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NOTES ON NEGLECTED AND UNDERUTILIZED CROPS

Characterization of *Prosopis cineraria* (L.) Druce germplasm with suitable horticultural traits from the hot arid region of Rajasthan, India

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Abstract Sixteen germplasm accessions of *Prosopis cineraria* with suitable horticultural traits were identified from north-western Rajasthan, India, propagated clonally by budding on seedling rootstock and maintained in the field gene bank. Morphological characterization of seven-year-old trees of these accessions by 21 traits indicated a lot of variation among the accessions tested. Higher number of flowers per raceme was found in accession CIAH/K2, higher width of ripened pod in CIAH/K5, higher number of seeds per pod in CIAH/K12 and a higher weight of seed per pod in CIAH/K6. Overall, CIAH/K16 was found to be a superior genotype for most of the useful traits. High significant positive correlation was obtained with traits useful for horticultural values. Out of 62 random decamer primers for random amplification (RAPD) reaction, and four minisatellite core sequence for direct amplification of minisatellite DNA (DAMD) screened with these accessions, 12 RAPD and 2 DAMD primers were found polymorphic. Average polymorphism resolved by these markers among the accessions was 93.2%. Genetic diversity revealed by Jaccard's co-efficient was between 0.11 and 0.77, and four major clusters were identified among these accessions by phylogenetic analysis using NTSYSpc-2.02e software. This study shows the existence of high genetic diversity within these accessions.

Keywords Genetic diversity · Horticulture · Morphology · *Prosopis cineraria* · RAPD

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Introduction

Prosopis cineraria (L.) Druce, commonly called "khejri" (synonyms: jambi, jambu, shumi, jandi) is a leguminous, multipurpose tree species distributed in the arid and semi-arid regions of India, Afghanistan, Pakistan, Iran and Arabia (Pareek 2002; Hanelt and IPK 2001). This tree is commonly found in the Thar Desert of India and Pakistan (Pasiecznik et al. 2004). In India, it is mostly found in the north-western parts of Rajasthan, Gujarat and Haryana. It is commonly used in dry land agro-forestry in India for various purposes like vegetable, fodder, firewood, timber, gum, medicine, fencing material and shade to the inhabitants of the Thar Desert (Dwivedi et al. 1997). Apart from these uses, it is extremely drought tolerant, promotes the fertility of the soil by increasing organic carbon, N, P and K under its canopy (Srivastava and Hetherington 1991).

Prosopis cineraria ordinarily attains heights of up to 10 m. The stem is commonly straight with a grey roughish bark, exfoliated in numerous thin flakes (Arshad et al. 2006). The branches are slender and

glabrous. Leaves are usually in two pairs with 7–10 pairs of leaflets. Flowers are greenish yellow in auxiliary spikes solitary or in terminal panicles. Pods are usually 10–20 cm long, rigid, cylindrical, glabrous, having thin exocarp, pulpy mesocarp and papery endocarp containing 10–15 seeds.

Prosopis cineraria is considered to be perennial vegetable crop. Flowering and fruiting of this tree occurs during the driest period from February to May. The pods are highly nutritious both at unripe and ripened stages (Gupta et al. 1974). Unripe green pods, commonly known as "sangari", are used as vegetable and is rich in protein (5.1%) and fibre (6.7%). Harvested unripe pods are boiled, dried and sold in the market as vegetable either single product or in combination with other local plant products such as *Panch-kutta*, a mixture of five species. The flour of mature pods is being used for making *chapatti* or mixing with biscuits and cookies preparation (Pareek 2002).

The pod of *P. cineraria* has two kinds of tastes; sweet (without any acrid) and acrid. The sweet pods are preferred for vegetable purpose. Therefore the trees yielding sweet pods are of horticultural use (Samadia et al. 2002). This is one of the ideotype characters of *P. cineraria* suitable for horticultural use (Dwivedi et al. 1997). Due to cross pollination, high degree of natural variation is observed in the morphology of this species which gives excellent opportunities to identify elite genotypes from the natural population. Various surveys have been conducted by Central Institute for Arid Horticulture (CIAH) from 1995 to 2002 in the arid region of Rajasthan and identified some of the promising genotypes of *P. cineraria* suitable for vegetable type (Pareek and Nath 1997; Nath et al. 2000; Samadia et al. 2002). More genetic diversity in the genus *Prosopis* (Goswami and Ranade 1999; Ferreyra et al. 2004; Juarez-Munoz et al. 2002) was earlier determined using randomly amplified polymorphic DNA (RAPD) by many researchers. Wide genetic variations have been reported in the species *P. cineraria* at both morphological and molecular level (Dwivedi et al. 1997; Arshad et al. 2006; Bahadur and Hooda 1995; Shukla and Kasera 2003; Sharma et al. 2010). Recently, PCR based single primer amplification reaction (SPAR) methods were more efficient in determining genetic diversity of this species (Sharma et al. 2010). Existence of genetic diversity in the natural population within the vegetable type *P. cineraria* genotypes has also been reported (Samadia et al. 2002). Therefore, the elite genotypes of *P. cineraria* suitable for horticultural purpose need to be conserved in the field gene bank by vegetative propagation and its characterization is essential to improve its horticultural traits for sustainable usage. Hence, the present investigation focused on morphological and molecular characterization of *P. cineraria* germplasm which are maintained at the field gene bank of CIAH for horticultural purpose.

Materials and methods

Survey, collection and maintenance of germplasm

Surveys were conducted in north-western region of Rajasthan, India to collect genotypes of *P. cineraria* suitable for perennial vegetable on the basis of the pod taste during March–April, 2002. During survey, individual 20–25-year-old trees were randomly selected and pods were tasted. Trees bearing sweet tasting pods were selected and five bud woods of 0.25–0.5 cm thickness of stem containing a minimum of 10 buds were selected from each tree. These bud woods were cut and packed in gunny bags and transported to the CIAH farm. These buds were clonally propagated by budding on the rootstock of *P. cineraria* seedlings by patch budding technique (Pareek and Purohit 2002). Buds of each tree were established on 10 seedling rootstocks and maintained in the field gene bank of CIAH (Table 1).

Morphological characters and data analysis

Sixteen accessions of 7 year old tree were selected for characterization. From each accession three trees were selected for replication. Twenty-one morphological characters (Table 1) including leaf, floral organ, tender & mature pod, pulp and seed were recorded as per the method described by earlier researchers (Samadia et al. 2002; Dwivedi et al. 1997; Arshad et al. 2006). Data were subjected to analysis of variance followed by post hoc test Duncan's Multiple Range Test (ANOVA-DMRT, $P < 0.05$) using the SPSS statistical software (14.0) for windows OS and coefficient of correlation and heritability was analyzed using INDOSTAT software, Hyderabad, India.

Table 1 Variations in morphological traits of *P. cineraria* germplasm used in this study

Accessions	Leaflet length (cm)	Leaflet width (cm)	Number of leaflet/pinna	Internodal length (cm)	Length of raceme (cm)
(a)					
CIAH/K1	1.50 ± 0.56^{d}	0.43 ± 0.06^{cd}	20 ± 4.0^{b}	3.5 ± 0.2^{cd}	9.53 ± 2.14^{bcd}
CIAH/K2	1.20 ± 0.10^{ab}	0.43 ± 0.06^{cd}	20 ± 4.0^{b}	4.53 ± 1.17^{e}	9.7 ± 1.13^{bcd}
CIAH/K3	1.07 ± 0.06^{ab}	0.4 ± 0.0^{cd}	18.7 ± 2.31^{a}	4.0 ± 0.87^{de}	12.07 ± 1.68^{cdef}
CIAH/K4	1.20 ± 0.10^{ab}	0.47 ± 0.06^{d}	18 ± 3.45^{a}	3.17 ± 0.06^{cd}	9.6 ± 0.10^{bcd}
CIAH/K5	1.10 ± 0.00^{ab}	0.3 ± 0.0^{ab}	22 ± 0.0^{c}	2.1 ± 0.36^{ab}	9.33 ± 2.83^{bc}
CIAH/K6	1.03 ± 0.15^{ab}	0.23 ± 0.06^{a}	22 ± 3.46^{c}	2.5 ± 0.5^{abc}	6.3 ± 0.20^{a}
CIAH/K7	1.30 ± 0.01^{bcd}	0.37 ± 0.06^{bc}	22 ± 5.29^{c}	3.37 ± 0.74^{cd}	13.9 ± 2.35^{f}
CIAH/K8	0.97 ± 0.06^{ab}	0.4 ± 0.0^{cd}	18 ± 2.0^{a}	3.0 ± 0.5^{bcd}	10.9 ± 2.35^{bcde}
CIAH/K9	1.17 ± 0.06^{ab}	0.4 ± 0.0^{cd}	18 ± 2.0^{a}	2.83 ± 0.29^{abc}	12.33 ± 1.60^{def}
CIAH/K10	1.10 ± 0.17^{ab}	0.47 ± 0.06^{d}	17.33 ± 1.55^{a}	1.87 ± 0.12^{a}	9.0 ± 0.4^{b}
CIAH/K11	1.2 ± 0.0^{ab}	0.23 ± 0.06^{a}	20.67 ± 1.15^{b}	3.4 ± 0.36^{cd}	12.83 ± 1.26^{ef}
CIAH/K12	1.13 ± 0.15^{ab}	0.4 ± 0.0^{cd}	20.67 ± 2.31^{b}	2.57 ± 0.40^{abc}	12.23 ± 1.75^{cdef}
CIAH/K13	1.10 ± 0.1^{ab}	0.3 ± 0.0^{ab}	21.33 ± 3.05^{c}	2.97 ± 0.57^{bcd}	8.73 ± 0.46^{ab}
CIAH/K14	1.07 ± 0.06^{ab}	0.47 ± 0.05^{d}	18.67 ± 1.15^{a}	2.93 ± 0.40^{bcd}	11.43 ± 0.40^{bcde}
CIAH/K15	0.90 ± 0.01^{a}	0.37 ± 0.05^{bc}	18 ± 0.0^{a}	3.13 ± 0.76^{bcd}	10.7 ± 0.26^{bcde}
CIAH/K16	1.60 ± 0.03^{d}	0.4 ± 0.0^{cd}	20.67 ± 4.62^{b}	3.3 ± 0.20^{cd}	20.66 ± 0.57^{g}
Mean ± SD	1.16 ± 0.23	0.38 ± 0.08	19.75 ± 2.79	3.07 ± 0.78	11.20 ± 3.32
Range	0.9–1.6	0.23–0.47	17.33–22.0	1.87–4.53	6.3–20.66
Heritability	0.66	0.89	0.16	0.76	0.93

Accessions	No. of buds/raceme	No. of flowers/raceme	No. of pods/raceme	No. of raceme/panicle	Length of tender pod (cm)
(a)					
CIAH/K1	50 ± 9.54^{a}	37.67 ± 9.86^{a}	4.33 ± 0.58^{abc}	5.0 ± 1.0^{b}	11.1 ± 0.46^{bcd}
CIAH/K2	64 ± 8.89^{a}	48.33 ± 10.21^{ab}	5.67 ± 2.08^{cd}	8.33 ± 2.08^{d}	12.5 ± 1.32^{cd}
CIAH/K3	55 ± 9.0^{a}	38.33 ± 14.74^{a}	3.33 ± 1.52^{b}	5.33 ± 0.58^{bc}	11 ± 1.48^{bcd}
CIAH/K4	60.67 ± 12.22^{a}	50.0 ± 5.57^{bc}	4.67 ± 1.15^{bcd}	6.0 ± 1.0^{bcd}	11.6 ± 0.95^{bcd}
CIAH/K5	96 ± 26.23^{bc}	55.33 ± 7.63^{c}	3.67 ± 0.57^{ab}	6.33 ± 1.53^{bcd}	9.47 ± 0.75^{abc}
CIAH/K6	56.67 ± 5.50^{a}	48 ± 10.40^{ab}	9.33 ± 2.31^{d}	6.0 ± 1.0^{bcd}	15.87 ± 1.18^{de}
CIAH/K7	78.33 ± 10.12^{ab}	45.67 ± 12.58^{a}	2.33 ± 0.57^{ab}	7.33 ± 3.21^{cd}	17.9 ± 1.97^{e}
CIAH/K8	72.33 ± 34.53^{ab}	46.33 ± 9.30^{a}	5.0 ± 1.00^{bcd}	6.0 ± 1.0^{bcd}	10.77 ± 1.25^{ab}
CIAH/K9	74.66 ± 8.02^{ab}	64.66 ± 7.50^{cd}	6.0 ± 2.00^{cd}	7.0 ± 1.0^{c}	8.57 ± 0.60^{ab}
CIAH/K10	98 ± 19.08^{bc}	63.67 ± 11.84^{cd}	1.67 ± 0.58^{a}	7.0 ± 1.73^{c}	8.3 ± 0.26^{ab}

Table 1 continued

Accessions	Leaflet length (cm)	Leaflet width (cm)	Number of leaflet/pinna	Internodal length (cm)	Length of raceme (cm)
CIAH/K11	99.67 ± 19.66^{bc}	72.66 ± 19.14 ^{cd}	4.67 ± 0.58^{bc}	1.67 ± 0.58^{a}	7.4 ± 4.98^{a}
CIAH/K12	100.33 ± 9.30 ^{cd}	81.67 ± 16.01^{d}	5.0 ± 1.0^{bcd}	6.67 ± 0.58^{bcd}	11.5 ± 1.73^{bcd}
CIAH/K13	70.67 ± 4.93^{ab}	62.66 ± 6.80^{bc}	5.0 ± 1.0^{bcd}	6.67 ± 1.154^{bcd}	13.33 ± 2.08^{d}
CIAH/K14	79.66 ± 14.15^{abc}	61 ± 17.34^{abc}	6.0 ± 2.65 ^{cd}	6.67 ± 0.58^{bcd}	14.97 ± 1.50^{d}
CIAH/K15	72.33 ± 9.07^{ab}	55 ± 5.57^{ab}	7.33 ± 0.58 ^{cd}	5.0 ± 1.0^{b}	13.37 ± 0.71^{d}
CIAH/K16	142.67 ± 15.28^{e}	103.33 ± 21.97^{e}	15.33 ± 2.52^{e}	7.67 ± 0.58 ^{cd}	19.63 ± 2.50^{ef}
Mean ± SD	79.43 ± 26.26	58.39 ± 19.51	5.58 ± 3.34	6.16 ± 1.84	12.32 ± 3.68
Range	50–142.67	37.67–103.33	1.67–15.33	1.67–8.33	7.4-19.63
Heritability	0.86	0.84	0.92	0.72	0.90

Accessions	Width of tender pod (cm)	Weight of tender pod (g)	Length of ripened pod (cm)	Width of ripened pod (cm)	Weight of pulp/pod (g)
(b)					
CIAH/K1	0.13 ± 0.06^{a}	0.55 ± 0.24^{ab}	12.83 ± 2.46 ^{cd}	0.5 ± 0.0^{ab}	3.55 ± 0.48^{d}
CIAH/K2	0.27 ± 0.05^{b}	0.75 ± 0.09^{bc}	15.2 ± 3.92^{de}	0.5 ± 0.0^{ab}	3.59 ± 0.42^{d}
CIAH/K3	0.3 ± 0.00^{bc}	0.64 ± 0.20^{b}	11.63 ± 1.70^{c}	0.53 ± 0.12^{b}	2.14 ± 0.71^{c}
CIAH/K4	0.23 ± 0.06^{b}	0.67 ± 0.08^{b}	12.8 ± 2.81 ^{cd}	0.57 ± 0.15^{bc}	1.03 ± 0.42^{ab}
CIAH/K5	0.33 ± 0.06^{c}	1.09 ± 0.26^{c}	6.0 ± 1.0^{a}	0.67 ± 0.12 ^{cd}	2.92 ± 0.83 ^{cd}
CIAH/K6	0.17 ± 0.05^{ab}	0.59 ± 0.14^{ab}	9.77 ± 3.29^{bc}	0.53 ± 0.06^{ab}	1.36 ± 0.65^{b}
CIAH/K7	0.23 ± 0.06^{b}	0.90 ± 0.16^{c}	13.77 ± 2.97 ^{cd}	0.6 ± 0.10^{bc}	3.61 ± 0.60^{d}
CIAH/K8	0.3 ± 0.0^{bc}	0.71 ± 0.20^{bc}	10.17 ± 2.75^{bc}	0.56 ± 0.06^{b}	2.11 ± 0.18^{c}
CIAH/K9	0.27 ± 0.06^{b}	0.64 ± 0.01^{b}	7.37 ± 2.17^{ab}	0.63 ± 0.06^{c}	2.33 ± 0.83^{c}
CIAH/K10	0.2 ± 0.00^{ab}	0.4 ± 0.03^{a}	8.5 ± 0.07^{abc}	0.5 ± 0.10^{ab}	1.81 ± 0.53^{bc}
CIAH/K11	0.17 ± 0.06^{ab}	0.48 ± 0.25^{a}	6.17 ± 0.91^{a}	0.37 ± 0.12^{a}	0.77 ± 0.08^{a}
CIAH/K12	0.17 ± 0.06^{ab}	0.49 ± 0.08^{ab}	17.53 ± 1.62^{ef}	0.5 ± 0.20^{ab}	2.33 ± 0.14^{c}
CIAH/K13	0.2 ± 0.0^{b}	0.77 ± 0.09^{bc}	14.13 ± 2.83^{de}	0.63 ± 0.06^{c}	1.38 ± 0.09^{b}
CIAH/K14	0.33 ± 0.06^{c}	1.18 ± 0.52^{c}	11.77 ± 1.70^{bc}	0.5 ± 0.10^{ab}	2.05 ± 0.59^{bc}
CIAH/K15	0.23 ± 0.06^{b}	0.81 ± 0.14^{bc}	16.13 ± 2.92^{ef}	0.53 ± 0.12^{ab}	1.83 ± 0.19^{bc}
CIAH/K16	0.33 ± 0.06^{c}	2.00 ± 0.84^{d}	18.17 ± 0.76^{f}	0.57 ± 0.06^{bc}	3.01 ± 0.51^{bcd}
Mean ± SD	0.24 ± 0.07	0.79 ± 0.44	11.99 ± 4.20	0.54 ± 0.10	2.23 ± 0.97
Range	0.13–0.33	0.4–2.0	6.0–18.17	0.37–0.67	0.77–3.61
Heritability	0.82	0.81	0.87	0.32	0.90

Table 1 continued

Accessions	Weight of seed/pod (g)	No. of seeds/pod	Seed weight at maturity (g)	Tender pod*		Color of mature pod#
				Shape	Color	
(b)						
CIAH/K1	0.27 ± 0.06^{ab}	9.0 ± 1.73^{c}	0.71 ± 0.18^{b}	F	G	GP
CIAH/K2	0.35 ± 0.18^{b}	11.67 ± 6.35^{d}	0.39 ± 0.19^{ab}	R	G	LG
CIAH/K3	0.16 ± 0.10^{a}	6.0 ± 1.0^{ab}	0.24 ± 0.07^{a}	R	G	G
CIAH/K4	0.21 ± 0.01^{ab}	6.0 ± 1.0^{ab}	0.3 ± 0.05^{ab}	R	G	G
CIAH/K5	0.17 ± 0.06^{a}	$13.33 \pm 6.81d^{c}$	0.65 ± 0.17^{b}	R	G	LG
CIAH/K6	0.58 ± 0.02^{c}	14.67 ± 6.02^{def}	0.72 ± 0.70^{b}	F	G	GP
CIAH/K7	0.36 ± 0.10^{b}	14.33 ± 4.04^{def}	0.59 ± 0.24^{b}	F	G	LG
CIAH/K8	0.4 ± 0.17^{bc}	14.33 ± 2.08^{def}	0.52 ± 0.05^{b}	R	G	LG
CIAH/K9	0.2 ± 0.1^{ab}	6.33 ± 2.51^{ab}	0.4 ± 0.07^{ab}	R	G	LG
CIAH/K10	$0.2 \pm 0.02a^{b}$	7.33 ± 3.21^{b}	0.40 ± 0.06^{ab}	F	G	G
CIAH/K11	0.15 ± 0.04^{a}	13.67 ± 3.78^{de}	0.42 ± 0.12^{ab}	F	G	G
CIAH/K12	0.16 ± 0.01^{a}	16.0 ± 1.0^{f}	0.60 ± 0.27^{b}	F	G	GP
CIAH/K13	0.44 ± 0.14^{bc}	12.7 ± 1.15^{d}	0.48 ± 0.06^{ab}	F	G	GP
CIAH/K14	0.22 ± 0.01^{ab}	8.0 ± 1.0^{bc}	0.40 ± 0.12^{ab}	R	G	G
CIAH/K15	0.34 ± 0.20^{b}	5.0 ± 1.0^{a}	0.31 ± 0.098^{ab}	R	LY	LGP
CIAH/K16	0.55 ± 0.25^{c}	15.67 ± 0.6^{f}	0.91 ± 0.9^{bc}	F	G	LG
Mean ± SD	0.29 ± 0.17	10.88 ± 4.76	0.50 ± 0.25	–	–	–
Range	0.15–0.055	5.0–15.67	0.24–0.91	–	–	–
Heritability	0.83	0.74	0.49	–	–	–

Mean value (n = 3) ± standard deviation from mean

Different letters (superscript) in a particular column differ significantly at 95% confidence level (ANOVA-DMRT, $P < 0.05$)

* *F* flat type, *R* round type, *G* green, *LY* light yellow

\# *G* green, *LG* light green, *GP* greenish pink, *LGP* light greenish pink

Genomic DNA extraction, PCR amplification and *In silico* analysis

Genomic DNA was isolated from 100 mg of young emerging leaves from each accession separately using QIAGEN DNA isolation kit as per manufacturer's instructions (Genetix Biotech Asia Pvt. Ltd., India). The quality of DNA was checked by electrophoresis in 0.75% agarose gel. Sixty-two random decamer primer series; 1–20 of OPBE & OPBA and 10–20 of OPA & OPN (Operon Technologies, USA) and four direct amplification of mini-satellite DNA (DAMD) markers (M13, HVR, HBV & INS) (custom synthesized from Chromous Biotech. Ltd., India) were used. Initially, the PCR protocol was optimized with varying concentrations of (i) template DNA (50, 100 and 150 ng) (ii) *Taq* DNA polymerase (0.5, 1.0 and 2.0U; MBI Fermentas) and (iii) $MgCl_2$ (1, 2 and 3 mM). 100 ng of template DNA with 1.0U of *Taq* DNA polymerase and 2.0 mM $MgCl_2$ gave reproducible and good quality bands. These concentrations were constantly used for all the reactions. Three randomly selected genotypes, CIAH/K1, CIAH/K5 and CIAH/K14, were screened with 62 random primers and four DAMD markers. On the basis of initial screening, 12 random primers and two DAMD primers (Table 2) were selected as they showed polymorphic and reproducible bands. Using the optimized PCR protocol these 14 primers were again utilized to screen all the samples. The total volume of PCR reaction was performed with 50 µl containing 100 ng of template DNA, 2 mM $MgCl_2$, 20 pmol random or 50 pmol DAMD primers, 1U *Taq* DNA polymerase, 0.2 mM dNTP (MBI Fermentas) with the following thermal profile: initial denaturation of 94°C for 5 min followed by 40 cycles of 94°C for 1 min, 36°C (in case of DAMD primers 55°C) for 1 min and 72°C for 2 min. Final extension was set at 72°C for 10 min. The PCR products were loaded on 1.2% agarose gel pre-stained with ethidium bromide, electrophoresed and viewed under a UV transilluminator. All the amplified bands were counted manually along with their size. The presence of band was scored as '1' and absence as '0' cumulatively for both RAPD and DAMD markers. A pair-wise matrix of genetic distances between genotypes was determined using the Jaccard similarity coefficient and a phylogenetic tree was constructed using NTSYSpc-2.02e version 2.0.1.5 software (Applied Biostatistics, Inc).

Table 2 Details of primers used in this study and their polymorphism

S. no.	Primer name	Primer sequence 5′–3′ (length)	Total no. of bands	No. of polymorphic bands	Per cent polymorphism
RAPD					
1.	OPA-11	CAATCGCCGT (10 mer)	5	4	80
2.	OPA-18	AGGTGACCGT (10 mer)	5	5	100
3.	OPN-10	ACAACTGGGG (10 mer)	7	7	100
4.	OPN-14	TCGTGCGGGT (10 mer)	7	7	100
5.	OPN-15	CAGCGACTGT (10 mer)	4	3	75
6.	OPBA-11	CCACCTTCAG (10 mer)	3	3	100
7.	OPBA-15	GAAGACCTGG (10 mer)	7	7	100
8.	OPBA-20	GAGCGCTACC (10 mer)	8	8	100
9.	OPBE-2	ACGCCTGTAG (10 mer)	6	6	100
10.	OPBE-4	CCCAAGCGAA (10 mer)	6	5	83.33
11.	OPBE-9	CCCGCTTTCC (10 mer)	5	4	100
12.	OPBE-11	GTCCTGCTGT (10 mer)	3	3	100
DAMD					
1.	M13	GAGGGTGGCGGTTCT (15 mer)	2	2	100
2.	HVR	CCTCCTCCCTCCT (13 mer)	5	4	80
Total			73	68	93.2

Results

Out of 418 *Prosopis cineraria* trees examined 16 (~4%) were found to be bearing sweet pods and useful for vegetable purpose. Analysis of morphological traits showed wide variations among the accessions (Table 1a, b). One accession, CIAH/K16, showed higher values for most of the traits such as length of raceme, number of buds and flowers per raceme, length and weight of tender pod, length of ripened pod, number of seeds per pod, weight of pulp and seed weight at maturity. Some accessions showed higher values for some of the traits, for instance, higher number of flowers per raceme (8.33) observed in accession CIAH/K2, broader ripened pod (0.67 cm) in CIAH/K5, more number of seeds per pod (16) in CIAH/K12 and higher weight of seeds per pod (0.58 g) in CIAH/K6. Correlation co-efficient analysis for the morphological traits revealed that leaflet length had positive and highly significant ($P < 0.01$) correlation with length of raceme, weight of pulp and weight of seed during maturity. Similarly, number of leaflets per pinna had positive and high significant correlation with weight of seed during maturity, length of raceme with number of buds and flowers per raceme and weight of tender pod, number of buds per raceme with number of flowers per raceme and weight of tender pod, number of flowers per raceme with number of pods per raceme, length of tender pod with weight of tender pod and length of ripened pod, and width of tender pod with its weight. Leaflet width, length of raceme, number of buds, flowers and pods per raceme, length of tender pod and ripened pod, and weight of pulp per pod were found to be highly heritable traits (Table 1a, b). Fifty percent of the accessions bearing flat shape tender pod and others are round shape, and all the accessions bearing green tender pods except for the accession CIAH/K15 (Fig. 1). However, color of mature pods was differing with accessions (Table 1b).

Among 66 bands amplified by 12 RAPD primers, 62 were found polymorphic (93.94%) and six were polymorphic out of seven bands amplified by two DAMD primers. Totally 68 out of 73 bands were polymorphic (93.2%) (Table 2). 100 per cent polymorphism was shown by nine RAPD (75%) primers. Maximum number of bands (8) was produced by OPBA-20 followed by OPN-10, OPN-14 and OPBA-15, each producing seven bands (Table 2) whereas the lowest number of bands and polymorphism (75%) was produced by OPN-15. Average number of bands per primer in RAPD was 5.5. The band size observed was 0.2–3.0 kb in RAPD and 0.6–1.6 kb in DAMD marker. Jaccard's similarity co-efficient among the accessions was 0.11–0.77. Highest similarity co-efficient was found between the accessions CIAH/K4 and CIAH/K8 (0.77) followed by CIAH/K6 and CIAH/K8 (0.66). Phylogenetic analysis showed that there were four clusters found among

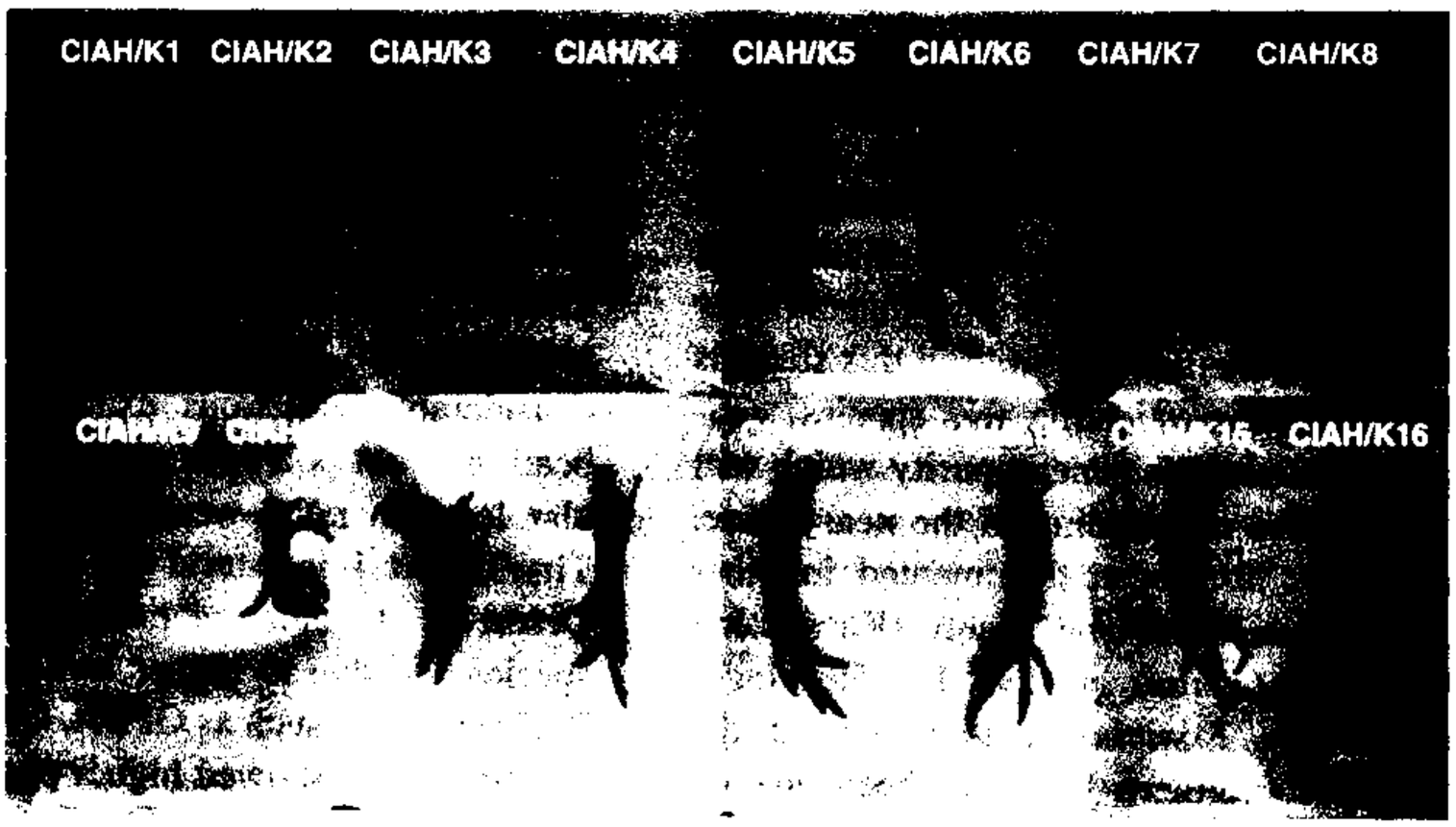

Fig. 1 Morphological diversity of tender pod among the sixteen accessions of *P. cineraria* suitable for horticultural use

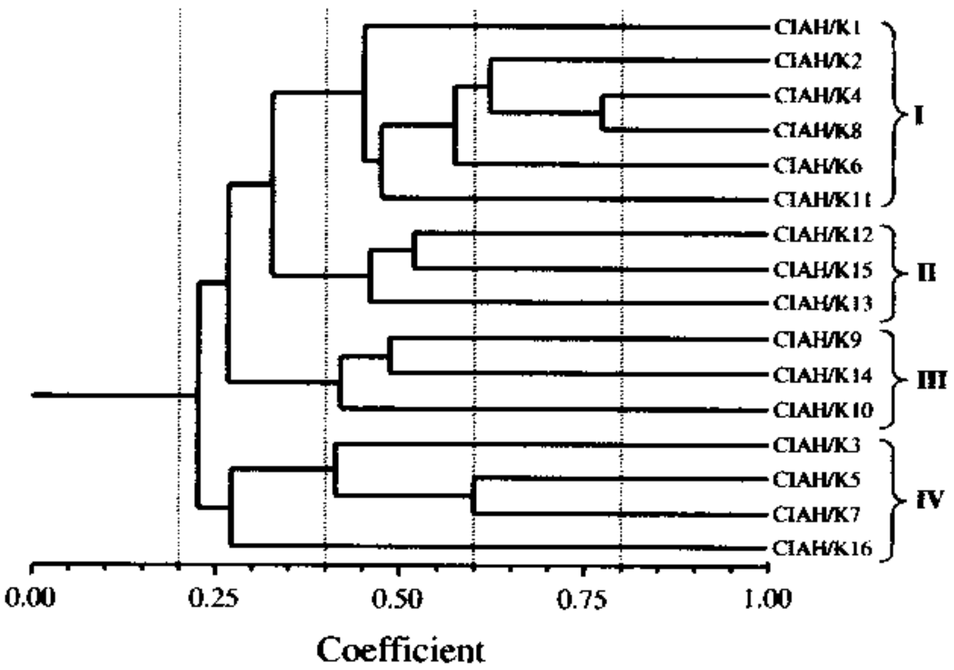

Fig. 2 Phylogenetic tree constructed based on sixteen accessions of *P. cineraria* germplasm analysed by RAPD and DAMD primers by UPGMA method and Jaccard coefficient using NTSYSpc-2.02e version 2.0.1.5 software. Vertical distance is arbitrary and horizontal distance indicates genetic distance. Clusters/groups among sixteen genotypes are indicated by *Roman numerals*

the accessions (Fig. 2). Cluster I had higher average similarity co-efficient of 0.57 and the lowest was cluster IV (0.42), while the other two clusters cluster II and III, had an average similarity co-efficient of 0.48 and 0.45, respectively. While comparing the clusters, the highest co-efficient was found between cluster II and III (0.35) and lowest between cluster III and IV (0.23).

Discussion

Conservation of *Prosopis cineraria* germplasm is essential as the population of this species is diminishing because it is threatened by the growing population, commercial usage of lands, reduction of ground water table (Pasiecznik et al. 2004), and mechanization of agricultural land. The vegetative or asexual propagation method followed in this study is certainly useful in domesticating this species, conserving and maintaining its genetic purity which will be used for purposes of breeding in the near future. Domestication has also been documented in other species like *Eryngium caucasicum* Trautv. (Khoshbakht et al. 2007) and *Lupinus pilosus* L. (Heistinger and Pistrick 2007). *Eryngium caucasicum* in the Elburz Mountains (northern Iran) has been characterized to its use for vegetable purpose and *Lupinus pilosus* in northern Italy has also been domesticated as coffee substitute. Various studies have demonstrated the genetic diversity of *P. cineraria* on natural population (Bahadur and Hooda 1995; Pareek and Nath 1997; Nath et al. 2000; Samadia et al. 2002; Arshad et al. 2006; Sharma et al. 2010). The advantage of the method described in this study is that, genotypes possessing useful traits can be maintained through vegetative propagation and used in future for improvement of this species particularly having horticultural values. The reason for the low frequency of trees yielding sweet pod in the natural population is not known and this trait does not seem to be linked with any of the traits tested. However, one monomorphic band was amplified by each RAPD markers; OPA-11, OPN-15, OPBE-4 and OPBE-9 for which linkage with sweetness along with genotypes yielding acrid pods will be studied in future. Many of the traits identified had high positive correlation with the traits useful for selecting genotypes suitable for vegetable purpose and they also showed high heritability. The accession, CIAH/K16, was found more suited for perennial vegetable crop and the institute has released this accession as a variety under the name of 'Thar Shoba'. This variety is being multiplied by vegetative means and distributed to the farmers of the arid region. Higher number of seeds per pod was observed in some of the accessions (CIAH/K6, CIAH/K7, CIAH/K8, CIAH/K12 and CIAH/K16). This trait is very useful as seeds may contain higher protein content (Samadia et al. 2002). This kind of identification and characterization of sweet pod bearing genotypes of *P. cineraria* will be the base of advanced breeding programme of this species to develop varieties/cultivars of high commercial value (Heistinger and Pistrick 2007). Intraspecific variation in *P. cineraria* was documented by Arshad et al. (2006) by morphological traits from Cholistan desert and Sharma et al. (2010) by SPAR method from Thar Desert. This study shows that considerable variations exist within the genotypes bearing sweet pod both at morphological and molecular level in north-western parts of Rajasthan, i.e., Thar Desert. It is natural to find variations in the Thar Desert as it could be the centre of origin of this species and due to higher level of cross pollination. RAPD primers such as OPBA, OPBE and OPN series identified here showed higher polymorphism and can be added to the polymorphic primers OPA and OPC series identified by Sharma et al. (2010). This will be

useful identifying the genetic diversity of this species. This seems to be the first report of characterization of germplasm accessions of *P. cineraria* for important horticultural traits.

References

Arshad M, Ashraf M, Arif N (2006) Morphological variability of *Prosopis cineraria* (L.) Druce, from the Cholistan desert, Pakistan. Genet Resour Crop Evol 53:1589–1596

Bahadur R, Hooda MS (1995) Genetic variability and correlation studies for some pod and seed traits in khejri (*Prosopis cineraria* (L.) Druce). Indian J For 18:161–164

Dwivedi NK, Bhandanri DC, Verma SK (1997) Collecting *Prosopis cineraria* (L.) Mac Bride Germplasm in Western India. IPGRI Newsletter for Asia. Pac Ocean 22:19

Ferreyra LI, Bessega C, Vilardi JC, Saidman BO (2004) First report on RAPDs patterns able to differentiate some Argentinean species of section Algarobia (Prosopis, Leguminosae). Genetica 121:33–42

Goswami M, Ranade SA (1999) Analysis of variations in RAPD profiles among accessions of Prosopis. J Genet 78:141–147

Gupta ML, Gandhi BM, Tandon BN (1974) An unconventional legume–Prosopis cineraria. Am J Clin Nutr 27:1035–1036

Hanelt P and Institute of Plant Genetics and Crop Plant Research (IPK) (eds) (2001) Mansfeld's encyclopedia of agricultural and horticultural crops. Springer, Berlin, pp 584–585

Heistinger A, Pistrick K (2007) 'Altreier Kaffee': *Lupinus pilosus* L. cultivated as coffee substitute in Northern Italy (Alto Adige/Südtirol). Genet Resour Crop Evol 54: 1623–1630

Juarez-Munoz J, Carrillo-Castaneda G, Arreguin R, Rubluo A (2002) Inter- and intra-genetic variation of four wild populations of *Prosopis* using rapd-pcr fingerprints. Biodivers Conserv 11:921–930

Khoshbakht K, Hammer K, Pistrick K (2007) *Eryngium caucasicum* Trautv. cultivated as a vegetable in the Elburz Mountains (Northern Iran). Genet Resour Crop Evol 54:445–448

Nath V, Pareek OP, Saroj PL, Sharma BD (2000) Biodiversity of khejri in arid region of Rajasthan: I–screening of khejri for culinary value. Indian J Soil Conserv 28:43–47

Pareek OP (2002) Topworking wild *Khejri* trees. Indian Hort 47:11–12

Pareek OP, Nath V (1997) Variability in horticultural traits of khejri in Thar desert. IPGRI Newsletter for Asia, the Pacific and Oceania 24:23–25

Pareek OP, Purohit AK (2002) Patch budding in khejri (*Prosopis cineraria*). Indian J Hort 59:89–94

Pasiecznik NM, Harris PJC, Smith SJ (2004) Identifying tropical *Prosopis* species: a field guide. HDRA, Coventry ISBN 0 905343 34 4

Samadia DK, Purohit AK, Pareek OP (2002) Genetic diversity in vegetable type *Khejri*. Indian J Agro For 4:132–134

Sharma SK, Rawat D, Kumar S, Kumar A, Kumaria S, Satyawada RR (2010) Single primer amplification reaction (SPAR) reveals intra-specific natural variation in *Prosopis cineraria* (L.) Druce. Trees 24:855–864

Shukla JK, Kasera PK (2003) Evaluation of *Prosopis cineraria* (Khejri) germplasm collected from diverse habitats of the Indian Thar Desert. Indian J Soil Conserv 31:52–56

Srivastava JP, Hetherington JC (1991) Khejri (*Prosopis cineraria*): a tree for the arid and semi-arid zones of Rajasthan. Int Tree Crops J 7:1–16