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Morphological Characterization of Soursop (*Annona muricata* L.) Germplasm in Sri Lanka

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ABSTRACT. *Annona muricata L. is an underutilized fruit tree species in Sri Lanka, which is mostly confined to homegardens. Despite the importance of A. muricata, collection, characterization and improvement of its germplasm is limited in Sri Lanka, hence hindered its effective conservation and utilization. Therefore, the objective of this research was to identify morphological variation of A. muricata populations in Sri Lanka. Multistage Sampling Survey was conducted in homegardens of Anuradhapura, Polonnaruwa and Hambantota dictricts in the Dry zone, Puttalama and Kurunagala districts in the AIntermediate zone, and Kalutara and Gampaha districts in the Wet zone. Random representative samples were also collected from existing germplasm collections at three national research centers. Morphological variation of A. muricata were observed on total of 315 samples collected from seven districts of three climatic zones and 133 samples collected from germplasm collections at three national research centers. Forty five morphological characters were recorded from 448 accesions and subjected to Principal Component Analysis (PCA) and Factor Analysis (FA), followed by Cluster Analysis. A dendrogram of evaluated characters showed nine distinguished clusters. Implications of findings are discussed in relation to utilization and conservation.*

Keywords: *Annona muricata, morphological variations, homegardens, factor analysis, cluster analysis*

INTRODUCTION

Annona muricata L. of the family Annonaceae (commonly known as Sour sop, *Katu anoda* or *Katu arrtha*) is one of the world's most exquisite, but less studied fruit species in Sri Lanka. The fruits are consumed widely as fresh form and the other plant parts are also a source of medicinal and other industrial products such as beverages, wine, jellies, jam and fruit-butter preserves and pure (Gleye *et al.,* 1997; Pinto *et al.,* 2005; Abbo *et al.,* 2006; Heenkenda *et al.,* 2011). The fruits contain vitamins, minerals, bioactive chemical substances. The other plant parts also contain numerous amounts bioactive chemical substances such as acetogenins, alkaloids, terpens, flavonoids, cyclopeptide annomuricatin and oils (Roblot *et al.*, 1993; Wu *et al.,* 1995; Ming *et al*., 1998; Gleye *et al.,* 1999; Pinto *et al*., 2005). These compounds are very useful medicines because some acetogenins have anti-tumoral, insecticidal, antibacterial, immuno-suppressant, pesticidal or antihelminthic properties (Kim *et al*., 1998; Yu *et al*., 1998). However, in Sri Lanka *A. muricata* is

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categorized as an underutilized fruit tree species (Heenkenda *et al.,* 2011). Only a limited amount of researches has been conducted on *Annona* species in Sri Lanka (Thantirige, 2001; Heenkenda *et al.,* 2011), though this species in general has high potential to diversify the farming system (Bowe and Haq, 2010).

Morphological characterization of a species is very useful in the separation of populations into different morphotypes and proper utilization of genetic resources in plant breeding programmes (Piyasundara *et al*., 2009). Better understanding of genetic diversity present and its distribution is essential for rational utilization of germplasm in crop improvement (Piyasundara *et al*., 2009). Study of plant genetic resources are also important to identify agrobiodiversity and this include primitive forms of cultivated plants, modern cultivars, *ex situ* collections and related wild species. Genetic diversity created in farmers' fields, homegardens over millennia (Upadhyaya *et al.,* 2008). Genetic relationships between nine *A. muricata* accessions using RAPD markers have been reported in Venezuela (Brown, *et al.*, 2003) where higher genetic variability has been reported according to the country of origin of plants. The objectives of the present research was identification of morphological variation of *A. muricata* population in homegardens and collection of germplasm centers in Sri Lanka

MATERIALS AND METHODS

Data collection and sampling method for *A. muricata* trees

Tree of *A. muricata* is found mainly in homegardens of many parts of Sri Lanka except in the higher elevations (Pinto e*t al.*, 2005; Anon, 2007) and four *ex situ A. muricata* gemplasm collections are located in Fruit Research Institute (FRI), Horana, Regional Agricultural Research and Development Centre (RARDC), Makadura, Horticultural Crop Research Institute (HORDI), Gannoruwa and Agricultural Research Station (ARS), Giradurukotte. Sampling of homegardens was done using the multistage sampling method to conduct the survey in large geographical area in the country. In order to increase the precision of sampling a large number of clusters were used as Thattil (1999) and Thattil and Samita, (2007) suggested. Sampling was carried out as shown in Fig.1.

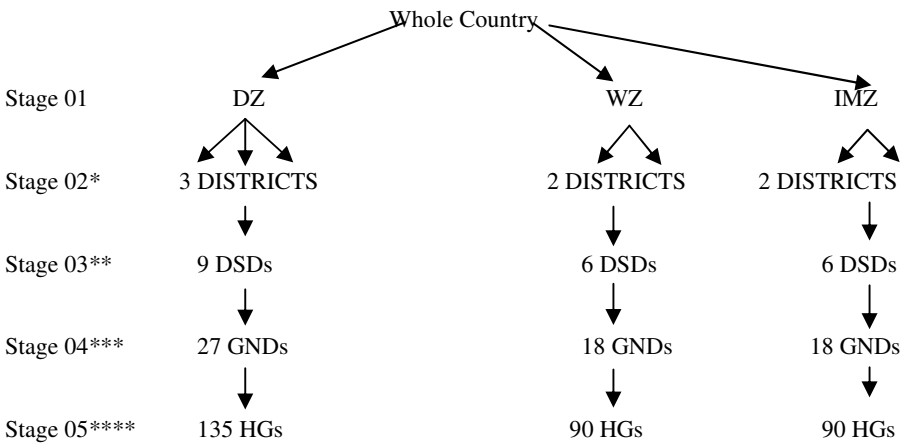

Fig. 1. Flow diagram of sample frame for *A. muricata* trees at homegardens.

Note: * Number of districts depends on size of the major climate zones; ** within each District, three Divisional Secretariat Divisions (DSDs) were selected randomly; *** within each DSD, three Grama Niladari Divisions (GNDs) were selected randomly; **** within each GND, five Homegardens (HGs) were selected randomly (Thattil, 1999).

The three main climatic zones were used in the first stage whereas administrative districts were randomly selected in the second stage. The number of districts depends on size, homogeneity and heterogeneity of the climatic zones. Accordingly three districts from the dry zone (Anuradhapura, Polonnaruwa, and Hambantota), two districts from the wet zone (Kalutara and Gampaha) and two districts from the intermediate zone (Puttalama and Kurunagala) were selected. In the third stage, Divisional Secretariat Divisions (DSDs) were considered and three DSDs were selected randomly from each district. In the fourth stage Grama Niladari Divisions (GNDs) were considered and three GNDs were selected randomly from each DSD. At the fifth level *A. muricata* tree at homegardens (HGs) were considered for sample collection. Five homegardens were visited in each GND and *A. muricata* trees were selected randomly if there were more than one plant at the considered homegarden (as shown in Fig. 1). Distribution of sampling sites (DSDs of relevant districts) is given in Fig. 2.

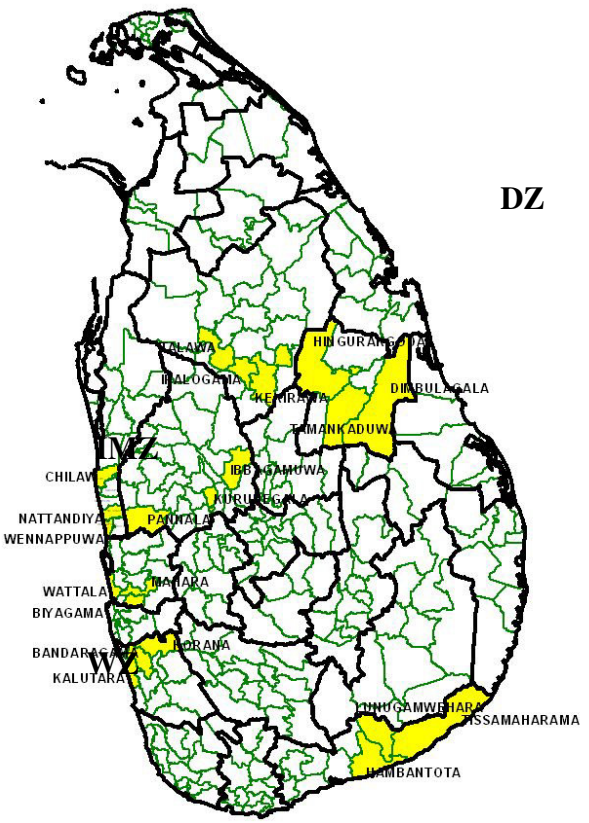

Fig. 2. Distribution of sampling sites (DSDs of selected districts) in Sri Lanka.

Since a descriptor list for *A. muricata* is not available, the descriptor list of *A. cherimola* Mill. (Cherimoya) compiled by the International Plant Genetic Resources Institute (IPGRI,

2008) was used in this study. Accordingly, 45 morphological descriptors were identified and assessed. Fourteen quantitative characters were measured including leaf length, leaf width, petiole length, petal length, petal width, fruit length, fruit diameter, weight of ripe fruit, peduncle length, weight of all fresh seeds, number of seeds, weight of fresh seed, seed length and seed width. From each tree ten fully expanded and healthy leaves, ten flowers and ten well developed fruits were randomly selected for measurements of characters. Thirty one qualitative characters were categorized and measured (Table 1). Colour chart of Royal Horticulture Society (RHS) was used to identify parameters such as trunck colour, leaf colour, flower colour, exocarp color, pulp colour and seed colour. Resistance to abrasion was recorded by thumb friction. Pulp oxidation was observed by pale colour of the pulp five minutes after cutting the fruit. Tenacity of the seed in its epithelium was observed by cutting of seed and observing the seed coat and the firmness of epithelium by removing cotyledon from seed coat.

Data analysis

Non parametric data were converted to scales as proposed by IPGRI in descriptors for *A. cherimola* (IPGRI, 2008). A total of 315 samples were analysed from dry, wet and intermediate zones of the country and 133 samples were collected from four Germplasm Collecting Centers. Among 45 characters, 22 morphological characters did not show any variance in 448 accesions and accordingly removed from the analysis (Table 1 show such variables using asteric*), leaving only 23 characters to be used in the primary analysis. As per Table 1 leaf length, petiole length, petal length, petal width, weight of fresh seed, seed length and seed width were also removed from the analysis due to low coefficient of variation (below 25% of CV, noted as **). Finally, only 15 characters were considered for analysis namely trunk colour, trunk ramification, suckering tendency, colour of young branches, leaf blade shape, average leaf width [mm], petal outer colour, location of fructification, fruit length [mm], fruit diameter [mm], weight of ripe fruit [g], peduncle length [mm], weight of all fresh seeds per fruit [g], number of seeds, pulp taste (Table 1).

Principal Component Analysis (PCA) and Factor Analysis (FA) were carried out using average values of descriptors. The results were used for cluster analysis. The analysis was conducted using Statistical Analysis System (SAS) for Windows Version 8e (Anon, 1999). The PCA and FA were used conducted for total data set and for data from districts and germplasm collecting centers, separately. Eigen values greater than 1.00 and cumulative proportion of variation explained were used to identify number of principal components (Thattil and Samita, 2007). The magnitudes of the component coefficients in Eigen vectors were used to measure the importance of each character to the particular principal component. Cluster analysis is the partitioning of a set of objects into groups so that objects within a group are similar and objects in different groups are dissimilar. It is efficient in grouping objects with similar characters (Hodgkin *et al*., 1995). The analysis was performed using the cluster procedure (method=average linkage) and the dendrogram with the tree procedure of SAS. Previous studies have shown that the average linkage method was used by many researchers in cluster analysis studies (Piyasiri *et al*., 2001; Ruckshanthi *et al*., 2002; Piyasundara *et al*., 2009). Clusters were defined based on their unique characters. In order to identify the relationship of accessions, they were plotted using variables (i.e. fruit weight vs. number of seeds in the fruit) as shown in Fig. 3 Fruit weight / number of seeds ratio was to identify clusters. Correlations among characters were identified using two dimensional plotting of factors.

Table 1. Variation of quantitative and qualitative characters measured and used in the analysis.

Quantitative Characters	Mean	SD	CV
Average Leaf width [mm]	51.46	12.87	25.01
Fruit length [mm]	157.16	42.23	26.87
Fruit diameter [mm]	109.12	29.05	26.62
Weight of ripe fruit [g]	509.72	480.77	94.32
Peduncle length [mm]	34.83	16.98	48.76
Weight of all fresh seeds per fruit [g]	12.75	7.46	58.51
Number of seeds	44.76	23.19	51.80
**Average Leaf length [mm]	135	17.47	12.94
**Petiole length [mm]	8.08	1.73	21.46
**Petal length [mm]	41.69	6.01	14.41
**Petal width [mm]	36.25	4.94	13.63
**Weight of fresh seed [g]	0.28	0.05	16.79
**Seed length [mm]	14	1.89	13.50
**Seed width [mm]	8.27	1.34	16.19
Qualitative Characters	Observed variation		
Trunk colour	1 Light grey (0%), 2 Grey (77%), 3 Dark grey (23%), 4 Other (0%)		
Trunk ramification	1 One branch (56%), 2 Two (26%), 3 Three or more (18%)		
Suckering tendency: number of suckers	0 Absent (83.1%), 1 ≤5 suckers (16%), 2 >5 suckers (0.9%)		
Colour of young branches	1 Light green (94%), 2 Green (6%), 3 Dark green (0%),		
Leaf blade shape	1 Ovate (15%), 2 Elliptic (0%), 3 Obovate (85%), 4 Lanceolate (0%), 5 Other (0%)		
Petal outer colour	1 Cream (9%), 2 Yellow (91%), 3 Green (0%), 4 Brown (0%), 5 Other (0%)		
Location of fructification	1 Base (17%), 2 Middle (66%), 3 Top of the crown (17%)		
Pulp taste	3 Bad (0%), 5 Average (4%), 7 Good (96%)		
*Pubescence of young branches	0 Absent (100%), 1 Present (0%)		
*Shape of leaf base	1 Acute (100%), 2 Rounded (0%), 3 Obtuse (0%), 4 Cordate (0%)		
*Shape of leaf apex	1 Acute (100%), 2 Rounded (0%), 3 Acuminate (0%)		
*Pubescence of leaf	0 Absent (100%), 1 Present (0%)		

Table continued on next page

*Colour of mature leaves	1 Light green (0%), 2 Green (0%), 3 Greyish green (0%), 4 Dark green (100%), 5 Other (0%)
*Colour of young leaves	1 Light green (100%), 2 Green (0%), 3 Dark (0%), 4 Other (0%)
*Leaf margin	1 Entire (100%), 2 Undulate (0%)
*Leaf blade venation	3 Submerged (0%), 5 Intermediate (100%), 7 Raised (0%)
*Colour of the internal petal base	1 Pink (0%), 2 Light red (0%), 3 Dark red (0%), 4 Cream (100%)
*Petal pubescence	0 Absent (100%), 1 Present (0%)
*Sepal pubescence	0 Absent (100%), 1 Present (0%)
*Fruit shape	1 Round (0%), 2 Oblate (0%), 3 Cordate (0%), 4 Broadly cordate (100%), 5 Oval (0%), 6 Other (0%)
*Uniformity in fruit size	0 No (100%), 1 Yes (0%)
*Fruit symmetry	0 No (100%), 1 Yes (0%)
*Exocarp type	1 smooth (0%), 1 slight depressions (0%), 3 small protrusions (0%), 4 medium protrusions (100%), 5 large protrusions (0%), 6 Other type (0%)
*Exocarp colour	1 Light green (0%), 2 Green (1%), 3 Dark green (0%), 4 Yellowish green (99%), 5 Yellow (0%), 6 Brownish green (0%), 7 Brown (0%), 8 Other (0%)
*Resistance to abrasion	0 No (0%), 1 Yes (100%)
*Pulp colour	1 White (100%), 2 Cream (0%), 3 Other (0%)
*Pulp texture	1 Watery (100%), 2 Creamy (0%), 3 Granular (0%), 4 Hard (0%), 5 Hard areas in the pulp (0%), 6 Other (0%)
*Pulp fibre content	0 Absent (0%), 1 Low (0%), 2 High (100%)
*Pulp oxidation	1 Poorly (0%), 2 Oxidized (100%), 3 Very oxidized (0%)
Seed coat colour *	1 Grey (1%), 2 Dark brown (99%), 3 Black,
Tenacity of the seed in its epithelium *	1 Cloaked (100%), 2 Semi-cloaked (0%), 3 Loose (0%)

Note: The highlighted (bold) 15 characters (7 quantitative and 8 qualitative characters) were considered for PCA and FA analysis and other morphological characters were removed due to lack of variation among accessions. (* indicated characters with no variation. ** indicated characters with low CV)

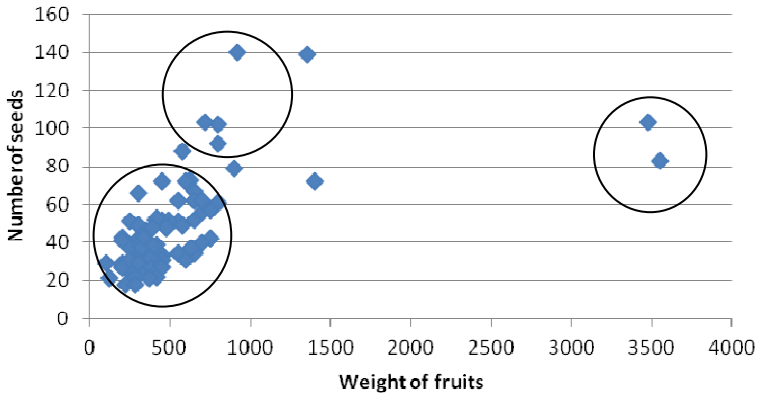

Fig. 3. Relationship between fruit weight and number of seeds of fruits in germplasm centers and seven districts

RESULTS AND DISCUSSION

The first five principal components explained 69%, 79%, and 76% of the accumulated variance of the total sampled germplasm collections (Table 2), samples collected from germplasm collecting centre (Table 3) and from seven districts (Table 4), respectively.

Table 2. Factor loading, eigen values and percentage of total (standardized) population variance explained by five factor model of 15 morphological characters

Morphological characters	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Comm unality
Trunk colour	-0.408	**0.789**	0.136	-0.008	-0.039	0.80
Trunk ramification	-0.100	-0.263	**0.709**	-0.031	-0.001	0.58
Suckering tendency	0.112	-0.222	**0.749**	0.299	0.077	0.71
Colour of young branches	-0.096	-0.285	**-0.447**	0.206	0.182	0.36
Leaf blade shape	0.339	**-0.630**	0.127	0.107	0.034	0.54
Average Leaf width [mm	0.369	**-0.776**	0.077	0.151	-0.017	0.76
Petal outer colour	-0.419	-0.087	0.038	**-0.518**	-0.113	0.46
Location of fructification	-0.056	0.173	-0.246	**0.751**	-0.346	0.77
Fruit length [mm	**0.659**	0.593	0.133	-0.029	-0.002	0.80
Fruit diameter [mm]	**0.702**	0.358	0.115	-0.091	0.119	0.65
Weight of ripe fruit [g]	**0.742**	0.312	0.017	-0.019	0.113	0.66
Peduncle length [mm]	-0.389	**0.589**	0.339	0.264	-0.124	0.69
Seed weight per fruit [g]	**0.905**	0.075	-0.055	-0.049	-0.068	0.83
Number of seeds	**0.876**	0.085	-0.083	-0.026	-0.164	0.80
Pulp taste	-0.099	0.194	-0.050	0.198	**0.893**	0.88
Eigen values	3.86	2.83	1.53	1.12	1.05	
Total population Variance explained (%)	26	19	10	7	7	
Cumulative total population Variance explained (%)	26	45	55	62	69	

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

Table 3. Eigen values and percentage of total (standardized) population variance explained by five factor model of 15 morphological characters for germplasm collected from germplasm collecting centers.

Factor	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
Eigen value	4.227	1.929	1.532	1.478	1.097
Total population Variance explained (%)	33	15	13	11	8
Cumulative Total population Variance explained (%)	33	48	60	71	79

In five factor model each character contributed a high percentage variation. The first principal component explained 26% of the variation and was associated with fruit characters such as length, diameter and weight of ripe fruit, weight and number of seeds per fruit. The second principal component explained 19% of the variation and was associated with trunk colour, leaf blade shape, average leaf width and peduncle length. The third principal component explained 10% of the variation and was associated trunk ramification, suckering tendency and colour of young branches. The 4^{th} and 5^{th} principal components explained variation 7% each. The 4^{th} principal component was associated with petal outer colour and location of ramification whereas 5^{th} principal component was associated with pulp taste (Table 2).

Table 4. Eigen values and percentage of total (standardized) population variance explained by five factor model of 15 morphological characters for germplasm collected from seven districts.

Factor	Factor1	Factor2	Factor3	Factor4	Factor5
Eigen value	3.706	3.194	1.638	1.061	1.041
Total population Variance explained (%)	26	23	12	8	7
Cumulative Total population Variance explained (%)	26	49	61	69	76

The characters can be used in the *A. muricata* improvement programmes as the knowledge of correlation among characters is useful in designing an effective breeding programme (Asudi *et al.,* 2010). Fruits collected from Giradurakotte Germplasm collecting center contain large fruit weight (3.355 kg and 3.48 kg) and fruits which contain less number of seeds are important for agricultural industries. These germplasms should be conserved and should be promoted for utilization. Fruits contain higher number of seed and low fruit weight can be utilized for future breeding programmes and need attention to conservation of those germplasm too.

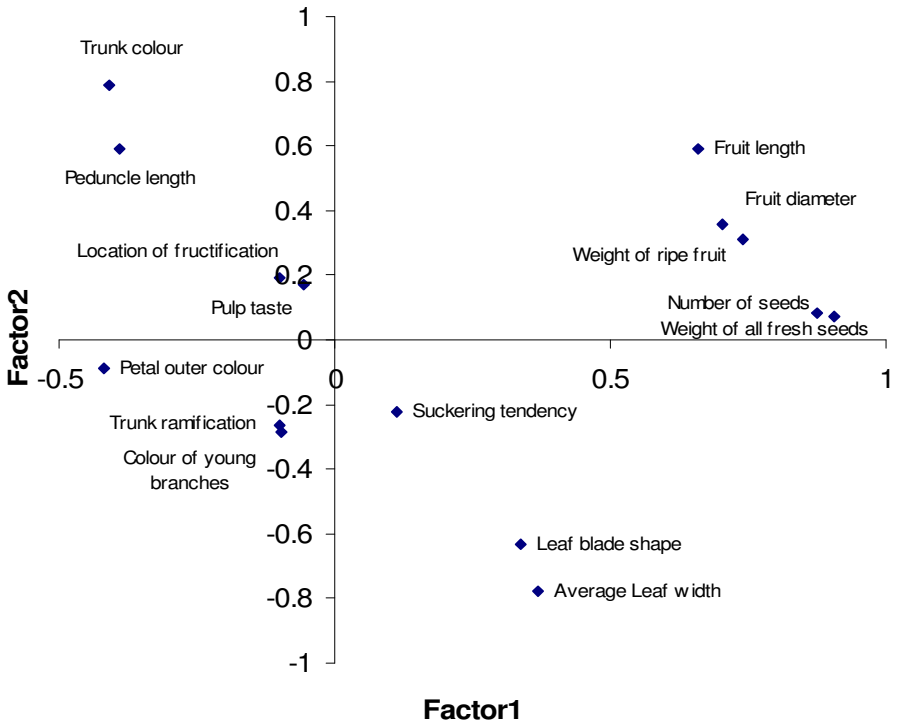

Fig. 4. Correlation among characters associated with first and second factor competent

Cluster analysis

The germplasm of *A. muricata* was grouped into nine distinct clusters at 1.00 linkage distance with each cluster containing accessions that are morphologically similar (Fig. 5). Distinct characters were indicated in Table 5. This indicates that the present collection probably contain duplicates. Molecular and chemical characterization of these individuals along with morphological characterization will provide the basis for utilization of fruits and conservation of individuals.

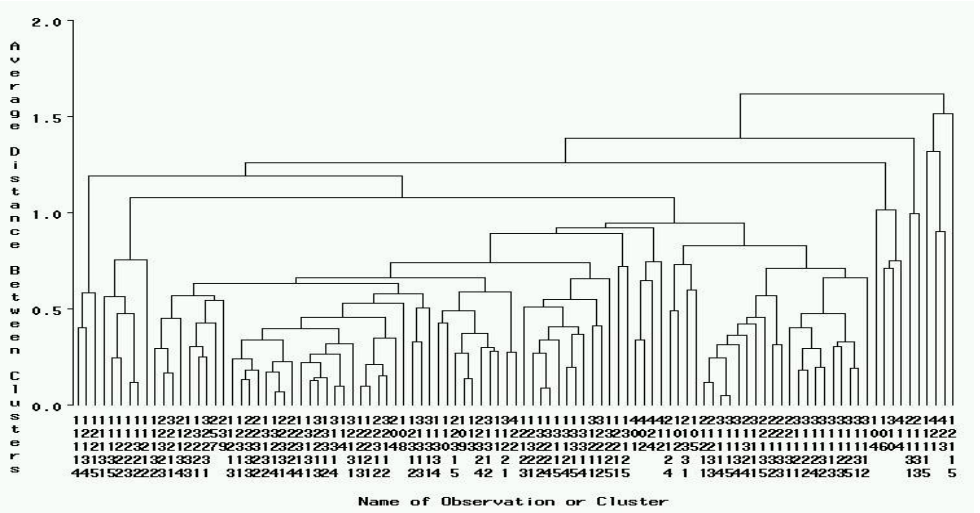

Fig. 5. Dendrogram obtained from average linkage cluster analysis for all accessions

Table 5. Cluster composition of all accessions.

Cluster	Accessions and distinct character
1	Cluster 1 is consisted of accession 12115 from Hambantota district with highest number of seeds and 15% seed / fruit weight ratio. This accession is not consumed and need chemical analysis because owners stated that once consumed people get fever and allergy conditions.
2	Cluster 2 comprises two accessions, 423 and 421 from Giradurukotte Germplasm collecting center. Those fruits are very large 3.355 kg and 3.48 kg. Seed / fruit weight ratio is 3–6%. The Officer in Charge of the Germplasm Center of Giradurukotte informed that those germplasm were collected in the year 1996 from the dry zone of Sri Lanka. These accessions can be used for the future breeding programs.
3	Cluster 3 comprises only one accession, 11115 which was collected from Kekirawa DS division of Anuradhapura district. It consists of medium sized fruit and seed / fruit weight ratio is 12%.
4	Cluster 4 comprises three accessions 21133, 21131. The plants located in the wet zone areas of Gampaha district, Biyagama DS division, Meegaswaththa GS division. Small size fruit, seed / fruit weight ratio is 8-9%.
5	Cluster 5 comprises the accessions 106, 310, 414 from germplasm collections centers at HORDI, Gannoruwa, Fruit Research Institute, Horana and Giradurukotte. Medium size fruits, seed / fruit weight ratio is 5-14%.
6	Cluster 6 comprises the accession 104, from germplasm collections centers in HORDI, Gannoruwa. Fruit is medium, seed / fruit weight ratio is 9%.
7	Cluster 7 comprises all other accessions which did not group in to other clusters.
8	Cluster 8 comprises accessions 11212, 11322, 11223, 11222, 11131, and consists of small size fruits in dry zone located in Anuradhapura district, seed / fruit weight ratio is 10-29%.
9	Cluster 9 comprises accessions 11114, 12134, 12215 those bears small size fruits in dry zone, districts where fruit weight to seed ratio is low (10-14%).

CONCLUSIONS

Results of the study revealed that five principle components from 15 characters explained 69% of total variability of *A. muricata* germplasm. Cluster analysis identified nine clusters with unique characters. Most prominent character is fruit weight to seed ratio. This factor is important in utilization of the fruit. Therefore, clusters which have higher and lower fruit weight to seed ratio are important to promote for utilization of breeding programmes and conservation of germplasms.

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