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Research Paper

Morphological, chemical and bioactive variation in selected Soursop germplasm

S.M.P.C. Padmini[1*], D.K.N.G. Pushpakumara[2] and R. Samarasekera[3]

[1]Sri Lanka Council for Agricultural Research Policy, Colombo, Sri Lanka
[2]Department of Crop Science, Faculty of Agriculture, University of Peradeniya, Sri Lanka
[3]Industrial Technology Institute, Colombo, Sri Lanka
*Corresponding Author: chandra_smp@yahoo.com

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Abstract: Soursop (*Annona muricata* L.) is an underutilized tropical fruit tree in Sri Lanka. The crop has shown variability for its fruit morphology and chemical constituents. Despite the importance of *A. muricata* collection, characterization and improvement of its germplasm has been limited in Sri Lanka, hindering its effective conservation through utilization. This study identified the morphological, chemical and bioactive variation of the selected germplasm of *A. muricata.* A multistage sampling survey was conducted in homegardens in the dry, intermediate and wet zones and random representative samples were collected from existing germplasm collections at the Research Centres in Sri Lanka. Morphological variations of *A. muricata* was observed in a total of 315 samples collected from three climatic zones and 133 samples obtained from germplasm collections at Research Centres. Although 45 morphological characters were recorded from 448 accessions only 15 characters were subjected to Principal Component Analysis, Factor Analysis and Cluster Analysis, due to lack of variability and low coefficient of variation. A dendrogram showed nine distinguishable clusters at 1% linkage distance. Further, cluster analysis by using chemical characters (total soluble solids, pH, reducing sugars, and DPPH free radical scavenging activity) showed three distinguishable clusters at 0.5% linkage distance. Accessions 104 and 423 with different traits such as fruit weight (0.7 - 3.5 kg), seeds (5 - 8 seeds / 100 g of fruit pulp), high total soluble solids, and moderate free radical scavenging capacity, can be considered as morpho-types with superior characters for selection and improvement of *A. muricata* in Sri Lanka.

Keywords: *Annona muricata*, bioactive characters, morphology, Sri Lanka, underutilized fruit

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Introduction

Soursop (*Annona muricata* L.) is a common tropical fruit tree in Sri Lanka belonging to the family Annonaceae (Anon, 2007a; Anon, 2007b). The fruits are consumed widely as fresh fruits and also used to prepare products such as beverages, wine, jellies, jams, fruit-butter preserves and puree (Abbo *et al.,* 2006). The fruit as well as plant parts are used for medicinal preparations. The fruits contain vitamins, minerals and bioactive chemical substances. However, commercial utilization of *A. muricata* is poor in Sri Lanka and is categorized as an underutilized fruit tree species

(Heenkenda *et al.,* 2011). Further, research on *Annona* species in Sri Lanka has been scanty (Heenkenda *et al.,* 2011; Thantirige, 2001; Padmini *et al.,* 2013), although this underutilized fruit tree has the potential to diversify the fruit basket and agroforestry farming systems (Bowe *et al.,* 2010; Anon, 2002a). The species has been considered as an excellent source of energy and medicine, and the trees are easy to grow, need little attention and have a commercial life of about 15 years (Anon, 2002a; Anon, 2002b).

In Sri Lanka, *A. muricata* is found in some of agricultural ecosystems such as, homegardens, horticultural farms and small crop-holdings. The genetic variability in *A. muricata* is mainly due to cross pollination, mutations, selections and environmental factors. Many cultivars, such as fibreless Soursop in Cuba, those with high productivity and high fruit weight in Costa Rica, and sweet and acid pulp types in Colombia, El Salvador, and Venezuela (Junior *et al.,* 1999). Accordingly, morphological and chemical characterization of the species is useful to identify different morpho-types and proper utilization of genetic resources in plant breeding programmes. Climate and soil also make a significant influence on the variation in growth, fruit set, fruit size and quality of fruits (Pinto *et al.,* 2005). Sri Lanka consists of three main climatic zones namely, dry, intermediate and wet, which are categorized based on rainfall (Panabokke, 1975). Biological evolution could take place within different climatic conditions and any genetic changes or modifications resulting in from adapting to different environments in a population are inherited over several generations (Karunagoda, 2009).

The fundamental objective of collecting plant genetic resources is to capture the maximum amount of genetic variation in the smallest number of samples (Upadhyaya *et al.,* 2008; Nass *et al.,* 2012). Exploration of *A. muricata*, which has vigorous and prolific plants that are resistant to cold condition, with abundant flowers, fertile pollen and excellent fruit quality with regards to flavour, pulp texture, fruit size and low number of seeds, are important to better utilization of fruits (Pinto *et al.,* 2005). Despite the importance of the crop, and although some germplasm has been collected in different research centers (Anon, 2007a; Anon 2007b), no morphological, chemical and bioactive characterization has been carried out for *A. muricata* in Sri Lanka. Therefore, the objectives of the study were to (i) identify morphological, chemical and bioactive variation in selected *A. muricata* germplasm in homegardens and the collection of germplasm in research centers in Sri Lanka and the contribution of such traits to the variation of *A. muricata* population, and (ii) identify potential parental stocks within the group for utilization and improvement in future breeding programs.

Materials and Methods

Sampling of germplasm

Random representative samples of Soursop fruits were collected from four *ex situ* collections of *A. muricata* germplasm in Sri Lanka located in the Regional Agricultural Research and Development Centre (RARDC) at Makandura (62 samples; intermediate zone), Agricultural Research Station (ARS) at Girandurukotte (24 samples; dry zone), Horticultural Crops Research and Development Institute (HORDI) at Gannoruwa (15 samples; wet zone), and Fruit Crops Research and Development Institute (FCRDI) at Horana (12 samples; wet zone) to observe the morphological variations. A multistage sampling method was used to collect morphological information of *A. muricata* plants. Three main climatic zones namely, dry zone (DZ; <1,750 mm annual rainfall), intermediate zone (IMZ; 1,750-2,500 mm annual rainfall) and wet zone (WZ; >2,500 mm annual rainfall) were considered in the first stage. A random selection of administrative districts within each climatic zone was done in the second stage depending on the size, homogeneity and heterogeneity of the climatic zones. Accordingly, three districts from the DZ (Anuradhapura, Polonnaruwa, and Hambantota), two from the WZ (Kalutara and Gampaha) and two from the IMZ (Puttlam and Kurunegala) were selected for the study. The third

stage consisted of three randomly selected Divisional Secretariat Divisions (DSDs) from each district, followed by three randomly selected Grama Niladari Divisions (GNDs) from each DSD (fourth stage). At the fifth stage, five homegardens (HGs) were selected randomly in each GND to select *A. muricata* trees. During the visits, one *A. muricata* tree was chosen randomly in cases where more than oone plant was present in a HG.

Sampling technique and data collection

Due to the non-availability of a descriptor list for *A. muricata*, the list compiled for *A. cherimola* Mill. (Cherimoya) by the International Plant Genetic Resources Institute (IPGRI, 2008) was used in this study. Accordingly, 45 morphological characters were identified and assessed. Fourteen quantitative characters including leaf length, leaf width, petiole length, petal length, petal width, fruit length, fruit diameter, weight of ripen fruit, peduncle length, weight of all fresh seeds, number of seeds, seed length and seed width were measured. From each tree, 10 fully-expanded and healthy leaves, five flowers and five mature fruits were randomly selected for the measurements of their characters. Five seeds per fruit were used to measure seed characters. Thirty one qualitative characteristics were measured. The colour chart of the Royal Horticulture Society (RHS) was used to identify parameters such as trunk 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 at five min. after cutting the fruit. Tenacity of the seed in its epithelium was observed by cutting the seed and observing the seed coat and the firmness of epithelium by removing the cotyledon from seed coat. Non-parametric data were converted to scales as proposed by IPGRI in descriptors for *A. cherimola* (IPGRI, 2008).

In a previous study, nine different clusters were identified by using morphological characters from selected samples of accessions (Padmini *et al.,* 2013). Accordingly, accessions were selected from nine different clusters for chemical and bioactive analysis. The study was carried out at the laboratory of Herbal Technology Section of the Industrial Technology Institute in Colombo, Sri Lanka. Mature fruits were harvested at the same maturity stage and allowed to ripen (2 - 3 days) at room temperature (30 ± 2.0 °C). During ripening, chlorophyll concentration of the *A. muricata* fruit skin declined and its colour changed from dark green to light green (RHS 139A – 144A).

Chemical analysis of *Annona muricata* fruit juice

The pH and total soluble solids (TSS) of the *A. muricata* fruit pulp were determined as physico-chemical properties (Cardozo *et al.,* 2012). Fruit juice was prepared by manually squeezing a portion of ripened fruits, without adding water. The pH meter (Adwa, Romania), which was calibrated with a buffer solution of pH = 4 and 7 at the room temperature (30 ± 2.0 ^{o}C), was used to measure the pH of fruit juice in triplicate. The total soluble solids (TSS) of the fruit juice was determined at room temperature (30 ± 2.0 ^{o}C) by using a hand held refractometer (Kyowa, Japan) and expressed in ^{o}Brix. The same fruit juice, which was used for pH measurements, was used to measure the TSS in triplicates.

Determination of reducing sugar content of fruit pulp

Reducing sugar content of *A. muricata* fruit pulp was determined by using Fehling's method described in Sri Lankan Standard SLS 586 (SLSI, 1982). The *A. muricata* fruit pulp (4.0 g) in a thimble and introduced to a soxhlet apparatus. Petroleum ether (300 ml) was added into a 500 ml round-bottom flask fixed with the Soxhlet apparatus and a condenser. The flask was heated using a heating mantle at 50 °C, for 4 h. After Soxhlet extraction, The fruit pulp was air-dried in the thimble. The fat-free fruit pulp was dissolved in a small quantity of distilled water and heated at 50 °C to dissolve the fruit pulp. The sample was cooled and gravity filtered through No. 40 Whatman filter paper and the filtrate was collected in a clean and dry flask. The filtrate was made up to 100 ml. The fat-free *A. muricata* fruit pulp solution (50.0 ml) was placed into the burette and the titration was carried out while boiling the Fehling's solution (10 ml in 250 ml conical flask) until the colour just changed from blue to brick red. Then, the Methylene blue indicator (1.0 ml) was added without interrupting boiling. The titration was continued till the blue colour

disappeared to colourless and the burette readings were recorded. Titration was carried out in triplicate.

Extraction of fruit pulp

The fruit pulp was extracted by using ripened fruits of *A. muricata.* Mature fruits were collected from accession 204 of the germplasm collection center at the Regional Agricultural Research and Development Center, Makandura and allowed to ripen for 2-3 days at room temperature (30 ± 2 °C) prior to extraction. Total ethanol extraction was used to separate the organic compounds from the sample. The ripened fruit pulp (387.80 g) was soaked with 300 ml of ethanol for 24 h and stirred for 1 h using a mechanical stirrer at room temperature. The ethanol was filtered through a Celite bed packed in a sinter funnel under vacuum. The same fruit pulp was subjected to the second and third extractions with 300 ml of ethanol. The filtrates obtained after each extraction were combined and concentrated using a rotary evaporator under vacuum (Buchi rotary evaporator, pressure 40 mbar and temperature 50 °C) to obtain a dark brown gummy extract (61.33 g) (Roesler *et al.,* 2007).

Determination of bioactivity of *Annona muricata* fruit pulp - Antioxidant activity

The antioxidant screening of fruit pulp were conducted using the 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging assay (Matthaus, 2002) The DPPH reagent (40 ppm) was prepared freshly by dissolving 0.002 g of DPPH in methanol (50 ml). Ethanol extract of *A. muricata* fruit pulp of cluster seven (0.06 g) was dissolved in methanol (10 ml) to prepare 6,000 ppm stock solution. The fruit pulp ethanol extract stock solution (40, 35, 30, 25 and 20 µl), methanol (60, 65, 70, 75, and 80 µl) and DPPH (200 µl) were introduced to micro wells (total volume of each micro well was 300 µl) and kept in the dark for 30 min at room temperature (30 ± 2.0 °C). A vitamin C solution (50 ppm) was used as the standard for the assay Where DPPH (200 µl), vitamin C (36, 30, 24, 18 and 12 µl) and methanol (64, 70, 76, 82 and 88 µl) were introduced to the micro wells and kept in the dark for 30 min at room temperature. Methanol (300 µl) was used as blank assay to correct any colour absorbance by the solvent. The control assay was carried out without adding the sample or extracts.

Percentage of inhibition was calculated by comparing with absorbance value of control assay. Three replicates were used for eah concentration of the sample to measure the absorbance at λ = 517 nm using a Spectra max micro plate reader. The free radical scavenging activity (antioxidant activity) was expressed as the fifty percent inhibition capacity (IC_{50}).

Data analysis

Preriliminary analysis was carried out for 45 morphological characters and those characters that did not show a significant variation, i.e. coefficent of variation (CV) <25%, were not subjected to any further analysis.

Principal Component Analysis (PCA) and Factor Analysis (FA) were carried out using average values of characters. The results were used for Cluster Analysis (CA). The analysis was conducted using Statistical Analysis System (SAS) for Windows Version 8e (Anon, 1999). The PCA and FA were used for the total data set and those collected from the districts and germplasm collecting centers, separately. Eigen values greater than 1.00 and the cumulative proportion of variation were used to identify the number of principal components (Anon, 1999). The magnitudes of the component coefficients in Eigen vectors were used to measure the importance of each character to the particular principal component.

The CA was performed separately for accessions collected from germplasm collection centres and accessions from HGs, using the cluster procedure (method=average linkage) and the dendrogram with the tree procedure of SAS. Correlations among the characters were identified using two dimensional plotting of factors 1 and factor 2 and observing the positive relationship of fruit characters such as fruit length, fruit diameter, weight of ripen fruit, number of seeds and weight of all fresh seeds.

Results and Discussion

Based on preriliminary analysis, among the 45 characters, 22 morphological characters did not show any singificant variance in 448 accesions. Accordingly, those characters were not subjected to further analysis, leaving only 23 characters to be used in the primary analysis. Further, eight characters were ecluded from the analysis due to low coefficient of variation (CV <25%).

Finally, only 15 characters 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 ripen fruit [g], peduncle length [mm], weight of all fresh seeds / fruit [g], number of seeds / fruit, pulp taste were considered for the analysis.

Principal component analysis

The correlation matrix of the PCA indicated the interrelationship of the traits. Positive correlations were observed within fruit length, fruit diameter, weight of ripen fruit, peduncle length, trunk colour, weight of all fresh seeds / fruit and number of seeds / fruit. Based on the Eigen values (Anon, 1999) the first five PCs (Table 1) were considered the most significant variables in the data set. The PC-1 explained the fruit diameter, weight of ripen fruit, weight of all fresh seeds / fruit, and number of seeds / fruit, while PC-2 explained trunk colour, leaf blade shape, average leaf width, petal outer colour, fruit length and peduncle length. The PC-3 explained trunk ramification, suckering tendency and colour of young branches and PC-4 explained location of fructification. The PC-5 explained Pulp taste.

Table 1. Eigen values for five principal components for 15 morphological characters of germplasm centres and seven districts by using principal component analysis.

Characters	PC-1	PC-2	PC-3	PC-4	PC-5
Trunk colour	-0.208	0.469	0.110	-0.008	-0.038
Trunk ramification	-0.051	-0.156	0.575	-0.029	-0.001
Suckering tendency	0.057	-0.132	0.607	0.283	0.076
Colour of young branches	-0.049	-0.169	-0.361	0.195	0.178
Leaf blade shape	0.173	-0.375	0.102	0.101	0.034
Average Leaf width [mm]	0.188	-0.461	0.062	0.142	-0.017
Petal outer colour	-0.214	-0.052	0.031	-0.489	-0.111
Location of fructification	-0.028	0.103	-0.199	0.709	-0.338
Fruit length [mm]	0.336	0.352	0.107	-0.027	-0.002
Fruit diameter [mm]	0.358	0.212	0.093	-0.086	0.116
Weight of ripen fruit [g]	0.378	0.185	0.013	-0.018	0.111
Peduncle length [mm]	-0.199	0.350	0.275	0.250	-0.121
Weight of all fresh seeds / fruit [g]	0.461	0.044	-0.045	-0.046	-0.067
Number of seeds	0.446	0.051	-0.067	-0.025	-0.160
Pulp taste	-0.051	0.115	-0.041	0.187	0.873

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

Factor analysis

In FA, each character in the five-factor model contributed a high percentage variation (Table 2). The first factor explained 26% of the variation and associated with the fruit characters such as length, diameter and weight of ripen fruit, weight and number of seeds / fruit while the second factor explained 19% of the variation and associated with the trunk colour, leaf blade shape, the average leaf width and peduncle length. The third factor explained 10% of the variation and associated with the trunk ramification, suckering tendency and colour of young branches. The fourth and fifth factors each explained 7% of the variation. The fourth factor was associated with the petal outer colour and location of ramification whereas the 5th factor was associated with the pulp taste (Table 2).

Table 2. Factor loading, Eigen values and percentage of total population variance explained by the five-factor model of 15 morphological characters

Morphological characters	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Communality
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 ripen 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
Seeds weight / fruit [g]	0.905	0.075	-0.055	-0.049	-0.068	0.83
Number of seeds / fruit	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 the selected characters of each factor

Factor analysis is more concerned with explaining the covariance structure of the variables than explaining the variances. The first five factors explained 69% of the accumulated variance of the total sampled germplasm collections and seven districts (Table 2). Communality estimates, which were noted in Table 2, explain the proportion of variance of variables accounted for the common factor (Anon, 1999).

Strong correlations were observed between fruit characters such as fruit length, fruit diameter, weight of ripen fruit, number of seeds and weight of all fresh seeds (Figure 1). The knowledge of correlation among characters is useful in designing an effective breeding programme (Padmini *et al.,* 2013; Asudi *et al.,* 2010). As per multivariate analysis based on genotypes of traits, the strong correlation between fruit characters and seed traits are coordinated by close genes (Asudi *et al.,* 2010).

Fruits collected from Girandurukotte germplasm collecting centre contained the highest fruit weight (3.35 kg and 3.48 kg).and contained relatively less number of seeds, which are important for agri-based industries These germplasm should be conserved and promoted for utilization. Fruits containing higher number of seeds and low fruit weight can be utilized for future breeding programmes and also need attention for conservation. Small, sweet Soursop fruits are recommended for the fresh market, while large acidic ones are more suitable for the processing industry (Pinto *et al.,* 2005). The commercial growers and breeders are interested in seedless or low number of seeds in *A. muricata* fruits (Pinto *et al.,* 2005). The genotypes having commercially important characters are good sources of germplasm for breeding programmes. Due to hermaphrodite and protogynous nature of the *A. muricata* flower, vegetative propagation is important to protect the genotype.

Cluster analysis

The germplasm of *A. muricata* was grouped into nine distinct clusters at 1.00 linkage distance with unique characters and each cluster contained accessions that are morphologically similar (Figure 2). The distinct characters of clusters are shown in Table 3. Molecular characterization of these individuals along with morphological

characterization will provide the basis for utilization of fruits and conservation of individuals. The distance of cluster separation indicated the variation level of the clusters. The clusters that separated at higher distance indicated that the species level separation and divisions within the species are low.

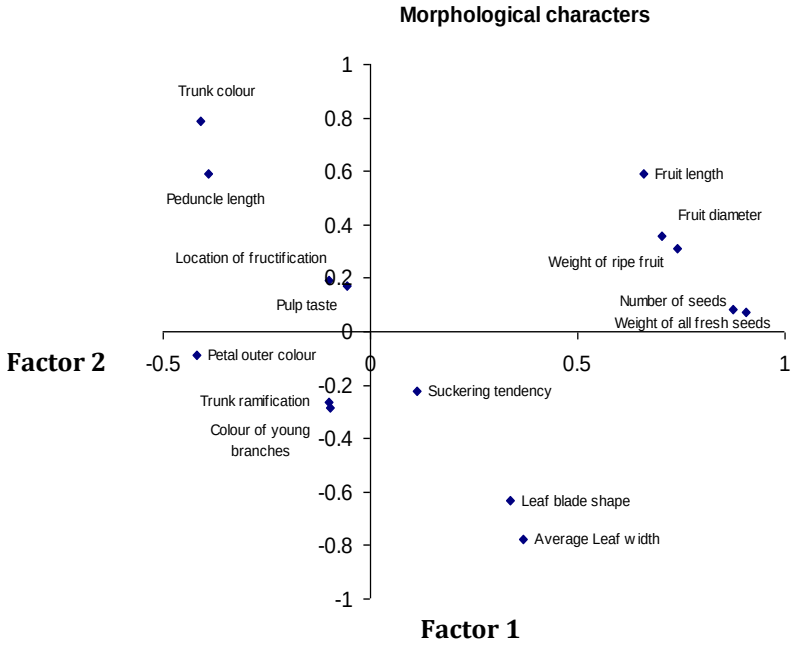

Figure 1. Correlation among characters associated with first and second factors.

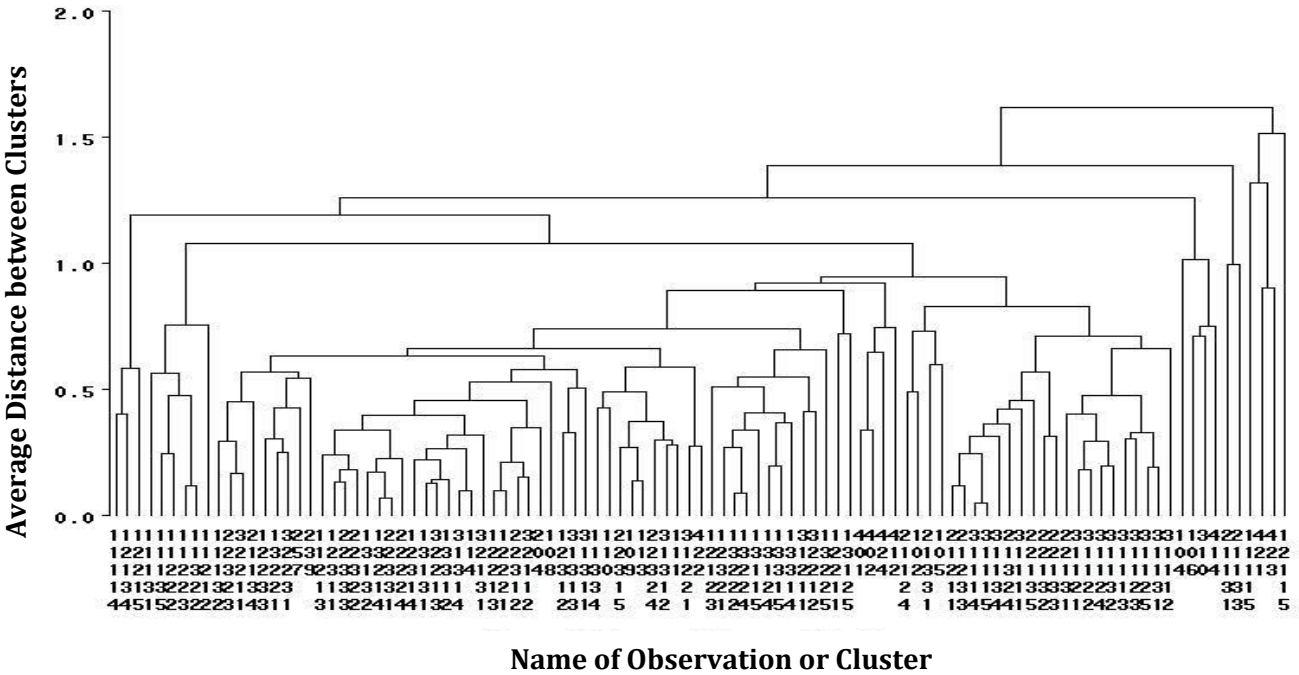

Figure 2. The average linkage cluster analysis with all accessions.

Table 3. Cluster composition of all accessions

Cluster	Accessions and distinct character
1	Cluster 1 consisted of accession 12115 from Hambantota district with highest number of seeds and number of seeds / 100 g of fruit pulp was 15. This accession's fruits were not consumed and need chemical analysis because owners stated that once consume they get caught to fever and allergy conditions.
2	Cluster 2 comprises with two accessions, 423 and 421 from Girandurukotte germplasm collection centre. Their fruits were very large (3.35 kg and 3.48 kg). Number of seeds / 100 g of fruit pulp were 3 - 6. The Officer in Charge of the germplasm collection centre, Girandurukotte informed that those germplasm were collected in 1996 from the DZ 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 in Anuradhapura district. It consists of medium sized fruit and number of seeds / 100 g of fruit pulp were 12.
4	Cluster 4 comprises with two accessions 21133, 21131. The plants located in the WZ areas in Gampaha district, Biyagama DS division, Meegaswaththa GS division. Small size fruit, number of seeds / 100 g of fruit pulp was 8-9.
5	Cluster 5 comprises with accessions 106, 310, 414 from germplasm collection centre at HORDI, Gannoruwa, FCRDI, Horana and Girandurukotte respectively. These fruits were medium in size; number of seeds / 100 g of fruit pulp was 5-14.
6	Cluster 6 comprises the accession 104, from germplasm collection centre in HORDI, Gannoruwa. Fruit was medium; number of seeds / 100 g of fruit pulp was 9.
7	Cluster 7 comprises all other accessions which did not group in to other clusters.
8	Cluster 8 comprises with accessions 11212, 11322, 11223, 11222, 11131 and consists of small size fruits in DZ located in Anuradhapura district, number of seeds / 100 g of fruit pulp was 10-29.
9	Cluster 9 comprises with accessions 11114, 12134, 12215 and located in DZ, bear small size fruits where number of seeds / 100 g of fruit pulp was 10-14

Factor analysis for chemical and morphological characters

A six-factor model was observed in the effort to identify the correlation among chemical and morphological characters using FA. The first factor explained 32% of the variation and associated with the chemical character, TSS and morphological characters such as trunk ramification, colour of young branches, length, diameter and weight of ripe fruit, weight and number of seeds per fruit. The second factor explained 18% of the variation and associated with the chemical character, reducing sugar content and morphological characters such as trunk colour, suckering tendency, leaf blade shape and pulp taste. The third factor explained 14% of the variation and associated with average leaf width, location of fructification and peduncle length. The fourth factor explained 13% variation and associated with chemical characters such as antioxidant activity and pH while the fifth and sixth factors explained 8% and 7% variations, respectively. Communality estimates showed the proportion of variance of variables accounted for the common factor.

Cluster analysis for chemical and morphological characters

Three groups were observed in the CA (Figure 3) by considering chemical and morphology characters. The clusters were grouped into three distinct clusters at 1.0 linkage distance with unique characters and each cluster containing accessions having similar traits. The accession 423 (collected from germplasm collection centre, Girandurukotte) clustered into a separate group. The fruits of the accession 423 showed the highest average fruit weight (3,550 ± 100 g) and the lowest number of seeds / 100 g of fruit pulp (5 ± 1.7). Fruits from this accession recorded a TSS of 17 ^{o}Brix, reducing sugar content of 4.44% ± 0.45 and a DPPH free radical scavenging activity of IC_{50} 591 ± 5.84 ppm. Therefore, the accession 423 had superior quality characters out of the three clusters (Table 4), and this cluster can be recommended for the breeding programmes.

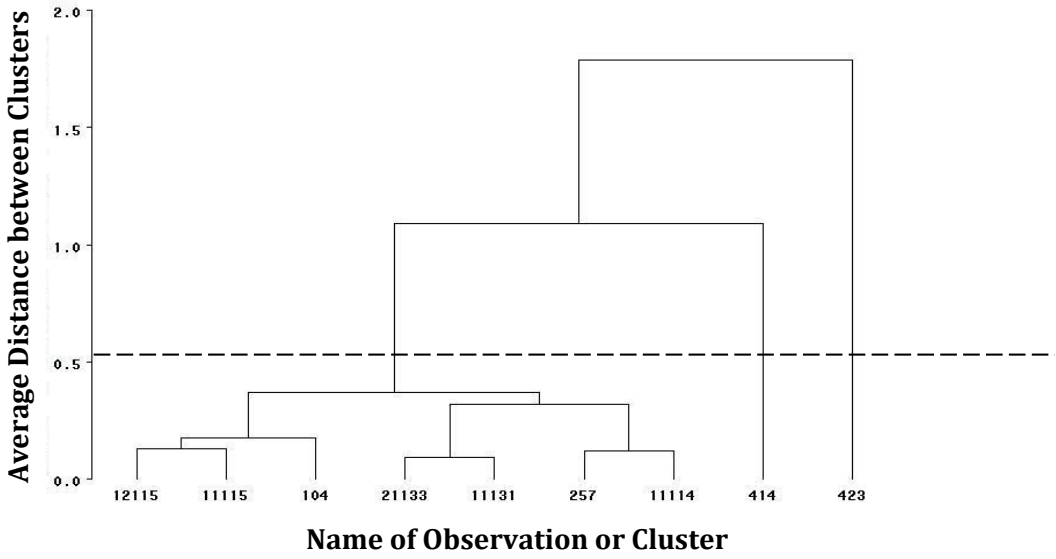

Figure 3. The average linkage cluster analysis for selected accessions by considering chemical and morphological characters

Accessions 414 (collected from germplasm collection centre, Girandurukotte) also clustered into a separate group based on morphological characteristics with accessions 106 and 310 (collected from germplasm collection centres in Gannoruwa and Horana, respectively), which are from different climatic regions. This close relationship among plants may be due to the historical relationship in sharing common ancestors. The fruits of the accession 414 showed an average fruit weight of 720 ± 80 g and 12 ± 2 numbers of seeds / 100 g of fruit pulp. In the physico-chemical, biochemical and bioactivity analysis, accession 414 recorded a TSS of 16 ^{o}Brix, reducing sugar content of 4.5% ± 0.47 and the lowest DPPH free radical scavenging activity (IC_{50} as 2452 ± 3.64 ppm). This cluster can also be considered for the breeding programmes with other positive characters.

Accessions 12115, 11115, 104, 21133, 11131, 257 and 11114 were grouped into one cluster in the CA (Figure 3). The morphological variations of those seven accessions clustered them into seven different groups. The accession 104, located in the germplasm collection centre at Gannoruwa grouped into a separate cluster and exhibited the highest reducing sugar content (5.82 ± 0.38%) among the selected accessions, TSS of 15 ^{o}Brix, and the DPPH free radical scavenging activity of IC_{50} as 592 ± 2.28. The average weight of fruit in the accession 104 was 700 ± 20 g and the number of seeds / 100 g of fruit was 8 ± 3. The accession 104 had small sized fruits with low number of seeds and sweet characters (Table 4). Therefore, the accession 104 is important to be used in breeding programmes and for conservation purposes due to the better fruit characteristics.

The highest DPPH free radical scavenging activity was detected in accessions 21133 and 11131 having IC_{50} of 224 and 316 ppm, respectively, and they grouped into a separate cluster. Fruits of these accessions showed a TSS content of 13 and 14 °Brix and reducing sugar content of 4.06% and 4.64%, respectively. The high DPPH free radical scavenging activity and low reducing sugar content of the fruits are important for medicinal purposes. The average fruit weight of these accessions was 300 ± 20 g and the number of seeds / 100 g of fruit was 8 ± 4.

Accessions 257 and 11114 were grouped together having relatively low fruit weights (370 ± 80 g and 200 ± 60 g, respectively) and moderate DPPH free radical scavenging activity (743 ± 3.36 and 844 ± 2.71 ppm, respectively). The accessions 12115 and 11115 grouped together having fruit weights 920

± 50 g and 800 ± 80 g and moderate DPPH free radical scavenging activity of 843 ± 2.84 and 733 ± 3.29, respectively.

Cluster analysis done using morphological, physico-chemical, biochemical and bioactivity characters showed that accessions 423 and 104 carry important traits to become superior commercial cultivars. *Annona muricata* fruits from accession 423 are large in size (fruit weight higher than 2.5 kg) having low number of seeds (5 ± 1.7 seeds/100 g of fruit pulp) and can be recommended to use in processing industry. The fruits from accession 104 are sweet and small in weight (0.8 - 2.5 kg) with low number of seeds (8 ± 3 seeds/100 g of fruit pulp) and can be recommended for fresh consumption. A group of different types of individuals of the same species in a population is considered as morpho-type. Accordingly accessions 423 and 104 can be considered as morpho-types with superior characters (Table 4).

Table 4. Superior cultivars of Sri Lankan *A. muricata* identified in the study

Characteristic	Accession Number	
	423	**104**
Average fruit weight (g)	3,550 ± 100	700 ± 20
No. of seeds / 100 g of fruit pulp	5±1.7	8 ± 3
pH	3.59 ± 0.0	3.78 ± 0.01
TSS (^{0}Brix)	17 ± 0.0	15 ± 0.0
Reducing sugar %	4.45 ± 0.45	5.82 ± 0.38
DPPH radical scavenging IC_{50} (ppm)	591 ± 5.86	592 ± 2.28

Note: Highlighted values of each column represented selected characters of each accession

Conclusion

The factor analysis based on morphological characterization revealed that five principal components namely, number of seeds and fruit weight, trunk colour, suckering tendency, location of fructification and pulp taste out of 15 characters studied, accounted for 69% of the total variability of collected *A. muricata* germplasm. Cluster analysis identified nine clusters with unique characters. The important morphological, physico-chemical, biochemical and bioactivity variations of the selected accessions were the fruit weight (0.7 - 3.5 kg), low number of seeds (5 - 8 seeds / 100 g of fruit pulp), high reducing sugar content (4.45 % - 5.82 %) and high TSS content of fruit pulp (15 - 17 °Brix). Accordingly, accessions 104 and 432 showed the best characters among those evaluated and these accessions are suitable for breeding programmes of *A. muricata*. The study identified different morpho-types of *A. muricata*. Further evaluation of the genetic diversity in *A. muricata* is suggested by using molecular techniques, to identify economically important morpho-types of the species.

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