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Morpho-phenological characterization of grape (*Vitis vinifera* L.) germplasm grown in northern zones of Punjab, Pakistan

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This endeavor includes thirty grapes' genotypes grown in northern zones of Punjab, Pakistan to determine similarities and dissimilarities in forty-four morphological traits according to the descriptor "International Plant Genetic Resources Institute (IPGRI)". The relation between the genotypes was determined by principal component analysis (PCA), and similarity was worked out by using cluster analysis. The dendrogram divided the genotypes into two main groups with classes and subclasses. The variation present within the class was up to 81.72% while the difference between classes was 18.28%. The morphological quantitative traits such as bunch length, bunch width, bunch weight, peduncle length, the weight of 10 berries, number of berry in a bunch, berry length, and berry width were in the following range 27.50-11 cm, 13.75-5 cm, 583.56-77.70 g, 6.50-1.55 cm, 53.70-9.70 g, 354-28, 27.37-11.40 mm and 18.06-10.41 mm respectively, which indicated a wide level of diversity in the selected genotypes. Based on phenological attributes, "Regina", "Perlet", and "Early White" were found early maturing genotypes to prevent berry rotting due to monsoon rains at the final stage of ripening. The data generated in this study would be helpful to preserve the existing germplasm and be available for designing future breeding programs.
Keywords: Cluster analysis; grapevines; IPGRI; morphology; principal component analysis.

INTRODUCTION

Grape (*Vitis vinifera* L.) is an important horticultural species that is cultivated worldwide for wine and table purposes. It is one of the most remunerative fruit crops of Pakistan, mainly grown for table purposes. Table grape is known as "European grape" that are emerged between Black and Caspian area (Uddin *et al.*, 2011). Grapes are included among rare fruits of the world which are cultivated from tropical to temperate zones of the world due to their huge diversification. It is believed that genus *Vitis* contains above than 100 species, out of 44 are considered ambiguous due to interspecific hybrids (Sajid *et al.*, 2006). Grapes are a good source of several minerals, vitamins, antioxidants and phenolic compounds required for human health (De Lorenzis *et al.*, 2015). These compounds have anti-inflammatory, anti-aging and antimicrobial properties which protect from cardiovascular diseases (Mattivi *et al.*, 2009; Lorrain *et al.*, 2011).

In Pakistan, the northern areas of Punjab, Baluchistan and KPK have great grapes diversity and it is cultivated on an area of 15 thousand hectares with 643 thousand tons' production (GOP 2013). However, these areas require characterization for good production (Sajid and Ahmed 2008). In Punjab province, the Potohar area is becoming famous for the commercial production of grapes' early mature genotypes due to suitable climate conditions of the region. In grapes, early mature cultivars are generally preferred for low altitude regions while late-maturing cultivars are grown at high altitudes (Sabir *et al.*, 2009). The climate of a particular place greatly affects the grapes' diversity and production of the crop (Akram *et al.*, 2019).

Globally, the knowledge of grape genetic resources has enormously increased the interests of scientists and researchers as germplasm is a valuable source for species conservation, understanding gene functions and development of new and improved lines (Khadivi *et al.*, 2019; Ates *et al.*, 2011; Khawale and Singh 2005). In grapes, ampelography is

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considered a scientific methodology to characterize grapes based on morphological, pomological and phenological traits (Ates *et al.*, 2011). It is a basic tool which is being used for a long time in genotypes certification programs, monitoring genetic quality and for the preservation of endangered species (Dilli *et al.*, 2014). Moreover, it is helpful to find the relationship among genotypes, recognizing genotypes, identifying pitfalls during collection, management strategies and identify agronomic interest genes (Khadivi *et al.*, 2019; Blanco *et al.*, 2007; Ergul *et al.*, 2006). For characterization in grapes, several multivariate techniques like principal components analysis and cluster analysis are being used for qualitative and quantitative analysis (Leão *et al.*, 2011). These techniques describe agronomical and morphological characters (Matheou *et al.*, 1995), check relationships among genotypes related to traits (Boselli *et al.*, 2000) and check the amount of variation between traits (Borges *et al.*, 2008; Coelho *et al.*, 2004).

The Potohar area is one of the enriched areas of Pakistan having grapes, olives and natural vegetation. Despite being a highly diverse area enriched with grapes diversity, it is considered a neglected area of Pakistan with low attention is given to characterize grapes found in this area. The grapes genotypes found in this area belong to different origins, some are wild native to this region while some are acclimatized from different regions of the world (Akram *et al.*, 2019). Moreover, pre-monsoon and monsoon rains at crop maturity and farmer priority towards exotic cultivars to earn an early high profit are causing the disappearance of the local genotypes.

Hence the present study was done to characterize grapes genotypes present in this area based on morphological qualitative traits, with the aim to check the morphological variation and relationship among exotic and local genotypes. Further, quantitative agronomic traits were also taken to evaluate superior genotypes and a phenology calendar was developed to evaluate early, mid and late genotypes. This detailed study of morphological qualitative and quantitative traits will also be helpful to prevent disappearing local cultivars, for the preservation of germplasm and future breeding programs.

MATERIALS AND METHODS

***Details of the experimental* site:** The genotypes collection was conducted from the Potohar region, located in the northern zone of Punjab, Pakistan. Potohar Zone lies in a range between 32.5°N to 34.0°N Latitude and 72°E to 74°E Longitude (Rashid and Rasul 2011). This region is present between the Jhelum and Indus rivers with altitudes 600 to 800 m. It extends from the north of the salt range towards the foothills of the Himalayas. The soil of this region is generally medium textured to clay-loam and has pH of 7.5 to 8.5 (Ahmad *et al.,* 1990). The region is considered as a sub-mountainous area having semiarid climatic conditions. The average annual temperature of the region is 22.3°C with an 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 were recorded during the study. The location of Potohar in Pakistan is mentioned in Fig.1.

***Plant materials*:** The study was carried out during 2017-2018 with 30 grapevine genotypes (exotic and local) obtained from the northern zone of Punjab, Pakistan. The genotypes included in the study were Regina, Kishmish, Early White, White Seedless, NARC Black, Saibi, Haita, Gola, Gol, Aesel, Taifi, Chakwal Selection, BRI-001, Italia, Perlet, King Ruby's, Vitro Black, Sultanina, Red Globe, Flame Tokay, Thomson Seedless, Flame Seedless, Chesselas-b, Superior, Muscat Hambourg, Danlas-b, Moscatol Romano, Sundar Khani, Cardinal and Italia Hybrid. All the exotic and local genotypes in the study were 5 to 6year old and their plants were developed from cutting. All the genotypes were collected from different sites of Potohar and were planted at Barani Agriculture Research Institute (BARI) for germplasm conservation. The management practices including pruning, plowing, irrigation, fertilizer, insecticides and pesticide application were similar for all plants.

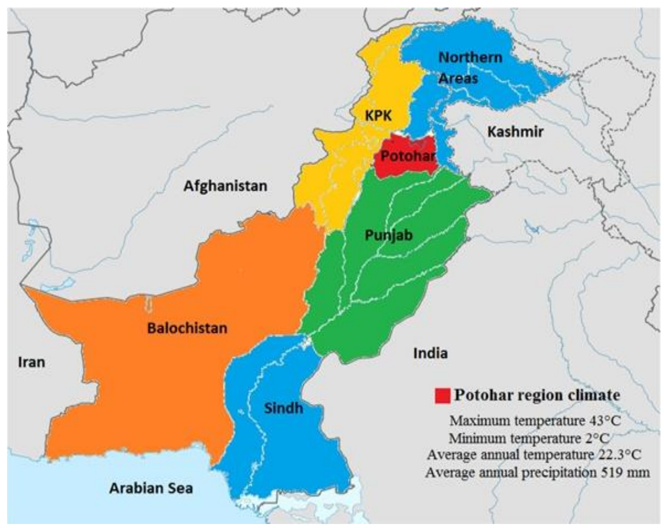

Figure 1. Map showing Potohar region of Pakistan and its climatic conditions.

***Amphelographic qualitative* traits:** The mean values from six plants of each genotype were recorded for consecutive two years 2017-18 based on 43 ampelographic traits that is shown in Table 1. These traits were selected from the given descriptors as prescribed by International Plant Genetic Resources Institute (IPGRI UPOV 1997).

***Quantitative traits*:** For the evaluation of superior agronomic traits, eight parameters including bunch length, bunch width, bunch weight, peduncle length, the weight of 10 berries,

Table 1. List of forty-three amphelographic traits selected from IPGRI, OIV descriptor

Amphelographic traits	Abbreviation	Amphelographic traits	Abbreviation
1. Young shoot: Form of tip	FT	23. Mature leaf: Shape of teeth	ST
2. Young shoot: Anthocyanin coloration of tip	ACP	24. Mature leaf: General shape of petiole sinus	GSPS
3. Young shoot: Density of prostrate hairs on tip	DPHT	25. Mature leaf: Tooth at petiole sinus	TPS
4. Young shoot: Density of erect hairs on tip	DEHT	26. Mature leaf: Petiole sinus limited by veins	PSLV
5. Shoot: Colour of dorsal side of internode	CDI	27. Mature leaf: Shape of upper lateral sinus	SULS
6. Shoot: Colour of ventral side of internode	CVI	28. Mature leaf: Depth of upper lateral sinus	DULS
7. Shoot: Colour of dorsal side of node	CDN	29. Mature leaf: Density of prostrate hairs between veins	ML: DPHBV
8. Shoot: Colour of ventral side of node	CVN	30. Mature leaf: Density of erect hairs between veins	ML: DEHBV
9. Shoot: Density of erect hairs on node	DEHN	31. Mature leaf: Density of prostrate hairs on main veins	ML: DPHMV
10. Shoot: Density of erect hairs on internode	DEHI	32. Mature leaf: Density of erect hairs on main veins	ML: DEHMV
11. Shoot: Density of prostrate hairs on node	DPHN	33. Mature leaf: Length of petiole compared to middle vein	LPCMV
12. Shoot: Density of prostrate hairs on internode	DPHI	34. Woody shoot: Surface	WS
13. Shoot: Number of consecutive tendrils	NCD	35. Shoot: Main Color	MC
14. Shoot: Length of tendril	LT	36. Bunch density	BD
15. Young leaf: Color of upper surface	CUS	37. Berry: Shape	BS
16. Young leaf: Density of prostrate hairs between veins	YL: DPHBV	38. Berry: Presence of seeds	PS
17. Young leaf: Density of erect hairs between veins	YL: DEHBV	39. Berry: Skin color	SC
18. Young leaf: Density of prostrate hairs on main veins	YL: DPHMV	40. Berry: Anthocyanin coloration of flesh	ACF
19. Young leaf: Density of erect hairs on main veins	YL: DEHMV	41. Berry: Juiciness of flesh	JF
20. Mature leaf: Shape of blade	SB	42. Berry: Ease of detachment from pedicel	EDP
21. Mature leaf: Number of lobes	NL	43. Berry: Taste	T
22. Mature leaf: Anthocyanin coloration of main veins on upper side of blade	ACVB		

number of berry in bunch, berry length and berry width were noted. Fruit bunches were collected from three trees of each genotype and all observations were taken as triplicate replicates for conformation of data analysis.

***Phenological* traits:** For the evaluation of early maturing genotypes, different phenology parameters were examined from bud burst to fruit harvest. The traits noticed were time of budburst, time of 1st leaf emergence, time of inflorescence emergence and time of harvest. All parameters were observed when 50% of the plants of each genotype showed its physiological responses. Based on these parameters phenological calendar was developed.

***Statistical analysis*:** The qualitative morphological traits were analyzed by principal component analysis (PCA) and cluster analysis was developed on the basis of dissimilarity using XLSTAT (2018) software. However, for the evaluation of superior genotypes based on qualitative traits, the data were subjected to analysis of variance (ANOVA) and Fisher's least significant differences were calculated following a significant ($P \leq 0.05$) F test.

RESULT

***PCA of grape genotypes based on qualitative* traits:** A scree plot was developed in PCA based on 44 morphological quantitative traits of 30 grapes genotypes as shown in Fig 2. The scree plot showed the estimated eigenvalues and cumulative variations found in grapes genotypes. In the scree plot, the eigenvalues up to the first seven factors decreased sharply while the cumulative variation increased sharply up to the first seven factors. Factor F1 had a maximum eigenvalue (8.141) with cumulative variability of 18.503%.

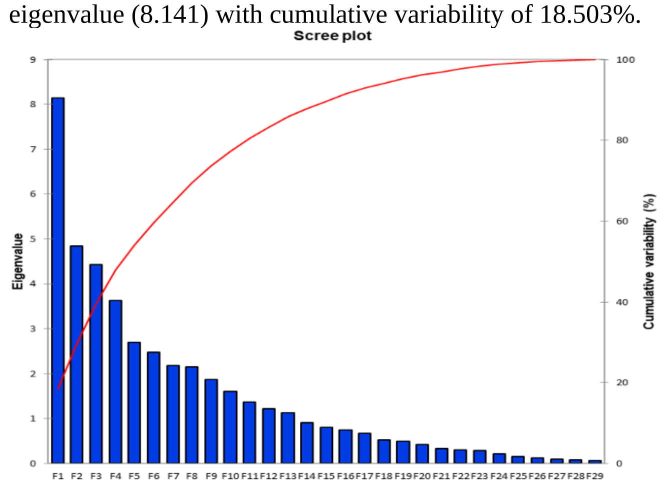

Figure 2. PCA of morphological qualitative traits of grape genotypes grown in northern zones of Punjab, Pakistan.

Similarly, F2, F3, F4, F5, F6 and F7 have eigenvalues (4.848), (4.436), (3.630), (2.707), (2.481) and (2.187) with their cumulative variability (29.522%), (39.603%), (47.853%), (54.006%), (59.644%) and (64.616%), respectively. After that, there was a steady increase in the cumulative variability up to 100% for F29. Phenotypic variations of mature leaf characters' ventral and dorsal sides are shown in Figures 3 and 4, respectively.

In the eigenvector analysis of grapes variables, high variability (64.616%) was controlled by the first seven vectors

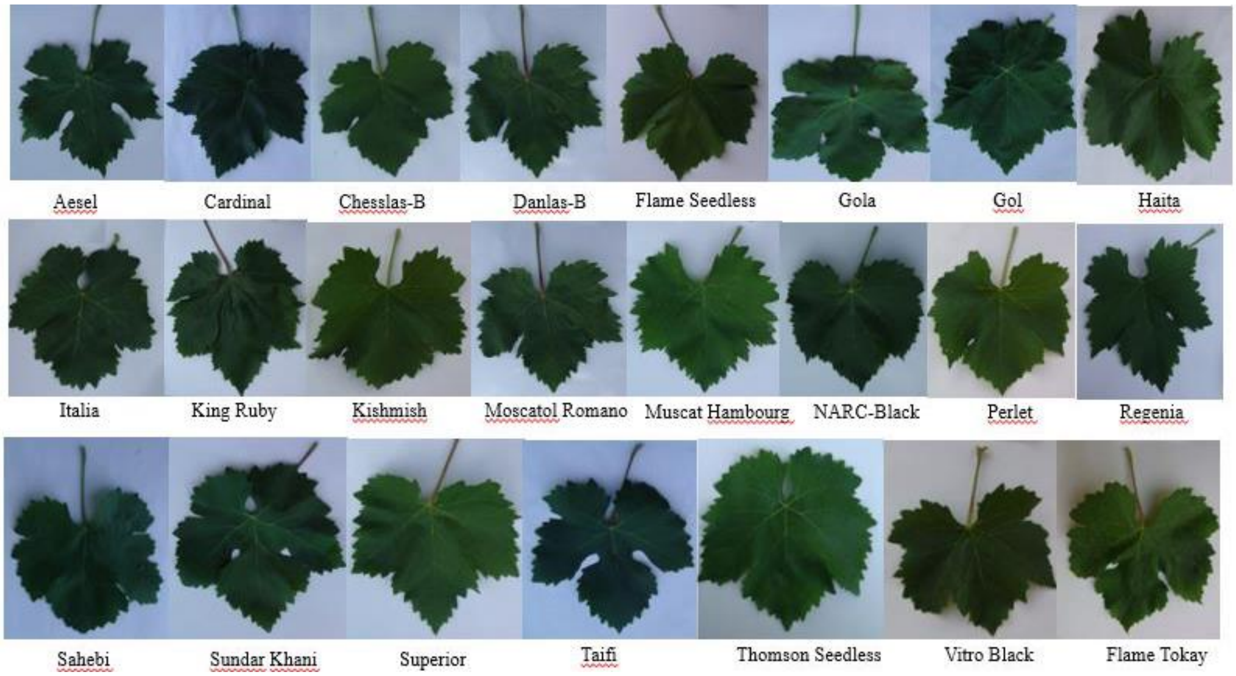

Figure 3. Phenotypic variations of characters observed in upper surface of mature leaves grapes genotypes grown in northern zones of Punjab, Pakistan.

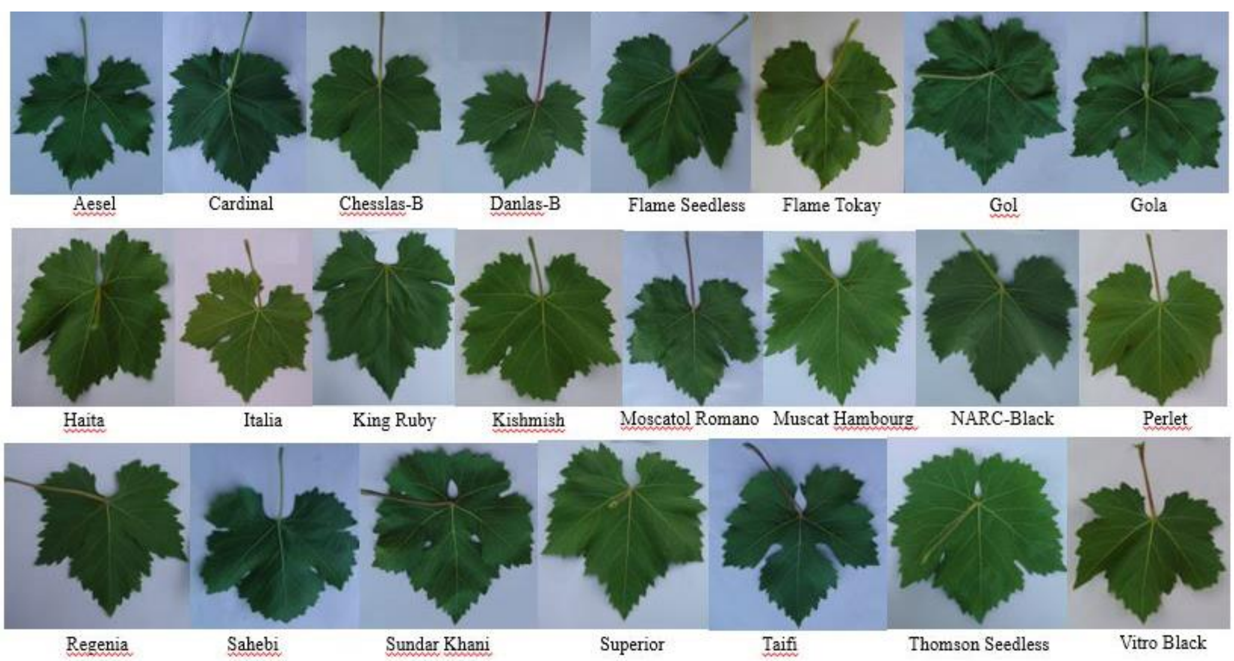

Figure 4. Phenotypic variations of characters observed in lower surface of mature leaves of grapes genotypes grown in northern zones of Punjab, Pakistan.

(PC-1 to PC-7). The first vector or PC-1 covered maximum diversity of 18.503%. It includes the contributory morphological qualitative parameters as shown in Table 2.

In PC-1 maximum diversity was controlled by mature leaf variables ML: DPHMV, ML: DPHMV, and ML: DEHMV. In young leaf characteristics, maximum contributions were of variables YL: DPHBV, YL: DPHMV and YL: DEHMV. While in shoot characteristics, S: DPHN and S: DEHN variables showed maximum contribution. Similarly, second eigenvector or PC-2 controlled (11.018%) diversity having

Table 2. PCA of forty-three morphological traits of 30 grapes genotypes grown in northern zones of Punjab, Pakistan.

	F1	F2	F3	F4	F5	F6	F7
YS: FT	-0.395	0.332	0.139	0.699	-0.101	-0.230	-0.125
YS: ACT	0.395	-0.645	-0.391	0.216	0.151	0.196	-0.004
YS: DPHT	0.358	0.697	-0.261	0.204	-0.297	-0.036	-0.048
YS: DEHT	0.325	0.575	-0.168	0.053	-0.414	0.212	-0.120
S: CDSI	0.110	-0.463	-0.618	-0.121	0.116	-0.067	0.058
S: CVSI	-0.085	-0.565	0.046	0.187	0.028	0.144	-0.034
S: CDSN	0.295	-0.370	-0.091	-0.126	-0.100	0.273	-0.146
S: CVSN	0.093	-0.585	-0.168	-0.092	-0.287	-0.032	-0.256
S: DEHN	0.626	0.260	-0.441	0.143	0.058	-0.006	0.328
S: EHI	0.449	0.538	-0.280	0.020	0.083	0.153	0.053
S: DPHN	0.674	0.234	-0.414	-0.284	0.001	0.146	0.083
S: DPHI	0.561	0.428	-0.564	-0.097	0.187	0.019	0.196
S: NCT	0.104	-0.182	0.437	0.367	0.148	0.044	0.586
S: LoT	0.434	-0.147	0.039	0.198	0.069	0.303	-0.502
YL: CUS	0.664	-0.447	-0.269	-0.003	-0.249	0.117	-0.083
YL: DPHBV	0.845	0.217	-0.156	0.178	0.023	-0.179	0.066
YL: DEHBV	0.572	-0.207	0.015	0.076	0.239	0.274	0.288
YL: DPHMV	0.784	-0.127	0.106	-0.053	-0.399	-0.123	0.005
YL: DEHMV	0.783	0.075	0.206	0.104	-0.326	0.027	-0.006
ML: SoB	-0.241	0.298	-0.229	-0.360	0.339	0.483	0.016
ML: NoL	0.036	-0.240	-0.473	0.223	-0.037	-0.470	0.250
ML: ACMVUSB	0.115	-0.060	-0.418	0.386	-0.019	-0.041	0.341
ML: SoT	0.095	-0.267	-0.026	-0.128	-0.030	0.533	0.381
ML: GSPS	-0.186	0.431	-0.280	-0.080	0.461	0.100	0.022
ML: TPS	-0.018	0.283	-0.418	0.372	0.473	-0.098	-0.049
ML: PSLV	-0.112	0.383	-0.053	-0.027	-0.141	0.512	-0.532
ML: SULS	0.027	0.052	0.062	-0.087	0.379	-0.144	-0.179
ML: DULS	-0.133	0.120	-0.052	0.710	-0.159	0.073	-0.164
ML: DPHBV	0.364	0.598	0.400	-0.224	-0.008	-0.212	-0.015
ML: DEHBV	0.535	0.220	0.371	-0.111	-0.040	-0.240	0.162
ML: DPHMV	0.823	-0.161	0.206	-0.171	0.222	-0.129	-0.283
ML: DEHMV	0.766	-0.120	0.339	-0.216	0.211	-0.239	-0.162
ML: DPHMV	0.818	-0.175	0.206	-0.197	0.190	-0.165	-0.289
ML: LPCMV	0.071	0.098	0.049	0.666	0.270	0.367	-0.078
WS: S	0.274	-0.371	0.284	0.051	0.268	0.035	0.130
WS: MC	0.559	-0.021	0.328	0.138	-0.056	0.347	0.078
Bh: Den	-0.181	0.053	0.269	-0.543	0.340	-0.201	0.166
Ber: Size	0.209	-0.218	-0.034	0.460	0.509	0.045	-0.274
Ber: Shp	-0.315	0.057	0.094	-0.375	-0.181	0.294	0.010
Ber: SC	0.239	0.158	0.756	0.178	0.151	0.266	0.141
Ber: ACF	0.079	0.139	0.743	0.305	0.096	0.226	0.211
Ber: JF	0.017	-0.104	0.077	0.402	0.162	-0.412	-0.299
Ber: EDP	0.165	0.031	0.158	0.218	-0.504	-0.130	0.161
Ber: Tst	-0.222	-0.380	-0.066	0.127	-0.332	0.145	0.148
Variability (%)	18.503	11.018	10.081	8.251	6.153	5.638	4.972

responsible variables YS: DPHT, ML: DPHBV, YS: DEHT and S: EHI, respectively. While PC-3 covered (10.081%) diversity with the highest loading variables including Ber: SC and Ber: ACF, respectively. The fourth component possessed 8.251% variability and a maximum contribution of ML: DULS, YS: FT and ML: LPCMV variables. The fifth vector revealed the maximum contribution of variables ML: TPS and ML: GSPS. However, the sixth vector showed the maximum contribution of variables ML: SOT and ML: PSLV while the seventh variable depicted maximum contribution of S: NCT as shown in Table 2. The variations present in bunches is presented in Figure 5.

PCA plots for qualitative morphological traits and grape genotypes: PCA plot developed based on 44 morphological traits divided the variables into four quadrants (Fig. 6). This plot depicts that grapes variables near to each other are closely

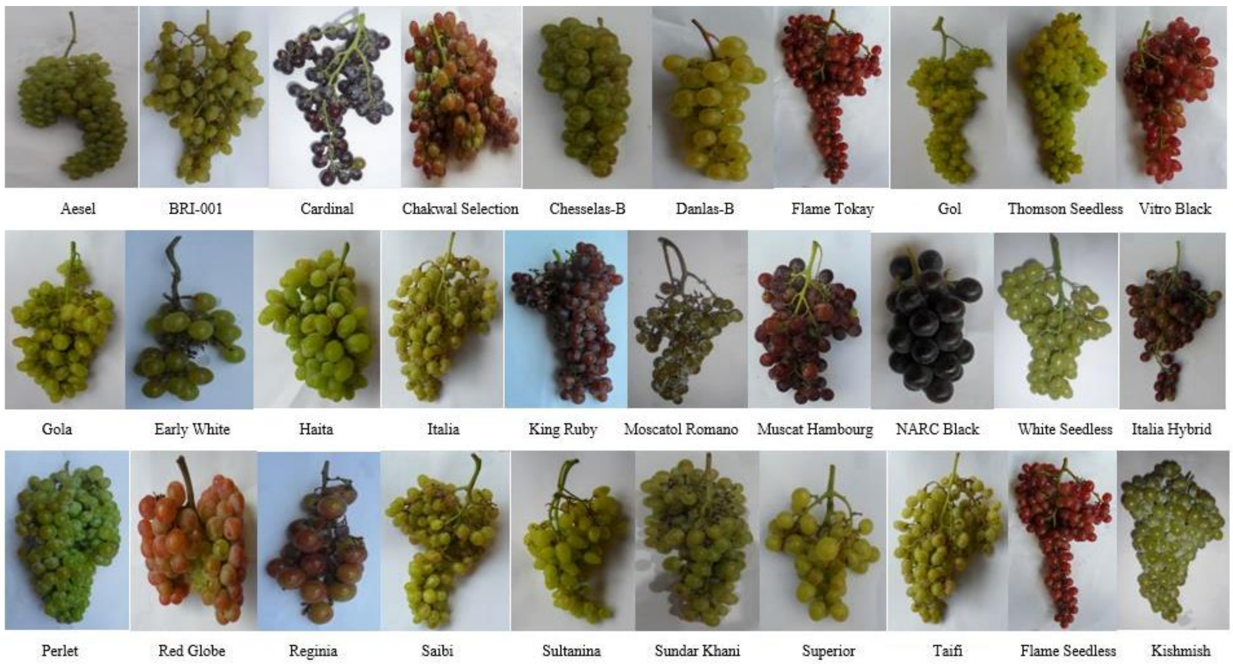

Figure 5. Variations observed in bunch characteristics (size, color, shape) of grape genotypes grown in northern zones of Punjab, Pakistan.

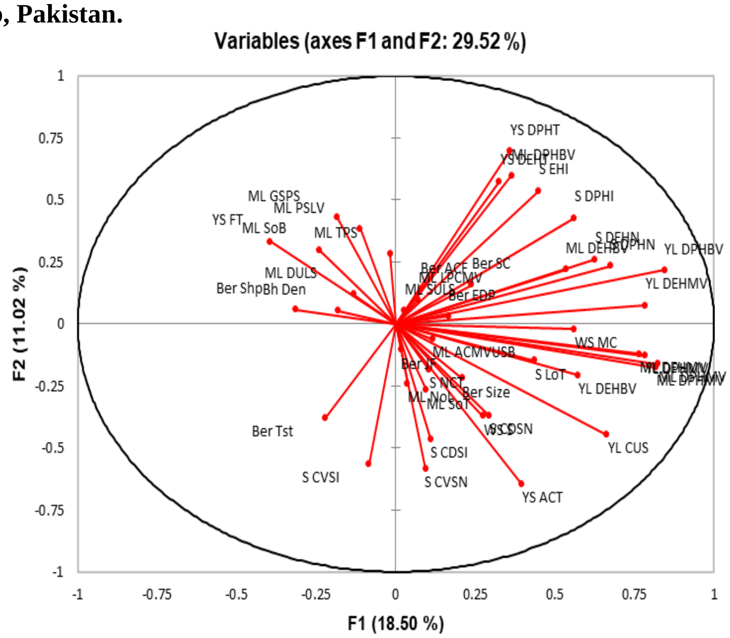

Figure 6. PCA plot of qualitative morphological traits for grape genotypes grown in northern zones of Punjab, Pakistan.

related to each other have similar characteristics and have less contribution in diversity. While variables away from the center of the axis and variables present away from each other showed a high level of variations in grapes genotypes. In the PCA plot of variables, there were 15 variables that were present at the upper right quadrant of the plot. Out of these 15 variables, five variables Ber: ACF, Ber: SC, ML: LPCMV, Ber: EDP and ML: SULS were present near to the center of the axis. While ten variables, YS: DPHT, YS: DEHT, ML: DPHBV, S: EHI, S: DPHI, S: DPHN, S: DEHN, ML: DEHBV, YL: DPHBV and YL: DEHMV were far from the center and are responsible for variations in the grapes genotypes.

There were only two variables Ber: Tst and S: CVSI which were present at the lower left quadrant of the plot. It showed that these two variables had close relation between them but have negative relations with the variables at the upper right side of the plan. There were 19 variables present in the lower right side of the quadrant that had a strong relation between them and was closely associated with each other. Similarly, there were eight variables present in the upper left side of PCA plot which was closely related to each other. However, they were present at less distance from the center which showed that these variables had less contribution in variation as compared to variables present in the upper right quadrant of the plot. Similarly, there were 19 variables present in the lower right quadrant of the plot which had a strong association among them. The variable YS: DPHT on the upper right side and YS: ACT at the lower right side distinguished the cultivars more as compared to other variables.

***PCA biplot of 44 qualitative morphological traits and 30 grape* genotypes:** A biplot (Fig. 7) showed the relationship between the genotypes and morphological variables. The results revealed that 15 qualitative traits or variables (Ber: ACF, Ber: SC, ML: LPCMV, Ber: EDP, ML: SULS, YS: DPHT, YS: DEHT, ML: DPHBV, S: EHI, S: DPHI, S: DPHN, S: DEHN, ML: DEHBV, YL: DPHBV and YL: DEHMV) had a strong correlation with genotypes Aesel, Early White, Sundar Khani, Flame Seedless, Muscat Hambourg and NARC Black and these characters had their positive contribution in these genotypes which included them in a similar group. In the opposite direction, at the left lower quadrant there were only two morphological traits Ber: Tst and S: CVSI which were mostly similar in genotypes White seedless, Haita, Chakwal Selection, Flame Tokay, Perlet, Saibi, Danlas- B, Regina and Vitro Black present in this quadrant.

The morphological variables present in the upper left side quadrant had a strong correlation with genotypes present in this quadrant. Similarly, the variables present on the lower

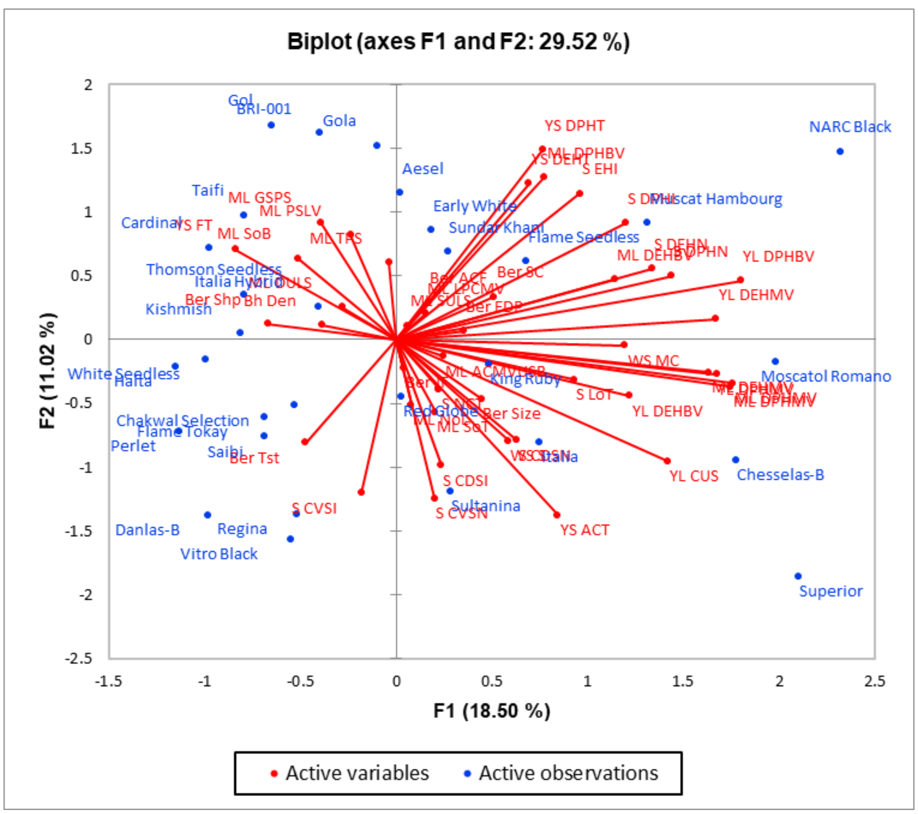

Figure 7. PCA biplot of 30 grape genotypes for qualitative morphological traits.

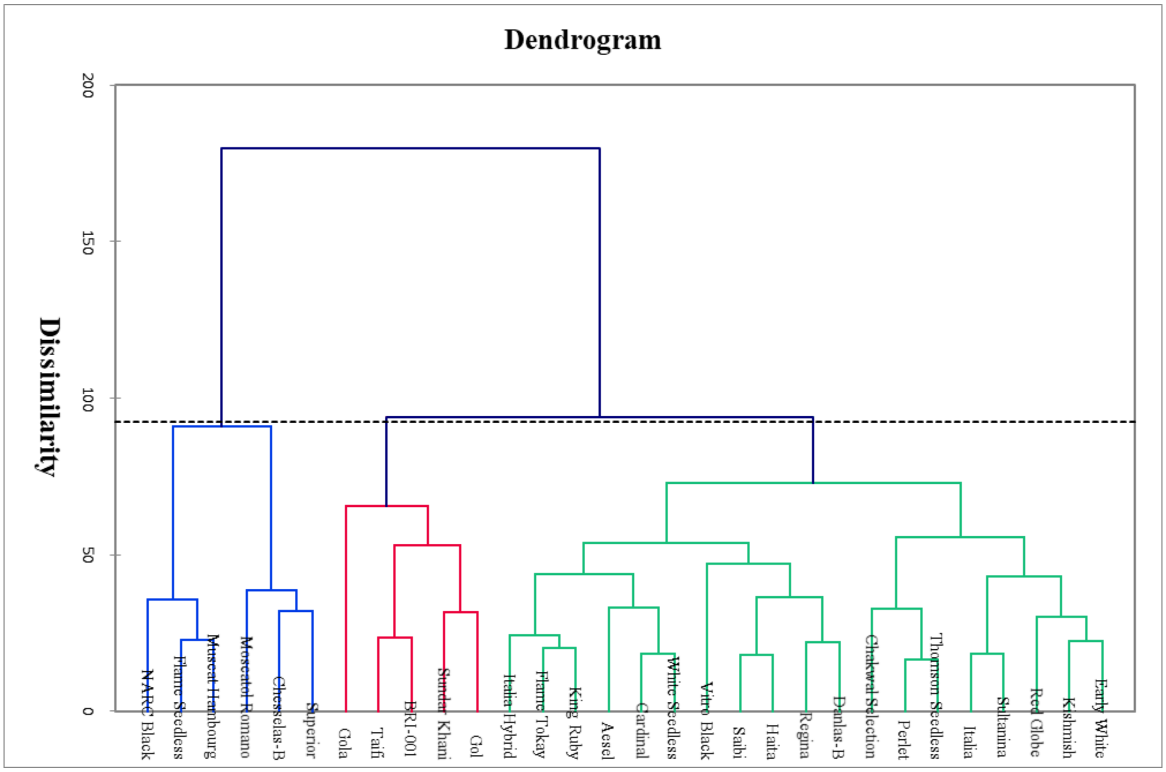

Figure 8. Dendrogram representing the relationship of 30 grapes genotypes based on morphological traits growing in northern zones of Punjab, Pakistan.

right side had a strong correlation with genotypes present in a similar quadrant. However, these two quadrants had a negative correlation between them. The results also told that grapes variables present near to the center of the axis were closely related to each other and had less contribution in diversity. While variables away from the center of the axis showed a high level of variations in grapes genotypes. Similarly, the genotypes close to each other were more similar in characteristics as compared to genotypes that were away like Chesslas-B which was present separately in the lower right quadrant (Fig. 7).

***Cluster analysis of 30 grape genotypes based on morphological qualitative* traits:** Based on 44 morphological qualitative traits, 30 grape genotypes present in the northern zone of Punjab, Pakistan was divided into two main groups Group-1 and Group-2 (Fig. 8). These groups were further divided into classes. Group-1 contained only one class with two subclasses. While Group-2 further contained two classes in it with further subclasses. The variations present within the class were 81.72% while the variations present between classes were 18.28%. In class 1, there were six genotypes which were further divided into two sub-classes and in each subclass, there were three genotypes. Subclass 1, consisted of NARC Black, Flame Seedless and Muscat Hambourg while subclass 2 consisted of Moscatol Romano, Chesslass-B and Superior. These six genotypes were similar to each other but were significantly different from Group 2 genotypes based on morphological quantitative traits.

In Group-2, there were two main classes present; Class 1 consisted of five genotypes Gola, Taifi, BRI-001, Sundar Khani and Gol. However, Gola showed little variation among these genotypes. In Group-2, Class 2 was the largest class having 19 genotypes in it. This class was further divided into two sub-classes and each subclass was further divided into two sub sub classes based on similarity. Group-2, sub-class 1 consisted of 11 genotypes. Sub sub-class 1 was comprised of six genotypes Italia Hybrid, Flame Tokay, King Ruby's, Aesel, Cardinal and White Seedless. While sub sub-class 2 was comprised of five genotypes: Vitro Black, Saibi, Haita, Regina and Danlas-B. However, Group-2, sub-class 2 consisted of eight genotypes. In sub sub class 1, there were three genotypes Chakwal Selection, Perlet and Thomson seedless while in sub sub-class 2, there were five genotypes Italia, Sultanina, Red Globe, Kishmish and Early White. The genotypes in each sub sub-class were most similar to each other.

***Evaluation of grape genotypes based on quantitative* traits:** As fruit agronomic traits are greatly affected by the environment, however, they are used for grapes classification and are also of great importance for breeding new cultivars. The quantitative fruit characters recorded were bunch length, bunch width, bunch weight, peduncle length, weight of ten berries, number of berry in bunch, berry length and berry width (Table 3). All characters were significantly different from each other. Bunch length varied from (27.50 to 11 cm) in the observed accessions. Large fruit bunches were found in Gola, Saibi, Sultanina-C and Thomson seedless respectively while the smallest bunch was of Haita cultivar. Similarly, maximum bunch width was noted in Taifi (13.75 cm) while minimum bunch width was found in Chesslas-B (5 cm). Bunch weight significantly varied from 583.56 to 77.10 gm. Maximum bunch weights were recorded in Kishmish (583.56 gm), Haita (579.50 gm) and Gola (556.50 gm) respectively. Peduncle lengths varied from 6.50 to 1.55 cm. The weight of ten berries was also done separately along with bunch weight. The maximum weight of ten berries was obtained in Gola (53.70 gm) as bunch weight was also maximum in this cultivar while minimum weight was examined in Thomson Seedless berries (9.70 gm).

Maximum number of berries varied from 354 to 28. Thomson seedless cultivar contained maximum berries which were small in size but large in number while NARC Black genotype has large berries in size but few in number. Maximum berry length was observed in Gola (27.37 mm) and Sultanina-C (25.19 mm) respectively. Berry diameter was found in the range from 18.06 to 10.75 mm where larger berry widths were noted in Danlas-B (18.06 mm), King's Ruby (18 mm) and Gola (17.98 mm) respectively.

Phenological calendar of 30 genotypes grown in northern zones of Punjab, Pakistan based on their phenological stages: For the evaluation of early, middle and late maturing genotypes, five phenological stages of each genotype were

Table 3. Fruit characteristics of grapes grown in northern zones of Punjab, Pakistan.

Varieties	Bunch length	Bunch width	Bunch weight	Peduncle length	Weight of 10 berries	Number of berry in bunch	Berry length	Berry width
F-value	36.32**	17.95*	151.00**	50.69**	65.06**	17.48**	11.78**	32.17**
Regina	17.00 d-g	7.00 e-g	150.65 j-m	2.25 g-m	42.45 b	28.33 kl	17.67 c	15.00 d-g
Kishmish	20.23 b-d	12.33 ab	583.56 a	2.50 f-l	26.35 cd	187.00 d	18.58 c	15.71 c-f
FlameTokay	12.83 i-k	9.93 b-e	193.90 g-k	3.00 d-g	14.80 g-i	86.33 gh	15.08 cd	14.84 e-g
Italia	14.00 f-k	9.75 b-e	271.10 ef	2.80 e-h	38.00 b	62.50 h-j	19.08 bc	17.85 ab
NARC Black	12.00 jk	6.00 fg	77.70 n	1.95 j-m	23.65 c-e	28.00 l	16.75 cd	16.04 b-f
Perlet	21.00 bc	12.50 ab	205.67 f-j	6.50 a	13.33 hi	180.00 de	16.00 cd	17.00 a-c
King Ruby	17.50 c-f	12.50 ab	196.70 g-k	1.90 k-m	15.70 g-i	146.00 f	17.00 cd	18.00 a
Vitro Black	17.33 c-f	9.90 b-e	227.85 f-i	3.85 bc	26.35 cd	72.50 hi	11.40 d	15.47 c-g
Sultanina	22.33 b	9.60 b-e	240.45 f-h	2.70 e-j	26.10 cd	151.50 f	25.19 a	14.28 fg
Red Globe	11.83 jk	5.75 fg	152.65 j-m	2.90 e-h	37.95 b	58.00 ij	16.52 cd	15.89 c-f
Early White	11.93 jk	5.55fg	140.00 j-n	3.90 b	36.67 b	43.67 j-l	18.00 c	15.87 c-f
White Seedless	14.00 f-k	6.00 fg	142.67 j-n	2.00 i-m	20.10 d-g	64.00 h-j	16.25 cd	16.04 b-f
Thomson Seedless	22.00 b	10.50 a-d	441.50 b	2.50 f-l	9.70 i	354.00 a	11.61 d	10.41 i
Flame Seedless	16.50 e-i	11.50 a-c	346.55 cd	2.85 e-h	23.95 c-e	162.50 d-f	16.79 cd	16.01 b-f
Saibi	22.40 b	12.00 ab	390.33 bc	3.23 b-f	27.33 c	145.50 f	18.21 c	15.09 d-g
Haita	11.00 k	11.70 a-c	579.50 a	3.45 b-e	40.45 b	160.00 ef	24.57 ab	16.74 a-d
Chesselas-b	13.15 h-k	5.00 g	130.00 k-n	3.90 b	19.15 e-h	74.00 hi	14.44 cd	15.09 d-g
Superior	14.00 f-k	10.05 b-e	125.70 l-n	1.80 lm	24.70 c-e	55.00 i-k	18.24 c	16.67 a-e
Gola	27.50 a	11.00 a-c	556.50 a	1.55 m	53.70 a	112.50 g	27.37 a	17.98 a
Muscat Hambourg	15.25 e-j	11.25 a-c	309.35 de	2.20 h-m	26.20 cd	112.50 g	16.50 cd	14.66 fg
Danlas-b	13.50 g-k	5.50 fg	167.50 i-l	1.75 lm	38.15 b	45.00 j-l	19.05 bc	18.06 a
Moscatol Romano	14.75 f-j	9.75 b-e	173.50 h-l	2.75 e-i	26.55 cd	64.50 h-j	16.30 cd	15.60 c-g
Sundar khani	18.70 b-e	8.65 c-f	439.05 b	2.20 h-m	23.00 c-f	159.00 ef	16.10 cd	13.80 gh
Aesel	17.00 d-g	11.50 a-c	350.55 cd	3.10 c-f	17.00 f-h	267.50 b	13.79 cd	10.75 i
Taifi	17.30 c-f	13.75 a	353.00 cd	2.60 f-k	23.50 c-f	143.00 f	18.35 c	15.75 c-f
Cardinal	12.20 jk	7.60 d-g	94.20 mn	1.55 m	20.00 d-g	48.50 i-l	15.40 cd	15.05 d-g
Gol	16.75 d-h	10.00 b-e	255.56 e-g	3.70 b-d	15.30 g-i	219.00 c	14.60 cd	12.15 hi
Italia Hybrid	20.25 b-d	10.25 b-e	172.10 h-j	2.65 d-i	19.95 e-h	87.50 hi	15.25 cd	14.05 f-h
Chakwal Selection	18.70 b-f	10.95 a-c	439.05 b	2.20 f-l	23.00 c-g	158.00 ef	16.10 cd	13.80 gh
BRI-001	19.80 b-e	11.75 a-c	385.00 bc	3.00 c-f	27.90 c	137.00 fg	17.85 c	14.20 e-g

*Means with same letters do not differ significantly at p 0.05 by Tukey HSD test; ** = Highly Significant (P≤0.01); * = Significant (P≤0.05); NS = Non-Significant (P≥0.05) recorded at a specific time, like time of budburst, first leaf emergence, time of inflorescence, cap fall to fruit set and time of harvesting. The data of each stage was recorded when more than 60% of plants showed stage symptoms. The time of bud burst indicated great variation in observed genotypes as mentioned in Fig. 9. The time of budburst started in observed genotypes from the 1st week of March and ends up to the 3rd week of March depending upon the specific genotype. Early white and White seedless were the accessions which started bud burst in 1st week of March. However, Italia hybrid was the only genotype that started bud bursting at the end of 3rd week of March. It was also recorded that several genotypes started and completed their bud burst stage in 2nd week of March (Kishmish, Italia, Vitro Black, Haita, Chesslas-B, Moscatol Romano and Chakwal Selection).

The first leaf emergence stage depicted great variations in leaf sprouting. This stage started from 2nd week of March and ends at the start of the 4th week depending upon the genotypes. Early White and White seedless genotypes started leaf emergence from the 2nd week of March. Early White completed its stage this week while White Seedless completed its stage at the start of 3rd week. In genotypes Flame Seedless, Aesel and Taifi, leaf emergence started from the 2nd week of March and remained up to the middle of the 4th week. Time of inflorescence was completed in Early White genotype up to start of 3rd week of March. It was the only accession that completed this stage earliest as compared to other accessions. This stage started from the start of 3rd week and ends in the 1st week of April depending upon the genotype.

Cap fall to fruit set was the longest stage which was found in grapes. In genotype Early White, this stage started earliest from 3rd week of March followed by genotypes Regina, Flame Tokay, NARC Black, King's Ruby and White Seedless. While in genotypes Red Globe, Superior, Moscatol Romano, Aesel, Taifi, Italia Hybrid and BRI-001 cap fall started late in the first week of April. The fruit set stage was included up to when berries attained their maximum size. There were great variations observed between the accessions at this stage. Some genotypes attained their maximum size at the start of June while other genotypes attained their maximum berry size up to the middle of July depending on their maturity behavior. Regina, Perlet and Early White were the earliest mature genotypes that were harvested in the second week of June followed by genotypes Flame Tokay, White Seedless and Flame Seedless which matured in the third week of June. Sundar Khani, Aesel and Taifi were genotypes that mature at the last as compared to other genotypes under study. These cultivated were harvested in the third week of July so they were late mature genotypes. Kishmish, Cardinal and Moscatol

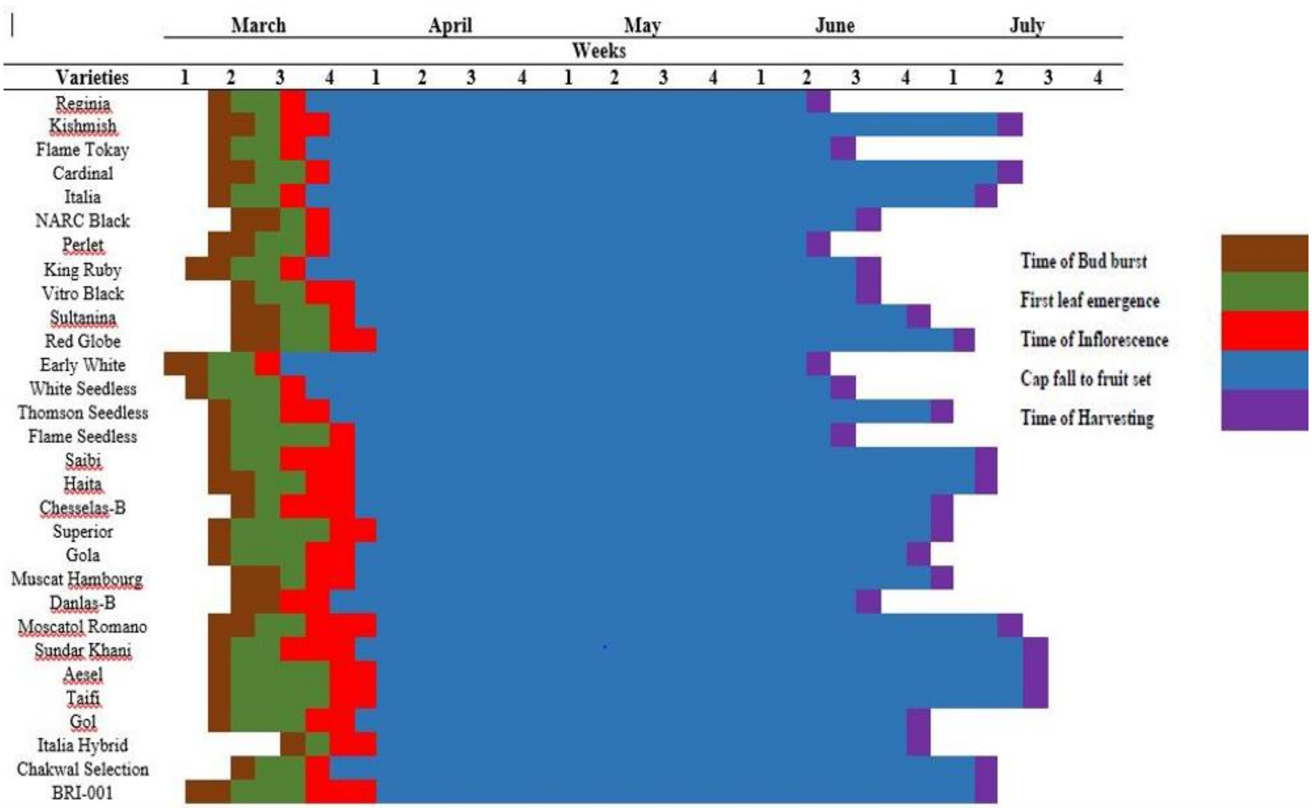

Figure 9. Phenological calendar of 30 genotypes grown in Potohar region of Pakistan based on different phenological stages.

Romano were also late mature genotypes and were harvested at the end of the 2nd week of July.

DISCUSSION

Morphological characterization of grapes genotypes based on qualitative and quantitative traits: Morphological characterization is one of the basic and simplest tools to differentiate genotypes based on visual observations. It is being used since Mendel's era and is still considered an important tool in this modern era for discrimination and separation of genotypes. Morphological characterization provides basic information about the traits. It is necessary to manage existing germplasm in vineyards for the development of new lines through breeding as breeding goals in fruits are to improve fruit weight, shape, color and inner quality (Guan *et al.,* 2020). Morphological characterization provides superior results as we can determine more characteristics as compared to other characterization. Morphological characterization dominance should be taken into account while interpreting the number of characteristics examined (Atak *et al.,* 2012).

In our study, morphological characterization was quite helpful to discriminate genotypes based on characteristics taken as prescribed by the descriptor of IPGRI. These characters showed variation among the grape genotypes grown in the Potohar region of Pakistan. The variables density of erect and prostrate hairs on young and mature leaves was the most prominent factor in our study which differentiated the genotypes based on dissimilarity. Similar findings were observed by (Dilli *et al.,* 2014) who found the density of erect and prostrate hairs on mature leaves responsible for the discrimination of grape genotypes in their findings. Similar results were reported by (Ates *et al.,* 2011) that the density of prostrate hair on young leaves played a significant role in the identification of grapevine genotypes. These characters showed significant variations in varieties grown in the northern zones of Punjab, Pakistan.

Based on these hair characteristics of young and mature leaves, genotypes in our study were divided into different sub-groups. Genotypes in each subgroup were very similar in their morphological characters. In this comprehensive study of descriptors, certain characters including the density of prostrate hairs on main veins; density of erect hairs on main veins; erect hairs on internode; density of prostrate hairs between veins and density of erect hairs between veins played their anticipated role in the identification of the grape genotypes. These characters also played a major role in the construction of a dendrogram to evaluate the phylogenetic relationships of genotypes. Similar findings were observed by Knezovic *et al*. (2017) who selected 16 characteristics from OIV descriptor to identify ten grapes genotypes. Likewise, Atak *et al*., (2014) determined 55 morphological traits to check variability in nine grapes cultivars.

Mature leaf characters are also powerful tools for the identification of grapevines (Ates *et al.,* 2011; Boselli *et al.,* 2000). Depth of upper lateral sinus which is different among all genotypes is a mature leaf character that provides discriminative data in this study. These findings were also similar to Santiago *et al.* (2007) and Ortiz *et al.* (2004) who reported that mature leaf characters provide discriminative data for the identification and separation of genotypes. However certain mature leaves characters in our study such as the number of lobes, shape of teeth, petiole sinus limited by veins and density of erect hairs between veins were very similar in several genotypes and did not play a significant role in discrimination of the genotypes.

Morphological characters provide basic information about the traits. Traits that are related to the production of a crop like the number of berries, bunch size, berry weight, berry length and berry width can only be measured by taking physical observations which is possible to measure only by morphological characterizations. Further, this information is important for breeders which helps them to improve genotypes. The fruits traits are the major factors that determine crop productivity and are required for crop improvement. These factors also play a major role in breeding selection criteria for grape species (Vafaee *et al*., 2017). Davies and Savolainen, (2006) reported that morphological characters like berry length are highly correlated with changes in genetic characters. Grapes bunch and berry characters have their significant role in quality assessment especially in table grapes (Dilli *et al.,* 2014). Variability related to bunch and berry was determined in the studied genotypes. The berry ripeness in grapes varies depending on their intent to use (Khan *et al.,* 2009). Often characters like bunch size, berry size, weight, flavor and sweetness are used as indicators of grape maturity. Color alone is not the sole parameter for grapes harvesting (Jauron *et al.,* 1997).

Berry size development directly depends upon water as it is the fundamental constituent of the grapes berry and it is 75-85% of the fruit weight (Khan *et al.,* 2009). Berry length, width, weight and number of berries are directly involved in the production of crops and these parameters directly depend upon the genotype selection and environmental factors. In our findings, the highest berry length, width and bunch weight was observed in Gola was the local cultivar of the region. The reason to attain maximum berry length and width is that this genotype has well adapted itself to climate and has genetic characteristics. Environmental factors like soil, precipitation, temperature, humidity and their combination greatly influence grapes' quality (Ubalde *et al.,* 2010). Quality includes the physical features like fruit shape, color and size which attract a consumer. The climatic variations in grape growing areas account for diversity in berry quality and other grapes products (Khan *et al.,* 2009). Berry and bunch characters are also of great importance for breeders as they play their direct role in variety development and improvement. Further, the evaluation of morphological and agronomical traits in grapes is helpful in adopting superior varieties and these findings are helpful in the selection of best-performing cultivars for a specific region based on fruit yield and quality attributes (Kuria *et al*., 2020).

***Phenology of grapes genotypes grown in Potohar region*:** Grapes is one of the most cultivated crops of the world and are highly responsive to climatic and environmental variations. It is included in temperate fruits as it requires specific chilling hours to break its dormancy. This crop goes through several phenological stages during its life cycle like dormancy, bud break, first leaf emergence, inflorescence emergence, fruit set and fruit harvest (Poudel *et al.,* 2010). Phenology is defined as a study of plant life cycle stages with its relation to climate (Schwartz 2013). It is also considered one of the key signs of climate change (Menzel *et al.,* 2005). In past, grapes phenological stages like bud break, flowering and fruit maturity were used to check the climate variability (Jones *et al.,* 2005)

Under the agro-climatic conditions of (Potohar) Pakistan, grapes go to dormancy during winter and the bud break starts in early March depending upon the genotypes. Phenology of grapes genotypes changes with climatic conditions. Temperature, precipitation, and humidity are responsible to change the phenology of grapes (Cleland *et al.,* 2007; Chuine *et al.,* 2003). The change in climatic conditions greatly affects the growth and yield of a plant. Under the climatic and environmental conditions of Potohar, it takes more than 90 days to mature. But at maturity, there is the problem of monsoon rains in the Potohar area of Pakistan which causes the berry rotting pathogens and splitting. The rains at maturity invite several fungal which ultimately destroy the yield. Our results were in harmony with other researchers who stated that narrowing the harvesting period in grapes is a severe problem that increases fungal problems in table grapes (Atak and Kahraman, 2012). Temperature changes during the phenological stage cause stress and can endanger a crop (Kalra 2008). Similar findings were observed by Singh *et al.,* (2007) who said that change in temperature at any vegetative or reproductive stage severely affects the vine yield. Other climatic factors like rainfall or precipitation during a phenological stage can cause instability of grape crops (Jackson and Schuster, 2001). So, it is necessary to know about the grapes' genotypes which types of grapes are suitable for the specific region under specific climatic conditions (Jones *et al.,* 2010). Climate greatly affects the physical and biological maturity of grapes (Akram *et al.,* 2020).

The number of days required to complete phenological stages also includes genetic factors. Different genotypes mature at different times under the same environmental conditions. The varieties which take fewer days from bud burst to maturity earlier mature genotypes. Similarly, varieties that took more days to mature are known as late mature genotypes. In our study, genotypes Regina, Flame Tokay, Early White, Perlet, White seedless and Flame seedless mature earlier in the middle of June. While Kishmish, Cardinal, Sundar Khani, Aesel and Moscatol Romano were late mature genotypes that mature at the end of July. The reason for these genotypes' maturation at different times is that early mature genotypes require less chilling hours to budburst as compared to late mature genotypes. Therefore, early mature genotypes take fewer days from bud burst stage to fruit harvest. Our finding had accordance with researchers Saniya *et al.,* (2017); Gupta *et al.,* (2015) and Mandelli *et al.,* (2003) who found that early mature genotypes took less time from bud burst to fruit set while studying different grapes genotypes.

The adaption of a specific grape genotype depends upon its genetics and on its phenology behavior. Grapes phenology is a major component found for variety adaption to a specific environment (Duchene *et al.,* 2010; Chuine, 2010). In grapes, it is considered that genotype or variety which adapts itself in a certain ecological condition had a high potential of yield while which are unable to adapt gave poor production and quality (Cvetkovic *et al.,* 1999). Hence it is necessary to know a variety of phenological stages and climate of the site before the plantation of a vineyard. The climatic conditions like sunlight, rainfall and evaporation reflect the phenological process and grapes' ability in a specific place and affect its berry quality (Van Leeuwen *et al.,* 2004).

Conclusion: According to morphological characters investigated in this study, great differences were noted among local and exotic genotypes. There were several characters, as described above, which played their specific part in the constitution of the morphological dendrogram. For example, characters from Mature leaf (density of prostrate hairs on main veins; density of erect hairs on main veins; density of prostrate hairs between veins; density of erect hairs between veins) characters from the shoot (erect hairs on internode; erect hairs on the node; density of prostrate hairs on node; density of prostrate hairs on internode) and characters from Young leaf (density of prostrate hairs between veins; density of erect hairs between vein; density of prostrate hairs on main veins; density of erect hairs on main veins) were greatly different among the studied genotypes. The results revealed that Regina, Perlet and Early White were the earliest mature genotypes that matured in the 2nd week of June followed by genotypes Flame Tokay, White Seedless and Flame Seedless which matured in the 3rd week of June. Local genotypes Sundar Khani, Aesel and Taifi matured in the last week of July. This study provided specific knowledge on some local grape genotypes, some of which are on the verge of extinction. Therefore, this study helps to prevent the disappearance of local varieties and preserve this collection of germplasm for future studies.

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