Research Article

Multivariate Clustering of Grapes Cultivars for Productivity and Quality Enhancement under Rainfed Conditions

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Abstract | Grape yield and quality traits are interlinked and understanding their interactions is highly critical. This challenge is more obvious under rain fed conditions which impose physical constraints for normal plant growth. The present study was designed to find interactions between yield and quality characters of grapes cultivars. Therefore, several grapevine cultivars like 'Kings Ruby', 'Flame Seedless', 'Perlette', 'Superior', 'Early White', 'Vitro Black' and 'Sultanina C' were tested in rain fed area of Punjab. Data of two seasons were collected by a randomized complete block design. Experiment and results were analyzed through correlation, principal component analysis (PCA), and a cluster analysis (CA). Grapes varieties indicated a distinct cluster grouping in terms of yield and quality characteristics. 'Superior' with a closely associated group of CVs; 'Sultanina C', 'Kings Ruby' and 'Flame Seedless' are highly suitable for district Chakwal in Punjab whereas' Perlette' and 'Early White' didn't show suitability for this region. The results also indicated that the berry size is positively correlated with berry weight and yield (group-I), cluster weight and TSS (group-II) and number of clusters/plant and titratable acidity (TA) (group-III). However, TSS and TA are negatively correlated to each other in a multivariate analysis. In most of the cases the annual pattern for grapevine yield and quality traits were almost similar. The findings of current study provide a better understanding of grapes yield and quality interactions.

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Introduction

Grapevines are the largest fruit crop with a high economic value in many parts of the world. It contributes to a total of 16 percent of global fruit production (Bhat *et al*., 2017). Grapes are a distinct perennial fruit crop and belong to family vitaceae that have a diverse group of grapevines around 1000 species and 17 genera. Among all the members of this family, *Vitis vinefere*, from the temperate zones, has become the world's chief contributor of total grapes production in about 90 different countries. There are around 24,000 names for grapevine cultivars including synonyms and homonyms, with around 4000 distinct cultivars (OIV, 2019; Rafique *et al*., 2023a). This delicious fruit is important for its nutritional and medicinal benefits. It helps in strengthening of cardiac muscles, reduces cholesterol in blood and impart in immunity to the human body (Ruel and Walker, 2006). They are commonly used as fresh for table grapes, raisins, wine production and to produce liquors (Kuhn *et al*., 2013, Ruel *et al*., 2006). Global vineyards cover an area of 7.4 million hectares with a production of 77.8 million tons annually. Wines grapes with a production of 57% are leading the viticulture industry, whereas table and dried grapes have a share of 36% and 7 % respectively (OIV, 2019).

In Pakistan, grapes production stands as 107.54 thousand tons from an area of 16.03 thousand hectares. Baluchistan province is largest producer with 106.37 thousand tons on an area of 15.70 thousand hacters (GoP, 2023). Baluchistan is considered as the most suitable area for grapes production area in Pakistan, due to low humidity in fruiting season. The grapes have been cultivated in this region since centuries as Persians played a key role in spread of grape cultivation in this area. Several varieties of fresh and processed grapes such as Manakkah, Sundarkhani and Chaman angoor, Kishmish are grown in this region since many decades. There are also some other potential grapes growing pockets in Pakistan including deserted districts of Punjab and Sindh where excellent irrigation system is existed linked with the mighty Indus River. Despite a lot of potential, there are lot of challenges for the spread of grapes production in Pakistan. However, two new zones are emerging in colder-north and warmer-south districts in Punjab, Pakistan (Rafique *et al*., 2023). Vine productivity and fruit yield and is significantly lower in Pakistan compared to other main producers. Among the other factors, lack of investment from public and private sector, lesser extension education services for grapes growers of these new regions and government support in terms of provision of productive plants are the main factors.

Various varieties of grapes berry have variable morphology, especially for berry size, berry color and berry shape (Khalil *et al*., 2023a). Berry size, color and shape have primary importance in determining the physical quality, market value and acceptability to consumers (Eyduran *et al*., 2015). Identification of promising cultivars is important to grapes growers, regulatory authorities and processing industry. Traditionally, the promising grapes varieties were selected by visual observation which is called as ampelography. However, the studies based solely on ampelography does not give all the information, and some variability in descriptor definition may occur due to environmental conditions, cultural practices and genetic variations as well (Mena *et al*., 2014). Pheno-physiological basis of categorizing grapevine cultivars valuable information regarding varietal suitability (Rafique *et al*., 2023d). The response regarding berry color, berry size and sugar contents are affected with the change in environmental conditions. Similarly, plant responses vary against various insects and diseases as well.

With growing interest in grapevine cultivation in Pakistan in selecting the correct and suitable grape-planting material is crucial. The profit of one acre of grapes can be higher up to three times, compared to groundnut and up to six times compared to that of wheat, that are major crops in Chakwal district and adjoining areas (Rafique *et al*., 2023b; 2023c). Owing to high profitability of grapes production, the viticulture industry has spread to northern and southern Punjab areas (Anjum *et al*., 2020; 2022; Rafique *et al*., 2021a; 2021b, Khalil *et al*., 2023b). Previous studies have established the physiological basis associated with phenology of grape cultivars in Pothwar (Rafique *et al*., 2023b; 2023c; 2023d). However, studies focusing on finding correlations between key quality variables and varietal categorization are still at initial stages thus need further investigation.

Recently some sophisticated techniques have been introduced for data analysis that can guide researchers for recommendation of varieties for commercial cultivation through Multivariate statistical analysis technique (Basile *et al*., 2021). It is impossible that

all desired attributes related to quality are present in single genotype thus it becomes difficult to categorize the cultivars based on simple statistical analysis. The multivariate analysis that shows the collective impression of given characters, thus helps in better categorization for suitability (Francis *et al*., 2017). PCA is a statistical technique that transforms a set of interrelated variables into a set of uncorrelated variables. The newly formed variables (PCs) are linear combinations of the original variables. This tool can indicate the variables that are the most influential on system variability. The Multivariate statistical method allows to take a wise decision for recommendation of a certain variety on the basis of key attributes. The present research was performed for the evaluation of varietal suitability of grapes varieties among a prominent collection of exotic varieties established at Barani Agriculture Research Institute (BARI), Chakwal and gradually spread to the progressive farmers of the area.

Materials and Methods

Experiments on variety evaluation were conducted in BARI-Agriculture Research vineyards at Chakwal during 2015 and 2016. Seven grapes cultivars namely; 'Kings Ruby', 'Flame Seedless', 'Perlette', 'Superior', 'Early White', 'Vitro Black' and 'Sultanina C' were investigated in multi-variety trials at Chakwal in Punjab. Vines were selected in RCBD experimental statistical design with three replications for each cultivar having three vines in a replicate. Vines were planted apart 8×10 feet during 2012-2013 in order to check their adaptability and yield potential. The regular cultural operations were adopted such as irrigation and fertilizer application at the grapes research areas. The mean responses were compared for evaluating correlation among cultivars, years and responses variables.

The number of clusters were counted on three replicated vines, a total of nine clusters were selected randomly from the tagged vines at harvest to analyze the physical and biochemical attributes. Berry and cluster traits such as number of clusters, cluster weight, berry size and berry weight were recorded for all cultivars during the study. Yield and quality traits including total soluble solids (TSS) and titratable acidity (TA) were also recorded at harvest. Thirty berries from different positions of a cluster were taken as one replicate thus a total of three berry samples replicates were drawn from nine tagged vines for recording mean berry weight and berry size. TSS for sweetness in juice were observed at harvest through a digital refractometer (RX 5000-ATAGO-Japan) and expressed as Brix°. TA (%) was determined by titration of fresh juice against NaOH, (Hortwitz, 1960). Calculations were made using following assumptions for TA.

$$\text{Titratable acidity (\%)} = \frac{\text{0.1 N NaOH used in titration x 0.0064}}{\text{Volume of sample used}} \times 100$$

Differences among the mean values were recorded for berry growth, quality and yield characters. Variables were analyzed by a multivariate analysis at p value of 0.01 (level of significance). The multivariate responses were evaluated following guidelines as described by Hair *et al*. (2009) using a principal component analysis (PCA) and a cluster analysis (CA). The first principal component, PC1, given by the eigenvector associated with the highest eigenvalue of the correlation matrix, explains the highest percentage of the system variability. The second principal component, PC2, corresponds to a lower proportion of the variance, and so on in this study, a PCA was performed on the seven cultivars defined by the corresponding average values in each of the variables considered. In addition, a cluster analysis (CA) was performed by defining metrics i.e., points of proximity among the cultivars and variables into possible groups. A hierarchical clustering method (UPGMA, Unweighted Pair Group Method with Arithmetic Mean) was used to identify similar clusters behavior. The grouping process first considers the individual objects as separate clusters. The similarities among groups were quantified through the linkage distance in x-axis (Hair *et al*., 2009). SAS statistical program was used for data analysis for scatter plots, correlation, PCA and hierarchical clustering.

Results

Scatter Plot and Correlation Matrix

The presented results as scatterplot matrix for the years 2015 and 2016 indicates that grapevine physical and biochemical traits are associated with each other as shown in (Figure 1 and b). Number of clusters are strongly correlated with cluster weight, berry size and berry weight during 2015 and with berry size and weight for the year 2016. The berry weight was correlated with berry size and clusters per plant and also with yield (for 2016). Cluster weight correlated

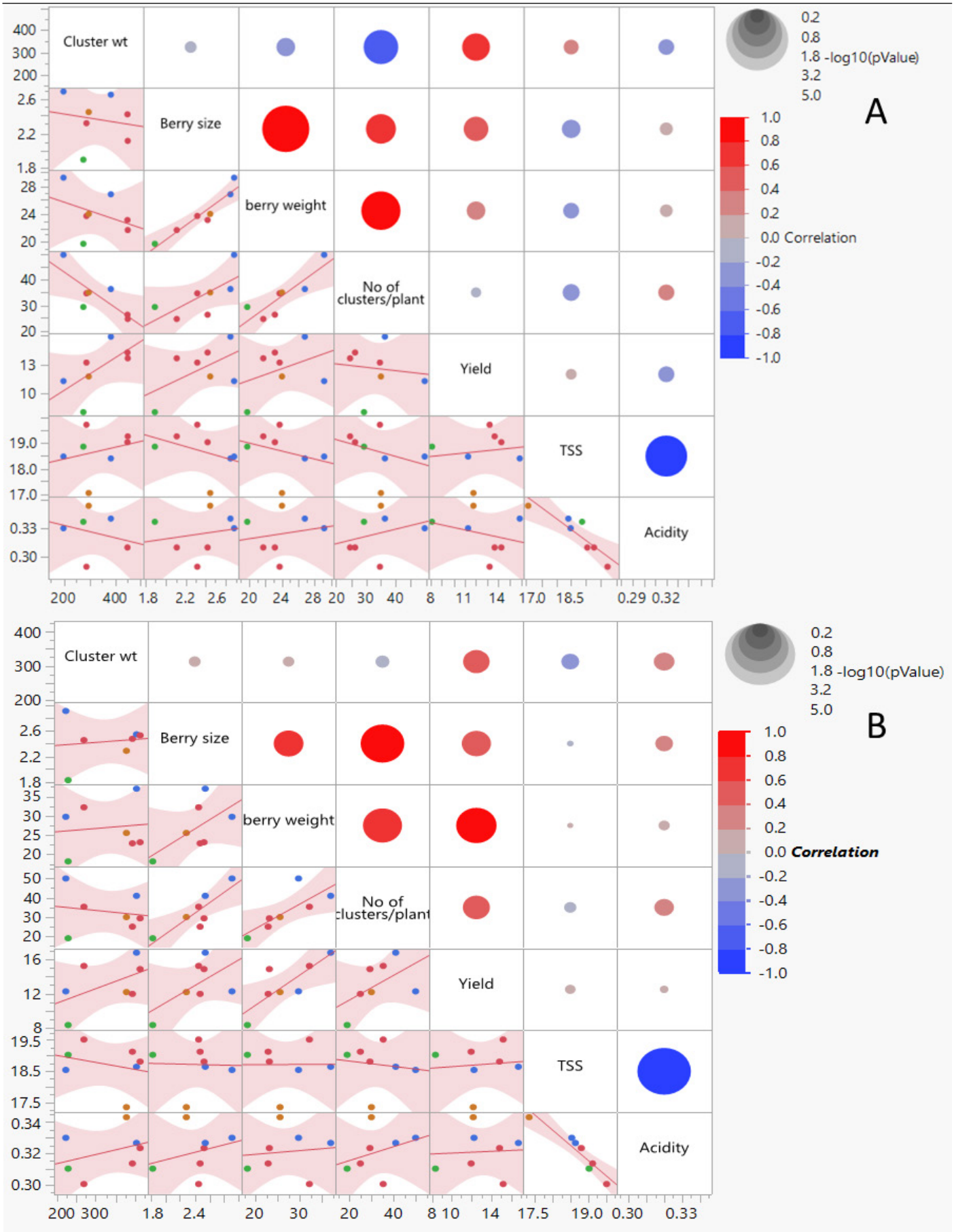

Figure 1: *Scatterplot matrix for various yield and quality attributes in grapes under rain fed conditions for the year 2015 (A) and 2016 (B). The circle size indicates the strength of correlation. whereas as red circles indicate positive and blue circles indicate negative correlations.*

Table 1: *Correlation among various yield and quality attributes in grapes under rainfed conditions for the year 2015.*

Trait		Cluster Weight	Berry Size	Berry Weight	Clusters Per Plant	Yield	TSS	Acidity
Cluster Weight	Correlation	1.000	-0.160	-0.374	-0.789	0.640	0.256	-0.281
	P-Value	<.0001	0.733	0.409	0.035	0.122	0.579	0.541
Berry Size	Correlation	-0.160	1.000	0.941	0.694	0.556	-0.374	0.196
	P-Value	0.733	<.0001	0.002	0.084	0.195	0.409	0.673
Berry Weight	Correlation	-0.374	0.941	1.000	0.862	0.374	-0.286	0.186
	P-Value	0.409	0.002	<.0001	0.013	0.408	0.534	0.690
Clusters per plant	Correlation	-0.789	0.694	0.862	1.000	-0.116	-0.323	0.290
	P-Value	0.035	0.084	0.013	<.0001	0.804	0.479	0.528
Yield	Correlation	0.640	0.556	0.374	-0.116	1.000	0.134	-0.290
	P-Value	0.122	0.195	0.408	0.804	<.0001	0.775	0.528
TSS	Correlation	0.256	-0.374	-0.286	-0.323	0.134	1.000	-0.893
	P-Value	0.579	0.409	0.534	0.479	0.775	<.0001	0.007
Acidity	Correlation	-0.281	0.196	0.186	0.290	-0.290	-0.893	1.000
	P-Value	0.541	0.673	0.690	0.528	0.528	0.007	<.0001

with fresh fruit yield. Similarly, TSS and acidity were strongly correlated (negative) with each other. During the year of 2016 the cluster weight was moderately correlated with berry size and number of clusters while it was strongly correlated with berry weight. Berry size was strongly correlated with berry weight and number of clusters as well. Berry weight was also strongly correlated with number of cluster and yield. The trend for biochemical characters i.e., TSS and acidity was almost similar.

The correlation analysis (p value = <0.0001) for 2015 revealed that cluster weight was correlated with cluster per plant (-0.79) and yield (0.64) as indicated in Table 1. However, this relation was negative for number of clusters per plant and positive for yield. The correlation analysis (p value= <0.0001) amongst various physical and biochemical and cluster weight indicated that it was negatively correlated with berry size, berry weight, clusters per plant however, it had a week positive correlation with yield and TSS. However, it did not give a clear indication of actual rise in yield. Berry size is strongly correlated berry weight (0.94), cluster/plant (0.69) and with total yield (0.56). The berry weight was strong positively correlated with berry size (0.94) and cluster/plant (0.862). The cluster/plant are correlated with cluster weigh (-0.79), berry size (0.69) and berry weight (0.862). In the same pattern, yield was observed to be strongly correlated with cluster weight (0.64). The biochemical attribute i.e., total soluble solids (TSS) was strong negatively correlated with acidity (-0.893). Similarly, acidity was negatively correlated with cluster wright and yield (Table 1).

The correlation analysis (p value= <0.0001) amongst various physical and biochemical attributes during second year (2016) showed many similarities with previous year as given in Table 2. It was revealed that cluster weight is positively correlated with yield (0.52) while, berry size was strongly correlated berry weight (0.60), cluster/plant (0.85). Berry weight also strongly correlated with cluster/plant (0.79) and yield (0.81). The cluster per plant were strongly correlated with berry size (0.85) and berry weight (0.79), while cluster per plant negatively interacted with cluster weight and TSS. Fruit yield was correlated with cluster weight (0.51), berry size (0.58), cluster per plant (0.53) whereas, it showed a strong positive correlation with berry weight (0.81). The biochemical attribute i.e., TSS followed the previous year's pattern showing a strong negatively correlation with acidity (-0.9423) and vice versa (Table 2).

Multivariate Analysis of Grapevine Cultivars

Bi-plots matrix of formed factors and original variables during the years of 2015 and 2016 indicated distinct variable responses. The formed factor competent i.e., PC 1 and PC 2 showed the cultivars and their attributes that showed 42.4 % of variation for the first component and 28.8% for second component. The third component showed that cultivars along their biochemical attributes accounted for 20.7% of the variation. These three components together

Table 2: *Correlation among various yield and quality attributes in grapes under rain fed conditions for the year 2016.*

		Cluster Weight	Berry Size	Berry Weight	Clusters Per Plant	Yield	TSS	Acidity
Cluster Weight	Correlation	1.0000	0.1229	0.1130	-0.1711	0.5190	-0.2805	0.3555
	P-Value	<.0001	0.7929	0.8093	0.7138	0.2326	0.5423	0.4339
Berry Size	Correlation	0.1229	1.0000	0.6012	0.8543	0.5852	-0.0270	0.2764
	P-Value	0.7929	<.0001	0.1534	0.0144	0.1675	0.9543	0.5484
Berry Weight	Correlation	0.1130	0.6012	1.0000	0.7893	0.8093	0.0057	0.1082
	P-Value	0.8093	0.1534	<.0001	0.0348	0.0274	0.9904	0.8174
Clusters per plant	Correlation	-0.1711	0.8543	0.7893	1.0000	0.5333	-0.1300	0.3246
	P-Value	0.7138	0.0144	0.0348	<.0001	0.2177	0.7812	0.4776
Yield	Correlation	0.5190	0.5852	0.8093	0.5333	1.0000	0.0929	0.0552
	P-Value	0.2326	0.1675	0.0274	0.2177	<.0001	0.8430	0.9065
TSS	Correlation	-0.2805	-0.0270	0.0057	-0.1300	0.0929	1.0000	-0.9423
	P-Value	0.5423	0.9543	0.9904	0.7812	0.8430	<.0001	0.0015
Acidity	Correlation	0.3555	0.2764	0.1082	0.3246	0.0552	-0.9423	1.0000
	P-Value	0.4339	0.5484	0.8174	0.4776	0.9065	0.0015	<.0001

The correlations are estimated by Row-wise method.

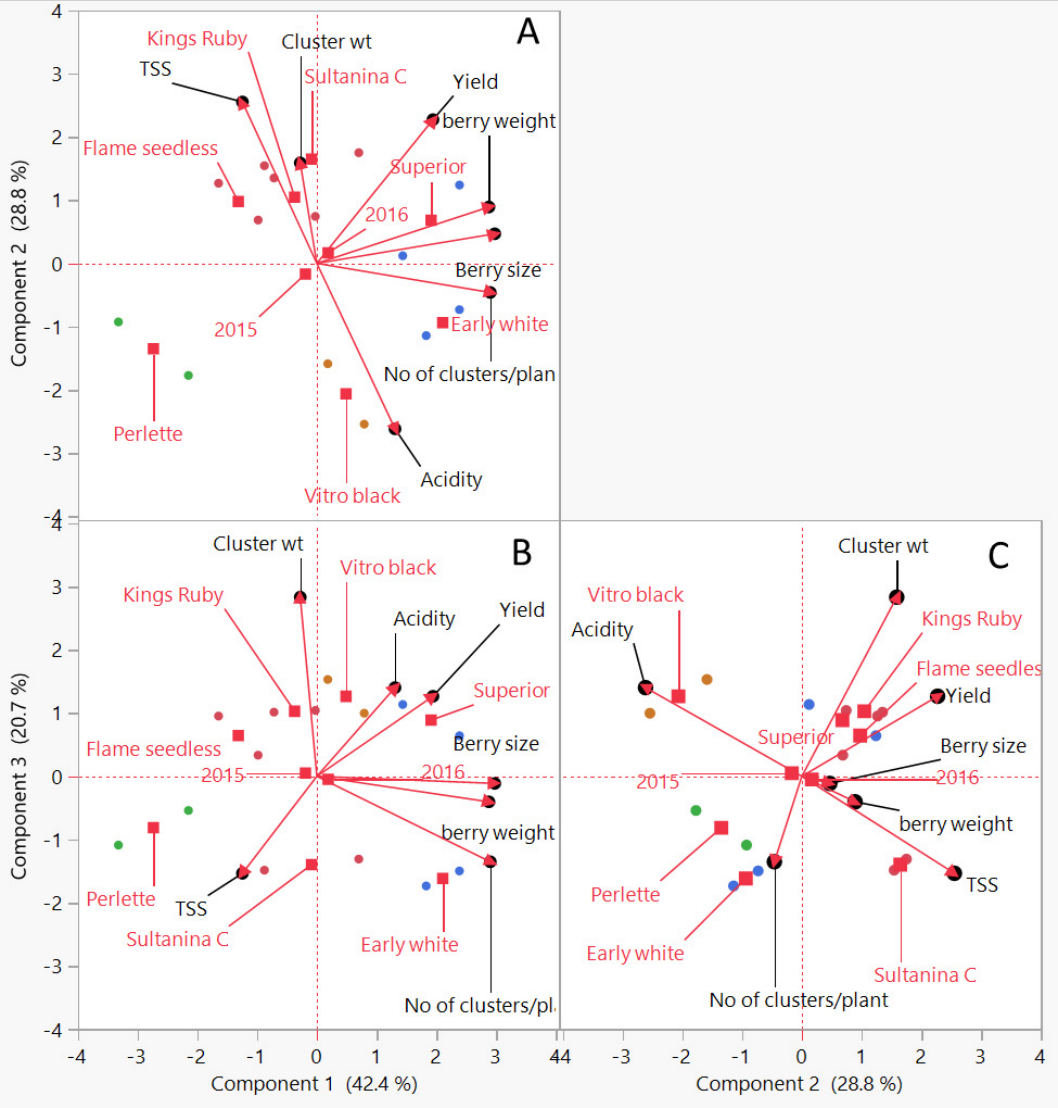

Figure 2: *Biplot matrix of first three components; PC1 and PC2 (A), PC1 and PC3 (B) and PC2 and PC2 (C) for various yield and quality attributes in grapes under rain fed conditions across the years 2015 and 2016.*

indicate a major portion of variability i.e., 92 % during the experiments (Figure 2). Among the total 71 % of variability is accounted for first two components (PC1 and PC2) as shown in Figure 2A. The analysis of these two components indicated four distinct groups of cultivars. The group-1 consisted of 'Superior' with a higher berry weight and yield, second group; 'Flame Seedless', 'Kings Ruby' and 'Sultanina C' having higher TSS and cluster weight, third group; consists of 'Perlette' with least responsiveness for grapevine attributes and the fourth group consists of 'Early While' and 'Vitro Black' with a higher berry size, clusters/plant and acidity. Although, there exists some variability between the years however, the mean variability is non-significant compared to variations arising from other sources.

The summary PCA plot of variable of various grapes cultivars during the years of 2015-16 indicated that two main components (PC1 and PC2) accounted for seventy one percent of total variability. The pooled data indicated four groups for cultivars; 'Superior' (group-1), closely associated with 'Sultanina C', 'Kings Ruby', 'Flame Seedless' (group-11),Perlette' (group-111), 'Early White' (group-1V). Cultivars in each group has some marked featured different from other cultivars (Figure 3). Furthermore, three discrete groups for grapevine physical and biochemical attributes were identified. Grapevine attributes in each of the groups; yield, berry weight and berry size (group-1), cluster weight and TSS (group-11) and number of clusters/plant and acidity (group-111) are closely associated with each other having distinct variations with another group as shown in Figure 3. The squared cosines values are also presented in different colours where the active individual is showed in the blue colour. The other colors showing the supplementary qualitative and quantities variable (red and green). The active individual showed the variation and fluctuation arising from varieties, attributes and years (Figure 4).

Hierarchical Clustering of Grapevine Cultivars

The dendrogram for the hierarchical cluster analysis revealed the grouping of plant attributes and cultivars (Figure 5). For 2015 season, three resembling groups of varieties are shown in figure 5A, where grapevine cultivars; 'Kings Ruby', 'Flame Seedless' and 'Sultanina C' formed the group-1 with better performance, 'Perlette' formed the group-II showing moderate performance. How ever cultivars; 'Early White', 'Vitro Black' and 'Superior' indicated group-III with least responsiveness (Figure 5A). The grapes attributes are separated in two groups; cluster weight, yield and TSS are associated with each other, whereas berry size, berry weight, clusters/plant and acidity are more associated with each other. The cluster for 2016 has some distinct features than for 2015 (Figure 5B) e.g., group-I consisted of 'Kings Ruby', 'Flame Seedless' and 'Vitro Black', group-II; 'Sultanina C', 'Early White' and 'Superior' and 'Perlette' formed group-III. The cluster for attributes indicated three closely associated groups; cluster weight and acidity are more associated with each other (group-I), berry size, berry weight and cluster per plant are related to each other

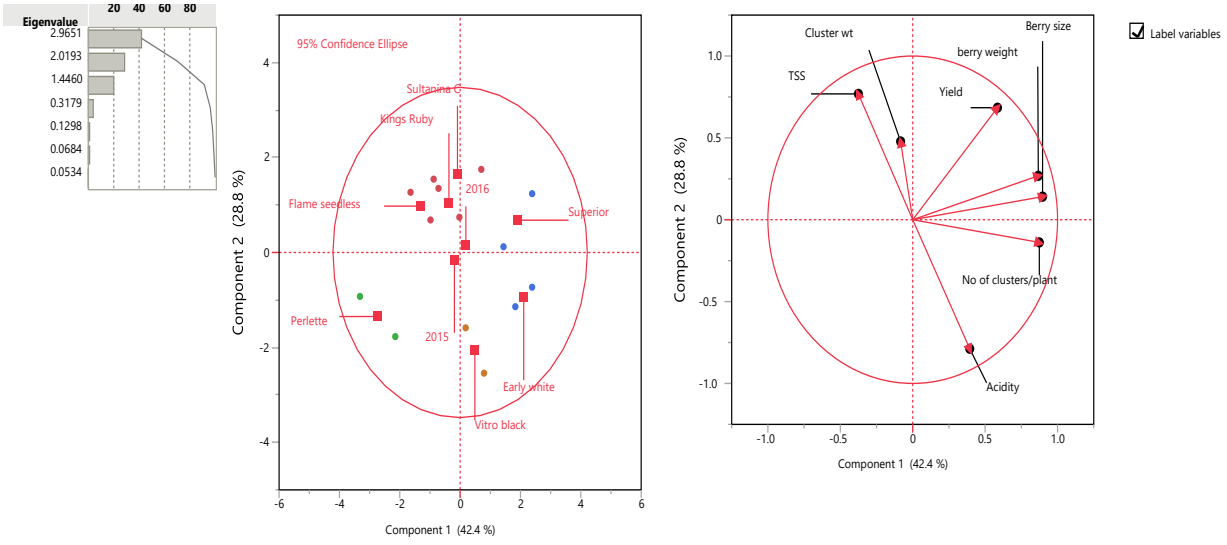

Figure 3: *Summary plot of PCA for various yield and quality attributes in grapes under rain fed conditions across the years 2015 and 2016.*

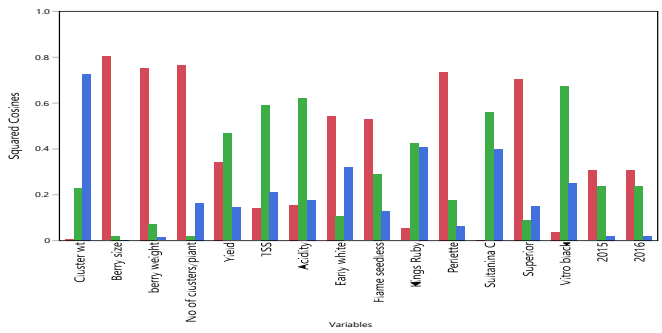

Figure 4: *Plot of Squared Cosines for various yield and quality attributes in grapes under rain fed conditions across the years 2015 and 2016.*

(group-II), TSS and yield are more distinct than other traits (group-III) are grouped alike while the plant attributes of cluster weight, yield and TSS are grouped separately compared with other attributes. The color scheme filled showed the strong correlation of cultivars with plant attribute as well. The high intensity of red color showing strong positive correlation of plant attribute and variety as well (Figure 5A and 5B)

Discussion

The results of this study indicated that grapevine varieties differed significantly for the morphological attributes such as berry physical attributes and biochemical attributes under semi-arid climate. Cultivar 'Superior' had more berry weight and a higher yield and, 'Flame Seedless', 'Kings Ruby' and 'Sultanina C' had more TSS and cluster weight. 'Early White' and 'Vitro Black' have a higher berry size, clusters/plant and TA. However, lesser yearly difference (for 2015 and 2016) were observed and almost similar trend was observed. Multiple factors affect berry quality, physical, and biochemical attributes and they respond differently for different cultivars and regions (Shah *et al.*, 2021; Rafique *et al.*, 2023a; Khalil *et al.*, 2023c). Rafique *et al.* (2021) also highlighted the variability in growth habit of grapevine cultivars.

Grapevine cultivars 'Superior', 'Flame Seedless', 'Kings Ruby' and 'Sultanina C' have better performance for most of the attributes in this study. Previously, 'Kings Ruby' and 'Sultanina C' have been potentially identified as better performing varieties in Punjab (Anjum *et al.*, 2020; Rafique *et al.*, 2023e). The early maturing varieties are considered more suitable for cultivation in Chakwal climatic conditions than late ripening. This may be due to heavy monsoon rains from July to August in this region. Rainfall or high humidity may reduce fruit set, hindering pollination by impeding the complete detachment of the calyptra. In addition, rains can also dilute the stigmatic fluid and thus interfere with the germination of pollen grains. Despite early maturing 'Perlette' and 'Early White' did not perform well in terms of yield and quality traits compared to other varieties. This necessitates finding some other adaptive features in addition to early maturity.

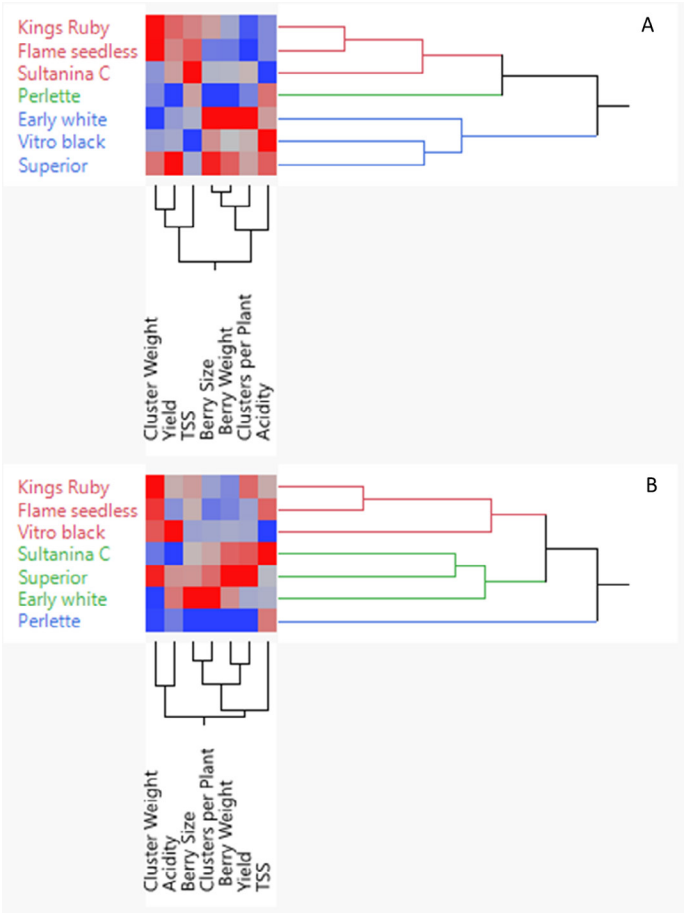

Figure 5: *Hierarchical clustering for various yield and quality attributes in grapes cultivars* **under** *rain fed conditions for the year 2015 (A) and 2016 (B)*

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The climate of Chakwal is supportive for good quality grapes production as most of the climatic variables are in good ranges for better growth, quality and production. Moreover, the temperature and other climatic factors are comparable with other commercial grape producing regions. In this region, temperature starts rising from early springs, this rise in temperature support better size of berry, more sweetness, low acidity, high number of clusters and optimum berry weight. These factors contribute to early and superior quality grapes are produced in Chakwal region of Pakistan.

TSS and TA are widely broadly recognized as key biochemical maturity indices for grapes (Rolle *et*

al., 2015). These attributes are strongly correlated with formation of sugar contents in fruit flesh (Shiraishi *et al*., 2010). The sugar contents of berries are mainly affected by genotype under the influence of climatic factors and vineyard management (Jackson and Lombard, 1993; Dai *et al*., 2011; Duchêne et al., 2012; Rafique *et al*., 2023e). The joint effect of temperature and rainfall alters the TA and other berry attributes, however during the present study the correlation of environmental factors with berry attributes was not observed. The reason for this might be the same location for both years and lesser variability for environments during the years 2015 and 2016. Relevantly low temperatures of rain-fed areas of Chakwal region increased TA that is strongly correlated with TSS (Easterling *et al*., 1997; Rafique *et al*., 2023e) that ultimately interacts with the sweetness of berries in rain fed areas. In a recent research study, Rafique *et al*. (2023e) observed a strong negative correlation between TSS and acidity.

Strong genotypic differences indicate that grapevine cultivars have varying ripening potential under the same agroclimatic conditions (Khalil *et al*., 2023c). Genotypic variability existed in grapes cultivars for different physical and biochemical characters. A similar diversity for sugars in berries of 45 grapevine genotypes was observed in north-eastern viticulture region of China (Yin shan *et al*., 2017). Henceforth, it is crucial to utilize this inherited diversity among different genotypes through crossbreeding for improving fruit quality. This variability in grapes varieties is supported by the findings of Muñoz-Robredo et al. (2011); Daniels *et al*., 2019 who noticed genotypic variability for grapes fruit quality. The genotypic variability points towards a genotype-specific vineyard management.

The multivariate analysis clustering of morphological, agronomic and varietal characters are applied for sorting of crop traits (Joshi *et al*., 2015). The cluster analysis form the groups of alike varieties and shows correlation amongst them on the basis of characters under consideration (Abu-Zahra, 2010). The berry yield is strongly correlated with number of clusters, cluster weight, berry weight and berry size. The results indicated that increasing cluster weight no doubt increased yield but to a limited extent, hence maintaining an optimum bunch size is good for good quality and better yield. The higher cluster weight will reduce the berry traits like berry size and weight. Another, reason for this might be higher number of bunches actually competing for reserves.

Our preliminary findings on these cultivars derived from a single location in this experiment with almost similar climatic conditions which needs to be tested in other areas as well. We propose future research on climate, phenology, physiology and yield in other region as well along with finding source-sink relation with berry traits. In this regard, application of dynamic approaches for predicting fruit quality as proposed by Hopf *et al*. (2022) is valuable particularly for the specialty crops. The results also emphasize the developing some adaptation strategies such as efficient rootstocks and photo-synthetically efficient cultivars (Nazir *et al*., 2022; Rafique *et al*., 2023ac).

Conclusions and Recommendations

The multivariate cluster analysis indicates 'Sultanina C', 'Kings Ruby' and 'Flame Seedless' are highly suitable in Chakwal region of Punjab. These varieties have superior traits in terms of cluster weight (g), berry weight (g), number of clusters, TSS and TA, these varieties can be recommend for cultivation in rain fed areas of Punjab. Our results indicated that quality traits act in conjugation with yield traits and point towards balanced yield and quality. Further testing is required to evaluate the varietal responses in hotter regions of Punjab such as Bahawalpur, Multan, Rahim Yar Khan and D.G. Khan for better fruit yield and profitability. Cultivating these recommended grapes varieties may help to enhance economic returns for grape growers in Punjab.

Acknowledgement

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Novelty Statement

The present applied research provides a multivariate approach for comprehensive understanding of grapes cultivars and helpful for grapes growers in Pothwar.

Author's Contribution

Monis Hussain Shah: Conducted the study
Naveed Anjum and Muhammad Asim: Helped first

author in the study.

Muhammad Aqeel Feroze, Muhammad Shahid Iqbal and Tehseen Ashraf: Statistical analysis
Bushra Zulfiqar: Review and data tabulation
Rizwan Rafique: Review and statistical analysis

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