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Research Article

Phenotypic diversity and clustering of germplasm accessions of cashew for utilization and conservation

A. C. Asna*, Jalaja S. Menon and M. S. Smitha

Cashew Research Station, Kerala Agricultural University, Madakkathara, Kerala, India-680651

*E-Mail: asna.ac@kau.in

Abstract

To promote the better utilization of genetic resources of cashew, 91 accessions maintained at Cashew Research Station, Madakkathara were assessed for 38 phenotypic traits. Frequencies of the phenotypic classes of each character were calculated. The Shannon diversity index (H') was used to estimate the magnitude of diversity. Polymorphism was high for tree habit (0.96), tree height (0.89), branching pattern (0.89), colour (0.80), ridges (0.93), apex (0.95), cavity (0.90) and weight (0.84) of cashew apple, attachment of nut to apple (0.85), flanks of nut (0.95) and cotyledonary grooves (0.99). The cluster analysis using the squared Euclidean distance method classified the accessions into 13 clusters at a 55 per cent similarity level. More than 50 per cent of the accessions were high yielding with moderate quality kernels. Grouping of the genotypes into agronomic groups revealed the existence of highly prolific accessions with high quality kernels that can be exploited for crop improvement programmes.

Key words: Cashew, Phenotypic diversity, Cluster analysis, Euclidean distance

INTRODUCTION

Cashew (*Anacardium occidentale* L.) belonging to the family Anacardiaceae, is an important dollar earning crop of India. According to Bailey (1958), *Anacardium* is a small genus of eight species indigenous to South America. The occurrence of a large number of wild species suggests that North-East Brazil is the site of origin for *Anacardium occidentale*, which is the only species in the genus that attained economic importance (Ascenso, 1986). Though cashew came to India as a gift from Portuguese travellers, it has now acclimatized to Indian conditions and is being widely cultivated mainly for its nutritious kernel in Kerala, Karnataka, Andhra Pradesh, Maharashtra, Tamil Nadu, Odisha, West Bengal and Goa (Salam and Peter, 2010).

As cashew is a highly cross-pollinated heterogeneous crop, there is great variability in vigour, productivity, sex ratio and fruiting behavior. This variability prevailing in cashew plantations offer a great scope of selection for improvement. Since the variability that exists in the cashew population is widespread, germplasm collection, evaluation

and selection are the primary breeding programmes. Considerable progress has been accomplished over the last 50 years in terms of developing high yielding varieties of cashew which exemplifies the importance of germplasm in crop improvement programmes. Keeping in view the changing global scenario research programme on cashew, genetic resources should be intensified to link the characters of accessions to meet the demand and challenge in the international market.

Cashew Research Station, Madakkathara is a research station under the Central Zone of Kerala Agricultural University at Madakkathara in the Thrissur district of Kerala, India. It is one of the 14 centers of the All India Coordinated Research Project on cashew and maintains a large germplasm collection of cashew. Clustering of these accessions based on morphological, biochemical or molecular markers will help in developing hybrid progenies with a desirable level of superiority. The utilization of diverse germplasm is important in a

breeding programme to improve the yield of the crop (Nandini *et al.*, 2020). The success of any hybridization programme, particularly in perennials depends upon the genetic divergence among the selected parental lines. Despite the merits of molecular and genetic markers, morphological descriptors in genetic diversity are still important when exploring the possibility of selecting germplasm for incorporation into breeding programmes (Sounigo *et al.*, 1997). Thus, phenotypic characterization is a pre requisite for the exploration of useful traits in plant breeding (Brandolini *et al.*, 2000). In this background, the present study which forms a part of an ongoing project "Germplasm collection, evaluation, characterization and cataloguing" at Cashew Research Station was taken up to facilitate the utilization of cashew genetic resources by assessing the diversity and clustering of germplasm accessions of cashew using phenotypic traits.

MATERIALS AND METHODS

Ninety-one clonal accessions of cashew comprising of nine exotic and 82 indigenous ones selected from the germplasm maintained by Cashew Research station, Madakkathara at a spacing of 4 m x 4 m formed the material for the study. Four grafts of all the accessions at the steady bearing stage of six years were evaluated for phenotypic characters.

Eight exotic accessions (IC 302045 to IC 302050, IC 250061 and IC 250062) were collected from Brazil, the native place of cashew. The indigenous accessions included 42 local germplasm collections (IC 302051 to IC 302054, IC 302069 to IC 302071, IC 302074 to IC 302083, IC 249792, IC 250126, IC 250127, IC 302044, IC 250079, IC 249789, IC 302094 to IC 302109, and IC 302111 to IC 302113), 13 germplasm accessions obtained from other centers (IC 249949 to IC 249954, IC 249928, IC 249961, IC 249920 from Karnataka, IC 302072 and IC 302116 from Andhra Pradesh, IC 302110 and IC 250047 from Maharashtra) and 28 hybrid lines (IC 302084 to IC 302093, IC 249809, IC 302055 to IC 302068, IC 250140, IC 250129, and IC 250128) developed at the station.

Data were recorded for 38 phenotypic characters including 30 qualitative and 8 quantitative traits using the minimum descriptors of cashew germplasm accessions developed by the Directorate of Cashew Research (Nayak *et al.*, 2014). This catalogue contains data on tree, leaf, apple, nut and kernel characters. It is essential to use the morphological descriptors to obtain basic information on existing morphological variability in cultivated species and their wild relative before the advanced plant breeding techniques are attempted in the genetic improvement of any species (Adebola and Morakinyo, 2006). To evolve improved cultivars, it is essential to systematically analyze the available morphological and genetic diversity.

The phenotypic frequency distribution of the characters was calculated for all the materials. The Shannon-Weaver

diversity index (H') was computed using phenotypic frequencies to assess the phenotypic diversity for each character. The Shannon-Weaver diversity index (1949) is as follows:

$$H = - \sum_{i=1}^n p_i \ln p_i$$

Where p_i is the proportion of accessions in the i^{th} class of an n class character and n is the number of phenotypic classes for a character. The standardized H' ranging from zero to one was obtained by dividing H by the \log_e of the total number of phenotypic classes as follows:

$$H' = \frac{H}{\ln n}$$

By pooling these traits across the accessions, the additive properties of H' was used to evaluate the genetic diversity of the traits between the accessions of cashew.

In order to better classify the accessions, all the 91 clonal accessions of cashew were subjected to cluster analysis based on complete linkage, Squared Euclidean distance method using the free online software 'Minitab' accommodating the phenotypic traits with high diversity index. A dendrogram depicting the grouping of genotypes into various clusters at different similarity levels was also drawn.

RESULTS AND DISCUSSION

The frequency distribution of 38 phenotypic traits for cashew accessions is presented in **Fig. 1**. The growth pattern of any perennial tree decides the subsequent architecture in the tree. In the case of cashew, the volume of tree canopy is very important as it indicates the bearing area and determines the tree spacing as well as population density per unit area (Ona *et al.*, 2017). The growth and canopy expansion rate also have an influence on pruning practices (Mangal, 2016).

The growth habit of cashew trees varied greatly with accessions and eco-types. Broadly tree growth habits can be classified into three *viz.*, trees with upright and compact canopy, a tree with an upright and open canopy and a tree with spreading canopy. Forty-three accessions had spreading growth habits followed by an upright and open canopy in 25 accessions and upright and compact canopy in 23 accessions (**Fig. 1**). The accessions with upright and compact canopy are suitable for high density planting as a greater number of trees can be accommodated per unit area. But the trees with spreading canopy cause interlocking of canopies of adjacent trees and are not suitable for this intensive cultivation. On a

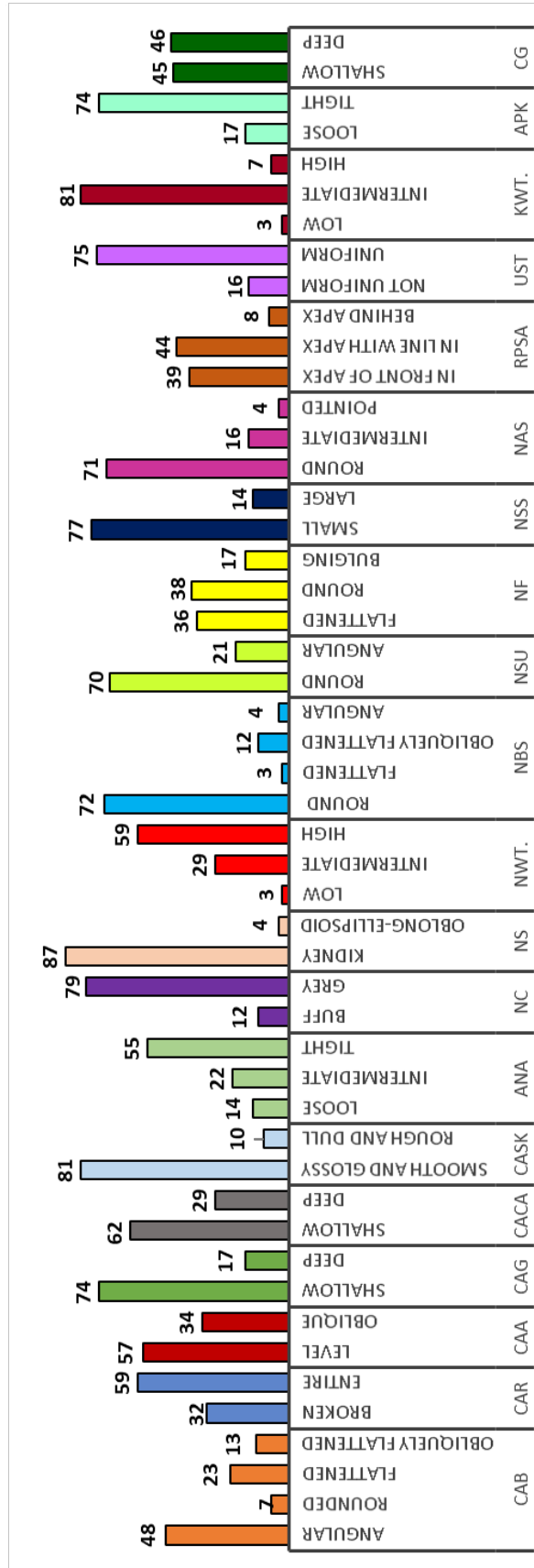
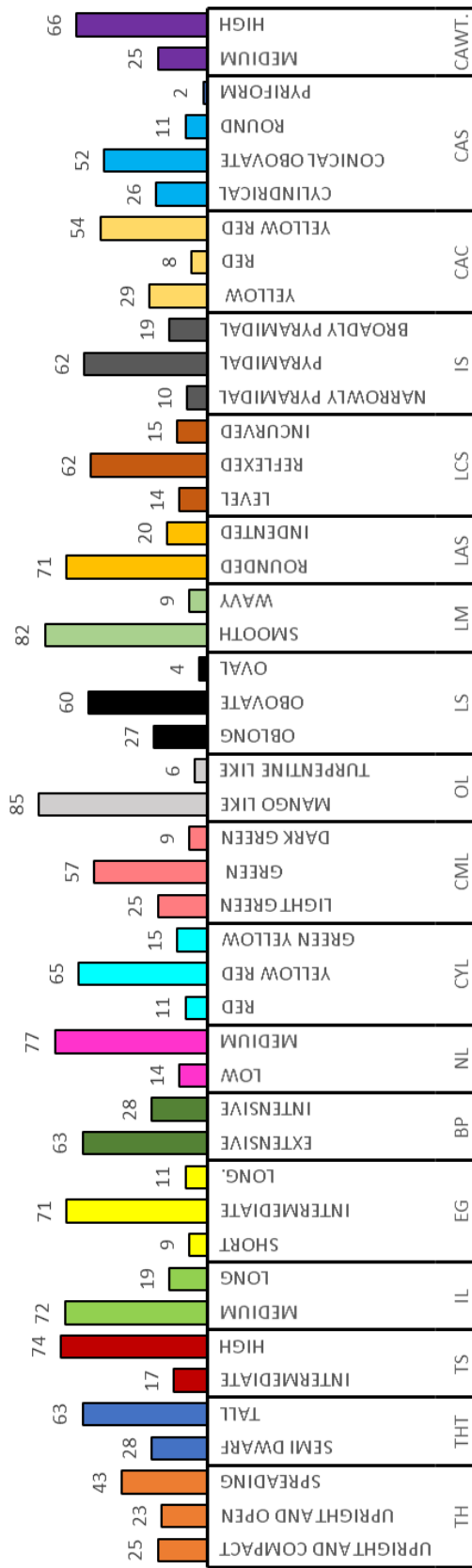


Fig. 1. Frequency distribution of 91 cashew accessions for 38 morphological traits (Shown in Table 1)

per tree basis, the productivity may be high in spreading type, on a per hectare basis it may be high in the upright and compact canopy (Salam and Peter, 2010). Upright and open canopy facilitates better penetration of solar radiation within the canopy.

Tree height is one of the indicators of the growth and development of cashew trees and is determined by plant genetics (Ona *et al.*, 2017). It always increases with the increasing age of the tree and is irreversible (Salam and Peter, 2010; Aliyaman and Indradewa, 2019). Based on the tree height, the 28 accessions of cashew with a height of 2.5 to 4 m were grouped as semi tall and 63 accessions above 4 m as tall (**Fig. 1**). None of the accessions was dwarf with a height below 2.5 m. A high spread of canopy (>6.0 m) was observed in 74 accessions, whereas 17 accessions exhibited tree spread between 3 m and 6 m (**Fig. 1**). The differential growth rates of cashew varieties/hybrids with respect to tree height and canopy spread were also reported by Naik *et al.* (1997), Swamy *et al.* (2000), Dorajeeroo *et al.* (2002) and Reddy *et al.* (2002).

Internode length of the twig was 1.0 cm to 2.0 cm and extension growth of twig was 9.0 cm to 19.0 cm in most of the accessions evaluated (**Fig. 1**). Compared with tall trees, the semi-tall trees showed no difference at 2 months, but at 8 months, seedlings were shorter with short internode length than those from tall trees. The branching pattern in cashew trees can be extensive or intensive (Damodaran *et al.*, 1965). Intensive type of growth pattern tends to give the bushy appearance to trees whereas, extensive type results in spreading tree habit. More than 60 per cent of intensive branches are seen in high-yielding trees whereas low-yielders possess less than 20 per cent intensive branches (Masawe, 2009). Even though, both types of branching were observed in all trees in the present study, extensive type dominated in the majority of the accessions.

Cashew flushes may vary in shape, size and color depending on the genotype (Masawe, 2006). Some cashew varieties can be easily identified by the type of leaves or flushes. Leaves may be red, yellow red, green yellow or purple. But young leaves were yellow red and mature leaves were green in most of the varieties evaluated (**Fig. 1**). Plant leaf area is an important determinant of light interception and consequently of transpiration, photosynthesis and plant productivity (Goudriaan and Van Laar, 1994). Leaf shape is a varietal character and can be broadly classified as oblong, obovate or oval. In the present study, the most frequently observed leaf shape was obovate with a smooth margin and round apex (**Fig. 1**). The leathery leaves of all the accessions produced a smell of mango when crushed except for 6 accessions where the odour of leaves was turpentine like (OL). Leaf cross section was reflexed in 62 per cent of accessions.

According to the descriptor list for cashew, the shape of inflorescence was observed as 'Broadly Pyramidal'

in 19 germplasm, 'Pyramidal' in 62 accessions and 'Narrowly Pyramidal' in the remaining 10 accessions (**Fig. 1**). Generally, the accessions with broadly pyramidal inflorescence shape had higher width compared to length. All the germplasm accessions of cashew showed loose inflorescence (open) with branches of cream-coloured flowers all around the main axis. In cashew, flower color may develop or change during the anthesis period (Weevers, 1952). Mangal (2016) and Sena *et al.* (1995) also observed the inflorescence shape of germplasm accessions of cashew. Unlike mango, which bears its crop on the past season's wood, the cashew produces flowers on the current season's flushes (Roe, 1994), after the growth flush at the end of the rainy season. However, some trees develop terminal inflorescences without any previous shoot growth (Ohler, 1979).

The cashew apple is an accessory fruit (sometimes called a pseudo or false fruit), a hypocarpium, that develops from the pedicel and the receptacle of the cashew flower (Varghese and Pundir, 1964). The cashew apple has a base at the point of attachment to the peduncle, ridges on its body and a cavity attached to the nut. Apples can be cylindrical, conical obovate, round or pyriform in shape depending on the variety. The base, apex, ridges, grooves, cavity and skin of cashew apple also showed great variation among the accessions. More than 50 per cent of accessions produced conical-obovate shaped apples with angular base, entire ridges, level apex, shallow grooves and cavity and smooth and glossy skin (**Fig. 1**). The cashew apples fell under two colours; red and yellow, although apples with intermediate colour (yellow red) were predominant among the accessions. The tannin content of red apples is relatively more compared to that in yellow apples (Salam and Peter, 2010). The apples of cashew differ in their size as well. Based on the weight of the cashew apple (CAWT), the accessions can be grouped into two *viz.*, 25 accessions with an apple weight of 27 to 52 g and 66 accessions with an apple weight more than 52 g (**Fig. 1**). The accessions with high apple weight (>50 g) are suitable for the processing industry for the extraction of juice and preparation of various processed products.

The nut consists of shell, testa and kernel. The shell has an outer epicarp, a mesocarp containing corrosive cashew nut shell liquid and an endocarp. The testa within the shell forms a protective cover to the kernel. The shape of cashew nut differed greatly with genotypes. Kidney shaped nuts were observed in all the accessions except in four, where oblong-ellipsoid shaped nuts were present. The base, apex, flanks and suture of nuts were found to be round shaped in the majority of the accessions (**Fig. 1**). Selections based on nut weight is an effective selection criterion for both quantitative and qualitative yield characters (Faluyi, 1987). In the present investigation, high nut (NWT) weight (>7 g) was observed in 65 per cent of the accessions. Only three accessions produced nuts with low weight *i.e.*, <5 g. Intermediate nuts were also

observed in 29 accessions. This shows the potential of germplasm accessions as a valuable source of variations to be used in further crop improvement programmes.

Cashew is mainly grown for its delicious and nutritious kernel. Unlike other crops, both the fruit as well as kernels are edible and nutritious in the perennial crop. Based on the weight of the kernel the accessions studied can be grouped as low (<1.2 g), intermediate (1.2 to 2.5 g) and high (>2.5 g). Only three accessions produced kernels with a weight less than 1.2 g. More than 80 per cent of the accessions belong to the intermediate kernel weight category (**Fig. 1**). Seven accessions produced kernels with a weight >2.5 g. The accessions with high kernel weight can be clonally propagated to release as improved cultivars to the farmers to meet the short-term need and/or undergo further evaluation across different locations through a long term national and/or regional cashew improvement programme. In addition, these materials can be used for the establishment of polyclonal seed gardens for both research and commercial uses.

The qualitative morphological diversity for individual traits over all accessions of cashew is shown in **Table 1**. The estimates of morphological diversity indices (H') for individual traits varied from 0.26 for nut shape to

0.99 for cotyledonary grooves, with an overall mean diversity index of 0.70. The standardized Shannon and Weaver (1949) diversity index was classified as low (0-0.33), intermediate (0.34 - 0.66) and high (0.67 - 1). The majority of the morphological traits *viz.*, habit, height and spread of the tree, internode length of twig, branching pattern, leaf apex shape, leaf cross section, colour of mature leaves, inflorescence shape, colour, shape, apex, grooves, cavity and weight of cashew apple, the shape of cashew apple base, attachment of nut to apple, nut weight, suture and flanks of nut, uniformity of shell thickness, attachment of peel to the kernel, cotyledonary grooves, the relative position of suture and apex were polymorphic having high genetic diversity (0.67 - 1). The only shape of nut had low genetic diversity. Leaf margin, stylar scar on nut, extension growth of twig, number of leaves per twig, colour of younger leaves, colour of mature nut shell, odour, shape and margin of leaves, kernel weight, the shape of nut apex and base and skin of cashew apple showed intermediate diversity index. The genetic diversity values for the qualitative traits revealed a wide genetic variability among the accessions evaluated. Thus, the diversity among cashew accessions was successfully revealed by the recorded traits. The Shannon index (H') increases with both the richness and the evenness of the species.

Table 1. Estimates of diversity indices for 38 morphological markers among cashew accessions

Traits	Diversity index (H')	Traits	Diversity index (H')
Tree habit (TH)	0.96	Ridges on cashew apple (CAR)	0.93
Tree height (THt.)	0.89	Cashew apple apex (CAA)	0.95
Tree spread (TS)	0.69	Grooves on cashew apple (CAG)	0.69
Internode length of twig (IL)	0.73	Cavity of cashew apple (CACA)	0.90
Extension growth of twigs (EG)	0.61	Skin of cashew apple (CASK)	0.49
Branching pattern (BP)	0.89	Attachment of nut to apple (ANA)	0.85
No. of leaves per twig (NL)	0.61	Colour of mature nut shell (NC)	0.56
Colour of young leaves (CYL)	0.45	Nut shape (NS)	0.26
Colour of mature leaves (CML)	0.79	Nut weight (NWt.)	0.68
Odour of leaves (OL)	0.35	Shape of nut base (NBS)	0.50
Leaf shape (LS)	0.57	Suture of nut (NSU)	0.77
Leaf margin (LM)	0.46	Flanks of nut (NF)	0.95
Leaf apex shape (LAS)	0.75	Stylar scar on nut (NSS)	0.61
Leaf cross section (LCS)	0.78	Shape of nut apex (NAS)	0.57
Inflorescence shape (IS)	0.75	Uniformity of shell thickness (UST)	0.67
Cashew apple colour (CAC)	0.80	Kernel weight (KWt.)	0.37
Cashew apple shape (CAS)	0.73	Attachment of peel to kernel (APK)	0.69
Cashew apple weight (CAWt.)	0.84	Cotyledonary grooves (CG)	0.99
Shape of cashew apple base (CAB)	0.83	Relative position of suture and apex (RPSA)	0.84

Average diversity index: 0.70

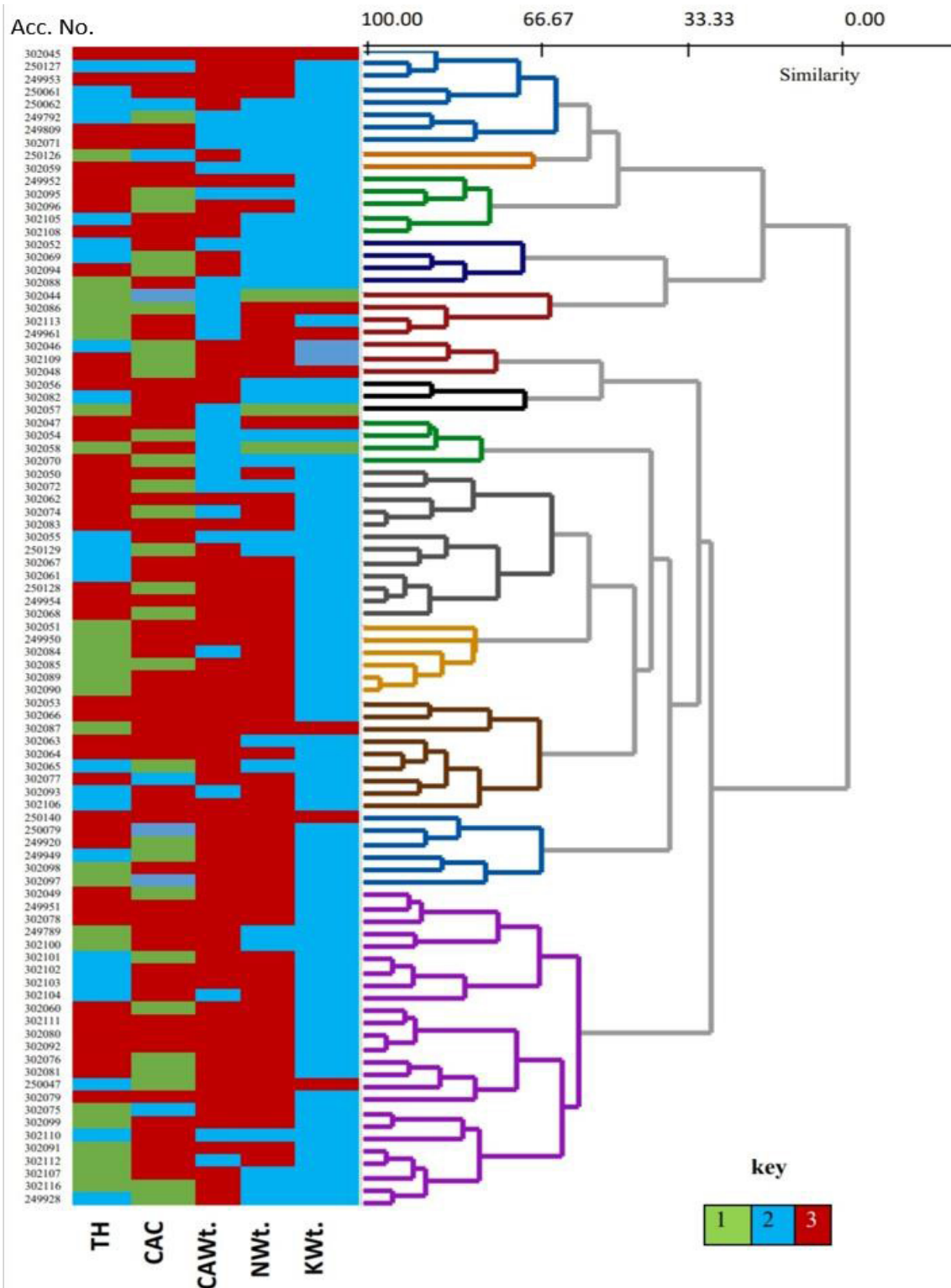


Fig. 2. Phenotypic variability and dendrogram based on 24 phenotypic traits of cashew accessions
Legends for the characters and key

TH: Tree habit (1-Upright and compact, 2-Upright and open, 3- Spreading); CAC: Colour of cashew apple (1-Yellow, 2-Red, 3-Yellow red); CAWt: Weight of cashew apple (1-Low(<27g), 2-Medium (27-52g), 3-High (>52g)); NWt: Nut weight (1-Low (<5g), 2-Intermediate (5-7g), 3-High (>7g)); KWt: Kernel weight (1-Low (<1.2g), 2-Intermediate (1.2-2.5g), 3-High (>2.5g))

Germplasm accessions of cashew were grouped into clusters using 24 phenotypic traits having high genetic diversity following the method of complete linkage Squared Euclidean distance. Clustering of accessions resulted in 13 distinct clusters (**Fig. 2**) at 55 per cent similarity level with considerable diversity within each cluster. The size of the individual clusters varied considerably. The largest cluster contained 25 accessions (**Fig. 2**). But clustering did not differentiate the accessions strictly according to the region of collection. Even the exotic collection, clusters in different groups. The hybrids developed by the station were spread across 13 different clusters that underlined the very diverse nature of the individual hybrids.

The candidate accessions can be divided into groups to facilitate the assessment of distinctness by using the characteristics like colour of young leaves, leaf shape, colour of mature apple, cashew apple shape, nut weight and shelling percentage, which are known from experience not to vary, or to vary only slightly, within a variety and which in their various states are evenly distributed across all varieties in the collection (Salam and Peter, 2010). Kanukullaya (2018) observed significant variations in the mean performance of 20 cocoa genotypes for 13 traits which revealed that the germplasm collections being maintained have high breeding value.

An overview of the phenotypic (genetic) variability of the accessions with respect to tree habit, cashew apple weight, cashew apple colour, nut weight and kernel weight are presented in **Fig. 2**. This trend does not only reflect enormous variability that could exist in a typical cashew field but brought into light the level of redundancy in cashew farms in terms of yield performance. Three sets of genotypes, one with high quality kernels and average nut weight; the second group with high yielding and moderate quality kernels and the third set having highly prolific fruiting types, were identified from this evaluation to facilitate the efficient utilization of better candidates

Seven accessions *viz.*, IC302045, IC250140, IC302086, IC302048, IC302047, IC302087 and IC250047, produced high quality kernels with an average nut weight of >10 g and kernel weight of >2.5 g. This category of cashew trees is often referred to as 'Bold nut types' having export quality. These trees can be a good source for the introgression of genes for high grade kernels in cashew breeding programmes. The Brazilian collections of this group were predominantly low yielding with fewer fruits, high volume of apple juice and nut weight of >15 g and are called Jumbo varieties by farmers. Since, a study on nut size and number trade-off in cashew (Aliyu and Awopetu, 2011) has shown poor yield in this category of cashew trees, such materials are not suitable for the establishment of investable cashew farms, but could be used as a good source of genetic resources for research and developments of better varieties/cultivars.

More than 50 per cent of the accessions were high yielding with moderate quality kernels. These trees were characterized by having an average nut weight of about 8 -10 g and individual kernel weight ranged from 2 to 2.5 g. Derivable kernels from these trees were a mixture of W240 and W210 grades with the latter being prominent. The fruit maturity was mostly in the middle of the season. These cashew accessions are mostly suitable for immediate use as planting materials because of the superior yield characteristic, but little improvement work on the kernel quality is needed to select candidates with stable kernels.

Three of the accessions (IC302057, IC302058 and IC302044) were characterized by trees with small and compact canopy, though they are highly prolific in fruiting the total output per tree was significantly influenced by the smallness of its nuts and kernels. These plant materials could be useful for future breeding of cashew cultivars that would be adaptable to high density planting in an effort to improve outputs per unit area. The evaluation also revealed that some of the trees were of low yield and poor in agronomic traits. However, because of the large canopy nature of these trees, they are good genetic resources for afforestation, land reclamation and erosion-controlled programmes in the arid regions and areas threatened by gully erosions. Further studies are needed to understand the poor correlation between tree size and nut yield in cashew (Masawe *et al.*, 1999; Aliyu and Awopetu, 2007).

Cashew germplasm studied in this paper presented an average diversity index of about 0.70 showing a relatively important phenotypic diversity of this collection. Following the grouping of the entries into their respective agronomic groups, highly prolific accessions with high quality nut and kernel yield were identified which have the potential to be released as high yielding varieties. A long-term recurrent selection strategy can be embarked on for the development of improved cashew varieties from the accessions with the medium quality kernel.

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