International Journal of Genetics and Molecular Biology Vol. 2(5), pp. 92-100, May 2010
Available online at http://www.academicjournals.org/IJGMB
ISSN2006-9863© Academic Journals

Full Length Research Paper

Genetic variation within and among three invasive *Prosopis juliflora* (Leguminosae) populations in the River Nile State, Sudan

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Accepted 18 March, 2010

The species of *Prosopis* (Leguminosae) are trees or shrubs well adapted to grow in arid and semi arid regions. In Sudan *Prosopis juliflora* was introduced in 1917. Currently, it has become a noxious weed spreading aggressively in natural and managed habitats. The structure of genetic diversity within and among *P. juliflora* populations infesting three forests in the River Nile State were assessed by RAPD technique. A total of 56 bands were obtained from seven primers. The mean percentage of polymorphic loci over all populations was (55.36%). Kulhuda population had the highest percentage of polymorphic loci (64.29%) and the highest number of private alleles (3). Makabrab population had the lowest percentage of polymorphic loci (46.43%) and two private alleles. Mean expected heterozygosity was (0.218). High genetic differentiation was found among populations (PhiPT = 0.328, P = 0.001). There was a genetic variation of 33% among the populations and within them 67% (AMOVA, P < 0.001). The mean Shannon information index was (I = 0.319, SE = 0.023). UPGMA clustering did not precisely reflect the geographic position of the populations. The results show the current structure of the populations and the similarities between groups of populations, might be due to the recent introduction of the species into Sudan, the limited seed source, the extensive endozoic dispersal seed system and limited pollen dispersal.

Key words: *Prosopis juliflora*, invasive, genetic variation, RAPD, Sudan.

INTRODUCTION

Mesquite trees belong to the family Mimosaceae, Subfamily Mimosoideae, genus *Prosopis* which includes 44 species grouped in 5 sections and 6 series, it occurs worldwide in arid and semiarid regions (Burkart, 1976).

Several *Prosopis* species possess remarkable colonising ability (Bessega et al., 2000a). Raven and Polhill (1981) reported that the dispersion and evolution of the genus *Prosopis* is thought to have taken place approximately 70 million years ago, earlier than the separation of the African and the South-American continents occurred. Two centres of diversity of *Prosopis* occur in the American continent, the Texan-Mexican centre and the Argentinean-Paraguayan centre (Burkart, 1976).

The sections Algarobia have been largely considered Obligate out-crossers (Simpson, 1977; Simpson et al., 1977). More recently, Bessega et al. (2000b) gave evidence that selfing may occur in some species. They are pollinated by insects and the seeds are endozoically dispersed by herbivores (Burkart, 1976).

Prosopis species have been widely introduced in several countries around the world over the past 150 years for the production of fuel wood, fodder and their ability to grow in the poorest soils and survive in areas where no other trees can survive (Pasiecznik et al., 2001). It is highly recognised for windbreaks, soil binders and sand stabilizers, moreover, provide shelter and food to animals that feed on its nectar, pollen, leaves and fruits (Golubov et al., 2001).

Prosopis juliflora (Swartz) DC is a leguminous, perennial phreatophyte. It is a deciduous, thorny tree (Burkart and Simpson, 1977). It belongs to the section Algarobia (Burkart, 1976). World wide, this dominant woody plant

Abbreviation: PCA, Principal coordinate analysis.

exists in about 45 million ha of grazing lands from sea level up to 1500 m. It is tolerant to very high temperatures (e.g. 48°C) and annual rainfall range of 150 - 750 mm (Darke, 1993; Geilfus, 1994). The roots penetrate to great depths in the soil and can grow in wide range of soils, such as saline, alkaline, sandy and rocky soils (George et al., 2007*). P. juliflora* is also tolerant to heavy metals (Sinha et al., 2005) and have been proposed to be suitable solution for treatment of soils contaminated with cadmium, chromium and copper (Senthilkumar et al., 2005). Recently, George et al. (2007) investigated the stress-induced genes in *P. juliflora* through analysis of expressed sequence tags. Their study reveals some insights into the genes responsible from abiotic stress tolerance in *P. juliflora* as some of the genes in their library known to play a significant role in stress tolerance.

Mesquite (*P. juliflora*) was introduced into Sudan in 1917 from South Africa and Egypt and planted in Khartoum (Brown and Massey, 1929). Ever since, there had been repeated introductions in several regions of the country.

Introductions in the River Nile State in Sudan were first reported in 1948 to act as shelter belts around Gandatu Agricultural project and continued later on to act as shelterbelts to protect Agricultural Projects from moving sand. In later years, several shelterbelts had been introduced in several villages east and western Nile. More recently (between 1985 - 1996) 23 belts were established around more villages.

Recently, in Sudan, it has been perceived as noxious weed rather than being useful for environmental amelioration. It has invaded diverse habitats, both natural and managed. Over the years this tree species has spread to northern, central and eastern Sudan, with over 90% of the invasion in the Eastern State. Between 1992 and 1996, it was estimated to have spread at a rate of 460 ha per year and by 2006 it covered approximately 230,000 ha of land (Babiker, 2006).

It has become a nuisance in agricultural lands as it forms impenetrable thickets that affect native vegetation community structure development and pastures. It constitutes a threat to biodiversity and affects livelihoods of populations who depend on livestock keeping and subsequent farming as their main sources for income generation (Elhouri, 1986). Consequently, there has been growing calls for its elimination, culminating in a presidential decree in 1996 and followed by several campaigns more recently for its eradication. However, complete eradication of mesquite was not successful due to i) nature of its infestations, ii) longevity of seeds, iii) free of natural enemies (Babiker, 2006) iv) competitive ability (Elsidig et al., 1998) and allelopathy (Mohamed, 2001).

As argued by Ward et al. (2008), there are very few published studies done to understand the genetic diversity and genetic structure within invasive plant populations and for limited number of invasive plants (Ward et al. 2008). No information exists on the genetic diversity of *Prosopis* species in Sudan based on molecular markers.

The objectives of this study were: (1) to investigate the structure and the distribution of genetic variability within and among *P. julflora*_populations infesting three forests in the River Nile State in Sudan; (2) to find the relationships between those populations based on the RAPDs fragment analysis. The findings should enable us to understand how these populations establish, adapt and expand in their environment.

MATERIALS AND METHODS

Population samplings and plant materials

Three populations of *Prosopis* growing in three different forests were sampled (Table 1, Figure 1). A total of 45 individuals of *Prosopis* were collected (15 per forest) in the River Nile State in Sudan. Two populations were collected along the Atbara River and one along the River Nile. From each sampled tree, leaves were collected and dried in silica gel and kept in ventilated room under shade until DNA extraction was done. Plants sampled were at least 100 m from each other, to avoid sampling of ramets of the same vegetative clone. Voucher specimens from each site are kept at the Department of Molecular Biology, Commission for Biotechnology and Genetic Engineering, NCR, Khartoum, Sudan.

DNA extraction

Genomic DNA was extracted from dry leaves of 45 individuals using modified CTAB method (Porebski et al., 1997). In this method the fine powdered plant materials were transferred into 13 ml Falcon tubes containing 6 ml of pre-warmed buffer solution. Tubes containing the samples were then incubated in a water bath at 65°C with gentle shaking for 30 min and left to cool at room temperature for 5 min. Isoamyl alcohol chloroform mixture (1:24) was added to each tube and the phases were mixed gently for 5 min at room temperature to make a homogenous mixture. The cell debris was removed by centrifugation at 5000 rpm for 15 min and the resulted clear aqueous phases (containing DNA) were transferred to new sterile tubes. The step of the chloroform: isoamyl alcohol extraction was repeated twice. The nucleic acids in the aqueous phase were precipitated by adding equal volume of deep cooled isopropanol. The contents were mixed gently and collected by centrifugation at 4000 rpm for 10 min. The formed DNA pellet was washed twice with 70% ethanol and the ethanol was discarded after spinning with flash centrifugation. The remained ethanol was removed by leaving the pellet to dry at room temperature. The pellet was dissolved in TE buffer (10 mM Tris, 1 mM EDTA, pH 8) and stored at -20°C for further use. The extracted DNA samples were observed under UV illumination after staining with ethidium bromide and agarose gel electrophoresis. The purity and the concentrations of the DNA were then spectrophotometrically assessed following Sambrook et al. (1989) method.

RAPD technique

The PCR was carried out in 25 µl final volume using 1 µl of genomic DNA (20 - 40 ng) containing, 2.5 µl of 10X Taq buffer, 1.5 µl MgCl2 (50 mM) , 2.5 µl dNTPs (2 mM/µl), 2 µl random primer (10 pmol/ µl), 0.5 units of Taq DNA polymerase (Vivantis). The mixture was made up to 25 µl by addition of sterilized distilled water. The mixture was amplified in a thermal cycler (Biometra) which was programmed for one cycle of initial denaturation at 94°C for 5 min, 40 cycles of 94°C for 1 min, followed by annealing 36°C for 1 min and ended by extension at 72°C for 1 min followed by a final extension cycle that

Table 1. Geographic information and area of the three forests of the sampled populations of *Prosopis juliflora*.

Forest name	Symbol	Area (ha)	Latitude (N)	Longitude (E)	Location
Makabrab	M	340	17.44854	33.89567	Eastern bank of the River Nile
Um Sayala	S	360.36	17.36925	34.44579	Eastern bank of the Atbara river
Kulhuda	K	840	17.33573	34.36975	Western bank of the Atbara river

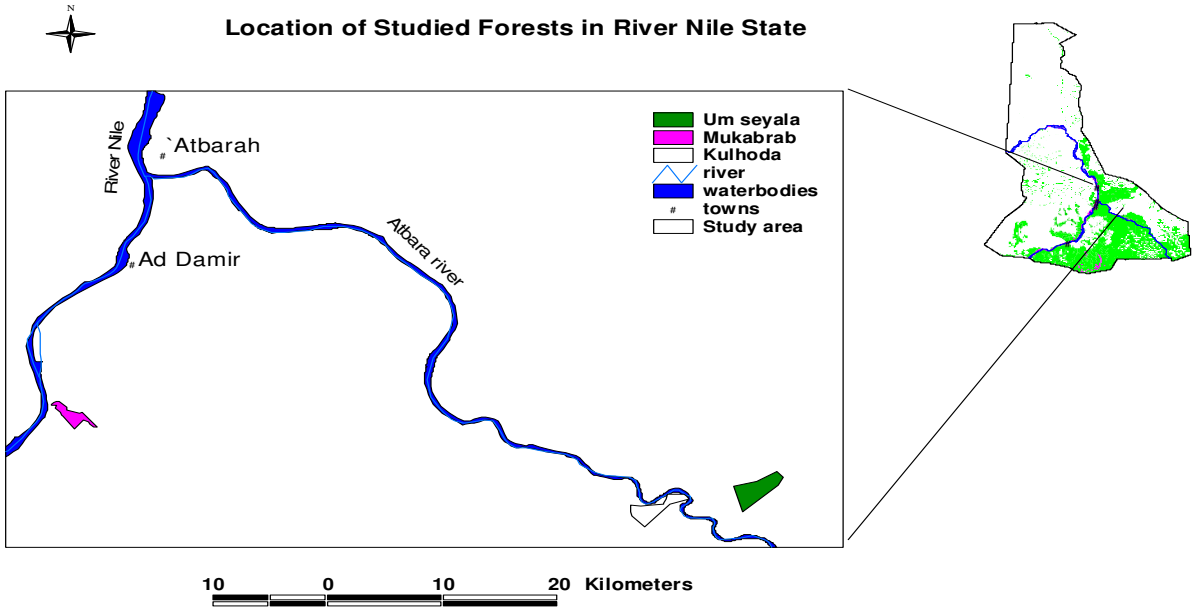

Figure 1. Map of the Nile River State indicating the three forests sites.

performed at 72℃ for 7 min. The PCR machine was adjusted to hold the product at 4℃.

Each amplified product was mixed with 3 µl of loading dye (12.5 bromophenol blue, 2 g sucrose) and spun briefly in a micro centrifuge before loading. The PCR products and 1 kp DNA ladder were electrophoresed on 2% agarose gel (stained with EtBr) at 80 volts. The separated fragments were visualized with an ultraviolet (UV) transilluminator. In this study, several RAPD primers from the University of British Columbia (UBC),Vancouver, British Columbia, Canada and from Operon Technologies Inc., Ala- meda, CA; were tested for their reproducibility and cautions were made to avoid unspecific amplification.

Statistical analysis

Analysis of genetic diversity

Each band in the RAPDs profile was treated as an independent locus with two alleles. The numbers of bands produced for each primer were scored manually for presence (1), or absence (0) and a binary matrix was generated and then used for analysis.

The PhiPT (analogue of F_{ST} fixation index) value for genetic variability, the percentage of polymorphism (% P), heterozygosity (he), number of observed alleles (Na), number of effective alleles (Ne) and Shannon's information index (I) were calculated for each population using GenALEx v. 6.1 (Peakall and Smouse, 2006).

With AMOVA, the variance components and their significance levels for variation among populations and within population using RAPD data of the 45 individuals was obtained. It was conducted in GenALEx v. 6.1. using the PhiPT "P value is calculated as the number of values ≥ observed value (Including Observed Value) ÷ (Number of Permutations + 1)".

Genetic structure

A principal coordinate analysis (PCA) was conducted with GenALEx v. 6.1 (Peakall and Smouse, 2006). This multivariate approach was chosen to complement the cluster analysis information, because cluster analysis is more sensitive to closely related individuals whereas PCA is more informative regarding distances among major groups (Hauser and Crovello, 1982).

Pairwise genetic distances between individuals were calculated by the percentage disagreement method. These data were used in cluster analysis with the unweighted pair-group method using arithmetic averages (UPGMA), in which samples are grouped based on their similarity with the aid of statistical software package STATISTCA- ver.6 (StatSoft, Inc., 2001).

RESULTS

After screening of a series of primers, a total of seven primers (10 mer) that produced strongly amplified polymorphic bands were selected for RAPD-PCR analysis

Table 2. Polymorphism detected by the use of seven random primers on three *Prosopis juliflora* populations.

Name of primer	Sequence of primer (5'-3')	Total number of bands	Number of polymorphic bands	% of polymorphic Bands
A-01	CAGGCCCTTC	7	7	100
B-8	GTCCACACGG	13	10	76.92
B-20	GGACCCTTAC	9	6	66.67
C-2	GTGAGGCGTC	4	4	100
C-10	TGTCTGGGTG	9	8	88.89
UBC-101	GCGGCTGGAG	7	7	100
UBC-122	GTAGACGAGC	7	6	85.71

Table 3. Gene diversity in the three *Prosopis juliflora* populations.

Population	Number of observed alleles (Na)	Number of effective alleles (Ne)	Shannon's information index (I)	Heterozygosity (He)	Polymorphism (%)
M	1.411(0.080)	1.293(0.050)	0.250 (0.039)	0.169 (0.027)	46.43
S	1.411(0.098)	1.438(0.057)	0.344 (0.043)	0.239 (0.030)	55.36
K	1.589(0.080)	1.427(0.051)	0.364 (0.039)	0.247 (0.027)	64.29

Numbers in parenthesis refers to standard error.

Table 4. Summary of the analysis of molecular variance (AMOVA) within and among *Prosopis juliflora* populations.

Source	df	SS	MS	Est.Var	%	Stat	Value	Probability
Among Pops	2	87.733	43.867	2.573	33			
Within Pops	42	221.200	5.267	5.267	67	PhiPT	0.328	0.001

The analysis is based on RAPD phenotypes consisting of 56 band states. Levels of significance are based on 1000 iterations.

Table 5. Summary of Nei pairwise population genetic distance (NeiP), genetic structure (PhiPT) values and their level of significance, estimates of georgraphic distances and Nm values between populations.

Population 1	Population 2	PhiPT	Geographic distance (Km)	Nei genetic distance (GD)
S	K	0.372(P<0.001)	9.180	0.203
S	M	0.260(P<0.001)	58.520	0.131
K	M	0.329(P<0.001)	52.130	0.180

(Table 2). The selected primers generated an appropriate amplification pattern with clear and consistent reproducible bands. A total of 56 bands, were obtained.

The seven informative primers were selected and used to evaluate the degree of polymorphism and genetic relationships within and between all individuals under study. The maximum number of band fragments was produced by the primer B-8 (13 bands) with 76.92% polymorphism while the minimum number of fragments was produced by the primer C-2 (4 bands) with 100% polymorphism. With an average of 6.86 bands per primer. Pattern of RAPD fragments produced by all the highest (0.247, SE = 0.027) (Table 3).

The mean population diversity using the Shannon information index (I) was 0.319 (SE = 0.023). Kulhuda population was the most diverse (I = 0.364) and lowest diverse population was Makabrab (I = 0.25) (Table 3).

The analysis of molecular variance (AMOVA) for populations showed significant differentiation (P < 0.001), with 67% of the differentiation attributed to within populations and 33% attributed to among primers is shown in (Table 2).

The highest number of effective alleles was in Umsayala population (1.438, SE = 0.057) and the lowest in Makabrab (1.293, SE = 0.05), with an average 1.386 (SE = 0.031) over all populations. The mean percentage of polymorphic loci over all populations was 55.36 (5.15%). Kulhuda population had the highest percentage

Table 6. Matrix of genetic distances based on percentage disagreements

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	K16	K17	K18	K19	K20	K21	K22	K23	K24	K25	K26	K27	K28	K29	K30	M31	M32	M33	M34	M35	M36	M37	M38	M39	M40	M41	M42	M43	M44	M45
S1	0.00																																													
S2	0.11	0.00																																												
S3	0.18	0.07	0.00																																											
S4	0.25	0.21	0.14	0.00																																										
S5	0.32	0.25	0.21	0.11	0.00																																								
S6	0.25	0.21	0.25	0.14	0.18	0.00																																							
S7	0.09	0.13	0.13	0.20	0.23	0.23	0.00																																						
S8	0.13	0.09	0.13	0.23	0.27	0.27	0.14	0.00																																					
S9	0.16	0.16	0.20	0.16	0.23	0.20	0.18	0.11	0.00																																				
S10	0.25	0.18	0.21	0.25	0.25	0.18	0.20	0.20	0.23	0.00																																			
S11	0.25	0.25	0.25	0.21	0.25	0.25	0.27	0.30	0.23	0.29	0.00																																		
S12	0.20	0.20	0.23	0.30	0.30	0.27	0.18	0.21	0.21	0.20	0.30	0.00																																	
S13	0.13	0.09	0.09	0.20	0.27	0.30	0.14	0.07	0.14	0.23	0.27	0.18	0.00																																
S14	0.18	0.14	0.11	0.14	0.21	0.25	0.13	0.16	0.16	0.21	0.21	0.23	0.13	0.00																															
S15	0.18	0.18	0.14	0.21	0.25	0.36	0.13	0.20	0.23	0.25	0.32	0.23	0.13	0.14	0.00																														
K16	0.34	0.27	0.30	0.30	0.34	0.30	0.36	0.32	0.29	0.30	0.38	0.29	0.29	0.34	0.30	0.00																													
K17	0.41	0.38	0.38	0.34	0.41	0.41	0.39	0.39	0.36	0.38	0.48	0.39	0.36	0.34	0.30	0.14	0.00																												
K18	0.45	0.38	0.38	0.38	0.38	0.41	0.43	0.39	0.39	0.38	0.48	0.39	0.39	0.38	0.34	0.18	0.14	0.00																											
K19	0.34	0.30	0.34	0.41	0.41	0.41	0.36	0.32	0.36	0.30	0.41	0.39	0.32	0.34	0.30	0.29	0.25	0.18	0.00																										
K20	0.25	0.21	0.25	0.32	0.32	0.32	0.27	0.23	0.27	0.29	0.32	0.34	0.23	0.29	0.25	0.27	0.41	0.27	0.16	0.00																									
K21	0.27	0.23	0.27	0.34	0.34	0.34	0.29	0.25	0.29	0.27	0.34	0.36	0.25	0.27	0.23	0.29	0.39	0.25	0.14	0.05	0.00																								
K22	0.38	0.30	0.30	0.27	0.27	0.30	0.36	0.32	0.29	0.38	0.38	0.32	0.32	0.30	0.30	0.18	0.25	0.14	0.32	0.23	0.21	0.00																							
K23	0.45	0.38	0.38	0.34	0.34	0.38	0.43	0.39	0.36	0.38	0.45	0.36	0.39	0.34	0.34	0.21	0.14	0.07	0.21	0.30	0.32	0.14	0.00																						
K24	0.29	0.25	0.32	0.36	0.36	0.32	0.30	0.27	0.27	0.25	0.39	0.30	0.30	0.29	0.29	0.27	0.30	0.20	0.13	0.18	0.16	0.23	0.20	0.00																					
K25	0.39	0.36	0.39	0.36	0.39	0.43	0.38	0.38	0.34	0.39	0.46	0.34	0.38	0.32	0.32	0.23	0.16	0.13	0.23	0.36	0.34	0.20	0.13	0.18	0.00																				
K26	0.46	0.43	0.46	0.39	0.43	0.36	0.48	0.41	0.38	0.39	0.39	0.38	0.45	0.46	0.50	0.38	0.45	0.38	0.45	0.36	0.38	0.30	0.34	0.36	0.32	0.00																			
K27	0.30	0.27	0.30	0.34	0.34	0.34	0.29	0.29	0.29	0.30	0.41	0.32	0.32	0.30	0.30	0.25	0.29	0.21	0.14	0.20	0.21	0.25	0.21	0.09	0.16	0.38	0.00																	
K28	0.38	0.38	0.38	0.38	0.41	0.41	0.36	0.36	0.36	0.38	0.45	0.36	0.36	0.30	0.30	0.21	0.14	0.11	0.18	0.30	0.29	0.25	0.14	0.27	0.13	0.38	0.21	0.00															
K29	0.29	0.18	0.25	0.29	0.32	0.25	0.30	0.23	0.23	0.21	0.32	0.27	0.23	0.29	0.32	0.13	0.23	0.20	0.27	0.25	0.27	0.23	0.23	0.18	0.21	0.29	0.20	0.23	0.00														
K30	0.38	0.30	0.30	0.34	0.38	0.38	0.36	0.36	0.32	0.34	0.41	0.32	0.32	0.34	0.30	0.11	0.11	0.14	0.21	0.30	0.32	0.21	0.18	0.27	0.20	0.41	0.25	0.14	0.16	0.00													
M31	0.16	0.13	0.16	0.27	0.30	0.27	0.18	0.18	0.21	0.20	0.30	0.21	0.14	0.23	0.16	0.18	0.32	0.29	0.21	0.13	0.14	0.25	0.32	0.16	0.34	0.41	0.18	0.32	0.16	0.25	0.00												
M32	0.20	0.16	0.23	0.27	0.30	0.23	0.21	0.21	0.18	0.16	0.27	0.18	0.21	0.23	0.23	0.21	0.36	0.32	0.25	0.16	0.18	0.25	0.32	0.13	0.30	0.34	0.14	0.36	0.16	0.29	0.07	0.00											
M33	0.21	0.14	0.18	0.32	0.36	0.32	0.23	0.20	0.23	0.21	0.32	0.16	0.16	0.25	0.18	0.16	0.30	0.27	0.27	0.18	0.20	0.23	0.30	0.21	0.32	0.36	0.23	0.30	0.18	0.23	0.05	0.09	0.00										
M34	0.21	0.14	0.18	0.32	0.36	0.32	0.23	0.20	0.23	0.21	0.32	0.16	0.16	0.25	0.18	0.16	0.30	0.27	0.27	0.18	0.20	0.23	0.30	0.21	0.32	0.36	0.23	0.30	0.18	0.23	0.05	0.09	0.00	0.00									
M35	0.18	0.14	0.18	0.29	0.36	0.32	0.20	0.16	0.20	0.29	0.36	0.23	0.13	0.21	0.14	0.20	0.30	0.30	0.30	0.18	0.16	0.20	0.34	0.25	0.32	0.36	0.27	0.30	0.21	0.27	0.09	0.16	0.07	0.07	0.00								
M36	0.25	0.18	0.25	0.29	0.29	0.21	0.23	0.23	0.23	0.14	0.29	0.20	0.23	0.25	0.29	0.27	0.41	0.38	0.30	0.21	0.20	0.27	0.38	0.18	0.36	0.29	0.20	0.41	0.18	0.34	0.13	0.05	0.14	0.14	0.18	0.00							
M37	0.21	0.14	0.18	0.32	0.36	0.32	0.23	0.20	0.23	0.25	0.36	0.20	0.16	0.25	0.18	0.20	0.30	0.27	0.23	0.18	0.16	0.23	0.30	0.25	0.36	0.39	0.27	0.27	0.21	0.20	0.09	0.16	0.07	0.07	0.07	0.18	0.00						
M38	0.16	0.16	0.20	0.30	0.38	0.30	0.18	0.18	0.21	0.27	0.34	0.18	0.14	0.20	0.16	0.21	0.29	0.29	0.25	0.20	0.21	0.29	0.32	0.27	0.34	0.41	0.29	0.21	0.23	0.21	0.11	0.18	0.09	0.09	0.09	0.23	0.05	0.00					
M39	0.16	0.09	0.13	0.23	0.30	0.27	0.18	0.11	0.18	0.23	0.30	0.21	0.07	0.16	0.13	0.21	0.29	0.32	0.29	0.20	0.21	0.25	0.32	0.27	0.34	0.38	0.29	0.29	0.20	0.25	0.11	0.18	0.09	0.09	0.05	0.20	0.09	0.07	0.00				
M40	0.25	0.18	0.18	0.32	0.36	0.32	0.23	0.16	0.23	0.29	0.36	0.23	0.20	0.29	0.21	0.27	0.41	0.34	0.34	0.21	0.23	0.27	0.38	0.25	0.36	0.39	0.27	0.38	0.25	0.34	0.16	0.20	0.14	0.14	0.14	0.25	0.18	0.20	0.16	0.00			
M41	0.18	0.14	0.18	0.29	0.32	0.32	0.20	0.13	0.20	0.25	0.36	0.20	0.13	0.21	0.14	0.20	0.27	0.27	0.23	0.18	0.20	0.23	0.27	0.21	0.29	0.36	0.23	0.23	0.21	0.23	0.09	0.16	0.07	0.07	0.07	0.21	0.07	0.05	0.05	0.14	0.00		
M42	0.23	0.20	0.23	0.30	0.34	0.38	0.25	0.21	0.32	0.27	0.34	0.29	0.18	0.27	0.23	0.32	0.43	0.39	0.32	0.23	0.25	0.36	0.43	0.27	0.38	0.38	0.29	0.39	0.27	0.39	0.14	0.18	0.16	0.16	0.20	0.20	0.23	0.21	0.18	0.27	0.16	0.00	
M43	0.16	0.16	0.20	0.27	0.30	0.34	0.18	0.18	0.25	0.27	0.34	0.25	0.14	0.23	0.16	0.25	0.36	0.32	0.25	0.16	0.14	0.25	0.36	0.20	0.30	0.38	0.21	0.32	0.23	0.32	0.07	0.14	0.13	0.13	0.09	0.16	0.13	0.14	0.14	0.20	0.09	0.11	0.00
M44	0.18	0.14	0.18	0.25	0.29	0.32	0.20	0.16	0.27	0.21	0.29	0.23	0.13	0.21	0.18	0.30	0.38	0.38	0.27	0.21	0.23	0.34	0.38	0.25	0.36	0.36	0.27	0.34	0.25	0.34	0.13	0.16	0.14	0.14	0.18	0.18	0.18	0.16	0.13	0.25	0.11	0.05	0.09	0.00	
M45	0.13	0.13	0.16	0.23	0.27	0.30	0.14	0.14	0.21	0.23	0.30	0.21	0.11	0.20	0.13	0.25	0.32	0.32	0.21	0.16	0.18	0.29	0.32	0.20	0.30	0.41	0.21	0.29	0.23	0.29	0.07	0.14	0.13	0.13	0.13	0.20	0.13	0.11	0.11	0.20	0.05	0.11	0.04	0.05	0.00

of polymorphic loci (64.29%) and the highest number of private alleles (3). Makabrab population had the lowest percentage of polymorphic loci (46.43%) and two private alleles. Mean expected heterozygosity was 0.218 (0.016), with Kulhuda having populations. The PhiPT estimates were high (0.328) and highly significant (P = 0.001) (Table 4).

Principal components analysis of molecular variance performed in GenAlex 6.1, based on individual band pattern. The first two axes explain 56.59% (Figure 3). The fixation index value of PhiPT over all populations was (0.328) and highly significant (P < 0.001). Between populations, the highest PhiPT value (0.372, P < 0.001) was found between Umsayala and Kulhuda and the lowest between Umsayala and Makabrab (0.260, P < 0.001), Table 5. The UPGMA and percent disagreement values (PDV) were used to estimate the degree of relationships between the individuals analysed based on common amplified fragments. Based on the matrix obtained (Table 6), the mean average percent disagreement values (PDV) for all individuals was 0.25 ranging from 0.04 to 0.46. The dendrogram (Figure 2) shows a trend of clustering of regional populations to an extent. Nevertheless, most of Kulhuda

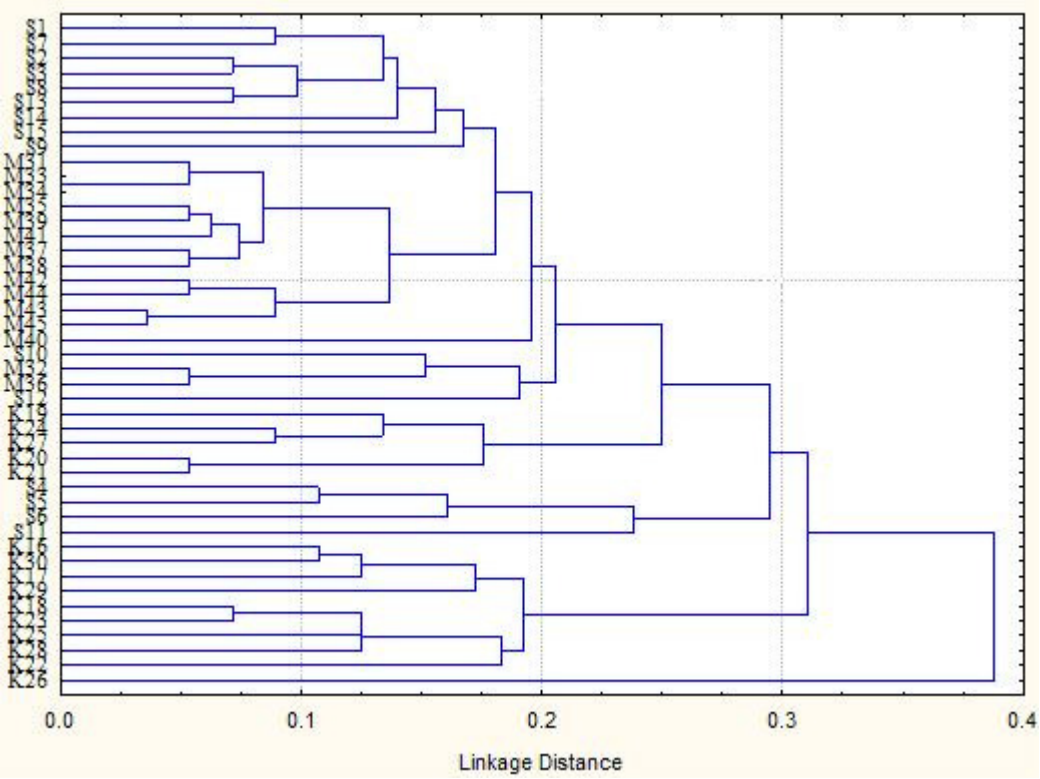

Figure 2. UPGMA tree showing relationships among three populations of *Prosopis juliflora* based on 54 RAPD loci.

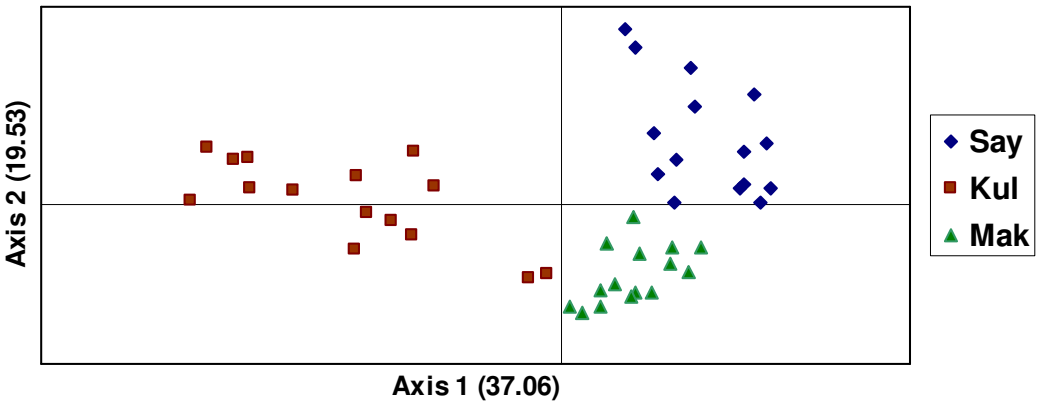

Figure 3. Principal coordinate analysis of the three *Prosopis juliflora* populations based on 56 random amplified polymorphic DNA loci. Note the separation between the Kulhuda individuals and the other two populations.

individuals formed a distant cluster from the other two populations. The pattern that is depicted by the PCA analysis differs from the UPGMA tree. The PCA seems to show three groups that correspond to the three populations, with two individuals of Kulhuda clearly differentiated (Figure 3). In accordance with the results obtained of higher gene diversity values in case of Kulhuda samples, the PCA plot also shows wide scattering when compared to Makabrab, which shows opposite trend both in the PCA plot and the corresponding gene diversity values. The first three coordinates explained 71.7% of the total variation.

Of the three regions, the Makabrab samples show more homogeneity as against the rest two regions (gene diversity measures).

DISCUSSION

A clear understanding of the genetic diversity with explicit analyses of genetic structure of the invasive populations is important; it can help in understanding the history of a particular invasion and their response to environmental changes (Ward, 2006). The mechanisms of their local spread and adaptation and predict the potential for populations of invasive species to evolve in response to evolution of resistance to herbicides or biological control agents based on the diversity levels (Sakai, 2001), which can lead to setting effective control plan for its eradication. The majority of the work done to understand the genetic diversity in the Prosopis genus used the isozyme markers (Saidman 1986, 1990; Saidman and Vilardi, 1987; Keys and Smith, 1994; Saidman et al., 1996, 1998). Later studies, used the Random Amplified Polymorphic DNAs (RAPDs) to study Prosopis species from Africa, South America and Asia (Saidman et al., 1998; Ramirez et al., 1999; Landreas et al., 2006) and to detect inter and Intra-specific genetic variability thanks to their ability to detect the polymorphism (Juárez-Muñoz et al., 2002).

Genetic variation

The He and P values obtained in the present study are similar to those obtained in previous studies on several Prosopis species using both isozymes and RAPDs (Saidman, 1986, 1990; Saidman et al., 1997, 1998, Saidman and Vilardi, 1987; Verga, 1995; Bessega et al., 2000c). In this study, the average number of fragments produced per primer (6.86) using seven primers is less than the one obtained by (Juárez-Muñoz et al., 2002) (8.6) by using five primers on four populations of P. juliflora, Propis laevigata and Propis glandulosa.

Population genetic structure, mating system and seed dispersal

PhiPT and FST are analogous standardizing measures of the degree of genetic differentiation among populations: scores for both measures range from 0 (no differentiation) to 1 (no alleles shared).

In this study, the PhiPT value obtained over all populations was (0.328) and highly significant ($P < 0.001$), suggesting structuring among the populations. Bessega et al. (2000b) argued that Prosopis species populations are expected to be structured because pollen and seed dispersal in species of *Algarobia* is limited, causing population substructure. Seeds dispersal distances could vary, from short to long which affect the population genetic structure (Hamrick et al., 1992).

There was a genetic variation of 33% among the populations and within them the estimated genetic variation was 67% (AMOVA, $P < 0.001$). This is in accordance with the findings of Juárez-Muñoz et al. (2002), as they obtained higher variation (92.85%) within the two populations of P. laevigata and lower variation among them (7.15). Nevertheless, when they compared four populations of P. juliflora, P. glandulosa and two of P. laevigata the molecular variance was significantly different and high among the populations (72.48%) and 27.52 within the populations. In their study (Ferreyra et al., 2007), using both RAPDs and isozyme in species of Prosopis, they reported that most genetic variation occurs within populations.

Saidman et al. (1998) observed non-significant levels of gene flow ($Nm < 1$) among populations of different species of Prosopis. Bessega et al. (2000c) studying P. glandulosa and P. velutina using both isozyme and RAPDS markers, found also low estimates of gene flow between species ($Nm=0.39$ and 0.60) for isozyme and RAPD loci, respectively.

The results of this study indicate that geographic proximity is not indicative of genetic similarity and hence, is not a guide for understanding the genetic structure of this species.

UPGMA cluster analysis showed a trend of clustering of regional populations. There was considerable overlap among Umsayala and Makabrab populations, which reflects their genetic closeness although they are 58.5 km distant. The main source for seed propagation and distribution in the River Nile State was Gandatu Agricultural project (Abdel Magid T.D, Ex-National Coordinator of Mesquite in the Forests National Corporation, personal communication, 2009).

The introduction of mesquite in the River Nile State in Sudan, prevailing drought, with extensive livestock and animal movement added to decrease in land use and over exploitation of natural vegetation have led to spread of Prosopis into various areas. Browsing animals such as camels, goats, donkeys, cattle and sheep are main agents in spreading it. Furthermore, human beings who collect the pods to feed their animals also contributed.

The fruit pods of mesquite trees are considered as a rich food for domestic animals and human beings in hot dry countries. The pods contain much sugar and a fair amount of protein (Abdel, 1986). Abdel (2001) found that pods contained 26% glucose and 9 to 14% protein and 55% carbohydrates. In Central America the pods are ground into a meal for use in concentrated rations (Laurie, 1974).

It has been reported by Brown and Archer (1987), who studied the dispersal of *P. glandulosa* var. glandulosa seeds by cattle in a savannah woodland in Texas, that 75% of dung pats of the cattle contained *Prosopis*

seedlings, with an average of 4.2 seedlings per pat and when the cattle were excluded, no establishment of Prosopis was reported. Reynolds (1954) reported a maximum dispersal distance of 50 m by kangaroo rats, whereas livestock and larger animals disperse it to a maximum distance of 4 to 6 km.

After germination, mesquite seedlings grow vigorously. The roots are fast in developing to deeper depth and the un-palatability of the green leaves by animals increase the survival chances of the seedlings, especially in areas that undergo heavy grazing (Mohamed, 2001).

In this case study, pollen dispersal mediated by insects is not expected to be distant as the main vectors of seed dispersal are herbivorous mammals, which are not expected to transport seeds over large distances.

Kulhuda population was genetically differentiated from the other two populations. That might be due to the special microclimate due to closeness of its position to the Atbara River, where, livestock visiting this forest would aid in transporting the seeds from other areas and dispose them in this forest. The moist and fertile soil will aid in the fast germination and establishment of such seeds. Also, in later stages, the selection pressures at this site will be less than in the other two populations.

Although Makabrab forest also lies very close to the eastern bank of the River Nile, it has less number of recently established mesquite individuals (3 - 4 years) than the other forests. The lower genetic diversity found can be explained by the same seed source in the area. Nevertheless, being lower in genetic diversity than the other populations, this population maintain 2 private alleles. Factors increasing inbreeding in Prosopis is being entomophilic where the pollen is usually unable to migrate large distances and having endozoic dispersal seed system (Genisse et al., 1990). Bessega et al. (2000b) study on mating system parameters of seven species of Algorobia, showed that out crossing rate ranges between 0.72 and 1, with an average of 0.85, indicating 15% of selfing may occur in natural populations.

It has been shown in previous studies that the genetic structure of populations affect the efficacy of control of invasives. The control of a population with a genetically homogeneous structure, due to asexual mode of reproduction can be easier with matching a biological control agent to the host genotype, where it is vulnerable to the biological enemies (Van Driesche and Bellows, 1996). However, in sexually reproducing weeds, the greater genetic variation leads to fast adaptive evolution and escape from the biological control agent (Sakai et al., 2001).

Sakai et al. (2001) suggested that if the eradication of invasive populations is impossible, then, setting control strategies to alter the population genetic structure in order to reduce adaptive variation.

Molecular markers have proven to be very useful tools in this study in estimating the genetic variation within and among Prosopis populations. This study area represents limited area where Prosopis cause problems in Sudan. Therefore, substantial genetic differentiation might be expected in this species when studying more populations from different regions.

In our study, high diversity within and similarities between groups of populations, were indicated by the RAPD markers. The recent introduction of the species into Sudan, the limited seed source introduced, the extensive endozoic dispersal seed system and limited pollen dispersal might have shaped the current structure of the populations.

Despite the problem caused by the Prosopis in Sudan, further information on the genetic background of the existing populations is not available. The results of the present investigation constitute the first effort to study the genetic variation within and among some selected populations. Therefore, more research in future is encouraged.

ACKNOWLEDGEMENTS

Thanks to the Forests National Corporation, especially Dr. Abdalla Gafar, Alhag A. Alawad, Abdelhafeez Kamal, Hafiz Habib and Osman Karar for field support, help in sampling and hosting. I am grateful to Habeballa R and Ismail A for help in laboratory work; as well to Dr. Tore Satersdal and Nile Basin Research Program (University of Bergen, Norway) for encouragement and support during the research stay at the University of Bergen.

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