

Ampelographic diversity assessment of local grape varieties grown in under Indian condition

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Abstract

Grapevine is well adapted to the agro climatic conditions of India, which makes it one of the major element of the Indian horticulture. However, there has been limited focus on assessing grapevine germplasm, and genetic resources have previously remained underutilized despite their potential for adapting to environmental changes. This study aims to evaluate the diversity of local (developed in India) grapevine accessions growing in different regions in India. A total of 20 local varieties were evaluated using 14 fruit traits previously developed by DUS. An important variability was revealed among the local varieties studied based on fruit characteristics. The most discriminant traits were bunch weight, berry diameter, bunch length, berry weight, berry shape, seed number per berry. Hierarchical clustering analysis showed three main clusters, each regrouping accessions of different named varieties. Although preliminary, our results indicate a potential of genetic diversity within the local varieties that should be further investigated in order to understand their performance and to evaluate them in selection programs.

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Keyword: Ampelography, *Vitis vinifera*, Local genotypes, diversity,

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Introduction

Grape (*Vitis vinifera* L.) is a significant horticultural crop, valued globally for their economic importance in various regions and their historical association with the development of human culture since ancient times (Somkuwar et al., 2024). India is globally recognized for its diverse regions suitable for viticulture due to different climatic conditions, a wide range of grape varieties, and diverse cultivation techniques. Our country has a history of viticulture of about 6000 years and there is rich grape biodiversity as a wild vine (*Vitis vinifera* L. subsp. *sylvestris*) and cultivated vine (*Vitis vinifera* L. subsp. *sativa*) (Samarth, 2016). In India, grape is grown on an area of about 1.62 lakh hectare with annual production of 3489.40 tons (Anonymous, 2023). Approximately 78% of the total production used for fresh consumption, around 17-20% dried for raisin production, and the remaining 2% processed into juice and wine (Somkuwar et al., 2020).

Comment [KM4]: This part seems poor. There are many unneeded information here, while important ones are not included. Economic value of grape? Ingredients? Health effects? Briefly why grape is important? What are the problems of grape cultivation and how these local genotypes could be beneficial?

I did not find the presentation of similar studies existing in scientific literature. Authors need to clarify what their study brings in novelty, compared to these similar studies found in scientific literature. It is very important to state what exactly you bring in novelty in order to express your originality.

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Comment [KM6]: Talk about grape instead.

Local grape cultivars vary in their morphological traits, cluster and berry sizes, phenology, harvest times, productivity, and quality measures (Ates et al., 2011; Eyduran et al., 2015; Sabir et al., 2010). Evaluating grape diversity is crucial for characterizing and conserving germplasm, which is essential for maintaining and enhancing crop productivity. Identifying local varieties accurately and conserving them can prevent their extinction and safeguard them for future requirements, enabling genetic, ecological, and agronomic diversity that can handle different diseases, enhance adaptation to local soil and climate conditions, and facilitate adjustments to future market shifts (Sancho-Galan et al., 2020). Traditionally, grape variety identification has relied on observing the morphological characteristics of both plant growth and reproductive parts. Morphological characteristics of fruits have traditionally served as a valuable tool for characterizing fruit trees due to their economic significance and ability to differentiate varieties (Chessa and Niedu, 2005). Local grape cultivars play a crucial role in maintaining agricultural diversity and are vital for ensuring food security, nutrition, and economic stability, especially for small-scale farmers and rural communities in marginal areas. The variation in local grapes helps mitigate risks of crop failure, provides unique ingredients for traditional local dishes, and meets specific dietary needs. Additionally, these diverse grape cultivars offer valuable locally adapted genetic resources for enhancing new grape varieties (Pallas et al., 2016; Sargolzaei et al., 2021). The main objective of this research work is focused on morphological identification of a 20 local varieties released from different research institute. In India different grapes are grown, a multitude of local varieties contribute to the global diversity of grapevines. These varieties exhibit significant variation within each type, highlighting the importance of accurately defining and identifying them in both scientific research and practical applications in modern viticulture.

Comment [KM7]: In these references, researches used varieties not local genotypes. First look into the articles then use them as a reference in suitable place. Apply it to whole article, I will check in the second round.!

Material and methods

Present investigation was carried at National Active Germplasm Site of ICAR-National Research Centre for Grapes, (latitude 18°32'N and longitude 73°51'E), Pune during 2023. Eight-year-old local varieties grafted on Dogridge rootstocks were specifically selected for this research (Table 1 and Fig 1). The vines were planted with a distance of 3.0 m between the rows and 1.83 m between the vines, thus accommodating 1800 vines per hectare. Due to the tropical conditions of the region, single cropping and double pruning approach is being adopted. As a result, the vines were pruned two times in a year - once after the crop harvest (known as back pruning) and a second time for fruit production (known as forward pruning). Fourteen fruit

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Pomological observations...
Chemical observations...
Statistical analysis... etc.

character were evaluated to study pomological variability of the local cultivars on the basis of **DUS** guideline. The fruit bunches were harvested when they reached full ripeness, which was determined by measuring total soluble solids, titratable acidity, as well as assessing taste and color. Bunch and berry parameter (length and width) were measured with digital caliper. Weight of cluster, berry and seed were measured by weighing balance. The grape juice was extracted and **the**

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(FIGURES WERE HERE)

total soluble solid was recorded using a refractometer (**Erma, Japan**) while the acidity was measured using 0.1 N NaOH by titration ~~method~~. The pH values were measured by pH meter.

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The standardized dataset was then used to determine the genetic diversity and relationships between cultivars. Analysis of variance (ANOVA) was performed for all ampelographic traits by SAS software (SAS Institute 1990) using one-way ANOVA. Mean and standard deviation (SD) were calculated for each data set. Also, coefficients of variation (CV %) were determined as indicators of variability. Relationships between cultivars were investigated by multivariate ANOVA (PCA) using SPSS statistics software. The distance matrix created from ampelographic data was utilized in cluster analysis to examine variations among cultivars, employing the unweighted pair-group method of arithmetic average (UPGMA).

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And did you standardize the data? How? Why?

Table 1. Information about the local grape varieties developed by different research institute.

Varieties	Parentage	Released by	Year
Arka Chitra	Angur Kalan x Anab-e-Shahi	IIHR, Bengaluru	1994
Arka soma	Anab-e-Shahi x Queen of Vineyards	IIHR, Bengaluru	1994
Arkavati	Black Champa x Thompson seedless	IIHR, Bengaluru	1980
Arka Krishna	Black Champa x Thompson Seedless	IIHR, Bengaluru	1994
Arka Neelmani	Black Champa x Thompson seedless	IIHR, Bengaluru	1992
Arka Hans	Bangalore Blue x Anab-e-Shahi	IIHR, Bengaluru	1980
Arka Kanchan	Anab-e-Shahi x Queen of Vineyards	IIHR, Bengaluru	1980
Arka Shweta	Anab-e-Shahi x Thompson Seedless	IIHR, Bengaluru	1994
Arka Majestic	Angur Kalan x Black Champa	IIHR, Bengaluru	1994

Arka Trishna	Bangalore Blue x Convent Large Black	IIHR, Bengaluru	1994
Arka Shyam	Bangalore Blue x Black Champa	IIHR, Bengaluru	1980
Pusa Seedless	Clonal selection from Thompson Seedless	IARI, New Delhi	1970
Pusa Swarnika	Hur x Cardinal	IARI, New Delhi	2014
Pusa Aditi	BanquiAbyad x Perlette	IARI, New Delhi	2014
Pusa Navrang	Madeleine Angevine x Rubi Red	IARI, New Delhi	1997
Manjari Medika	Pusa Navrang X Flame Seedless	NRCG, Pune	2018
Manjari Naveen	Clonal selection from Centennial Seedless	NRCG, Pune	2008
Manjari Kishmish	Mutant derived from KishmishRozavis	NRCG, Pune	2019
Manjari Shyama	Black Champa x Thompson Seedless	NRCG, Pune	2019
H-516	Catawba x Beauty Seedless	ARI, Pune	2008

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Result and discussion

The fruit characteristics among the studied local varieties showed significant variability. Table 2 provides some descriptive statistics namely: the mean, standard deviation (SD), coefficient of variation (CV), minimum and, maximum values, and coefficient of variation (CV) for these fruit traits. Average bunch weight ranged from 129.90g (Arkavati) to 270.50g (ManjariMedika), with a coefficient of variance (CV) of 38.97%. Cluster length ranged from 10.40cm (Manjari Naveen) to 28.30cm (Pusa Aditi). The CV for the cluster length was calculated as 25.47%. Berry length varied from 13.0mm (PusaNavrang) to 21.0mm for Arka Soma. Additionally, some varieties like Pusa Seedless, Arkavati, ArkaNeelmani, Manjari Naveen, Manjari Kishmish were seedless, while other contained one to four seeds per berry. Somkuwar et al. (2023) similarly observed one to four seeds per berry in Indian grape while Vafae et al. (2017) noted that seed number per berry ranged from zero to three. The coefficient of variations (CV) for the juice characteristics in the berries of the studied vines were 5.35% for pH, 7.77% for TSS (Total Soluble Solids), and 15.78% for TA (Titratable Acidity). TSS levels ranged from 16.50% to 22.60%, with a mean of 20.07%. The pH values ranged between 3.08 and 3.67, averaging 3.36. Titratable Acidity varied from 0.41% to 0.78%, with a mean of 0.57%.

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Table 2. Descriptive statistics for measured fruit characteristics between the local grape varieties.

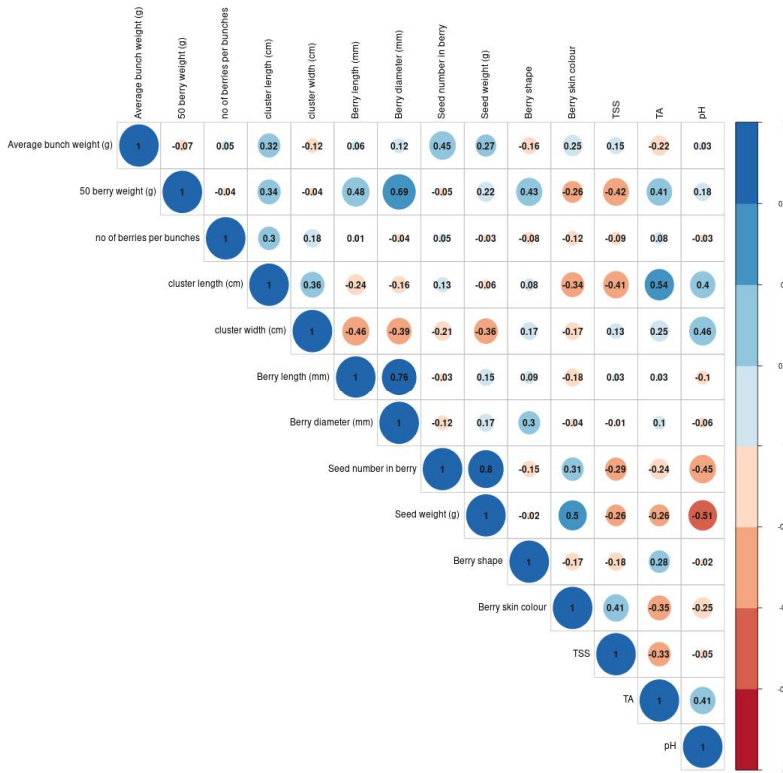
Characteristics	Minimum	Maximum	Mean	SD	CV (%)
Average bunch weight (g)	129.90	270.50	138.39	61.04	44.10
50 berry weight (g)	61.00	201.00	138.08	59.39	43.01
No of berries per bunches	40.00	125.30	76.56	24.47	31.96
Cluster length (cm)	10.40	28.30	15.27	3.89	25.47
Cluster width (cm)	3.50	13.50	8.88	2.55	28.71
Berry length (mm)	13.00	21.00	16.11	2.19	13.59
Berry diameter (mm)	13.20	20.30	15.97	1.89	11.83
Seed number in berry	0.00	4.00	1.60	1.46	91.25
Seed weight (g)	0.00	9.78	4.11	3.36	81.75
Berry shape	1.00	4.00	2.45	0.82	33.46
Berry skin colour	1.00	6.00	3.30	2.51	76.06
TSS (°Brix)	16.50	22.60	20.07	1.56	7.77
TA (%)	0.41	0.78	0.57	0.09	15.78
pH	3.08	3.67	3.36	0.18	5.35

Comment [KM19]: Which characteristics were found statistically significant? Make multiple comparison test and add into this article just as a * or ns (non-significant) and only talk about significant ones. There are many characteristics here but most of them ignored by you. Why? We have to understand this as a reviewer or reader...

Correlation analysis was conducted to examine the relationships among the traits (Figure 2). Bunch weight showed positive correlation with cluster length ($r = 0.32$) and seed number in berry ($r = 0.45$). Bunch length exhibited positive correlations with bunch width ($r = 0.85$). Additionally, larger berries generally occur on larger bunches. Berry length was positively correlated with berry width ($r = 0.76$), consistent with previous research (Leao et al., 2011; Khadivi-Khub et al., 2014; Vafaei et al., 2017; Migicovsky et al., 2017), suggesting that berries larger in one dimension tend to be larger in the other. Berry diameter showed negative bunch length and width. These insights could be valuable for future plant breeding program, such as conducting crosses between cultivars with large berry size and low seed count to achieve high yield and superior quality cultivars. Seed number per berry was negatively correlated with berry length and berry width, while Khadivi-Khub et al., (2014) reported positive correlation between seed number per berry was positively with berry length and berry width. A negative correlation was found between TSS and TA ($r = -0.33$). Our results are in accordance with the result of Leao et al. (2011) who noted a negative correlation between TSS and TA in grape. The correlation coefficient can offer information on the traits that are most significant in evaluating genotypes (Norman et al. 2011). This information could be very useful for future plant breeding programs.

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For example, to achieve high yields and superior quality cultivars, breeders might cross cultivars with large berry sizes, high total

soluble solids (TSS), and low seed numbers.

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Fig 2. Correlation between different bunch and berry parameters in studied local varieties.

PCA using the correlation matrix was conducted to assess fruit differentiation among cultivars and to determine if the data reduction achieved through the new set of variables (principal components) uncovered a variation pattern that aligns with grouping, especially when the major components of overall variance are due to differences between groups. For each factor, a PC loading of more than 0.52 was considered as being significant, indicating five components and explaining 77.18% of the total variance (Table 3). The first three PCs explained 59.62% of the variance (23.84, 20.31 and 15.47%, respectively), showing that these attributes exhibit the greatest variation among the cultivars and have the most significant effect on differentiating them. The highest loading on the PC1 (Khadivi-Khub et al.2014). The highest loadings on the PC1 axis were associated with attributes such as cluster length and cluster width. The variables with the highest loadings on the second principal component (PC2) axis included berry diameter and berry weight. Meanwhile, the highest loadings on the third component (PC3) were cluster length and seed number per berry. The remaining components accounted for less variability. Variables that had a strong correlation with PC1 can be regarded as respective of berry size. While berry traits are crucial for distinguishing and analyzing breeding materials in the morphological characterization of grapes (Leao et al. 2011). Correlations between traits identified through PCA might indicate either a genetic linkage between the loci controlling these traits or a pleiotropic effect.

Table 3. Eigenvalues and proportion of total variability and eigenvectors of five principal components (PCs) for studied local grape cultivars.

Traits	PC1	PC2	PC3	PC4	PC5
Average bunch weight	-0.322	0.071	0.424	0.731**	-0.074
Berry weight (g)	0.37	0.831**	0.055	0.043	-0.194
No of berries	0.149	-0.034	0.319	0.177	0.581**
Cluster length	0.559**	0.083	0.719**	0.246	0.015
Cluster width	0.557**	-0.46	0.205	0.091	-0.31
Berry length	-0.104	0.739	-0.4	0.261	0.273
Berry diameter	-0.027	0.832**	-0.368	0.296	-0.038

Comment [KM21]: Structure fail.

Comment [KM22]: Correlation part is the best part of your article. But needs improvement still. You should talk about the possible physiological aspect of relations.

You might look articles below for improvement of correlation and PCA analysis.

Mertoğlu, K., Durul, M. S., Korkmaz, N., Polat, M., Bulduk, I., & Esatbeyoglu, T. (2024). Screening and classification of rosehip (*Rosa canina* L.) genotypes based on horticultural characteristics. *BMC Plant Biology*, 24(1), 345.

Akkurt, E., Mertoğlu, K., Evrenosoğlu, Y., & Alpu, Ö. (2024). Pollinizer potentials of reciprocally crossed summer apple varieties by using ANOVA and resampling based MANOVA. *Applied Fruit Science*, 66(1), 25-34.

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Seed number per berry	-0.593	0.19	0.698**	-0.073	-0.025
Seed weight	-0.667	0.437	0.467	-0.128	-0.183
Berry shape	0.334	0.402	-0.08	-0.266	-0.511
Berry skin	-0.67	-0.176	0.034	0.189	-0.46
TSS	-0.275	-0.434	-0.534	0.491	-0.186
TA	0.724	0.242	0.173	-0.036	-0.095
pH	0.696	-0.163	0.004	0.414	-0.149
Eigenvalue	3.339	2.844	2.167	1.33	1.125
Percent of variance	23.849	20.312	15.476	9.522	8.039
Cumulative %	23.849	44.161	59.476	69.159	77.198

****Eigenvalues significant ≥ 0.52**

A multivariate statistical analysis of the data was performed using PCA. Figure 2 was plotted according to the correlation between Local cultivars and morphological parameters. PC1 accounted for 23.8% of the total variance (44.16%), and PC2 accounted for 20.31%. The position of each variable in the loading plot describes its relationship with the other variables. Variables that are close to each other have high correlations. Variables on the same side of the origin (0.0) are positively correlated and those on the opposite side of the origin are negatively correlated. Local grape varieties could be discriminated on the PCA plane. PC1 was positively related to berry weight, berry shape, cluster length and titratable acidity. PC2 was related to average bunch weight, seed weight, berry diameter and berry length. Arka majestic, Arka Chitra, Arka Kanchan, Manjari Naveen, Pusa Aditi, Pusa Seedless, Arkavati and Arka Shweta were located on positive side of PC1. On the other hand, Arka Soma, Arka Hans, Arka Krishna, Manjari Medika, Pusa Navrang, Arka Neelmani were located on negative side of PC2.

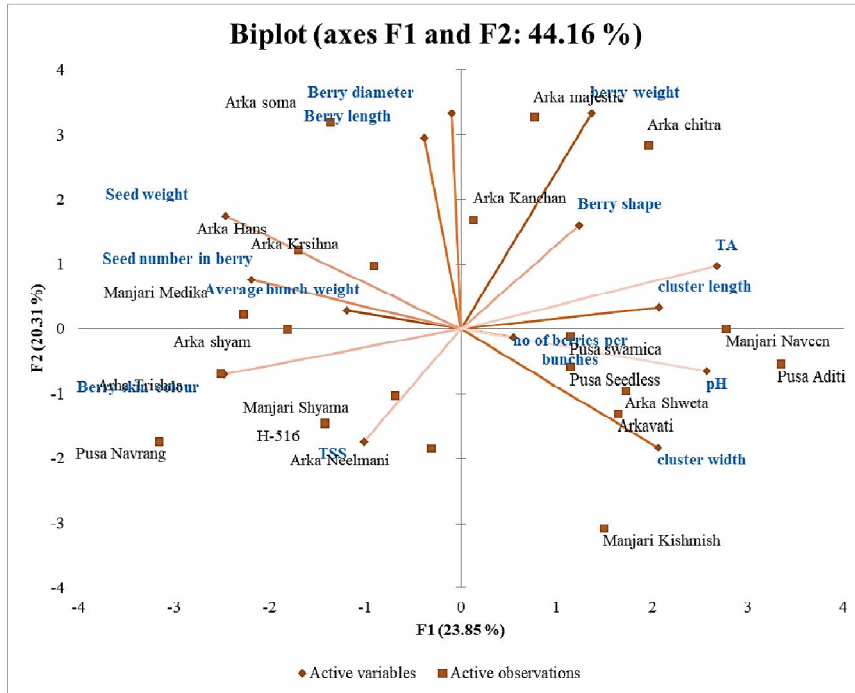


Figure 3. Biplot of the first two principal components (44.16 % of the total variability) based on fruit parameters in local grape varieties.

Cluster analysis

Based on their phenotypic data, Euclidean distances applied through the UPGMA method, served as the metric for cluster analysis to assess the dissimilarity and similarity among the studied local cultivars (Fig.4). The dendrogram revealed three clusters. The first cluster (I) contained 4 cultivars, second cluster (II) contained 15 cultivars, while the third cluster (I) included only on cultivar. The obtained data revealed the morphological diversity within the studied local cultivars. High dissimilarity level among the studied cultivars showed high fruit variability in the germplasm. Natural hybridization and human selection may have contributed to this variation (Sefc et al., 2003).

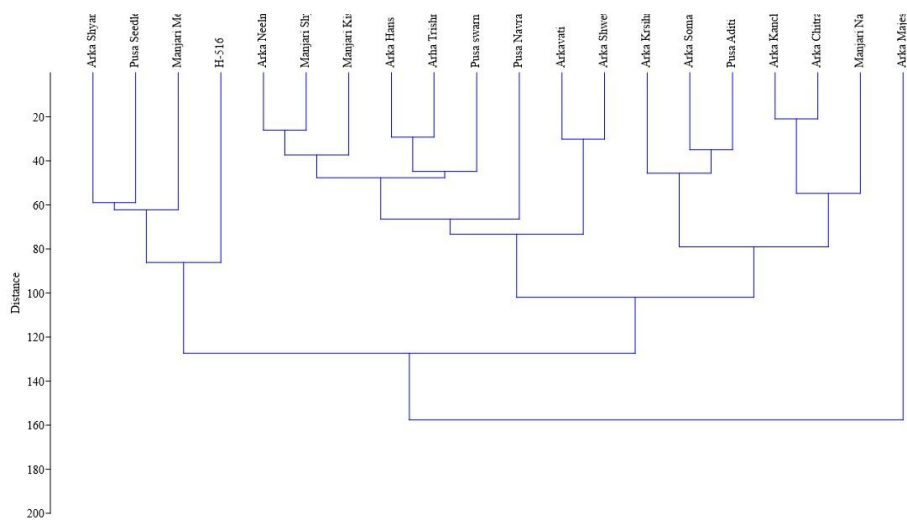


Fig 4. UPGMA cluster analysis of the studied local grape cultivars based on morphological traits using Euclidean distance.

Conclusion

Our findings show a significant morphological variability within the local grape variety. In future research on the morpho-agronomic assessment of grapes, the traits of fruits, particularly those related to berry characteristics, could be taken into account. The high morphological variability of Indian local grape cultivars might be considered as characterizing the large gene pool that contributed to the domestication process of grape. This morphological characterization should be further completed with molecular analysis through DNA markers. Genetic analysis of these resources will help establish the genetic distances between accessions, enabling the selection of diversity to preserve, distinguishing local cultivars from international germplasm, and informing their application in future breeding programs.

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