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Molecular assessment of genetic diversity in *Acacia senegal*

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ABSTRACT

Acacia senegal is well adapted to arid environment of western Rajasthan and has a potential to restore soil fertility and sand dune stabilization. There is a scope of improvement by exploiting geographical genetic diversity. It is a drought- tolerant multipurpose leguminous African tree species and also an important forest resource for gum Arabic, fuel, food and fodder. Thirteen selected plants showing significantly high and low seed yields from Rajasthan and exotic locations, viz Niger, Mali, Senegal and Sudan, transplanted in 1988 at Central Arid Zone Research Institute (CAZRI), Jodhpur, were subjected to randomly amplified polymorphic DNA (RAPD) analysis. Six random primers generated a total of 86 scorable loci and exhibited 77.77 to 94.73% polymorphism. Unweighed pair group method using arithmetic averages (UPGMA) dendrogram obtained from cluster analysis using Jaccard's similarity coefficient delineated all the 13 population samples representing seven geographical populations. The results clearly revealed existence of genetic diversity within and among geographical populations of *A. senegal*. The Indian population exhibited the maximum genetic diversity from rest of the African populations.

Key words: *Acacia senegal*, African tree species, Diversity, Geographical populations, Polymorphism, RAPD

Acacia senegal (L.) Willd, is highly drought-tolerant multipurpose leguminous African tree species that belongs to the sub-genus Aculeiferum (Arce and Blanks 2001). It is an important forest resource of gum Arabic, fuelwood, human food and fodder for livestock (Aoki *et al.* 2007). Jindal *et al.* (2000) studied the performance of different accessions of *A. senegal* for tree height at rocky rangelands of the Thar Desert. The morphological variability of the seed characteristics (Jindal and Singh 2003) and interrelationship of shoot dry weight with component traits have been studied under Indian conditions (Jindal *et al.* 1987).

The morphological traits are influenced by environmental factors and developmental stages of plant, so the results do not provide true assessment of genetic diversity. Molecular markers offer several advantages over the conventional breeding tools for selection of diverse parents. Randomly Amplified Polymorphic DNA (RAPD) uses arbitrary 10-base pair primers to amplify the random portion of genome (Williams *et al.* 1990). The data from RAPD analysis have indicated greater diversity than allozymes in plant species (Esselman *et al*. 2000). It is high throughput marker technology, which allows the analysis of large number of individuals with different markers in relatively short time, as only a few primers allow the generation of sufficient data to obtain a robust estimate of diversity index and have allowed the resolution of complex taxonomic relationships (Casiva *et al.* 2002).

To our knowledge there is a little information on the structure of genetic diversity in *A. senegal*. Hence, efforts are required to document, conserve and utilize the diverse genetic resources for desired elite traits. The present investigation is an attempt towards the assessment of genetic diversity within and among accessions of *A. senegal* representing different geographical origins. The geographical genetic diversity information shall then be exploited for improvement in Indian populations of *A. senegal*.

MATERIALS AND METHODS

Seeds of five accessions from Niger (EC 87/7490), Mali (EC 87/7493, EC 87/7497 and EC 87/7499) and Senegal (EC 87/7500) were procured from CIFT Nogent Sur Marne. Seedlings of these accessions along with the accessions of Sudan (Sudan/87) and local collections from Rajasthan (Raj/87) were transplanted in 1988 at Central Research Farm of Central Arid Zone Research Institute, Jodhpur. The plants showing significantly high and low seed yields from each accession were selected for molecular diversity analysis in 2009. The details of accession

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numbers, origin and environmental conditions are shown in Table 1.

The total DNA was extracted from 100 mg of fresh leaves of 13 selected plants of *A. senegal* crushed with 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 13 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/ml); dNTP mix, 2 µl (2 mM each of dATP, dGTP, dCTP and dTTP from MBI, Fermentas); $MgCl_2$,1 µl (25 mM, MBI, Fermentas); Taq DNA polymerase, 0.5 µl (5U/µl, 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 4 µl of genomic DNA (approx 40–60 ng). RAPD-PCR amplifications 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 for presence (1) and absence (0) of scorable bands with the assumption of positional homology. To establish the genetic relationship within and among the populations of *A. senegal*, similarity coefficients were used to construct dendrograms depicting the genetic relationship implying the UPGMA Algorithm (Unweighted Pair Group

Table 1 Details of *Acacia senegal* geographic populations used for molecular diversity analysis

Tree no.	Accession no.	Seed yield	Origin	Longitude	Latitude	Altitude (m)	Precipitation (mm)
3	Sudan/87	Low	Sudan	30°00'E	15°00'E	400	161
35	Sudan/87	High	Sudan	30°00'E	15°00'E	400	161
68	EC87/7490	Low	Niger	8°00' E	16°00'N		150
78	EC 87/7490	High	Niger	8°00' E	16°00'N		150
113	EC87/7497	High	Mali	10°22'W	14°37'N	150	650
224	EC87/7493	High	Mali	11°35'W	14°57'N	100	560
235	EC87/7499	Low	Mali	07°10'W	15°08'N	270	450
244	EC87/7499	High	Mali	07°10'W	15°08'N	270	450
266	EC87/7493	Low	Mali	11°35'W	14°57'N	100	560
296	EC87/7500	Low	Senegal	14°49' W	15°40'N	60	400
407	EC87/7500	High	Senegal	14°49' W	15°40'N	60	400
411	Raj/87	Low	India	73°08'E	26°18'N	241	317
442	Raj/87	High	India	73°08'E	26°18'N	241	317

Table 2 Growth and yield patterns of different geographic populations *Acacia senegal*

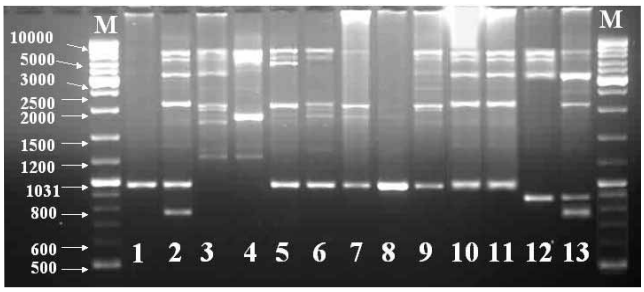
Tree no.	Accession no.	Collar diameter (cm)	Diameter at breast height (cm)	Tree height (cm)	Canopy length (cm)	Canopy width (cm)	Net pod weight (g)	Net seed weight (g)
3	Sudan/87	6.8	2.13	450	350	492.5	210	50
35	Sudan/87	16.4	12.2	680	605	847.5	6 370	2510
68	EC87/7490	6.8	4.80	440	320	330.0	100	30
78	EC 87/7490	18.6	18.55	840	660	807.5	7 890	1995
113	EC87/7497	14.8	15.00	710	535	605.0	6 880	1285
224	EC87/7493	20.8	20.46	840	660	945.0	7 080	1980
235	EC87/7499	12.4	13.74	620	360	472.5	3 240	730
244	EC87/7499	16.2	15.42	680	500	775.0	6 600	1850
266	EC87/7493	6.8	5.30	410	300	427.5	70	20
296	EC87/7500	15.2	10.00	605	385	685.0	45	15
407	EC87/7500	12.8	11.40	600	455	625.0	4 825	1160
411	Raj/87	4.8	4.40	340	215	515.0	170	60
442	Raj/87	9.6	7.20	540	390	435.0	2 280	690

Method using Arithmetic Averages) of the NTSYS-pc, Version 2.02 h programme (Rohlf 1997, Sneath and Sokal 1973).

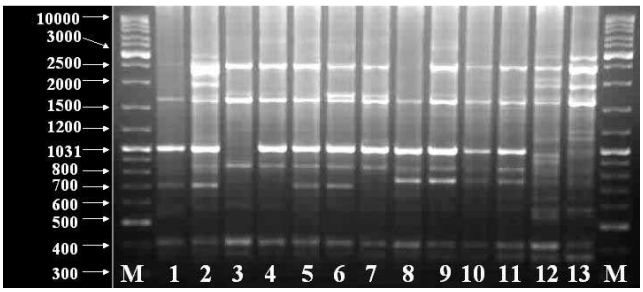
RESULTS AND DISCUSSION

The growth and yield patterns of different geographical populations of *A. senegal* are presented in Table 2. The collar diameter and diameter at breast height (DBH) of the accessions varied significantly within and among geographical locations. It is clear from the data that high seed-yielding accessions of exotic locations were recorded with greater biomass in terms of collar diameter, DBH and canopy than the low seed yielding accessions. The maximum collar diameter, DBH and canopy were recorded in the accession EC 87/7493 (tree no. 224) of Mali. Whereas, the maximum seed weight was harvested from the accession Sudan/87 (tree no. 35).

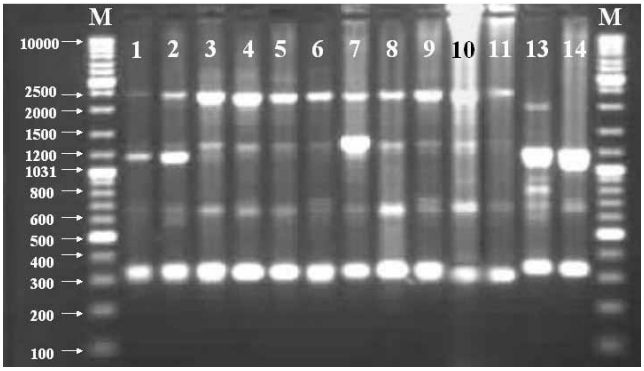
Out of 13 RAPD primers initially screened for their polymorphism, reproducibility and capacity to differentiate among 13 samples representing seven accessions of *A. senegal*, six primers were selected to access the genetic diversity. The other primers gave indistinct, sub-tropical or monomorphic amplification products. The six selected primers namely, OPA 9, OPA 13, OPP 7, OPP 9, OPP 12 and OPP 16 generated a total of 86 amplicons of which 75 were polymorphic (87.21%) and exhibited high degree of marker index ranging from 77.77 to 94.73% polymorphism in banding pattern. (Table 3). The number of PCR amplified



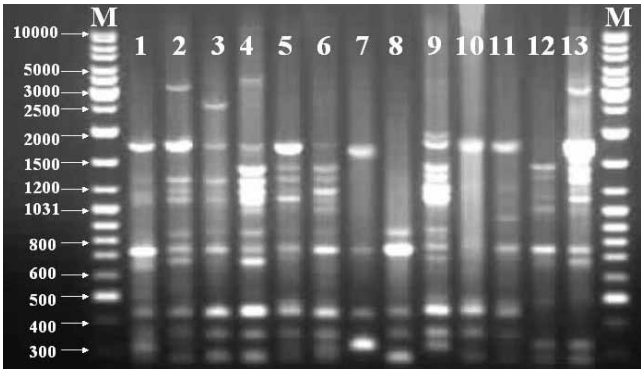
(a) OPP 12



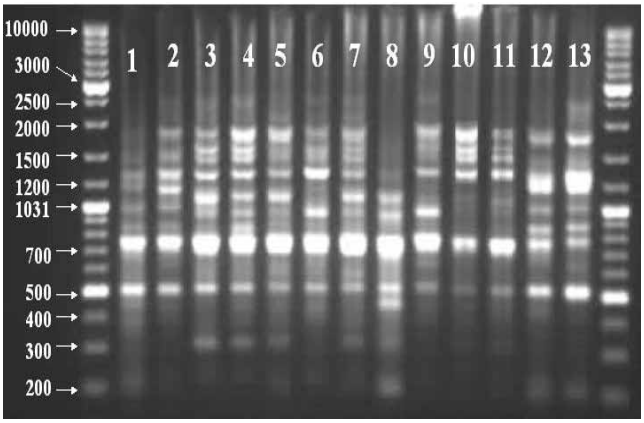
(b) OPP 16



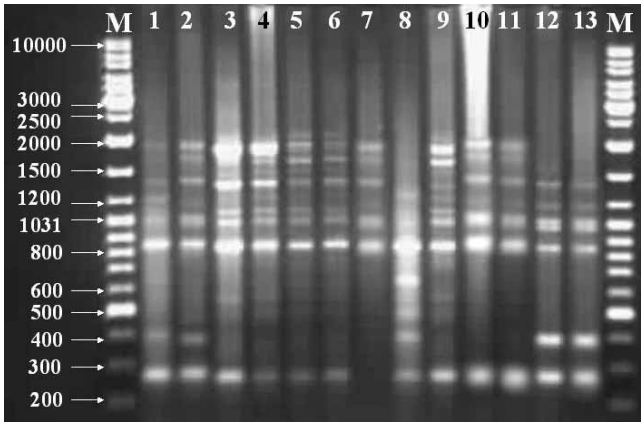
(c) OPA 9



(d) OPP 9



(e) OPA 13



(f) OPP 7

Fig 1 (*a-f*) RAPD profiles showing polymorphism in banding patterns of *A. senegal* using six random primers

Table 3 Primer code, sequence, GC content, number of polymorphic bands and per cent polymorphism of each RAPD primer used

Primer code	Primer sequence	GC content (%)	No. of amplified bands	No. of polymorphic bands	Polymorphic bands (%)
OPA 9	5'-GGG TAA CGC C-3'	70%	9	7	77.77
OPA 13	5'-CAG CAC CCA C-3'	70%	16	14	87.50
OPP 7	5'-GTC CAT GCC A-3'	60%	15	13	86.66
OPP 9	5'-GTG GTC CGC A-3'	70%	19	18	94.73
OPP 12	5'-AAG GGC GAG T-3'	60%	10	9	90.00
OPP 16	5'-CCA AGC TGC C-3'	70%	17	14	82.35
	Total		86	75	87.21

products formed ranged from 9 (OPA 9) to 19 (OPP 9) with an average of about 14 bands/primer. The RAPD profiles generated by six primers are shown in Fig 1 (a-f).

The UPGMA dendrogram obtained from the cluster analysis using Jaccard's similarity coefficient clearly delineated all the 13 population samples representing 7 geographical populations. The combined dendrogram of the entire six random primer data matrix yielded three major clusters (Fig 2). Cluster 1 included sample 1 and 8, cluster 2 included the maximum samples 3, 4, 5, 6, 9, 10, 11 with two out group populations of number 2 and 7, whereas, cluster 3 included samples 12 and 13.

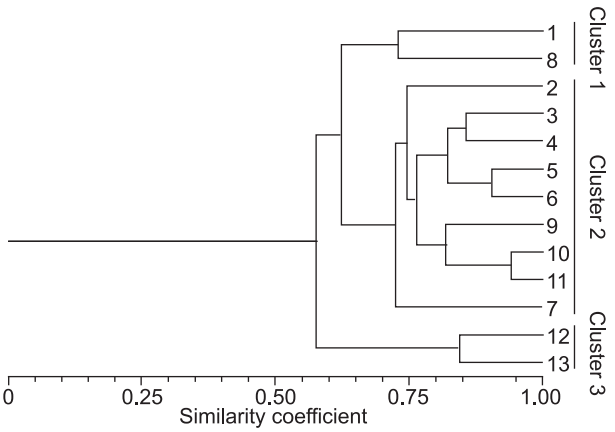


Fig 2 Dendrogram showing genetic diversity in geographical populations of *A. senegal*

All accessions from within and amongst different geographical locations exhibited 7 to 43% genetic diversity. The maximum genetic similarity of 93% was recorded between low and high seed-yielding trees of accession EC 87/7500 from Senegal. The low seed yielding trees of accession Sudan/87 from Sudan showed about 74% genetic similarity with high seed-yielding population of accession EC 87/7499 from Mali. The low and high seed-yielding samples representing EC 87/7490, EC 87/7493, EC 87/7499 and EC 87/7500 accessions exhibited the maximum similarity within and amongst themselves and grouped together in cluster 2. Whereas, the maximum genetically diverse accession Raj/87 representing Rajasthan, exhibited 43% genetic diversity from rest of the African populations of *A. senegal*.

High degree of polymorphism (77.77 to 94.73%) under present investigation in RAPD banding patterns is attributed to the efficiency of technique as it randomly detected variation in the whole genome. The higher levels of polymorphism than allozyme analysis and faster and easier analysis than micro satellites, RAPDs have been widely used in studies of plant populations (Nybom 2004, Nybom and Bartish 2000).

The maximum genetic diversity of Indian population of *A. senegal* from rest of the accessions from African countries is attributed to the differences in the geographical conditions (longitude, latitude, altitude). Whereas, the genetic similarity of Cluster 2 could be due to similar environmental ecology (Table 1). Genetic similarity of Sudan/87 accession with EC 87/7499 of Mali (cluster 1) and out grouping of Sudan/87 and EC 87/7499 (cluster 2) is due to cross pollination and out crossing nature of *A. senegal* whereby pollen from other populations also contribute to the gene pool of the seed (Morris *et al.* 2002). Simultaneously, separation of low seed-yielding accession of Sudan/87 (cluster 1) and out grouping of high seed-yielding population of Sudan/87 within cluster 2 can be due to separation of Sudan from Niger, Mali and Senegal by Chad lake and significant differences in longitude (Table 1)

Chiveu *et al*. (2008) studied the genetic diversity in Kenyan populations of *A. senegal* using RAPD and ISSR markers and recorded separation of regional population into two groups and suggested geographic sub-structuring of the genetic diversity. High genetic divergence between geographic regions in the highly out crossing *Acacia* species have also been reported (Shrestha *et al.* 2002.).

The gum chemical composition based on source of origin has been reported to vary within some Kenyan population of *A. senegal* (Chikamai and Odera 2002). Geographical variations in chemical composition, morphological and molecular characteristics between Ugandan and Sudanese population of *A. senegal* have been studied by Fagg and Allison (2004). Brenan (1983) grouped *A. senegal* into several varieties on the basis of morphological attributes. However, these groups showed limited levels of inter and

intra-varietal polymorphisms and are not sufficient to account for all the diversity in the species. A great amount of genetic variability is expected due to diversified environmental conditions among the population of the species in different geographical locations (Oleghe and Akinoufesi 1992).

Present study has clearly revealed existence of genetic diversity within and across geographical populations of *A. senegal*. More intense monitoring of diversity ought to be conducted to identify candidate population for *in situ* and *ex situ* conservations of *A. senegal* germplasm.

It is concluded that all high seed yielding exotic accessions had greater biomass than the Indian populations of *A. senegal.* The RAPD dendrogram also exhibited substantial genetic diversity within and among exotic populations *vis-a-vis* Indian populations. The plus tree nos. 35, 78, 224, 244, 407 and 441 may be used as one of the genetic diverse parents for cross pollination to induce heterosis with Indian populations to increase biomass and greater adaptability in arid environment of Rajasthan.

REFERENCES

Aoki H, Katayama T, Ogasawara T, Sasaki Y, Al-Assaf S and Phillips G O. 2007. Characterization and properties of *Acacia senegal* (L.) Willd var. *senegal* with enhanced properties (*Acacia* (*sen*) SUPER GUM ™) : Part 5. Factors affecting the emulsification of *Acacia senegal* and *Acacia* (*sen*) Super Gum ™. *Food Hydrocolloids* **21**: 353–8.

Arce L R and Banks H. 2001. A preliminary survey of pollen and other morphological characters in Neotropical *Acacia* subgenus Aculeiferum (Leguminosae: Mimosoideae). *Botanical Journal of The Linnean Society* **135**: 263–70.

Brenan J P M. 1983. *Manual of taxonomy of Acacia species*. FAO Forestry Division, Rome, pp–3.

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

Chikamani B N and Odera J A. 2002. *Commercial Plants, Gums and Resins in Kenya*. pp. 47–52, Nairobi, Kenya.

Chiveu C J, Dangasuk O G, Omunyin M E and Wachira F N. 2008. Genetic diversity in Kenyan populations of *Acacia senegal* (L.) Willd revealed by combined RAPD and ISSR markers. *African Journal of Biotechnology* **7** (14) : 2333–40.

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.

Fagg CW and Allison G E. 2004. *Acacia senegal and gum Arabic trade*. pp. 42, Oxford Forestry Institute, Tropical Forestry.

Jindal S K and Singh Manjit 2003. Morphological variability for seed characters in *Acacia senegal*. (*in*) *Advances in Arid Legumes Research*, pp 155–8. Henry A, Kumar D and Singh N B (Eds). Scientific Publishers (India), Jodhpur.

Jindal S K, Kackar N L and Solanki K R. 1987. Interrelationship between shoot dry weight and component in *Acacia senegal* (L.) Willd., Progeny. *Nitrogen Fixing Tree Research Reports* **5**: 16–7.

Jindal S K, Singh Manjit, Pancholy Anjly and Kackar N L. 2000. Performance of *Acacia senegal* (L.) Willd. accessions for tree height at rocky rangelands of the Thar Desert. *Journal of Arid Environments* **45**: 111–8.

Morris A B, Baucom R S and Cruzan M B. 2002. Stratified analysis of the soil seed bank in the cedar glade endemic *Astragalus bibullatus*: evidence of historical changes in genetic structure. *American Journal of Botany* **89**: 29–36.

Nybom H and Bartish I V. 2000. Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants. *Perspectives in Plant Ecology Evolution and Systematics* **3** (2) : 93–114.

Nybom H. 2004. Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. *Molecular Ecology* **13**: 1143–55.

Oleghe P E and Akinoufesi F K. 1992. Gum yield of *Acacia senegal* as affected by soil and water potential and season of tapping. *Nitrogen Fixing Tree Research Reports* **10**: 106–9.

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

Shrestha M K, Goldhirsh A G and Ward David 2002. Population genetic structure and the conservation of isolated population of *Acacia raddiana* in the Negev Desert. *Biological Conservation* **108** (1) : 119–27.

Sneath P H A and Sokal R R. 1973. *Numerical Taxonomy*. W.H. Freeman, San Francisco, California.

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