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Isozyme and RAPD studies in *Prosopis glandulosa* and *P. velutina* (Leguminosae, Mimosoideae)

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Abstract

Allozyme and random amplified polymorphic DNA (RAPD) techniques have been compared for their usefulness for genetic and taxonomic studies in *Prosopis glandulosa* and *P. velutina* populations. Isozymes and RAPDs yielded similarly high estimates of genetic variability. Genetic structure and differentiation were analyzed through non-hierarchical Wright's F_{DT}. For all populations considered, both markers produced low gene flow (Nm < 1) estimates. When only *P. glandulosa* populations were analyzed, isozyme data yielded higher gene flow estimates (Nm > 1), in agreement with that expected for conspecific populations. However, in RAPD data the expected reduction in F_{DT} and the increase in Nm were not observed. Correlation between F_{DT} and geographical distance matrices (Mantel test) for all populations was significant (P = 0.02) when based on isozymes, but not so (P = 0.33) when based on RAPDs. No significant associations among genetic and geographical or climatic variables were observed. Two isoenzyme systems (GOT and PRX) enabled us to distinguish between *P. glandulosa* and *P. velutina*, but no diagnostic band for recognition of populations or species studied here were detected by RAPD. However, RAPD markers showed higher values for genetic differentiation among conspecific populations of *P. glandulosa* and a lower coefficient of variation than those obtained from isozymes.

INTRODUCTION

The genus *Prosopis* occurs worldwide in arid and semiarid regions and includes about 44 species grouped in 5 sections and 8 series (Burkart, 1976). Many species of this genus, particularly those belonging to the Algarobia Section, have economic and ecological potential, often being major components of native North and South America ecosystems wherein they offer shade, firewood, food, and forage for wildlife and livestock (Leakey and Last, 1980; Habit *et al.*, 1981; Joshi and Nimbkar, 1991). Some species, like *P. juliflora* and *P. flexuosa*, are being used for reforestation of severely degraded ecosystems (Felker, 1984; Cony, 1995). By contrast, some other species colonize and invade areas altered by human activity, particularly *P. glandulosa* (honey mesquite) in Texas, Mexico, and New Mexico, *P. velutina* (velvet mesquite) in Arizona, and *P. ruscifolia* (vinal) in the Chaco Region, Argentina (Morello *et al.*, 1971; Burkart, 1976; Simpson and Solbrig, 1977; Hennessy *et al.*, 1983; Brown and Archer, 1987; Archer *et al.*, 1988; Scanlan and Archer, 1991). In North America, mesquite encroachment damages grasslands, especially when combinated with other factors such as overgrazing, drought, climate shifts and fire (Fisher, 1977; Schlesinger *et al.*, 1990). It also decreases rangeland productivity through competition and by increasing soil erosion.

Exploiting the ecological potential of this species requires selection for and combination through interspecific crosses of advantageous characteristics exhibited by different species. This, in turn, demands genetic markers possibly associated with desirable characters. Biochemical and molecular markers have been used to enhance understanding of plant population genetic structure (Soltis *et al.*, 1992). Among several efficient methods for revealing genetic variability within and among plant populations, some of the most widely applied are isozyme electrophoresis (Hamrick and Allard, 1972; Hamrick and Godt, 1990), random amplified DNA polymorphism - RAPDs - (Wolff and Peters-van Rijn, 1993; Wachira *et al.*, 1995; Brummer *et al.*, 1995; Swoboda and Bhalla, 1997; Palacios and Gonzalez-Candelas, 1997), and restriction fragment length polymorphisms - RFLPs - (Keim *et al.*, 1989; Hong *et al.*, 1993; Yanesita *et al.*, 1997). In the genus *Prosopis*, previous isoenzymatic studies in some Algarobia Section species have shown low differentiation among species and populations coupled with high variability within populations (Saidman, 1985, 1986, 1990, 1993; Saidman and Vilardi, 1987; Verga, 1995). Among the species of this section so far studied only *P. caldenia* (Saidman, 1990) and *P. velutina* (Bessega 1997) are recognizable by patterns of few loci. New molecular techniques seem necessary for obtaining genetic markers useful in species and population recognition.

Here, for studying genetic variation distribution within and among populations of two native North American species of the Section Algarobia, *P. glandulosa* and *P. velutina*, we compare isozyme and RAPD technique adequacy as tools for providing genetic markers useful in 1) quantify-

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ing genetic variability within a population; 2) measuring differentiation among populations and 3) analyzing the relationships among these markers and climatic and geographic variables.

MATERIAL AND METHODS

Species and populations

To estimate differentiation among *P. glandulosa* populations, four populations were sampled: Weslaco (WE), La Copita (LC), Frio Co. (FC), and Bell Co. (BC) (Figure 1, Table IA). We estimated the differences between *P. glandulosa* and *P. velutina* by comparing the former populations with the Santa Rita (SR) population of the latter species. Materials studied, kindly donated by Dr. G.J. DeLoack (Grassland Research Station USDA), were collected in July-September 1994.

Mesquite forests form a continuum of shrubs or trees with contiguous canopies. Five maternal parent trees were sampled in each population. Presence of an adequate seed crop was the first selection criterion; they also had to be separated from each other by at least 50 m. With these restrictions, the "pollen cloud" proceeding from the neighboring canopies over each mother plant was assumed to be of different origin, based on empirical observations (see Saidman and Vilardi, 1993) indicating that these isolated Argentinean Algarobia tree species present highly reduced seed crops.

Number of pods collected ranged from 50 to 400 per tree. Seeds obtained from all pods of each tree were lumped together and from this pool seeds were randomly chosen for isoenzyme or RAPD analyses. Allelic frequency estimations were based on equal numbers of seeds from each mother tree.

Despite relatively low number of mother plants collected per population, most alleles are expected to be represented in the sampled tree seeds taking into account the following considerations: species belonging to Section Algarobia are protogynous (Burkart, 1976) and have been considered obligate outcrossers (Simpson, 1977); recent estimates of outcrossing rates ranging from 0.6 to 1 in *P. velutina* (Keys and Smith, 1994) seem to confirm a cross pollination tendency in natural populations, explaining the many genotypes sampled within seeds collected from single mother trees.

Electrophoretic technique

Nine isoenzymatic systems (esterases, peroxidases, super oxide dismutase, glutamate oxalacetate transaminase, aminopeptidases, alcohol dehydrogenase, isocitric dehydrogenase, shikimic dehydrogenase and 6-phosphogluconic acid dehydrogenase) were studied. Homogenates were ob-

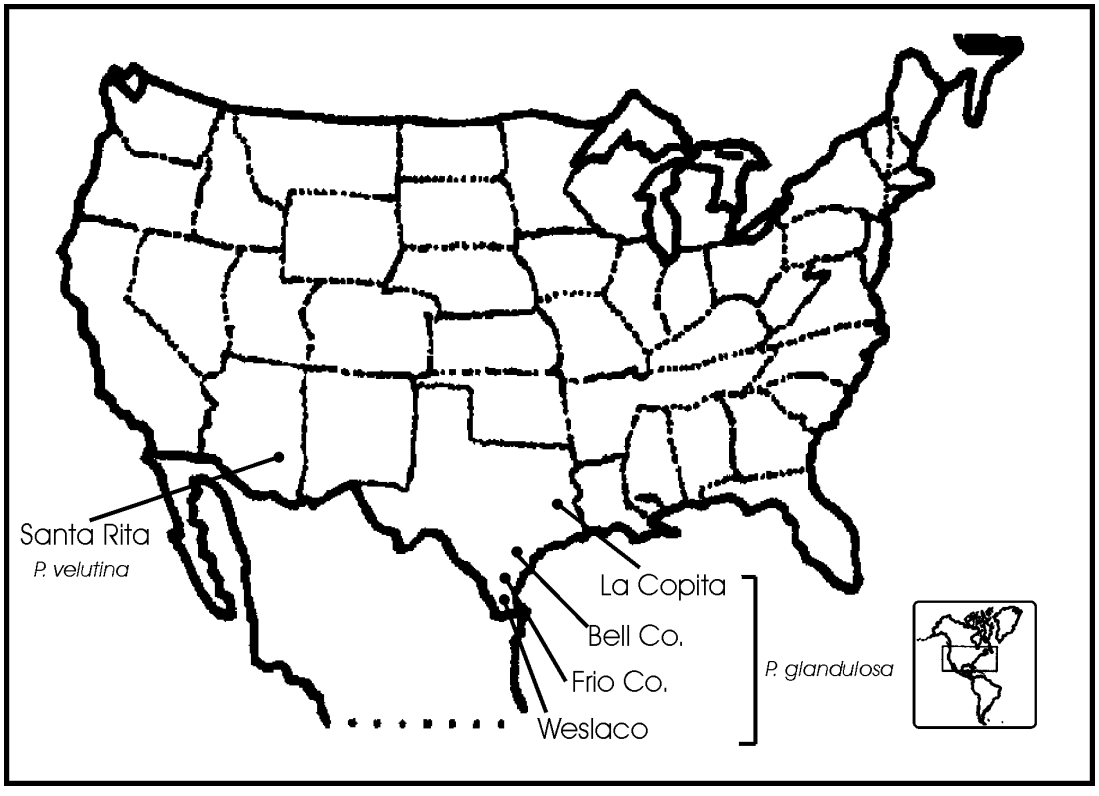

Figure 1 - Localization of sampled populations.

Table I-A - Geographic localization of sampling sites and closest meteorological experimental stations where climatic variables were obtained from.

Species	Population	Latitude	Longitude	Experimental station	Latitude	Longitude	Distance from sample site
P. glandulosa	Bell Co.	31°05' N	97°21' W	Waco	31°18' N	97°06' W	32 km
	Frio Co.	29°02' N	99°01' W	San Antonio	29°12' N	99°18' W	71 km
	Weslaco	26°09' N	98°00' W	Mc. Allen	26°12' N	98°14' W	26 km
	La Copita	27°45' N	98°04' W	Corphus Christie	27°30' N	97°25' W	71 km
P. velutina	Santa Rita	32°05' N	110°57' W	Tucson	32°14' N	110°57' W	35 km

Table I-B - Geographic distances among the populations sampled (in km).

	P. vel SR	*P. gla* FC	*P. gla* LC	*P. gla* BC
P. gla FC	1200			
P. gla LC	1312	173		
P. gla BC	1280	288	381	
P. gla WE	1415	338	174	541

P. gla, *Prosopis glandulosa*; *P. vel*, *P. velutina*; FC, Frio Co.; LC, La Copita; BC, Bell Co.; WE, Weslaco; SR, Santa Rita.

tained from cotyledons of 7-day-old seedlings for all systems but ADH where whole seeds soaked for 24 h were used. Electrophoresis was performed on 5 or 7% acrylamide horizontal gels. Buffers and electrophoretic conditions are described elsewhere (Saidman, 1985, 1986, 1990; Saidman and Vilardi,1987; Verga, 1995; Bessega, 1997).

Homology relationships among bands, genes, and alleles were determined by their correspondence to other species of Algarobia previously studied (Saidman, 1985, 1986, 1988, 1990, 1993; Saidman and Vilardi, 1987; Bessega, 1997). The mean number of individuals (seedlings) studied per locus is shown in Table I (slight differences in number of individuals studied in different loci are due to missing data).

RAPD technique

Total genomic DNA was extracted from cotyledons of 7-day-old seedlings using a modification of the protocol described by Dellaporta *et al.* (1983). PCR reactions involving arbitrary primers (Promega) contained approximately 50 ng of genomic DNA, 100 μM each dNTP, 60 ng primer, 1X Taq polymerase buffer (10 mM Tris-HCl, pH 9, at 25ºC, 50 mM KCl, 0.1% Triton X-100), 2 mM Cl_2Mg and 2.5 units of Taq Polymerase (Promega) in a final volume of 50 μl. The thermal cycler (Techne Progene) was programmed for one cycle of 94ºC for 1 min, 40 cycles of 92ºC for 1 min, 36ºC for 2 min, 72ºC for 2 min, followed by a final extension step of 72ºC for 6 min. Products were resolved on 1.4% agarose gels. Re-amplifications were performed routinely to ensure reproducibility of banding patterns and usual cautions observed to prevent contaminations of PCR experiments with previously amplified fragments. The necessary negative and positive controls were done to test PCR products reliability.

Ten arbitrary primers were screened for suitability on a small number of representative individuals. They varied in ability to detect polymorphisms and in reproducibility. Four primers which revealed informative patterns were chosen for analysis of all populations. Their sequences were: BO1: 5' - TCGAAGTCCT - 3'; BO7: 5' - AGATGCAGCC - 3'; BO8: 5' - TCACCACGGT - 3'; B10: 5' - CAGGCACTAG - 3'.

RAPD bands were scored as present (1) or absent (0). In order to assure reliability of data matrix, photo interpretations were made by two different members of our group. The mean numbers of individuals studied per primer in each population are indicated in Table II (slight differences in number of individuals studied in different loci are due to missing data).

Data analysis

Isoenzymatic genotypic data were converted into allelic frequencies (Table II) following the genetic interpretation previously published (Saidman, 1985, 1990; Saidman and Vilardi, 1987; Verga, 1995; Bessega, 1997).

The RAPD data matrix of band presence-absence was transformed into a frequency table (Table III) using the method of Lynch and Milligan (1994) through the RAPDBIOS program of the RAPD package by Black IV (1996). Linkage between RAPD loci was estimated using the program RAPDLD from the RAPD package. RAPDLD uses Ohta's (1982) method of partitioning the variance in disequilibrium in the total sample into within and among population components. The within population component is a measure of the proportion of total variance in disequilibrium due to epistasis; the among population component is a measure of the proportion of the total variance due to Wahlund's effect or genetic drift among populations. The sum of these components measures variance in disequilibrium in the total sample.

Table II - Allelic frequencies of polymorphic loci in studied populations of *Prosopis glandulosa* (*P. gla*) and *P. velutina* (*P. vel*). M and D denote monomeric and dimeric enzymes, respectively. D* indicates dimeric enzymes exhibiting allelic and nonallelic interactions. N indicates sample sizes. For *Got-3*, *Got-4*, *Prx-4* and *Prx-5* the lack of variation within populations did not allow a hypothesis on the number of polypeptide subunits in the active enzyme.

Locus	Allele	*P. gla* Weslaco	*P. gla* La Copita	*P. gla* Bell Co.	*P. gla* Frio Co.	*P. vel* Santa Rita
N		45	35	37	35	31
Adh-1	1^{30}	0.936	0.950	0.950	1.000	0.738
D*	1^{28}	0.064	0.050	0.050	0.000	0.262
	1^{24}	0.000	0.000	0.000	0.000	0.000
Amp-2	2^{88}	0.417	0.405	0.250	0.381	0.794
M	2^{76}	0.467	0.392	0.455	0.548	0.206
	2^{70}	0.116	0.203	0.295	0.071	0.000
Est-1	1^{93}	0.094	0.067	0.289	0.379	0.100
M	1^{92}	0.469	0.433	0.500	0.190	0.767
	1^{91}	0.437	0.500	0.211	0.431	0.133
Est-2	2^{87}	0.750	0.757	1.000	0.673	0.351
M	0	0.250	0.243	0.000	0.327	0.649
Est-3	3^{85}	0.388	0.160	0.423	0.433	0.239
M	0	0.612	0.840	0.577	0.567	0.761
Est-4	4^{81}	0.177	0.030	0.024	0.134	0.487
M	0	0.823	0.970	0.976	0.866	0.513
Got-1	1^{72}	0.108	0.098	0.250	0.205	0.116
D	1^{69}	0.387	0.455	0.544	0.346	0.081
	1^{71}	0.000	0.000	0.000	0.000	0.802
	1^{70}	0.505	0.446	0.206	0.449	0.000
Got-2	2^{48}	0.627	0.821	0.929	0.903	0.000
D	2^{40}	0.373	0.179	0.071	0.097	0.000
	2^{54}	0.000	0.000	0.000	0.000	1.000
Got-3	3^{49}	0.000	0.000	0.000	0.000	1.000
Got-4	4^{44}	0.000	0.000	0.000	0.000	1.000
Idh-1	1^{100}	0.054	0.071	0.384	0.211	0.167
D*	1^{70}	0.920	0.714	0.593	0.611	0.833
	1^{63}	0.027	0.214	0.023	0.178	0.000
6Pgd-2	2^{23}	0,000	0,028	0,022	0,000	0,000
	0	1.000	0.972	0.978	1.000	1.000
Prx-1	1^{36}	0.190	0.520	0.065	0.068	0.000
M	0	0.810	0.480	0.935	0.932	0.000
Prx-2	2^{29}	1.000	0.667	0.842	0.719	0.000
M	0	0.000	0.333	0.158	0.281	0.000
Prx-3	3^{21}	0.561	0.194	0.275	0.111	0.000
M	0	0.439	0.806	0.725	0.889	0.000
Prx-4	4^{39}	0.000	0.000	0.000	0.000	1.000
Prx-5	5^{32}	0.000	0.000	0.000	0.000	1.000
Skd-1	1^{24}	0.093	0.190	0.250	0.158	0.531
M	1^{22}	0.651	0.429	0.659	0.711	0.469
	1^{19}	0.256	0.381	0.091	0.132	0.000

Allelic frequencies from both isoenzyme and RAPD markers were used separately to estimate Wright's (1978) non-hierarchical F_{DT}, Nei's (1978) genetic identities, mean heterozygosity (Nei, 1978), percentage of polymorphic loci and mean number of alleles per locus using the computational program Biosys-1 (version 1.7) (Swofford and Selander, 1981).

Nei's (1987) genetic distance matrices (GDM) were estimated from allelic frequencies of both isozyme and RAPD data. From GDM, a phenogram was obtained by the unweighted pair group method using arithmetical means. Multidimensional scaling plots (MDS) were also obtained from GDM.

Phenogram reliability was tested by bootstrapping the allelic frequency data set. For each genetic marker (isozymes and RAPDs), 100 bootstrapped pseudoreplicates were obtained using the Seqboot program of the PHYLIP package (Felsenstein, 1993). These pseudoreplicates were used to obtain 100 UPGMA trees (based on Nei's genetic distances) and the corresponding consensus tree for each kind of marker using the programs Gendist, Neighbor and Consense of the same package.

The 100 bootstrapped GDMs were also used to estimate genetic distance error through the coefficient of variation (CV) by dividing the standard deviation of genetic distance by the corresponding bootstrap sample mean.

Table III - Band sizes (bp) and allelic frequencies of the 46 RAPDs loci for the *Prosopis glandulosa* and *P. velutina* populations. (Only presence frequencies are shown). N: Sample size.

Locus	Size (bp)	*P. gla* LC	*P. gla* FC	*P. gla* BC	*P. gla* WE	*P. vel* SR
	N	24	7	13	12	15
01-01	2094	0.074	0	0	0.083	0
01-02	915	0.167	0.375	0.077	0	0
01-03	893	0.556	1	0.692	0.292	0.714
01-04	809	0.074	0	0.038	0	0.179
01-05	694	0.056	0	0.154	0.25	0.464
01-06	626	1	1	1	1	1
01-07	528	0.019	1	0.115	0.083	0
01-08	508	0.019	0.25	0.385	0	0
01-09	489	0.037	1	0.038	0	0
01-10	455	0.426	1	1	1	0.071
01-11	440	0.556	0	0	0.25	0.286
01-12	420	1	0.25	1	1	0.536
07-01	2238	0.024	0	0.038	0	0
07-02	1580	0.048	0.2	0.192	0.167	0.7
07-03	1260	0.762	0.5	0.577	1	0.167
07-04	1000	0	0.1	0.077	0	0
07-05	950	1	1	0.5	1	0
07-06	803	0.548	0.2	1	0.583	0
07-07	708	1	0.2	1	1	0.7
07-08	630	1	1	1	1	0.367
07-09	562	0	0	0	0	0.033
07-10	500	1	0.5	1	1	0.7
07-11	447	0.262	0.3	0.692	0.667	0.033
07-12	355	0.405	1	0.692	0.667	0
07-13	316	0.136	0.3	0	0	0
08-01	1000	0	0.063	0.042	0.042	0.1
08-02	891	0.269	0.25	0.083	0.125	0
08-03	708	0.115	1	0.417	0.417	0.067
08-04	562	0.135	0.5	0.5	0.25	0.3
08-05	501	0.308	0.375	0.042	0.167	0.067
08-06	398	0.769	1	0.667	1	0.7
08-07	316	1	1	0.667	1	1
10-01	3162	0.352	0.063	0.115	0.042	0.464
10-02	2239	0.352	0.063	0.115	0.042	0.464
10-03	1540	0.704	0.5	1	1	1
10-04	1259	0.426	0.5	0.269	0.5	0.036
10-05	1122	0.019	0	0.077	1	0.036
10-06	1000	0.481	1	0.385	0	0.036
10-07	977	0.444	0.375	0	0	0.143
10-08	932	0.778	1	1	1	1
10-09	724	1	1	1	1	0.179
10-10	660	1	1	1	1	0.321
10-11	549	0.704	1	1	0.667	0.536
10-12	331	0.185	0.125	0.385	0.417	0.036
10-13	301	1	1	0.692	0.667	0.607
10-14	275	0.019	0	0	0	0

For abbreviations see legend to Table I.

Gene flow was estimated following the expression:

$$Nm = \frac{1 - F_{ST}}{4F_{ST}}$$ (Wright, 1951).

F_{DT} indices were used to study isolation by distance using the subprogram ISOLDE of the program GENEPOP (Raymond and Rousset, 1995). This program compares geographic distance matrix structure with that of F_{DT} or D indices by Mantel test. Distances between sample sites were estimated using the Microsoft Encarta World Atlas measuring tool (Spanish version) (1995-1996) (Table IB).

The consistency between the relationships among populations obtained from isoenzyme and RAPD data was analyzed by comparing the corresponding matrices of genetic identities and F_{DT} by Mantel's test.

Spearman's rank correlations between allelic frequencies and each of 16 climatic and geographic variables were estimated using the program STATISTICA (StatSoft, Inc., 1995). Geographical and climatic variables used correspond to all data recorded by the closest station (Table IA) to each population, available at NOAA-CIRES Climate Diagnostics Center (http://www.cdc.noaa.gov/cas/Climo): latitude, longitude, elevation, maximum and minimum temperatures, mean annual rain and snowfall, annual average chance of precipitation, mean wind speed, annual average percentage of available sun, maximum and minimum July temperatures, maximum and minimum January temperatures, July and January rainfall.

RESULTS

Isozyme distribution

Analysis of nine isoenzymatic systems revealed 27 loci. Previous results (Bessega, 1997) indicated that nine of these loci (*Amp-1*, *Adh-2*, *Idh-2*, *6Pgd-1*, *Sod-1-Sod-5*) are invariable for the five analyzed populations. By contrast, *Got-3*, *Got-4*, *Prx-4*, and *Prx-5* are the only diagnostic loci which allow differentiating the Santa Rita population of *P. velutina* from the species of Algarobia so far studied (see Bessega, 1997). For the remaining loci, populations and species differ only in allelic frequencies (Table II).

RAPD product distribution

The four arbitrary primers chosen for the present analysis revealed 46 bands that were consistently and unambiguously scorable in the five populations studied. These bands were treated as genetic loci. Of the 46 RAPD loci, 45 were polymorphic. No diagnostic bands for population or species recognition were detected (Table III).

After applying Ohta's (1982) method to estimate linkage between RAPD markers, only 7 out of 990 pairs of bands (0.71%) suggested epistasis disequilibrium.

Genetic diversity

Both isozymes and RAPDs yielded similar genetic variability estimates (Table IV). The H and P values obtained in the present study are also similar to those obtained from previous isozyme studies (Saidman, 1985, 1986, 1990; Saidman *et al.*, 1997, 1998b, Saidman and Vilardi,1987; Verga, 1995; Bessega, 1997) in other species of Section Algarobia. Indeed, the differences among estimates are not statistically significant since confidence intervals overlap.

Genetic structure and differentiation

Genetic differentiations estimated through non-hierarchical Wright's (1978) F_{DT} from isozyme and RAPD data (0.388 and 0.290, respectively) were similar when all populations were included in the analysis. The correlation between F_{DT} matrices (r = 0.566) was significant according to Mantel's test (P = 0.02). Consequently, in both cases gene flow estimate (Nm = 0.39 and 0.60 for isoenzyme and RAPD loci, respectively) was less than one individual per generation.

However, results of isoenzyme and RAPD analysis are very different when *P. velutina* is excluded (r = 0.08; P = 0.69). In this case, F_{DT} and Nm estimates from isozyme data (0.071 and 3.27, respectively) agree with the expected for conspecific populations (Nm > 1). For RAPD data, exclusion of *P. velutina* does not yield the expected reduction in F_{DT} (0.283) and increase in Nm (0.63).

The CV of Nei's (1978) genetic distance (GD) estimates was higher for isozyme (0.33 ± 0.01) than for RAPD (0.27 ± 0.01) data, suggesting higher accuracy of RAPD markers. However, this result should be interpreted with caution considering the GD range observed for each data set. GD estimates for isozyme loci vary from 0.01 to 0.49 when all populations are considered, but the range dropped dramatically (0.01-0.03) if *P. velutina* was excluded. Conversely, GD range for RAPDs was narrower for all populations (0.06-0.35) but not so strikingly reduced when only *P. glandulosa* populations were considered (0.06-0.23) (Table V). As a consequence, cluster analyses from isoenzyme and RAPD loci did not completely agree. The GD matrices' correlation is borderline significant whether or not the *P. velutina* data are considered (P = 0.06 and P = 0.08, respectively). In the phenograms and MDS plots obtained from both data sets (Figure 2), *P. glandulosa* populations clustered together, but in the plots based on isozyme data they appeared to be more homogeneous and further separated from *P. velutina*. This fact was demonstrated through the analysis based on bootstrapping allele frequencies of both marker types. The trees obtained from isozyme data showed high consistency: the populations of *P. glandulosa* clustered together and separated from *P. velutina* in 100% of the cases. On the contrary, only 53% of the trees obtained from RAPD data separated *P. glandulosa* populations from that of *P. velutina*. The main cause for this inconsistency is due to the fact that Frio Co. and Santa Rita are almost equally differentiated from the remaining *P. glandulosa* populations for RAPD data, as can be clearly seen in the MDS plot for RAPD data.

Isolation by distance

Relation between F_{DT} and geographical distances was tested through Mantel's (1967) test. The correlation between F_{DT} and geographical distance matrices including all populations was significant (P = 0.02) when genetic data

Table IV - Measures of genetic variability estimated from isozyme and RAPD loci. P: Percentage of polymorphic loci; H: mean heterozygosity; n: mean number of alleles per locus; SE: standard errors.

	Isozymes			RAPD		
	n (SE)	P (SE)	H (SE)	n (SE)	P (SE)	H (SE)
P. glandulosa La Copita	1.8 (0.2)	48.1 (9.6)	0.203 (0.048)	1.7 (0.1)	55.6 (7.4)	0.215 (0.030)
P. glandulosa Frio Co.	1.6 (0.1)	48.1 (9.6)	0.177 (0.043)	1.4 (0.1)	44.4 (7.4)	0.186 (0.034)
P. glandulosa Bell Co.	1.7 (0.2)	40.7 (9.4)	0.161 (0.043)	1.6 (0.1)	48.9 (7.4)	0.192 (0.031)
P. glandulosa Weslaco	1.7 (0.2)	44.4 (9.5)	0.187 (0.047)	1.4 (0.1)	37.8 (7.2)	0.151 (0.030)
P. velutina Santa Rita	1.4 (0.1)	33.3 (9.1)	0.127 (0.036)	1.6 (0.1)	48.9 (7.4)	0.192 (0.030)

Table V - Matrices of Nei's (1978) genetic distances estimated from isozyme (below diagonal) and RAPD data (above diagonal). Coefficients of variation of each estimate are indicated in parentheses.

	P. gla LC	*P. gla* FC	*P. gla* BC	*P. gla* WE	*P. vel* SR
P. gla LC		0.18(0.29)	0.07(0.21)	0.07(0.36)	0.16(0.27)
P. gla FC	0.03(0.39)		0.17(0.25)	0.23(0.24)	0.35(0.24)
P. gla BC	0.03(0.32)	0.01(0.36)		0.06(0.35)	0.19(0.26)
P. gla WE	0.02(0.38)	0.03(0.32)	0.03(0.31)		0.23(0.26)
P. vel SR	0.44(0.30)	0.49(0.28)	0.47(0.30)	0.44(0.30)	

For abbreviations see legend to Table I.

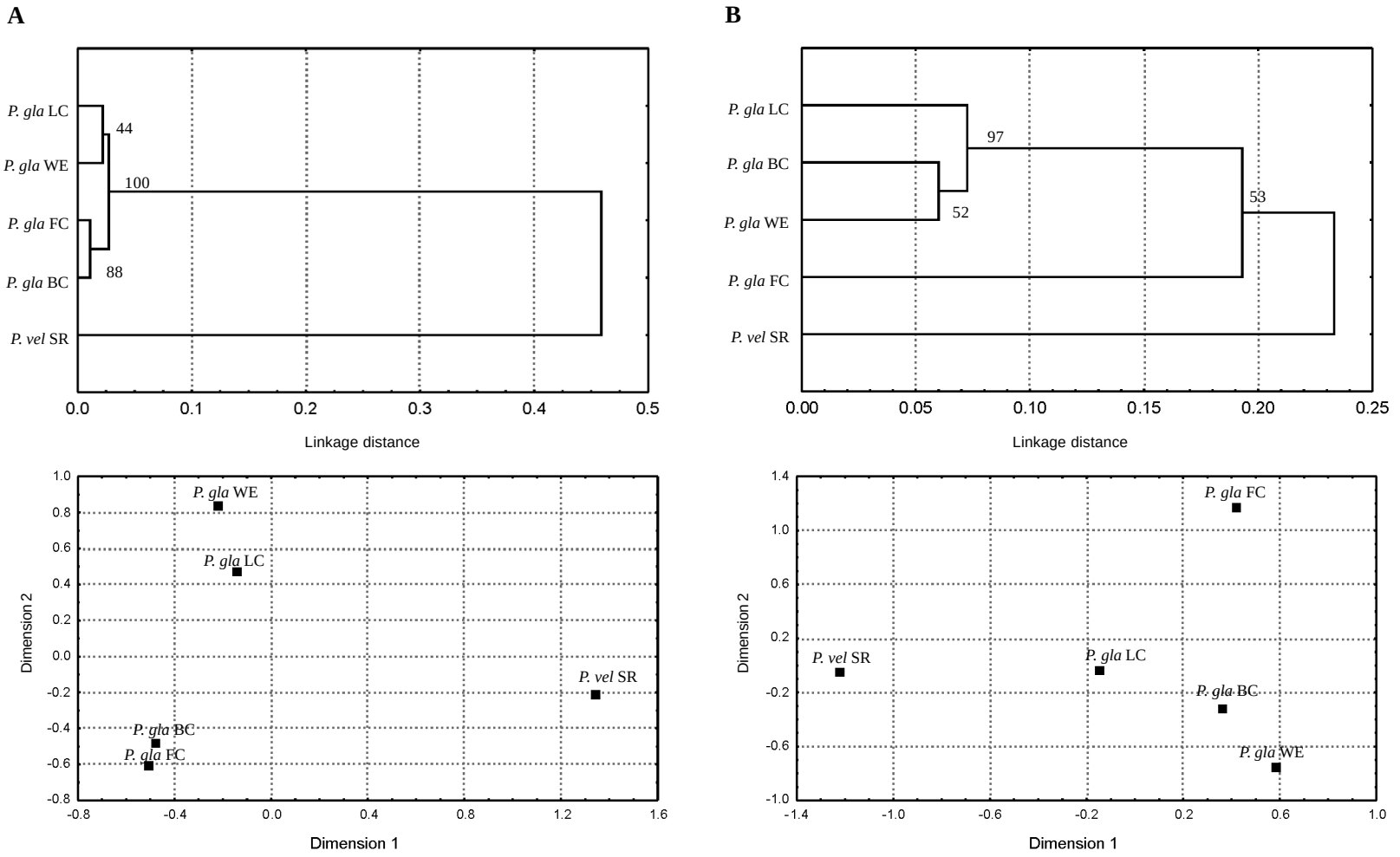

Figure 2 - Phenograms (top) and multidimensional scaling plots (bottom) obtained from Nei's (1978) genetic distances among populations for isozymal data (A) and RAPD loci (B). The numbers at the forks indicate percentage of times the group consisting of species to the left of that fork occurred among bootstrapped trees. For abbreviations see legend to Table I-B.

were based on isozymes but nonsignificant (P = 0.33) when based on RAPDs. The same tendency (higher correlation for isozyme-based F_{DT}s) was observed if the analysis was restricted to *P. glandulosa* populations although in this case neither correlation was significant (P = 0.07 and P = 0.68, respectively, for isoenzyme and RAPD-based F_{DT}s).

Correlation between climatic data and molecular markers

Possible dependence of genetic variables (45 RAPD bands and 47 allozymes) on sixteen geographical and climatic variables was analyzed through the estimate of Spearman's rank correlations. From 1472 correlations estimated, only 68 (4.6%) yielded significant values ($P < 0.05$), none significant matrix-wide, suggesting that associations among genetic and physical variables have little if any influence on relationships estimated among populations.

DISCUSSION

The taxonomy of Algarobia Section of genus *Prosopis* is complicated because of profuse interspecific hybridization in wide areas of sympatry (Hunziker *et al.* 1975, 1977; Palacios and Bravo, 1981). Previous isoenzymatic studies of South American species (Saidman, 1985, 1986, 1990, Saidman and Vilardi, 1987; Saidman *et al.*, 1997; Verga, 1995) showed relatively high levels of heterozygosity within but low differentiation among populations. Diagnostic loci for species identification are virtually absent and most alleles are shared by South American species so far studied. Virtual lack of clear reproductive barriers as well as high genetic similarity among morphologically good taxonomic species were considered evidence for assuming that several species of this section may constitute a syngameon (Palacios and Bravo, 1981; Saidman, 1985, 1988; Saidman and Vilardi, 1987, 1993; Saidman *et al.*, 1998a,b).

Although hybridization might be considered a plausible explanation for high genetic similarities among species, available information is contradictory. Saidman *et al.* (1998a) observed that genetic similarity among populations of different species is independent of sympatry and hybridization opportunity. Moreover, Bessega (1997) observed high levels of genetic similarity among South American species and *P. glandulosa* despite geographical isolation. By contrast, the only *P. velutina* population so far studied exhibited several diagnostic allozymes.

The first results obtained by RAPD technique in "pure" populations (i.e., without evidence of hybridization) and hybrid swarms of South American species of Algarobia (Saidman *et al.*, 1998b) were consistent with those of isozymes. Genetic variability within populations was high and genetic distances among species were very low. However, a *P. alba* x *P. nigra* hybrid population showed private RAPD bands at a low frequency not observed in either parent (Saidman *et al.*, 1998b). These results seem to indicate that RAPDs might constitute a useful tool for analyzing genetic relationships and consequences of hybridization among *Prosopis* species.

In the present work isozyme and RAPD analyses were applied to the North American Algarobia species *P. glandulosa* and *P. velutina*, comparing the information produced by each methodology. The number of RAPD bands varied from 2 to 10 amplification products per primer. This range agrees with predictions for 10-mer primers in plants (Waugh and Powell, 1992). Sampling variance of genetic distances based on allozyme and RAPD data was measured through coefficient of variation. With the number of loci studied here, CV estimates did not differ statistically between markers and were consistent with estimates expected according to other studies (Thormann *et al.*, 1994; Hallden *et al.*, 1994; Spooner *et al.*, 1996).

The present estimates of genetic variability with both techniques resemble each other and values observed for South American species of Algarobia (Saidman *et al.*, 1998a,b). This result indicates that, despite the relatively low number of mother plants, most alleles present in populations were represented in the seed samples analyzed, as a consequence of the predominantly outcrossing system of these species. The high heterozygosity estimates are also consistent with a tendency to high outcrossing rates.

Some discrepancies in results attained with both techniques were observed in the estimate of differences among populations. The isozyme-based F_{DT} and genetic distances seem to reflect taxonomic status better than RAPD-based indices do. In the phenogram and multidimensional scaling plots based on isozymes, all *P. glandulosa* populations formed a tighter group. Pictures obtained from RAPDs showed that Frio Co. was differentiated from the remaining *P. glandulosa* populations. Moreover, while isozymal differentiation level among populations may be partially explained by geographical distances, for RAPD markers isolation analysis by distance yielded nonsignificant results.

Differences between results from the different techniques cannot be attributed to sampling error differences because CVs, as stated above, were very similar. One explanation might be that RAPD band homology between species is overestimated due to co-migration of nonhomologous DNA fragments, a possibility testable by hybridizing Southern blots of RAPD products with labelled fragments extracted from replicate gels.

Another possible explanation relates to the nature of the markers. Although we have no evidence of selective effects of any polymorphic loci studied here (correlation with climatic and physical variables were nonsignificant), isozyme markers are more likely to respond to adaptive constraints than RAPD loci. If this were the case, conspecific populations might show greater isozyme similarity because of occupying the same ecological niche. Testing this possibility would require study of more environmental variables and biotic factors.

According to the present results, RAPD markers tend

to show higher values of genetic differentiation among conspecific populations than those obtained from isozymes, and might be more useful in screening genetic variability within species. Isozymes have shown greater ability to differentiate the North American species of Algarobia, mainly by GOT and PRX patterns. However, these conclusions cannot be extended to other species, where only allelic frequency differences have been detected (Saidman, 1985, 1990; Saidman and Vilardi, 1987, 1993; Saidman *et al.*, 1997, 1998a,b) for isozyme loci.

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RESUMO

Técnicas de análise por alozimas e DNA polimórfico aleatoriamente amplificado (RAPD) foram comparadas quanto a sua utilidade para estudos genéticos e taxonômicos em populações de *Prosopis glandulosa* e *P. velutina*. Isozimas e RAPD deram estimativas igualmente elevadas de variabilidade genética. A estrutura genética e a diferenciação foram analisadas pelo índice F_{DT} não hierárquico de Wright (1978). Para todas as populações consideradas, ambos os marcadores produziram estimativas baixas do fluxo de genes (Nm < 1). Quando apenas as populações de *P. glandulosa* foram analisadas, os dados de isozima deram estimativas do fluxo gênico mais elevadas (Nm > 1), em concordância com o esperado para populações co-específicas. Contudo, com os dados de RAPD a redução esperada em F_{DT} e o aumento em Nm não foram observados. A correlação entre F_{DT} e as matrizes de distância geográfica (teste de Mantel) para todas as populações foi significante (P = 0,02) quando baseada em isozimas, mas não tanto (P = 0,33) quando baseada em RAPD. Não foram observadas associações significantes entre variáveis genéticas e geográficas ou climáticas. Dois sistemas isoenzimáticos (GOT e PRX) possibilitaram a distinção entre *P. glandulosa* e *P. velutina*, mas não foram detectadas por RAPD bandas diagnósticas para o reconhecimento de populações ou espécies aqui estudadas. Contudo, os marcadores de RAPD mostraram valores mais elevados para a diferenciação genética entre populações co-específicas de *P. glandulosa* e um coeficiente de variação menor do que aqueles obtidos a partir de isozimas.

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