

## Characterization of karonda (*Carissa carandas*) accessions under arid region

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### Abstract

*Carissa carandas*, native to India is an underutilized fruit crop with tremendous phyto-therapeutic and nutritive importance. Seven diverse accessions and a released variety were evaluated for morphological, biochemical and molecular diversity. The test accessions varied significantly with regard to all the morphological characters except plant height and number of stipules per node. The accession, CZK2011 and CZK 2031 recorded 30 and 3% higher fruit yield over the variety Pant Manohar while other accessions gave almost equal fruit yield to variety Pant Manohar. The accessions CZK2012, CZK2021 and variety Pant Manohar were found precocious due to first fruiting at three years of age. Seven primers detected low intra-specific variation amounting to 25 % polymorphism and exhibited 11.1 to 57.1% polymorphism in banding pattern indicating narrow genetic base in the available germplasm. The accessions CZK2011, CZK2022 and CZK2031 may be recommended for cultivation in arid zone on account of their higher yield and bigger sized fruits.

**Key words:** Karonda, genetic diversity, RAPD, accessions, precocity

### Introduction

Karonda (*Carissa carandas* L.) of family Apocynaceae is an underutilized fruit plant which thrives well throughout tropical and subtropical climate. It is native to India and also grown widely in other parts of world like Nepal, Afganistan, South Africa, Malaysia, Indonesia, Sri Lanka and Australia *etc.* In India, it grows wild in states of Bihar, West Bengal, Uttar Pradesh, lower, outer and middle Himalayas, Uttarakhand, Maharashtra, Rajasthan and parts of southern India (Malik *et al.*, 2010). Fruits are edible and rich in minerals such as iron, calcium, magnesium and phosphorus. The presence of vitamin C and anthocyanin enhances the antioxidant properties of karonda fruit (Sawant and Godghate, 2013). The fruit is used for preparation of jelly, pickle, beverage and preserve. Its fruits and seed latex are used for treating rheumatoid arthritis, piles, cardiac diseases and nerving disorder. The roots are useful in stomach disorder, intestinal worms, scabies, diabetic, ulcer and pruritus. The phyto-therapeutic significance of karonda has been described by Maheshwari *et al.* (2012). Germplasm of *Carissa* species have been widely collected from Kolhapur district of Maharashtra with high degree of genotypic and phenotypic variability (Sawant *et al.*, 2003). Variation in number of flower buds per umbel, length of petals and pedicels, petal number and flower colour was also recorded by Karale *et al.* (1990).

*C. carandas* is usually propagated by seeds which need immediate sowing after extraction as they are recalcitrant type. Vegetative propagation has been attempted using air layering but rarely used for propagation. Singh and Ravishankar (2010) attempted softwood grafting with success rate of 40-50%. The present investigation is an attempt to explore the extent of genetic diversity based on nuclear ribosomal DNA internal transcribed spacer and RAPD polymorphism in *C. carandas* for selection and crop improvement.

### Materials and methods

**Germplasm evaluation:** Seven germplasm accessions from different parts of Rajasthan, Gujarat and Uttarakhand and a released variety (Pant Manohar) were collected and planted at a spacing of 4 x 4 m in the field genebank at horticulture block of Central Arid Zone Research Institute, Jodhpur during 2000-2002. Ten year old plants were evaluated for vegetative growth, flowering, fruiting, morphological characters of leaves, fruits and stipules. Twenty randomly selected leaves were taken for measurement of length, width, leaf area and other morphological traits. Twenty mature fruits were harvested randomly from each accession to record observation on physico-chemical 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.

**DNA fingerprinting:** The genomic DNA was extracted from one gram of composite fresh leaves of each *C. carandas* test sample. A hybrid protocol for genomic DNA isolation developed using initial steps of CTAB method (Doyle and Doyle, 1990) and subsequently columns and solutions of the plant genomic DNA Purification spin kit (Hi-media Company) developed by Singh *et al.* (2013) was followed. The genomic DNA was finally eluted using 200 µL of Tris-EDTA buffer at 10000 rpm for one minute to obtain high molecular weight pure DNA for fingerprinting. DNA was quantified with UV/VIS spectrophotometer by measuring OD<sub>260</sub> and OD<sub>280</sub>. The quantified DNA samples were diluted in TE buffer to make a final concentration of 50ng/µL for PCR reactions.

**RAPD analysis:** The decamer random primers of OPA and OPB series (Operon Technologies) were used for screening of 8

samples of *C. carandas*. Based on the reproducibility of scorable bands the RAPD was performed using 7 decamer arbitrary primers. Amplification was performed in a total reaction mixture of 25 µL. Each reaction mixture contained: decamer primer, 50 pmol; dNTP mix, 0.25-mM (Bangalore Genei); MgCl<sub>2</sub>, 2.5-mM (Sigma Chem); *Taq* DNA polymerase 1U (Sigma Chem); 10× PCR buffer, 5.0-mM (Tris-HCl, pH 8.3, 15 mM MgCl<sub>2</sub>), 250 mM KCl), genomic DNA 40 ng in dH<sub>2</sub>O). RAPD-PCR amplification were performed in a thermal cycler (Corbett Research, USA) with initial denaturation step of 94 °C for 3 min followed by 34 amplification cycles of 94 °C for 40 sec, 50 °C for 40 sec and 72 °C for 2 min and final elongation at 72 °C for 7 min.

Amplicons were separated on a 1.4% agarose gel pre-stained with ethidium bromide solution using 1× TAE buffer. The gels were run for 3 h at 60 V. The size of the amplified fragments was determined using 1 kb ladder (MBI Fermentas). All RAPD reactions were performed twice to test the reproducibility of the amplicon profiles.

**Molecular analysis of RAPD:** The RAPD amplification products were scored as present (1) and absent (0) of scorable loci for each primer accession combination. Molecular data were entered into a binomial matrix and were used to determine Jaccard's similarity coefficient with NTSYS-pc software (Rohlf, 1997; Sneath and Sokal, 1973). Most informative primers were selected based on high polymorphism information content (PIC) value of individual primers.

$$PIC = \sum_{i=1}^n 2F(1-F)$$

Where F is frequency of presence of marker band, *i* is discrimination rate (DR) estimated to test the efficacy of individual primers in distinguishing the isolates, employing the formula DR = number

Table 1. Plant growth, fruiting, yield and leaf characteristics of *C. carandas* accessions

Accession No.	Plant height (m)	Canopy area (m <sup>2</sup> )	Age at first fruiting (years)	Fruit yield (kg plant <sup>-1</sup> )	Leaf length (cm)	Leaf width (cm)	Leaf area (cm <sup>2</sup> )	Stipules	
								Per node	Length (cm)
CZK2000	2.87	9.28	4	12.5	6.51	4.09	22.34	2.0	1.91
CZK2011	2.91	13.32	5	16.7	6.52	3.32	19.86	1.93	2.31
CZK2012	2.55	9.31	3	11.5	6.49	3.03	17.34	2.0	1.80
CZK2013	2.60	9.94	6	10.5	6.48	3.30	20.21	1.93	2.29
CZK2021	2.74	9.18	3	11.6	6.37	3.63	22.78	2.0	1.45
CZK2022	2.56	6.97	4	12.6	5.73	4.26	20.98	1.8	1.46
CZK2031	2.54	7.37	6	13.2	6.33	3.91	20.94	2.0	1.55
Pant Manohar	2.63	9.61	3	12.8	5.88	3.71	19.39	2.0	2.11
LSD ( <i>P</i> =0.05)	0.27	0.77	1	0.70	0.15	0.12	0.44	0.21	0.29

Table 2. Physico-chemical characteristics of fruits of *C. carandas*

Accession	Fruit size (mm)		Fruit weight (g)	Fruit volume (cc)	Number of seeds/fruit	TSS (°Brix)	Acidity	Dry matter (%)	Vitamin C (mg 100 <sup>-1</sup> g)
	Length	girth							
CZK2000	17.37	14.32	2.60	1.9	3.5	9.2	2.90	13.56	34.32
CZK2011	21.79	16.35	3.74	3.8	6.3	9.4	2.82	12.85	35.88
CZK2012	23.05	18.29	2.96	3.2	3.6	9.5	2.92	13.19	24.96
CZK2013	23.52	19.22	4.86	4.3	6.1	8.5	2.84	12.31	43.68
CZK2021	19.29	16.05	2.76	3.1	6.6	8.0	2.75	13.83	31.20
CZK2022	22.47	19.14	4.18	4.5	4.9	8.5	2.14	12.77	31.22
CZK2031	22.66	19.57	5.01	4.7	5.4	8.7	2.95	12.96	37.45
Pant Manohar	21.52	17.15	3.34	3.4	3.5	9.0	2.90	13.00	31.28
CD (5%)	0.77	0.68	0.30	0.2	0.21	0.2	0.17	0.22	0.48

of pairs of isolates differentiated/ total number of pairs.

## Results

**Morpho-physiological characterization:** The results of morpho-physiological characterization data of eight accessions of *C. carandas* are presented in Table 1. It is clear from the data that the karonda accessions varied significantly with regard to most of the morphological characters except plant height and number of stipules per node. The canopy area varied from 7.37 m<sup>2</sup> to 13.32 m<sup>2</sup>, leaf length from 5.73 to 6.52 cm, leaf width from 3.03 to 4.26 cm, leaf area from 17.34 to 22.78 cm<sup>2</sup>, stipule length from 1.45 to 2.29 cm. The germplasm lines showed great variation in fruit yield. Most of the accessions gave fruit yield equal to or greater than the check variety *i.e.* Pant Manohar. The fruit yield ranged from 10.5 kg per plant in accession CZK2013 to 16.7 kg per plant in accession CZK2011. The germplasm also exhibited diversity in precocity ranging from 3-6 years (age) of different accessions. The accessions CZK2012, 2021 and variety Pant Manohar were precocious being able to start fruiting at the age of three years.

The morphological and chemical characteristics of fruits of *C. carandas* are shown in Table 2. It is evident from the data that TSS (°Brix), acidity and dry matter exhibited significant variations but range was not wide. Whereas, fruit length, fruit girth, fruit weight, fruit volume, number of seeds per fruit and vitamin C contents varied quite significantly among the accessions tested.

**RAPD analysis:** Out of 18 decamer random primers, seven primers detected intra-specific variation generating scorable amplicons reproducible patterns and generated 60 marker bands in the range of 250 bp to 2.5 kb (Table 3). Among these, 15 markers were polymorphic amounting to 25 % polymorphism and exhibited 11.1 to 57.1% polymorphism in banding pattern.

Table 3. Details of primer code, GC content and per cent polymorphism of 7 RAPD primers used for molecular profiling of *C. carandas* accessions

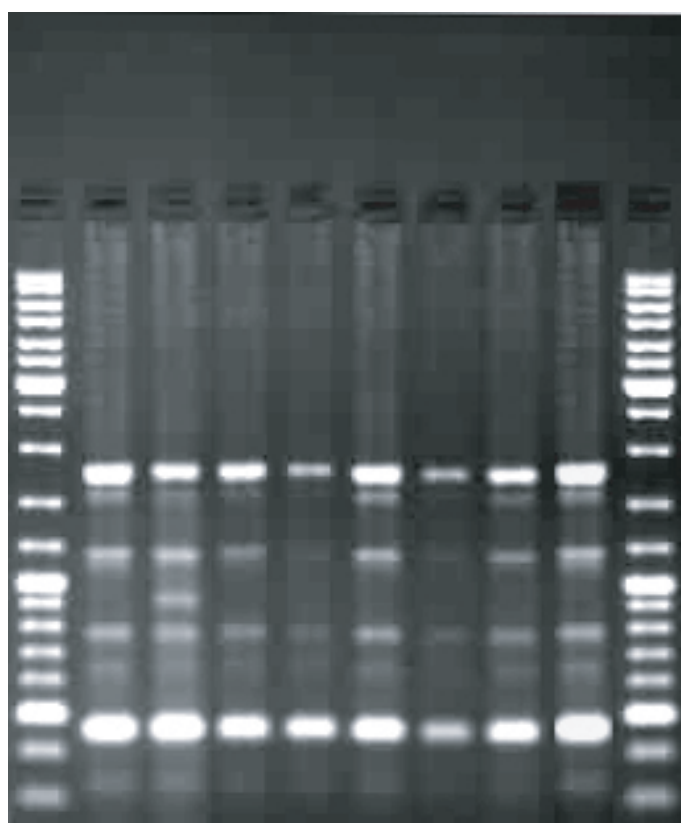
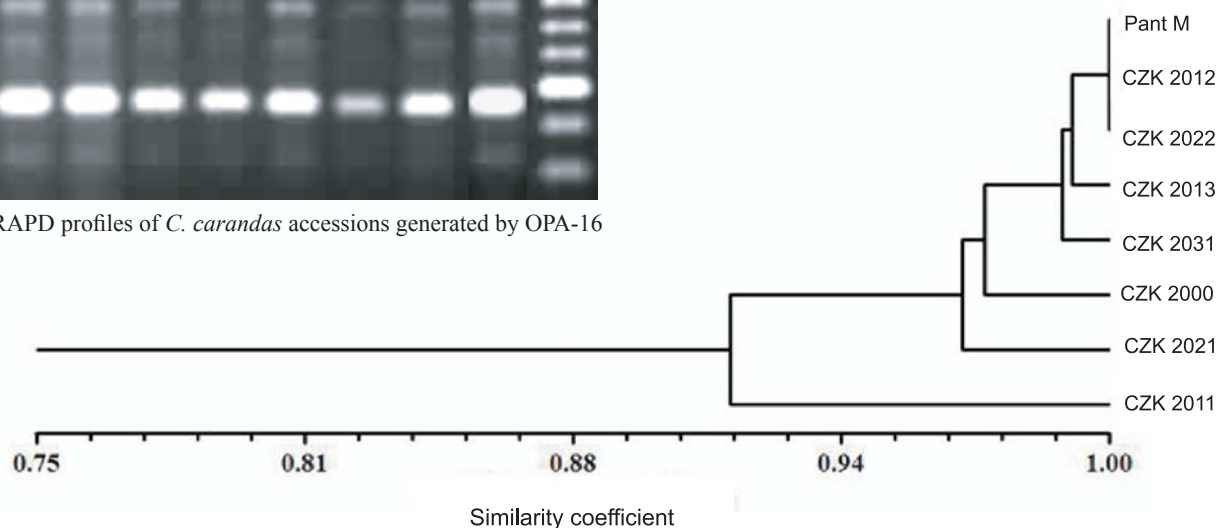
Primer	Primer Sequence	GC (%)	Number of Bands	Number of polymorphic bands	Polymorphism (%)	PIC value (%)
OPA-02	TGC CGA GCT G	70	12	3	25.0	92
OPA-09	GGG TAA CGC C	70	9	2	22.2	87
OPA-13	CAG CAC CCA C	70	9	1	11.1	89
OPA-16	AGC CAG CGA A	60	7	4	57.1	84
OPA-17	GAC CGC TTG T	60	4	1	25.1	75
OPB-05	TGC GCC CTT C	70	9	1	11.1	89
OPB-10	CTG CTG GGA C	70	10	3	30.0	90
Total			60	15	25.0	87

The number of PCR amplified products formed ranged from 4 (OPA-17) to 12 (OPA-02). The RAPD profiles generated by OPA-16 showing the maximum polymorphism is depicted in Fig 1. The PIC values ranged from 75 (OPA-17) to 92% (OPA-02). The dendrogram obtained from cumulative cluster analysis of 7 primers is shown in Fig. 2. The combined dendrogram of seven random primer data matrices delineated eight accessions in three main clusters. Cluster I had four accessions (CZK2021, CZK2022 and CZK2013) and the released variety Pant Manohar. Cluster II comprised of two accessions CZK2000 and CZK2021 and Cluster III had only one accession CZK2011.

## Discussion

The germplasm lines showed significant variation in fruit yield. Most of the accessions gave fruit yield equal to or greater than the check variety *i.e.*, Pant Manohar that exhibits the potentials of new accessions. Despite significant variations in the morphological and or chemical characters, there was no consistency among yield attributing traits. For example, the fruit yield ranged from 10.5 kg per plant in accession CZK2013 to 16.7 kg per plant in accession CZK2011 but shared similar ranges of plant height, leaf length, leaf width, leaf area, stipules per node, stipule length, number of seeds per fruit, acidity, dry matter *etc.* The germplasm also exhibited diversity in precocity behaviour ranging from 3-6 years (age) of different accessions but did not show any consistency *vis-à-vis* other characters. The data on leaf and stipular morphology did not show much variation among different accessions. Similar variation in morphological characteristics of leaf, stipules and growth habit among promising selections of karonda from hills of Maharashtra was recorded by Sawant *et al.* (2003). However, variation in number of flower bud per umbel, length of petals and pedicels, petal number and flower colour was also recorded by Karale *et al.* (1990). Present study reports that the accessions CZK2012, CZK2021 and variety Pant Manohar were more precocious being able to start fruiting at the age of three years.

Rich diversity in the morphological and chemical characteristics of the fruits of different accessions validates the existence of genetic diversity in *C. carandas* accessions developed at field genebank in CAZRI. Variations in the germplasm of Karonda with regard to physico-chemical characters, maturity period and

Fig. 1. RAPD profiles of *C. carandas* accessions generated by OPA-16Fig. 2. Dendrogram obtained from cumulative cluster analysis of *C. carandas* accessions

yield components have also been reported from Punjab (Bal, 2003). High degree of genotypic and phenotypic variability have been reported from Maharashtra by Sawant *et al.* (2003). The perusal of morpho-chemical *vis-à-vis* RAPD markers exhibited no consistency in grouping of *C. carandas* accessions with similar morphological traits as phenotypically similar accessions were genetically catalogued into different clusters of RAPD dendrogram.

The morphological characterization based on fruit yield and yield attributing traits also appear to be arbitrary as the high yielding accession CZK2031 with 13.2 kg fruit yield per plant exhibited closest proximity with lowest yielding accession CZK2013 with 10.5 kg fruit yield per plant. Similarly, phenologically very distinct accessions *i.e.*, Pant Manohar, CZK2022 and CZK2013 with 3, 4 and 6 years age at first fruiting were grouped together (cluster I) in RAPD dendrogram.

Qi *et al.* (2009) constructed a dense genetic linkage map and identified six QTLs to explain phenotypic variation by using AFLP and RAPD powerful and robust genetic markers in Chinese jujube. The molecular markers such as RAPD and AFLP have also been applied for characterization, evaluation and utilization of tree germplasm resources and developing conservation strategies (Wang *et al.*, 2009). RAPD analysis has revealed presence of polymorphic sites in the genomic DNA among the accessions of *C. carandas* studied. However, a low level of polymorphism amounting to only 25% among *C. carandas* accessions by RAPD markers revealed low genetic diversity among the accessions analysed indicating its narrow genetic base in the available germplasm.

Based on the performance of the germplasm over a period of eight years for fruit yield and other desirable attributes, the accessions CZK2011, CZK2022 and CZK2031 may be recommended for cultivation in arid zone on account of their higher yield and bigger sized fruits.

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