

# Analysis of morphological, biochemical and molecular diversity in karonda (*Carissa carandas* L.) germplasm

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## Summary

**Introduction** – An attempt was made to analyze the morphological, biochemical and molecular diversity in karonda (*Carissa carandas* L.) populations maintained at the Indian Institute of Horticultural Research in Bangalore, India, using a multivariate analysis approach. **Materials and methods** – Morphological and biochemical traits were recorded and evaluated for variance and mean comparisons. Sequence Related Amplified Polymorphism (SRAP) molecular markers were used for generating information on genetic variation and relationships among the accessions. Mantel test was used for comparing the distance matrices derived from morphological, biochemical and molecular markers. **Results and discussion** – Significant variation was found in fruit-related morphological characters and fruit quality traits. The combined analysis used in this study successfully categorized the different karonda accessions into various clusters based on genetic diversity and also established relatedness among them. A high correlation was found between Euclidean distance matrices of biochemical and morphological data. **Conclusion** – This work will be useful for the breeders working on this future crop for characterization, genotype identification, and selection of parents.

## Keywords

genetic diversity, Mantel test, SRAP markers

## Introduction

Karonda (*Carissa carandas* L.;  $2n=2x=22$ ) belongs to family Apocynaceae which comprises of more than 25 species in the genus *Carissa*, of which five species are indigenous to India, including *C. carandas*. It is an evergreen, spiny shrub which is found growing wild in India, Sri Lanka, Indonesia, Myanmar, Pakistan, Malaysia and South Africa. It is a hardy, drought-tolerant species which does well in a wide range of soil conditions. This crop is well adapted for arid tropics and sub-tropics since it can withstand high temperatures, thrive well as a rainfed crop, and gives yield with the minimum management. It is grown for its attractive colored edible fruits and also used as live fencing around the orchards due to the presence of spines which provide protection against stray and wild animals. The mature fruits

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## Significance of this study

*What is already known on this subject?*

- Karonda is a hardy fruit crop grown for its attractive colored edible fruits which differ for fruit colour, fruit size, sweetness and astringency of fruit, antioxidant activity, polyphenol, flavanol and anthocyanin content. It has several pyrotherapeutic properties and it is a useful multipurpose crop, well adapted to arid sub-tropics and tropics.

*What are the new findings?*

- The study on genetic diversity and relatedness using morphological, biochemical and molecular markers was successful in categorizing karonda accessions into different groups based on sweetness, color of fruits and geographical origin. The combination of all the three systems of characterization provided evidence that significant diversity exists among the accessions.

*What is the expected impact on horticulture?*

- The analysis done in this study will be useful for selecting superior accessions which can be used as parents in breeding programs.

are consumed which have taste varying from acidic to sweet, containing a high amount of pectin which makes it useful for the preparation of different products such as jelly, jam, squash, sauce and syrup. Sour and astringent unripe fruits are traditionally used for preparation of pickles and chutney. The fruits can also be candied like cherry or substituted for raisins. The crop has the potential for its utilization as a source of iron, vitamin C and phytochemicals to meet the human dietary requirements and preparation of several ayurvedic medicines. Karonda has several phyto-therapeutic properties as described by Maheshwari *et al.* (2012). In view of its diversified uses, it is expected that karonda will gain higher importance in future as a beneficial crop under arid and semi-arid conditions.

As karonda is indigenous to India, an immense wealth based on variable morphological and biochemical qualities is available which warrants for appropriate addressing and documentation of the germplasm. Plant breeding programs depend on the efficient selection of parental genotypes. In this regard, the analysis of genetic diversity and relatedness among genotypes becomes very useful. Morphological traits (both quantitative and qualitative) have been used conventionally, for assessment of diversity

and still are a widely used approach (Kumar *et al.*, 2015). These markers are easy to score and useful but could be ambiguous, particularly for quantitative traits, which are controlled by multigene (Bartolozzi *et al.*, 1999). In karonda, on the basis of fruit colour, three categories are recognized: pink-white, greenish pink, and reddish purple. The fruits of pink variety are white and change to pink at maturity. The colour of reddish-purple fruits is green at immature stage, which changes to reddish purple at maturity. Another criterion of classification followed in karonda is based on utilization: pickle types consist of varieties which produce sour fruits suitable for pickle and chutney preparation and table types which produce sweet fruits. This species shows a high degree of genotypic and morphological variability as reported by Sawant *et al.* (2003) who studied collections made from Kolhapur district of Maharashtra. Karale *et al.* (1990) also reported variation in number of flower bud per umbel, length of petals and pedicels, petal number and flower colour. In recent years characterization of germplasm using nonconventional approaches such as biochemical markers and DNA markers has received attention. Since genomic resources are not available in karonda, the Sequence Related Amplified Polymorphism (SRAP) technique was utilized in the present study. This technique is highly reproducible and relatively simple for both mapping and gene tagging in plants where genomic sequence information is not available (Li and Quiros, 2001). Several studies have shown that SRAP marker is more informative than other PCR-based techniques in detecting genetic diversity (Budak *et al.*, 2004). It has been successfully used in several species for assessment of diversity and relationship studies (Da Silva *et al.*, 2016; Abedian *et al.*, 2012). The present work was undertaken to demonstrate the usefulness of morphological, biochemical and SRAP markers for studying the genetic diversity and relationships among a diverse collection of karonda sourced from different regions of India.

## Materials and methods

### Morphological evaluation

Fifty-four germplasm accessions from different parts of Gujarat and Karnataka were collected and planted at a spacing of 4 × 4 m in the field gene bank at Indian Institute of Horticultural Research, Bengaluru. Ten-year-old plants were evaluated for vegetative growth, flowering, and fruiting, morphological characters of leaves, flowers and fruits for two years (2016 and 2017). Twenty randomly selected leaves were taken for measurement of length, width and other morphological traits. Twenty mature fruits were harvested randomly from each accession to record observation on physicochemical parameters. Fruit size (length and girth) was measured with the help of digital Vernier caliper while weight was taken by digital top pan balance. The total soluble solids (TSS) were determined with Erma Hand Refractometer (0–32 °Brix). The titratable acidity (%) and ascorbic acid content were determined by AOAC methods. Ten fruits from each accession were weighed and volume estimated by water displacement method.

### Biochemical evaluation

After harvesting 30–40 fruits were washed with distilled water and grinded. A known quantity (5 g) of fruit pulp was incubated in acidic methanol (1:99 v/v) for 48 h and ground thoroughly in a pestle and mortar. The extract was filtered through two layers of muslin cloth and this was

repeated twice and volume was made up to 50 mL. Ten mL of the extract was centrifuged at 10,000 rpm for 5 min and used further for analysis of antioxidant activity, phenol, flavonoid and anthocyanin content. Total phenol content of the methanol extract was estimated according to the Folin-Ciocalteu method (Singleton and Rossi, 1965) and expressed as gallic acid equivalents. Total flavonoid in the methanol extract was determined as per Chun *et al.* (2003). Total anthocyanin was estimated by measuring the absorbance of the extract at 540 nm (Fuleki, 1969). Total sugar and reducing sugar content were estimated using the method suggested by Somogyi (1952).

For all quantitative traits, descriptive statistics were calculated and the genetic distance among the lines was worked out using D2 statistics (Rencher, 1995) on the basis of multiple characters. The clustering of genetic groups was done by the method suggested by Tocher (Rao, 1974). The means of all the characters were subjected to Squared Euclidian Cluster analysis and a dendrogram was derived using Ward's method (Rencher, 1995). PROC CLUSTER and PROC PRINCOMP of SAS V 9.3 were used to construct a Pearson correlation matrix from the Euclidean distances, and a Principal Components Analysis was then conducted on the correlation matrix (SAS, 2012). The first three principal components were plotted on a three-dimensional graph.

### DNA extraction and SRAP markers

For SRAP analysis genomic DNA was extracted from young leaves and its quality was checked and adjusted to 50 ng  $\mu\text{L}^{-1}$  for PCR reactions. Good quality DNA was obtained from 30 accessions and these were subjected to molecular analysis. Initial screening was carried out on a panel of 10 morphologically diverse genotypes with fifty primer combinations (PC) which resulted in selection of eleven PCs giving good and reproducible amplification. Standard PCR reaction and conditions were used as suggested by Li and Quiros (2001). The PCR product was analyzed on 2% agarose gel with ethidium bromide staining in 1× TBE (Tris-borate-EDTA) buffer. A 100 bp DNA ladder as was used as the fragment size marker.

Soring was done manually for intense and clearly resolved PCR amplified bands. SRAP matrix data sheet was prepared with the presence of band (1) or absence of band (0). In order to estimate the band size, a medium range DNA ruler (100 bp) was run along with amplified products. The number of alleles and polymorphic information content was calculated according to the formula given by Smith *et al.* (1997). Jaccard's coefficient of similarity was used for assessing the genetic relatedness among the accessions. Using the binary data and R software (R Core Team, 2013) a dendrogram constructed based on Unweighted Pair Group Method with Arithmetic Mean (UPGMA). GenAlEx 6.5-Genetic Analysis in Excel (Peakall and Smouse, 2012) was used for analysis of molecular variance.

### Relationship among morphological, biochemical, and genetic distances

Mantel test, which uses sampled randomization technique to ascertain correlations between distance matrices (Sokal and Rohlf, 1995), was carried out to compare Euclidean distance matrices for the morphological, biochemical and molecular data using GenAlEx Software for Excel (Peakall and Smouse, 2006).

TABLE 1. Morphological traits evaluated in karonda population.

| Name        | Area of collection | Leaf length (cm) | Leaf width (cm) | Fruit wt. (g) | Fruit length (cm) | Fruit diam. (cm) | Fruit colour | Fruit shape | Flesh colour   | Taste | Seed wt. (g) | No. of seed/fruit | TSS  | Acidity (%) |
|-------------|--------------------|------------------|-----------------|---------------|-------------------|------------------|--------------|-------------|----------------|-------|--------------|-------------------|------|-------------|
| Karonda -1  | Western Ghats      | 5.14             | 2.47            | 5.9           | 2.65              | 1.33             | Greenish red | Oblong      | Greenish white | Sour  | 0.35         | 2.0               | 10.5 | 1.65        |
| Karonda -2  | Western Ghats      | 4.89             | 2.37            | 3.1           | 1.48              | 0.85             | Greenish red | Oblong      | Greenish white | Sour  | 0.35         | 2.0               | 11.2 | 1.60        |
| Karonda -3  | Western Ghats      | 4.59             | 2.32            | 6.4           | 2.46              | 1.17             | Greenish red | Oblong      | Greenish white | Sour  | 0.40         | 2.0               | 11.0 | 1.53        |
| Karonda -4  | Western Ghats      | 6.38             | 4.24            | 3.2           | 1.1               | 1.1              | Dark purple  | Round       | Greenish white | Sour  | 0.3          | 2.0               | 10.2 | 1.40        |
| Karonda -5  | Western Ghats      | 4.90             | 3.33            | 3.5           | 1.47              | 1.47             | Dark purple  | Round       | Greenish white | Sour  | 0.35         | 2.5               | 10.5 | 1.51        |
| Karonda -6  | Western Ghats      | 5.72             | 3.35            | 3.8           | 1.37              | 1.36             | Dark purple  | Round       | Greenish white | Sweet | 0.40         | 2.5               | 10.4 | 1.43        |
| Karonda -7  | Chettali           | 5.72             | 3.35            | 15.4          | 2.67              | 3.13             | Dark purple  | Oblong      | Whitish pink   | Sweet | 0.51         | 2.1               | 11.0 | 1.20        |
| Karonda -8  | Chettali           | 7.31             | 3.95            | 15.7          | 2.73              | 2.89             | Dark purple  | Round       | Whitish pink   | Sweet | 0.40         | 2.0               | 11.6 | 1.20        |
| Karonda -9  | Chettali           | 7.10             | 3.75            | 12.71         | 2.37              | 2.81             | Dark purple  | Oblong      | Whitish pink   | Sweet | 0.35         | 1.9               | 12.5 | 1.15        |
| Karonda -10 | Chettali           | 7.11             | 3.85            | 16            | 2.93              | 2.6              | Dark purple  | Round       | Whitish pink   | Sweet | 0.45         | 1.4               | 8.8  | 0.9         |
| Karonda -11 | Chettali           | 7.25             | 3.90            | 16.2          | 2.85              | 3.21             | Dark purple  | Oblong      | Whitish pink   | Sweet | 0.3          | 2.4               | 10.3 | 0.60        |
| Karonda -12 | Chettali           | 6.35             | 3.65            | 8.66          | 2.3               | 2.23             | Dark purple  | Round       | Whitish pink   | Sweet | 0.30         | 2.1               | 16.3 | 1.5         |
| Karonda -13 | Chettali           | 7.15             | 3.90            | 16.57         | 2.86              | 3.28             | Dark purple  | Oblong      | Whitish pink   | Sweet | 0.55         | 2.30              | 13.0 | 1.05        |
| Karonda -14 | Chettali           | 7.25             | 3.68            | 17            | 2.81              | 3.21             | Dark purple  | Oblong      | Whitish pink   | Sweet | 0.45         | 2.1               | 12.7 | 1.25        |
| Karonda -15 | Chettali           | 6.85             | 3.55            | 6.3           | 2.09              | 2.08             | Dark purple  | Round       | Whitish pink   | Sweet | 0.5          | 2.0               | 13.3 | 0.78        |
| Karonda -16 | Chettali           | 5.92             | 3.45            | 3.55          | 1.45              | 1.52             | Dark purple  | Round       | Whitish pink   | Sweet | 0.25         | 2.0               | 13.5 | 0.64        |
| Karonda -17 | Chettali           | 7.20             | 3.72            | 19.33         | 2.87              | 3.55             | Dark purple  | Oblong      | Whitish pink   | Sweet | 0.35         | 1.5               | 12.5 | 1.55        |
| Karonda -18 | Chettali           | 5.95             | 3.52            | 4.33          | 1.63              | 2.0              | Dark purple  | Round       | Pink           | Sweet | 0.25         | 1.1               | 16.2 | 1.1         |
| Karonda -19 | Chettali           | 7.10             | 3.85            | 15.8          | 2.52              | 3.08             | Dark purple  | Oblong      | Whitish pink   | Sweet | 0.30         | 1.2               | 13.4 | 1.3         |
| Karonda -20 | Chettali           | 7.15             | 3.75            | 16            | 2.73              | 3.27             | Dark purple  | Oblong      | Whitish pink   | Sweet | 0.35         | 1.3               | 11.0 | 1.3         |
| Karonda -21 | Chettali           | 6.55             | 3.65            | 4.98          | 1.77              | 1.83             | Dark purple  | Round       | Pink           | Sweet | 0.30         | 1.4               | 16.7 | 1.75        |
| Karonda -22 | Chettali           | 6.45             | 3.55            | 3.0           | 1.41              | 1.54             | Dark purple  | Round       | Whitish pink   | Sweet | 0.30         | 1.4               | 14.2 | 1.6         |
| Karonda -23 | Chettali           | 5.95             | 3.45            | 9.11          | 2.35              | 2.22             | Dark purple  | Round       | Whitish pink   | Sweet | 0.25         | 1.1               | 16.2 | 1.1         |
| Karonda -24 | Chettali           | 6.56             | 3.85            | 11.39         | 2.55              | 2.4              | Dark purple  | Round       | Whitish pink   | Sweet | 0.25         | 1.2               | 15.3 | 1.3         |
| Karonda -25 | Western Ghats      | 4.85             | 3.35            | 3.6           | 1.59              | 2.16             | Dark purple  | Round       | White          | Sour  | 0.4          | 2.0               | 11.2 | 1.9         |
| Karonda -26 | Western Ghats      | 5.83             | 3.78            | 2.1           | 1.36              | 1.37             | Dark purple  | Round       | Pink           | Sweet | 0.24         | 1.4               | 18   | 1.1         |
| Karonda -27 | Western Ghats      | 4.84             | 3.4             | 2.6           | 1.42              | 1.41             | Dark purple  | Round       | Pink           | Sweet | 0.4          | 1.7               | 14.1 | 1.2         |
| Karonda -28 | Western Ghats      | 5.64             | 4.16            | 2.9           | 1.47              | 1.47             | Dark purple  | Round       | Creamy white   | Sweet | 0.3          | 1.8               | 15.3 | 1.15        |
| Karonda -29 | Western Ghats      | 5.67             | 3.8             | 2.4           | 1.44              | 1.44             | Dark purple  | Round       | Pink           | Sweet | 0.4          | 2.2               | 11.5 | 1.1         |
| Karonda -30 | Western Ghats      | 6.0              | 3.95            | 2.7           | 1.64              | 1.64             | Dark purple  | Round       | White          | Sweet | 0.42         | 3.3               | 13.8 | 1.22        |
| Karonda -31 | Western Ghats      | 6.2              | 4.37            | 1.9           | 1.32              | 1.32             | Dark purple  | Round       | Light pink     | Sweet | 0.3          | 2.9               | 15.2 | 1.25        |
| Karonda -32 | Western Ghats      | 5.49             | 3.77            | 2.5           | 1.57              | 1.57             | Dark purple  | Round       | Pink           | Sweet | 0.5          | 3.2               | 15.3 | 1.11        |
| Karonda -33 | Western Ghats      | 5.76             | 3.78            | 2.4           | 1.21              | 1.34             | Dark purple  | Round       | Pink           | Sweet | 0.5          | 3.5               | 16.6 | 1.21        |

TABLE 1. Continued.

| Name        | Area of collection | Leaf length (cm) | Leaf width (cm) | Fruit wt. (g) | Fruit length (cm) | Fruit diam. (cm) | Fruit colour  | Fruit shape | Flesh colour | Taste      | Seed wt. (g) | No. of seed/fruit | TSS  | Acidity (%) |
|-------------|--------------------|------------------|-----------------|---------------|-------------------|------------------|---------------|-------------|--------------|------------|--------------|-------------------|------|-------------|
| Karonda -34 | Western Ghats      | 4.61             | 3.38            | 2.7           | 1.25              | 1.25             | Dark purple   | Round       | Red          | Sweet      | 0.4          | 3.4               | 16.1 | 1.09        |
| Karonda -35 | Western Ghats      | 5.53             | 3.45            | 2.9           | 1.22              | 1.22             | Dark purple   | Round       | Creamy white | Sweet      | 0.3          | 3.5               | 15.8 | 1.10        |
| Karonda -36 | Western Ghats      | 4.5              | 3.35            | 2.5           | 1.22              | 1.22             | Dark purple   | Round       | White        | Sweet      | 0.2          | 2.8               | 14.6 | 1.15        |
| Karonda -37 | Western Ghats      | 5.26             | 3.36            | 2.3           | 1.27              | 1.21             | Dark purple   | Round       | Pink         | Sweet      | 0.4          | 3.0               | 13.2 | 1.21        |
| Karonda -38 | Western Ghats      | 5.1              | 4.42            | 2.8           | 1.25              | 1.6              | Dark purple   | Oblong      | Pink         | Sweet      | 0.3          | 3.2               | 15.6 | 1.18        |
| Karonda -39 | Western Ghats      | 4.2              | 3.2             | 2.4           | 1.3               | 1.1              | Dark purple   | Round       | Pink         | Sweet      | 0.5          | 2.6               | 15.2 | 1.20        |
| Karonda -40 | Western Ghats      | 5.5              | 3.7             | 1.8           | 1.1               | 1.0              | Dark purple   | Round       | Pink         | Very sweet | 0.3          | 2.4               | 15.3 | 1.01        |
| Karonda -41 | Western Ghats      | 4.7              | 5.6             | 2.2           | 1.3               | 1.2              | Dark purple   | Round       | Pink         | Sweet      | 0.2          | 2.2               | 15.3 | 1.10        |
| Karonda -42 | Western Ghats      |                  |                 |               |                   |                  | Dark purple   | Round       | Pink         | Sweet      | 0.4          | 2.8               | 12.6 | 1.20        |
| Karonda -43 | Western Ghats      | 5.0              | 3.0             | 3.2           | 1.5               | 1.2              | Dark purple   | Round       | Creamy white | Sweet      | 0.2          | 2.3               | 16.6 | 1.20        |
| Karonda -44 | Bangalore          | 6.10             | 3.25            | 3.6           | 1.56              | 1.56             | Dark purple   | Round       | Creamy white | Sweet      | 0.4          | 2.7               | 14.2 | 1.12        |
| Karonda -45 | Bangalore          | 4.15             | 2.75            | 4.0           | 2.05              | 1.54             | Dark purple   | Oblong      | White        | Sour       | 0.3          | 3.0               | 13.2 | 1.5         |
| Karonda -46 | Bangalore          | 6.53             | 2.58            | 4.46          | 2.0               | 1.53             | Dark purple   | Oblong      | White        | Sweet      | 0.2          | 2.6               | 15.0 | 1.21        |
| Karonda -47 | Bangalore          | 3.5              | 2.5             | 3.1           | 1.3               | 1.5              | Dark purple   | Round       | White        | Sweet      | 0.3          | 2.0               | 14.5 | 1.12        |
| Karonda -48 | Gujarat            | 5.3              | 3.8             | 6.9           | 1.90              | 2.53             | Reddish black | Oblong      | Whitish red  | Sour       | 0.3          | 2.0               | 14.0 | 1.15        |
| Karonda -49 | Gujarat            | 5.1              | 3.2             | 3.9           | 3.8               | 1.9              | Red           | Oblong      | White        | Sour       | 0.3          | 2.1               | 11.0 | 1.45        |
| Karonda -50 | Gujarat            | 4.5              | 2.3             | 2.0           | 1.8               | 1.2              | Red           | Oblong      | White        | Sour       | 0.25         | 2.0               | 11.4 | 1.40        |
| Karonda -51 | Bangalore          | 5.6              | 3.2             | 4.6           | 2.0               | 1.9              | Black         | Round       | Whitish red  | Sour       | 0.3          | 2.2               | 11.8 | 1.40        |
| Karonda -52 | Bangalore          | 6.1              | 3.0             | 3.5           | 1.57              | 1.56             | Black         | Round       | Whitish red  | Sweet      | 0.3          | 2.1               | 12.4 | 1.10        |
| Karonda -54 | Bangalore          | 5.02             | 3.25            | 3.2           | 2.3               | 1.7              | Bright red    | Oblong      | White        | Sour       | 0.35         | 2.1               | 11.4 | 1.35        |
| Karonda -55 | Bangalore          | 5.54             | 3.53            | 3.4           | 2.4               | 1.7              | Bright red    | Oblong      | White        | Sour       | 0.4          | 2.2               | 11.5 | 1.42        |

## Results and discussion

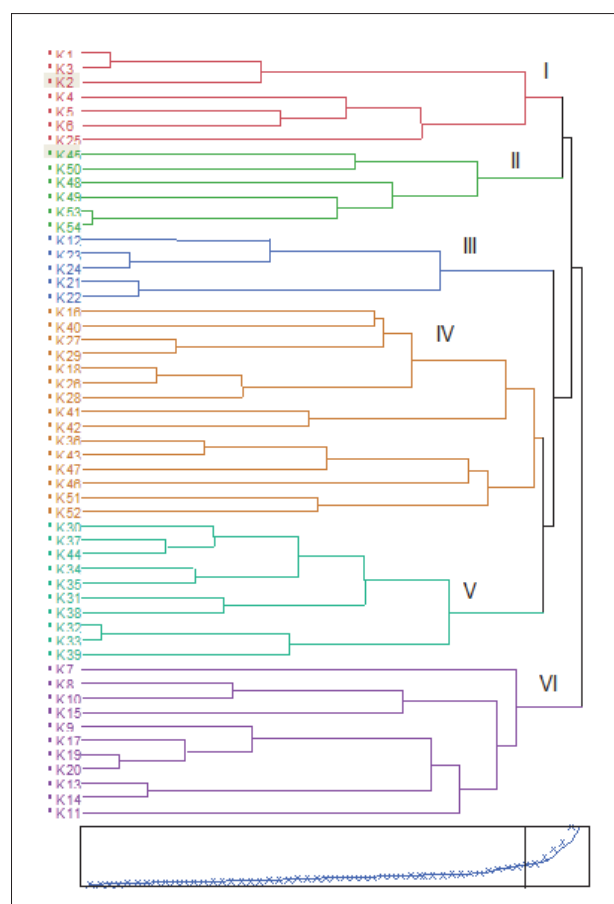
### Morpho-physiological characterization

Karonda genotypes collected from Western Ghats region, Chetalli, and around Bangalore were studied to decipher the morphological, biochemical and genetic variation available in the germplasm which will be useful for the breeders in the selection of promising parents for karonda improvement program. Characterization and diversity studies are important for meeting the goal of conservation and utilization of germplasm by hybridization (Uddin and Boerner, 2008). The results of morphophysiological characterization data of fifty-four accessions of *C. carandas* is presented in Table 1. Accession number 53 was removed from analysis since there were some missing data for this accession. It is clear from the variance analysis (Table 2) that karonda accessions showed significant differences with regard to most of the morphological characters which can be exploited through selection. For each of the nine traits evaluated, descriptive statistics were worked out including mean, median, range, variance with their coefficient of variation.

The germplasm lines showed high variation in fruit weight (g plant<sup>-1</sup>) and TSS (°Brix) which ranged from 1.8 to 19.33 with a mean value of 6.16 and from 1 to 18 with a mean of 13.3 respectively. The germplasm also exhibited diversity in fruit length, fruit width, fruit weight, seed weight and the number of seeds per fruit of different accessions showing large differences between maximum and minimum values. It is clear from the results that the karonda accessions varied significantly with regard to the majority of the morphological characters. In a previous study by Patil *et al.* (2017) significant difference among the karonda genotypes were reported with respect to quality parameters such as titratable acidity, reducing sugar, non-reducing sugar and total sugar which was attributed to the genetic makeup of genotypes and the prevailing climatic conditions. Meghwal *et al.* (2014) also reported significant variations in fruit length, weight, volume, diameter, number of seeds, and vitamin C content in karonda genotypes collected from different regions of Rajasthan, Gujarat and Uttaranchal. However, the range for variation was not wide for traits such as TSS, acidity and dry matter.

### Cluster analysis and phenetic relationships

The genetic distance was the lowest between accessions K55 and K54 (0.98), K32 and K33 (1.06) and K1 and K3 (1.07). Maximum genetic distance was recorded between, K41 and K7 (8.92), K7 and K26 (8.57) and K7 and K36 (8.46). Using the nine morphological traits (Table 2), a dendrogram (Figure 1) was generated highlighting relationships among studied karonda population. At least six main groups can be

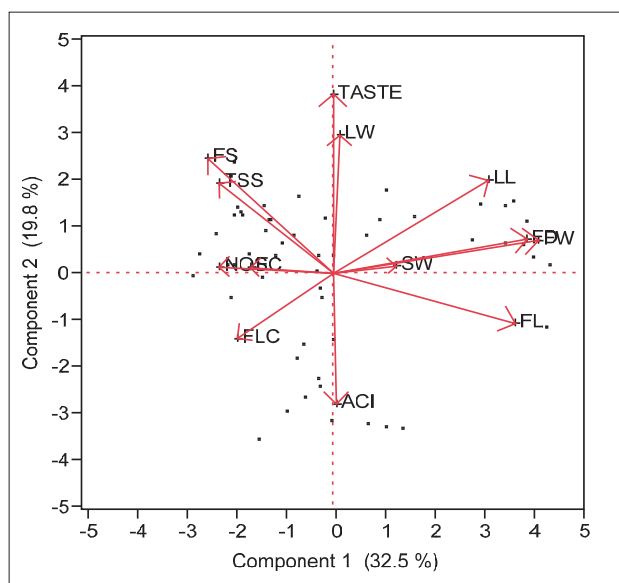


**FIGURE 1.** Dendrogram depicting relationships among 53 karonda populations using Euclidean distances from morphological data.

distinguished in this tree. Group I includes 7 accessions: all are sour type with medium fruit weight (3.1 to 6.4 g). The fruit colour of three accessions is greenish red while remaining acquire dark purple skin colour on maturity. Group II, located between Groups I and III, is small with only six accessions; all the fruits are the sour type, with colour varying from dark purple to bright red, the shape of all fruits is oblong and the weight is medium (2–4 g fruit<sup>-1</sup>). Group III is the smallest group encompassing just 5 accessions which are sweet, round in shape and dark purple skin colour. The TSS in this group varies from 14.2 to 16.7 °Brix; Group IV is the largest and comprises of all the sweet types with the highest TSS from 11.5 to 18. The sweetest accession, K40, belongs to this group. The fruits of all accessions are round in shape with dark purple skin colour, except K46 which is oblong.

**TABLE 2.** Descriptive statistics for morphological traits in karonda.

| Name   | Leaf length (cm) | Leaf width (cm) | Fruit wt. (g) | Fruit length (cm) | Fruit diam. (cm) | Seed wt. (g) | No of seed/fruit | TSS      | Acidity (%) |
|--------|------------------|-----------------|---------------|-------------------|------------------|--------------|------------------|----------|-------------|
| Min.   | 3.5              | 2.3             | 1.8           | 1.1               | 0.85             | 0.2          | 1.1              | 1        | 0.6         |
| Max.   | 7.31             | 5.6             | 19.33         | 3.8               | 3.55             | 0.55         | 3.5              | 18       | 1.9         |
| Range  | 3.81             | 3.3             | 18.98         | 2.7               | 2.7              | 0.35         | 2.4              | 17       | 1.3         |
| Mean   | 5.7826           | 3.544902        | 6.163529      | 1.8788            | 1.8824           | 0.344615     | 2.205769         | 13.31346 | 1.231538    |
| Median | 5.74             | 3.55            | 3.55          | 1.63              | 1.57             | 0.3          | 2.1              | 13.65    | 1.2         |
| SD     | 0.961404         | 0.520625        | 5.4376        | 0.635663          | 0.709725         | 0.089902     | 0.646501         | 2.747339 | 0.23649     |
| SE     | 0.115739         | 0.062676        | 0.65461       | 0.076525          | 0.085441         | 0.010823     | 0.07783          | 0.330741 | 0.02847     |
| CV     | 0.166258         | 0.146866        | 0.882222      | 0.338334          | 0.377032         | 0.260875     | 0.293095         | 0.206358 | 0.192028    |



**FIGURE 2.** A two-dimensional scatter plot depicting relationships among karonda accessions using the first two principal components from morphological data.

The next group, *i.e.*, Group V, comprises of 10 accessions which are all sweet type, with dark purple skin colour and round shape of the fruits. Group VI includes 11 accessions,

all sweet type, varying in shape from oblong to round with dark purple skin colour. The accession with the highest fruit weight, K17, is present in this group.

**Principal components analysis**

With the eigenvalues greater than 1.0, the four first principal components together explained 76.2% of the total variation of morphological traits. The first two components which collectively explained 52.2% of the total variation are depicted as two-dimensional biplot arrangement (Figure 2). The first principal component has an eigenvalue of 4.2, which explained 32.4% of the whole variation. Fruit-related traits such as fruit weight, fruit length and fruit diameter largely contributed to variation for this principal component. The second principal component explained 19.7% of total variation with an eigenvalue of 2.5. Vegetative traits such as leaf width and quality, fruit taste contributed to the dissimilarity of this principal component. The third principal component explained 13.3% of the total variation with traits such as fruit and flesh colour contributing maximum. The fourth principal component had an eigenvalue of 1.3; contributing 10.6% of total variation. Seed weight and seed number explained most of the variation.

**Biochemical characterization**

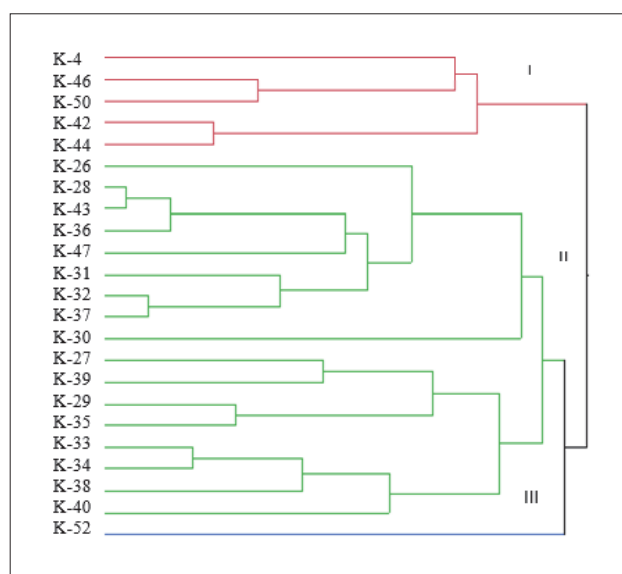
The biochemical characterization for fruits of 23 genotypes is presented in Table 3. Phenols ranged from 427.15 to 1027.11 with a mean of 715 mg 100 g<sup>-1</sup>. Accession num-

**TABLE 3.** Descriptive statistics for biochemical traits in karonda.

| S. No. | Samples | Phenols (mg 100 g <sup>-1</sup> ) | Flavonoids (mg 100 g <sup>-1</sup> ) | FRAP (mg 100 g <sup>-1</sup> ) | DPPH (mg 100 g <sup>-1</sup> ) | Anthocyanin (mg 100 g <sup>-1</sup> ) | Redusug (mg 100 g <sup>-1</sup> ) | Tot Sug (mg 100 g <sup>-1</sup> ) |
|--------|---------|-----------------------------------|--------------------------------------|--------------------------------|--------------------------------|---------------------------------------|-----------------------------------|-----------------------------------|
| 1      | K-4     | 514.52                            | 126.05                               | 500.132                        | 279.61                         | 42.47                                 | 8.33                              | 9.465                             |
| 2      | K-26    | 829.55                            | 220.50                               | 758.15                         | 263.27                         | 138.39                                | 11.20                             | 12.490                            |
| 3      | K-27    | 778.10                            | 196.25                               | 701.70                         | 254.79                         | 172.46                                | 19.74                             | 20.132                            |
| 4      | K-28    | 745.09                            | 178.97                               | 714.31                         | 270.95                         | 103.57                                | 12.95                             | 14.092                            |
| 5      | K-29    | 767.42                            | 184.12                               | 736.04                         | 254.34                         | 185.42                                | 15.46                             | 16.437                            |
| 6      | K-30    | 552.39                            | 137.08                               | 545.92                         | 271.84                         | 59.38                                 | 22.06                             | 22.636                            |
| 7      | K-31    | 785.38                            | 220.50                               | 719.55                         | 276.04                         | 95.80                                 | 13.47                             | 15.343                            |
| 8      | K-32    | 726.64                            | 173.09                               | 676.48                         | 270.86                         | 116.78                                | 14.95                             | 16.940                            |
| 9      | K-33    | 892.17                            | 239.24                               | 771.93                         | 261.93                         | 141.10                                | 14.77                             | 15.733                            |
| 10     | K-34    | 1011.09                           | 235.94                               | 786.86                         | 264.25                         | 125.30                                | 16.85                             | 17.416                            |
| 11     | K-35    | 926.63                            | 196.98                               | 735.26                         | 246.40                         | 219.49                                | 15.66                             | 17.448                            |
| 12     | K-36    | 760.62                            | 145.16                               | 715.67                         | 268.09                         | 146.91                                | 14.36                             | 14.819                            |
| 13     | K-37    | 771.30                            | 202.86                               | 726.92                         | 266.13                         | 114.69                                | 15.25                             | 17.468                            |
| 14     | K-38    | 860.61                            | 221.97                               | 740.50                         | 268.54                         | 114.81                                | 18.12                             | 18.691                            |
| 15     | K-39    | 725.19                            | 171.62                               | 709.26                         | 271.49                         | 132.83                                | 17.90                             | 20.001                            |
| 16     | K-40    | 1027.11                           | 273.05                               | 814.41                         | 261.84                         | 131.35                                | 19.82                             | 21.136                            |
| 17     | K-42    | 498.99                            | 97.02                                | 600.62                         | 269.07                         | 118.88                                | 14.67                             | 15.062                            |
| 18     | K-43    | 787.80                            | 166.11                               | 701.89                         | 270.77                         | 109.62                                | 13.58                             | 14.664                            |
| 19     | K-44    | 543.16                            | 121.28                               | 581.81                         | 273.18                         | 122.09                                | 12.12                             | 12.746                            |
| 20     | K-46    | 427.15                            | 99.59                                | 445.81                         | 250.15                         | 54.94                                 | 9.52                              | 9.804                             |
| 21     | K-47    | 608.69                            | 131.20                               | 711.01                         | 279.25                         | 109.87                                | 12.29                             | 13.073                            |
| 22     | K-50    | 468.90                            | 96.29                                | 443.68                         | 254.70                         | 37.16                                 | 12.33                             | 13.210                            |
| 23     | K-52    | 836.83                            | 173.83                               | 764.75                         | 207.56                         | 368.50                                | 6.51                              | 6.955                             |
|        | C.D.    | 133.287                           | 35.488                               | 77.337                         | 14.682                         | 23.049                                | 3.207                             | 3.014                             |
|        | SE (m)  | 45.28                             | 12.056                               | 26.272                         | 4.988                          | 7.83                                  | 1.089                             | 1.024                             |
|        | SE (d)  | 64.035                            | 17.049                               | 37.155                         | 7.054                          | 11.074                                | 1.541                             | 1.448                             |
|        | C.V.    | 8.743                             | 9.719                                | 5.477                          | 2.679                          | 8.599                                 | 10.674                            | 9.361                             |

ber K46 recorded the lowest and K40 recorded the highest amount of phenol. The flavonoid content in all genotypes varied from the lowest of 96.29 in K50 to the highest of 239.24 mg 100 g<sup>-1</sup> in K33. The mean value was 170.35 and the coefficient of variation was 9.71. FRAP content varied from 443.68 in K50 to 814.41 in K40 while DPPH in K52 was the lowest at 207.56, and the maximum in K47 at 279.25. Anthocyanin was lowest in K50 at 37.16 while it was maximum in K 52 at 368.5. Reducing sugars were found to be the lowest in K52 at 6.51, and the highest in K30 at 22.06, with the coefficient of variation of 10.67. The total sugars also followed the same trend with the lowest recorded in K52 at 6.95 and the highest in K30 at 22.63, with the coefficient of variation of 9.36.

In a study conducted by Dutta *et al.* (2016), it has been reported that the green-fruited karonda contains a high amount of antioxidants, phenols and flavonoids compared to white ones. However, in our study, we have found that the pink fruited accessions had a high content of these nutritive phytochemicals. Genotypic variation has been observed in the phenol and other biochemical components in karonda by Hiregoudra *et al.* (2012). In a study conducted by Krishna *et al.* (2017), it has been reported that that addition of red food colorant derived from karonda variety CIAH Selection-1 to any colorless lemon-based beverage significantly improved the anthocyanin, phenol and flavonoid content of the drink. In our study genotypes, *viz.* K33, K40, and K52, which have dark-purple to blackish appearance, were found to be high in these compounds and could be a source of red food colorant. The PCA analysis of biochemical traits agreed with the groups formed by cluster analysis and similar reports are available in other fruit crops such as dates (Bedjaoui *et al.*, 2018) and caprifig (Essid *et al.*, 2015), where PCA and CA together add to explain the relationship among the genotypes. The advantage of PCA is that it reduces the number of variables which explain the maximum variation (Härdle and Simar, 2003) while cluster analysis is better for visualizing the relationships among various genotypes. The first two PC contributed to 84% variation of the total observed variation which would help the breeders select a limited number of populations for identifying highly variable genotypes for selection of parents.



**FIGURE 3.** Dendrogram depicting relationships among 23 karonda populations based on Euclidean distances from biochemical data.

### Cluster analysis

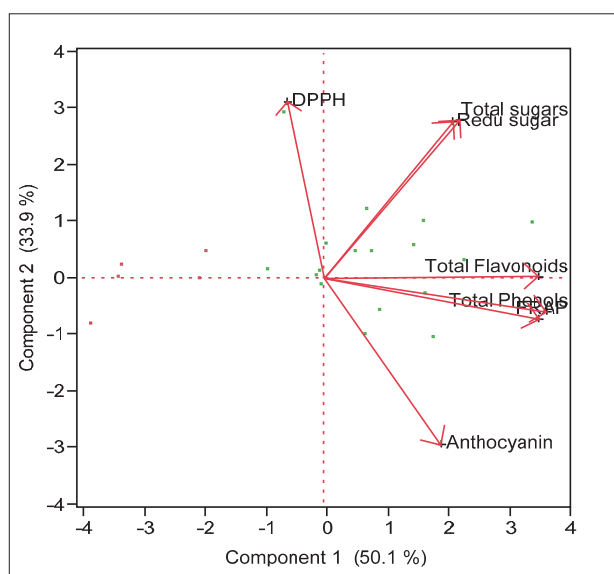
The genetic distance based on biochemical traits was the lowest between accessions K28 and K43 (0.46), K32 and K43 (0.84) and K36 and K43 (0.78). Maximum genetic distance was recorded between K30 and K52 (9.01), K7 and K26 (8.57) and K7 and K36 (8.46). Cluster analyses based on the combined biochemical data (Figure 3) defined two clusters and one single population. The dendrogram in Figure 3 clusters together some groups of similar accessions based on the amount of reducing sugar and total sugars present. The I<sup>st</sup> group comprises of the accessions with low amount of reducing sugar and total sugars. The accession in the single population is K52 which has recorded the lowest amount of reducing sugar and total sugars. The remaining accessions are all grouped in II<sup>nd</sup> cluster and have a high of amount of reducing sugar and total sugars including the accession with the highest amount, *i.e.*, K30.

### Principal components analysis

Maximum variation (84.03%) of biochemical traits is explained by the first two principal components having eigenvalues greater than 1.0 shown as a two-dimensional arrangement of biochemical data in Figure 4. The variation of the first principal component was contributed by traits such as total phenols, flavonoids and FRAP. The second principal component explained 33.8% of total variation with an eigenvalue of 2.3. Traits responsible for fruit taste *viz.* reducing sugars, total sugars and DPPH contributed strongly to the variation of this principal component.

### Molecular analysis

Eleven SRAP markers produced clear, reproducible polymorphic bands (Table 4). The primer combination for polymorphic bands, the total number of bands produced, number of polymorphic bands produced, the percentage of polymorphic bands (PPB) and polymorphic information content (PIC) are shown in Table 5. In total 71 bands were amplified using 11 SRAP markers across the 30 karonda accessions, of which 60 (57.04%) were polymorphic. The total number of bands ranged from 4 (Primer Me4F-Em12R and Primer Me1F-Em13R) to 9 (Primer Me3F-Em15R). Among the prim-



**FIGURE 4.** A two-dimensional scatter plot depicting phenetic relationships among karonda accessions based on the first two principal components from biochemical data.

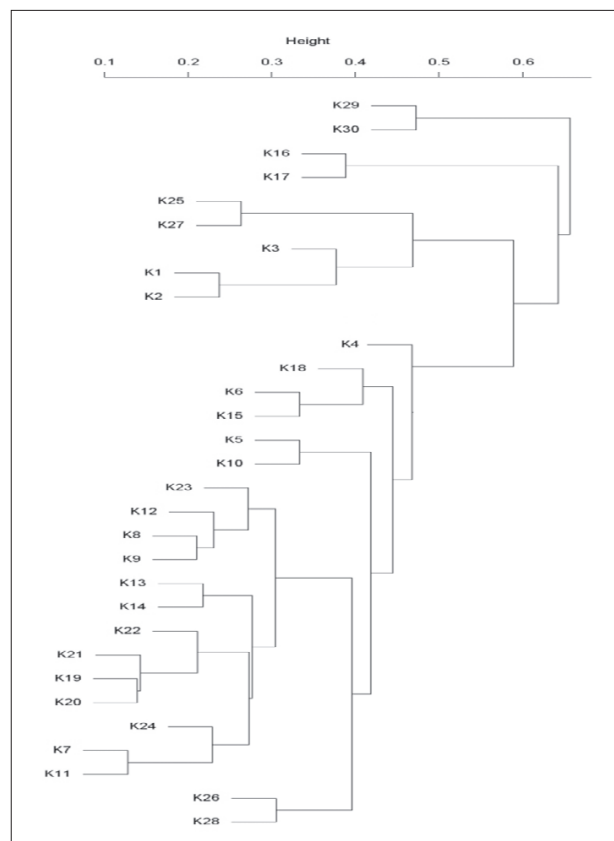
**TABLE 4.** List of SRAP primers used in the study.

| Sl. No. | Primer name | Sequence (3' - 5')      |
|---------|-------------|-------------------------|
| 1       | Me 1F       | TGA GTC CAA ACC GGA TA  |
| 2       | Me 2F       | TGA GTC CAA ACC GGA GC  |
| 3       | Me 3F       | TGA GTC CAA ACC GGA AT  |
| 4       | Me 4F       | TGA GTC CAA ACC GGA CC  |
| 5       | Em 1R       | GAC TGC GTA CGA ATT AAT |
| 6       | Em 3R       | GAC TGC GTA CGA ATT GAC |
| 7       | Em 6R       | GAC TGC GTA CGA ATT GCA |
| 8       | Em 7R       | GAC TGC GTA CGA ATT CAA |
| 9       | Em 11R      | GAC TGC GTA CGA ATT CTA |
| 10      | Em 12R      | GAC TGC GTA CGA ATT CTC |
| 11      | Em 13R      | GAC TGC GTA CGA ATT CTG |
| 12      | Em 14R      | GAC TGC GTA CGA ATT CTT |
| 13      | Em 15R      | GAC TGC GTA CGA ATT GAT |

er combinations, Me4F-Em3R generated the lowest percentage of polymorphic bands (40%); 4 primers (Me1F-Em11R, Me1F-Em14R, Me3F-Em15R, Me4F-Em16R) produced 100% polymorphic bands. The discriminatory power of the various SRAP markers was revealed by the PIC values which ranged from the highest 0.39 obtained for Me3F-Em15R combination to the lowest of 0.28 for primer combination Me4F-Em3R. The mean PIC value for all markers was 0.30.

**Cluster analysis**

The genetic distance was lowest between K26 and K30; K38 and K39; K38 and K40; K39 and K40 (all accessions from the Western Ghats) while the highest distance was observed between K1/K2 (collected from the Western Ghats) and K55 collected from the Bangalore. UPGMA algorithm was used to generate a dendrogram using the SRAP data (Figure 5). The 30 karonda accessions were found to cluster into four groups (Group I, Group II, Group III and Group IV). These results on the grouping of accessions on the basis of genetic distance were generally consistent with the results from the morphological analysis. Group I contained 2 accessions with bright red, sour fruits collected from the Bangalore region. Group II also contained 2 accessions with sweet taste collected from the Western Ghats region. Group III comprised of 5 accessions of which two are from the Bangalore region



**FIGURE 5.** Dendrogram generated from SRAP data showing relationships of 30 karonda accessions based on Unweighted Pair-Group Method of Arithmetic Averages (UPGMA).

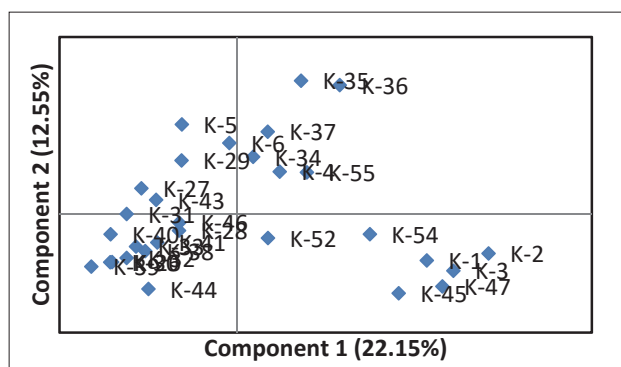
and the remaining from Western Ghats. Group IV was further divided into five subgroups (a, b, c, d and e). Subgroup IV included a single accession from the Western Ghats. Subgroup IVb comprised of 3 sweet accessions from the Western Ghats. Subgroup IVc included 2 accessions while subgroup IVd contained 13 accessions mostly from the Western Ghats area. The last subgroup IVe comprised of 2 accessions from the Bangalore region. In general, it was found that genotypes derived from identical or neighboring areas clustered into the same group or subgroup. In an earlier study conducted by

**TABLE 5.** Polymorphic information content (PIC) value and incidence for 11 SRAP PCs.

| Sl. No. | Primer combination | No. of total bands | No. PB | PPB (%) | PIC      |
|---------|--------------------|--------------------|--------|---------|----------|
| 1       | Primer 1F-7R       | 8                  | 7      | 87.5    | 0.346389 |
| 2       | Primer 1F-11R      | 8                  | 8      | 100     | 0.286111 |
| 3       | Primer 1F-13R      | 4                  | 3      | 75      | 0.285185 |
| 4       | Primer 1F-14R      | 5                  | 5      | 100     | 0.292444 |
| 5       | Primer 2F-4R       | 6                  | 5      | 83.3    | 0.367037 |
| 6       | Primer 3F-11R      | 7                  | 5      | 85.71   | 0.357143 |
| 7       | Primer 4F-1R       | 7                  | 5      | 85.71   | 0.338519 |
| 8       | Primer 3F-15R      | 9                  | 9      | 100     | 0.392099 |
| 9       | Primer 4F-3R       | 5                  | 2      | 40      | 0.284444 |
| 10      | Primer 4F-6R       | 8                  | 8      | 100     | 0.288056 |
| 11      | Primer 4F-12R      | 4                  | 3      | 75      | 0.15     |
| Total   |                    | 71                 | 60     |         | 3.38     |
| Mean    |                    |                    |        | 84.74   | 0.307    |

PB- Polymorphic Bands; PPB- Percentage of Polymorphic Bands; PIC- Polymorphic Information Content.





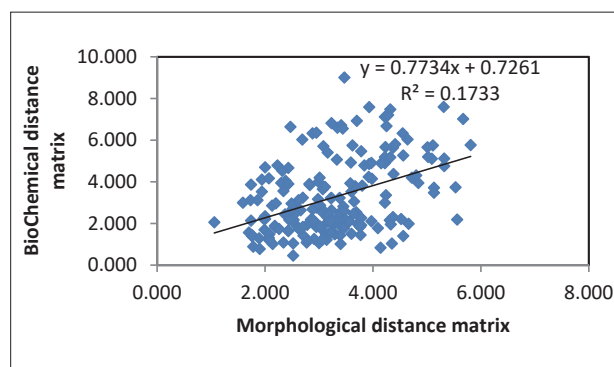
**FIGURE 6.** Scatter plot obtained from principal coordinate analysis using 11 SRAP markers in 30 karonda accessions.

Meghwal *et al.* (2014) using RAPD markers, no consistency in grouping of *C. carandas* was reported as morphologically similar accessions were genetically catalogued into different clusters. In our study, the UPGMA dendrogram analysis and PCoA plot shared a similarity in the grouping of the accessions. The subpopulations IVb, IVC and IVD could be discriminated clearly while the accessions from mixed subpopulation were placed in the middle of the three subpopulations.

**Principal coordinates analysis (PCoA) and analysis of molecular variance (AMOVA)**

As shown by the two-dimensional scatter plot (Figure 6), the first two PCoA axes account for 22.15% and 12.55% of the genetic variation, respectively. The UPGMA dendrogram analysis and PCoA plot share a similarity in the grouping of the accessions. The subpopulations IVb, IVC and IVD could be discriminated clearly while the accessions from mixed subpopulation were placed in the middle of the three subpopulations.

Evaluation of within and among subpopulation diversity was performed by analysis of molecular variance (AMOVA). Based on the overall PhiPT = 0.097 (P < 0.01) values, it was observed that the variance within subpopulations was the major contributor accounting for 90% of the total variation, while differences among subpopulations contributed to 10% of total variation (Table 6). This could be due to the method of propagation followed in karonda which is propagated vegetatively by cuttings. This crop is highly adaptable to diverse soil and climate conditions, resulting in its widespread cultivation in different parts of the country. The presence of a high level of genetic diversity within populations and the lower level between populations suggests that there is a high level of migration and gene flow among regions due to the movement of genetic material. SRAP marker was found to be useful for studying the genetic diversity and population structure, since genomic resources such as SSR and SNP markers are lacking in this crop. SRAP markers are comparable to AFLP markers for characters like reproducibility, the number of bands and polymorphism with the advantage that it targets the gene-rich regions. These markers have been successfully used in several crops such as citrus (Amar *et al.*,



**FIGURE 7.** Correlation between distance matrices of morphological and biochemical data.

2011), persimmon (Jing *et al.*, 2013a), almond (Jing *et al.*, 2013b) and passion fruit (Oluche *et al.*, 2018), which indicates the potential use of these markers for molecular characterization of the available germplasm.

**Correlations among morphological, biochemical, and genetic distances**

Weak correlations were found between Euclidean distance matrices of morphological data and molecular data from SRAP (r<sub>xy</sub> = 0.228) and between biochemical and molecular distance matrices (r<sub>xy</sub> = 0.166). However, higher correlation was found between Euclidean distance matrices of biochemical and morphological data (r = 0.416) (Figure 7).

Human selection for certain desirable plant phenotypes could have played some role in fruit biochemical traits. Our study did not identify significant correlations between molecular and biochemical data sets. However, such correlations have been reported in other plant taxa such as potato (Sulli *et al.*, 2017), chilli peppers (Torres *et al.*, 2012) and coriander (Lopez *et al.*, 2008).

**Conclusions**

The study on genetic diversity and relatedness using morphological, biochemical and molecular markers was successful in categorizing karonda accessions into different groups based on sweetness, colour of fruits and geographical origin. The combination of all three systems of characterization provided important information about this minor fruit crop. The fruit breeders largely depend on the morphological and biochemical characterization for understanding the variation present in the accessions, however, molecular markers provide the genetic basis for the same. Through this work we came to know that significant diversity exists among the accessions. This can be further broadened by selecting a greater number of accessions from different geographical areas. This work will help in parental selection, genotype identification, conservation and enhancing future karonda improvement programs.

**TABLE 6.** Summary analysis of Molecular Variance (AMOVA).

| Source of variation | Sum of squares | Estimated variation | % variation |
|---------------------|----------------|---------------------|-------------|
| Among populations   | 23.643         | 1.123               | 10          |
| Within populations  | 293.057        | 10.466              | 90          |
| Total               | 316.700        | 11.589              |             |

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## Conflict of interest

The authors declare that they have no conflict of interest.

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