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AMPELOGRAPHIC AND GENETIC CHARACTERIZATION OF GRAPES GENOTYPES COLLECTED FROM POTOHAR REGION OF PAKISTAN

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The ampelographic and genetic analysis of grapes (*Vitis vinifera* L.) genotypes is important for their breeding or any crop improvement program. This information has proved significantly helpful in maintaining germplasm and to develop new superior genotypes by selecting suitable planting material. This study revealed the morphological (qualitative & quantitative) traits and genetic diversity for 30 grapes genotypes, which were collected from Potohar (salt range) region of Pakistan. The data information for ampelographic (qualitative & quantitative) traits were recorded for consecutive 3 years and data was analyzed using principal component analysis (PCA) method. Further, twelve SSR markers were used for grapes genotyping. Two SSR primers VVS4 and VVMD25 showed monomorphism in these genotypes while remaining ten SSR primers VVMD7, VVMD24, VMC4H6, VrZAG21, VVMD32, VrZAG25, VrZAG79, VMC4F3, VMC4A1 and VrZAG62 showed polymorphism in 30 grapes genotypes. The highest PIC value and maximum number of alleles were observed in primers VMC4A1, VVMD32 and VrZAG62. The result also identified a synonym genotype which showed 95.83% similarity between genotypes, BRI-001 and Sundar Khani. The Euclidean distance metric and the Ward's agglomeration method were used in an unsupervised hierarchical cluster analysis of all cultivars. This cluster analysis divided the genotyped into two main groups having further classes and subclasses between them, which revealed high potential for specific breeding goals. Hence, these data could be used for protection or patenting processes of existing or new grapes cultivars.
Keywords: *Vitis*, genetic resources, exotic germplasm, genetic variation, arid region, molecular markers, multivariate analysis

INTRODUCTION

Grapes have rich amount of genetic diversity as this fruit is grown all over the world. According to an estimate there are more than 10,000 cultivars of grapes that exist in nature (Teixeira *et al*., 2013). Further, the genus Vitis contained above than 100 species, among them 65 are supposed to original species of the genus whereas more than 44 are under suspect as they may be hybrids between species (Sajid *et al*., 2006). The production area of grapes in Pakistan is about 15 thousand hectares and the production is around 643 thousand tons (GOP, 2013). The European grapes are largely grown in Pakistan especially for table purposes.

In Punjab Pakistan, Potohar is included in salt range area which is sub mountainous area having medium textured to clay-loam soil with pH 7.5 to 8.5 (Ahmad *et al*., 1990). It is an arid region and mostly cultivation depends upon the rainfall. However, this area is considered rich in plant species diversity due to natural vegetation present in this area. The grapes present in Potohar area have different origins and are evolved from different places, some of them are wild and native to Potohar while others are exotic from different parts of the world. The knowledge available for the genetic variation is helpful for breeders to improve a character by breeding so the genetic variations of species are considered as a valuable resource to improve existing or to develop new cultivars (Khawale and Singh, 2005; Nafees *et al*., 2015, 2016). Maximum number of exotic and indigenous grapes germplasm is growing in this area but it requires identification and characterization on the base of morphology and genetic characters for the improvement of these cultivars (Sajid and Ahmad, 2008; Haider *et al*., 2015). Besides being an important area of grapes, there is no studies reported about grapes characterization grown of this region on the base of morphologically or genetically. Authentic and accurate characterization is helpful in breeding programs and germplasm management (Boz *et al*., 2011; Shahzad *et al*., 2013; Mahmood *et al*., 2016).

In this regard, multivariate analysis is considered as an efficient approach to analyze large data of qualitative and quantitative phenotypic traits to identify patterns and relationships among powerful statistical techniques, such as the principal component analysis (PCA) and the cluster analysis (Ganopoulos *et al*., 2015; Mahmood *et al*., 2013, 2014). The importance of molecular markers has been widely accepted for grapes identification (Thomas *et al*., 1994) and

molecular characterization has given superiority over morphological characterization for assessment of genetic diversity (Doligez *et al*., 2006), for clone identification (Ye *et al*., 1998) and for endangered species characterization (Bocccacci *et al*., 2005). In previous studies, synonyms and homonyms cultivars were identified by using the molecular techniques (Karatas *et al*., 2007). The objective of this study was to (1) determine variation in grapes genotypes based on phenotypic qualitative and quantitative traits, (2) find correlation between the grapes quantitative traits, (3) fingerprinting and characterization of grapes genotypes based of molecular techniques by using SSR markers, (4) 1st time documentation of grapes qualitative and quantitative traits grown under Potohar (Salt range) region of Pakistan and (5) provide useful information to breeders about local and exotic genotypes for future breeding programs.

MATERIALS AND METHODS

***Plant materials*:** The study was carried out during three consecutive years 2015-2017 on 30 grapevine genotypes (exotic and local) obtained from Potohar region of Pakistan. The details of grapes genotypes including genotype name, collection site and their longitude, latitude and elevation is presented in Table 1. Potohar region is considered as sub mountainous area having the arid subtropical climactic conditions. The average annual temperature of region is 22.3°C with average precipitation of 519 mm annually (http://en.climate-data.org/location/1308/). However minimum temperature in winter 2°C and maximum temperature 43°C in summer was recorded during the study.

***Ampelographic analysis*:** Total thirty, exotic, local and wild grapes genotypes present in Potohar region were included in this study. Six plants of each grapevine genotype were selected and fruit morphological qualitative and quantitative data was analyzed by taking three bunches from each selected grapevine. For quality traits physical attributes; Bunch: Density, Berry: Size, Berry shape, Berry: Skin color, Berry:

Table 1. Details of grapes genotypes collection site.

Sr.#	Genotypes	Area	Location	Latitude	Longitude	Elevation
1	Reginia	Talagang	Pipli	32.54	72.38	517
2	Kishmish	Talagang	Pipli	32.56	72.38	517
3	Flame Tokay	Balksar	Balksar	32.55	72.40	538
4	Cardinal	Balksar	Balksar	33.06	72.97	541
5	Italia	Balksar	Durabi Dam	32.66	72.60	600
6	NARC Black	Balksar	Durabi Dam	32.77	72.70	644
7	Perlet	Balksar	Durabi Dam	32.59	72.40	496
8	King Ruby	Jhelum	A Village	32.93	72.70	539
9	Vitro Black	Jhelum	A Village	32.94	72.41	515
10	Sultanina	Jhelum	A Village	32.54	72.39	513
11	Red Globe	Kalar Kahar	Bhaun	32.74	72.70	742
12	Early White	Kalar Kahar	Bhaun	32.56	72.42	534
13	White Seedless	Kalar Kahar	Khandoyah	32.74	72.73	764
14	Thomson Seedless	Choa Saidan Shah	Kot Rajgan	32.43	72.50	733
15	Flame Seedless	Choa Saidan Shah	Kot Rajgan	32.44	73.00	627
16	Saibi	Choa Saidan Shah	Kot Rajgan	32.44	75.55	737
17	Haita	Talagang	KK Farm Izhar	32.43	72.57	693
18	Chesselas-B	Talagang	KK Farm Izhar	32.56	72.42	534
19	Superior	Dhudial	Dhudial	32.40	73.03	314
20	Gola	Dhudial	Dhudial	33.03	72.58	542
21	Muscat Hambourg	Chak Malook	Chak Malook	32.38	73.00	294
22	Danlas-B	Chak Malook	Chak Malook	32.46	73.00	294
23	Moscatol Romano	Chakri	Chakri	32.00	73.03	510
24	Sundar Khani	Chakri	Chakri	32.55	72.45	513
25	Aesel	Chakwal	Thoa bahadur	32.33	72.39	217
26	Taifi	Chakwal	Thoa bahadur	33.17	72.45	349
27	Gol	Chakwal	Murid	32.54	72.30	415
28	Italia Hybrid	Chakwal	Murid	32.90	72.50	415.6
29	Chakwal Selection	Chakwal	BARI Res. Station	32.55	72.43	521
30	BRI-001	Chakwal	BARI Res. Station	32.58	72.40	521

Anthocyanin coloration of flesh, Berry: Juiciness of flesh, Berry: Ease of detachment from pedicel, Taste and Transverse ridges on seed were studied. Ten quantitative yield base traits included Bunch Length, Bunch Width, Bunch Weight, Peduncle Length, Weight of ten berries, Berries in a bunch, Seed Length, Berry Length, Berry Width and Number of seeds were also studied.

***Leaf selection and collection*:** Healthy and disease-free plants were selected for leaf DNA extraction. From each genotype, young and tender leaf tissues were collected, washed thoroughly with distilled water and were dried. After drying, leaves were packed in zipper bags and were tagged properly. The zipper bags were then placed in ice box and were transferred to citrus sanitation laboratory, UAF. The leaves were then placed in -80°C freezer until further use.

***DNA extraction*:** For DNA extraction Doyle and Doyle (1990) protocol was followed with little modifications in it. In this procedure, 300 mg of leaf sample was taken after measuring on weighing balance. Then it was placed in sterilized mortar and pestle for grinding. Grinding of leaves were done by adding 1 mL of CTAB solution. The grinded mixture was transferred to two eppendorf tubes. The eppendorf tubes were placed in incubator for 45 min at 65°C. Then 660 µL of Chloroform: Isoamyl (24:1) per tube was added and centrifuged the mixture at 10000 rpm for 10 minutes. After centrifuged, a layer developed inside the tube. The upper portion of the layer was transparent and was called supernatant while the remaining green color called debris. The supernatant was picked up carefully with the help of pipette and was poured into other eppendrof tube. In supernatant 660 µL chilled absolute ethanol was added and kept at room temperature for half an hour. After incubation at room temperature prepared mixture of supernatant was centrifuged at 10000 rpm for 10 minutes. Then discard the ethanol and again wash pellet developed in eppendrof with 70% ethanol at 1000 rpm for 15 minutes. After washing, allowed the pellet to dry at room temperature for 24 hours. Then pour 50 µL deionized distilled water (D_3H_2O) and let the pallet dissolved in it. The prepared DNA solution was stored at -20^{o}C till further use.

***PCR mixture and conditions*:** PCR reaction mixture (20 µL final volume) was consisted a 30 ng of template DNA, 2.0 µL of 10x PCR buffer, 4 µL of dNTPs, 3.0 µL of MgCl2 (25mM), 1.0 µL of each forward and reverse primer and 1 U of Taq DNA Polymerase (Fermentas, USA). Thermocycler (Bio-Rad T-100) was used to conduct amplification by setting initial denaturation at 94°C for 4 min, followed by denaturation at 94°C for 1 min, annealing temperature for 1 min (50-60°C) depending upon primer, extension for 1 min at 72°C (35 cycles) and a final elongation of 10 min at 72°C.

Total 12 SSR markers were used in study were selected from previous study. Out of them, two primers VVS4 and VVMD25 were proved monomorphic in our study. While all other remaining primers used in study including namely VVMD7, VVMD32, VrZAG21, VrZAG25, VrZAG79, VrZAG62, VMC4F3 (Wang *et al*., 2015), VVMD24, VMC4H6, VMC4A1 (Hadadinejad *et al*., 2011), and VMC4A1 were proved as polymorphic. In order to check results, PCR product was run on 3% agarose gel having 100 ml (0.5 X) TAE buffer. The number of alleles were clearly observed in each gel picture. As SSR markers bands are in set of binary form. So, during band counting, the presence of allele was given (1) score while absent allele was given (0) score.

***Statistical analysis*:** Principal component analysis (PCA) was performed using XLSTAT (2018) software and cluster analysis was performed to find relationship between the selected 30 accessions. Principal component analysis (PCA) was performed using XLSTAT (2018) software and correlation matrix analysis, the Spearman coefficient was performed to find relation between quantitative variables (Somers, 1986). To find dissimilarity in genotypes Euclidean distance was chosen and Ward's method was used for the agglomerative hierarchical clustering (AHC) to find dissimilarity in genotypes. To analyze allelic data, DARWin6 (Perrier *et al*., 2006) and GenALEx (Peakall and Smouse, 2012) were used. Allele size and allele number were determined by GenALEx. However, dendrogram of genotypes relation was developed by DARWin6.

RESULTS

***Phenotypic traits of grapes genotypes*:** The most important grapes bunch and berry qualitative traits are shown in Table 2. Grapes phenotypic diversity was observed based on qualitative traits as described by International Plant Genetic Resources Institute (IPGRI). The trait bunch density was observed in range from very loose to very dense. The genotypes Cardinal, Sultanina, Saibi, Superior, Muscat Hambourg, Moscatol Romano, Aesel, Taifi and Chakwal Selection had very loose bunches while Kishmish, Perlet, Red Globe, Thomson Seedless, Flame Seedless and Gol had very dense bunches. In berry size trait, local genotypes Haita and Gola were very large in size as compared to other genotypes while the Thomson Seedless was only genotype which was very small in berry size. Most of the berries found in Potohar region had predominately round and Obtuse-ovate shape. The fruit shape was predominantly green-yellow (Kishmish, Italia, Perlet, Sultanina, Early White, White Seedless, Thomson Seedless, Saibi, Haita, Chesselas-B, Superior, Gola, Moscatol Romano, Sundar Khani, Aesel, Taifi, Gol & Italia Hybrid) such as red (Regenia & Cardinal), blue-black (NARC black & Vitro black), red grey (King Ruby, Red Globe, Flame Seedless, Muscat Hambourg & Chakwal Selection) and rose

Table 2. Grapes genotypes with their main bunch and berry traits analyzed in this study.

Genotypes	Bunch: density	Berry: size	Berry: shape	Berry: skin color	Berry: anthocyanin coloration of flesh	Berry: juiciness of flesh	Berry: ease of detachment from pedicel	Taste	Transverse ridges on seed
Reginia	Medium	Large	Oblate	Red	Very strongly colored	Very juicy	Difficult	Little sour	Absent
Kishmish	Very Dense	Medium	Obtuse-ovate	Green-yellow	Very slightly colored	Very juicy	Difficult	Sweet	Absent
Flame Tokay	Dense	Small	Obtuse-ovate	Rose	Very strongly colored	Slightly juicy	Difficult	Sweet	Absent
Cardinal	Very loose	Small	Round	Red	Very strongly colored	Slightly juicy	Very easy	Sweet	Absent
Italia	Loose	Large	Elliptic	Green-yellow	Very slightly colored	Very juicy	Slightly easy	Sweet	Absent
NARC Black	Dense	Large	Round	Blue-black	Very strongly colored	Slightly juicy	Slightly easy	Sweet	Absent
Perlet	Very Dense	Small	Round	Green-yellow	Very slightly colored	Slightly juicy	Slightly easy	Little sour	Absent
King Ruby	Dense	Medium	Round	Red-grey	Very strongly colored	Slightly juicy	Very easy	Sweet	Absent
Vitro Black	Medium	Large	Ovate	Blue-black	Very strongly colored	Slightly juicy	Slightly easy	Little sour	Absent
Sultanina	Very loose	Large	Oblong	Green-yellow	Very slightly colored	Slightly juicy	Slightly easy	Sweet	Absent
Red Globe	Very Dense	Large	Oblate	Red-grey	Strongly colored	Slightly juicy	Difficult	Sweet	Absent
Early White	Medium	Medium	Round	Green-yellow	Very slightly colored	Very juicy	Difficult	Very sweet	Absent
White Seedless	Medium	Medium	Round	Green-yellow	Very slightly colored	Very juicy	Slightly easy	Very sweet	Absent
Thomson Seedless	Very Dense	Very Small	Obtuse-ovate	Green-yellow	Very slightly colored	Slightly juicy	Slightly easy	Sweet	Absent
Flame Seedless	Very Dense	Medium	Round	Red-grey	Very strongly colored	Slightly juicy	Difficult	Sweet	Absent
Saibi	Very loose	Medium	Ovate	Green-yellow	Very slightly colored	Very juicy	Slightly easy	Little sour	Absent
Haita	Dense	Very large	Obtuse-ovate	Green-yellow	Very slightly colored	Very juicy	Slightly easy	Very sweet	Absent
Chesselas-B	Dense	Medium	Oblate	Green-yellow	Very slightly colored	Very juicy	Very easy	Very sweet	Absent
Superior	Very loose	Medium	Round	Green-yellow	Very slightly colored	Very slightly juicy	Difficult	Sweet	Absent
Gola	Loose	Very large	Oblong	Green-yellow	Very slightly colored	Very juicy	Difficult	Very sweet	Absent
Muscat Hambourg	Very loose	Medium	Round	Red-grey	Very strongly colored	Very juicy	Slightly easy	Very sweet	Absent
Danlas-B	Loose	Large	Round	Green-yellow	Very slightly colored	Very juicy	Slightly easy	Sour	Absent
Moscatol Romano	Very loose	Medium	Round	Green-yellow	Very slightly colored	Very juicy	Very easy	Little sour	Absent
Sundar Khani	Loose	Medium	Obtuse-ovate	Green-yellow	Very slightly colored	Very slightly juicy	Difficult	Sweet	Absent
Aesel	Very loose	Small	Oblong	Green-yellow	Very slightly colored	Very juicy	Very easy	Sweet	Absent
Taifi	Very loose	Medium	Obtuse-ovate	Green-yellow	Very slightly colored	Very slightly juicy	Difficult	Sour	Absent
Gol	Very Dense	Small	Obtuse-ovate	Green-yellow	Slightly colored	Very slightly juicy	Slightly easy	Very sweet	Absent
Italia Hybrid	Loose	Medium	Elliptic	Green-yellow	Very slightly colored	Very juicy	Slightly easy	Sour	Absent
Chakwal Selection	Very loose	Medium	Round	Red-grey	Very strongly colored	Slightly juicy	Slightly easy	Sweet	Absent

(Flame Tokay). The trait transverse ridges on seed were absent in all grapes genotypes found in Potohar.

***Correlation analysis of quantitative traits*:** Significant correlation was recorded in fruits morphological quantitative traits of grapes genotypes as shown in Table 3. In the grapes bunch qualitative characteristics, bunch width showed a highly positive correlation (r = 0.633) with bunch weight and with number of berries in a bunch (r = 0.596). Similarly,

Table 3. Pearson's correlation coefficients of morphological quantitative traits in 30 grapes accessions found in Potohar region of Pakistan.

Variable	BL	BW	BWE	PL	WT	NB	SL	BEL	BEW	NS
BL	1.000									
BW	0.543	1.000								
BWE	0.526	0.633	1.000							
PL	0.034	0.159	-0.031	1.000						
WT	0.053	-0.069	0.328	-0.279	1.000					
NB	0.512	0.596	0.637	0.218	-0.353	1.000				
SL	-0.048	-0.124	0.207	-0.085	0.639	-0.431	1.000			
BEL	0.238	0.128	0.354	-0.217	0.726	-0.159	0.482	1.000		
BEW	-0.198	-0.102	-0.159	-0.072	0.563	-0.632	0.484	0.508	1.000	
NS	-0.176	-0.120	-0.203	0.080	0.248	-0.534	0.614	0.139	0.459	1.000

Abbreviates: BL; Bunch Length; BW: Bunch Width; BWE: Bunch Weight; PL: Peduncle Length; WT: Weight of ten berries; NB: Number of berries in a bunch; SL: Seed Length; BEL: Berry Length, BEW: Berry Width; NS: Number of seeds

Table 4. Descriptive statistics analysis of quantitative morphological traits of 30 grapes genotypes found in Potohar region of Pakistan.

Variables	Minimum	Maximum	Mean	Std. deviation	CV
Bunch Length	11.00	27.50	16.76	3.98	23.75
Bunch Width	5.00	13.75	9.65	2.48	25.70
Bunch Weight	77.70	583.55	274.26	149.37	54.46
Peduncle Length	1.55	6.50	2.77	0.98	35.38
Weight of ten berries	9.70	53.70	24.91	9.18	36.85
Berries in a bunch	28.00	354.00	120.43	74.65	61.98
Seed Length	0.20	0.90	0.61	0.17	27.86
Berry Length	11.40	27.37	17.27	3.45	19.97
Berry Width	10.42	18.06	15.26	1.87	12.25
Number of seeds	0.00	4.00	2.53	0.86	33.96

bunch weight also showed strong positive correlation (r = 0.637) with number of berries, however bunch weight showed slightly negative correlation (r = -0.031) with peduncle length. In grapes berry traits, weight of ten berries had highly strong positive correlation (r = 0.726) with berry length followed by (r = 0.639) and (r = 0.563) for seed length and berry width respectively. In seed traits, strong positive correlation (r = 0.614) was between seed length and seed number. In negative correlations, maximum negative correlation (r = -0.632) was observed in number of berries in a bunch with berry width followed by number of seeds (r = -0.534).

***Statistical description and PCA of grapes quantitative traits*:** Ten fruit quantitative traits were studied on the base of morphology attributes for the estimation of grapes diversity. In the studied genotypes, a large extent of diversity was observed (Table 4). Maximum coefficient of variation (CV) as observed in berries in a bunch (61.98%), followed by bunch weight, weight of ten berries and peduncle length (54.46, 36.85 and 35.38%, respectively). While the least CV (12.25%) was observed in berry width. The bunch length, bunch width and bunch weight were varied from 11 to 27.50 cm, 5 to 13.75 cm and 77.70 to 583.55 g, respectively. The peduncle length was varied from 1.5 to 6.5 cm in studied genotypes. The weight of ten berries in grape accessions was recorded in range from 9.70 to 53.70 g. The grapes berries number were found in the range of 28 to 354 which showed highest variation. In grapes berry characters, berry length and berry width was found in the range from 11.40 to 27.37 mm and 10.42 to 18.06 mm, respectively. While seed length range was from 0.20 to 0.90 cm and seed number found in studied genotypes were in range from 0 to 4.

The first vector or PC-1 covered maximum diversity of 35.53% (Table 5). It includes the contributory quantitative parameters berries in a bunch, bunch width, bunch length, bunch weight and peduncle length, respectively, as shown in Table 5. Similarly, second eigenvector or PC-2 controlled (27.32%) diversity having responsible variables bunch weight, berry length, bunch length, bunch width and weight of ten berries respectively. While PC-3 covered (12.129%) diversity with highest loading including peduncle length, number of seeds and bunch width, respectively. However, PC-4 and PC-5 controlled 6.985% and 6.225% diversity, respectively. In fourth factor maximum contribution was showed by berry width, peduncle length and berry length while in fifth factor maximum contribution in variation was showed by peduncle length, bunch weight, weight of ten berries and seed length.

Table 5. PCA of quantitative morphological traits in 30 grapes accessions found in Potohar region of Pakistan

Parameters	F1	F2	F3	F4	F5
Bunch Length	0.397	0.660	0.062	-0.017	-0.333
Bunch Width	0.465	0.639	0.295	0.095	-0.331
Bunch Weight	0.296	0.863	-0.012	-0.185	0.229
Peduncle Length	0.246	-0.100	0.816	0.332	0.359
Weight of ten berries	-0.674	0.576	-0.212	0.064	0.226
Berries in a bunch	0.863	0.390	0.039	-0.056	0.147
Seed Length	-0.729	0.389	0.226	-0.375	0.202
Berry Length	-0.495	0.687	-0.186	0.288	0.045
Berry Width	-0.791	0.161	0.125	0.429	-0.200
Number of seeds	-0.656	-0.019	0.555	-0.360	-0.255
Variability (%)	35.530	27.320	12.129	6.985	6.225

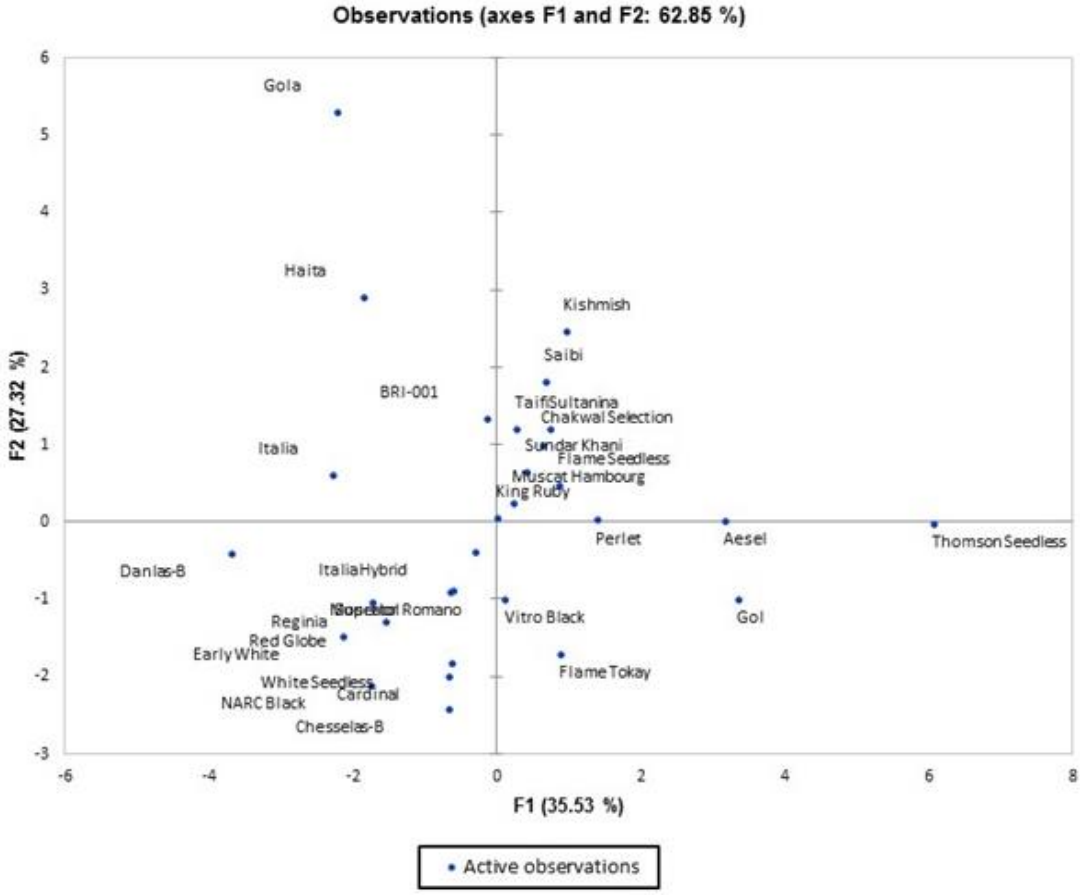

Figure 1. PCA plot for grapes genotypes found in Potohar region of Pakistan.

PCA plot developed for grapes accessions showed similarities and dissimilarities between the accessions (Fig. 1). The genotypes near to the center of axis were less diverse on the base of quantitative morphological variables. The genotypes which were present on upper right side of plane were similar with one another. It includes King Ruby, Muscat Hambourg, Flame Seedless, Sundar Khani, Chakwal Selection, Taifi, Sultanina, Taifi and Kishmish. However, Kishmish and King Ruby were away from each other in the plane which showed that these varieties contained more variations between them on the base of quantitative variables as compared to King Ruby and Muscat Hambourg which were present very close to each other. Similarly, the genotypes Gola, Haita, Italia and BRI-001 were present on upper left side of the plane which showed similarity among them. But in comparison of the upper left side and the lower right side, it showed that varieties

Table 6. Genetic diversity for twelve SSR primers estimated in grapes accessions of Potohar, Pakistan

Sr.#	Primers	Allele size (bp)	Allele number	Hobs*	Hexp*	PIC*
1	VVMD7	260-270	2	0.555	0.439	0.338
2	VVMD24	230-250	2	0.517	0.470	0.355
3	VMC4H6	180-190	2	0.896	0.503	0.372
4	VrZAG21	160-210	3	0.379	0.511	0.428
5	VVMD32	190-480	4	0.520	0.724	0.662
6	VrZAG25	240-250	2	0.655	0.448	0.343
7	VrZAG79	170-180	2	1.000	0.508	0.375
8	VMC4F3	180-190	2	0.333	0.499	0.370
9	VMC4A1	180-350	5	0.689	0.715	0.654
10	VrZAG62	180-350	3	0.428	0.650	0.565

Hobs* = Observed Heterozygosity; Hexp* = Estimated Heterozygosity; PIC* = Polymorphic Information Content

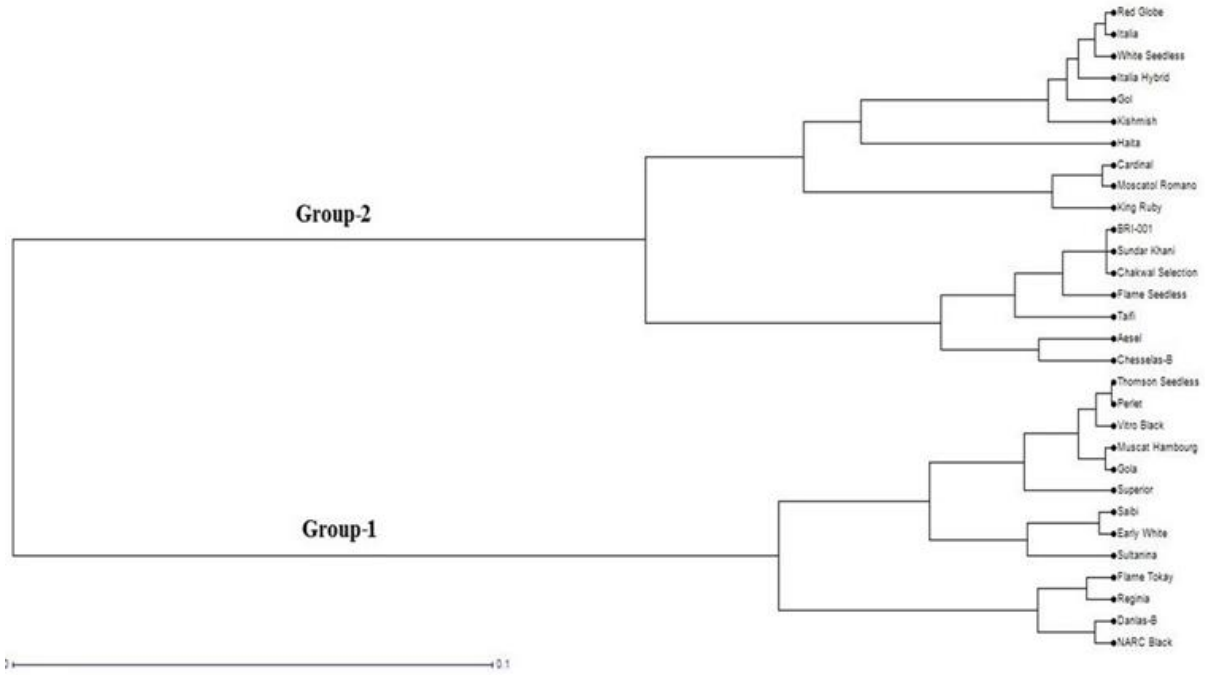

Figure 2. Dendrogram showing the cluster patterns of 30 grapes genotype.

present in opposite planes were highly diverse from each other on the base of quantitative variables.

***Microsatellite analysis*:** The overall expected heterozygosity (Hexp) observed in grapes genotypes was in range from 0.00 to 0.724 (Table 6). While observed heterozygosity (Hobs) was in range from 0.0 to 1.00. The maximum observed heterozygosity 1.00 was observed in primer VrZAG79 followed by primers VMC4H6, VMC4A1 and VrZAG25 having observed heterozygosity 0.896, 0.689 and 0.655, respectively. The polymorphic information content (PIC) of the primers used were in range from 0.00 to 0.662. The highest PIC value 0.662 was observed in primer VVMD32 followed by primers VMC4A1 and VrZAG62 with their PIC values 0.654 and 0.565, respectively. The maximum number of alleles observed in primers VMC4A1, VVMD32 and VrZAG62 were 5, 4 and 3, respectively.

***Cluster analysis*:** On the base of molecular analysis, 30 grapes genotypes present in Potohar region of Pakistan were divided into two main groups Group-1 and Group-2 (Fig. 2). These groups were divided into classes and classes were further divided into sub classes. Group-1 contained two classes with total thirteen genotypes in it. In Group-1, class 1 was consisted of only one sub class having four genotypes NARC-black, Danlas-B, Reginia and Flame Tokay in it. While Group-1, class 2 was further consisted of two sub classes having nine genotypes. In class 2 sub class 1 there were three genotypes of Sultanina, Early White and Saibi while in sub class 2 there were six genotypes Superior, Gola,

Muscat Hambourg, Vitro Black, Perlet and Thomson seedless in it. While Group-2 further contained two classes with seventeen genotypes in it. In Group-2, class 1 was consisted of two sub classes having seven genotypes in it. In subclass 1, there were two genotypes Chesslas-B, and Aesel while in subclass 2 there were five genotypes Taifi, Flame Seedless, Chakwal Selection, Sundar Khani and BRI-001 in it. However, in Group-2, class 2 was consisted of three sub classes in it. The sub class 1 was consisted of three genotypes King Ruby, Moscatol Romano and Cardinal. Sub class 2 was consisted of only one genotype Haita in it. While in sub class 3, there were six genotypes Kishmish, Gol, Italia Hybrid, White Seedless, Italia and Red globe. The genotypes in each sub classes were most similar to each other as compare to classes.

PCA plot and dendrogram was developed to analyze relation in genotypes on the base of molecular markers. The dendrogram developed on the base of dissimilarity divided genotypes in to two main groups with further classes and sub classes in it. The genotypes Sundar Khani and BRI-001 were found synonyms in our study. Both PCA and dendrogram revealed the similar results as both genotypes were present at least distance with each other. Similarly, most of our local source cultivars like Gol, Kishmish, Haita, Chakwal Selection, Taifi and Haita were present in similar group which showed their similarity on the base of genetic level. In our study, our local genotype NARC-black showed its genetic relation with imported genotypes instead of local genotypes which is suspicious to be mislabeled.

DISCUSSION

Traits related to yield like bunch size, berry weight, number of berries, berry length and berry width can be measured only by taking quantitative observations which is possible only through morphological characterizations. The information regarding quantitative fruits characteristics is of significant importance for breeders which can help them to improve genotypes and varietal development. Similar findings were observed by Dilli *et al*. (2014) who reported that grapes bunch and berry quantitative characters have their significant role in quality assessment of grapes especially in table grapes. Variability related to bunch and berry quantitative parameters was observed in our studied genotypes. The berry and bunch parameters like their length, width, weight and number of berries were observed more in our local genotypes as compared to imported genotypes. Our results were also in accordance with Fahmi *et al*. (2012) who found cluster length more in local Taifi cultivars. These findings were also showed an agreement with the findings of researcher Marwad (2002) who worked with different grapes cultivars. Berry length, width, weight and number of berries are directly involved in the production of crops and these crop yield parameters directly depends upon the cultivar selection and on environmental factors. Further, berry increase may be due to genotype character or due to adaptation of these cultivars under a specific climate of Potohar, Pakistan. Similarly, results were reported by Davies and Savolainen (2006) that bunch length varied with genotypes and Mattia *et al*. (2007) who found variations in grapes morphological traits due to environmental factors. The results were also in line with Khan *et al*. (2011) who found that climatic variations in grapes growing areas accounts diversity in berry quality and other grapes products. Our findings were also in harmony with other researcher who worked on different grapes cultivars (Wahab, 2011; Sabry *et al*., 2009).

The health of a plant played its major role in gaining maximum fruit size. The plant grown in stress condition result less production as compared to plant grown in ideal environment of proper irrigation, sunlight and nutrition. In grapes, berry size development directly depends upon water as it is the fundamental constituent of the grapes berry and is 75 to 85% of total fruit weight. The increase in grapes berry size and its quality is directly related to water content available in soil (Khan *et al*., 2008). Environmental factors like soil, precipitation, temperature, humidity and their combination greatly influence grapes quality and quality is defined as the traits governing the fruit attractiveness. For consumers, traits like fruit size, shape and color are the first quality traits which attract consumers. It is generally observed that deficient water reduces the grapes berry size as it reduced cell expansion and division during development. In short, environmental factors played their significant role in crop production. Similar results were also reported by Delrot *et al*. (2001) and Conde *et al*. (2007).

In our study, the reported primers for previous study proved highly efficient in showing polymorphism in grapes accessions grown under Potohar climatic conditions. Our results were in range with Fatahi *et al*. (2003) who obtained allele size in 120-472 bp range during characterization of Iranian grapes. Allele length obtained in our findings were also in range with Mattia *et al*. (2007) and Rao *et al*. (2014) who found allele length in range from 121-268 bp and 130-230 bp, respectively during grapes characterization. Higher number of alleles amplified means high genetic variation.

Most of our primers revealed observed heterozygosity similar with Fatahi *et al*. (2003) who found observed heterozygosity level in range between 0.47- 0.86; however, in our study heterozygosity was up to 1. The reason for increase in this value was due to primer VrZAG79 which showed highest polymorphism in our selected genotypes. Our findings were also in line with Guo *et al*. (2013) who observed heterozygosity from 0.00 to 0.844 in grapes by using SSR markers. Similarly, Mattia *et al*. (2007) and Rao *et al*. (2014) found maximum H_{obs} values 0.951 and 0.947 grapes germplasm during work on grapes diversity. The observed heterozygosity results were also in line with Tangolar *et al*.

(2009) who found maximum Hobs value 0.901 in SSR markers during Turkish grapevine genetic analysis.

The primer showing higher PIC values is considered highly valuable for allelic variation. The PIC value varies with primers and genotypes. In our study, its value was from 0.00 to 0.662. Tangolar *et al*. (2009) and Boz *et al*. (2011) found lowest value 0.065 and 0.074 with primer VVMD5 during grapes characterization. However, the highest PIC value 0.830 was observed in grapes by using SSR primers (Leao *et al*., 2011; Maryam *et al*., 2016). While in our results highest PIC value was 0.662. This reason for low value of PIC may be due to change of SSR primer and genotypes used in our study. Our findings of PIC values, heterozygosity and allele size revealed that grapes grown in Potohar region have wide diversity and has potential to improve by crossing elite genotypes.

Synonyms and homonyms in grapes genotypes is a major issue due to its broad diversity of nature. Same genotype has its several names at different places of world. This issue limits the breeding and this factor arises due to absence of morphological characterization of accessions (Mattia *et al*., 2007). Moreover, environmental and ecological conditions of a land is responsible to suppress certain characteristics of a genotype which may be the reason of false attribution. Similarity analysis or authentication of 30 grapes genotypes was calculated by analysis of their genetic SSR profiles. There was only one case found which showed similarity in genotypes grown in Potohar, Pakistan. The results revealed that there was 95.83% similarity in genotype BRI-001 and Sundar Khani. In genetic comparison of these two accessions, there were total 24 alleles out of which 23 were similar in both. The results revealed that it may be mislabel cultivars of the region. Grapes have enormous biodiversity due to its existence in diverse ecological and climatic conditions. The assessment of its diversity can be analyzed as it is successfully grown in tropical, subtropical and temperate regions of the world. Due to its diverse nature, there exists several synonyms and homonyms of its genotypes. Hence, there is need to characterize grapes on genetic level for conservation and crop improvement.

For characterization, SSR markers or microsatellites markers are considered a powerful tool to analyze genetic relationship between grapes genotypes (Riaz *et al*., 2004). SSR markers provides accurate results as compared to other molecular markers due to polymorphic and codominant nature (Rao *et al*., 2014). These markers are used to determine progeny relation in grapes (Bowers *et al*., 1999) and are also used for cultivar identification (Lamboy and Alpha, 1998). SSR markers played their role as fingerprinting tool and are used to identify synonyms or homonyms in grapes (Selli *et al*., 2007; This *et al*., 2004). The difference in morphological and biochemical characteristics of these genotypes may be due to climatic factors and due to cultural practices. Tangolar *et al*. (2009) also separated three homonyms genotypes by using SSR markers. Mattia *et al*. (2007) separated several homonyms and synonyms genotypes of Sardania by using DNA fingerprinting by SSR primers. Several homonyms and synonyms of grapes were also identified in Anatolia six provinces by using SSR marker (Boz *et al*., 2011).

***Conclusion*:** The multivariate techniques are helpful to study genetic diversity in continuously variable characters which are interrelated with each other. The phenotypic characteristics of grapes are helpful for breeders to develop new desired cultivars. In quantitative traits correlation, positive correlation was observed among bunch width, bunch weight and number of berries while these traits had negative correlation with peduncle length. In molecular analysis, the highest PIC value and maximum number of alleles were observed in primers VMC4A1, VVMD32 and VrZAG62. Hence these primers should be used for accurate fingerprinting of grapes genotype. These primers proved helpful for synonym genotype identification. The result also identified a synonym genotype which showed 95.83% similarity between genotypes (BRI-001 and Sundar Khani). The cluster analysis performed in study showed the genetic relation between the genotypes. This work will also be helpful for grapes breeders in future for breeding point of view.

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