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Genetic Structure of Cactus Pear (*Opuntia ficus indica*) in Moroccan Collection

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Abstract: Recently, a large plantation has been established in Morocco, to reduce water and wind erosion, rangeland degradation, sand movement and to enhance the restoration of the vegetation cover. However, this plant material has unknown genetic characterization. In addition, several local classifications and morphological descriptions were used. The objective of this study was to analyze the genetic diversity using RAPD markers in a collection of 13 provenances of Moroccan *Opuntia ficus indica* (L.). Based on 13 random primers, the result showed that the level of diversity (h) and polymorphism varied according to the provenance. A high genetic differentiation was found between the provenances (Gst = 0.29), thus some loci were characteristic of certain provenances. These results can be used to characterize genetic resources of Morocco cactus pear and to initiate a program of genetic improvement and selection.

Key words: *Opuntia ficus indica*, RAPD, genetic diversity, genetic resources

INTRODUCTION

The genus Opuntia belongs to the Cactaceae family (subfamily Opuntioideae) and includes several species that originated in North and South America, some of which were relatively recently distributed throughout the world. The number of species included in the genus is unknown (Chavez-Moreno *et al*., 2009). The cactus was introduced to the Mediterranean during the 16th century (Barbera, 1995). In Morocco, *Opuntia ficus* represented particularly in arid and semi-arid areas, from Oriental (Oujda, Figuig) to South (Sidi Ifni) through the arid plateaus of Haouz. Cactus pear constitutes, in these areas a resource to control water and wind erosion in eroded soils. The taxonomy of cacti has been traditionally based on comparative observation of morphological and biogeographic data (Metzing and Kiesling, 2008). The knowledge of the detailed descriptions are needed in the use of germplasm, because in this germplasm may have valuable genes for use in breeding programs.

Due to the multiple uses and the ability of cacti to thrive in arid and semiarid environments, it has become increasingly important to describe and characterize these valuable resources. The latter is a challenging goal since up to now; knowledge regarding the amount of genetic variation and genetic relationship by means of molecular tools is missing in Moroccan Opuntia ficus indica. In fact, though this crop is widely cultivated in the country, the majority of research works were especially oriented towards the characterization of the nutritional value of the cladodes as an important fodder crop in arid areas, independently from their genetic potential (Boujghagh and Chajia, 2001). At present, molecular markers have been proved to be valuable tools in the characterization and evaluation of genetic diversity within and between species and populations. It has been shown that different markers might reveal different classes of variation (Russell *et al*., 1997). The advent of the Polymerase Chain Reaction (PCR) favored the development of different molecular techniques (Saiki *et al*., 1988). These molecular markers had been successfully used in Opuntia genus for detecting genetic diversity and relationships (Arnholdt-Schmitt *et al*., 2001; Labra *et al*., 2003). Of these techniques, RAPD has several advantages, such as simplicity of use, low cost and the use of small amount of plant material, etc.

The success of cactus plantations in arid and sub-arid environments insists on leading researches on the management of Moroccan genetic resources, which will permit a well knowledge of the planted cacti. At the present, no study is yet available based on genetic variation and relationship by molecular tools in *Opuntia ficus indica* of Morocco.

The objectives of the present study were to assess the usefulness of RAPD to differentiate *Opuntia* ecotypes and to investigate genetic relationships among different

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ecotypes and to set up rational decisions concerning the establishment of a national reference collection. Indeed, though this crop is widely cultivated in the country, collection repositories are missing.

MATERIALS AND METHODS

The Cladodes of *Opuntia ficus indica*, representing 13 provenances were collected from 13 localities in Morocco (Table 1) and planted in Faculty of Sciences Agadir, this material were used for molecular polymorphism (RAPD) research. For each accession, an external slice of the cladode was taken for analysis. A piece of about 1 g of the chlorenchyma was cut using a scalpel and taking care not to include areole. The protocol of DNA extraction used here is that of (Saghai-Maroof *et al*., 1984) modified. DNA was quantified by visual comparison with lambda DNA molecular marker on ethidim bromide stained agarose gels.

The amplification is performed according to the protocol (Arnholdt-Schmitt *et al*., 2001), 14 oligonucleotide (decamer) were used for the amplification of random DNA (OPA-03, OPA-08, OPA-09, OPA-10, OPA-11, OPA-12, OPA-13, OPA-14, OPA-15, OPA-16, OPA-17, OPA-18, OPA-19, OPA-20). PCR reactions were performed in a 25 mL reaction mixture containing: 2.5 ng of template DNA, 2.5 mL of Go Taq buffer (Promega), 3.5 mL of dNTPs, 2 mL of 25 mM $MgCl_2$, 25 pmol of primer and 0.5 U of Go Taq DNA polymerase (Promega). The PCR was performed in a Thermoblock thermocycler (Techne).

PCR amplification was performed using the following profile: 5 min at 95°C; 45 cycles of denaturation (1 min at 95°C), annealing (1 min at 36°C) and extension (5 min at 72°C); then a final step for 5 min at 72°C.

Products of the PCR were separated by electrophoresis in 2% agarose gels using a volt range of 2 Vcm^{-1} during 3 h. The gel was finally fixed with ethidium bromide and photographed under UV light. Size of alleles was defined using the molecular weight marker 100 bp DNA leaders.

Table 1: Geographic localization of several provenances of *Opuntia ficus indica* under study

Provenance	Vernacular names	Latitude W	Longitude N	Altitude (m)
Ait baha	Achefri	30°10'	9°14'	222
Tafraout	Achefri	29°53'	9°00'	1215
Sidi-Ifni M	Moussa	29°20'	10°08'	66
Sidi-Ifni A	Aissa	29°20'	10°08'	65
Tamri	Achefri	30°40'	9°52'	36
Ben guerir	Aknari	32°30'	7°53'	434
Chaoun	Zaaboul	35°08'	5°16'	555
Hoceima	Delahia	30°57'	4°17'	1100
Nador	Hndiya	35°15'	3°40'	70
Berkane	Hndiya	34°51'	2°36'	200
Meknès	Hndiya	33°47'	5°29'	700
Mohammedia	Hndiya	33°41'	7°19'	64
Safi	Aknari	32°40'	9°04'	56

Different RAPD amplified bands were scored for their presence (1) or absence (0) and the resulting data matrices were computed with Nei coefficient to provide a similarity matrix. Different parameters have been estimated and the ability of primers to distinguish between individuals was calculated by evaluating the resolving power, POPGENE32 software was used to treat the binary matrices obtained to estimate genetic parameters intra-provenances (%P: percentage of loci polymorphic, h: Nei's genetic diversity (Nei, 1973) and inter-provenance (Gst: coefficient of genetic differentiation and genetic distance (Nei, 1978).

RESULTS AND DISCUSSION

Photometric measurements at 260 and 280 nm of the nucleic acid extracts of the various accessions of Barbary fig indicated a quotient (260/280) between 1.82 and 2.02. Thus, the obtained DNA is of high quality.

Among the 14 primers used to assess polymorphism in the tested ecotypes, 13 have revealed unambiguously scorable bands, these mentioned primers generated multiple banding each produced two to twenty readable tapes and are reproducible (Table 2). OPA-13 primer was abandoned because of the difficulty of reading the gel. A total of 105 bands were obtained which 55 were monomorphic and 50 were polymorphic. The average number of bands per primer was 8.07. All bands reported 73 different electrophoretic profiles. The resolving power of primers varied from 0 for the primer OPA-18 to 7.68 for primer OPA-14 with an average value of 1.55. The primer OPA-14, followed by primer OPA-11 and OPA-12, primers seem to be the most efficient to assess the genetic diversity, since they presented relatively high resolving power rates among the prickly pear in Morocco.

Genetic diversity (h) has a nil value for the accessions analyzed of Meknes Mohammedia and Safi and a value ranges from 0.01 to 0.11 for other provenances (Fig. 1). Thus, the ecotypes tested from Meknes, Mohammedia and Safi are monomorphic, while other ecotypes have different levels of polymorphism. The ecotype of Chaoun is the most highly polymorphic (h = 0.11).

Gst coefficient has a value of 0.29 if we takes into account all the loci studied and 0.75 if we limit only to polymorphic loci. It seems that in both cases, studied provenances have a significant structuring and gene flows are very limited. Gst furthermore the Gst coefficient has the greatest value for loci OPA-11-01-03 and OPA-04

Table 2: Parameters of genetic diversity evaluated on the RAPD markers in Moroccan cacti

Primers	No. of bands	No. of polymorphic bands	Polymorphic bands (%)	Number of RAPD profiles	Resolving power (Rp)
OPA-03	4	1	25	1	0.24
OPA-08	4	3	75	4	1.66
OPA-09	2	1	50	2	0.04
OPA-10	9	4	44.44	6	1.24
OPA-11	9	8	88.88	14	2.98
OPA-12	7	5	71.42	7	2.2
OPA-13	-	-	-	-	-
OPA-14	20	18	90	22	7.68
OPA-15	13	2	15.38	4	1.02
OPA-16	6	1	16.66	2	0.24
OPA-17	7	2	28.57	3	0.76
OPA-18	7	0	0	1	0
OPA-19	10	4	40	5	2.1
OPA-20	7	1	14.28	2	0.1
Total	105	50	-	73	20.26
Means	8.07	3.84	-	-	1.55

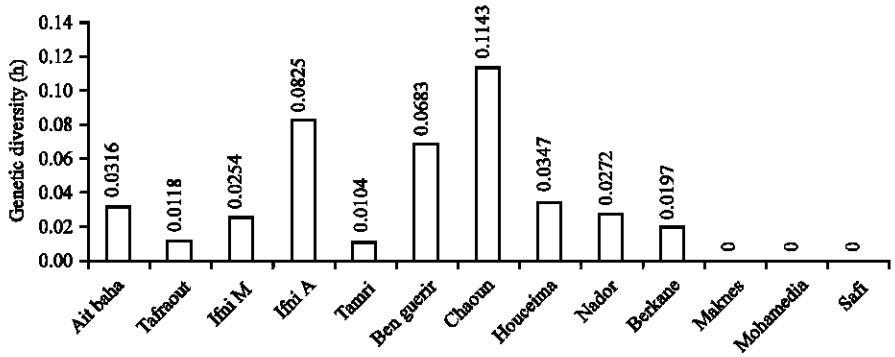

Fig. 1: Nei's Genetic diversity (h) within provenances

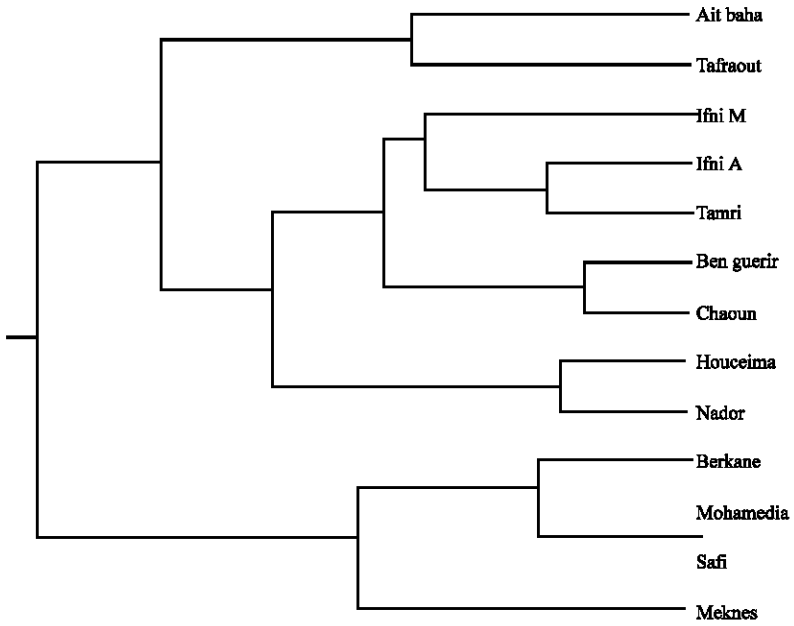

Fig. 2: Dendrogramme of 13 provenances of *Opuntia ficus indica* throughout Nei's genetic distance

(Gst = 1). Thus, these loci characterize provenances of Ait Baha and Tafraout. The genetic distance of (Nei, 1978) in terms of dendrogram (Fig. 2, 3) shows different levels of similarities and dissimilarities between studied

Pop	Ait baha	Tafraout	Ifni M	Ifni A	Tamri	Ben grir	Chaoun	Hoceima	Nador	Berkane	Meknes	Mohmadia	Safi
Ait baha	****												
Tafraout	0.0632	****											
Ifni M	0.0985	0.1028	****										
Ifni A	0.1011	0.0990	0.0415	****									
Tamri	0.1127	0.0952	0.0867	0.0339	****								
Ben grir	0.0769	0.0784	0.0723	0.0445	0.0628	****							
Chaoun	0.1000	0.1139	0.0718	0.0613	0.0972	0.0253	****						
Hoceima	0.1491	0.1646	0.0656	0.0809	0.1179	0.0595	0.0635	****					
Nador	0.1691	0.2067	0.1068	0.1062	0.1541	0.0996	0.0764	0.0307	****				
Berkane	0.1974	0.1714	0.1004	0.1112	0.1430	0.1276	0.0986	0.0775	0.0719	****			
Meknes	0.1685	0.1787	0.0847	0.1231	0.1867	0.1303	0.0897	0.1012	0.0855	0.0432	****		
Mohmadia	0.2308	0.2166	0.1529	0.1707	0.1885	0.1814	0.1275	0.1218	0.1170	0.0363	0.0896	****	
Safi	0.2308	0.2166	0.1529	0.1707	0.1885	0.1814	0.1275	0.1218	0.1170	0.0363	0.0896	0.0000	****

Fig. 3: Matrix of genetic distances between 13 Moroccan provenances of prickly, based on RAPD data (Nei, 1978)

provenances. Therefore, apart from provenances of Safi and Mohamedia which are similar, all others provenances shows different cases, including the isolation of Ait Baha and Tafraout on the one hand and Berkane and Meknes on the other hand. These results showed a disparity between the varieties named Moussa (late) and Aissa (early) from Sidi Ifni. Also, the geographical distribution of the studied provenances is partially respected throughout the genetic similarities.

CONCLUSION

The results presented in this study are the first contributions to the application of molecular markers to assess the genetic diversity of prickly pear in Morocco. Exploring RAPD showed that provenances studied are genetically quite divergent. The loci analyzed participated differently to the description of this divergence. Thus, some loci were polymorphic and easily characterized some provenances.

These results may contribute to the establishment of a conservation program and management of the genetic diversity of prickly pear in Morocco. They can be developed by analyzing other markers with a larger sample. This work could also be combined to agro morphological traits to start a breeding program and a genetic selection.

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