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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 5 (9): 983-987, 2002

Molecular Characterization of *Plantago albicans* L. Populations in Response to Edaphic Variations

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Abstract: Molecular characterization of *Plantago albicans* L. accessions in sites of different edaphic characters in the north western Mediterranean region of Egypt was carried out to assess the range of genetic diversity of this species. The results indicated the necessity of using all types of data together to clarify the interpopulation relationships. Populations I (Burg El-Arab) and II (Omayed) were suggested to be two different varieties of *P. albicans* (variety "Typica pilger" of subspecies *albicans* and variety "nana" respectively). Population II was considered endangered and needs urgent conservation. Population VIII (Ras El-Hekma) was considered an ecotype.

Key words: *Plantago albicans* L., biodiversity, Nei's genetic distance, taxonomic distance

Introduction

Plantago albicans L. is a widely distributed species in the western coastal region of Egypt. It is of high grazing (El-Kadi, 1987) and medicinal value (Brigges *et al.*, 1977). Heterogeneity between its populations with chromosomal differences was studied by Amin and El-Bakatoshi (1998). This type of heterogeneity reflects the genetic content which influences the process by which particular kind of enzymes can be synthesized. However, the interaction between environment and genetic content acts on phenotypes. These interactions are integrated into the physiological and biochemical functions of the organism within an ecological framework. It is now firmly established that molecular evidences as obtained by isozyme electrophoresis are reliable in assessing the genetic divergence (Tankesly, 1983; Thorpe, 1983; Bon, 1996; Yang *et al.*, 1996). In addition, these data can be used in measuring genetic variation, genetic distance and gene flow within and among populations and species (Nei, 1978; Hedrick, 1984; Slatkin, 1985).

The present study was carried out to detect and measure the genetic variations among and within populations of *Plantago albicans* in sites of different edaphic characters of the north western desert of Egypt, through the study of molecular polymorphism of isozymes.

Materials and Methods

Sampling of field specimens: Eight populations of *Plantago albicans* L. were selected in 1995, from eight habitats (sites) along the Mediterranean coastal strip (37 to 230 km west of Alexandria; Burg El-Arab, Omayed, Dabaa Fuka and Ras El-Hekma; Fig. 1). Description of the selected sites as well as the associated common species were recorded (Table 1). At least seven widely spaced (to avoid repeated samples from the same clone) mature individuals representing each population were randomly selected from each site. Three replicates of soil samples covering the major topographic variations were collected from freshly exposed surface to a depth of 25 cm under the selected individuals of each site.

Tissue extracts and enzyme electrophoresis: Scratched and sterilized seeds from each individual were germinated at 20 ± 1°C. Homogenous seedlings (eight or ten days old) were macerated each in 1% saline solution (0.8% NaCl + 0.2 $NaNO_3$) (Brewbaker *et al.*, 1968) then centrifuged at 12,000 rpm for 20 min. Thirty microliter of the supernatant was used to analyze three isozyme systems: acid phosphatase (AP), esterase (Est) and catalase (Cat). Electrophoresis was carried out following the method of Laemmli (1970) using 7.5% acrylamide vertical slaps. Gels were stained for the desired enzymes according to Soltis *et al.* (1983). Since no stained bands of catalase were found in all specimens, its activity was tested using the procedure of Beers and Sizer (1952) and it

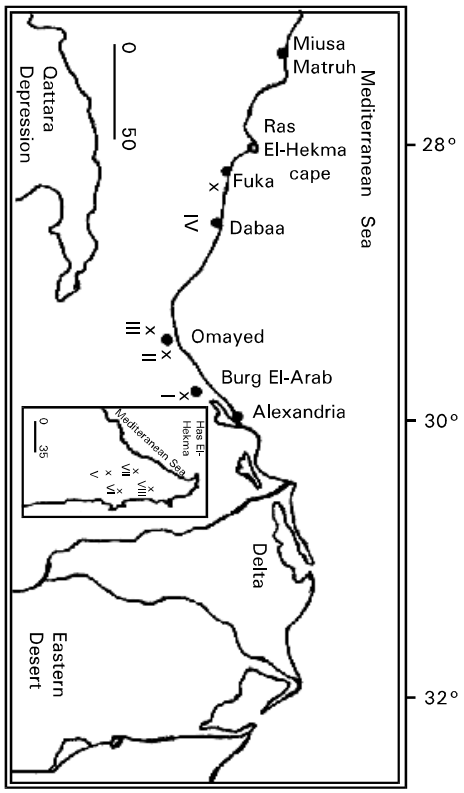

Fig. 1: Map of the Mediterranean coastal region of Egypt indicating locations of different populations from I to VIII.

was found to be as low as 4.472 unit/mg in all samples. The designation of loci (in numerals) and alleles (in alphabets) was done for all studied specimens sequentially from anodal to cathodal direction.

Soil analysis: Air dried soil samples finer than 2 mm were analyzed for physical and chemical characters according to Allen (1989) and Jackson (1958). Soil texture was determined by Bouyoucos hydrometer method whereby the percentages of sand, silt and clay were calculated. Soil water extracts (1:5 v/v) were prepared to estimate pH and electric conductivity (EC) using a glass electrode pH-meter (Crison pH/mv-506) with a calomel reference

electrode (Ingold 4455), and conductivity meter (WIWLF 56, Germany), respectively. Available soil elements were extracted from fixed weights of air-dried soil by using ammonium acetate pH 9. The concentration of sodium, potassium and calcium cations was estimated by clinical flame photometer (CORNING).

Data analysis: Allele frequency of different loci, mean number of alleles per locus (A), proportion of polymorphic loci (P), observed and theoretical average heterozygosity (H_{obs} and H_{th}, respectively), were calculated according to Nei (1978) and Hedrick (1984). Nei's genetic distances (D) between the studied populations were computed. Standardized data were used to calculate taxonomic distance coefficient between populations. The average taxonomic distance was computed using NTSYS-pc version 1.50 computer program of Rohlf (1990). Cluster analysis based on the unweighed pair-group method arithmetic average (UPGMA) using genetic and taxonomic distance matrices was applied for constructing phenograms (Sneath and Sokal, 1973). Cytological data of the studied populations were extracted from Amin and El-Bakatoshi (1998) and used with the isozyme and edaphic data of the present study in the UPGMA cluster analysis. The relationship between isozyme, cytology and soil characters were assessed applying the correlation coefficient analysis.

Results

Isozyme analysis of the studied populations are presented in Table 2. Only one seed of population III succeeded to germinate in spite of having many seeds per spike. So, it would be difficult to carry out the isozyme analysis for III. For other populations, the analysis of acid phosphatase (AP) revealed four loci. AP1 locus was detected only in populations VI and VIII, and it was monomorphic. Both AP2 and AP3 were diallelic loci in all studied populations except populations II and IV for AP2 and population VII for AP3. However, locus AP4 was monomorphic in all populations except population VI. Five esterase (Est) loci were also detected. Est1 locus was dimorphic in populations IV and VI, while it was monomorphic in the other populations. Est2 locus was monomorphic in all populations, and absent in population VII. Est3 locus was dimorphic in all populations except population II and VII. Est4 and Est5 loci were characterized by expressing three alleles. However, two alleles (a and c) of Est4 and Est5 were expressed in populations II and VIII respectively, while allele "c" only of Est5 was expressed in populations VII and VIII.

Populations II and VII exhibited the lowest allele frequency per locus, while population VI exhibited the highest one (Table 3). A similar trend was observed when the proportion of the polymorphic loci was considered. The observed average heterozygosity (H_{obs}) per locus fluctuated from 0.2 in population VII to 0.8 in population VI. The highest value for theoretical average heterozygosity was observed in population VII, while the lowest value was observed in population I.

The UPGMA clustering based on Nei's genetic distance (D) divided the studied populations into two main groups at D = 0.60 (Fig. 2a). Group 1 included population VI mainly due to the highest values of mean number of alleles per locus, proportion of polymorphic loci and observed average heterozygosity. Group 2 was further subdivided into two subgroups at D = 0.42. Subgroup "A" included population VII only, mainly due to the lowest value in H_{obs}, while subgroup "B" was subdivided into two clusters at D = 0.36. Cluster "B1" contained population VIII only, while cluster "B2" included populations I, II, IV and V. Populations II and IV were separated at D = 0.20 while populations "I and V" were separated at D = 0.15.

The description of vegetation and climate features of different selected sites are presented in Table 1. Wide range of variation in soil elements and texture and moderate ranges of salinity (expressed as electric conductivity) and pH are presented in Table 4. The lowest value of EC was recorded for the nonsaline depression habitat "site II", while the highest value (~ double) was recorded for inland habitat "site I".

Table 1: Description of the selected localities as well as the dominant species.

No.	District	Population site from Alexandria	Site description	*Climate: Annual temperature Min.	Annual temperature Max.	Annual rainfall (mm)	Annual RH (mm)	Dominant species
I	Burg El-Arab	37 Km		15.20	25.00	120.00	67.40	*Thymelaea hirsuta Plantago albicans Asphodelus microcarpus.*
II	Omayed	69 Km	Non saline depression road side gentle slope at north direction.	15.20	25.00	120.00	67.40	*Labulania arabica, Launaea residifolia, Plantago albicans Cutandia dichotoma, Carduncellus eriocephalus.*
III		88 Km	Transitional area between sand dunes and saline depression.	15.20	25.00	120.00	67.40	*Crucianella maritima, Plantago albicans, Ammophila arenaria, Echinops spinosissimus, Silene succulenta, Salvia lanigera, Hyoseris lucida.*
IV	Dabaa-Fuka	180 Km	Dabaa- Fuka alluvial plain (in Fig. orcards).	14.50	24.00	148.00	66.00	*Thymelaea hirsuta, Echinops spinosissimus, Plantago albicans*
V	Ras El-Hekma	230 Km	Ras El-Hekma cap, sandy slope under Fig tree	14.50	24.00	148.00	66.00	*Thymelaea hirsuta, Pituranthos- tortuosus, Plantago albicans*
VI			Ras El-Hekma cap, fixed coastal dunes on calcarous bed rock.	14.50	24.00	148.00	66.00	*Thymelaea hirsuta, Silene succulenta, Echinops spinosissimus, Echium sericeum, Echiochilon fruticosum, Plantago albicans.*
VII			Ras El-Hekma cap between rocks of the coastal elevation.	14.50	24.00	148.00	66.00	*Onoins vaginalis Crucianella maritima, Plantago albicans.*
VIII			Ras El-Hekma cap, sand deposite on the lower slope of rocky coastal elevation.	14.50	24.00	148.00	66.00	*Onoins vaginalis, Crucianella maritima, Plantago albicans.*

*Climatic data from Bidak (1993) and Ahmed and Hussein (1994).

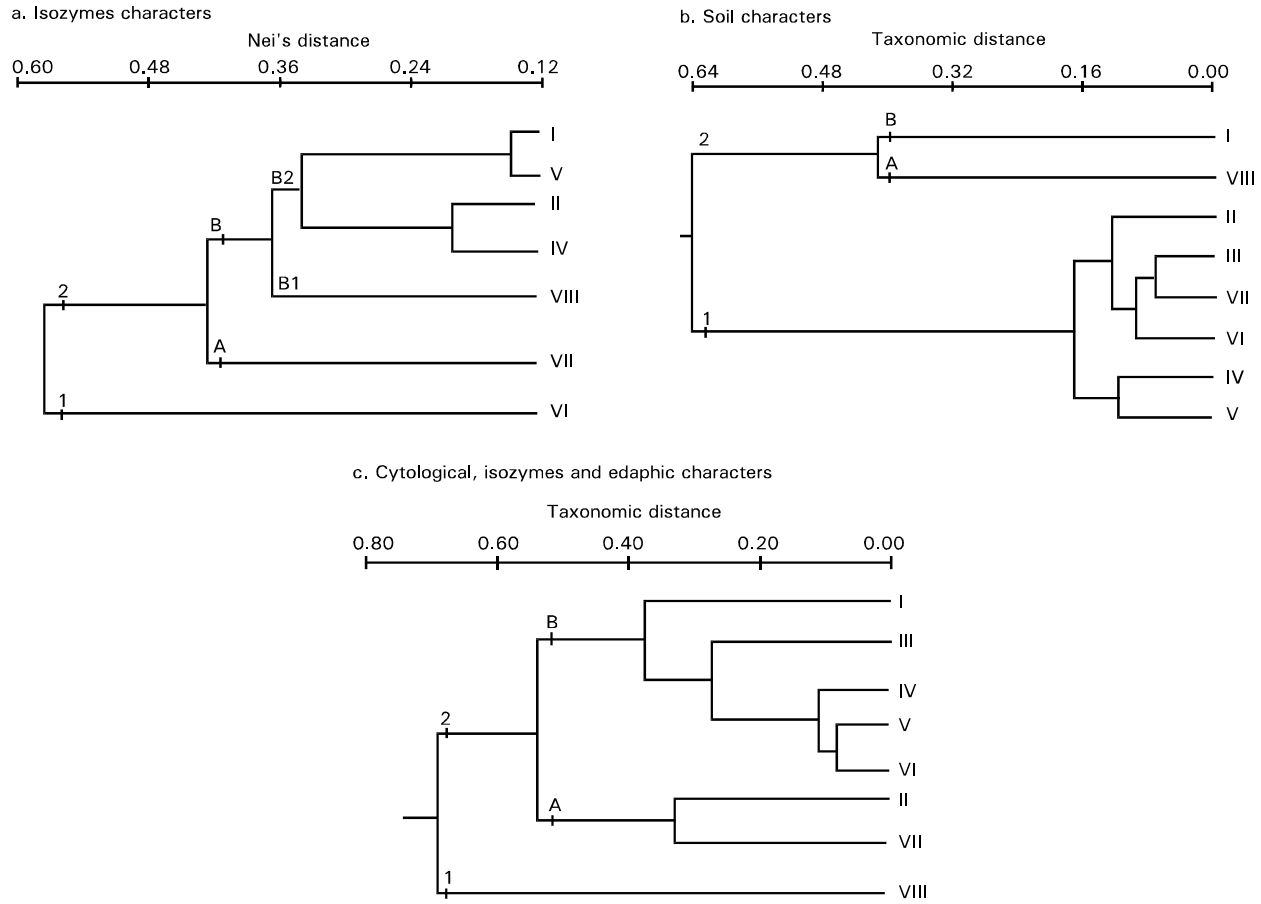

Fig. 2: Phenograms of the studied characters resulted from UPGMA analysis (1 & 2 refer to groups; A & B to subgroups; B1 & B2 to clusters).

Table 2: Allele frequency of the polymorphic loci in population of *P. albicans*

Population number								
Locus	Genotype	I	II	IV	V	VI	VII	VIII
AP1	a	0.000	0.000	0.000	0.000	1.000	0.000	1.000
	b	0.000	0.000	0.000	0.000	0.000	0.000	0.000
AP2	a	0.800	0.000	0.000	0.800	0.250	5.000	5.000
	b	0.200	1.000	1.000	0.200	0.750	0.500	0.500
AP3	a	4.000	0.750	0.750	0.700	0.500	1.000	0.500
	b	0.600	0.250	0.250	0.300	0.500	0.000	0.500
AP4	a	0.000	0.000	0.000	0.000	0.830	0.000	0.000
	b	1.000	1.000	1.000	1.000	0.170	1.000	1.000
Est1	a	1.000	1.000	0.917	1.000	0.944	1.000	1.000
	b	0.000	0.000	0.083	0.000	0.056	0.000	0.000
Est2	a	1.000	1.000	1.000	1.000	1.000	0.000	1.000
	b	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Est3	a	0.188	0.000	0.667	0.800	0.277	0.500	0.375
	b	0.812	1.000	0.333	0.200	0.723	0.500	0.625
Est4	a	0.438	0.375	0.250	0.300	0.389	0.250	0.500
	b	0.375	0.000	0.333	0.300	0.333	0.500	0.250
	c	0.187	0.625	0.417	0.400	0.278	0.250	0.250
Est5	a	0.187	5.000	0.420	0.300	0.222	0.000	0.000
	b	0.563	0.000	0.333	0.400	0.444	0.000	0.000
	c	0.250	0.500	0.250	0.300	0.333	1.000	1.000

Table 3: Genetic variability measures for populations of *P. albicans*

Population number	I	II	IV	V	VI	VII	VIII
Sample Size (N)	13.00	6.00	10.00	10.00	15.00	4.00	8.00
Mean alleles/locus(A)	1.667	1.222	1.667	1.667	2.00	1.220	1.667
Proportion of polymorphic loci (P)	0.625	0.375	0.625	0.625	0.778	0.429	0.556
Average heterozygosity (observed) H_{obs}	0.667	0.444	0.556	0.556	0.778	0.222	0.333
(theoretical) H_{th}	0.414	0.448	0.454	0.501	0.425	0.541	0.523

Table 4: Soil characters of different habitats of the studied populations of *P. albicans*

Population number	EC (µ mhos/cm) x 10	pH	Soil texture (%)			Elements (ppm)		
			Clay	Silt	Sand	Na^+	Ca^{++}	K^+
I	31.00	7.71	6.70	7.30	86.40	10.70	75.00	4.00
II	16.62	7.33	2.50	8.00	89.50	4.40	28.75	4.50
III	20.87	7.36	1.50	2.50	96.00	3.40	26.87	4.50
IV	30.00	8.01	2.00	10.00	88.00	2.20	27.50	5.40
V	25.00	7.31	0.00	12.00	88.00	2.20	32.50	7.20
VI	23.00	6.57	6.00	4.00	90.00	1.20	32.50	7.20
VII	24.00	7.97	2.00	2.00	96.00	4.20	32.50	5.40
VIII	23.00	7.05	2.00	2.00	96.00	39.20	125.00	32.60

The phenogram of the soil characters (Fig. 2b) separated populations I and VIII from all other populations at 0.64 dissimilarity distance and they were separated at 0.45 dissimilarity distance. The differences between the latter populations were very low.

The phenogram based on isozyme, soil and cytological characters (Fig. 2c) separated population VIII as an out-group. Populations II and VII formed a separate group at a dissimilarity distance of 0.54 in spite of the differences in the cytological data. All other populations were grouped together with relatively narrow differences in dissimilarity distance.

Discussion

The cluster analysis based on isozyme and soil analysis in the present study, separated between seven populations of *Plantago albicans* collected from habitats of different edaphic characters in the western coastal desert of Egypt.

Population I was very closely related to population V in the phenogram based on isozyme data, to population VIII in the phenogram based on soil characters, and to populations IV, VI and VII in the phenogram based on cytological data (Amin and El-Bakatoshi, 1998). However, the UPGMA analysis of isozyme, cytology and soil data collectively revealed the separation of population I as a subgroup of its own. This population expressed a high value of polymorphic loci (5 polymorphic out of 8) and relatively high values of heterozygosity per locus. The relatively high values of the average observed heterozygosity (molecular heterozygosity) coincided with the intrapopulation morphological variation (Ahmed and El-Bakatoshi, unpublished) and the occurrence of well-established relatively dense population. This molecular heterozygosity could be attributed to the high ability for cross-pollination occurring between different accessions of this population and the survival of the new progeny. This large population with high genetic diversity inhabiting the most saline habitat would resist anthropogenic disturbance and extinction and would lead to subsequent evolutionary change (Bawa *et al.*, 1991). This population could be considered as a variety Typica pilger of subspecies *albicans* as was suggested by Alapetite (1981).

Population II was grouped with population IV in the phenogram based on isozyme data due to the theoretical average heterozygosity. But, when isozyme, soil and cytological characters were analyzed together, the phenogram resulted in grouping populations II and VII together. This is evidently due to the similarity of the molecular data (isozymes) and the narrow differences in soil characters in spite of the wide cytological variation between these two populations (Amin and El-Bakatoshi, 1998). However, phenograms of cytological data only (Amin and El-Bakatoshi, 1998) and that of morphological, cytological, isozyme and soil data together (Ahmed and El-Bakatoshi, under publication) delimited population II as a group of its own. This result is evidently more realistic, since both morphological and cytological variations are very wide. This population is the only one, which survives in a habitat with the lowest EC value and the highest silt percent, has a diploid chromosome number of 2n= 10 (Amin and El-Bakatoshi, 1998). Moreover, it is characterized by having the lowest values of both mean alleles per locus and polymorphic loci (3 out of 8 loci). This low polymorphism and being represented by fewer individuals than all other studied populations may indicate that it is endangered and that its conservation is urgent. The present results are in good agreement with the cytological results of Amin and El-Bakatoshi (1998) of a population found in Tunisia by Puech (1987 and 1992), which he named "nana" (Boiss) form.

Populations V, VI and VII were grouped differently, in different phenograms. In the isozyme phenogram each one formed a separate lineage in a different cluster. The soil phenogram or cytology phenogram only (Amin and El-Bakatoshi, 1998) or that based on isozymes, soil and cytological characters together placed populations VI and VII in one group with a small difference in dissimilarity distance, and population V in an other group with a high dissimilarity distance. However, they were intermingled in the phenograms based on all data including those of morphology (Ahmed and El-Bakatoshi, under publication). This indicated that, in spite of the differences in isozyme data and low variations in cytological data, the similarity in soil and morphological data placed these three populations together in one group. This might be attributed to either the genetic stimulation of certain loci for facing sudden environmental stress during the stages of seed formation (Human and Stegemann, 1989), or that these populations probably had arisen from the cross pollination system of this species and the high dispersal of seeds (by means of grazing animals) between these three nearby populations, which insured high gene flow between them (Lesins and Lesins, 1979).

Population VI, exhibiting the highest value of polymorphic loci and the highest mean number of alleles per locus, was separated as a group of its own at a high genetic distance in the phenogram based on isozyme data. This may be attributed to the highest value of chromosomal translocation (Amin and El-Bakatoshi, 1998). Moreover, the wide variation in the mean number of alleles between populations V, VI and VII might be due to the Null mutation arising by transposition events through translocation (Rieger *et al.*, 1991). In the current study, this suggestion is supported by the finding of positive correlation between mean number of alleles per locus and percent of translocation.

Population VIII formed a separate cluster in all phenograms and an

out group in two phenograms, based on isozyme, soil and cytological characters and on all data including morphological characters (Ahmed and El-Bakatoshi, under publication). Accordingly, it is evident that soil characters were the major factors in the formation of this out group. Soil samples of this population contained the highest percentages of Na^+, Ca^{++} and K^+, which were significantly correlated (-ve or + ve) with some morphological and cytological characters (Amin and El-Bakatoshi, 1998). The difference in dissimilarity distance between population VIII and all other studied populations based on all data was 0.38 (Ahmed and El-Bakatoshi, under publication). This indicates that most of the characters which distinguish this population, seem to be adaptive to its environment that influenced its distribution within its habitats (Hamrick, 1989). Accordingly, this population may represent an ecotype besides being distinguished by Amin and El-Bakatoshi (1998) as a cytotype of *P. albicans*.

In conclusion, this study emphasizes the importance of multidisplanary studies on biodiversity of *P. albicans* populations and indeed of those of other species. Some populations have distinct phenotypic characters which are genetically controlled. Population I and II are suggested as different varieties (and different cytotypes), and population II is considered to be endangered and needs urgent conservation. Population VIII is considered an ecotype and a cytotype of *P. albicans*.

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