Analysis of variations in RAPD profiles among accessions of *Prosopis*

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Abstract

The genetic variability among accessions of *Prosopis* was determined using randomly amplified polymorphic DNA (RAPD) profiles. Similarities of profiles were determined using the algorithm of Jaccard, and UPGMA and neighbour joining trees were generated from the similarity data. The average similarity was highest among the accessions of *P. glandulosa* (0.52 ± 0.18) and least in the accessions of *P. juliflora* (0.37 ± 0.15), indicating that the latter species has greater diversity among accessions. Our observations suggest that RAPD analysis could help in identifying genetic variations among different accessions of *Prosopis*.

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Introduction

The genus *Prosopis* is one of the commercially important genera of legumes with multifarious benefits for mankind. For arid climatic zones, species of *Prosopis* are among the most important multipurpose leguminous trees, and are used for revegetation, agroforestry, apiculture, fodder and firewood (Leakey and Last 1980). As a genus *Prosopis* includes about 44 species that have been described using morphological criteria and these are in turn grouped into five sections. Further, these five sections include eight series. However, this classification does not seem to be rigid (Burkart 1976; Schinini 1981). At the morphological level, leaves and flowers are more or less similar while in case of fruit there is a development from straight to curved and spirally coiled loments. This latter character has been considered to be important for distinguishing plants at the species level (Burkart 1976). There is also a marked vegetative diversification in the presence or absence of spines, which also provides the foundation for a sectional subdivision of the genus (Burkart 1976). Similarly variation of characters such as isozymes (Saidman and Vilardi 1987, 1993), seed lipids (Lamarque *et al.* 1994), and mineral, crude protein and structural carbohydrate (Arya *et al.* 1995) have also been studied for distinguishing species, at least within a section and series. Other than the above reports of interspecies variations, not much is known about intraspecific genetic variability in *Prosopis* species, especially at the molecular level.

Molecular techniques have been found to be more useful and accurate for determination of both interspecies and intraspecies genetic variation in plants. Randomly amplified polymorphic DNA (RAPD) markers, in particular, have been successfully employed for determination of intraspecies genetic diversity in several plants. These include date palm (Corniquel and Mercier 1994), papaya (Stiles *et al.* 1993), poplars (Bradshaw *et al.* 1994) and amaranths (Ranade *et al.* 1997). In contrast fewer reports are available on determination of interspecies diversity in plants using the RAPD technique. Analysis of interspecies diversity in plants has invariably yielded information about the phylogenetics and/or systematics of the plant, as in *Juniperus* (Adams and Demeke 1993) and mahoganies (Chalmers *et al.* 1994). We have reported elsewhere on how the sectional classification of the genus *Prosopis* is not reflected in the molecular data on the basis of interspecies affinities of *Prosopis* species using RAPD profiles (S. A.

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Table 1. Species and accessions of *Prosopis* from which genomic DNA was isolated for RAPD studies.

Sample no.	Species	Section	Series	Origin
1	*P. affinis*	Algarobia	Pallidae	NA
2, 5	*P. chilensis*	Algarobia	Chilensis	NA
6, 7	*P. flexuosa*	Algarobia	Chilensis	NA
8–11	*P. glandulosa*	Algarobia	Chilensis	NA
12–18	*P. pallida*	Algarobia	Pallidae	NA
19, 20	*P. siliquastrum*	Algarobia	Chilensis	NA
21	*P. pubescens*	Strombocarpae	Strombocarpae	NA
22	*P. alba*	Algarobia	Chilensis	NA
23	*P. velutina*	Algarobia	Chilensis	NA
24	*P. cineraria*	Algarobia	Prosopis	NA
25	*P. juliflora*	Algarobia	Chilensis	Washington
26	*P. juliflora*	Algarobia	Chilensis	Oxford
27	*P. juliflora*	Algarobia	Chilensis	Israel
28	Unknown genotype	ND	ND	Jodhpur
29	*P. juliflora*	Algarobia	Chilensis	Kanpur
30	*P. juliflora*	Algarobia	Chilensis	Lucknow
31	*P. juliflora*	Algarobia	Chilensis	Lucknow (plus tree)
32	*P. juliflora*	Algarobia	Chilensis	Lucknow (plus tree)
33	*P. juliflora*	Algarobia	Chilensis	Lucknow (plus tree)
34	*P. juliflora*	Algarobia	Chilensis	Lucknow (plus tree)

NA, Information about the exact accession or origin either not available or not known; ND, no data about the section and series for the unknown genotype.
Source of all the plant material is Biomass Research Centre of National Botanical Research Institute, Lucknow, at Banthra.

Ranade and M. Goswami Interspecies affinities in the genus *Prosopis* based on RAPD profiles. In *Proceedings of symposium on advances in legume research in India.* (ed. R. R. Rao), in press). The present paper describes the RAPD profile variation among the different accessions of species of *Prosopis* as well as variation between different species of *Prosopis* and determination of the relative heterogeneity of the species. This is perhaps the first report for species of *Prosopis* on determination of RAPD-based intraspecies variations.

Materials and methods

Plant material: The Biomass Research Centre of National Botanical Research Institute, Lucknow, has a collection of *Prosopis* species and accessions (table 1). These were used in the present study. Leaves were collected from selected trees, placed into separate plastic bags, frozen in liquid nitrogen and stored at $-70°C$.

DNA isolation: Frozen leaves were powdered in a pre-chilled mortar using liquid nitrogen, and the DNA was then extracted by the method of de Kochko and Hamon (1990), with minor modifications as already described elsewhere (Ranade *et al.* 1997). The DNA recovered from the plant material, after purification by treatment with RNAase A, was reprecipitated with double volume of absolute ethanol, washed with 70% ethanol, and finally dissolved in TE buffer. Concentration of DNA was measured using Hoechst 33258 as a fluorochrome on a DyNA Quant 200 fluorometer (Pharmacia-Hoefer). The quality of DNA was checked by electrophoresis through 1% agarose gel in $0.5\times$ TBE according to Sambrook *et al.* (1989).

RAPD amplification and agarose gel electrophoresis: DNA amplification using arbitrary-sequence primers was standardized for *Prosopis* DNA. The reactions were essentially according to Williams *et al.* (1990). On the basis of this optimization with two or three DNA preparations (data not shown), all reactions were performed in 25-µl final volume and contained 10 pmol of the 10-mer primer (Operon Technologies, Inc., USA), 25 ng template, 200 µM each dNTP (Pharmacia), 3.5 mM Mg^{++}, and 1 unit *Taq* DNA polymerase (Pharmacia). Reactions were cycled 45 times at 94°C for 60 s, at 35°C for 90 s and at 72°C for 90 s, with a final extension at 72°C for 5 min, in a DNA Robocycler (Stratagene GmbH, Germany). After completion of the amplification 2.5 µl $10\times$ blue dye was added to the samples, and the amplified DNA was analysed on 1.4% agarose gel in $0.5\times$ TBE buffer (electrophoresis at constant current of 5 A overnight).

Data analysis: The fragment sizes of amplification products were estimated from the gel by comparison with standard molecular weight marker (lambda DNA double-digested with *Hin*dIII and *Eco*RI, Bangalore Genei, India). For each primer, a matrix of all the bands present in different DNAs was generated using '1' when the band was present and '0' when the band was absent. Similarities of profiles were determined using the algorithm of Jaccard (1901) in the RAPDistance software package (ver 1.03, Armstrong *et al.* 1994). A UPGMA dendrogram was generated from the

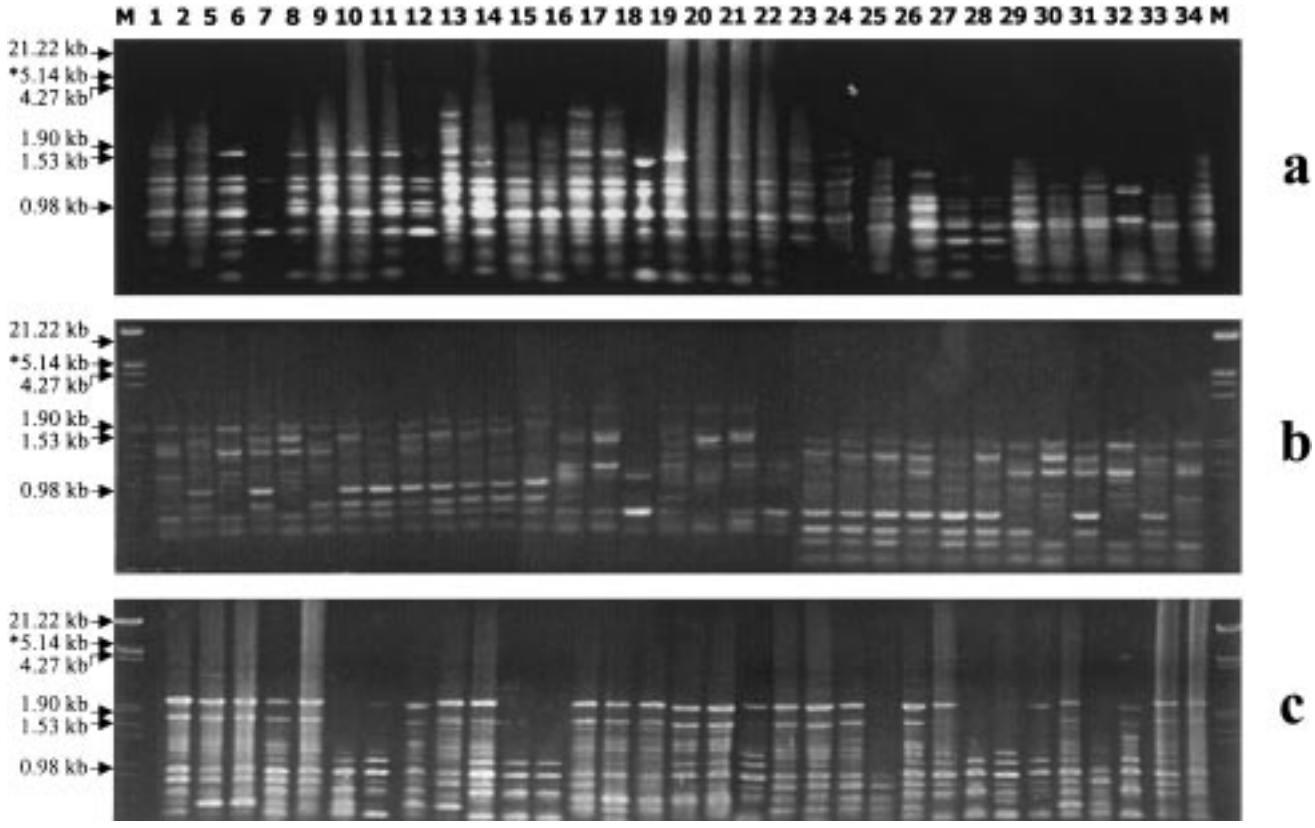

Figure 1. RAPD agarose gel electrophoresis profiles of *Prosopis* species and accessions using primers (a) OP-B12, (b) OP-B18 and (c) OP-A01. Lane M contains λ DNA double-digested with *Hin*dIII and *Eco*RI as molecular weight marker. Sizes of the marker DNA bands are indicated on the left. The band marked *5.14 kb is actually a double band of sizes 5.14 and 4.97 kb. Lanes 1 to 34 are template DNA from the appropriate species or accession as listed in table 1.

similarity data following the method of Sokal and Sneath (1963), and the neighbour-joining (NJ) tree was generated according to Saitou and Nei (1987).

Results

In the present study 32 plants of *Prosopis*, of different species as well as different accessions of the same species (table 1), were analysed by RAPD analysis. Initially, 101 primers were tested with three different DNAs from among the 32 DNAs prepared for the present study. Of these 101 primers, 47 produced some RAPD patterns while the rest of the primers resulted in either no amplification or smeared profiles in as many as two out of the three DNAs tested. Therefore only those 47 primers were used with all 32 DNAs. Further, 14 out of these 47 primers resulted in consistently reproducible banding patterns (similar patterns of prominent bands in duplicate reactions) in at least 27 of the 32 DNAs under consideration. Band data from these 14 primers only were considered for the cumulative analysis. The RAPD profiles with three of these primers, namely OP-B12, OP-B18 and OP-A01 are shown in figure 1. A total of 357 bands were generated by these 14 primers. The size range of the amplification products was 200 bp to 5600 bp. The pairwise similarities of the RAPD profiles were calculated according to Jaccard (1901) cumulatively for all the primers, and the data are given in figure 2. The similarity indices for the multiple accessions of *P. glandulosa, P. pallida* and *P. juliflora* were averaged (simple arithmetic averaging) and these are given in table 2. The extent of polymorphism (similarity index) varied with the primer used. From the table it is clear that the average similarity per primer was least among the *P. juliflora* accessions. Further, primer OP-E04 resulted in identical profiles for all the accessions of *P. glandulosa* (figure 3). The primers OP-D12 and OP-D20 (average similarities 0.18 with both primers) in case of the accessions of *P. pallida*, and OP-E18 (average similarity 0.19) in case of the accessions of *P. juliflora* resulted in mostly dissimilar profiles among the accessions.

The UPGMA dendrogram for the *Prosopis* species and accessions was computed from the values of similarity indices for the RAPD profiles and is depicted in figure 4a, while the NJ tree computed from the same data set is shown in figure 4b. Twelve different species of *Prosopis* have been analysed in this study. These included *P. affinis* and *P. pallida* (section Algarobia, series Pallidae); *P. velutina*, *P. chilensis*, *P. juliflora*, *P. flexuosa*, *P. glandulosa*, *P. siliquastrum* and *P. alba* (section Algarobia, series Chilensis); *P. pubescens* (section and series Strombocarpae); an indigenous species

Matrix of pair-wise similarity values computed from RAPD data

1.00
0.12 1.00
0.20 0.22 1.00
0.41 0.08 0.19 1.00
0.49 0.10 0.17 0.43 1.00
0.33 0.13 0.14 0.27 0.39 1.00
0.34 0.11 0.16 0.28 0.37 0.42 1.00
0.35 0.14 0.15 0.27 0.37 0.57 0.51 1.00
0.34 0.16 0.16 0.24 0.30 0.37 0.41 0.42 1.00
0.28 0.08 0.15 0.31 0.27 0.26 0.31 0.28 0.30 1.00
0.31 0.11 0.18 0.30 0.35 0.30 0.34 0.33 0.32 0.41 1.00
0.34 0.16 0.17 0.29 0.35 0.27 0.30 0.28 0.29 0.29 0.55 1.00
0.37 0.16 0.15 0.34 0.34 0.28 0.32 0.29 0.31 0.35 0.54 0.64 1.00
0.31 0.18 0.16 0.26 0.27 0.22 0.29 0.22 0.32 0.27 0.39 0.47 0.57 1.00
0.29 0.11 0.15 0.28 0.32 0.24 0.27 0.18 0.23 0.22 0.35 0.33 0.38 0.43 1.00
0.29 0.19 0.16 0.24 0.27 0.23 0.26 0.23 0.26 0.22 0.35 0.38 0.37 0.40 0.51 1.00
0.15 0.23 0.17 0.14 0.11 0.17 0.18 0.16 0.26 0.21 0.19 0.17 0.22 0.26 0.24 0.30 1.00
0.11 0.30 0.14 0.12 0.07 0.11 0.09 0.12 0.15 0.13 0.11 0.10 0.13 0.14 0.12 0.19 0.35 1.00
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0.13 0.24 0.28 0.10 0.11 0.12 0.19 0.14 0.18 0.09 0.15 0.20 0.16 0.17 0.15 0.18 0.23 0.13 0.23 0.19 0.20 0.18 0.31 0.19 0.21 0.32 0.26 0.37 0.45 0.47 1.00
0.08 0.30 0.25 0.08 0.06 0.07 0.11 0.08 0.09 0.05 0.11 0.13 0.14 0.14 0.09 0.12 0.14 0.15 0.17 0.12 0.15 0.21 0.23 0.16 0.26 0.32 0.25 0.33 0.38 0.34 0.47 1.00

Figure 2. Pairwise similarity indices for the 32 samples of table 1 given as a left triangular matrix. The similarity indices were calculated between pairs of genotypes among the 32 accessions and species of *Prosopis* as listed in table 1 from the RAPD band data for all the primers cumulatively.

Table 2. Average similarity indices (SI) for accessions of *P. glandulosa*, *P. pallida* and *P. juliflora*.

Primer	Average similarity index ± S.D.		
	P. glandulosa (4)	*P. pallida* (7)	*P. juliflora* (5)
OP-A01	0.62 ± 0.07	0.75 ± 0.14	0.72 ± 0.12
OP-B12	0.52 ± 0.15	0.60 ± 0.18	0.37 ± 0.24
OP-B18	0.54 ± 0.24	0.40 ± 0.15	0.20 ± 0.28
OP-C02	0.60 ± 0.20	0.58 ± 0.15	0.28 ± 0.29
OP-D03	0.48 ± 0.05	0.38 ± 0.20	0.32 ± 0.37
OP-D05	0.63 ± 0.18	0.24 ± 0.23	0.50 ± 0.24
OP-D07	0.37 ± 0.12	0.84 ± 0.12	0.52 ± 0.18
OP-D08	0.54 ± 0.21	0.62 ± 0.15	0.41 ± 0.26
OP-D11	0.34 ± 0.17	0.38 ± 0.25	0.20 ± 0.20
OP-D12	0.28 ± 0.08	0.18 ± 0.16	0.38 ± 0.13
OP-D20	0.38 ± 0.12	0.18 ± 0.20	0.31 ± 0.12
OP-E04	1.00 ± 0	0.22 ± 0.25	0.52 ± 0.29
OP-E07	0.62 ± 0.23	0.43 ± 0.25	0.29 ± 0.15
OP-E18	0.36 ± 0.16	0.40 ± 0.15	0.19 ± 0.21
Average SI per primer	0.52 ± 0.18	0.44 ± 0.20	0.37 ± 0.15

Similarity indices were calculated as described in Materials and methods and the values have been averaged from data of the 14 informative primers selected for the present studies. The figures in parenthesis after the species name are numbers of accessions studied. Sl values were taken from the matrix shown in figure 2.

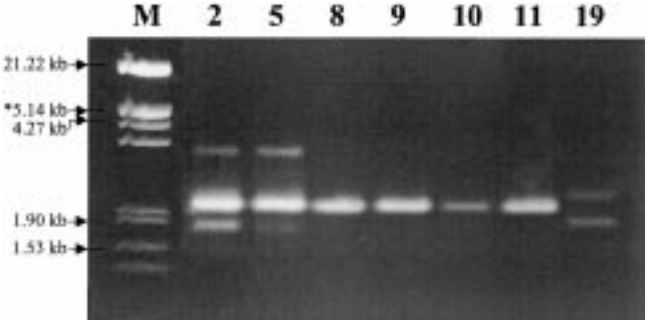

Figure 3. RAPD profile of two *P. chilensis* accessions (lanes 2 and 5), four *P. glandulosa* accessions (lanes 8–11), and one accession of *P. siliquastrum* (lane 19) obtained with primer OP-E04. The single band in lanes 8–11 suggests monomorphy in case of the *P. glandulosa* accessions. The lane numbers correspond to the list in table 1. Lane M contains λ DNA double-digested with *Hin*dIII and *Eco*RI as molecular weight marker. The band marked *5.14 kb is actually a double band of sizes 5.14 and 4.97 kb.

P. cineraria (section Algarobia, series Prosopis); and an unknown species from Jodhpur. The dendrogram (figure 4a) indicates that *P. juliflora*, *P. siliquastrum*, *P. chilensis*, *P. alba*, *P. velutina*, *P. cineraria* and *P. pubescens* form one major cluster, while *P. affinis*, *P. flexuosa*, *P. glandulosa* and *P. pallida* form the other major cluster. Further, within the clusters accessions most related to each other are subclustered together, as in case of *P. juliflora*, *P. pallida*, *P. glandulosa* and *P. flexuosa*. Contrary to this trend, *P. chilensis* seems to be more heterogeneous since the two accessions of this species do not group together. The unknown species is close to the Israel accession of *P. juliflora* and is also well

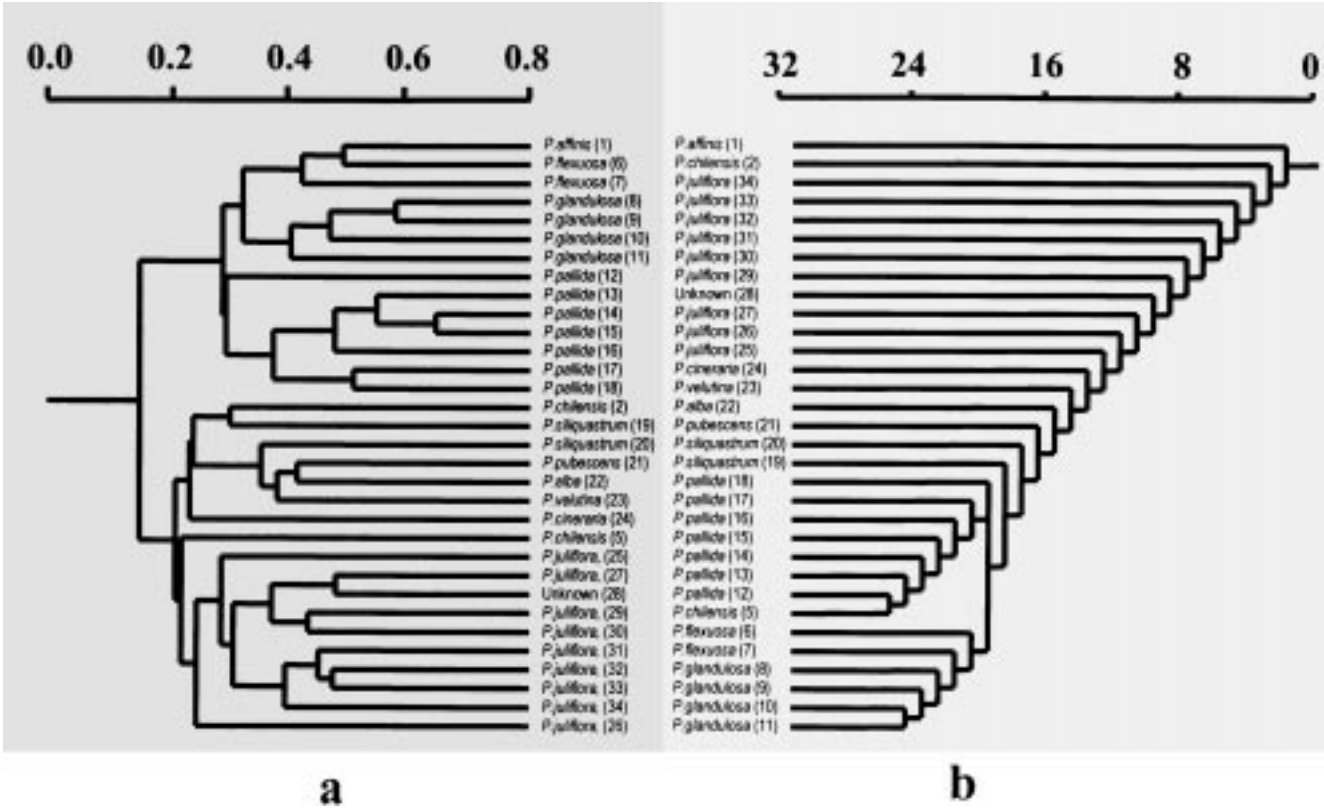

Figure 4. (a) UPGMA and (b) NJ dendrograms generated from the total RAPD data for *Prosopis* species and accessions. The numbers in parenthesis after the species name correspond to the list in table 1.

within the *P. juliflora* cluster. We have therefore, on the basis of RAPD profiles, putatively identified it to be a *P. juliflora* accession. When we analysed this unknown species as included among the accessions of *P. pallida*, *P. glandulosa* and *P. flexuosa*, it always clustered separately from the other accessions (data not shown), thereby reaffirming its affinities to *P. juliflora*.

Discussion

Taxonomy of the genus *Prosopis* has been problematic, with many authors suggesting different subdivisions of the genus (Schinini 1981; Hunziker *et al.* 1986). The main causes of disagreement in the classification based on morphological characters are hybridization and introgressions of genes among the species, which create newer phenotypes. Studies on the analysis of the chromosomes (karyomorphological analysis) did not provide any solution to the taxonomy of *Prosopis* (Hunziker *et al.* 1986). Similarly the biochemical analysis of free amino acids (Carman *et al.* 1974), phenols (Naranjo *et al.* 1984) and seed lipids (Lamarque *et al.* 1994) showed that the similarity within a section was higher than that expected. However, none of these could resolve the taxonomic problems. Consequently, no species-diagnostic markers were found among such characters. In such a situation the RAPD technique may have greater value in the determination of genetic variability in *Prosopis*. All the species in the present study belong to section Algarobia except *P. pubescens* which belongs to section Strombocarpae. If this sectional classification reflects a rigid genetic distinction between the various species, it would be logical to expect *P. pubescens* to cluster separately from all other species and accessions included in the present studies. Contrary to this expectation we observe that *P. pubescens* (section Strombocarpae, series Strombocarpae) falls in a subcluster along with *P. juliflora*, *P. siliquastrum*, *P. chilensis*, *P. alba* and *P. velutina* (all belonging to section Algarobia, series Chilensis) and *P. cineraria* (belonging to section Algarobia, series Prosopis). We have earlier shown on the basis of interspecies comparison of RAPD profiles that the sectional classification of the genus *Prosopis* is not rigidly followed (S. A. Ranade and M. Goswami Interspecies affinities in the genus *Prosopis* based on RAPD profiles. In *Proceedings of symposium on advances in legume research in India.* (ed. R. R. Rao), in press). Both the UPGMA and NJ trees determined in the present study for the multiple accessions of a species have further indicated the non-rigidity of the sectional system of classification. Some of the species are more heterogeneous than others, as in the case of *P. chilensis* and *P. siliquastrum*.

The average similarity index values among the accessions of three species of *Prosopis* for all the informative primers (14 primers for which RAPD data were used for analysis)

are given in table 2. All three species are relatively heterogeneous since the average similarities were 0.37 ± 0.15 (*P. juliflora* accessions), 0.44 ± 0.20 (*P. pallida* accessions) and 0.52 ± 0.18 (*P. glandulosa* accessions). Of these three, the *P. juliflora* group can be considered to be more heterogeneous. One possible reason for this is that the accessions of *P. juliflora* were from different geographical regions while in the case of the other two species all the accessions were from the same geographical region. The overall trend of small similarities among the accessions is in contrast to the results of Saidman and Vilardi (1987) who determined species affinity on the basis of isoenzymatic studies. In their study, the percentage of polymorphic loci and heterozygosity ranged from 38 and 0.132 respectively (for *P. ruscifolia*) to 50 and 0.223 respectively (for *P. flexuosa*). They also found that the genetic distance among species within a section was lower, indicating high intrasection similarity. However, the above example is of the intrasection interspecies variability. To our knowledge there are no reports available about the extent of genetic similarity or dissimilarity among accessions within a species. Ours is perhaps the first report addressing the question of intraspecific variability in *Prosopis*. An interesting result was obtained in case of all accessions of *P. glandulosa*, where the profiles obtained with primer OP-E04 were identical in each case (figure 3). These RAPD products may therefore generate a *P. glandulosa*-specific primer or probe when sequenced completely (sequence characterized amplified region, or SCAR, marker). Such SCAR markers may prove to be invaluable for species distinction. Considering that besides morphological characters no species diagnostic markers have been found, the above results are significant.

The present study also included a collection of five accessions of *P. juliflora* from different geographic regions; the accessions of other species were from the same region. For taxonomic identification of the one unknown species, we compared the RAPD profiles with those of the different species and found that this unknown species was best grouped with *P. juliflora* (S. A. Ranade and M. Goswami Interspecies affinities in the genus *Prosopis* based on RAPD profiles. In *Proceedings of symposium on advances in legume research in India.* (ed. R. R. Rao), in press). The various accessions seem to cluster according to geographical origins (figure 4a). Thus, relative to the Indian accessions, the 'Oxford' and 'Washington' accessions of *P. juliflora* clustered separately. Further, among the Indian accessions, accession numbers 31, 32, 33 and 34 are 'plus trees' from a Lucknow population and cluster together. These plus trees were selected from the Lucknow population on the basis of their better biomass performance and, thereafter, propagated vegetatively. These observations suggest that RAPD analysis could help in tracing the affinities of different accessions within a species.

On the basis of the similarity values computed, we conclude that there is high interspecies variability and low intraspecies variability. These results are in good agreement with the cross-pollinated nature of the trees as well as the fact that *Prosopis* is self-incompatible. Further, we have been able to putatively assign the unknown species from Jodhpur to the *P. juliflora* group on the basis of the RAPD patterns. The analysis of interspecies and intraspecies affinities in *Prosopis* has implications for studying the biodiversity of this genus. The identification of a primer that can apparently generate species-specific profiles is significant for further taxonomic and phylogenetic studies.

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