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RESEARCH ARTICLE

Morphological and fruit variability of grape (*Vitis vinifera* L.) germplasm under sub-tropical condition

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Abstract

The research was carried out at ICAR-National Research Centre for Grapes in Manjri, Pune, focusing on 24 grape germplasms. These germplasms were assessed for morphological and qualitative traits. During October 2023 to March 2024, data on vegetative growth, bunch characteristics, yield and quality were collected in line with Distinctness, Uniformity and Stability (DUS) guidelines and descriptors from International Plant Genetic Resources Institute (IPGRI), International Union for the Protection of New Varieties of Plants (UPOV) and International organization of Vine and Wine (OIV 2007). The mean and standard deviation (SD) were computed for all morphological traits and coefficients of variation (CV %) were used to indicate variability levels. Principal Component Analysis (PCA) was performed using SPSS statistical software to identify correlations between qualitative traits. The findings revealed significant variability among the grape germplasms for various morphological traits. The time of bud burst showed considerable variation, with most germplasms exhibiting medium days to bud burst. Shoot tip opening and young leaf color also demonstrated diverse forms, indicating the genetic diversity within the germplasms. Most varieties displayed an erect growth habit, which is advantageous for vineyard management. Mature leaf characteristics, such as width and shape, provided crucial identification markers. The study noted a predominance of pentagonal leaf shapes and observed anthocyanin coloration on the main vein in a subset of varieties. Additionally, variations in leaf hair density and petiole sinus opening were important discriminative features. Bunch characteristics such as density, shape and uniformity of berry size were recorded, with medium density and conical bunch shapes being the most common. Berry shape and color varied significantly, with short elliptical and green-yellow berries being prevalent. The absence of anthocyanin coloration in the berry mesocarp was noted in all germplasms, which is relevant for table grape quality. This variability underscores the genetic diversity present in the grape germplasm, which is essential for breeding and conservation efforts. Overall study demonstrated the effectiveness of morphological characterization in assessing genetic diversity and identifying distinct grapevine genotypes.

Keywords: germplasms; IBPGR; morphology; Principal Component Analysis (PCA); vineyard

Introduction

Grapevines (*Vitis spp.*) is one of the major important fruit crops with wide range of autochthonous cultivars. It comprises around 60 interbreeding wild species spread across Asia, North America and Europe, thriving in subtropical, mediterranean and continental temperate climates (1). *Vitis vinifera* L. contributes to over 90 % of the world's annual grape production, due to its extensive genetic diversity and relatively low chilling requirements to break bud dormancy (2). Over the centuries, extensive cultivation, natural hybrids, vegetative reproduction, somaclonal variations and spontaneous mutations have all contributed to the genetic diversity of this species.

The world vineyard surface area is estimated to be 7.2 Mha, with the production of 27.9 million metric tonnes. Major grape producing countries are China, Italy, France, Spain, USA, Turkey and India (3). In the II advance estimates of 2023, an area of 175 thousand ha was covered under grapevines and production was 3896 thousand tons in India (4). The grapes are mainly grown in tropical regions of country. Maximum area and production were recorded in Maharashtra state followed by Karnataka and Tamil Nadu. India is known as table grape producing country and only about 2 % of total production is being utilized for juice or wine purpose (5). This segment is globally important due to its rich bioactive compounds with health benefits, its role in scientific research and its significant economic impact. Wine production supports trade, tourism and rural development, while its by-products are valuable in food, cosmetics and sustainable industries further enhance its global value.

India has a long history of grape cultivation. It is believed that cultivated grapes were introduced to India around 1300 AD by Muslim invaders from Iran and Afghanistan (6). Grapes were introduced to South India in 14th century. After the partition of India, much of the primary grape-growing region in northwest India became part of Pakistan, leading to restricted cultivation in northern India. Today, grapes in India are primarily grown for table consumption, with small amounts used for raisins and juice. Due to large number of table grape cultivars in commercial production, germplasm collections and thorough characterization is crucial for several reasons.

Assessing diversity plays an important role in characterizing and conserving grapes germplasm. This is essential for sustaining and enhancing agricultural production, promoting sustainable development and alleviating poverty (7,8). Understanding the morphological traits such as bud burst timing, leaf shape, leaf lobes, véraison and ripening, along with genotype resistance or tolerance to biotic and abiotic stress, is essential for quality-focused cultivation practices. Additionally, detailed ampelographic records play a vital role in documenting different vineyard techniques like pruning and harvest criteria (9). The International Organization of Vine and Wine reports that there are currently over 150 descriptors used for characterizing and identifying grapevine genotypes. These descriptors encompass a variety of morphological traits, molecular genetic markers and phenological characteristics (10). Scientists and researchers around the world are more interested in grape genetic resources now that they are aware of them since germplasm is essential for species conservation, understanding gene functions and creating new and improved lines (11).

The purpose of this study was, to evaluate the diversity within the available germplasm to aid in selecting suitable varieties for specific purposes and developing cultivars with targeted traits suitable under tropical condition.

Materials and Methods

Plant materials

Field gene bank of grapes is maintained at the ICAR-National Research Centre for Grapes, Manjri, Pune. (Latitude 18°32 N and longitude 73°51 E). The vineyard was seven years old, thriving with good health and yielding regular crops. Y trellis system was selected for the study. Double pruning and single cropping are being practiced. The vines were maintained according to standard recommended agricultural practices. twenty-four different grape germplasm are presented in Table 1.

Ampelographic qualitative traits

Twenty-four grape germplasms were examined for various morphological characters. The observations on various vegetative growth, bunch and bunch quality, were recorded based on the DUS guidelines during the year 2023-24. In

Table 1. List of twenty-four grape germplasms

Sr. no	Germplasms	Parents	Origin	Breeder
1	Anab- E- Shahi	It was introduced from Middle- East in 1890	India	Identified by Shankar Pillai (1943) horticulturist of erstwhile Hyderabad, India.
2	Pandhari Sahebi	It was introduced into Deccan in 1338 from Afghanistan.	Afghanistan	Not available
3	Clone 2A	Selection from Thompson seedless	USA	Not available
4	Italia	Bicane and Muscat Hamburg	Italy	Not available
5	Muscat of Alexandria	-	Greece	Not available
6	TAS-A-Ganesh	A clonal sel. from Thompson seedless	India	Sel: M/s Arve Bro's, Borgaon, Tasgaon, Sangali Dist. MS
7	Hussain Kadu		Afghanistan	
8	Golden queen	Black Alicante × Ferdinand De Lesseps	UK	Bred by J Pearson (1876) from Notting-ham, UK
9	Red globe	(Hunicia × Emperor) × (Hunicia × Emperor) × Nocera)	California	Bred by H P Olmo (1982),
10	Beauty seedless	Queen of Vineyards × Black Manukka	California	Bred by H P Olmo (1941)
11	Carolina black rose	Aurelia × Black Rose	USA	Not available
12	Arka shyam	Bangalore Blue × Black Champa	India	Bred by Dr S S Negi from IIHR B'luru.
13	Sarita seedless	Selection from kishmish Chernyi	India	Identified by Nanasaheb Kale from Nannaj, Solapur, Maharashtra during 1996-97
14	Nath seedless	Selection from Kishmish Chernyi,	India	Identified by Vithal Nivrutti Thorat of kalamb, Pune
15	EC-552114	Not available	India	Not available
16	Ribier	Not available	France	Not available
17	Gulabi	Not available	India	Not available
18	Foster seedling	Not available	Australia	Not available
19	Kishmish red	Not available	Azerbeijan	Not available
20	Red Nihalson	Muscat of Alexandria X Hybrid selection	Japan	Not available
21	Crimson seedless	Not Available	USA	Not Available
22	Nana Saheb purple	Selection from Kishmish Chernyi	India	Identified by Nanasaheb Kale of Nan-naj, Solapur, Maharashtra during 1998-1999
23	Krishna seedless	A clonal selection from Kishmish Chernyi,	India	Sel: Vittal Mali, Bedag, Sangli Dist. MS.
24	Kishmish chernyi	Not available	Russia	Not available

addition, the catalogues of International Plant Genetic Resources Institute (IPGRI), International Union for the Protection of New Varieties of Plants (UPOV), International organization of Vine and Wine (OIV) was also used for the observations (12). Qualitative characteristics were evaluated using a descriptor list specific to *Vitis* species, involving rating and coding**.** Various quantitative measurements were also taken using laboratory instruments such as a vernier caliper, weighing scale, measuring tape and weighing balance. Sampling involved selecting grape berries from bunches on both sides of the row, with attention given to the top, middle and bottom sections of each bunch.

Statistical analysis

For all the morphological traits, the mean and Standard Deviation (SD) were calculated for each data set. Additionally, coefficients of variation (CV %) were determined to indicate the level of variability. PCA was conducted to determine the correlation between qualitative characteristics by using SPSS statistical software**.**

Results and Discussion

In the present study, twenty-five different morphological parameters studied are presented in Table 2 and showed in Fig. 1, 2 and 3. According to DUS guidelines and OIV, the time of bud burst indicated great variation in observed germplasms. The duration for bud burst varied in most of the germplasms late to medium (7) and fewer in early (2). Opening of shoot tip was observed in three distinct forms ranging from half-open to fully closed. Fully opened young shoot tip was most common among the germplasms studied. Colour of upper side of leaf blade was (11) varieties with yellow colour, while (7) germplasms recorded yellow with bronze spot. Most of the varieties had erect growth habit (22). The highest coefficient of variation among 55 grape varieties was observed (13). As previously reported, a strong correlation between traits

Table 2. Frequency distribution of different morphological characters

Characterization	Frequency and code (No. of germplasms)					
Time of bud brust	Early (2)	Medium (15)	Late (7)			
Young shoot: opening of shoot tip	Closed (1)	Half open (9)	Fully open (14)			
Young leaf: colour of upper side of blade	Green with bronze spot (5)	Yellow (11)	Yellow with bronze spot (7)	Copper (1)		
Shoot attitude: (Growth habit)	Erect (22)	Semi erect (2)				
Mature leaf: width of blade (cm)	Medium (1)	Large (12)	Very large (11)			
Mature leaf: shape of blade	Cordate (2)	Wedge shaped (4)	Pentagonal (9)	Circular (8)	Kidney (1)	
Mature leaf: number of lobes	Single (1)	Three (2)	Five (13)	Seven (7)	More than seven (1)	
Mature leaf: anthocyanin coloration of main vein on lower side of blade	Absent (12)	1st bifurcation (4)	Point (8)			
Mature leaf: shape of teeth	Wedge (7)	Convex (17)				
Mature leaf: degree of opening / overlapping of petiole sinus	Closed (4)	Open (13)	Overlapped (2)	Strongly overlapped (4)	Very wide open (1)	
Mature leaf: prostrate hairs between veins on lower side of blade	Very low (5)	Low (4)	Medium (2)	High (3)	Very high (3)	Absent (7)
Mature leaf: erect hairs between veins on lower side of blade	Very low (4)	Low (0)	Medium (0)	High (0)	Very high (0)	Absent (20)
Mature leaf: ratio of length of petiole compared to mid vein	Short (23)	Equal (1)				
Inflorescence: average number of inflorescences per shoot	<1 (11)	1 to <2 (7)	2 to <3 (5)	3 or more (1)		
Time of full bloom (DAP)	Medium (3)	Late (9)	Very late (12)			
Bunch: berry density/ compactness in table grapes	Loose (4)	Medium (13)	Compact (7)			
Bunch: Shape/type	Globular (6)	Cylindrical (1)	Conical (17)			
Bunch: uniformity of berry size	Non uniform (1)	Uniform (23)				
Berry shape	Oblate (1)	Round (6)	Short elliptical (12)	Long elliptical (4)	Ovate (1)	
Berry: colour of skin (without bloom)	Red (2)	Blue-black (3)	Pinkish Red (1)	Black (10)	Green yellow (8)	
Berry: anthocyanin coloration of mesocarp	Absent (24)	Present (0)				
Berry: flavor	Neutral (13)	Muscat (5)	Foxy (3)	Others (2)		
Berry: length of pedicel	Very short (10)	Short (13)	Medium (1)			
Berry attachment with pedicle	Loose (9)	Firm (15)				

Fig. 1. The pictures of bunches of studied grape germplasms.

Fig. 2. The pictures of berry of studied grape germplasms.

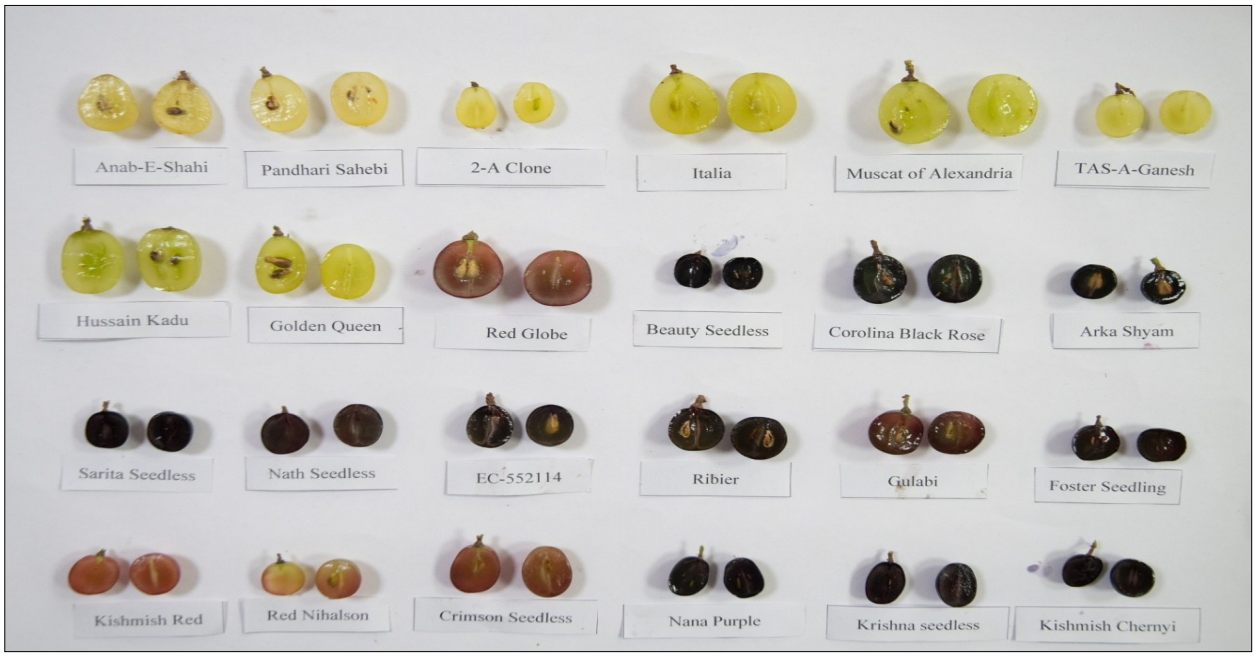

Fig. 3. The pictures of cut berries of studied grape germplasms.

can be advantageous, as selecting for one desirable trait may also promote the presence of another desirable trait within the germplasm (14).

Other useful tools for grapevine identification are mature leaf characteristics. In the present investigation, 12 varieties recorded largest width of blade, while only 1 germplasm recorded medium width of leaf blade. The most common leaf shape recorded was pentagonal (9), followed by circular (8), wedge (4), cordate (2) and kidney (1). In most of the germplasms, five lobes were observed in the mature leaf. Anthocyanin coloration on the main vein on the lower of the leaf blade was absent in 12 germplasms while in remaining germplasms, anthocyanin coloration was present at the point (8) and at the first bifurcation (4) respectively. The teeth shape of the mature leaf was primarily a mix of both sides being straight and both sides being convex. In most germplasms, the overlapping of the petiole sinus was open, although some germplasms exhibited a closed petiole sinus. In most germplasms, prostrate hairs (7) and erect hairs (20) were absent. In this comprehensive study of descriptors, specific characteristics such as the density of prostrate hairs on the main veins, the density of erect hairs on the main veins, played their expected role in identifying the grape genotypes. In mature leaf, ratio of length of petiole compared to mid vein was ranging from short to equal, however only one germplasm recorded equal ratio of length of petiole compared to mid vein. (15) studied 16 characteristics from OIV descriptor to identify ten grapes' genotypes. The mature leaf characteristics offer discriminative information was reported in previous works (16). Less than one inflorescences were recorded in each shoot. There were maximum germplasms occurred in the time required for full blooming, while 12 germplasms took maximum days for blooming. The bunch: berry density compactness in table grapes in most of the genotypes was medium (13) followed by compact (7) and loose (4). Different types of bunch shape were observed including conical (17), globular (6) and cylindrical (1). Most of the germplasms had uniform sized berries in a bunch. Berry shapes were predominantly short elliptical (12), round (6), long elliptical (4) while oblate and cylindrical shapes were observed in only one germplasm each. Different berry shapes and a wide range of berry skin colours were document in earlier works (17). Eight germplasms recorded green, yellow colour of skin followed by black (10) and red (3). Anthocyanin coloration of the berry mesocarp was absent in all studied germplasms. Out of the 24 germplasms studied, 13 germplasms had neutral berry flavour, while muscat (5) and foxy (3) flavour also occurred. Short pedicel length was observed in 13 germplasms which were closely followed by very short (10). In most of the germplasms, 15 berries were firmly attached. Morphological characterization offers superior results because it enables the determination of a greater number of characteristics compared to other methods of characterization (18). The works on fifty-five grape germplasms and reported greater variability and suggested to integrate the present data into future studies and investigating the genetic diversity of grapes from other regions (19).

Out of the twenty-five different morphological parameters, Berry anthocyanin coloration of mesocarp received the highest CV (122.47 %), followed by berry flavour (92.78 %) while the lowest CV was recorded for the time of full bloom (7.15 %), followed by uniformity of berry size (11.95 %) and berry attachment with pedicle (12.76 %), 20 morphological characteristics for varietal identification were studied (20). A maximum coefficient of variance was reported in 31 grape accessions and 55 grape cultivars, respectively (13). (Table 3).

Principal Component Analysis (PCA)

PCA was performed for qualitative characterization of different grapes germplasms. Nine components were expressed with 100 % of the total variance during the PCA analysis (Table 4) PC1 accounted for 28.13 % of the total variance and has

Table 3. Morphological characterization of grape germplasm

Sr. No	Characters	Unit	Min.	Max	Mean	SD	CV %
1	Time of bud brust	Code	8	14	10.45	1.77	31.83
2	Young shoot: opening of shoot tip	Code	1	9	7.60	2.33	16.91
3	Young leaf: colour of upper side of blade	Code	2	6	3.21	0.93	29.03
4	Shoot Attitude: (Growth habit)	Code	1	3	1.17	0.56	48.40
5	Mature leaf: width of blade (cm)	Code	5	9	7.83	1.17	14.90
6	Mature leaf: shape of blade	Code	1	5	3.08	1.01	33.01
7	Mature leaf: number of lobes	Code	1	11	5.33	1.78	33.48
8	Mature leaf: anthocyanin coloration of main vein on lower side of blade	Code	1	9	3.66	3.04	83.06
9	Mature leaf: shape of teeth	Code	3	5	3.58	0.92	25.91
10	Mature leaf: degree of opening / overlapping of petiole sinus	Code	1	9	4.58	2.43	53.02
11	Mature leaf: prostrate hairs between veins on lower side of blade	Code	1	9	3.43	3.07	89.45
12	Mature leaf: erect hairs between veins on lower side of blade	Code	1	3	1.33	0.76	57.10
13	Mature leaf: ratio of length of petiole compared to mid vein	Code	1	5	1.17	0.83	71.05
14	Inflorescence: average number of inflorescences per shoot	Code	1	7	2.42	1.82	75.14
15	Time of full bloom (DAP)	Number	32	45	41.17	2.94	7.15
16	Duration of flowering	Number	3	10	5.75	1.80	31.30
17	Bunch: berry density/ compactness in table grapes	Code	1	7	4.92	2.00	40.64
18	Bunch: Shape/type	Code	1	3	2.46	0.88	35.94
19	Bunch: uniformity of berry size	Code	3	7	6.83	0.82	11.95
20	Berry shape	Code	1	6	2.96	1.02	34.55
21	Berry: colour of skin (without bloom)	Code	1	7	3.96	2.31	58.28
22	Berry: anthocyanin coloration of mesocarp	Code	1	9	1.35	1.67	122.47
23	Berry: flavor	Code	1	9	2.65	2.46	92.78
24	Berry: length of pedicel	Code	1	5	2.25	1.15	51.18
25	Berry attachment with pedicle	Code	7	9	7.75	0.99	12.76

Table 4. PCA of morphological characteristics of 24 grapes germplasms

Variables	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
Average bunch weight (g)	0.196	-0.547	0.031	-0.263	0.17	-0.526	0.341	0.222	0.348
50 berry weight (g)	-0.398	0.022	0.361	0.28	0.531	0.08	0.438	-0.375	0.11
No of berries per bunch	0.447	0.235	-0.127	-0.274	0.461	-0.186	0.133	-0.193	-0.593
Bunch width (mm)	-0.089	0.071	-0.601	0.653	-0.007	-0.33	0.229	0.159	-0.115
Bunch peduncle length (mm)	-0.253	0.048	-0.649	-0.458	-0.01	0.16	0.152	-0.412	0.29
Berry diameter (mm)	-0.521	0.015	-0.046	-0.317	0.137	0.172	0.208	0.646	-0.341
Berry length (mm)	-0.483	-0.213	0.008	-0.069	0.115	-0.509	-0.582	-0.236	-0.222
TSS (°Brix)	0.165	-0.464	-0.261	0.182	0.535	0.465	-0.367	0.127	0.059
Titrable acidity (%)	0.018	0.614	-0.01	-0.058	0.399	-0.205	-0.286	0.296	0.499
Eigen Value	2.532	1.924	1.244	1.016	0.79	0.496	0.438	0.313	0.247
Percentage of variance	28.13	21.372	13.827	11.286	8.782	5.513	4.868	3.478	2.743
Cumulative percentage	28.13	49.502	63.329	74.616	83.398	88.91	93.779	97.257	100

positively correlated with number of berries per bunch (0.447) and negative loadings from berry diameter (-0.521) and berry length (-0.483). This suggests that PC1 represents a contrast between the number of berries per bunch and the size of individual berries. PC2 explains 21.37 % of the variance with strong positive loadings from titrable acidity (0.614) and negative loadings from average bunch weight (-0.547). PC2 appears to represent the acidity and the weight of bunches. PC3 accounts for 13.82 % of the variance, with significant negative loadings from bunch width (-0.601) and bunch peduncle length (-0.649). This suggests PC3 captures the size and length characteristics of the bunch. PC4 explains 11.28 % of the variance, primarily influenced by bunch width (0.653) and negative contributions from bunch peduncle length (-0.458), representing opposing characteristics in bunch structure. The morphological characters, such as berry length, are highly correlated with changes in genetic characters were reported (21). This finding highlights the connection between physical traits and underlying genetic variations, suggesting that observable characteristics can be strong indicators of genetic differences or similarities. These characters are also important in determining the breeding requirements for grape species (22). The bunch and berry characteristics of grapes play an important role in determining quality, particularly in table grapes (23).

Conclusion

This study focused on the morphological characterization and evaluation of genetic diversity among 24 grape germplasms. In the present study, significant differences were observed between different grapes germplasms. The morphological characterization and PCA results highlighted the distinct qualitative characteristics that differentiate grape germplasms. These findings offer a comprehensive understanding of the morphological diversity within the studied grape germplasms, facilitating better identification and classification. The study emphasizes the importance of preserving and maintaining valuable germplasm for future research and breeding programs.

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Authors' contributions

PSG conducting the research, data acquisition, data analysis, statistical analysis and final manuscript drafting. RGS performed the conceptualization, experimental design and manuscript editing. PKA done the data analysis and manuscript drafting. NG done the data acquisition and statistical analysis assistance. PBK helps with experiment execution and data collection. PSK done the data collection. All authors read and accepted the manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare no competing financial interests or personal relationships that could influence the work reported in this paper.

Ethical issues: None

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