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Assessment of Genetic Diversity by Using Multivariate Analysis for Morphological Qualitative Traits of Bullock's Heart (*Annona reticulata* L.) Genotypes in the Konkan Plains of Western India

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Abstract

An experiment was carried out to assess diversity of bullock's heart (*Annona reticulata* L.) in Ratnagiri and Sindhudurg districts of Maharashtra. Sampling of home gardens was done using multistage sampling method and samples of 100 elite genotypes were collected from different tehsils of surveyed districts. A total of seventy-eight morphological characters were measured and analyzed in the study. Among them, five morphological characters did not show any variance in the studied genotypes and hence, were removed from the analysis. Eleven morphological qualitative traits were identified and assessed for multivariate analysis. Results demonstrated the presence of substantial variability among the evaluated genotypes for morphological traits. The traits appointed to five principal components of qualitative traits explained 62.15% of total variability. Factor analysis captured more variation within the genotypes as compared to other and five factors from 11 traits explained 150.85% of total variability. Variations among genotypes in fruit shape showed significant positive correlation with fruit symmetry, and fruit symmetry with uniformity in fruit size and colour. Eleven clusters were formed and indicated that the genotypes selected from cluster XI represented highest cluster mean for most of the traits like uniformity in fruit size, fruit symmetry, fruit shape and ripe fruit colour and the same was confirmed by principal component analysis. The genotypes DP-5, VL-22, KL-7, KL-9, SW-12, DP-16, DP-4, VL-7, SW-20 and VL-16 showed potentially good characteristics for international markets. Result suggested that the factors other than geographical separation are responsible for divergence, and genotypes originated from same place may have different genetic architecture or vice-versa.

Key words: *Annonaceae, correlation, diversity, morphological traits, PCA*

Introduction

Bullock's heart (*Annona reticulata* L.) belongs to the family Annonaceae and is a tropical and subtropical fruit tree, widely distributed in Asia, Africa and the America (Nakasone and Paul, 1998). There are about 119 species in the family Annonaceae, of which only six are of commercial importance (Popenoe, 1974). *A. reticulata*, a diploid species with chromosome number 2n=2x=14 and 16 (Darlington and Wylie, 1956), is native of tropical America but its exact native range is unknown and is thought to be in Caribbean from where it has distributed to Mexico and tropical America (Popenoe, 1974). The species varies widely in fruit quality, flavor, habitat, and insect susceptibility (Safford, 1914). In India, it is extensively grown next to custard-apple under diverse conditions from the plains up to 1200 m elevation. It also runs almost wild as an escape, especially in the vicinity of old forts, temples, chapels, villages, etc. throughout the moist as well as drier hot parts of the peninsular, central, western, north-eastern, eastern regions and southern India (Saraswat et al., 2006). It has become intensively naturalized to the extent as to have often appeared being indigenous to India. In some parts of West Bengal, it is planted for utilization of waste land, particularly in heavy soils. In Maharashtra, it is cultivated in Pune, Ahmednagar, Aurangabad, Beed, Dhule and in Nagpur districts, whereas, considerable production is also found in forests and wastelands. The fruits are mainly used for fresh consumption, generally being considered as a 'Fruit of Poor People'. Bullock's heart, which is deciduous in nature, synchronizes the flowering and fruiting during periods of water availability.

In Konkan region of Maharashtra, bullock's heart is commonly grown as a crop of kitchen garden and is found to be regular bearing and does not require any special horticultural practice. The region has potential areas for large-scale bullock's heart cultivation in the future, provided that market outlets are clearly defined (Ghavale et al., 2016). As existing plantation of bullock's heart trees in the Konkan region are of seedling origin and being a cross pollinated crop, large variation in morphological parameters is observed. However, no efforts have been made in selection of superior genotypes of bullock's heart grown in the Konkan region of Maharashtra. By taking into consideration the future importance of this crop under changing agro-climatic conditions, the survey and characterization of bullock's heart genotypes grown under Konkan agro-climatic conditions was made to assess the variability using morphological qualitative traits.

Material and methods

The present investigation was carried out at Department of Horticulture, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Maharashtra, India during 2012 to 2013 to study the diversity of bullock's heart in Ratnagiri and Sindhudurg districts. During survey, samples from 100 genotypes were collected from different tehsils of Ratnagiri and Sindhudurg using multistage sampling method for morphological characterization (Table 1). In order to increase the precision of sampling, a large number of clusters were used according to the suggestions given by Thattil and Samita (2007). Scion sticks of all the studied trees were given to the nursery of Horticulture department for further grafting and conservation. Since a descriptor list for *A. reticulata* is not available, the descriptor list of *A. cherimola* compiled by the IPGRI, 2008 was used in this study. A total of seventy-eight morphological characters were measured and used to analyze this study. Among these, 5 morphological characters did not show any variance in 100 genotypes and accordingly removed from the analysis (Table 2). From each tree ten fully expanded and healthy leaves, ten flowers from four directions and two well-developed fruits were randomly selected for measurements of characters. Eleven morphological qualitative traits were identified and assessed for multivariate analysis (Table 2). Munsell Color System chart published by the *Azalea Society of America* (Anon., 1999) was used to record the parameters such as trunk colour, colour of young branches, leaf colour, flower colour, exocarp color, pulp colour and seed colour.

Table 1. List of experimental material used for multivariate analysis in bullock's heart

Genotypes	Number of collections	Place of collection
SW-1, SW-2, SW-3, SW-4, SW-5, SW-6, SW-7, SW-8, SW-9, SW-10, SW-11, SW-12, SW-13, SW-14, SW-15, SW-16, SW-17, SW-18, SW-19, SW-20, SW-21, SW-22, SW-23, SW-24	24	Sawantwadi, Sindhudurg
VL-1, VL-2, VL-3, VL-4, VL-5, VL-6, VL-7, VL-8, VL-9, VL-10, VL-11, VL-12, VL-13, VL-14, VL-15, VL-16, VL-17, VL-18, VL-19, VL-20, VL-21, VL-22, VL-23, VL-24, VL-25	25	Vengurla, Sindhudurg
KL-1, KL-2, KL-3, KL-4, KL-5, KL-6, KL-7, KL-8, KL-9, KL-10	10	Kudal, Sindhudurg
MN-1, MN-2	2	Malwan, Sindhudurg
KN-1, KN-2	2	Kankawali, Sindhudurg
DV-1, DV-2, DV-3, DV-4	4	Devgad, Sindhudurg
VB-1, VB-2, VB-3, VB-4, VB-5	5	Vaibhavwadi, Sindhudurg
DP-1, DP-2, DP-3, DP-4, DP-5, DP-6, DP-7, DP-8, DP-9, DP-10, DP-11, DP-12, DP-13, DP-14, DP-15, DP-16, DP-17, DP-18	18	Dapoli, Ratnagiri
KD-1, KD-2	2	Khed, Ratnagiri
CN-1, CN-2, CN-3, CN-4, CN-5, CN-6, CN-7	7	Chiplun, Ratnagiri
GH-1	1	Guhagar, Ratnagiri

Table 2. Variation of qualitative characters measured and used in the analysis

Qualitative parameters	Observed Variation
*Trunk colour	1. Light grey (43%), 2. Dark grey (40%), 3. Pale grey (17%).
Tree crown shape	1. Ellipsoid (11%), 2. Spheroid (9%), 3. Oblong (42%), 4. Irregular (38%).
Tree growth habit	1. Erect (64%), 2. Spreading (8%), 3. Semi-spreading (28%) and 4. Drooping (0%).
*Colour of young branches	1. Light green (37%), 2. Dark green (24%), 3. Moderate green (18%), 4. Pale green (21%).
*Defoliation at the end of fructification phase	0. Absent (0%), 1. Partial (82%). 3. Complete (18%).
Leaf blade shape	1. Ovate (15%), 2. Elliptic (19%), 3. Obovate (0%), 4. Lanceolate (66%).
*Shape of leaf base	1. Acute (87%), 2. Rounded (13%), 3. Obtuse (0%) and 4. Cordate (0%).
*Shape of leaf apex	1. Acute (85%), 2. Rounded (0%) and 3. Acuminate (15%).
*Colour of mature leaves	1. Light green (23 %), 2. Brilliant green/Green (23 %), 3. Greyish green (0%) and 4. Dark green (54 %).
*Leaf margin	1. Entire (100%) or 2. Undulated (0%).
*Leaf blade venation	3. Submerged (0%), 5. Intermediate (100%) or 7. Raised (0%).
Petal outer colour	1. Yellowish green (55.0 %), 2. Light yellowish green (30.0 %), 3. Deep yellowish green (15.0 %) and 99. Other (0%).
*Colour of the internal petal base	1. Pink (63%), 2. Light reddish purple (11%), 3. Dark red (26%) and 4. Deep reddish purple (0%).
Location of fructification	1. Base of the crown (10%), 2. Middle of the crown (71 %) and 3. Top of the crown (19%).
Fruit shape	1. Round (10%) 2. Oblate (6%) 3. Cordate/Heart (60%) 4. Broadly cordate (17%) 5. Oval (7%).
Fruit symmetry	0. Asymmetric (40 %) or 1. Symmetric (60 %).
Uniformity in fruit size	0. No (48%) or 1. Yes (52%).
Fruit exocarp type	1. *Laevis* (smooth) (65%), 2. *Impressa* (slight depressions) (33%), 3. *Umbonata* (small protrusions) (2%), 4. *Tuberculata (*medium protrusions) (0%) and 5. *Mamillata* (large protrusions) (0%).
Ripe fruit colour	1. Reddish yellow (32%), 2. Reddish brown (52%), 3. Reddish green (13%) and 4. Pink (3%).
*Pulp colour	1. White (0%) and 2. Creamy white (100%).
Pulp texture	1. Watery (46%), 2. Creamy (30%), 3. Granular (24%), 4. Hard (0%) and 5. Hard areas in the pulp (%).
*Seed coat colour	1. Grey (0%), 2. Brownish black/Dark brown (28%), 3. Black (72%) and 99. Other (0%).

(* indicated characters with no variation)

Statistical analysis

The data collected on individual characters were tabulated and subjected to statistical analysis and two seasons' data for fruits characters was pooled to arrive at a proper conclusion (Panse and Sukhatme, 1985).

Multivariate analysis

Non-parametric data were converted to scales as proposed in descriptors for *A. cherimola* (IPGRI, 2008). Multivariate analysis *viz*., Principal Component Analysis (PCA), Factor analysis (FA), Principal Co-ordinate Analysis (PCO), Canonical Vector Analysis (CVA), Biplot, Score plot, Scree plot, 2D scatter plot, correlation coefficients, covariance matrix, D^2-analysis and Hierarchical cluster Analysis (HCA), as developed by Mahalanobis (1936), were performed using the mean data for each character following the widely used Windostat version 9.1 and JMP@10.0.2 statistical computer software packages program. For multivariate analysis, total 65 characters were used out of 78 characters and remaining 13 characters were also removed from the analysis due to low coefficient of variation. Furthermore, among 65 characters, 11 morphological qualitative traits were analyzed separately. The collected data were summarized and subjected to diversity analysis.

Results and discussion

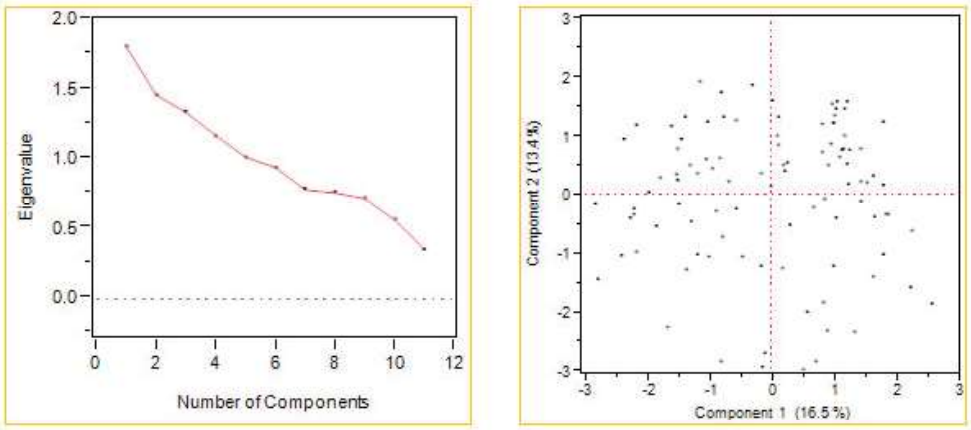
Principal Component Analysis

Results revealed that the 1st principal component (PC) largely accounted for variation among genotypes which contributed to 16.52% of the variation followed by 2nd PC (13.38%). PCA identified 5 PCs with Eigen values >1 explaining 62.15% of total variation. Most of the variability of analyzed genotypes has been explained by first 3 PCs. Results suggested that the 1st PC represented mainly uniformity in fruit size, fruit symmetry, tree growth habit, fruit shape, exocarp types and location of fructification. 2nd PC represented mainly exocarp type, fruit symmetry, leaf blade shape, fruit shape, tree crown shape, location of fructification, tree growth habit and fruit colour, while 3rd PC represented location of fructification, fruit shape, tree crown shape, petal outer colour, leaf blade shape, exocarp type, fruit symmetry and uniformity in size (Table 3). The scatter diagram distributed genotypes into 11 groups and showed that the 10 genotypes occupied distinct position, out of which 8 genotypes were far from the origin while 2 were near to the origin of scatter plot (Fig. 1). A 2D representation of the relative position of the genotypes in the biplot graph was found adequate and indicated the structure of population (Fig. 2). Biplot analysis revealed significant positive associations among uniformity in fruit size, fruit symmetry, ripe fruit colour, fruit shape (Fig. 2). 2D plot demonstrated that genotypes of divergent clusters scattered far apart, while genotypes of similar clusters were placed close to one another (Fig. 3). The 2D PCA plot was consistent with the grouping of genotypes obtained using cluster analysis. Results of PCA-I revealed that the traits responsible for genetic divergence in major axis of differentiation were uniform fruit size, symmetry, tree growth habit and fruit colour (Fig. 4). In PCA-II, the traits having a major role in determining genetic divergence in the 2nd major axis of differentiation were fruit exocarp type, fruit symmetry, leaf blade shape, petal outer colour, fruit shape and tree crown shape. Positive values in both vectors across two axes indicating the important component of genetic divergence among the studied characters were tree crown shape, location of fructification, fruit shape, fruit symmetry and ripe fruit colour, while negative values of both vectors for pulp texture indicated lowest contribution towards divergence. This was also confirmed by relative character contribution percentage towards genetic diversity and rank distribution, i.e. uniformity in fruit size (20%), fruit symmetry (18%), ripe fruit colour (16%), fruit shape (13%) and exocarp type (11%). Aforementioned 5 major traits together accounted for 78% contribution towards divergence (Fig. 4). These results are in accordance with the observations reported by Padmini *et al.* (2013), where the 5 PCA from 15 characters explained 69% of total variability of *A. muricata* germplasm. The findings analogous to this observation have also been reported by Rahman and Al Munsur (2009) in lime, Sudha et al. (2013) in papaya, Majumder et al. (2013) in mango and Rajasekhar et al. (2013) in sapota.

Table 3. Eigen/Latent vectors (V), loading matrix (L), eigen values, Bartlett test and percentage of total population of variance explained by 7 principal components of 11 morphological qualitative traits of 100 bullock's heart genotypes

Sl. No.	Morphological qualitative characters	PC I		PC II		PC III		PC IV		PC V		PC VI		PC VII	
		Vector	Loading	Vector	Loading	Vector	Loading	Vector	Loading	Vector	Loading	Vector	Loading	Vector	Loading
1	Tree Crown shape	0.036	0.048	**0.233**	**0.283**	**0.419**	**0.486**	**0.421**	**0.457**	**-0.417**	**-0.422**	-0.002	-0.002	0.080	0.071
2	Tree growth habit	**-0.447**	**-0.603**	0.069	0.083	-0.030	-0.035	0.125	0.136	**0.355**	**0.359**	**-0.406**	**-0.396**	0.114	0.102
3	Leaf blade shape	**-0.242**	**-0.327**	**0.406**	**0.493**	0.138	0.159	-0.187	**-0.203**	0.162	0.164	**0.590**	**0.575**	0.083	0.074
4	Petal outer colour	0.082	0.111	**-0.425**	**-0.516**	**0.326**	**0.378**	0.168	0.182	0.084	0.085	**0.355**	**0.346**	**0.644**	**0.572**
5	Location of fructification	0.011	0.015	0.121	0.147	**-0.509**	**-0.590**	**0.338**	**0.367**	**0.398**	**0.403**	**0.383**	**0.373**	-0.026	-0.023
6	Fruit shape	0.182	**0.245**	**0.234**	**0.283**	**0.430**	**0.498**	-0.197	**-0.214**	**0.527**	**0.534**	**-0.341**	**-0.332**	0.144	0.128
7	Fruit symmetry	**0.496**	**0.669**	**0.414**	**0.502**	0.042	0.049	0.113	0.122	**0.238**	**0.241**	0.036	0.036	0.073	0.065
8	Uniformity in fruit size	**0.602**	**0.812**	-0.043	-0.052	0.009	0.010	0.054	0.059	-0.029	-0.029	0.036	0.036	**-0.316**	**-0.281**
9	Fruit exocarp type	0.135	0.182	**-0.584**	**-0.709**	0.092	0.106	-0.139	-0.151	**0.353**	**0.357**	0.085	0.083	-0.195	-0.173
10	Ripe fruit colour	**0.269**	**0.363**	0.033	0.040	**-0.494**	**-0.573**	-0.141	-0.153	-0.145	-0.146	**-0.246**	**-0.240**	**0.626**	**0.556**
11	Pulp texture	-0.025	-0.034	-0.103	-0.125	-0.010	-0.011	**0.732**	**0.795**	0.163	0.165	-0.168	-0.164	-0.046	-0.041
Eigen values		1.82		1.47		1.34		1.18		1.02		0.95		0.79	
Total population variance explained (%)		16.52		13.38		12.22		10.72		9.31		8.64		7.17	
Cumulative percentage		16.52		29.90		42.12		52.84		62.15		70.78		77.95	
Chi Square		94.04		69.18		54.38		40.92		30.80		24.03		17.53	
DF		55.03		47.15		39.31		32.05		25.27		19.06		13.56	
Prob>ChiSq		0.001*		0.02*		0.06		0.14		0.21		0.20		0.20	

Highlighted values of each column represented selected characters of each principal component



Scree plot (a) **Scatter plot (b)**

Fig. 1. Principal component analysis scree plot (a) and scatter plot (b) depicting the genetic diversity based on PC scores of morphological qualitative data of 100 bullock's heart genotypes

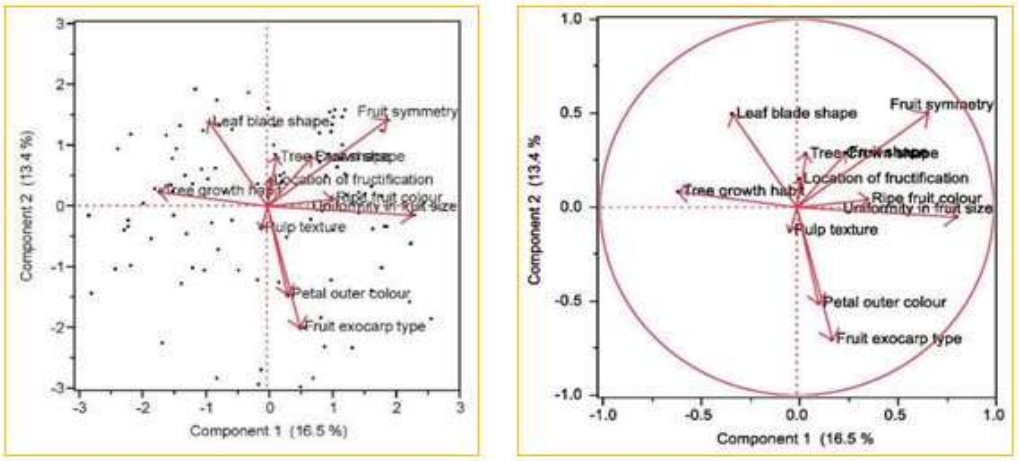


Fig. 2. Principal component analysis (PCA) Biplot (a) and loading plot (b) of PC1 and PC2 factor loadings for genotypes-by-traits and correlation analysis among various morphological qualitative traits using cumulative data of 100 bullock's heart genotypes

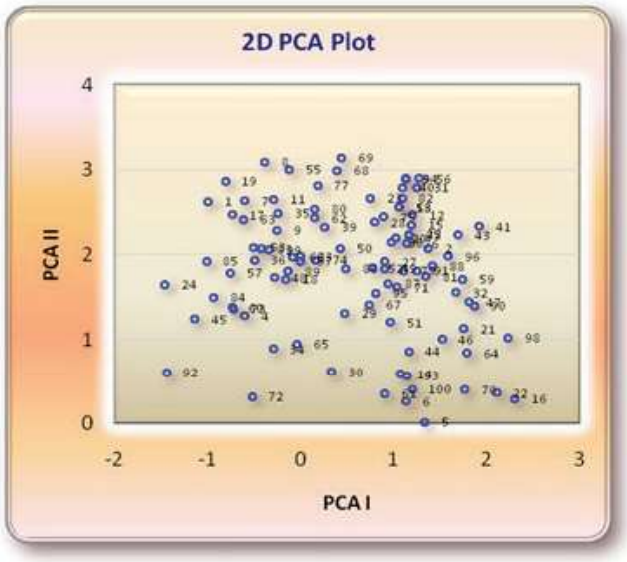


Fig. 3. Scattered diagram: Two dimensional ordination showing the relative position of 100 bullock's heart genotypes based on PCA scores (PC 1 and PC 2) of morphological qualitative traits

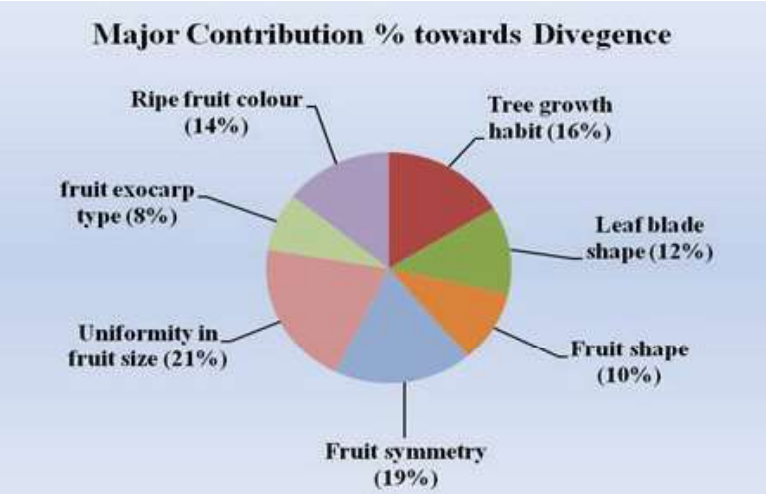


Fig. 4. Graphical representation of relative proportionate contribution of studied major traits (in parentheses value) out of 11 morphological qualitative traits towards genetic divergence of 100 bullock's heart genotypes

Correlation studies

Tree crown shape registered significant positive correlation with fruit symmetry and positively associated with pulp texture, fruit shape and petal outer colour (Table 4). Tree growth habit positively associated with pulp texture, leaf blade shape and location of fructification. Leaf blade shape positively associated with fruit shape, fructification, fruit symmetry. Petal outer colour showed significant positive correlation with fruit exocarp type and positively associated with pulp texture and uniformity in fruit size. Location of fructification recorded significant positive correlation with fruit symmetry, pulp texture and ripe fruit colour. Fruit shape had significant positive correlation with the fruit symmetry and positively associated with fruit exocarp type and uniformity in fruit size. Fruit symmetry had significant positive correlation with uniform fruit size and fruit colour. Uniformity in fruit size showed significant positive correlation with fruit exocarp type and ripe fruit colour and positively associated with pulp texture (Table 4). These results are in line with the observations reported by Verma et al. (2012) in pomegranate, Majumder et al. (2013) in mango and Rajasekhar et al. (2013) in sapota.

Table 4. Correlations coefficients among 11 morphological qualitative traits of 100 bullock's heart genotypes

Characters → ↓	Tree Crown shape	Tree growth habit	Leaf blade shape	Petal outer colour	Location of fructification	Fruit shape	Fruit symmetry	Uniformity in fruit size	Fruit exocarp type	Ripe fruit colour	Pulp texture
Tree Crown shape	**1.000**										
Tree growth habit	-0.053	**1.000**									
Leaf blade shape	0.025	0.048	**1.000**								
Petal outer colour	0.040	-0.067	-0.082	**1.000**							
Location of fructification	-0.092	0.048	0.039	-0.087	**1.000**						
Fruit shape	0.062	-0.002NS	0.072	-0.014	**-0.147***	**1.000**					
Fruit symmetry	**0.103***	**-0.170***	0.014	-0.028	**0.138***	**0.279***	**1.000**				
Uniformity in fruit size	0.008NS	**-0.344***	**-0.216***	0.049	-0.026	0.050	**0.441***	**1.000**			
Fruit exocarp type	**-0.153***	**-0.101***	**-0.181***	**0.229***	-0.012	0.063	**-0.164***	**0.144***	**1.000**		
Ripe fruit colour	**-0.129***	**-0.146**	**-0.144***	-0.095	**0.106***	-0.047	**0.132***	**0.128***	-0.031	**1.000**	
Pulp texture	0.098	0.071	-0.096	0.071	**0.116***	-0.038	-0.020	0.011	0.003NS	-0.048	**1.000**

Cell Contents= Simple correlation; NS=Non Significant; Bold value and * indicates significant at p=5 % level (significant at p<0.05).

Factor Analysis: Varimax Rotation

Factor analysis identified 6 factors retained by positive Eigen value criterion, which explained 154.82% of the total genotypes variation. Most of the variability of the analyzed genotypes was explained by first 3 factors (Table 5). First factor with Eigen value of 1.10 accounted for 62.70% of the variation and was primarily related to uniformity in fruit size, fruit symmetry, tree growth habit and ripe fruit colour, while uniformity in fruit size, fruit symmetry, and ripe fruit colour showed highest positive correlation. The second factor that accounted for 37.35% of the total variance was mainly loaded by fruit exocarp type, fruit symmetry, leaf blade shape and petal outer colour, while fruit symmetry and leaf blade shape revealed highest positive correlation. The third factor accounted for 25.96 % of the total variation and was mainly associated with location of fructification, ripe fruit colour, fruit shape and tree crown shape, while fruit shape and tree crown shape showed highest positive correlation. The communality values ranged from 0.49 to 0.13. Even so, the factor analysis identified traits which contributed most to the variation of the analyzed genotypes and could serve as a useful tool for facilitating the selection of desirable characteristics in bullock's heart breeding. The findings are in accordance with those reported by Manigandan and Vijayakumar (2014).

Table 5. Factor loading (unrotated and rotated factor), Eigen values, cumulative variance, percentage of total (standardized) population of variance explained by 6 factor model and communalities of the 11 morphological qualitative traits of 100 bullock's heart genotypes from factor analysis

Sr. No.	Morphological qualitative characters	Factor I		Factor II		Factor III		Factor IV		Factor V		Factor VI		Communalities
		UFL	RFL	UFL	RFL	UFL	RFL	UFL	RFL	UFL	RFL	UFL	RFL	
1	Tree Crown shape	0.048	0.034	0.171	-0.044	0.242	0.064	**0.221**	**0.395**	-0.165	-0.050	-0.009	0.026	0.17
2	Tree growth habit	**-0.409**	**-0.461**	0.118	-0.086	-0.009	0.015	0.084	0.011	0.175	0.098	-0.106	0.008	0.23
3	Leaf blade shape	-0.193	-0.167	**0.325**	-0.189	0.096	0.089	-0.105	0.054	0.030	-0.026	**0.166**	**0.342**	0.19
4	Petal outer colour	0.053	0.034	**-0.295**	**0.376**	0.208	-0.008	0.123	0.099	0.031	-0.038	0.079	-0.041	0.16
5	Location of fructification	0.022	0.022	0.098	-0.116	**-0.353**	-0.070	0.154	**-0.111**	0.169	**0.407**	0.100	0.011	0.20
6	Fruit shape	0.191	0.049	0.173	0.019	**0.324**	**0.455**	-0.118	0.055	**0.199**	-0.116	-0.077	0.062	0.23
7	Fruit symmetry	**0.595**	**0.483**	**0.350**	-0.195	0.019	**0.414**	0.056	0.114	0.107	0.189	0.011	0.017	0.49
8	Uniformity in fruit size	**0.663**	**0.620**	-0.117	0.120	-0.010	0.161	0.032	0.011	-0.043	0.048	0.006	-0.170	0.46
9	Fruit exocarp type	0.092	0.056	**-0.492**	**0.482**	0.091	0.002	-0.059	**-0.200**	0.170	-0.052	0.039	-0.120	0.29
10	Ripe fruit colour	**0.230**	**0.256**	-0.013	-0.163	**-0.326**	-0.064	-0.114	**-0.235**	-0.033	0.085	-0.075	-0.144	0.18
11	Pulp texture	-0.021	-0.067	-0.052	0.098	-0.023	-0.045	**0.349**	0.196	0.058	**0.234**	-0.037	-0.144	0.13
Eigen values		1.10		0.66		0.46		0.26		0.17		0.07		-
Total population variance explained (%)		62.70		37.35		25.96		14.91		9.93		3.97		-
Cumulative percentage		62.70		100.05		126.01		140.92		150.85		154.82		-

UFL- Unrotated Factor Loading; RFL- Rotated Factor Loading and 6 factors will be retained by the positive Eigen value criterion.

Non-hierarchical clustering

Non-hierarchical clustering was done by using correlation coefficients and covariance matrix where100 bullock's heart genotypes were grouped into 11 clusters. By application of this non-hierarchical clustering pattern of the genotypes, the PCA was confirmed. The clustering patterns obtained through different techniques coincided with the grouping patterns done by PCA suggesting that the results obtained through PCA were established by non-hierarchical clustering.

Hierarchical Cluster Analysis (HCA)

Cluster contributions based on dendrogram

The genotypes based on qualitative traits were grouped into 11 distinct clusters with each cluster containing genotypes that were morphologically similar

(Table 6 and Fig. 5). The number of genotypes in the different groups ranged from 3-21. The distribution pattern indicated that the majority of the genotypes in cluster-II, cluster-I and cluster-VIII, whereas the minimum genotypes were in cluster-XI. The clusters II, I, VIII and X together accounted for 59% of total genotypes.

Table 6. Cluster composition of all genotypes regarding morphological qualitative traits based on dendrogram by Ward's minimum distance method with their source of collection

Clusters	Genotypes and distinct characters
I	Cluster-I is consisted of fourteen genotypes, SW-1, SW-19, CN-5, SW-8, KL-6, SW-7, DP-5, SW-9, SW-18, KL-4, KN-2, SW-11, DP-12 and DP-13 from Ratnagiri and Sindhudurg districts which had highest mean values for most of the studied traits such as tree growth habit (-1.04), followed by uniformity in fruit size (-0.99) and fruit symmetry (-0.88) and almost lowest for fruit shape (0.18).
II	Cluster-II comprising of twenty one genotypes, SW-3, SW-12, SW-15, VL-1, VL-18, DP-4, KD-1, VB-4, SW-13, KL-9, VL-16, VL-7, KL-7, DP-10, DP-3, VL-11, VB-2, DP-2, DP-17, VL-19 and VB-1 from Sawantwadi, Vengurla, Vaibhavwadi and Kudal tahsils of Sindhudurg districts and Dapoli and Khed tahsils of Ratnagiri district which had the extreme mean values for location of fructification (0.81).
III	Cluster-III comprising of four genotypes, VL-5, VL-15, VL-20 and DP-11 from Ratnagiri and Sindhudurg districts which had the extreme mean values for tree crown shape (1.35) and fruit shape (1.33).
IV	Cluster-IV comprising of eight genotypes, SW-23, VL-4, KN-1, VL-25, CN-2, VL-14, KL-10 and GH-1 from Ratnagiri and Sindhudurg districs which had highest mean values for leaf blade shape (1.73) and location of fructification (1.45) other than tree crown shape (1.35) and fruit shape (1.33).
V	Cluster-V comprising of five genotypes, SW-2, SW-20, DP-8, KL-3 and DV-4 from Ratnagiri and Sindhudurg districts which had the extreme mean values for fruit shape (2.48) other than tree crown shape (2.50).
VI	Cluster-VI comprising of seven genotypes, SW-17, VL-21, KL-8, VL-24, DP-14, VB-5 and KD-2 from Ratnagiri and Sindhudurg districts which had the extreme mean values for location of fructification (2.09) other than tree crown shape (3.07), leaf blade shape (3.17) and fruit shape (3.05).
VII	Cluster-VII comprising of seven genotypes, SW-4, VL-12, SW-24, VL-9, CN-7, DV-3 and VL-10 from Ratnagiri and Sindhudurg districts which had the extreme mean values for ripe fruit colour (2.32) other than leaf blade shape (3.89), tree crown shape (3.64) and fruit shape (3.62) and which were also greater than mean value of all eleven clusters.
VIII	Cluster-VIII comprising of fourteen genotypes, SW-5, SW-22, VL-2, VL-13, SW-14, VL-6, KL-5, DP-6, SW-21, VL-8, CN-3, DV-1, VB-3 and KL-2from Ratnagiri and Sindhudurg districts which had possessed first position in case of leaf blade shape (4.61) followed by tree crown shape (4.22) and fruit shape (4.20).
IX	Cluster-IX comprising of three genotypes,SW-10, MN-2 and VL-22, had almost lowest for fruit symmetry and uniformity in fruit size, no remarkable feature was noticed in this cluster for these two traits, while highest mean values for leaf blade shape (5.33), tree crown shape (4.79) and fruit shape (4.77).
X	Cluster-X comprising of ten genotypes, VL-17, DP-18, DP-16, CN-4, CN-6, DP-9, VL-23, DP-15, SW-6 and SW-16 from Ratnagiri and Sindhudurg districts which had the extreme mean values for leaf blade shape (6.05), tree crown shape (5.36), fruit shape (5.35), tree growth habit (3.78) and pulp texture (3.77).
XI	Cluster-XI comprising of seven genotypes, MN-1, DP-1, VL-3, DP-7, CN-1, KL-1 and DV-2 from Ratnagiri and Sindhudurg districts which had the extreme mean values for leaf blade shape (6.76) followed by tree crown shape (5.94) and fruit shape (5.92) and also the remarkable feature was noticed in this cluster for location of fructification, fruit symmetry and uniformity in fruit size.

[Hierarchical clustering]
Method=Ward
No. of clusters=11

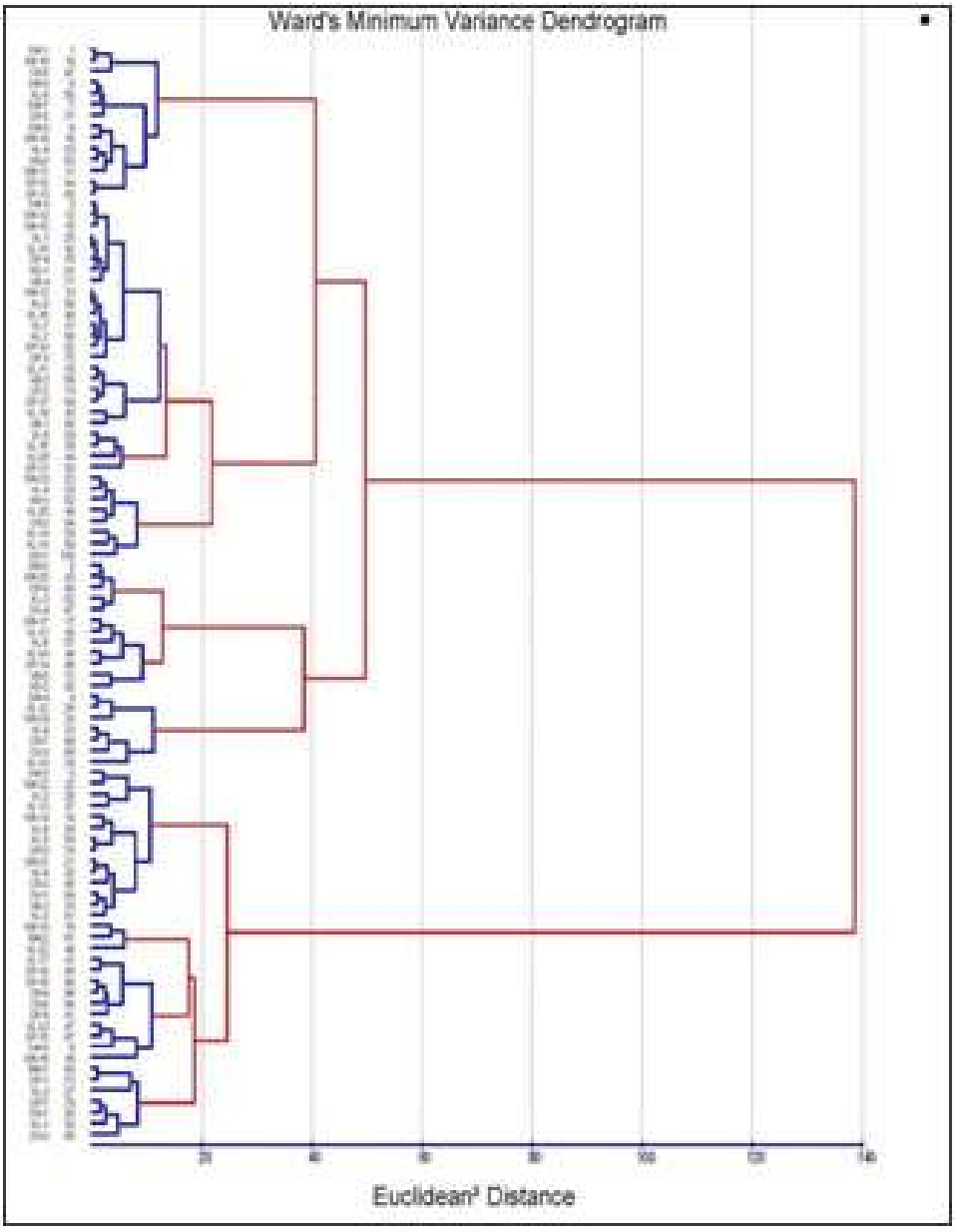


Fig. 5. Consensus tree diagram (Dendrogram) representing relationships of 100 elite bullock's heart genotypes produced by Ward's minimum distance cluster analysis based on 11 morphological qualitative traits (Scale: Euclidean^2 distance)

Cluster means with distinct characters

Differences among the genotypes for leaf blade shape, tree crown shape, fruit shape and location of fructification were more pronounced as compared to the other traits. Some of these characters, though not very significant in our study, could be effectively exploited in future crop improvement. In particular, the comparison between non-hierarchical clustering and HCA revealed the uniformity in fruit size to be the most important character its contribution was maximum (21%) in the genetic divergence (Fig. 4). Cluster-XI and cluster-X had the highest cluster mean values for most of the traits (Table 7). Based on the cluster means, the important clusters were cluster-I for uniformity in fruit size and fruit symmetry, and cluster-XI and cluster-X for fruit shape and pulp texture.

Table 7. Cluster means of 100 bullock's heart genotypes for 11 clusters with respect to eleven studied morphological qualitative traits based on dendrogram by Ward's minimum distance method

Morphological qualitative characters	<-------Clusters mean------>											Mean
	I (14)*	II (21)	III (4)	IV (8)	V (5)	VI (7)	VII (7)	VIII (14)	IX (3)	X (10)	XI (7)	
Tree Crown shape	0.20	**0.78**	**1.35**	**1.92**	**2.50**	**3.07**	**3.64**	**4.22**	**4.79**	**5.36**	**5.94**	**3.07**
Tree growth habit	**-1.04**	-0.50	0.03	0.57	1.10	1.64	2.18	2.71	3.25	3.78	4.32	**1.64**
Leaf blade shape	-0.42	0.29	1.01	1.73	**2.45**	**3.17**	**3.89**	**4.61**	**5.33**	**6.05**	**6.76**	**3.17**
Petal outer colour	-0.62	-0.17	0.27	0.71	1.16	1.60	2.04	2.49	2.93	3.37	3.82	**1.60**
Location of fructification	0.49	**0.81**	1.13	1.45	1.77	2.09	2.41	2.73	3.05	3.37	3.69	**2.09**
Fruit shape	0.18	**0.75**	**1.33**	**1.90**	**2.48**	**3.05**	**3.62**	**4.20**	**4.77**	**5.35**	**5.92**	**3.05**
Fruit symmetry	-0.88	-0.58	-0.29	0.01	0.30	0.60	0.90	1.19	1.49	1.78	2.08	**0.60**
Uniformity in fruit size	**-0.99**	-0.69	-0.38	-0.08	0.22	0.52	0.82	1.12	1.42	1.73	2.03	**0.52**
Fruit exocarp type	-0.21	0.11	0.42	0.74	1.05	1.37	1.69	2.00	2.32	2.63	2.95	**1.37**
Ripe fruit colour	-0.37	0.08	0.52	0.97	1.42	1.87	2.32	2.77	3.22	3.66	4.11	**1.87**
Pulp texture	-0.65	-0.17	0.32	0.81	1.29	1.78	2.27	2.75	3.24	3.73	4.21	**1.78**

Highest values with bold figure in column indicating important characters of the cluster and * indicating number of genotypes in the cluster

Principal Co-ordinate Analysis

Intra-cluster distances ranged from 4.38 to 9.33 (Fig. 6). Highest intra-cluster distance was observed in cluster-IX indicating that the genotypes belonging to this cluster were far diverged from cluster-VII. Minimum intra-cluster distance was observed in cluster-II which included maximum number of genotypes. Cluster-II was far diverged with the rest of the clusters indicating that these genotypes could be crossed with other genotypes in order to incorporate the desired characters like fruit shape, fruit symmetry, uniformity in fruit size, fruit exocarp type, ripe fruit colour and pulp texture into the cultivated types.

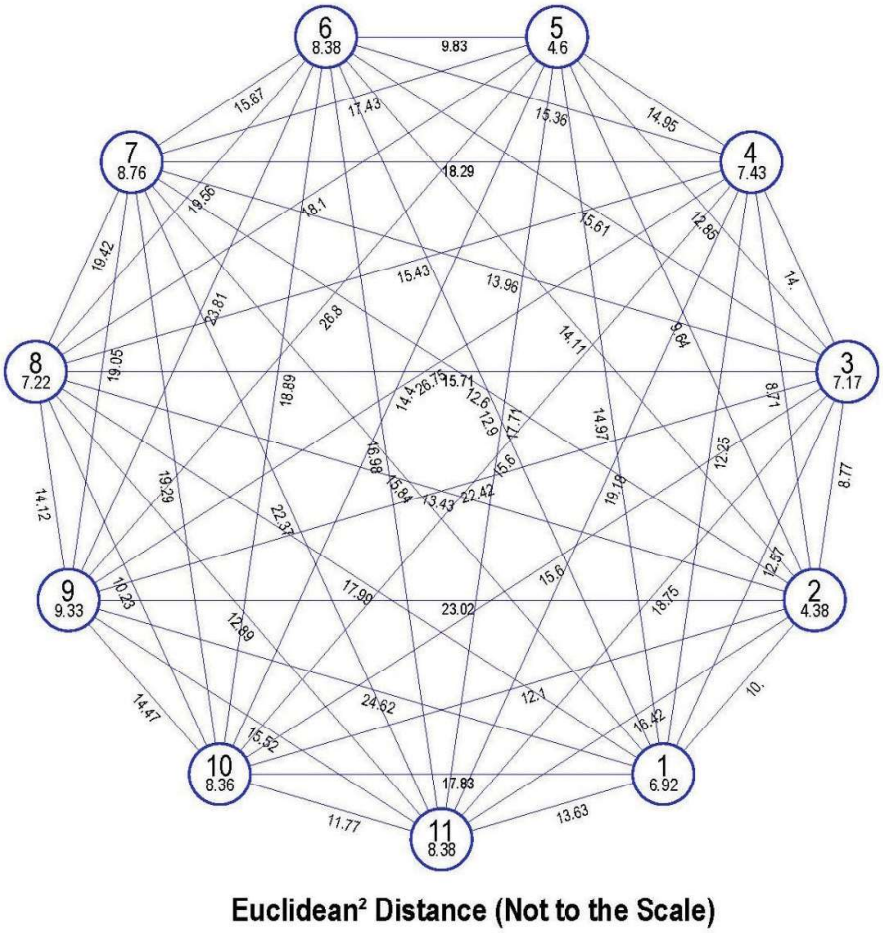


Fig. 6. Cluster diagram showing the average intra and inter cluster distance ($D = \sqrt{D^2}$) based on Euclidean² distance for 11 morphological qualitative traits in 11 clusters of 100 bullock's heart genotypes (The values along the lines indicate inter cluster distances and the values within the circle indicate intra cluster distances)

Canonical Vector Analysis

Results revealed that inter-cluster distances ranged from 8.71 to 26.80 (Fig. 6). Maximum inter-cluster distance was observed between cluster-V (SW-2, SW-20, DP-8, KL-3, DV-4) and cluster-IX (SW-10, MN-2, VL-22) indicating wide range of genetic diversity between these two clusters, followed by cluster-IV and IX, cluster-I and IX, cluster-VI and IX and cluster-II and IX. Lowest inter-cluster distance was between cluster- II and cluster- IV meaning more genetic similarity. The putative parents for a systematic crossing programme should belong to diverse clusters characterized by a large inter-cluster distance. The hybridization among the genotypes drawn from widely divergent clusters with high yield potential is likely to manifest maximum heterotic combinations as well as new recombination with desired traits. From the observations, it was apparent that there was a considerable degree of divergence at inter-genetic stock (between genotypes), inter-cluster (between clusters), and intra-cluster (within cluster) levels of diversification in *A. reticulata.* These results are in accordance with the observations reported by Rajasekhar et al. (2013) in sapota and Sharma et al. (2013) in apple.

Conclusion

Results of morphological diversity analysis demonstrated the presence of substantial variability among the evaluated bullock's heart genotypes for the studied traits and it was enough to distinguish between them. All desired characteristics were not found in one unique genotype, although some genotypes DP-5, VL-22, KL-7, KL-9, SW-12, DP-16, DP-4, VL-7, SW-20 and VL-16 (Fig. 7) had showed potentially good characteristics for international markets. Hierarchical and non-hierarchical algorithms, based on the multivariate statistical techniques, are common methods used by breeders to identify diverse genotypes for developing varieties that suit the target environment. Application of these three methods was useful for classification, documentation and characterization of bullock's heart genotypes. Results suggested that the genotypes originated from same place may have different genetic architecture or vice-versa. It is therefore recommended that the genetic conservation and improvement of bullock's heart based on the selected materials should be ecouraged.

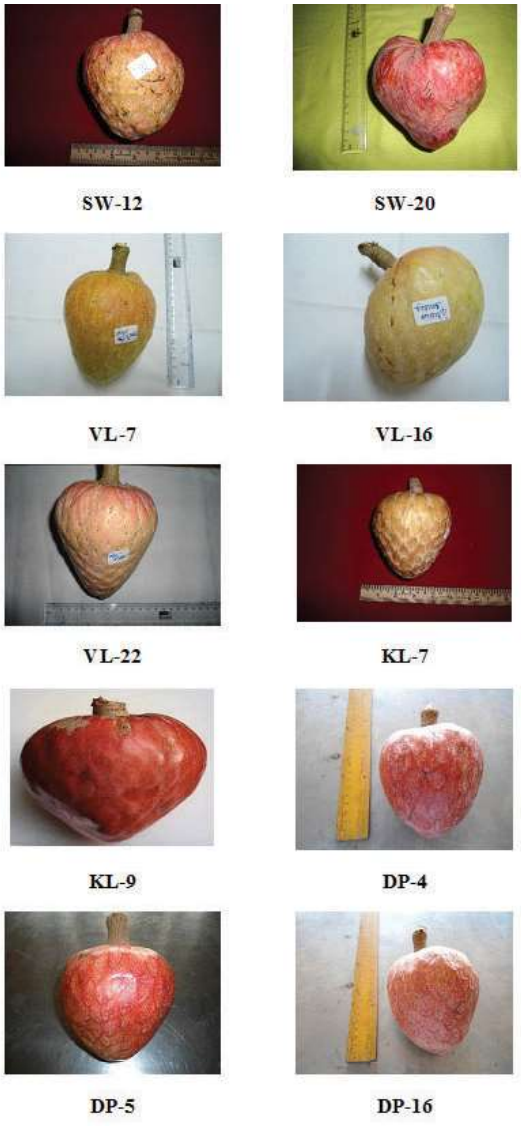


Fig. 7. Variability in fruit characteristics in selected genotypes of bullock's heart

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