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EVALUATION OF GENETIC DIVERSITY BETWEEN SOME *NITRARIA RETUSA* POPULATIONS USING MOLECULAR MARKERS

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Abstract

Nitraria retusa (Nitrariaceae) is a true xerophyte plant grows in most deserts of Egypt, especially in wadies habitat and dry salt marshes. This study aims to evaluate any possible genetic diversity among samples of *Nitraria retusa* grown under different habitats using Random Amplified Polymorphic DNA (RAPD) markers, protein electrophoresis and isozyme electrophoresis. For such goal, the ecological aspects of the selected habitats were investigated with the view of finding correlations among these markers and some ecological parameters. Five collected samples of *Nitraria retusa* were collected from Wadi Hof (sunny and shady), Oyun Musa, Kattamyia and Hammam Faraon. The obtained results showed that differences in the levels of genetic variations detected by the tested markers agree with those observed at the morphological levels. The data obtained from the tested primers resulted in PCR amplified products that differentiate the shady sample of Wadi Hof (with broad leaves) from the other investigated samples. Thus, the results obtained from the present study consider DNA and protein fingerprints for the studied populations.

Keywords: RAPD markers, Protein electrophoresis, Isozymes, Ecological parameters and *Nitraria retusa*.

Introduction

Family Nitrariaceae includes only one species which is *Nitraria retusa*. This plant grows in most deserts of Egypt, especially in wadies habitat and dry salt marshes **(Zahran &Willis, 1992).**

The technology of molecular biology has been developed over the past 20 years and provided new methods for observing the genetic differences among species. These techniques offer many advantages over the conventional methods **(Rajapakse and Ballard, 1997).**

RAPDs and DNA profiles are used to study genetic relatedness / diversity among cultivars, construct phylogenies and calculate genetic distances. RAPDs are useful in the verification of existing taxonomy, based mostly on morphology, and in elucidating ambiguous areas in plant systematics. DNA profiles are also used to assess the origin of hybrids, to construct genetic linkage maps and to check for mutations **(Williams *et al.*, 1990)**.

Staub *et al.*, 1997 on studying *Cucumis* plants reported that RAPD markers, in conjugation with isozyme and morphological markers, can allow for more rigorous

assessment of germplasm management stratigies and can assist in the planning of collection expeditions. Also, **Li *et al.*, 2001** used RAPD markers in their study to investigate the genetic variation and the relationships among *Choix* germplasm accessions. The results indicated that classification by RAPD data reflected the differences in geographic origins and evolution in *Choix*.

Bertozo and Valls, 2001 studied seed protein profiles to evaluate the genetic diversity of a germplasm collection of the *Caulorrhizae* section of genus *Arachis* collected from different Brazilian river basins. The study showed that protein variation was much higher between accessions than within accessions.

The genetic diversity and interrelationships among twelve domesticated and three wild mulberry species using PCR based assays including RAPD and ISSR (Intersample Sequence Repeat) were reported **(Awasthi *et al.*, 2004)**. They used 19 random primers generated 128 discrete markers ranging from 500-3000 bp in size. One-hundred-ninteen of these were polymorphic (92%), with an average of 6.26 markers per primer. Among these a few putative species-specific amplification products which could be useful for germplasm classification and introgression studies. Their results suggest that RAPD and ISSR markers are useful for mulberry genetic diversity analysis and germplasm characterization.

This study aims to evaluate any possible genetic diversity among samples of *Nitraria retusa* collected from different habitats using RAPD markers, protein and isozyme electrophoresis with the view of finding correlations among these markers and some ecological parameters.

Materials and methods

Five stands, with wide ecological amplitude and different growth forms, were chosen to collect *Nitraria retusa* **(Table 1)**. Herbarium specimens were prepared and deposited in herbarium of Botany Department, Faculty of Science (Girls), Al-Azhar University.

Table (1). Code number, localities and morphotypes of the selected populations of *Nitraria retusa*.

Code No.	Localities	Morphological type
N1	Wadi Hof (sun)	Small leaves
N2	Wadi Hof (shade)	Large leaves
N3	Oyun Musa	Small leaves
N4	Hammam Faraon	Large leaves
N5	Kattamya	Small leaves

Methods

I- Ecological study

Ecological study included the following parameters

1- Climate

Data of climatic particulars of the studied areas were obtained from the metreological stations as the following:

1- Wadi Hof (shade and sun): data represented by the climate of Wadi Hof (after Emad Eldin 1990).
2- Kattamia-Ain Sukhna road (1 stand): represented climatically by Suez station (mean of 20 years from 1975-1994).
3- Oyun Musa and Hammam Faraon stands : represented by the climate of Ras Sudr station (mean of 20 years from 1976-1995).

2- Soil analysis (Physical and chemical analysis)

Soil samples, of each selected stand, were taken to a depth of 30cm, air dried, sieved and used for mechanical analysis of soil particles.

Particle size analysis of the different soil samples was accomplaished according to **Piper, 1950**. Moisture measurements of soil reaction and the determination of total soluble salts were done according to **Jackson, 1967**.

Calcium and magnesium contents were determined according to the procedure of **Richard, 1954**. Chloride extract from soil pastes and the carbonate percentage were determined according to **Jackson, 1967**. Sulphate estimation by turbidimeter method was done according to the **Standard Method, 1989**. The total organic carbon was determined as described by **Piper, 1950**.

3- Vegetation analysis

Vegetation parameters included density (D), relative density (RD), frequency percentage (F%), relative frequency (RF), abundance (A) and relative abundance (RA). Importance value (IV) was determined and calculated according to **Shukla and Chandel, 1998**. Identification and naming of the plant species with 10 quadrates in each site were done with the help of local floras (e.g. **Taeckholm, 1974**).

4- Description of *Nitraria retusa* plants

This was fulfilled considering mean length and diameter of the plants as well as mean length and size of the leaves of the species in the studied habitats during the growing season.

II- Molecular biology study

The molecular biology study included the following parameters

1- Random amplified polymorphic DNA (RAPD) analysis

RAPD assay was done at the Agriculture Genetic Engineering Research Institute (AGERI), Giza, Egypt.

Genomic DNA of *Nitraria retusa* was extracted from bulked leaf materials that were randomly collected from the wild investigated populations. Genomic DNA was isolated using cetyltrimethyl ammonium bromate (CTAB) extraction and the amplification conditions followed those described by **Williams *et al.*, 1990**. Eleven decamer primers (Operon Technologies, Inc., USA) of arbitrary sequences were used for PCR amplification **(Table 2)**. The DNA amplification reaction was performed in a volume of 12.5 µl reaction mixture, containing 1x buffer, 3mM $MgCl_2$, 40 pmol primers, 200 µ M $dNTP_{(s)}$, 25 ng of genomic DNA, 2.5 µ Taq DNA polymerase. The amplification was performed in a DNA thermal cycler (Perkin Elmer Cetus), programmed as follows: 1 cycle of 94°C for5 min. (initial strand separation), followed by 40 cycles for 1 min. at 94°C (denaturation), 1 min. at 36°C (annealing), 2 min. at 72°C (elongation), and finally 1 cycle for 7 min at 72 °C (final extension).

RAPD data were scored as present (1) or absent (0). Genetic relationships among the studied populations, based on Euclidean distances, were used for hierarchical grouping of the populations by Unweighted Pair Group Method Analysis (UPGMA).

Table (2). Oligonucleotide primers used in the present study.

Primer	Nucleotide sequence 5`—3`
A06	GGTCCCTGAC
B04	GGACTGGAGT
B11	GTAGACCCGT
B12	CCTTGACGCA
B14	TCCGCTCTGG
B18	CCACAGCAGT
B20	GGACCCTTAC
Z11	CTCAGTCGCA
Z14	TCGGAGGTTC
Z18	AGGGTCTGTG
Z19	GTGCGAGCAA

2- SDS- Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Extraction of protein of *Nitraria retusa* was performed on the bulk leaf samples collected from the studied wild populations. Sodium Dodecyle Sulphate Polyacrylamide Gel Electrophoresis was performed according to the slab-gel-electrophoresis method of **Laemmli, 1970**.

3- Isozyme electrophoresis

Leaves of *Nitraria retusa* from the selected wild populations of each of the sites listed in **Table 1** were stored at -20°C in plastic bags for one day after collection, then analyzed using starch gel electrophoresis (**Cheliak, and Pitel. 1984**). Extraction, gel preparation, electrophoresis, staining followed the standard procedure. The enzyme system tested were: alcohol dehydrogenase (ADH), glutamate oxaloacetate transaminase (GOT), esterase (EST), isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), β. glucosidase (β. GLU) and acid phosphatase (ACP).

Results

I- Ecological study

1- Climate of the studied sites

The climate of Egyptian deserts is characterized by extreme aridity and high temperature. There are wide ranges of temperatures and humidity both annual and diurnal. Rainfall is scanty and varies in the different years. Generally, the climate of the inner desert areas is of the extreme arid types.

1.1 Wadi Hof microhabitat

In sunny and shady microhabitat, air temperature ranged from 26.5°C to 11.0°C and 21.5°C to 11.0°C respectively in March.

In June, the minimum rate of evaporation was 0.22 mm/h in the sunny microhabitat, while it was 0.13 mm/h in the shady one.

The mean value of wind velocity in the sunny microhabitat was 4.3 Km/h and 3.4 Km/h in March and June, respectively. On the other hand, in the shady microhabitat it was 0.12 Km/h and 0.76 Km/h in March and June, respectively.

The highest values of rainfall (4.0 mm) were recorded in January and there are five months without rainfall.

1.2 Ras Sudr and Hammam Faraon road

The maximum air temperature was recorded in July (35.3°C) while mean of the minimum was recorded in January (8.2 °C)**.**

The maximum relative humidity was recorded in October (62%), while the minimum was recorded in May (50%).

The maximum wind velocity was recorded in September (12.4 Km/hr), while the minimum was recorded in January and December (7.7 Km/h)**.**

The highest rainfall value was recorded in March (4.5 mm) and there were four months without rainfall**.**

1.3 Suez road and Kattamya Ain-Sukhna road

The maximum air temperature reached 36.3°C in July, while the minimum value was in January (9.4°C) indicating the marked seasonal fluctuation in air temperature of the area.

The maximum relative humidity was recorded in January (70.6%), while the minimum relative humidity was recorded in May (54%) indicating a moderate dry climatic conditions.

The highest evaporation rate recorded was 13.7 mm/day in June decreased to 5.1 mm/day in January**.**

The highest wind velocity was recorded in June (16.2 Km/h), meanwhile the minimum value was in December and January (9.2 Km/h).

It was noticed also that the highest value of rainfall was 6.1 mm. in February and there are five months without rainfall.

2- Soil analysis of the studied areas

2.1 Physical properties of soil samples

The mechanical analysis of the five studied stands showed remarkable variation in the soil texture, which are mainly due to differences in soil types of the studied regions **(Table 3)**.

2.2 Chemical analysis

Chemical analysis of the studied samples showed obvious variations among the studied sites **(Table 3)**.

Table (3). Soil analysis of the studied stands

Stand No.	Mechanical analysis					Moisture contents (%)	T.S.S (%)	pH	Organic Carbon (%)	Carbonate contents (%)	Soluble anions (me/L)		Soluble cations (me/L)	
	Gravel	Coarse sand	Fine sand	Silt	Clay						Cl^-	$SO4^-$	Ca++	Mg++
1	6.0	46.7	32.6	5.7	8.92	2.88	0.4	7.9	0.480	57.9	2.6	26	4.84	2.20
2	54.5	33.9	8.7	1.8	1.10	5.15	2.1	7.8	0.360	58.2	67.2	27.5	34.10	9.90
3	22.5	28.5	35.5	7.3	6.20	10.71	24.9	6.9	0.594	51.0	400.0	32.2	176.00	105.6
4	1.84	16.6	43.1	1.4	36.91	3.52	1.4	7.5	0.540	58.8	60.0	27.2	22.00	3.3
5	14.3	8.7	17.2	13.9	45.53	3.80	1.0	7.7	0.510	58.2	2.6	29.5	15.40	6.60

3- Vegetation

It is evidenced that genomic structure in any organism is controlled in the first place by inheritance and in the second place by the prevailing environmental conditions. Vegetation characteristics such as species composition in each of the studied stands may explain in part the differences observed in DNA and protein banding of the tested five populations of *N. retusa*.

Vegetation analysis included total number of species, their densities, frequencies, abundances, their relative counterparts and their importance value (I.V.) for every individual species. These parameters were investigated for each selected stand **(Table 4)**.

1- Wadi Hof (sunny and shady microhabitats): In sunny microhabitat the characteristic community is *Zilla spinosa* (I.V. 48.57), while in shady microhabitat the characteristic community is represented by *Lycium shawii* (I.V. 40.49).

2- In Oyun Musa the main community is represented by *Alhagi maurorum* (I.V. 227.23).

3- In Hammam Faraon the dominant species is *Zygophyllum album* (I.V. 62.63) and the co-dominant species is *Hamada elegans* (I.V. 54.26).

4- In Kattamya road the characteristic species is *Zilla spinosa* (I.V. 46.34).

4- Description of *Nitraria retusa* populations

Nitraria retusa is a common shrub of the coastal and inland salt marshes of Egypt. It is also common on the silt terraces of the wadies of the limestone desert where it may form scrubland subject to destruction for fuel but not to serious grazing.

Table (4). Total number of individuals of species and their importance value (I.V.) indices in the different studied stands.

Species name	Wadi Hof (sun)		Wadi Hof (shade)		Oyun Musa		Hammam Faraon		Kattamya	
	1	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**
Achillea fragrantissima	8	14.59	-	-	-	-	-	-	25	26.05
Alhagi maurorum	-	-	-	-	257	227.2	-	-	-	-
Anabasis articulata	-	-	-	-	-	-	5	14.75	16	18.95
Anabasis setifera	40	45.36	7	29.36	-	-	-	-	-	-
Artemisia judaica	1	4.14	-	-	-	-	3	11.6	4	8.19
Asteriscus graveolens	1	4.14	-	-	-	-	-	-	3	6.81
Astragalus spinosus	-	-	-	-	-	-	-	-	1	3.7
Atriplex halimus	1	4.14	6	26.51	-	-	-	-	22	23.49
Calligonum comosum	-	-	-	-	-	-	17	33.57	-	-
Capparis cartilaginea	-	-	-	-	-	-	17	33.57	-	-
Cistanche phelypaea	-	-	-	-	-	-	-	-	16	19.51
Cynodon dactylon	-	-	3	16.69	-	-	-	-	-	-
Deplotaxis acris	-	-	3	16.69	-	-	-	-	-	-
Deplotaxis harra	-	-	1	8.465	-	-	-	-	-	-
Deverra tortuosa	2	6.37	3	16.68	-	-	-	-	-	-
Echinops spinosissimus	1	4.17	3	16.68	-	-	-	-	3	6.73
Fagonia arabica	1	4.13	-	-	-	-	-	-	-	-
Farsetia aegyptia	3	7.88	-	-	-	-	-	-	4	6.69
Gymnocarpos decandrum	7	13.95	-	-	-	-	-	-	8	12.38
Hamada elegans	14	24.56	2	13.79	-	-	34	54.26	-	-
Heliotropium arbainense	3	7.88	-	-	-	-	-	-	-	-
Hyoscyamus muticus	-	-	-	-	-	-	1	5.96	-	-
Iphiona mucronata	8	14.99	-	-	-	-	-	-	-	-
Lavandula stricta	6	12.92	-	-	-	-	-	-	-	-
Limonium pruinosum	2	6.37	-	-	-	-	-	-	-	-
Lycium shawii	-	-	11	40.49	-	-	-	-	6	10.13
Nitraria retusa	4	11.52	4	20.15	9	36.86	12	26.88	17	19.7
Ochradenus baccatus	2	6.37	-	-	-	-	-	-	-	-
Peganum harmala	-	-	-	-	-	-	-	-	2	5.26
Penisitum divisum	1	4.13	4	20.15	-	-	-	-	-	-
Pergularia tomentosa	1	4.13	-	-	-	-	-	-	1	3.7
Phoenix dactylifera	-	-	-	-	7	27.44	-	-	-	-
Reaumuria hirtella	5	10.99	-	-	-	-	-	-	1	3.7
Retama raetam	-	-	-	-	-	-	-	-	13	17.1
Scrophularia deserti	3	8.59	-	-	-	-	-	-	-	-
Stachys aegyptiaca	2	6.61	2	13.79	-	-	-	-	-	-
Tamarix nilotica	-	-	1	8.465	1	8.47	1	5.96	5	9.01
Zilla spinosa	44	48.57	3	16.68	-	-	-	-	57	46.34
Zygophyllum aegyptium	-	-	-	-	-	-	-	-	24	24.88
Zygophyllum album	-	-	-	-	-	-	45	62.63	-	-
Zygophyllum coccineum	16	23.5	9	35.41	-	-	28	50.82	28	27.68

1= Total No. of plant species , **2**= I.V.

Variation in the morphological characters of *Nitraria retusa* growing in different habitats is summarized in the following:

- Leaf area ranged from 0.5 cm^2 to 2.8 cm^2.
- Plant height ranged from 130 cm to 200 cm.
- Plant length ranged from 200 cm to 450 cm.
- Plant width ranged from 190 cm to 320 cm. **(Table 5 & Plate 1)**.

Table (5). Measurements parameters of *Nitraria retusa* growing in the studied stands.

Samples	Leaf area cm^2	Height of the plant cm	Length of the plant cm	Width of the plant cm
N1	0.5	130	400	280
N2	2.0	140	450	320
N3	1.0	200	220	200
N4	2.8	170	200	190
N5	1.3	150	270	210

-Results represent average values

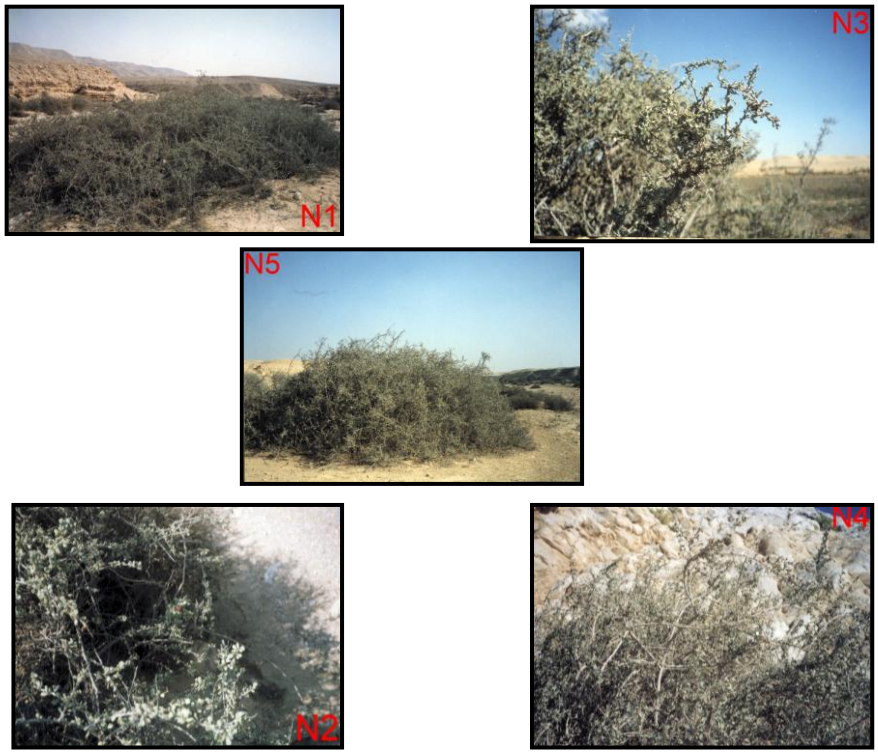

Plate (1): Variation in the morphological characters of *Nitraria retusa* plants in their studied sites.

II- molecular biology study of *Nitraria retusa* samples

1- RAPD analysis

Eleven decamer random primers were tested for their efficiency in initiating PCR product formation for the five selected samples of *Nitraria retusa*. Nearly all primers tested produced well- defined products as visualized in ethidium bromide-stained agarose gels. Primers varied in their ability to yield reproducible banding patterns with *Nitraria* template DNA. **Table 6**.

A total of 100 reproducible bands were generated, using the tested primers, and of these, 77 bands were polymorphic. The number of polymorphic bands ranged from 1 to 14 for each of the tested primers **(Table 6)**. A considerable degree of variability in RAPD markers was observed in *Nitraria* with approximately 77% of the 100 markers generated being polymorphic. Number of bands varied with primer but averaged 9 bands per primer.

Table (6). Nucleotide sequences of the 11 oligonucleotide primers and the total number of RAPD markers generated from different samples of *Nitraria retusa* .

Primer	*Sequence* 5`—3`	G-C cont.%	No. of scorable amplification products	No. of polymorphic products	%of polymorphic products
A06	GGTCCCTGAC	70	3	3	100
B04	GGACTGGAGT	60	10	8	80
B11	GTAGACCCGT	60	12	7	58.33
B12	CCTTGACGCA	60	12	8	66.66
B14	TCCGCTCTGG	70	1	1	100
B18	CCACAGCAGT	60	10	8	80
B20	GGACCCTTAC	60	15	14	93.33
Z11	CTCAGTCGCA	60	11	6	54.54
Z14	TCGGAGGTTC	60	1	1	100
Z18	AGGGTCTGTG	60	13	11	84.61
Z19	GTGCGAGCAA	60	12	10	83.33
		Total	100	77	77

The RAPD profiles for all samples generated amplification product sizes ranged from 72 bp to 4.03Kbp, most of them fall in the range 194 bp to2.03 Kbp **(Fig. 1)**. This figure show typical RAPD profiles obtained by several selected primers.

The RAPD profiles for all samples generated with Z11, B12 and B11 show apparent monomorphic bands, although there was a considerable variation among the studied samples **(Fig. 1)**. Obvious RAPD variation among the studied samples was observed with B20, Z18, Z19 and B18 primers **(Fig. 1)**.

We were able to distinguish *Nitraria* shady (N2) sample with large leaves from those of smaller leaves (sunny sample) N1 of Wadi Hof area based on the polymorphic bands generated by B18, B20, B11 and B04 **(Fig. 1)**.

One band was specific to Oyun Musa sample (lane 3) manifested with primer B18, while three bands were peculiar to Hammam Faraon sample (lane 4) generated by B12 and B11 **(Fig. 1)**. Two bands produced by Z18 primer, which were present in Kattamya sample, proved to be absent in all the studied samples. **(Fig. 1, lane 5).**

Pair-wise genetic distance for the studied samples based on RAPDs, generated from the used primers, ranged from 4.69 to 7.75 **(Table 7)**. Samples of Hammam Faraon and Oyun Musa stands (N4 and N3) had the closest genetic distance values. Wide genetic distances were recorded between Wadi Hof shady sample (N2) and the remaining studied ones **(Table 7)**. The UPGMA cluster analysis generated by RAPD data from the used primers **(Fig. 2)** placed all the studied samples, except N2, in one group. *Nitraria* sample (N2) which displayed distinct morphotype clustered apart from the other samples **(Fig. 2)**. The UPGMA cluster analysis generated by several primers e.g. B12, B18 and B20 confirmed the previous observation.

Table (7). Euclidean distance based on RAPDs showing the variability among the studied populations of *Nitraria*.

Stat. Cluster analysis	**Euclidean distances**				
Variable	N1	N2	N3	N4	N5
N1	.00	6.16	6.16	5.83	5.48
N2		.00	7.75	7.62	7.07
N3			.00	4.69	5.29
N4				.00	4.90
N5					.00

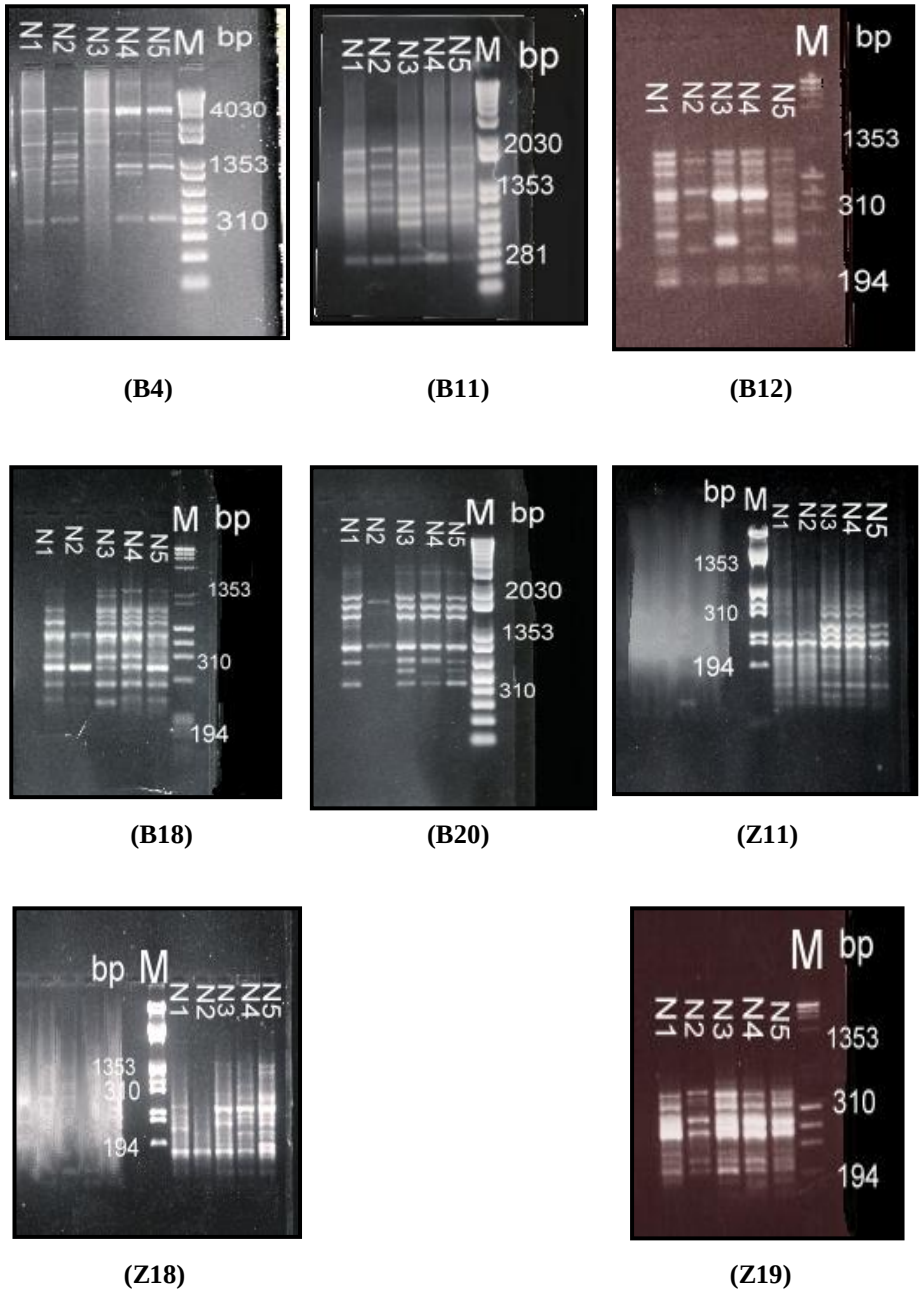

Fig. (1). Shows the amplification profiles of the five investigated samples of *Nitraria retusa* generated by eight primers. Lanes 1-5 represent the tested samples

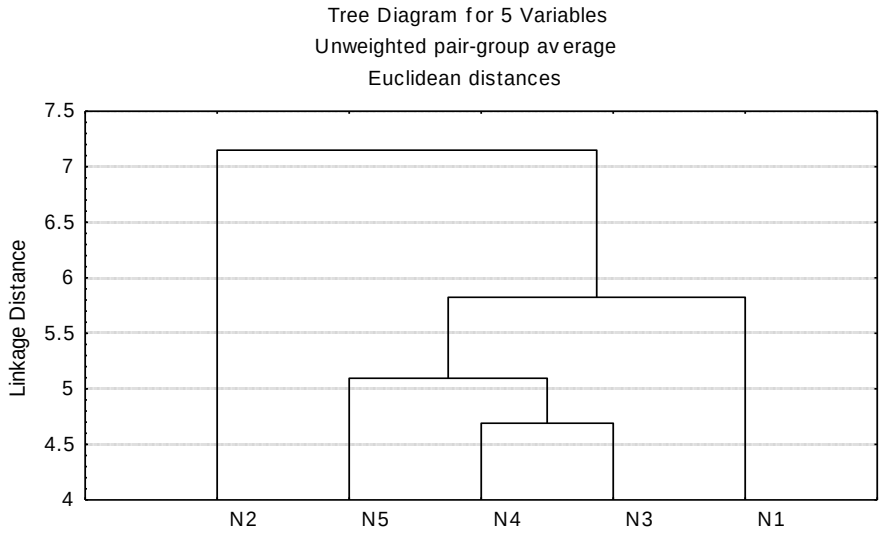

Fig. (2). UPGMA dendrogram based on data generated from 11 tested primers showing the genetic distance (linkage distance) among the studied populations.

2- Protein Electrophoresis

Crude protein from *Nitraria* leaves was investigated using sodium dodecyl sulphate- polyacrylamide gel electrophoresis. The banding patterns of the studied samples, their molecular weight (MW), relative front (RF), number of bands and average optical density (OD) are given in **Table 8**. The number of polypeptide bands varied from 9 (N4 and N5) to 11 (N2) and the MW of separated polypeptides ranged from 12.63 to 42.21 KDa. Remarkable variation in the amount of each protein fraction, which was indicated by the OD, was recorded among the selected studied samples **(Table 8)**.

Three obvious bands with approximately similar MW values were recorded in N3, N4 and N5 samples from Oyun Musa, Hammam Faraon and Kattamya. The amount of the above protein fractions varied among these three samples **(Table 8)**. Similarity between N3 and N4 samples, which was previously mentioned by RAPD data, was shown due to the presence of at least 5 bands of approximately similar MW values **(Table 8)**.

3- Isozyme investigation

The enzymes investigated in the studied *Nitraria* samples were: ADH, GOT, EST, IDH, MDH, β- GLU and ACP. Among the tested enzymes, ACP and MDH give faint band indicating low activities.

Table (8). Electrophoretic analysis of leaf protein of sampled populations of *Nitraria retusa* collected from different sites.

Lane number	Band Number	Relative front	Mol.Wt. KDa	Average OD	Lane number	Band Number	Relative front	Mol.Wt. KDa	Average OD
M	1	0.124	175	81.155	3	2	0.443	34.823	76.393
M	2	0.188	83	81.174	3	3	0.468	32.984	76.38
M	3	0.323	45	88.24	3	4	0.518	30.065	78.073
M	4	0.475	32.5	103.893	3	5	0.58	26.915	89.963
M	5	0.622	25	107.997	3	6	0.599	26.046	95.648
M	6	0.773	16	73.548	3	7	0.677	21.255	102.382
1	1	0.353	42.206	94.137	3	8	0.697	20	107.907
1	2	0.404	37.867	124.302	3	9	0.725	18.441	110.304
1	3	0.417	36.764	125.545	3	10	0.805	14.555	116.803
1	4	0.493	31.451	121.635	3	11	0.846	12.887	112.289
1	5	0.53	29.455	131.405	4	1	0.394	38.697	79.564
1	6	0.573	27.248	140.04	4	2	0.446	34.557	87.919
1	7	0.635	24.006	145.634	4	3	0.515	30.252	85.627
1	8	0.661	22.285	153.666	4	4	0.561	27.876	90.664
1	9	0.695	20.136	156.752	4	5	0.6	26.003	97.222
1	10	0.794	15.055	127.12	4	6	0.661	22.234	100.887
2	1	0.378	39.978	83.557	4	7	0.7	19.825	107.007
2	2	0.427	36.046	85.379	4	8	0.719	18.783	109.06
2	3	0.443	34.823	86.133	4	9	0.81	14.341	109.298
2	4	0.516	30.188	87.745	5	1	0.366	41.05	98.912
2	5	0.573	27.248	98.664	5	2	0.384	39.466	99.078
2	6	0.592	26.368	102.632	5	3	0.451	34.219	105.19
2	7	0.649	23.052	104.468	5	4	0.487	31.774	106.265
2	8	0.693	20.272	113.406	5	5	0.542	28.803	112.137
2	9	0.716	18.947	117.651	5	6	0.568	27.536	134.981
2	10	0.794	15.055	121.577	5	7	0.723	18.532	167.534
2	11	0.853	12.628	97.651	5	8	0.735	17.917	173.456
3	1	0.39	39.004	68.501	5	9	0.799	14.833	157.564

Discussion

One convenient method for identifying genetic polymorphism among natural populations is random amplified polymorphic DNA (RAPD). RAPD is a fingerprinting method that uses oligonucleotide primers to search for variation in the entire genomic DNA **(Williams *et al.*, 1990)**. RAPD technique has been widely used for population genetic studies of so many plants, for example, *Vigna luteola* and *V. marina* (**Sonnante *et al.*, 1997**); *Vanda* species (**Lim *et al.*, 1999**) and *Olea europaea* (**Besnard *et al.*, 2001**). RAPD markers have given the opportunity to screen an almost unlimited number of highly polymorphic markers which can be successfully used to assess genetic diversity within species **(Link *et al.*, 1995)** and among related species **(Ruas *et al.*, 2001)**.

The objective of the current study is to explore the pattern of genetic variations using *Nitraria retusa* samples collected from different regions of Egypt with varying morphotypes. The ecological aspects of the selected regions were investigated with view of finding correlations between the molecular results and some ecological parameters. In this respect, **Ayana *et al.*, 2000** reported that samples in germplasm collections across diverse environments and based on morphological variation have been considered to be a more efficient means for capturing genetic diversity.

In this study, genomic DNA extracted from bulked leaves, proteins and isozymes, also extracted from bulked samples, adopting the views of **Staub *et al.*, 1997**. They reported that as number of random measurements increases, the distribution of values taken from a subset of the populations become more uniform.

Morphological observation, in the present study, showed a marked level of variation in leaf size of the shady sample of *Nitraria retusa* collected from Wadi Hof (N2).

For the precise estimation of genetic distances, it has been recommended that at least 50 different polymorphic loci should be used **(Nei, 1978** and **Jain *et al.*, 1999)**. In the present study, the tested 11 primers generated a number of polymorphic bands that were around the recommended range.

PCR amplification of the genomic DNAs of the five samples of *Nitraria* with the tested primers showed detectable variation among the studied samples. The data obtained from the 11 primers as well as those from many tested primers including B20, Z19, B12 and B18 resulted in PCR amplified products that differentiate sample of N2 from the other studied ones.

Cluster analysis grouped samples with greater genetic similarities together; the cluster did not necessarily include all the populations from the same or nearby sites. The dendrogram based on RAPD data indicated that samples of populations from Wadi Hof (sunny), Oyun Musa, Hammam Faraon and Kattamya are the most related ones, whereas shady sample (N2) of Wadi Hof is the most distant one. Similar results were reported by **Loo *et al.* (1999)** in their study which included three populations of palm *Licuala glabra* (morphologically differed). They found that the phenogram generated by UPGMA of the RAPD data grouped the studied individuals of the three populations under three clusters; this is in agreement with the morphological grouping.

Electrophoretic protein analysis is in agreement with RAPD data in the presence of some variations in protein fractions between sunny and shady samples of Wadi Hof.

The tested enzymes (ADH, GOT,EST, IDH, β-GLU and ACP) did not produce interpretable bands throughout the material and were, therefore, omitted. Scarcely, fainter and ambiguous bands of MDH did not permit interpretation also.

Using the previous technique and the other traditional ones enabled us to record many different ecotypes for the studied species which have a degree of diversity in both morphological and genetic aspects. The effect of ecological determinants on these aspects was clearly obvious in this study. In many cases, pattern of trait variation across environmental gradients has suggested hypotheses concerning their adaptive significance **(Jonas and Geber, 1999).**

The size of population and gene flow may also partly explain the results obtained in our study. The relationship between population size and genetic variation was also investigated in many studies. **Fischer and Matthies, 1998** found a positive correlation between genetic variation and population size and between genetic variation and number of seeds per plant in the rare plant *Gentianella germanica* based on RAPD profile. Also, RAPD variation among and within small and large populations of rare clonal plant *Ranunculus reptans* was estimated by **Fischer *et al.*, 2000.** They indicated that samples from large populations were genetically more variable than those representing comparable areas of smaller populations.

Román *et al.*, 2003 used RAPD markers to study variation among 20 taxa in the genus *Orobanche*. They reported that the pattern of interspecific variation obtained is in general agreement with previous taxonomic studies based on morphology, and

the partition into two different sections (*Trionychon and Orobanche*) is generally clear.

Pluess and Stöcklin, 2004 used RAPD-PCR for a study of the genetic diversity within and among 20 populations of *Geum reptans*, an outcrossing clonal plant species in the Swiss Alps, with distance among populations ranging from 0.2 to 208 Km. Their results indicated considerable gene flow among populations within the same regional area, and they found no indication for genetic depletion during succession or in peripheral habitats.

Barik *et al.*, 2006 used random amplified polymorphic DNA (RAPD) for identification and determination of genetic variation within the two species of *Hibiscus* and 16 varieties of *Hibiscus rosa-sinensis* L. They came to the conclusion that these RAPD markers have the potential for identification of species/ varieties and characterization of genetic variation within the varieties.

From the present study we could differentiate clearly the different ecotypes diversity of each studied species. Though during the evolution they form new interfertile forms, which posses different genetic compositions and genotypes that arise through mutations, hybridization and isolation. Therefore, the ecotypes are the products of genetic responses of populations to their habitats. Our results indicated that RAPD is the most efficient marker for assessing genetic variation among the selected populations of the studied plants. Thus, the results obtained from the present study consider DNA and protein fingerprints for the studied populations, besides the ecological amplitude of the species.

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الملخص العربي

تقييم الأختلافات الوراثية فى نبات الغرقد (*Nitraria retusa*) باستخدام دلائل الحمض النووى

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يهدف هذا البحث الى فحص بعض عينات من نبات الغرقد النامية فى بيئات مختلفة وهى وداى حوف ، وعيون موسى ، والقطامية ، وحمامات فرعون وذلك باجراء دراسة بيئية كاملة على هذا النبات.

أيضا يشتمل هذا البحث على الدراسة الوراثية لهذا النبات وذلك بتحليل دلائل التكبير العشوائى للحمض النووى (RAPD), و مشابهات الإنزيمات, و التفريد الكهربى للبروتين و ذلك لدراسة العلاقة بين هذه الدلائل و المتغيرات البيئية.

أظهرت الدراسة وجود اختلافات مظهرية بين العينات باختلاف مناطق تجميعها . وباستخدام البادئات المختبرة تم الحصول على نواتج تؤكد تميز عينات الظل من وادى حوف (و التى تميزت بأوراق عريضة) عن باقى العينات . هذا وقد توافقت الاختلافات على المستوى الوراثى باستخدام الدلائل المختبرة مع تلك الملاحظة على المستوى المظهرى .