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Molecular assessment of inter-specific genetic diversity in selected species of *Prosopis* revealed by RAPD*

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Species of the genus *Prosopis* (Fabaceae Mimosoideae) are well adapted to grow in arid and semi-arid regions of the world. They are among the most important multipurpose leguminous trees and are used for re-vegetation, agro-forestry, apiculture, fodder and fire wood (Joshi and Nimbkar 1991). This genus contains 44 species of trees and shrubs, the majority of which originate in the Latin America. The species of genus *Prosopis* are grouped into five Sections and eight Series on the basis of morphological characteristics (Burkart 1976). However, this classification does not seem to be rigid (Schinini 1981). The morphological characters are quite homogenous across Sections and Series throughout the genus *Prosopis* as leaves, flowers and or pods are more or less similar. Further, the sectional subdivisions of the genus are fundamentally based on presence, absence and or orientation of spines and development of loments. There has been much taxonomic chaos over the identification of species of genus *Prosopis* with regard to specific and distinct characteristics.

The *Prosopis* species *P. juliflora* and *P. pallida* belong to different Series within the Section Algarobia. The diversification of the group formed by species of Series Chilensis and Pallidae is inferred to have started in the Pliocene, showing a high diversification rate. The moment of diversification within the major lineage of Latin American species of *Prosopis* is coincident with the spreading of arid areas in the Latin Americans, suggesting a climatic control for diversification of the group (Catalano *et al.* 2008). Similarly, *P. chilenis* and *P. flexuosa* are very closely related species and referred to as *P. chilensis-P. flexuosa* complex (Verga and Gregorious 2007) and revealed a broad cross-species affinity (Mottura *et al.* 2005). Earlier studies have suggested that the species belonging to the Section Algarobia are not natural groups and suggested that a few species would have originated in different founder events (Bessega *et al.* 2005).

The analysis of interspecies affinities in *Prosopis* has implications for studying the biodiversity of the genus. Exploitation of ecological potential of species of *Prosopis* demands genetic markers for crop improvement associated with desirable characters. Among several efficient methods for revealing genetic variability within and among plant populations, some of the most widely applied randomly amplified polymorphic DNA analysis-RAPDs (Esselman *et al.* 2000) and restriction fragment length polymorphisms-RFLPs (Yanesita *et al.* 1997).

RAPD uses 10-base pair primer to amplify the random portion of genome (Williams *et al.* 1990). The data from RAPD analysis have indicated greater genetic diversity than allozymes in plant species (Esselman *et al.* 1999). It is high through put marker technology, which allows the analysis of individual with large number of markers in relatively short time and have allowed the resolution of complex taxonomic relationships (Casiva *et al.* 2002).

The common species of *Prosopis* Section, Algarobia, viz *P. pallida*, *P. articulata* (Series Pallidae), *P. chilensis*, *P. juliflora*, *P. nigra*, *P. flexuosa, P. glandulosa* and *P. alba* (Series Chilensis) are well adapted to arid and semi-arid environments in India (Harsh *et al.* 1996). These species are being promoted as an integral part of agroforestry, sand dune stabilization, enrichment of soil fertility, wood, fodder and industrial purpose (Pasiecznik *et al.* 2001). We investigated the interspecific genetic diversity in eight *Prosopis* species belonging to Section Algarobia representing Series Pallidae and Chilensis using RAPD analysis.

Eight exotic accessions of *Prosopis,* viz *P. pallida, P. articulata, P. glandulosa, P. chilensis, P. nigra, P. flexuosa, P. juliflora* and *P. alba* belonging to the section Algarobia were obtained from Texas A and I University, Kingsville, Texas, USA were used for this study (Table 1).

The total DNA was extracted from 100mg of fresh leaves of each of the eight species of *Prosopis* was crushed with

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pestle and mortar in liquid nitrogen. The Plant Genomic DNA Purification spin kit 'Hi Pura' of Hi-media Company and protocols suggested by the manufacturer were followed for genomic DNA isolation. Finally, 200 µl of genomic DNA was eluted in Tris-EDTA buffer (TE) for DNA fingerprinting.

Multilocus genotyping by RAPD was performed using decamer arbitrary primers supplied by Operon Technologies. Amplification was performed in a total reaction mixture of 25 µl. Each reaction mixture contained: decamer primer, 2 µl (50 pmol µ/litre); dNTP mix, 2 µl (2 mM each of dATP, dGTP, dCTP and dTTP from MBI, Fermentas); $MgCl_2$,1µl (25 mM, MBI, Fermentas); Taq DNA polymerase, 0.5µl (5U µ/litre, Sigma chem); 10× PCR buffer, 2.5 µl (100 mM, Tris-HCl, pH-8.3, 15 mM $MgCl_2$, 250 mM KCl), 13.0 µl of dH_2O and 4µl of genomic DNA (approx 40–60 ng). RAPD-PCR amplification were performed in a gradient thermal cycler (Corbett Research, USA) with lid heating option at 110°C with initial denaturation step of 94°C for 3 min, followed by 36 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 10 min.

PCR amplification products were electrophoretically separated on 1.6% agarose gel (Sigma) prepared in 1× TAE (Tris –Acetic acid-EDTA). The gel was run for 3 h at 50 V. The staining was done with ethidium bromide and visualized under 300 nm UV light and photographed. The gel photographs were scored and similarity coefficients were used to construct dendrograms depicting the genetic relationship implying the UPGMA Algorithm (Unweighted Pair Group Method using Arithmetic Averages) of the NTSYS-pc, Version 2.02 h programme (Rohlf 1997).

Eight species of genus *Prosopis* (Section Algarobia) belonging to two Series Pallidae and Chilensis were subjected to RAPD analysis. Seven RAPD primers, viz OPA-9, OPA-10, OPA-13, OPA-16, OPP-7, OPP-9 and OPP-16 generated a total of 106 scorable amplicons of which 97 were polymorphic (91.5%) and exhibited very high degree of marker index ranging from 84.61 to 93.75% polymorphism in banding pattern (Table 2). The number of PCR amplified products formed ranged from 11 (OPA-16) to 22 (OPP-16) with an average of about 15 bands per primer (Fig 1). All the informative random primers generated RAPD profiles with one to several bands specific to a particular species (Table 3).

The combined dendrogram of the seven random primer data matrix delineated eight species into two clusters and three species as out groups. Cluster I included *P. pallida*, *P. articulata* and *P. glandulosa*, cluster II included *P. chilensis* and *P. nigra*. Whereas three out grouped species *P. flexuosa*, *P. juliflora* and *P. alba* exhibited significant genetic diversity from cluster I and cluster II. *P. alba* showed the maximum genetic diversity of 60%, *P. juliflora* 55% and *P. flexuosa* about 49% from rest of the *Prosopis* species.

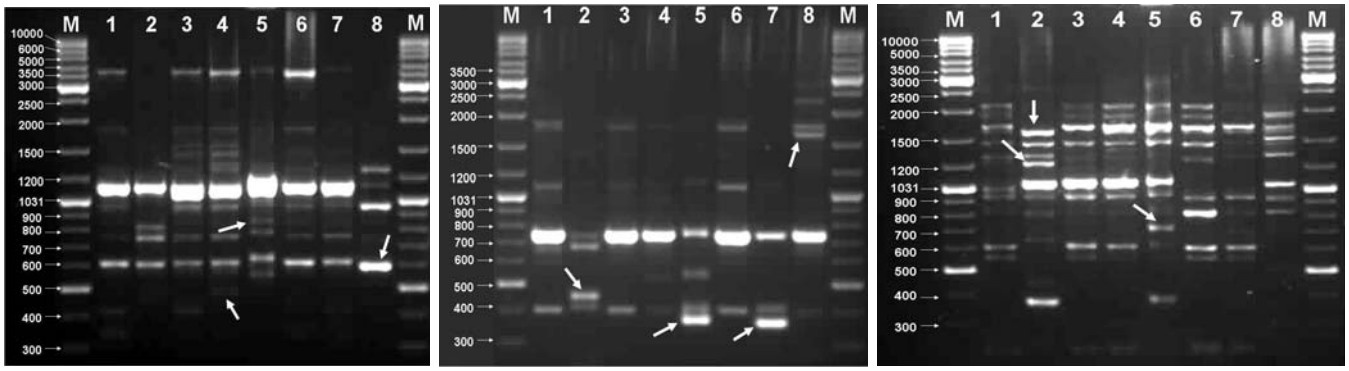
Table 1 Details of *Prosopis* species belonging to Section Algarobia

Accession no.	Prosopis species	Series	Morphological characters
Trujillo Prov. Proto Prac, Peru EC 308211	*P. pallida*	Pallidae	Leaflets 6-15 pairs / pinna, spines small, axillary, sometimes absent, legumes fleshy, straight and sweet in taste
Introduced from Israel in 1968	*P. articulata*	Pallidae	Leaflets 6–20 pairs / pinna, spines axillary, legume dry, flattened, sweet in taste
Mexico EC 1208/83	*P. glandulosa*	Chilensis	Leaflets 6-17 pairs / pinna, spines axillary, uninodal, long and mostly solitary, legumes straight or moniliform, sweet in taste
Capayan, Catamarca, Argentina EC 308189	*P. chilensis*	Chilensis	Leaflets 10–29 pairs / pinna, spines axillary, uninodal, hard up to 6cm long. Legumes linear compressed with parallel margins, straight, falcate or sub falcate, sugary and edible
San martin, Catamarca, Argentina EC 308029	*P. nigra*	Chilensis	Leaflets 20 -30 pairs / pinna, spines big, legumes straight or sub falcate, sub moniliform legumes, fleshy, compressed, rounded, edible and sweet
Anilaco, Catamarca, Argentina EC 308072	*P. flexuosa*	Chilensis	Leaflets 12–29 pairs / pinna, spines small, legumes straight to sub falcate, sweet and edible
Introduced from Mexico in 1917	*P. juliflora*	Chilensis	Leaflets 11-15 pairs / pinna, spines axillary, divergent, paired, legumes straight with incurved apex sometimes falcate, legumes bitter in taste
Neon Yacanta, Cardoba EC 308151	*P. alba*	Chilensis	Leaflets 25-50 pairs / pinna, spine scare and small only on strong shoots, legumes falcate to ring-shaped, compressed, sweet in taste

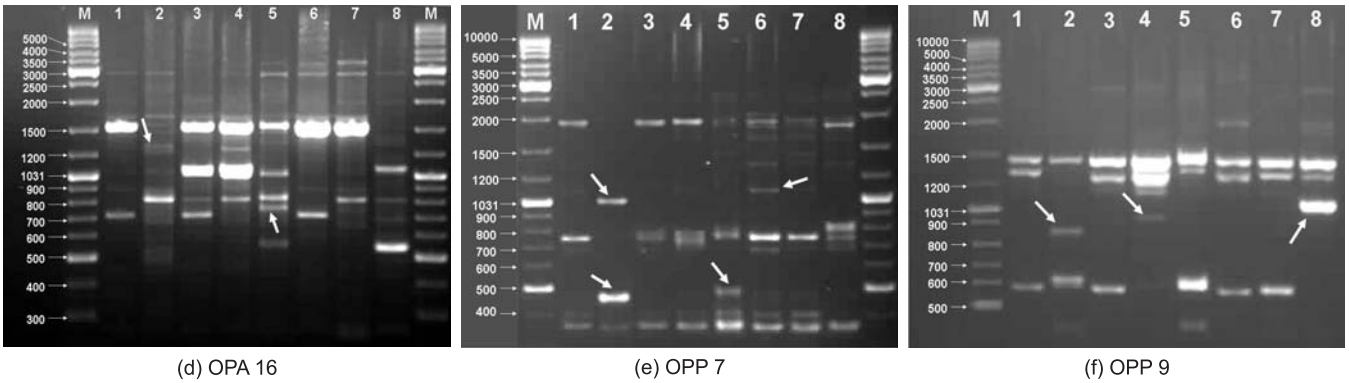
Burkart 1976; Pasiecznik *et al.* 2004

Table 2 Details of primer code, sequence, GC content and per cent polymorphism of RAPD primers used

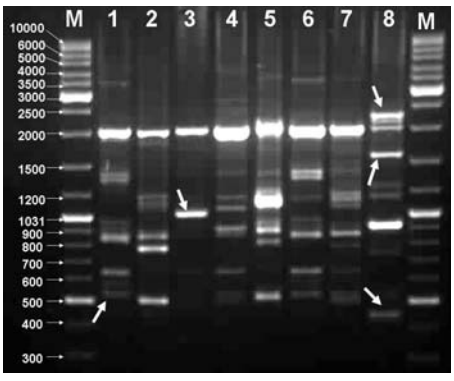
Primer	Primer sequence (5'-3')	GC content (%)	No. of amplified bands	No. of polymorphic bands	Polymorphism (%)
OPA-9	GGG TAA CGC C	70	16	15	93.75
OPA-10	GTG ATC GCA G	60	13	12	92.30
OPA-13	CAG CAC CCA C	70	16	15	93.75
OPA-16	AGC CAG CGA A	60	13	11	84.61
OPP-7	GTC CAT GCC A	60	15	14	93.33
OPP-9	GTG GTC CGC A	70	11	10	90.90
OPP-16	CCA AGC TGC C	70	22	20	90.90
Total			106	97	91.50



(a) OPA 9 (b) OPA 10 (c) OPA 13



(d) OPA 16 (e) OPP 7 (f) OPP 9



(g) OPP 16

Fig 1 (*a-g*) RAPD profiles showing polymorphism in banding patterns of *Prosopis* species using seven random primers

Table 3 Species-specific diagnostic RAPD markers bands

Primer	Bands size (bp)	*Prosopis* species	Primer	Bands size (bp)	*Prosopis* species
OPA-9	850	*P. flexuosa*	OPP-7	1110	*P. articulata*
	575	*P. juliflora*		1031	*P. alba*
	500	*P. nigra*		500	*P. flexuosa*
OPA-10	1750	*P. juliflora*		475	*P. alba*
	450	*P. alba*	OPP-9	1031	*P. juliflora*
	375	*P. flexuosa*		950	*P. nigra*
	350	*P. glandulosa*		875	*P. alba*
OPA-13	1700	*P. alba*	OPP-16	2250	*P. juliflora*
	1200	*P. alba*		1600	*P. juliflora*
	700	*P. flexuosa*		1031	*P. Chilensis*
OPA-16	1300	*P. alba*		525	*P. pallida*
	775	*P. flexuosa*		425	*P. juliflora*

Both the species of *Prosopis* belonging to Series Pallidae, ie *P. pallida* and *P. articulata* exhibited the maximum genetic similarity of about 82% and formed a separate cluster. Three species of *Prosopis* belonging to the Series Chilensis, viz *P. glandulosa, P. chilensis* and *P. nigra* showed more genetic similarities with species of Series *Pallidae* rather than their own Series *Chilensis*.

Taxonomy of the genus *Prosopis* has been complicated with many authors suggesting different sub divisions of the genus. The major reasons of disagreement in the classification based on morphological characters are interspecific hybridization and introgressions of genes among the species, which create newer phenotypes. Under present study, we observed that *P. glandulosa, P. chilensis* and *P. nigra* belonging to the Series Chilensis exhibited more genetic similarities and affinities with the species of Series Pallidae rather than linage with own Series Chilensis. Lack of clear reproductive barriers as well as high similarity among morphologically good taxonomic species was considered evidence of assuming that several species of this Section may constitute a syngameon (Saidman *et al.* 1998a; Saidman *et al.* 1998b).

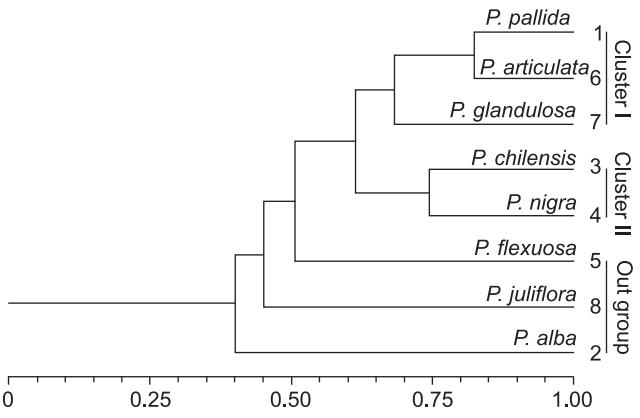


Fig 2 Dendrogram showing genetic diversity in species of genus *Prosopis*

P. chilensis and *P. flexuosa* have been reported very closely related species and referred to as species complex (Mottura *et al.* 2005), whereas under present study, *P. chilensis* exhibited more affinities with *P. nigra* and cluster I (Series *Pallidae*) than *P. flexuosa.* Our observations suggest that RAPD analysis could help in identifying genetic variations among different species of *Prosopis.* Studies on analysis of the chromosomes did not provide any solution to the taxonomy of *Prosopis* (Trenchard *et al.* 2008). Goswami and Ranade (1999) performed RAPD analysis among accessions of *Prosopis* and concluded that the identification of a primer that can apparently generate species-specific profiles is significant for further taxonomic and phylogenetic studies. Under present study a number of RAPD primers showed species-specific banding pattern with very high polymorphism ranging from 84.61 to 93.33% and can serve as useful genetic markers for identification of species belonging to the Section Algarobia.

SUMMARY

We report interspecific genetic diversity in eight species of genus *Prosopis* belonging to the Section Algarobia representing two Series, Pallidae and Chilensis using RAPD analysis. Seven random primers generated 106 scorable amplicons of which 97 were polymorphic (91.5%). All these informative primers generated unique RAPD profiles with one to several species specific bands. RAPD dendrogram delineated all the eight *Prosopis* species. *P. pallida* and *P. articulata* belonging to the Series Pallidae formed a separate cluster. Whereas, three species belonging to the Series Chilensis, viz *P. glandulosa, P. chilensis* and *P. nigra* showed more genetic affinities with species of Series Pallidae rather than their own Series. These species-specific bands may provide unique primers that could help in resolving taxonomic chaos and address the problems of species complex in the genus.

REFERENCES

Bessega C, Saidman B O and Vilardi J C. 2005. Genetic relationship among American species of *Prosopis* (Leguminosae) based on enzymes markers. *Genetic and Molecular Biology* **28** (2): 277–86.

Burkart A. 1976. A monograph of the genus *Prosopis* (Leguminosae, Subfamily Mimosoideae). *Journal of Arnold Arbor* **57**: 219–49.

Casiva P V, Saidman B O, Vilardin J C and Cialdella A M. 2002. First comparative phonetic studies of Argentinean species of *Acacia* (Fabaceae) using morphometric isozymal and RAPD approaches. *American Journal of Botany* **89**: 843–53.

Catalano S A, Vilardi J C, Tosto D and Saidman B O. 2008. Molecular phylogeny and diversification history of *Prosopis* (Fabaceae: Mimosoideae). *Botanical Journal of The Linnean Society* **93**(3): 621–40.

Esselman E J, Crawford D, Brauner S, Stussy I F, Anderson G J and Silva M O. 2000. RAPD marker diversity within and divergence among species of *Dendroseris* (Asteraceae: Lactuaceae). *American Journal of Botany* **87**: 591–6.

Esselman E J, Jianqiamg L, Crawford D J, Windus J L and Welfe A D. 1999. Clonal diversity in the rare *Calamagrostis porteri* spp insperata (Poaceae): Comparative results for allozymes and random amplified polymorphic DNA and inter-simple sequence repeat markers. *Molecular Ecology* **8**: 443–53.

Goswami M and Ranade S A. 1999. Analysis of variations in RAPD profiles among accessions of *Prosopis. Journal of Genetics* **78**: 141–7.

Harsh L N, Tewari J C, Sharama N K and Felker Peter. 1996. Performance of *Prosopis* species in arid regions of India. *(in) Prosopis: Semi-arid Fuelwood and Forage Tree Building Consensus for Disenfranchised Center for Semi-arid Forest Resources,* pp 4–21,34. Peter Felker and James Moss (Eds). Texas A & M University-Kingsville, Texas.

Joshi A B and Nimbkar N. 1991. Evaluation of the productivity of *Prosopis* species as a biomass source in semi arid region of western Maharashtra. *Final Technical Report*. Department of Non-conventional Energy sources (DNES), Ministry of Energy, Government of India, New Delhi.

Mottura MC, Finkeldey R, Verga AR, Gailing O. 2005. Development and characterization of minisatellite markers for *Prosopis chilensis* and *P. flexuosa* and cross species amplification. *Molecular Ecology Notes* **5** (3): 487–9.

Pasiecznik N M, Felker P, Harris P J C, Harsh L N, Cruz G, Tewari J C, Cadoret K and Maldonado L J. 2001. *The Prosopis juliflora-Prosopis pallida complex: A Monograph*. 162 pp. HDRA Conventry, UK.

Rohlf F J. 1997. *NTSYS pc: Numerical Taxonomy and Multivariate Analysis System* Version 2.02h. Exeter software, New York

Saidman B O, Vilardi J C, Montoya S, Dieguez M J and Hopp H E. 1998 a. Molecular markers: A tool for the understanding of the relationships among species of *Prosopis* (Leguminosae, Mimosoideae). *(in) Tree Improvement: Applied Research and Technology Transfer Science,* pp 311–24. Puri S (Ed.). Publishers Inc., New Hampshire, USA.

Saidman B O, Bessega C, Ferreyra L and Vilardi J C. 1998 b. Random amplified polymorphism DNA (RAPDs) variations in hybrid swarms and pure populations of genus *Prosopis* (Leguminosae). *(in) Proceeding of the Workshop on Recent Advances in Biotechnology for Tree Conservation and Management*, Florianopolis, pp 122–34. International Foundation for Science (IFS).

Schinini A. 1981. Contribucion a la flora del Paraguay. *Bonplandia* **5**: 101–8.

Trenchard L J, Harris P J C, Smith S J and Pasiecznik N M. 2008. A review of ploidy in the genus *Prosopis* (Leguminosae). *Botanical Journal of The Linnean Society* **156** (3): 425–38.

Verga A and Gregorious H R. 2007. Comparing morphological and genetic distances between populations: a new method and its application to the *Prosopis chilensis-P. flexuosa* complex. *Silva Genetica* **56**(2): 45–51.

Williams J G K, Kubelik A R, Livak K J, Rafalski J A and Tingey S V. 1990. DNA polymorphism amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* **18**: 6531–5.

Yanesita M, Nagasawa R, Engelke M C and Sasakuma T. 1997. Genetic variation and interspecific hybridization among natural populations of Zoysia grasses detected by RFLP analysis of chloroplast and nuclear DNA. *Genes and Genetic Systems* **72**: 173–9.