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GENETIC DIVERSITY OF NATURAL POPULATIONS OF *MORINGA PEREGRINA* IN EGYPT

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ABSTRACT

The genetic diversity of two populations of *Moringa peregrina* in South Sinai was performed by inter-simple sequence repeat markers (ISSR) using ten primers. The investigation was carried out as a primary step towards developing effective conservation strategies for the protection of *Moringa peregrina* germplasm. The aim of this study was to assess the genetic diversity within and among twenty individuals of *Moringa peregrina* collected from two populations in Wadi Zaghra and Wadi Feiran, South Sinai. The ISSR marker analysis showed a significant level of genetic variation within and among the populations, reached 95.45% of polymorphism. Cluster analysis was performed to construct a dendrogram using UPGMA. The dendrogram clustered the individuals into two major clusters, the first one for Wadi Zaghra individuals (about 78% polymorphism) and the second one for Wadi Feiran individuals (about 75% polymorphism). The obtained results indicate the high genetic diversity between the individuals of *Moringa peregrina* in South Sinai.

Kay words: Moringa, ISSR, population genetics, polymorphism

INTRODUCTION

Moringa peregrina (Forssk.) Fiori. is a green deciduous tree with sweet and oil rich white seeds. It belongs to the Moringaceae family that has only one genus called *Moringa* with 13 species **(Steinitz *et al.*, 2009)**. The most famous *Moringa* species are *Moringa oleifera* and *Moringa peregrina* **(Lalas *et al.*, 2012)**. *Moringa peregrina* "Al Yassar" is a wild plant grown in the eastern desert mountains and South Sinai in Egypt **(El-Alfy *et al.*, 2011)**. *Moringa peregrina* is one of the most valuable and economically important species in the Egyptian desert and became one of the most endangered plants. The plants are surrounded by tents of the local Bedouins, who would use every green pit of the plant for their goats and used any source of wood available to get worm in winter as well as to cook their food causing unmanaged grazing and overcollection. The plant could soon become one of the most valuable plants in the world.

Moringa peregrina occurs in tropical and non-tropical areas. Egypt, Ethiopia to Somalia, Sudan, the Red Sea region, Palestine, and Jordan are the main centers of distribution **(Lalas *et al.*, 2012)**. It has been shown that *Moringa peregrina* has high aridity adaptation with short germination time after irrigation.

Traditionally, *Moringa peregrina* has both medicinal and industrial uses. In Greece, Egypt, and Romania its seed oil is used to make perfume and its wood is used in building because of its resistance to termites. In addition, *Moringa peregrina* is used as medicine for abdominal pain, headache, and skin protection as well as a

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laxative **(Steinitz *et al*., 2009)**. Seeds contain potential anti-inflammatory and antioxidant factors **(Koheil *et al*., 2011)**. They are the source of oil used by ancient Egyptians and currently are used in folk medicine **(Abd El-Wahab *et al.*, 2004)**. Seeds and leaves of *Moringa peregrina* contain several amino acids, mineral, vitamins and protein and have different economic and medicinal importance (**Al Rawashdeh *et al*., 2016)**. Seeds are a good source of vitamin A, B and C and among the best plant sources of minerals **(Price, 2000)**. *Moringa peregrina* is becoming an endangered plant species all over the world because of unmanaged grazing and slow regeneration rate after browsing **(Steinitz *et al*., 2009; Gomma and Picó, 2011)**. Many studies were aiming to find efficient cultivation methods to save *Moringa peregrina* from extinction **(Zaghloul *et al*., 2010)**.

Understanding the genetic diversity within and among populations of rare and endangered plant species is essential when developing management strategies for both *in situ* and *ex situ* conservation activities of plants **(Hogbin and Peakall, 1999)**. Thus, genetic diversity estimation of the wild populations of *Moringa peregrina* is very important to clarify the relationships between individuals within and among the different populations, which has a great impact on the conservation management of the plant.

Inter-simple sequence repeat (ISSR) analysis involves PCR amplification of genomic DNA using a single primer that targets the repeats with 1-3 bases that anchor the primer at 3′ or 5′ end. The ISSR analysis is technically simpler than many other marker systems. The method provides highly reproducible results and generates abundant polymorphisms in many systems. Also, ISSR markers show high level of repeatability and have been used as useful molecular marker in studying genetic diversity and species relationships. Also, it does not require a prior knowledge of the DNA sequence and provides a genome-wide screening of a high number of loci **(Zietkiewicz *et al*., 1994; Pharmawati *et al*., 2004; Dogan *et al*., 2007)**.

ISSR markers have been widely applied in population genetics of various plants **(Hassel and Gunnarsson, 2003; Vanderpoorten *et al*., 2003; Werner *et al*., 2003; Natcheva and Cronberg, 2007; Buczkowska, 2010; Pla´sˇek and Sawicki, 2010)**.

The objective of the present study was to investigate the genetic diversity within and among two wild populations of *Moringa peregrina* from two locations in South Sinai using ISSR analysis.

MATERIALS AND METHODS

This study was carried out in the Molecular Genetics Laboratory, North Sinai Research Station (El-Sheikh Zuwayed), Desert Research Center (DRC), El-Matareya, Cairo, Egypt.

Genetic diversity was studied within and among two populations of *Moringa peregrina* collected twice from two locations at South Sinai (Wadi Zaghra and Wadi Feiran, 10-15 km apart).

Samples Collection

Leaf samples were collected from ten plant individuals of each site. Wadi Zaghra has 800 ft altitude and located at latitude 28° 39' 338" N and longitude 34° 17' 623" E, and Wadi Feiran also has altitude 800 ft and located at latitude 28° 42' 214" N and longitude 33° 39' 556" E (Fig. 1).

DNA fingerprinting of collected samples was carried out using ISSR-PCR analysis. Fresh leaves from the new twigs of *Moringa peregrina* individuals were packed in plastic zipper bags and stored in an ice cooler, then finally stored in a -80ºC freezer for DNA extraction.

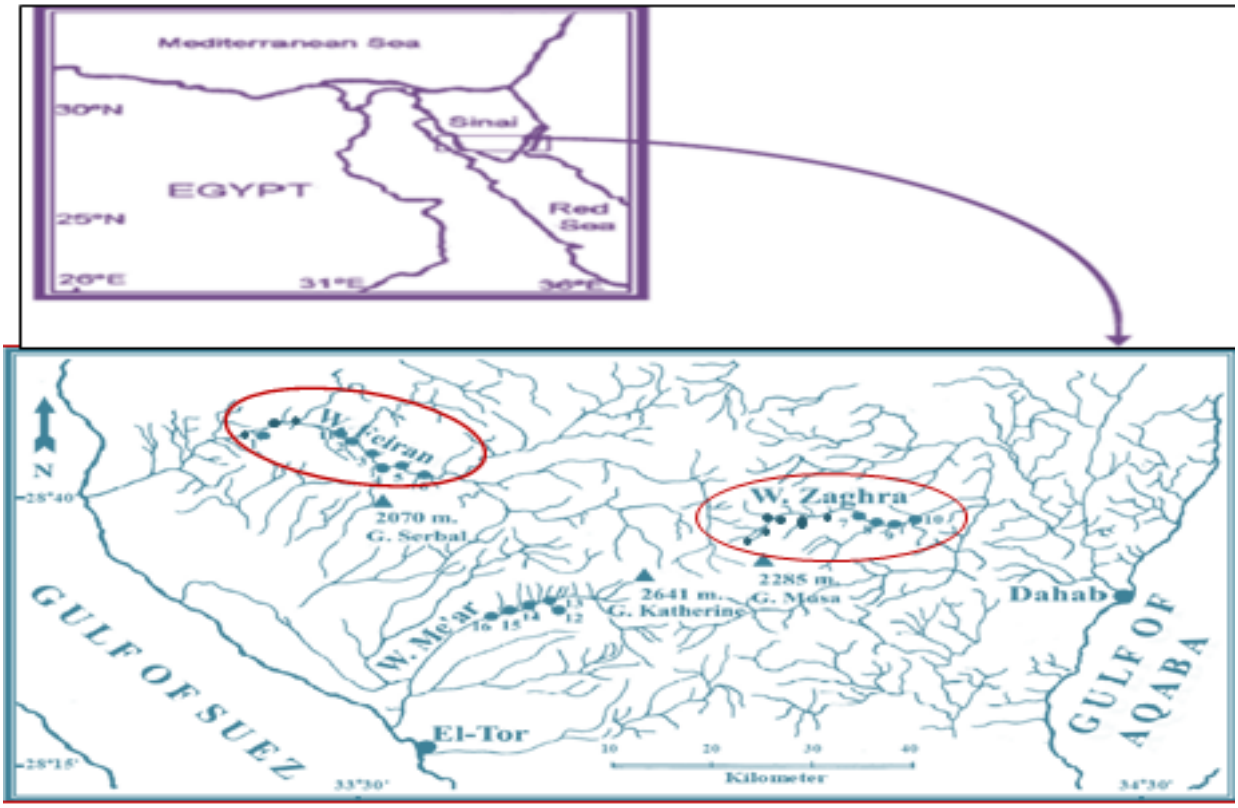


Fig. (1): A map showing the geographic location of *Moringa peregrina* studied populations at Wadi Zaghra and Wadi Feiran, South Sinai.

Extraction of Genomic DNA and PCR Amplification

Genomic DNA extraction from fresh leaves of the twenty *Moringa peregrina* individuals was done by employing the CTAB methodology as described by **Doyle and Doyle (1987)** with slight modifications.

DNA concentration and quality were assessed by a UV-spectrophotometer and gel electrophoresis. Ten ISSR primers were used for preliminary screening. PCR amplification was carried out in a total volume of 20 µl containing 1X PCR buffer [75 mM Tris /HCl, 50 mM KCl, 2.0 mM $MgCl_2$, and 20 mM $(NH_4)_2SO_4$]; 0.3 mM each of dATP, dTTP, dCTP and dGTP; 0.6 mM primer as shown in Table 1; 1 uml Taq DNA polymerase (Amplicon) and 25 ng/µl template DNA and water. Amplification was carried out in Stratagene RoboCycler Gradient 96, which was programmed for 45 cycles as follows: denaturation (one cycle) 94^{o}C for 2 min, followed by 30 cycles as follows; 94^{o}C for 30 s, 44^{o}C for 45 s, annealing at 72^{o}C for 1 min and 30 s, and finally one cycle of extension at 72^{o}C for 20 min, then 4^{o}C. Amplified products were resolved by electrophoresis on 1.5% agarose gel in 1X TBE buffer at 90 V for 2 h, then stained with ethidium bromide. The DNA fragments were visualized under UV light using a gel documentation system (Bio- RAD Gel Doc $^{TM}XR^{+}$). PCR reactions were repeated twice to check the reproducibility of the banding patterns. A 1 kbp DNA ladder was used as the molecular standard to confirm the appropriate ISSR markers.

Table (1): ISSR primers name and their sequence.

Primer name	Sequence	Primer name	Sequence
807	$(AG)_8$ T	HB4	$(GACA)_4$
814	$(CT)_8$TG	HB15	$(GTG)_3$GC
844A	$(CT)_8$ AC	17898A	$(CA)_6$ AC
844B	$(CT)_8$ GC	17898B	$(CA)_6$GT
HB1	$(CAA)_5$	17899B	$(CA)_6$GG

Data Analysis

The genetic polymorphism between individuals was analyzed by using the NTSYS pc 2.1 Software **(Rohlf, 2000)**. The binary data score was used to construct a dendrogram. The pairwise genetic relationship between individuals were determined by calculating Jaccard's similarity coefficient. The similarity coefficients were used for cluster analysis and a dendrogram was constructed by the Unweighted Pair-Group Method (UPGMA) according to **Sneath and Sokal (1973)**. Polymorphism information content (PIC) values were calculated according to **Smith (1989)** using the following formula:

$$PIC = 1 - \Sigma pi^2$$

pi^2 is the frequency of the allele. PIC provides an estimate of the discriminatory power of a locus by considering not only the number of alleles, but also the relative frequencies of those alleles. PIC values vary from 0 (monomorphic) to 1 (very highly discriminative, with many alleles in equal frequencies). To compare the efficiency of markers among *Moringa peregrina*, individuals, several parameters were estimated for each assay unit.

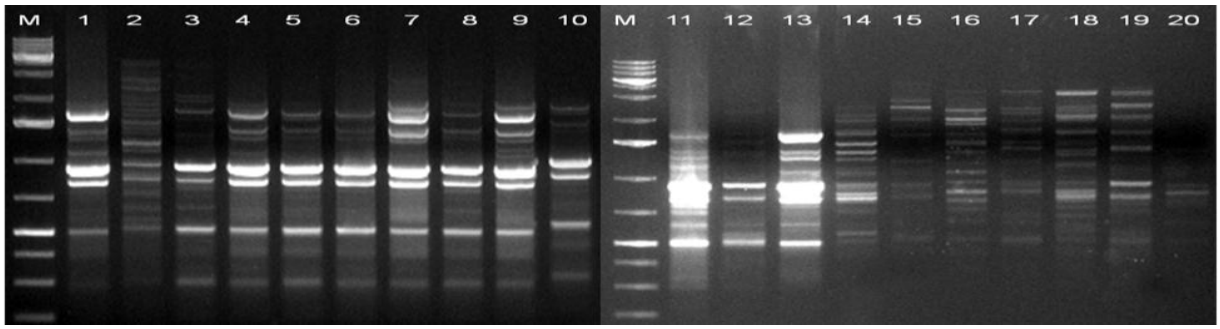
RESULTS AND DISCUSSION

Assessment of genetic diversity is very important for the conservation of plant genetic resources in their natural habitat. The genetic diversity between twenty naturally grown *Moringa peregrina* individuals in two populations; Wadi Zaghra and Wadi Feiran in South Sinai using ISSR technique were evaluated in the present study.

Ten ISSR primers were used to produce DNA fingerprint profiles (Fig. 2-5 and Table 2). The primers generated a total of 198 bands of which 189 bands were polymorphic, representing 95.54%. The total bands in Wadi Zaghra were 167 and the number of polymorphic bands was 133 (78.15% polymorphism). While in Wadi Feiran, the total bands were 132 and 102 bands were polymorphic (74.65%).

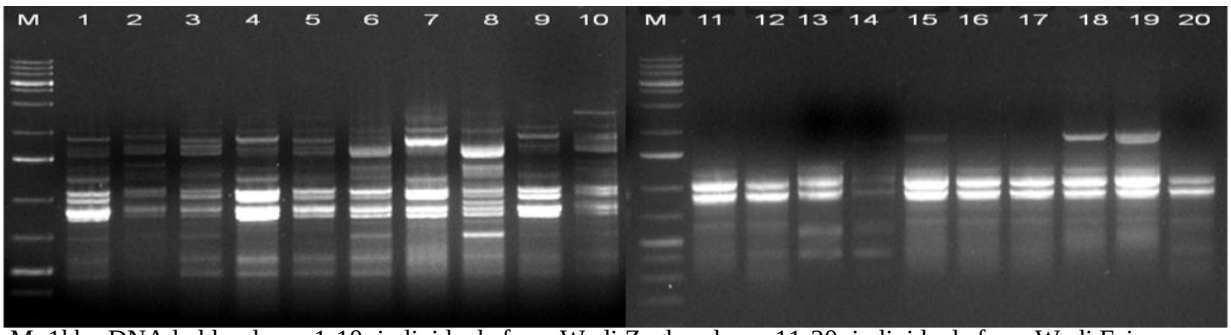
The level of polymorphism from each primer represented by PIC value ranged between 0.397 and 0.667. The results showed that the primers 807 (Fig. 2), 844A, HB1, HB15, 17898A, 17898B and 17899B produced 100% polymorphism, and the primer 814 (Fig. 4) produced the highest monomorphic bands of about 21.05% between individuals with the lowest polymorphism (78.95%) as represented in Table 2.

The results in Table 3 show the characteristics of ISSR analysis of the two studied populations of *Moringa peregrina*. The maximum number of effective alleles (17.29) was found between the individuals of Wadi Zaghra and the lowest number (15.59) was detected within the population of Wadi Feiran. The level of polymorphism (PIC value) was greater among all individuals of the two populations (0.595),



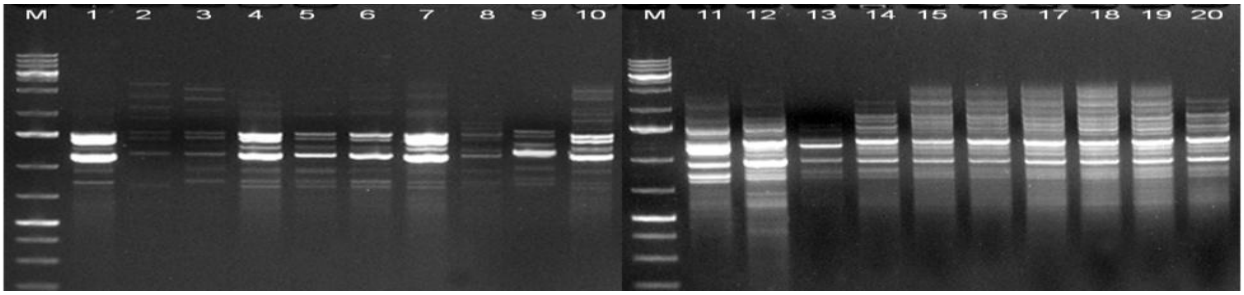
M: 1 kbp DNA ladder, lanes 1-10: individuals from Wadi Zaghra, lanes 11-20: individuals from Wadi Feiran.

Fig. (2): ISSR profile of *Moringa peregrina* individuals' DNA generated by primer 807 (100% polymorphism).



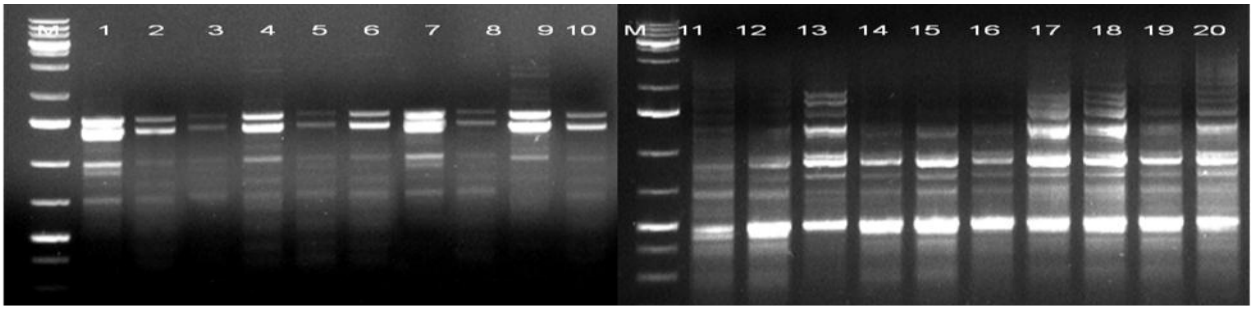
M: 1kbp DNA ladder, lanes 1-10: individuals from Wadi Zaghra, lanes 11-20: individuals from Wadi Feiran.

Fig. (3): ISSR profile of *Moringa peregrina* individuals' DNA generated by primer 844B (88.24% polymorphism).



M: 1kbp DNA ladder, lanes 1-10: individuals from Wadi Zaghra, lanes 11-20: individuals from Wadi Feiran.

Fig. (4): ISSR profile of *Moringa peregrina* individuals' DNA generated by primer HB4 (87.5% polymorphism).



M: 1 kbp DNA ladder, lanes 1-10: individuals from Wadi Zaghra, lanes 11-20: individuals from Wadi Feiran.

Fig. (5): ISSR profile of *Moringa peregrina* individuals' DNA generated by primer 814 (78.95% polymorphism).

Table (2): ISSR analysis of the DNA of all studied *Moringa peregrina* individuals *via* 10 primers.

No.	Primer name	Total no. of amplified bands	Bands length range (bp)	No. of polymorphic bands	Percentage of polymorphism (%)	PIC*
1	807	31	4000-300	31	100.00	0.623
2	814	19	2750 -300	15	78.95	0.471
3	844A	19	3500 -600	19	100.00	0.667
4	844B	17	2500 -500	15	88.24	0.524
5	HB1	16	3500 -350	16	100.00	0.518
6	HB4	24	3500-500	21	87.50	0.535
7	HB15	19	2370 – 400	19	100.00	0.582
8	17898A	19	2800-380	19	100.00	0.397
9	17898B	20	3000-350	20	100.00	0.568
10	17899B	14	2000 -500	14	100.00	0.473
Wadi Zaghra		167		133	78.15	
Wadi Feiran		132		102	74.65	
Total bands		198		189	95.45	

*__PIC:__ Polymorphism information content

Table (3): Characteristics of ISSR markers amplification in *Moringa peregrina* individuals.

Properties of markers		Wadi Zaghra individuals	Wadi Feiran individuals	All individuals
No. of markers	U	10	10	10
No. of non-polymorphic bands	n_{np}	34	30	9
No. of polymorphic bands	n_p	133	102	189
Average no. of polymorphic bands/assay unit	n_p/U	13.3	10.2	18.9
No. of loci or bands	L	167	132	198
No. of loci/assay unit	n_u	8.35	6.6	9.9
Total no. of effective alleles	N_e	17.29	15.59	22.23
Min of PIC	PIC	0.157	0.031	0.397
Max of PIC	PIC	0.567	0.548	0.667
PIC value	PIC	0.359	0.361	0.595
Fraction of polymorphic loci	B	0.7964	0.7727	0.9545
Assay efficiency index	A_i	1.729	1.559	2.223
Effective multiples ratio	E	10.484	8.54	10.372
Marker Index	MI	3.763	3.08	9.900
Total Banding pattern	Bp	109	81	165
Effective no. of patterns/assay unit	P	10.9	8.1	16.5

The dendrogram showing the relationship between the examined *Moringa peregrina* individuals based on the data obtained by the ten ISSR primers is represented in Fig. 6. Dice's similarity coefficients were calculated to assess the genetic resemblance among the individuals and the similarity coefficient matrix was used for UPGMA cluster analysis. The dendrogram is divided into two major clusters, the first cluster representing individuals of Wadi Zaghra and the second one for individuals of Wadi Feiran. Each cluster was divided into two sub-clusters according to the degree of similarity between the individuals within the populations of *Moringa peregrina.*

The plant populations evaluated in this study were characterized by a high genetic diversity level (95.54%) between individuals either within or among the two populations. This may be caused by cross pollination between *Moringa peregrina* plants in the nature or they may by originated from different geographical origins and under various habitat conditions in South Sinai. **Karron (1991)** reported that the high genetic variation within a population may be caused by the outcrossing pollination phenomenon.

The present study supports the use of ISSR molecular marker as a suitable and accurate tool for population genetic diversity detection. It is widely used and is accepted as a tool in population genetic studies of both wild and cultivated plants **(Roy and Chakraborty, 2009)**. It is a simple, fast, efficient, and highly reproducible technique of low-cost use **(Zietkiewicz *et al.*, 1994 and Roy and Chakraborty, 2009)**.

In the present study, the genetic diversity was higher among the two studied populations than within each one, which could by contributed to the geographic distance between the two populations and the differences in the ecological properties of each. The high genetic diversity (percentage of polymorphism) between the individuals of *Moringa peregrina* is in harmony with that of *Rhodiola crenulata* natural populations as reported by **Lei *et al.* (2006)**. A percentage of polymorphism of 97.83% was detected among nine populations in eastern Tibet and northern Yunnan using 12 ISSR primers. Also, the results agree with the findings of **Singh *et al.* (2007)**, who studied the genetic diversity of *Ziziphus mauritiana* using ISSR markers and found a high polymorphism (98.28%) between the selected individuals. Similar results were recorded by **Aabd *et al.* (2015)**, who found a percentage of polymorphism of 75.58 to 82.56%, between 150 trees of *Argania spinosa* using rice ISSR primer. Also, **Alansi *et al.* (2016)** reveled 93.4% polymorphism between 34 accessions of *Ziziphus spina-christi* by 11 ISSR primers. Recently, **Nilkanta *et al.* (2017)** used ISSR marker analysis to analyze genetic variations within the populations of *Melocanna baccifera* (bomboo) and found 80.58% of genetic variation was exhibited within the populations.

Estimating the genetic diversity between *Moringa peregrina* individuals is critical for the protection and conservation of the plant for it is long-term availability both in the term of ecological biodiversity and medicinal related uses. Accurate estimates of genetic diversity are useful for optimizing sampling strategies and for conserving and managing the genetic diversity of trees **(Godt and Hamrick, 1996)**. The population genetic structure of a species is affected by multiple evolutionary factors including the mating system, gene flow, mode of reproduction and natural selection **(Hamrick and Godt, 1989)**.

The high genetic diversity between individuals as shown in the present study is an advantage because it means that *Moringa peregrina* plant can survive and tolerate the climate changes because of the high level of polymorphism detected. This also indicated the urgent necessity of conserving and protecting all the existing individuals in the region to preserve this genetic diversity.

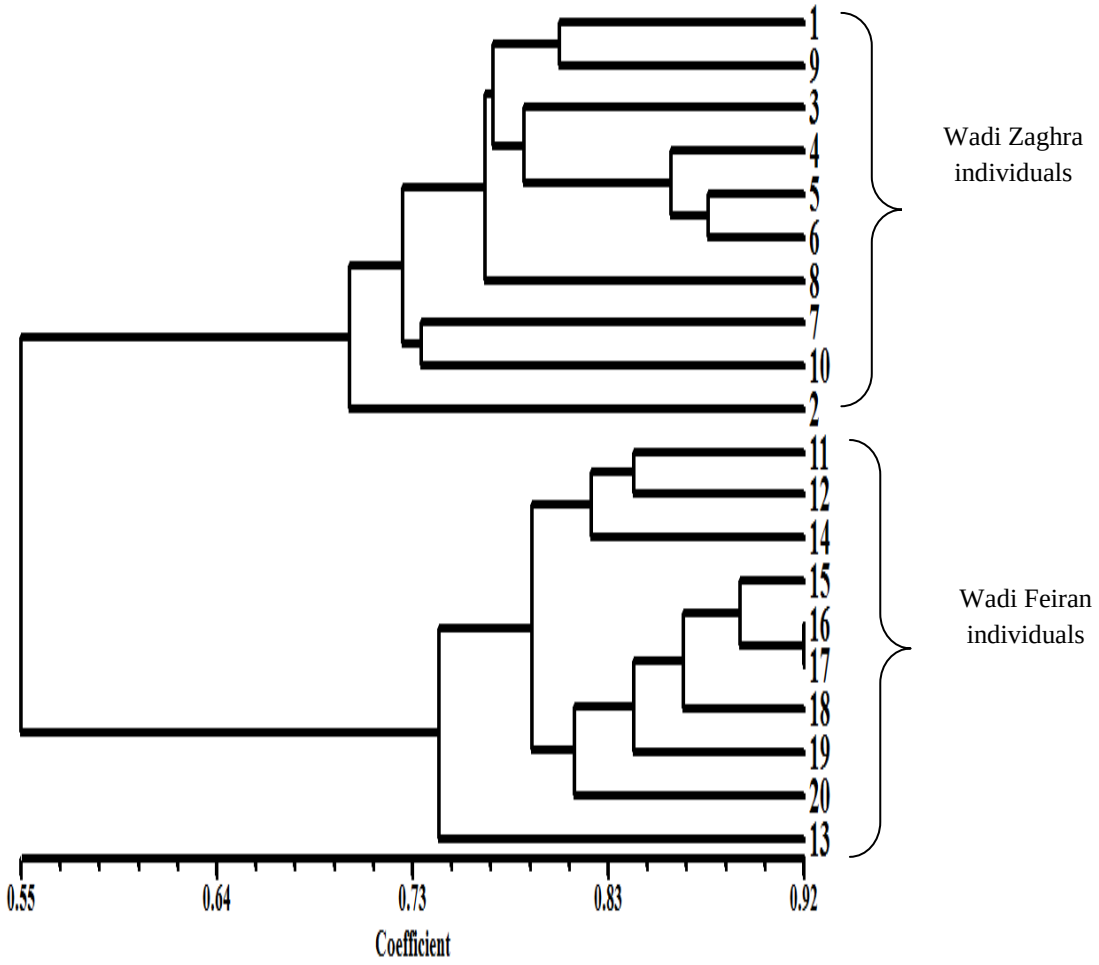


Fig. (6): A dendrogram showing the relationship between the examined *Moringa peregrina* individuals based on the data obtained by the ISSR technique.

This study should be made appropriately by larger sampling and more techniques to represent all the diversity existing on the scale of the natural distribution of *Moringa peregrina*.

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الملخص العربي

التنـوع الوراثي في عشـائـر نبـات اليســار النــامي طبيعيــًا في مصــر

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تم إختبار التنوع الوراثي لعشيرتين من نبات اليسار النامي طبيعيًا في جنوب سيناء، حيث تم إستخدام البصمة الوراثية بتقنية ISSR من خلال عشرة بادئات، وتعتبر خطوة في إتجاه الحفاظ على المصادر الوراثية لهذا النبات. الهدف من هذا البحث هو دراسة التنوع الوراثي لنبات اليسار بين أفراد العشيرة الواحدة وبين أفراد العشيرتين، حيث تم تجميع العينات النباتية من عشرة أفراد من وادي زغرة وأخرى من وادي فيران. أوضحت النتائج أن نسبة الإختلاف بين أفراد عشيرة وادي زغرة كانت حوالي ٧٨٪ وبين أفراد عشيرة وادي فيران كانت حوالي ٧٥٪ وبين الأفراد النباتية في العشرتين بالواديين كانت حوالي ٩٥٪. أوضحت النتائج أن أفراد النبات أكثر تشابهًا فيما بينها داخل العشيرة الواحدة ويقل هذا التشابه عند مقارنة الأفراد بين العشائر المختلفة، وهذا التنوع الوراثي بين أفراد نبات اليسار يؤثر إيجابيًا على بقاء النوع النباتي، وبالتالي الحفاظ عليه من الإنفراض يتطلب المحافظة على هذا التنوع بشتي الطرق الممكنة.

الكلمات الإسترشادية: البصمة الوراثية، المورينجا، اليسار، جنوب سيناء