	18.70 ± 0.29^{cd}	0 ± 0.00^{f}
8	Centennial Seedless	430.00 ± 16.28^{b}	18.38 ± 0.31^{b}	22.44 ± 0.18^{b}	20.60 ± 0.41^{ab}	39.26 ± 0.18^{c}	6.37 ± 0.26^{efgh}	3.38 ± 0.13^{ef}	23.77 ± 1.03^{a}	0 ± 0.00^{f}
9	Thompson Seedless	356.48 ± 15.10^{d}	20.54 ± 0.56^{a}	18.93 ± 0.09^{cd}	16.24 ± 0.38^{e}	13.68 ± 0.15^{h}	7.12 ± 0.06^{cd}	3.69 ± 0.03^{abcde}	18.35 ± 0.41^{cd}	0 ± 0.00^{f}
10	Angoor Kalan	216.67 ± 2.93^{fg}	16.03 ± 0.06^{de}	17.67 ± 0.43^{de}	16.37 ± 0.27^{e}	16.46 ± 0.27^{g}	6.50 ± 0.19^{efg}	3.82 ± 0.13^{a}	18.87 ± 0.83^{cd}	45.33 ± 0.65^{c}
11	Spin Sahebi	403.33 ± 15.63^{c}	14.51 ± 0.19^{f}	21.87 ± 0.17^{b}	20.05 ± 0.36^{cb}	32.59 ± 0.32^{d}	8.27 ± 0.10^{b}	3.67 ± 0.10^{abcde}	21.40 ± 0.40^{b}	55.47 ± 0.31^{a}
12	JaosBelyi	290.00 ± 2.35^{e}	14.50 ± 0.34^{f}	19.71 ± 0.64^{c}	18.21 ± 0.46^{d}	31.70 ± 0.61^{d}	5.77 ± 0.17^{hi}	3.41 ± 0.14^{def}	18.80 ± 0.36^{cd}	50.67 ± 0.78^{b}
13	Manjri Naveen	389.21 ± 6.67^{c}	9.95 ± 0.25^{g}	21.81 ± 0.28^{b}	15.27 ± 0.63^{e}	27.32 ± 0.71^{e}	6.02 ± 0.09^{fghi}	3.73 ± 0.08^{abc}	17.87 ± 0.26^{d}	0 ± 0.00^{f}
14	Black Round	129.45 ± 3.58^{h}	8.99 ± 0.16^{g}	13.17 ± 0.37^{f}	12.53 ± 0.28^{f}	6.20 ± 0.10^{i}	7.20 ± 0.12^{cd}	3.72 ± 0.15^{abcd}	21.60 ± 0.33^{b}	42.61 ± 1.57^{d}
15	Christmas Rose	340.21 ± 5.83^{d}	17.79 ± 0.03^{bc}	23.98 ± 1.02^{a}	21.50 ± 0.47^{ab}	21.94 ± 0.61^{f}	6.60 ± 0.04^{def}	3.79 ± 0.13^{ab}	18.73 ± 0.68^{cd}	54.96 ± 0.55^{a}
16	Sonaka	349.67 ± 7.25^{d}	18.91 ± 0.02b	20.19 ± 0.67^{c}	18.03 ± 0.00^{d}	16.67 ± 0.51^{g}	5.93 ± 0.05^{fgh}	3.57 ± 0.05^{abcde}	22.33 ± 0.91^{ab}	0 ± 0.00^{f}
	Mean	303.02	15.63	19.11	17.08	24.05	6.87	3.61	19.28	19.76
	F value	458.26	291.66	202.35	156.03	935.7	269.15	8.41	72.08	2761.14
	Pr > F	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001
	C.V. %	3.06	2.41	2.6	2.9	2.7	2.95	2.86	2.81	4.01

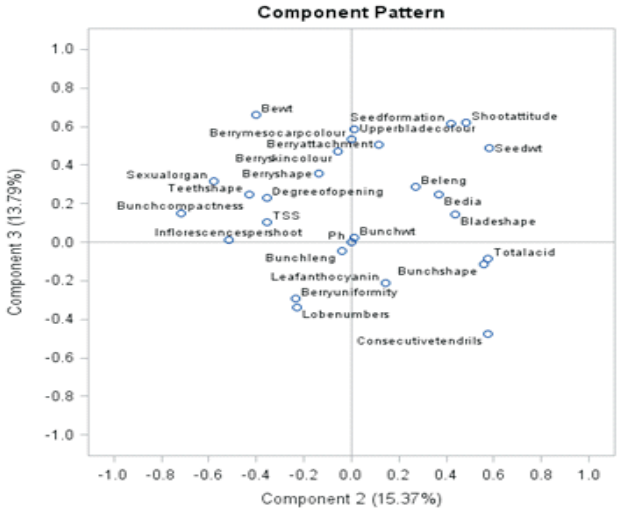

Figure 3: Variability contribution by morphological traits in PCA1 and PCA3

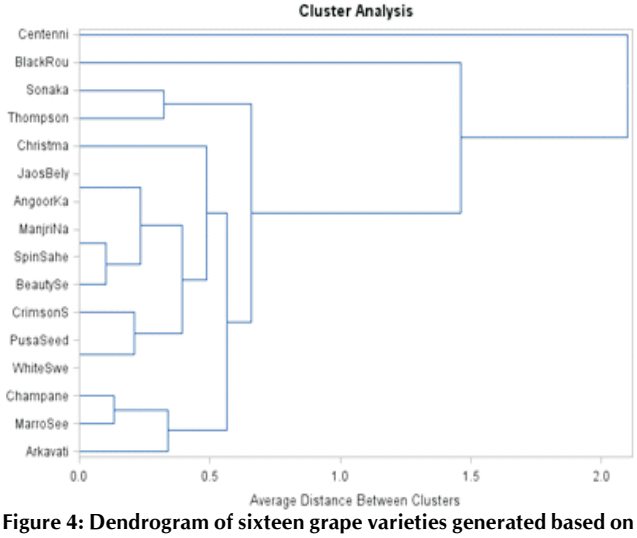

Figure 4: Dendrogram of sixteen grape varieties generated based on ampelographic traits and molecular markers

with 73.78% and 71.88% of allelic diversity. But all markers could able to discriminate the varieties.

Dendrogram was generated using a combine approach of morphological descriptors and molecular markers. On analyzing the genetic relationship among varieties, total fourteen were identified in single clusters (Fig. 4). Two separate clusters were formed by varieties Black Round and Centennial Seedless individually.

DISCUSSION

Ampelographicparameters is a unique tool for characterization adapted since centuries to till now.Sixteen varieties were considered for the characterization. PCA was performed to identify the traits contributing major role in detecting variation. Berry related traits such as berry attachment, berry weight, berry weight, berry diameter and total soluble solids

Table 5: Molecular characterization of sixteen grape accessions using five microsatellite markers

Marker	Number of Individuals	Number of Alleles	Polymorph In fo Content	Heterozy gosity	Allelic Diversity	Test for HWE Chi-Square	Pr > ChiSq
VrZag62	13	4	0.6374	0.0000	0.6864	39.00	<.0001
VrZag79	16	6	0.7234	0.0000	0.7578	80.00	<.0001
VVII52	13	5	0.5475	0.0000	0.5799	52.00	<.0001
VVMD7	15	5	0.6944	0.0000	0.7378	60.00	<.0001
VVMD27	16	4	0.6675	0.0000	0.7188	48.00	<.0001

contributed major role in variability among the grape genotype studied.

Other traits such as pentagonal blade shape, three lobed leaves, no anthocyanin colouration of mid vein of leaf, semi-erect habit of shoot, cylindrical/conical bunch shape, no mesocarp pigmentation of berries, uniform berries within clusters, globose berry shape were dominating catagories within their respectivetrait.Similarly, Alba *et al.,* 2014 revealed that the main veins, anglesbetween main veins and petior sinus were effective in differentiating the cultivars. Boselli *et al.*, 2000 carried out ampelographic characterization of white grape varieties using 29 adult leaf parameters as per OIV codes.Rusjan *et al.*, 2015 used ampelometric characterization to decipher origin of varieties like Refošk,Refosco, Teran and Terrano. The work on characterization, also helped in identification of varieties with useful traits such as bold berries, loose bunches, etc. Information generated through this study benefit in parental selection for breeding programme. Principle component analysis depicts the higher weightage of berry related traits like berry attachment, bunch weight, bunch length, berry diameter and total soluble solids. As these traits directly influence growers demand and market value of the product (Karibasappa, 2013). Bold berries fetches higher price in the market. Seven grape varieties were identified with naturally bold berries. Also growers needs varieties with loose bunches. Unavailability of manual power for bunch or berry thinning and wages to manual power has increased the cost of cultivation. Among the sixteen varieties, two were found with loose bunch compactness.Sweetness is one of criteria of consumers demand (Keller *et al.*, 2015). Further, utilization of ampelographic traits for varietal characterization has been studied widely by Ates *et al.*, 2011; Dilli *et al.*, 2014; Ferraj *et al.*, 2014; Hassan *et al.*, 2011; Merkouropoulos *et al.*, 2015; Sabir *et al.*, 2009 and Goto-Yamamoto *et al.*, 2006.

The literature indicates the reliability of microsatellite markers for varietal identification, genome mapping and in marker assisted selection (Yamamoto *et al.*, 2013). Worldwide many microsatellite markers were developed for *Vitis* species (Arroyo-Garcia and Martinez-Zapater, 2004; Di Gaspero *et al.*, 2005 and Goto-Yamamoto, Mouri *et al.*, 2006). Use of molecular marker was also done for deciphering the genetic relationship and evolution of the *Vitis*species (Crespan, 2010; Nagaty and El-Assal, 2011 and Liu *et al.*, 2013). Function of molecular markers for genetic diversity analysis/ varietal characterization was widely taken out in grapes (Alba *et al.*, 2014; Guo *et al.*, 2013 andKaratas *et al.*, 2013).In the present study, five microsatellite markers (table 5) were used for discrimination of varieties. Molecular markers thus can be use in trait specific marker assisted selection programme by differentiating the recipient and donar parents and also screen the population at early growth stage for trait of interest (Panigrahi *et al.*, 2013 and Pradiprao *et al.*, 2015). The genetic variability was evaluated on the basis of the presence of specific bands in a number of individuals, number of alleles generated, polymorphism information content, heterozygosity and allelic diversity. The data generated indicating the presence of a high genetic variability in the varieties. The combined cluster analysis has shown the distant relation of Centennial Seedless and Black Round with the remaining fourteen varieties.

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