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Characterization of genetic diversity of cactus species (*Opuntia spp.*) in Morocco by morphological traits and molecular markers

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Abstract

The genetic diversity within and among 124 accessions of *Opuntia spp*. collected from different regions of Morocco was assessed using morphological descriptors and molecular markers. Based on 10 morphological traits, the accessions were separated into 3 main clusters; each cluster was containing accessions from different regions and species. Polymerase chain reaction (PCR) was then performed on 22 accessions from different regions and species, with 10 inter-simple sequence repeat (ISSR) primers and one random amplified polymorphic DNA (RAPD) primer. ISSR primers produced 66 bands overall, 64 (96.9 %) of which were polymorphic while 6 bands were generated by the RAPD marker, all polymorphic. The polymorphic information content (PIC) values ranged from 0.62 to 0.97, with an average of 0.82. The dendrogram of genetic differences generated using the unweighted pair-group method using arithmetic averages (UPGMA) method showed 7 different clusters at a similarity of 0.76, which was confirmed by the principal component analysis (PCA). The main conclusion of our work is the high genetic similarity between *Opuntia ficus indica* and *Opuntia megacantha* species in Morocco. Our results will be useful for plant breeding and genetic resource conservation programs.

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Introduction

The cactus pear (*Opuntia spp.*) is a plant genus native to arid and semi-arid regions of Mexico[1]. It belongs to the family Cactaceae, which is consisting of about1500 species[30] and it is widely distributed throughout temperate, subtropical and cold regions (Feugang et al. 2006).In Morocco, the cactus pear is one of the most important crop plants in the semi-arid zones. It covers an area of about 120000 ha[5] and it is represented mainly by the species *Opuntia ficus indica*. The accessions of this species are classified into three distinct populations: (i) the Christians' nopal with prickly cladodes used as a field fence; (ii) the Muslims' nopal with inermis cladodes used as a green fodder for cattle and (iii) the Moses' nopal with large inermis cladodes that produce big pears[20]. The cactus pear population is present throughout the country, from Eastern to Southern Morocco[18], with predominance in Guelmim-Sidi Ifni and Haouz-El Kelâa des Sraghnas regions[5]. Increasing the cactus pear area of cultivation is one of the strategies of the Moroccan Ministry of Agriculture. The cactus pear is a tree with high economic and ecologic value and various uses. It is cultivated for human consumption since it is characterized by important nutritional components[42]. The cactus pear has also many beneficial effects on health, including anti-cancer effect, anti-viral effect and anti-diabetic-effect among others[23]. On the other hand, the cactus pear may be utilized as forage due to its nutritional characteristics; along this line, Andrade-Montemayor et al. (2011)[4] reported that cactus cladodes could be used for goats feeding in semi-arid regions. The cactus pear was also successfully used for feeding Santa Inês lambs[13] and dromedary camel[15]. Furthermore, it was reported that some cactus constituents could be extracted and used as food additives or for pharmaceutical and cosmetic purposes[32].The process of agricultural intensification and modern agricultural practices has led to genetic diversity degradation and gene pools loss[2-28]. Determining the level of genetic diversity among and within plant populations is primordial for genetic resources conservation and breeding programs[24-28]. The use of morphological descriptors is of great help for genetic diversity assessment[14]. In the recent years, molecular markers have been proven powerful to determine the genetic diversity among and within species[12] and could be used as complementary methods to morphological descriptors[14]. Along this line, inter-simple sequence repeat (ISSR) and random amplified polymorphic DNA (RAPD) markers have been widely used to assess the genetic diversity in many species. Both the techniques are characterized by their simplicity, rapidity, ability to show high polymorphism with no prior knowledge of the genome studied[36-41]. In the recent years, several studies have been carried out on the genetic diversity of the cactus pear. For example, Valadez-Moctezuma et al. (2014)[39] studied the genetic diversity within and among Mexican *Opuntia* species using RAPD and ISSR markers. In Italy, Labra et al. (2003)[27] evaluated the genetic diversity in *Opuntia* species with cpSSR and AFLP markers. Zoghlami et al. (2007)[44] have assessed the genetic diversity of *Opuntia ficus indica* L. in Tunisia using RAPD markers. More recently, Bendhifi et al. (2013)[6] published another work on the genetic diversity of the same species in Tunisia using morphological descriptors and RAPD markers. Despite the high importance of the cactus pear in Morocco, its genetic diversity has been scarcely studied. Therefore, the aim of this investigation was to assess the genetic diversity within and among *Opuntia spp*. from different regions of Morocco, using morphological descriptors as well as ISSR and RAPD markers.

Materials and Methods

Plant Material

In the following investigation, we used 124 *Opuntia spp*. accessions belonging to different species: *O. ficus indica; O. megacantha; O. robusta; O. aequatorialis; O. dillenii; O. leucotricha and O. inermis* (identified for the first time in Morocco; Table 1). The accessions are planted in the experimental site called Ain Nzagh of National Institute of Agronomic Research-Regional Research Centre of Settat, (INRA-CRRA Settat, Morocco). Originally, the accessions were collected from different geographical sites of Morocco as shown in Table 1 and Figure 1 then planted in the experimental site in June 2011. All the accessions have undergone the same culture edaphoclimatic conditions.

Morphological Analysis

In this study, 10 quantitative morphological traits including plant height, plant diameter, average number of cladodes per plant, cladode height, cladode width, index of cladode shape (calculated as the ration of one-year-old cladodes length divided by their width), cladode thickness, mean distance between areoles, average number of spines per areole and mean length of the longest spine per areole. Data were recorded from 4 randomly chosen plants (2 years old) for each accession. Morphological data were subjected to analysis of variance (ANOVA) and means were separated with the Student-Newman-Keuls test at a 0.05 probability level. A proximity matrix was generated using Squared Euclidean distance then clustering of accessions was performed using Ward's method. All statistical analyses were performed with SPSS v 16.0 software (IBM, Chicago, IL, USA).

Molecular Analysis

DNA Extraction

In this study, we used 22 ecotypes from different geographic origins in Morocco, and which represent seven species of *Opuntia* identified from the morphological traits; for example, for O. ficus indica, we used 8 accessions, each one was collected from a different region. Total genomic DNA was extracted according to the protocol of Doyle and Doyle (1990)[17] with some slight modifications, using 1 g of lyophilized and ground cladode tissue. The DNA concentration was then diluted to 20 ng/µL with TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8) using a biophotometer (Eppendorf).

Issr Analysis

Fourteen ISSR primers (Table 2) purchased either from Operon Technologies Inc. (Alameda, CA, USA) or Qiagen (Valencia, CA, USA) were tested in this study; and 2.5 µL of each primer was added to a PCR mixture containing 3 µL DNA template, 12.3 µL sterile distilled water, 3.5 µL (25 mM) MgCl2, 1 µL(10 mM) dNTPs, 0.2 µL(5u/µL) Taq polymerase and 2.5 µL Taq buffer (All purchased from Promega, Madison, WI, USA).DNA amplifications were performed using a DNA thermal cycler (Techne) with the following parameters: initial denaturation at 94°C for 5 min, 45 cycles of 15 s of denaturation at 94 °C, 30 s of annealing at the specific temperature of each primer (Table 2), and 60 s of extension at 72°C; then a final extension at 72 °C for 7 min.

Rapd Analysis

For RAPD analysis, 34 RAPD primers (Table 2) purchased from The University of British Columbia (UBC; Vancouver, Canada) were tested. The PCR mixture was containing the same above-mentioned components for ISSR primers, but with substituting ISSR primers with 0.4 µM of each RAPD primer. Amplification conditions were as follows: initial denaturation at 94°C for 5 min followed by 30 cycles of denaturation at 94 °C for 60 s, annealing at 36 °C for 60 s, and extension at 72 °C for 90 s; with a final extension at 72 °C for 15 min.

Issr and Rapd Gel Electrophoresis

After amplification, 5 µL bromophenol blue (Sigma, St. Louis, MO, USA) was added to each amplification product. Afterwards, 8 µL of each sample was electrophoresed at 90 V, 5 W for 1 h on 1.8 % agarose gel (Promega, Madison, WI, USA) in 100 mL TBE buffer (90 mL sterile distilled water, 10 mL TBE buffer 10X; Promega, Madison, WI, USA) supplemented with 6.6 µL ethidium bromide (Sigma, St. Louis, MO, USA). Molecular weight of the amplified bands was estimated by comparison with lambda DNA/*EcoR* I and Hind III markers (Promega, Madison, WI, USA) as standard. The samples were then visualized and photographed with a gel documentation system (Fisher Scientific).

Statistical Analysis of Molecular Data

For each gel, we recorded the presence (1) or absence (0) of bands in individual lanes; consequently, a binary data matrix was created. For each marker, we calculated the polymorphic

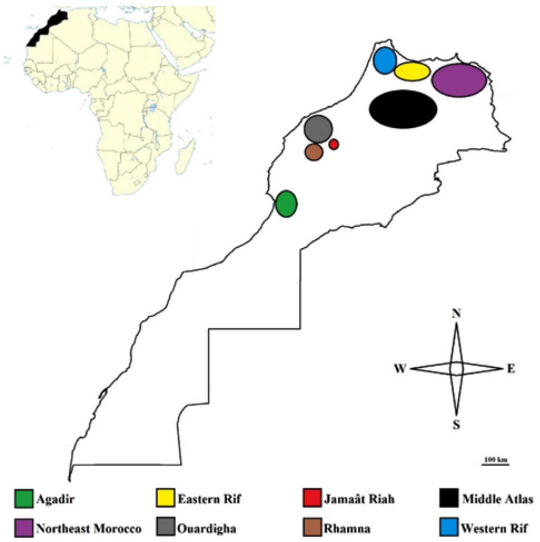

Fig. 1: Map of Morocco showing the geographic origin of the 124 accessions of *Opuntia spp*. used in this study.

information content (PIC) using the formula developed by Botstein et al. (1980)[7] :

$$\mathrm{PIC} = 1 - \sum_{i=1}^{n} pi^2 - 2\sum_{i=1}^{n-1}\sum_{j=i+1}^{n} pi^2\, pj^2$$

where pi and pj are the frequencies of the ith and jth alleles and n is the total number of such alleles.

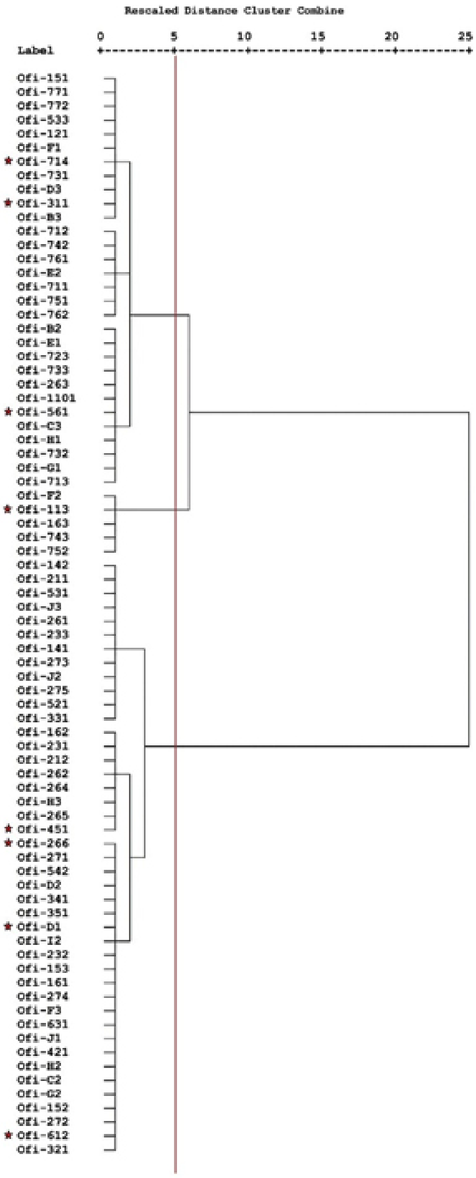

Fig. 2: Dendrogram grouping 78 accessions of *Opuntia ficus indica* based on 10 morphological traits and Ward's method. The red stars indicate accessions used for molecular analysis.

The Dice's coefficients were calculated and a dendrogram was generated based on the similarity matrix and the SAHN module using unweighted pair-group method of arithmetic analysis (UPGMA). Afterwards, a principal component analysis (PCA) was performed. All these analyses were conducted using NTSYSpc v 2.1 software[34].

Results and Discussion

Morphological Traits to Assess Genetic Variability

Genetic Variability Within Opuntia Ficus Indica

Our investigation showed that *Opuntia ficus indica* is present in all the studied areas. However, our results demonstrated clear differences among the 78 accessions with regard to morphological descriptors. For example, the plant height was ranging from 21.33 cm in Ofi-531 (from Eastern Rif) to 48.25 cm in Ofi-762 (from Agadir) and the plant diameter was ranging from 16.43 cm in Ofi-275 (from Ouardigha) to 71.38 in Ofi-163 (from Rhamna). In some cases,

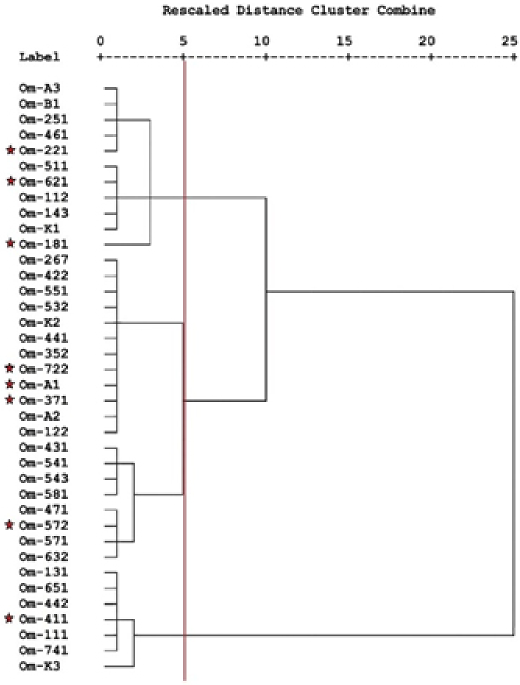

Fig. 3: Dendrogram grouping 38 accessions of *Opuntia megacantha* based on 10 morphological traits and Ward's method. The red stars indicate accessions used for molecular analysis.

Table 1: Means (+ SD) of morphological traits in different accessions of Opuntia spp. in Morocco
***Mean values with the same letters are not significantly different (p> 0.05)**
(-): Absence of spine

Species	Geographic origin	Accession	Plant height (cm)	Plant diameter (cm)	Average number of cladodes per plant	Cladode height (cm)	Cladode width (cm)	Index of cladoe shape (cm)	Cladode thickness (cm)	Mean distance between areoles (cm)	Average no. of spines per areole	Mean length of the longest spine per areole (cm)
O. ficus indica	Jamaât Riah	Ofi-B2	36.80 +3.16 abcd	45.08 +1.92 abcdefghi	3.00 +2.16 cdef	26.63 +2.36 bcdefghijk	13.63 +2.41 bc	0.51 + 0.09 bcdef	0.83 + 0.22 abc	2.70 + 0.36 bcdefg	-	-
	Jamaât Riah	Ofi-B3	37.10 +2.68 abcd	47.13 +9.91 abcdefghi	2.75 +0.96 cdef	21.98 +5.54 defghijkl	10.93 +2.44 bcd	0.50 +0.06 bcdef	0.68 +0.21 abc	2.58 +0.15 cdefg	-	-
	Jamaât Riah	Ofi-C2	33.38 +8.84 abcd	37.93 +16.12 abcdefghi	3.00 +0.82 cdef	21.80 +0.98 defghijkl	13.10 +0.33 bc	0.59 +0.01 bcdef	0.98 +0.05 abc	2.90 +0.08 bcdefg	-	-
	Jamaât Riah	Ofi-C3	36.25 +9.81 abcd	37.00 +6.77 abcdefghi	1.75 +0.50 ef	24.88 +1.84 bcdefghijk	13.25 +1.56 bc	0.56 +0.02 bcdef	0.73 +0.13 abc	2.93 +0.50 bcdefg	-	-
	Jamaât Riah	Ofi-D1	29.50 +4.55 abcd	28.38 +5.76 fghi	2.50 +0.58 cdef	20.08 +1.05 efghijkl	11.35 +0.29 bcd	0.57 +0.02 bcdef	1.10 +0.00 abc	2.45 +0.10 defg	-	-
	Jamaât Riah	Ofi-D2	30.13 +14.77 abcd	32.38 +14.51 cdefghi	2.00 +0.82 def	28.90 +3.53 bcdef	13.19 +3.05 bc	0.47 +0.05 bcdef	1.20 +0.00 abc	2.68 +0.49 bcdefg	-	-
	Jamaât Riah	Ofi-D3	36.38 +3.42 abcd	51.38 +7.61 abcdefghi	3.75 +0.50 bcdef	26.63 +3.38 bcdefghijk	13.85 +1.64 bc	0.52 +0.03 bcdef	0.90 +0.47 abc	2.68 +0.31 bcdefg	-	-
	Jamaât Riah	Ofi-E1	37.10 +10.54 abcd	43.33 +4.19 abcdefghi	2.25 +1.50 def	25.13 +2.78 bcdefghijk	13.30 +1.72 bc	0.62 +0.16 bcde	0.80 +0.29 abc	3.05 +0.33 bcdefg	-	-
	Jamaât Riah	Ofi-E2	42.75 +4.86 abc	52.25 +25.16 abcdefghi	3.75 +1.89 bcdef	27.43 +1.92 bcdefghij	13.80 +0.68 bc	0.50 +0.05 bcdef	0.75 +0.41 abc	2.90 +0.29 bcdefg	-	-
	Jamaât Riah	Ofi-F1	27.45 +12.12 abcd	51.95 +32.28 abcdefghi	3.50 +1.73 bcdef	28.25 +3.77 bcdefg	14.28 +1.81 bc	0.49 +0.04 bcdef	0.75 +0.41 abc	3.75 +0.29 ab	-	-
	Jamaât Riah	Ofi-F2	37.25 +7.58 abcd	66.00 +12.93 abc	4.00 +1.15 bcdef	28.15 +4.60 bcdefgh	14.25 +1.94 bc	0.51 +0.04 bcdef	0.78 +0.39 abc	3.18 +0.62 bcdefg	-	-
	Jamaât Riah	Ofi-F3	31.88 +1.89 abcd	34.00 +5.58 cdefghi	2.25 +0.50 def	22.10 +2.68 defghijkl	12.50 +0.71 bcd	0.55 +0.05 bcdef	0.60 +0.18 abc	2.78 +0.26 bcdefg	-	-
	Jamaât Riah	Ofi-G1	41.25 +14.31 abcd	37.28 +25.85 abcdefghi	3.50 +2.08 bcdef	27.55 +0.90 bcdefghij	10.13 +2.25 cd	0.41 +0.08 def	1.05 +0.12 abc	3.05 +0.29 bcdefg	-	-
	Jamaât Riah	Ofi-G2	32.63 +1.70 abcd	30.13 +4.01 cdefghi	1.50 +0.58 ef	23.75 +3.77 cdefghijkl	13.15 +2.21 bc	0.55 +0.02 bcdef	0.63 +0.13 abc	2.80 +0.24 bcdefg	-	-
	Jamaât Riah	Ofi-H1	39.38 +6.63 abcd	39.68 +1.89 abcdefghi	2.50 +0.58 cdef	26.45 +4.79 bcdefghijk	13.73 +1.39 bc	0.52 +0.08 bcdef	0.98 +0.32 abc	3.10 +0.20 bcdefg	-	-
	Jamaât Riah	Ofi-H2	30.00 +7.67 abcd	35.75 +5.74 bcdefghi	1.50 +0.58 ef	24.00 +2.04 cdefghijk	12.85 +0.53 bc	0.53 +0.02 bcdef	1.10 +0.16 abc	2.65 +0.04 bcdefg	-	-
	Jamaât Riah	Ofi-H3	26.30 +6.53 abcd	37.25 +2.33 abcdefghi	2.00 +0.00 def	21.60 +1.96 defghijkl	10.60 +1.55 bcd	0.48 +0.03 bcdef	1.20 +0.08 abc	2.60 +0.24 cdefg	-	-
	Jamaât Riah	Ofi-I2	29.88 +1.03 abcd	29.00 +11.05 efghi	1.75 +0.96 ef	18.17 +6.18 ghijkl	11.60 +1.10 bcd	0.72 +0.25 b	0.70 +0.24 abc	2.80 +0.43 bcdefg	-	-
	Jamaât Riah	Ofi-J1	30.93 +2.69 abcd	33.00 +8.77 cdefghi	2.50 +1.00 cdef	21.33 +1.43 defghijkl	10.43 +1.25 cd	0.49 +0.04 bcdef	0.70 +0.22 abc	2.73 +0.19 bcdefg	-	-
	Jamaât Riah	Ofi-J2	24.90 +10.25 bcd	27.23 +11.98 fghi	3.00 +0.82 cdef	19.50 +0.41 fghijkl	10.50 +1.22 cd	0.53 +0.05 bcdef	0.90 +0.08 abc	2.50 +0.41 defg	-	-
	Jamaât Riah	Ofi-J3	22.83 +4.28 cd	30.50 +3.67 cdefghi	2.75 +0.50 cdef	18.83 +1.09 fghijkl	9.88 +2.29 cd	0.53 +0.14 bcdef	0.98 +0.22 abc	2.55 +0.13 cdefg	-	-
	Rhamna	Ofi-113	35.00 +3.34 abcd	62.88 +15.93 abcdef	4.25 +1.50 bcdef	25.95 +3.09 bcdefghijk	13.33 +1.48 bc	0.51 +0.03 bcdef	0.75 +0.41 abc	2.75 +0.31 bcdefg	-	-
	Rhamna	Ofi-121	29.83 +2.17 abcd	46.75 +10.20 abcdefghi	6.25 +1.71 ab	25.28 +3.05 bcdefghijk	12.58 +1.26 bcd	0.50 +0.02 bcdef	0.88 +0.43 abc	2.90 +0.22 bcdefg	-	-
	Rhamna	Ofi-141	25.50 +0.00 bcd	26.50 +3.27 ghi	1.00 +0.00 f	16.30 +0.24 kl	9.50 +0.41 cd	0.57 +0.05 bcdef	0.90 +0.08 abc	2.60 +0.16 cdefg	-	-
	Rhamna	Ofi-142	24.75 +6.50 bcd	28.03 +4.01 fghi	1.00 +0.00 f	22.60 +1.96 cdefghijkl	12.85 +2.16 bc	0.56 +0.05 bcdef	1.15 +0.04 abc	2.65 +0.53 bcdefg	-	-
	Rhamna	Ofi-151	33.93 +3.99 abcd	45.45 +3.32 abcdefghi	2.25 +1.26 def	26.70 +2.52 bcdefghijk	12.00 +1.36 bcd	0.45 +0.08 bcdef	0.78 +0.22 abc	3.00 +0.14 bcdefg	-	-
	Rhamna	Ofi-152	31.48 +5.12 abcd	29.93 +1.34 defghi	1.50 +0.58 ef	24.50 +3.39 bcdefghijk	11.30 +2.43 bcd	0.45 +0.04 bcdef	0.80 +0.38 abc	2.83 +0.46 bcdefg	-	-
	Rhamna	Ofi-153	27.10 +8.23 abcd	32.78 +20.66 cdefghi	2.00 +0.82 def	22.14 +3.60 defghijkl	11.29 +2.47 bcd	0.47 +0.05 bcdef	1.00 +0.24 abc	3.23 +0.66 bcdefg	-	-
	Rhamna	Ofi-161	26.28 +5.33 abcd	31.33 +4.49 cdefghi	1.25 +0.50 f	20.43 +3.81 efghijkl	11.60 +2.05 bcd	0.57 +0.06 bcdef	0.78 +0.36 abc	2.78 +0.32 bcdefg	-	-
	Rhamna	Ofi-162	27.25 +2.30 abcd	43.05 +11.40 abcdefghi	2.50 +0.58 cdef	22.37 +1.69 cdefghijkl	12.77 +0.38 bc	0.57 +0.03 bcdef	0.80 +0.28 abc	2.80 +0.14 bcdefg	-	-
	Rhamna	Ofi-163	42.13 +5.39 abcd	71.38 +5.02 a	4.75 +1.89 bcde	29.13 +4.21 bcdef	14.53 +1.75 bc	0.50 +0.05 bcdef	0.88 +0.17 abc	2.85 +0.87 bcdefg	-	-
	Rhamna	Ofi-1101	38.00 +4.32 abcd	36.50 +7.08 bcdefghi	2.25 +0.50 def	27.70 +2.95 bcdefghi	13.48 +1.19 bc	0.49 +0.04 bcdef	0.95 +0.33 abc	3.00 +0.56 bcdefg	-	-
	Ouardigha	Ofi-211	25.38 +9.62 bcd	24.98 +9.72 ghi	1.00 +0.00 f	22.25 +1.02 defghijkl	12.00 +0.00 bcd	0.54 +0.02 bcdef	1.15 +0.12 abc	2.40 +0.08 efg	-	-

Ouardigha	Ofi-212	26.63 +3.68 $_{abcd}$	36.00 +6.16 $_{bcdefghi}$	1.50 +0.58 $_{ef}$	24.40 +2.57 $_{bcdefghijk}$	12.17 +1.03 $_{bcd}$	0.50 +0.05 $_{bcdef}$	1.13 +0.17 $_{abc}$	2.93 +0.45 $_{bcdefg}$	-	-
Ouardigha	Ofi-231	29.60 +3.93 $_{abcd}$	42.13 +12.68 $_{abcdefghi}$	2.50 +0.58 $_{cdef}$	21.10 +4.66 $_{defghijkl}$	11.05 +3.14 $_{bcd}$	0.52 +0.04 $_{bcdef}$	0.58 +0.21 $_{abc}$	2.53 +0.62 $_{defg}$	-	-
Ouardigha	Ofi-232	28.25 +3.97 $_{abcd}$	31.00 +4.32 $_{cdefghi}$	2.00 +0.82 $_{def}$	18.00 +1.63 $_{ghijkl}$	10.30 +0.24 $_{cd}$	0.57 +0.06 $_{bcdef}$	0.70 +0.16 $_{abc}$	2.80 +0.16 $_{bcdefg}$	-	-
Ouardigha	Ofi-233	24.13 +9.08 $_{bcd}$	29.00 +5.55 $_{efghi}$	1.75 +0.96 $_{ef}$	18.57 +1.41 $_{fghijkl}$	9.10 +0.65 $_{cd}$	0.49 +0.02 $_{bcdef}$	0.73 +0.12 $_{abc}$	2.57 +0.49 $_{cdefg}$	-	-
Ouardigha	Ofi-261	22.30 +3.19 $_{cd}$	29.45 +6.44 $_{defghi}$	1.00 +0.00 $_{f}$	19.60 +3.59 $_{fghijkl}$	10.65 +1.67 $_{bcd}$	0.54 +0.02 $_{bcdef}$	0.85 +0.12 $_{abc}$	2.70 +0.16 $_{bcdefg}$	-	-
Ouardigha	Ofi-262	26.70 +3.85 $_{abcd}$	35.63 +4.17 $_{bcdefghi}$	1.50 +0.58 $_{ef}$	22.81 +3.35 $_{cdefghijkl}$	11.43 +0.52 $_{bcd}$	0.49 +0.03 $_{bcdef}$	0.97 +0.34 $_{abc}$	2.73 +0.21 $_{bcdefg}$	-	-
Ouardigha	Ofi-263	39.13 +4.21 $_{abcd}$	44.60 +4.33 $_{abcdefghi}$	1.75 +0.50 $_{ef}$	30.45 +1.56 $_{abcde}$	13.50 +0.71 $_{bc}$	0.50 +0.12 $_{bcdef}$	0.93 +0.46 $_{abc}$	3.15 +0.24 $_{bcdefg}$	-	-
Ouardigha	Ofi-264	28.33 + 4.44 $_{abcd}$	36.88 + 9.19 $_{abcdefghi}$	2.50 +1.00 $_{cdef}$	23.90 +1.34 $_{cdefghijk}$	11.27 +0.56 $_{bcd}$	0.47 +0.03 $_{bcdef}$	0.47+0.10 $_{c}$	2.40 + 0.26 $_{efg}$	-	-
Ouardigha	Ofi-265	24.25 +0.61 $_{bcd}$	35.25 +5.92 $_{bcdefghi}$	3.00 +0.82 $_{cdef}$	23.20 +0.16 $_{cdefghijkl}$	10.20 +0.16 $_{cd}$	0.43 +0.08 $_{cdef}$	1.10 +0.08 $_{abc}$	2.90 +0.73 $_{bcdefg}$	-	-
Ouardigha	Ofi-266	29.88 + 5.02 $_{abcd}$	27.75 +1.32 $_{fghi}$	1.50 +0.58 $_{ef}$	27.23 +6.65 $_{bcdefghij}$	13.77 +1.45 $_{bc}$	0.52 +0.06 $_{bcdef}$	0.77 +0.09 $_{abc}$	3.33 +0.24 $_{bcdef}$	-	-
Ouardigha	Ofi-271	27.17 +7.71 $_{abcd}$	28.83 +8.66 $_{efghi}$	1.50 +0.58 $_{ef}$	26.20 +0.82 $_{bcdefghijk}$	12.45 +0.37 $_{bcd}$	0.47 +0.00 $_{bcdef}$	1.05 +0.04 $_{abc}$	3.00 +0.00 $_{bcdefg}$	-	-
Ouardigha	Ofi-272	35.88 +11.35 $_{abcd}$	30.75 +8.07 $_{cdefghi}$	2.00 +0.82 $_{def}$	22.48 +3.26 $_{cdefghijkl}$	12.18 +0.96 $_{bcd}$	0.54 +0.06 $_{bcdef}$	1.03 +0.10 $_{abc}$	2.68 +0.28 $_{bcdefg}$	-	-
Ouardigha	Ofi-273	24.33 +5.09 $_{bcd}$	27.38 +12.15 $_{fghi}$	1.50 +0.58 $_{ef}$	17.53 +2.50 $_{hijkl}$	9.87 +0.26 $_{cd}$	0.57 +0.10 $_{bcdef}$	0.63 +0.05 $_{abc}$	2.47 +0.39 $_{defg}$	-	-
Ouardigha	Ofi-274	28.38 +6.80 $_{abcd}$	35.00 +9.70 $_{bcdefghi}$	2.00 +0.82 $_{def}$	19.73 +6.88 $_{fghijkl}$	10.25 +1.19 $_{cd}$	0.56 +0.18 $_{bcdef}$	0.73 +0.10 $_{abc}$	2.43 +0.40 $_{efg}$	-	-
Ouardigha	Ofi-275	21.55 + 8.86 $_{cd}$	16.43 +3.51 $_{i}$	1.50 +0.58 $_{ef}$	18.55 +3.23 $_{fghijkl}$	12.30 +0.90 $_{bcd}$	0.67 +0.07 $_{bcd}$	1.15 +0.04 $_{abc}$	2.70 +0.24 $_{bcdefg}$	-	-
Middle atlas	Ofi-311	35.68 +7.92 $_{abcd}$	50.88 +3.45 $_{abcdefghi}$	2.25 +0.50 $_{def}$	24.15 +6.68 $_{bcdefghijk}$	11.65 +1.08 $_{bcd}$	0.50 +0.08 $_{bcdef}$	0.65 +0.10 $_{abc}$	2.53 +0.10 $_{defg}$	-	-
Middle atlas	Ofi-321	38.50 +19.07 $_{abcd}$	29.13 +12.80 $_{efghi}$	3.75 +0.96 $_{bcdef}$	23.47 +2.17 $_{cdefghijkl}$	12.87 +1.62 $_{bc}$	0.54 +0.03 $_{bcdef}$	0.70 +0.14 $_{abc}$	3.20 +0.14 $_{bcdefg}$	-	-
Middle atlas	Ofi-331	31.88 +8.23 $_{abcd}$	22.25 +1.66 $_{ghi}$	1.00 +0.00 $_{f}$	13.70 +0.00 $_{l}$	10.70 +0.00 $_{bcd}$	0.65 +0.11 $_{bcd}$	0.80 +0.00 $_{abc}$	2.55 +0.04 $_{cdefg}$	-	-
Middle atlas	Ofi-341	30.63 +6.94 $_{abcd}$	31.38 +7.06 $_{cdefghi}$	1.50 +0.58 $_{ef}$	19.27 +6.73 $_{fghijkl}$	13.00 +0.50 $_{bc}$	0.51 +0.05 $_{bcdef}$	0.83 +0.17 $_{abc}$	3.13 +0.26 $_{bcdefg}$	-	-
Middle atlas	Ofi-351	31.00 +8.13 $_{abcd}$	30.13 +10.01 $_{cdefghi}$	1.75 +0.50 $_{ef}$	21.07 +2.08 $_{defghijkl}$	11.93 +0.99 $_{bcd}$	0.56 +0.01 $_{bcdef}$	0.77 +0.29 $_{abc}$	3.03 +0.48 $_{bcdefg}$	-	-
Northeast Morocco	Ofi-421	31.00 +7.43 $_{abcd}$	32.68 +9.46 $_{cdefghi}$	1.50 +0.58 $_{ef}$	23.48 +1.07 $_{cdefghijkl}$	11.53 +1.02 $_{bcd}$	0.52 +0.06 $_{bcdef}$	0.73 +0.21 $_{abc}$	2.45 +0.26 $_{defg}$	-	-
Northeast Morocco	Ofi-451	24.50 +1.78 $_{bcd}$	39.63 +12.96 $_{abcdefghi}$	2.50 +0.58 $_{cdef}$	23.60 +1.31 $_{cdefghijkl}$	11.30 +0.41 $_{bcd}$	0.47 +0.01 $_{bcdef}$	0.70 +0.00 $_{abc}$	2.40 +0.08 $_{efg}$	-	-
Eastern Rif	Ofi-521	21.88 +6.51 $_{cd}$	21.50 +10.01 $_{hi}$	1.00 +0.00 $_{f}$	17.00 +1.63 $_{jkl}$	12.00 +1.63 $_{bcd}$	0.70 +0.16 $_{bc}$	1.10 +0.08 $_{abc}$	3.50 +0.41 $_{abcde}$	-	-
Eastern Rif	Ofi-531	21.33 +6.55 $_{cd}$	23.33 +7.32 $_{ghi}$	1.00 +0.00 $_{f}$	22.50 +0.41 $_{cdefghijkl}$	12.00 +1.63 $_{bcd}$	0.53 +0.02 $_{bcdef}$	1.00 +0.16 $_{abc}$	2.60 +0.33 $_{cdefg}$	-	-
Eastern Rif	Ofi-533	32.40 +14.02 $_{abcd}$	42.30 +23.93 $_{abcdefghi}$	3.75 +2.36 $_{bcdef}$	26.93 +1.37 $_{bcdefghij}$	12.90 +0.51 $_{bc}$	0.48 +0.04 $_{bcdef}$	0.73 +0.21 $_{abc}$	4.17 +1.31 $_{a}$	-	-
Eastern Rif	Ofi-542	26.38 +15.21 $_{abcd}$	29.75 +21.63 $_{defghi}$	2.00 +0.82 $_{def}$	29.05 +1.02 $_{bcdef}$	14.85 +0.78 $_{bc}$	0.51 +0.01 $_{bcdef}$	0.85 +0.04 $_{abc}$	3.70 +0.08 $_{abc}$	-	-
Eastern Rif	Ofi-561	37.25 +5.84 $_{abcd}$	34.93 +14.14 $_{bcdefghi}$	1.75 +0.96 $_{ef}$	26.20 +6.49 $_{bcdefghijk}$	12.75 +3.52 $_{bc}$	0.48 +0.05 $_{bcdef}$	0.78 +0.30 $_{abc}$	2.70 +0.48 $_{bcdefg}$	-	-
Western Rif	Ofi-612	36.25 +8.87 $_{abcd}$	33.63 +9.32 $_{cdefghi}$	2.75 +0.50 $_{cdef}$	23.10 +2.21 $_{cdefghijkl}$	11.57 +1.05 $_{bcd}$	0.50 +0.03 $_{bcdef}$	1.03 +0.09 $_{abc}$	2.37 +0.45 $_{efg}$	-	-
Western Rif	Ofi-631	30.20 +9.41 $_{abcd}$	33.88 +13.14 $_{cdefghi}$	3.50 +0.58 $_{bcdef}$	21.00 + 0.41 $_{defghijkl}$	12.25 +0.20 $_{bcd}$	0.58 +0.00 $_{bcdef}$	0.75 +0.04 $_{abc}$	2.80 +0.00 $_{bcdefg}$	-	-
Agadir	Ofi-711	41.70 +5.77 $_{abcd}$	50.80 +9.72 $_{abcdefghi}$	2.75 +1.26 $_{cdef}$	28.38 +2.84 $_{bcdefg}$	12.90 +2.37 $_{bc}$	0.49 +0.15 $_{bcdef}$	0.55 +0.19 $_{bc}$	3.03 +0.90 $_{bcdefg}$	-	-
Agadir	Ofi-712	40.17 +6.54 $_{abcd}$	57.50 +20.00 $_{abcdefgh}$	4.25 +2.63 $_{bcdef}$	26.03 +2.53 $_{bcdefghijk}$	15.47 +2.17 $_{bc}$	0.56 +0.04 $_{bcdef}$	0.87 +0.17 $_{abc}$	2.70 +0.22 $_{bcdefg}$	-	-
Agadir	Ofi-713	45.43 +4.64 $_{ab}$	38.75 +8.21 $_{abcdefghi}$	2.25 +1.26 $_{def}$	34.00 +3.58 $_{ab}$	20.23 +10.73 $_{a}$	0.58 +0.25 $_{bcdef}$	0.83 +0.26 $_{abc}$	3.00 +0.08 $_{bcdefg}$	-	-
Agadir	Ofi-714	35.78 +6.63 $_{abcd}$	47.43 +8.25 $_{abcdefghi}$	2.50 +0.58 $_{cdef}$	25.15 +9.11 $_{bcdefghijk}$	15.78 +3.26 $_{bc}$	0.67 +0.21 $_{bcd}$	0.86 + 0.21 $_{abc}$	2.85 +0.24 $_{bcdefg}$	-	-
Agadir	Ofi-723	39.03+11.53 $_{abcd}$	43.88 +15.29 $_{abcdefghi}$	2.25 +0.96 $_{def}$	28.45 +2.19 $_{bcdefg}$	15.08 +0.87 $_{bc}$	0.53 +0.04 $_{bcdef}$	1.00 +0.16 $_{abc}$	3.05 +0.10 $_{bcdefg}$	-	-
Agadir	Ofi-731	36.85 +4.98 $_{abcd}$	48.38 +7.62 $_{abcdefghi}$	2.00 +0.82 $_{def}$	27.43 +1.54 $_{bcdefghij}$	14.13 +1.26 $_{bc}$	0.51 +0.03 $_{bcdef}$	0.88 +0.28 $_{abc}$	2.95 +0.54 $_{bcdefg}$	-	-
Agadir	Ofi-732	42.68 +8.01 $_{abc}$	40.58 +8.39 $_{abcdefghi}$	2.50 +0.58 $_{cdef}$	29.00 +3.24 $_{bcdef}$	14.80 +0.99 $_{bc}$	0.51 +0.03 $_{bcdef}$	1.10 +0.22 $_{abc}$	3.13 +0.25 $_{bcdefg}$	-	-
Agadir	Ofi-733	38.25 + 10.44 $_{abcd}$	42.65 +16.01 $_{abcdefghi}$	2.25 +0.50 $_{def}$	27.65 +8.00 $_{bcdefghi}$	13.48 +2.67 $_{bc}$	0.48 +0.05 $_{bcdef}$	1.13 +0.56 $_{abc}$	3.00 +0.28 $_{bcdefg}$	-	-
Agadir	Ofi-742	42.93 +9.94 $_{abc}$	56.25 +23.09 $_{abcdefgh}$	3.75 +1.71 $_{bcdef}$	27.00 +2.27 $_{bcdefghij}$	13.33 +1.68 $_{bc}$	0.49 +0.07 $_{bcdef}$	0.75 +0.24 $_{abc}$	2.98 +0.24 $_{bcdefg}$	-	-
Agadir	Ofi-743	39.75 +10.43 $_{abcd}$	69.88 +26.42 $_{ab}$	4.25 +3.30 $_{bcdef}$	25.98 +4.29 $_{bcdefghijk}$	12.95 +1.32 $_{bc}$	0.50 +0.06 $_{bcdef}$	0.63 +0.29 $_{abc}$	3.03 +0.40 $_{bcdefg}$	-	-
Agadir	Ofi-751	45.75 +2.72 $_{ab}$	50.88 +19.84 $_{abcdefghi}$	4.00 +1.63 $_{bcdef}$	32.68 +10.79 $_{abc}$	13.13 +1.11 $_{bc}$	0.44 +0.18 $_{cdef}$	1.00 +0.26 $_{abc}$	3.05 +0.33 $_{bcdefg}$	-	-

	Agadir	Ofi-752	46.18 +3.94 ab	64.63 +6.42 abcde	5.25 +0.50 bcd	31.15 +2.56 abcd	15.63 +0.75 bc	0.50 +0.06 bcdef	0.73 +0.24 abc	2.88 +0.48 bcdefg	-	-
	Agadir	Ofi-761	40.00 +10.58 abcd	55.38 +19.74 abcdefgh	3.50 +1.29 bcdef	22.45 +6.94 cdefghijkl	13.43 +3.06 bc	0.63 +0.22 bcde	0.73 +0.40 abc	2.68 +0.24 bcdefg	-	-
	Agadir	Ofi-762	48.25 + 5.12 a	45.00 +13.20 abcdefghi	3.00 +1.41 cdef	23.65 +3.49 cdefghijkl	13.25 +1.46 bc	0.56 +0.08 bcdef	0.88 +0.34 abc	2.68 +0.30 bcdefg	-	-
	Agadir	Ofi-771	33.38 +3.25 abcd	46.00 +5.07 abcdefghi	3.00 +1.41 cdef	25.83 +1.72 bcdefghijk	12.05 +1.46 bcd	0.46 +0.05 bcdef	0.90 +0.16 abc	2.75 +0.65 bcdefg	-	-
	Agadir	Ofi-772	33.93 +3.00 abcd	47.07 +12.40 abcdefghi	2.00 +0.82 def	25.73 +1.46 bcdefghijk	12.80 +1.12 bc	0.49 +0.05 bcdef	0.73 +0.25 abc	2.63 +0.19 bcdefg	-	-
O. mega	Jamaât Riah	Om-A1	30.43 +3.54 abcd	42.05 +15.92 abcdefghi	2.75 +0.96 cdef	25.38 +3.50 bcdefghijk	12.38 +0.48 bcd	0.49 +0.07 bcdef	0.80 +0.18 abc	2.68 +0.39 bcdefg	4.25 + 1.50 abc	2.70 +0.22 ghijklmno
cantha	Jamaât Riah	Om-A2	32.15 + 2.83 abcd	43.05 +13.93 abcdefghi	2.00 +0.00 def	25.15 +1.65 bcdefghijk	14.67 +1.31 bc	0.58 +0.03 bcdef	0.90 +0.16 abc	2.90 +0.16 bcdefg	3.75 +0.50 cd	3.00 +0.50 cdefghijklm
	Jamaât Riah	Om-A3	29.03 +5.85 abcd	36.60 +6.13 bcdefghi	2.75 +0.96 cdef	25.40 +1.55 bcdefghijk	12.80 +0.98 bc	0.50 +0.01 bcdef	1.05 +0.04 abc	3.10 +0.33 bcdefg	3.00 +0.00 d	2.15 +0.29 nop
	Jamaât Riah	Om-B1	29.03 +5.85 abcd	36.60 +6.13 bcdefghi	1.75 +0.50 ef	25.25 +1.56 bcdefghijk	12.35 +1.11 bcd	0.49 +0.02 bcdef	0.98 +0.10 abc	2.95 + 0.37 bcdefg	3.00 + 0.00 d	2.38 +0.39 jklmno
	Jamaât Riah	Om-K1	25.00 +10.39 bcd	21.65 +12.88 hi	1.00 +0.00 f	23.53 +0.38 cdefghijkl	13.00 +1.63 bc	0.55 +0.04 bcdef	1.30 +0.08 abc	3.00 +0.41 bcdefg	4.00 +0.82 bcd	2.10 +0.08 op
	Jamaât Riah	Om-K2	33.93 +9.72 abcd	41.88 +10.36 abcdefghi	3.50 +1.00 bcdef	26.30 +3.50 bcdefghijk	12.83 +2.42 bc	0.48 +0.03 ef	1.40+1.33 a	2.90 +0.36 bcdefg	3.00 +0.82 d	3.07 +0.51 cdefghijk
	Jamaât Riah	Om-K3	32.63 +7.87 abcd	65.38 +14.10 abcd	4.25 +0.96 bcdef	26.28 +1.21 bcdefghijk	14.05 +1.17 bc	0.53 +0.03 bcdef	0.68 +0.33 abc	3.05 +0.17 bcdefg	3.50 +0.58 cd	2.60 +0.66 hijklmno
	Rhamna	Om-111	29.50 + 3.27 abcd	50.05 +3.23 abcdefghi	2.50 +0.58 cdef	24.85 +1.51 bcdefghijk	11.00 +0.00 bcd	0.44 +0.02 cdef	0.60 +0.00 abc	2.85 +0.12 bcdefg	4.00 +0.00 bcd	2.70 +0.00 ghijklmno
	Rhamna	Om-112	26.38 +2.06 abcd	30.13 +7.88 cdefghi	1.50 +0.58 ef	19.83 +4.19 fghijkl	9.40 +1.04 cd	0.48 +0.06 bcdef	0.53 +0.05 c	2.67 +0.47 bcdefg	3.00 +0.00 d	2.27 +0.12 mnop
	Rhamna	Om-122	28.90 +5.32 abcd	42.58 +8.63 abcdefghi	3.00 +1.41 cdef	23.17 +2.05 cdefghijkl	14.33 +3.52 bc	0.51 +0.01 bcdef	0.80 +0.33 abc	2.70 +0.50 bcdefg	3.00 +0.00 d	2.77 +0.46 ghijklmno
	Rhamna	Om-131	37.75 +3.88 abcd	55.38 +20.27 abcdefgh	3.75 +2.36 bcdef	23.75 +4.17 cdefghijkl	13.30 +2.03 bc	0.56 +0.03 bcdef	1.00 +0.47 abc	3.03 +0.67 bcdefg	4.00 +0.00 bcd	3.80 +0.58 ab
	Rhamna	Om-143	24.67 +0.62 bcd	34.07 +3.35 cdefghi	2.50 +1.73 cdef	22.70 +3.84 cdefghijkl	9.75 +0.20 cd	0.45 +0.09 bcdef	0.70 +0.16 abc	2.55 +0.04 cdefg	3.50 +0.58 cd	2.30 +0.08 lmnop
	Rhamna	Om-181	27.28 +4.30 abcd	32.88 +5.42 cdefghi	1.00 +0.00 f	37.00 +1.63 a	12.50 +0.41 bcd	0.33 +0.02 f	0.90 +0.08 abc	3.00 +0.08 bcdefg	4.00 +0.82 bcd	1.70 +0.16 p
	Ouardigha	Om-221	25.13 +8.13 bcd	39.25 +7.71 abcdefghi	2.00 +0.82 def	20.63 +1.25 defghijkl	10.88 +0.75 bcd	0.52 +0.03 bcdef	0.75 +0.26 abc	2.38 +0.15 efg	3.50 +0.58 cd	2.98 +0.66 defghijklm
	Ouardigha	Om-251	29.03 + 4.45 abcd	37.75 +11.26 abcdefghi	2.50 +0.58 cdef	21.70 +0.73 defghijkl	12.50 +0.82 bcd	0.57 +0.02 bcdef	1.00 +0.08 abc	3.00 +0.00 bcdefg	3.00 +0.00 d	2.30 +0.00 lmnop
	Middle atlas	Om-267	31.63 +2.17 abcd	43.38 +8.36 abcdefghi	2.75 +2.36 cdef	20.93 +2.81 defghijkl	11.15 +1.35 bcd	0.53 +0.04 bcdef	0.70 +0.18 abc	2.60 +0.34 cdefg	3.50 +1.00 cd	2.75 +0.80 ghijklmno
	Middle atlas	Om-352	31.88 +6.76 abcd	42.15 +13.78 abcdefghi	2.75 +0.50 cdef	22.83 +2.25 cdefghijkl	12.70 +0.92 bc	0.55 +0.02 bcdef	0.77 +0.26 abc	2.90 +0.43 bcdefg	3.50 +1.00 cd	2.83 +0.40 efghijklmn
	Middle atlas	Om-371	32.50 +6.54 abcd	42.38 +12.26 abcdefghi	2.25 +1.26 def	25.50 +3.97 cdefghijkl	12.75 +1.85 bc	0.57 +0.18 bcdef	0.83 +0.15 abc	2.75 +0.33 bcdefg	5.00 +1.15 a	3.08 +0.49 cdefghijk
	Northeast Morocco	Om-411	39.73 +8.94 abcd	58.50 +7.93 abcdefg	3.25 +0.50 cdef	25.30 +2.89 bcdefghijk	13.95 +2.42 bc	0.55 +0.05 bcdef	0.73 +0.24 abc	3.15 +0.83 bcdefg	4.25 +0.50 abc	3.13 +0.57 cdefghi
	Northeast Morocco	Om-422	30.63 +2.02 abcd	44.63 +7.38 abcdefghi	2.50 +1.73 cdef	22.88 +1.11 cdefghijkl	11.85 +2.28 bcd	0.52 +0.07 bcdef	0.80 +0.32 abc	2.73 +0.25 bcdefg	3.75 +0.50 cd	3.35 +0.13 bcdefg
	Northeast Morocco	Om-431	38.85 +7.35 abcd	40.95 +19.31 abcdefghi	3.00 +0.82 cdef	23.67 +1.43 cdefghijkl	13.23 +0.76 bc	0.55 +0.03 bcdef	0.80 +0.22 abc	2.75 +0.21 bcdefg	4.50 +0.58 abc	3.57 +0.39 abcd
	Northeast Morocco	Om-441	36.63 + 7.23 abcd	43.35 +10.61 abcdefghi	1.75 +0.96 ef	26.25 +4.52 bcdefghijk	12.97 +0.54 bc	0.47 +0.06 bcdef	1.20 +0.85 abc	2.63 +0.26 bcdefg	3.00 +0.00 d	2.97 +0.12 defghijklm
	Northeast Morocco	Om-442	35.25 +3.57 abcd	54.50 +7.33 abcdefgh	2.00 +0.00 def	26.43 +2.32 bcdefghijk	13.60 +0.90 bc	0.51 +0.01 bcdef	0.75 +0.21 abc	2.85 +0.39 bcdefg	3.75 +0.50 cd	3.28 +1.03 bcdefgh
	Northeast Morocco	Om-461	29.80 +12.09 abcd	34.15 +15.09 cdefghi	2.50 +0.58 cdef	22.55 +2.59 cdefghijkl	12.40 +1.73 bcd	0.54 +0.02 bcdef	0.87 +0.17 abc	2.43 +0.31 efg	3.50 +0.58 cd	2.53 +0.34 ijklmno
	Northeast Morocco	Om-471	35.00 +5.72 abcd	33.00 +8.16 cdefghi	1.00 +0.00 f	23.75 +1.84 cdefghijkl	13.75 +1.02 bc	0.57 +0.01 bcdef	0.85 +0.04 abc	3.05 +0.04 bcdefg	3.50 +0.58 cd	2.80 +0.08 fghijklmno
	Eastern Rif	Om-511	28.13 +8.93 abcd	30.20 +10.41 cdefghi	3.50 +0.58 bcdef	24.25 +3.06 bcdefghijk	12.90 + 1.96 bc	0.52 +0.01 bcdef	1.10 +0.00 abc	2.50 +0.00 defg	4.00 +0.00 bcd	2.85 +0.53 efghijklmn
	Eastern Rif	Om-532	36.50 +6.16 abcd	45.40 +20.76 abcdefghi	3.00 +1.15 cdef	20.98 +3.81 defghijkl	12.40 +2.09 bcd	0.59 +0.05 bcdef	0.80 +0.20 abc	2.75 +0.21 bcdefg	4.00 +0.00 bcd	2.43 +0.22 ijklmno
	Eastern Rif	Om-541	37.38 +6.14 abcd	40.75 +15.02 abcdefghi	2.00 +0.82 def	22.75 +3.84 cdefghijkl	12.38 +1.60 bcd	0.55 +0.06 bcdef	0.80 +0.29 abc	2.73 +0.46 bcdefg	3.50 +0.58 cd	2.43 +0.49 ijklmno
	Eastern Rif	Om-543	39.13 + 6.14 abcd	38.58 +9.14 abcdefghi	2.25 +0.50 def	20.90 +2.31 defghijkl	11.03 +1.86 bcd	0.52 +0.06 bcdef	0.85 +0.13 abc	2.13 +0.25 g	3.75 +0.50 cd	2.88 +0.25 efghijklmn
	Eastern Rif	Om-551	31.75 +5.39 abcd	44.50 +7.82 abcdefghi	2.50 +1.73 cdef	20.50 +4.02 efghijkl	13.85 +7.67 bc	0.70 +0.19 bc	0.90 +0.16 abc	2.30 +0.38 fg	4.00 +0.00 bcd	2.35 +0.64 klmno

	Eastern Rif	Om-571	40.33 +5.37 $_{abcd}$	35.50 +10.45 $_{bcdefghi}$	2.50 +1.73 $_{cdef}$	23.80 +2.03 $_{cdefghijkl}$	13.23 +1.34 $_{bc}$	0.55 +0.04 $_{bcdef}$	0.95 +0.10 $_{abc}$	2.33 +0.15 $_{fg}$	4.25 +0.50 $_{abc}$	2.40 +0.12 $_{ijklmno}$
	Eastern Rif	Om-572	37.50 + 11.02 $_{abcd}$	35.00 +4.49 $_{bcdefghi}$	1.00 +0.00 $_{f}$	25.35 +4.20 $_{bcdefghijk}$	13.85 +3.55 $_{bc}$	0.53 +0.05 $_{bcdef}$	1.05 +0.20 $_{abc}$	2.30 +0.41 $_{fg}$	3.50 +0.58 $_{cd}$	2.30 +0.08 $_{lmnop}$
	Eastern Rif	Om-581	35.63 +9.07 $_{abcd}$	40.38 +7.80 $_{abcdefghi}$	4.00 +1.83 $_{bcdef}$	21.13 +1.18 $_{defghijkl}$	11.40 +0.86 $_{bcd}$	0.54 +0.03 $_{bcdef}$	0.85 +0.06 $_{abc}$	2.05 +0.31 $_{g}$	4.25 +0.50 $_{abc}$	2.85 +0.10 $_{efghijklmn}$
	Western Rif	Om-632	37.45 +4.59 $_{abcd}$	30.68 +7.81 $_{cdefghi}$	3.00 +0.82 $_{cdef}$	17.30 +4.87 $_{ijkl}$	11.50 +1.35 $_{bcd}$	0.55 +0.06 $_{bcdef}$	0.70 +0.20 $_{abc}$	2.48 +0.13 $_{defg}$	4.00 +0.00 $_{bcd}$	2.30 +0.14 $_{lmnop}$
	Western Rif	Om-621	29.00 +7.82 $_{abcd}$	28.13 +7.20 $_{fghi}$	2.50 +1.29 $_{cdef}$	21.75 +0.20 $_{defghijkl}$	11.00 +0.00 $_{bcd}$	0.65 +0.12 $_{bcd}$	0.90 +0.08 $_{abc}$	2.35 +0.29 $_{efg}$	3.50 +0.58 $_{cd}$	2.35 +0.12 $_{klmno}$
	Western Rif	Om-651	38.13 + 7.40 $_{abcd}$	54.00 +6.87 $_{abcdefgh}$	2.75 +0.50 $_{cdef}$	22.93 +3.20 $_{cdefghijkl}$	13.40 +2.09 $_{bc}$	0.58 +0.02 $_{bcdef}$	0.80 +0.22 $_{abc}$	2.43 +0.38 $_{efg}$	4.00 +0.00 $_{bcd}$	2.95 +0.50 $_{defghijklm}$
	Agadir	Om-722	31.98 + 4.04 $_{abcd}$	41.50 +8.50 $_{abcdefghi}$	3.50 +0.58 $_{bcdef}$	24.23 +1.93 $_{bcdefghijk}$	12.56 +1.70 $_{bcd}$	0.51 +0.03 $_{bcdef}$	0.80 +0.24 $_{abc}$	2.63 +0.26 $_{bcdefg}$	4.00 +0.00 $_{bcd}$	3.03 +0.05 $_{cdefghijkl}$
	Agadir	Om-741	31.63 +3.12 $_{abcd}$	57.43 +6.95 $_{abcdefgh}$	3.75 +1.26 $_{bcdef}$	23.75 +1.44 $_{cdefghijkl}$	11.95 +1.14 $_{bcd}$	0.50 +0.04 $_{bcdef}$	0.85 +0.47 $_{abc}$	2.08 +0.61 $_{g}$	3.75 +0.50 $_{cd}$	2.63 +0.62 $_{hijklmno}$
O. robusta	Northeast Morocco	Or-412	29.28 +6.52 $_{abcd}$	42.20 +6.37 $_{abcdefghi}$	2.75 +0.96 $_{cdef}$	17.90 +3.59 $_{ghijkl}$	17.33 +3.45 $_{ab}$	0.97 +0.10 $_{a}$	1.38 +0.05 $_{ab}$	3.60 +0.45 $_{abcd}$	4.50 +0.58 $_{abc}$	4.03 +0.05 $_{a}$
O. aequatorialis	Rhamna	Oa-171	39.88 +6.94 $_{abcd}$	34.63 +4.70 $_{cdefghi}$	3.25 +0.50 $_{cdef}$	30.63 +2.95 $_{abcde}$	14.03 +9.99 $_{bc}$	0.48 +0.41 $_{bcdef}$	1.08 +0.39 $_{abc}$	2.43 +0.33 $_{efg}$	5.00 +0.82 $_{a}$	3.48 +0.32 $_{abcdef}$
O. dillenii	Ouardigha	Od-242	19.85 +2.16 $_{d}$	28.50 +3.67 $_{efghi}$	7.75 +3.50 $_{a}$	13.75 +1.84 $_{l}$	6.25 +0.86 $_{d}$	0.45 +0.00 $_{bcdef}$	0.65 +0.04 $_{abc}$	2.90 +0.16 $_{bcdefg}$	-	-
O. leucotricha	Middle atlas	Ol-361	37.63 +3.35 $_{abcd}$	53.33 +9.51 $_{abcdefgh}$	4.00 +0.82 $_{bcdef}$	24.75 + 3.10 $_{bcdefghijk}$	14.38 +0.25 $_{bc}$	0.66 +0.19 $_{bcd}$	1.13 +0.31 $_{abc}$	2.70 +0.26 $_{bcdefg}$	5.00 +0.82 $_{a}$	3.85 +0.54 $_{ab}$
	Western Rif	Ol-633	42.55 +9.17 $_{abc}$	42.93 +18.69 $_{abcdefghi}$	2.25 +1.26 $_{def}$	24.40 +5.63 $_{bcdefghijk}$	13.18 +2.19 $_{bc}$	0.55 +0.10 $_{bcdef}$	0.93 +0.32 $_{abc}$	2.90 +0.12 $_{bcdefg}$	4.50 +1.29 $_{abc}$	3.53 +1.01 $_{abcde}$
	Western Rif	Ol-611	45.88 +1.11 $_{ab}$	50.50 +6.81 $_{abcdefghi}$	4.00 +1.63 $_{bcdef}$	27.30 +3.50 $_{bcdefghij}$	13.63 +1.49 $_{bc}$	0.50 +0.02 $_{bcdef}$	0.85 +0.24 $_{abc}$	2.73 +0.38 $_{bcdefg}$	4.25 +0.50 $_{abc}$	3.65 +0.84 $_{abc}$
	Western Rif	Ol-641	40.98 +8.04 $_{abcd}$	51.95 +13.07 $_{abcdefghi}$	3.25 +0.50 $_{cdef}$	23.95 +5.17 $_{cdefghijk}$	13.45 +2.09 $_{bc}$	0.54 +0.03 $_{bcdef}$	0.85 +0.24 $_{abc}$	2.62 +0.26 $_{bcdefg}$	4.75 +0.96 $_{ab}$	3.10 +1.05 $_{cdefghij}$
O. inermis	Rhamna	Oi-191	35.63 +8.94 $_{abcd}$	50.00 +8.99 $_{abcdefghi}$	5.75 +0.96 $_{bc}$	20.73 +3.26 $_{defghijkl}$	9.60 +1.82 $_{cd}$	0.46 +0.02 $_{bcdef}$	0.78 +0.28 $_{abc}$	2.33 +0.25 $_{fg}$	-	-

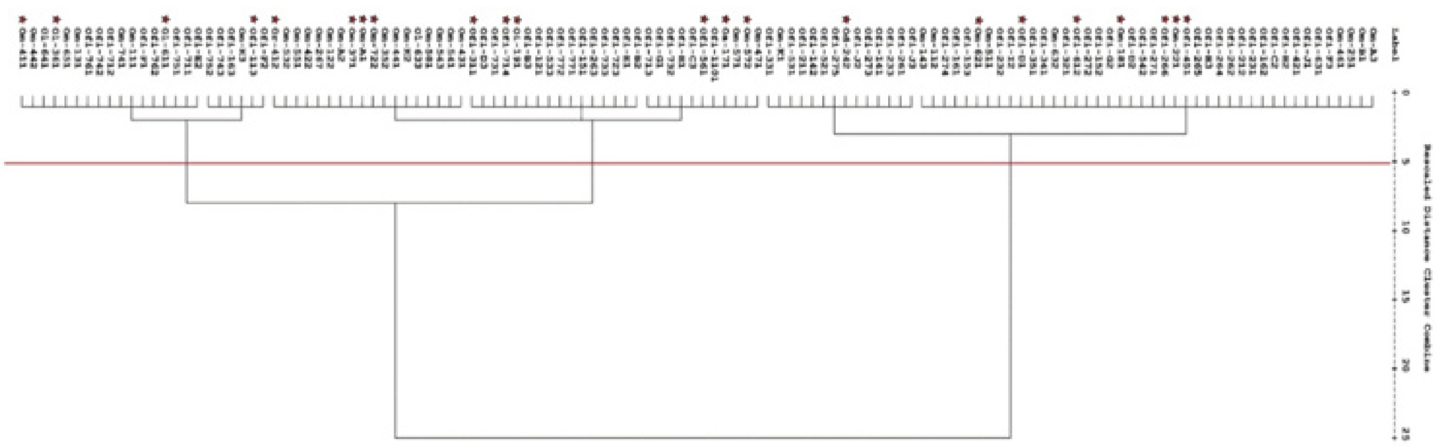

Fig. 4: Dendrogram grouping 124 accessions of *Opuntia spp.* based on 10 morphological traits and Ward's method. The red stars indicate accessions used for molecular analysis.

accessions from the same geographical site exhibited significantly different measures of morphological traits; for example, in accessions from Rhamna, cladode length was ranging from 16.30 cm in Ofi-141 to 29.13 cm in Ofi-163 (Table 1). The *O. ficus indica* accessions showed similarity coefficients ranging from 0.00 to 1.00 (proximity matrix not shown). The lowest similarity coefficient was noticed between accessions Ofi-212 and Ofi-262 from Ouardigha and between accessions Ofi-771 and Ofi-772 from Agadir while the highest similarity coefficient was observed between accessions Ofi-163 (from Rhamna) and Ofi-275 (from Ouardigha). In some cases, accessions from different regions show very low similarity coefficient (e.g. Ofi-C3 from Jamaât Riah and Ofi-561 from Eastern Rif displayed a similarity coefficient of 0.001). The dendrogram presented in Figure 2 suggested 3 major clusters. The first cluster contains 30 accessions (38.46 % of the total accessions); the second cluster contains 5 accessions (6.41 % of the total accessions) and the third cluster is comprised of 43 accessions (55.13% of the total accessions). Surprisingly, the three clusters contain accessions collected from different geographical sites.

Genetic Variability Within Opuntia Megacantha

The plant height of *O. megacantha* accessions ranged from 24.67 cm to 40.33 cm, plant diameter from 21.65 cm to 65.38 cm, the average number of cladodes per plant from 1.00 to 4.25, cladode height from 17.30 cm to 37.00 cm, cladode width from 9.40 cm to 14.67 cm, the index of cladode shape from 0.33 to 0.70, cladode thickness from 0.53 cm to 1.40 cm, the mean distance between areoles from 2.05 cm to 3.15 cm, the average number of spines per areole from 3.00 to 5.00 and the mean length of the longest spine per areole from 1.70 cm to 3.80 cm. Here again, some accessions from the same region displayed significantly different measures of morphological traits (Table 1). The range of similarity coefficients was from 0.00 to 1.00 (proximity matrix not shown). The lowest similarity was between Om-B1 and Om-A3, both from Jamaât Riah, while the highest similarity was between Om-K1 and Om-K3, from Jamaât Riah too. The dendrogram (Figure 3) shows 3 clusters, the first cluster consisted of 11 accessions (28.95 % of the total accessions); the second cluster contains 20 accessions (52.63 % of the total accessions) whereas the third cluster comprised 7 accessions (18.42 % of the total accessions). Interestingly, accessions collected from different regions could be found within the same cluster.

Genetic Variability Among Opuntia Spp.

Taking into consideration all the studied accessions of *Opuntia spp.*, the plant height ranged from 19.85 cm in Od-242 to 48.25 cm in Ofi-762, the average number of cladodes per plant from 1.00 to 7.75 in Od-242, with cladodes of different heights (from 13.70 in Ofi-331 to 37.00 in Om-181) and widths (from 6.25 cm in Od-242 to 20.23 in Ofi-713). Cladode thickness varied from 0.47 cm in Ofi-264 to 1.40 cm in Om-K2 (Table 1). Among the studied species, *O. ficus indica, O. dillenii* and *O. inermis* are spineless. The similarity coefficients were ranging from 0.00 to 1.00. The dendrogram shows three different clusters (Figure 4), and accessions from different species or geographic origins could be gathered into the same cluster. This shows that the clusters obtained do not fit with the species or geographical sites evaluated.

Molecular Markers to Assess Genetic Diversity

After screening 14 ISSR and 34 RAPD primers, 10 ISSR and 1 RAPD primers were selected for producing unambiguous, reproducible and intense bands. The ISSR primers generated a total of 66 bands, 64 (96.9 %) of which were polymorphic. The number of bands observed per locus varied from 4 to 9, with an average of 6.6. The RAPD primer (UBC-571) gave 6 bands, all polymorphic (Table 2). Based on the PIC values, the markers were highly informative. Indeed, the PIC values varied from 0.62 to 0.97, with an average of 0.82. The highest PIC value was observed in primer ISSR-8 while the lowest value was observed in primer ISSR-29 (Table 2). The dendrogram displayed 7 clusters at a similarity of 0.76 (Figure 5). *O. inermis* was separated from all the other species. The two accessions of *O. leucotricha* (Ol-361 and Ol-611) were placed into two different groups while accessions Or-412 (*O. robusta*) and Oa-171 (*O. aequatorialis*) were gathered into the same cluster. Interestingly, 14 accessions belonging to *O. ficus indica* and *O. megacantha* were gathered into the same group. This was confirmed by PCA (Figure 6), which results were similar to those obtained by the dendrogram.

Discussion

In the present study, the genetic diversity of 124 accessions of *Opuntia spp*. was evaluated based on 10 morphological descriptors. The use of morphological descriptors to determine genetic diversity within plant populations is simple, rapid and inexpensive[9]. Thus, such descriptors were used in many plant species such as date palm[19], hexaploid oat[16] and indigenous rice[43]. To the best of our knowledge, this is the first work to assess genetic variability within and among *Opuntia* populations in Morocco using morphological descriptors. Studies on the genetic variability in *Opuntia* species using morphological descriptors are scarce. Peña-Valdivia et al.(2008)[31] assessed genetic diversity within 55 accessions of *Opuntia spp*. in Mexico based on 65 morphological descriptors; and indicated that only few morphological traits showed significantly different measures within the studied populations. In a more recent study, Helsen et al. (2009)[25] highlighted a clear differentiation between *Opuntia* species using descriptors based on fruit, flower, seed, spine and trunk characteristics. In our case, the 124 accessions showed significant differences in the measured parameters and were distributed into 3 main distinct clusters based on Ward's method, containing 56, 45 and 23 accessions, respectively. Interestingly, accessions classified in the same species could be located in different clusters and vice versa, even though some species contain spines while others do not. This may be explained by several factors including the environmental effect and evolutionary change[11-40]. Our results are in good agreement with Valadez-Moctezuma et al. (2014)[39] who supported the hypothesis about the existence of a smaller number of *Opuntia* species compared to those currently described, but with high intraspecific genetic variation; and with Labra et al. (2003)[27] who suggested that the presence of spines should not be considered as a parameter in *Opuntia* taxonomy. Our results showed the influence of geographical origins on the morphological traits within the same species. Indeed, in some cases, accessions of the same species but from different regions were gathered into different clusters. This might be due to the environment effect[37-38]. Our results confirm previous findings on *Opuntia spp*. in Sardinia and Corsica islands, which show that environmental factors such as elevation, soil drainage, temperature and rainfall affect plant morphology[21]. As far as *Opuntia ficus indica* is concerned, the use of morphological or agro-morphological traits to assess genetic diversity within its accessions is very scarce. Felker et al. (2005)[22] compared genotypes of *O. ficus indica* from different origins based on yield and fruit characters, and significant differences were observed. In another

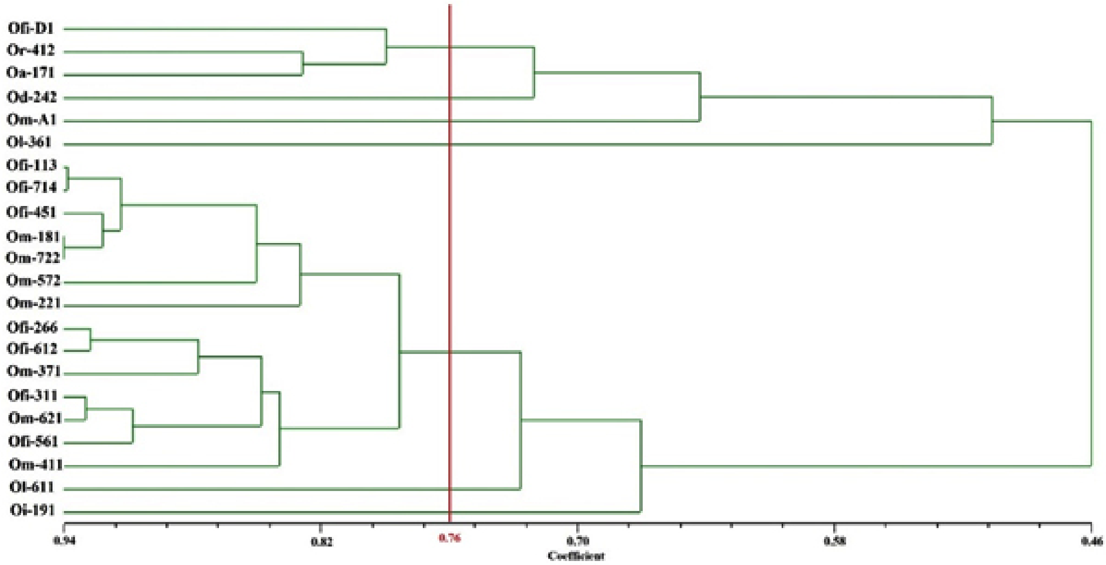

Fig. 5: Dendrogram grouping 22 accessions of Opuntia spp. based on 10 ISSR and 1 RAPD markers.

investigation, Bendhifi et al. (2013)[6] compared 28 Tunisian accessions of *O. ficus indica* using 20 descriptors based on plant, cladode and fruit characteristics. Here again, significant differences were observed between accessions. In our case, the 10 morphological traits showed significant differences between the 78 accessions. Remarkably, some accessions from the same geographical origin displayed significantly different measures of some traits. The use of morphological markers to assess genetic diversity within populations is simple and inexpensive. However, such markers can be affected by the environment[3]. Therefore, in the recent years, molecular markers have been widely used to assess genetic diversity within plant populations. Indeed, it was reported that molecular markers cover a larger proportion of the genome than the morphological ones and are less influenced by the environment since genetic variability is evaluated based on genotype rather than phenotype[26-38]. In addition, molecular markers have been proven powerful to detect variability when it could not be possible with morphological markers; i.e. a given morphological marker can affect other traits and the usefulness of morphological markers is restricted by their limited number[3]. Along this line, molecular markers have been applied alone or in association with morphological descriptors to assess genetic variability in many plant species; e.g. in Phoenix *dactylifera* L.[35], *Olea europaea* L.[8], *Citrus sinensis* L.[29]. Concerning the cactus pear, *Labra* et al. (2003)[27] evaluated the genetic diversity among 11 *Opuntia* species from Italy using chloroplastic simple sequence repeat (cpSSR) and amplified fragment length polymorphism (AFLP) markers, and a high genetic similarity between *O. ficus indica* and *O. megacantha* was revealed. Indeed, these authors suggested that *O. ficus indica* should be considered as a domesticated form of *O. megacantha.* Caruso et al. (2010)[10] assessed the genetic diversity among 62 accessions belonging to 16 *Opuntia* species from different origins, using simple sequence repeat (SSR) primers, and found that the *O. ficus indica* accessions did not cluster separately from some *Opuntia* species, including *O. megacantha*. In a more recent study, Valadez-Moctezuma et al. (2014)[39] assessed the genetic variability among 12 Mexican *Opuntia* species (52 cultivars) with ISSR and RAPD markers, and reported that *O. ficus indica* and *O. megacantha* might have a common ancestry. Our results are consistent with all these authors, since a high genetic similarity was observed between O. ficus indica and *O. megacantha*. On the other hand, Valadez-Moctezuma et al. (2014)[39] screened 120 RAPD primers, of which five were able to generate bands (4.16 %). In our case, only one RAPD primer over 34 (2.94%) generated bands. Our findings indicated that ISSR markers are the most efficient and informative in *Opuntia* species. Recently, ISSR markers have been used to assess the genetic and

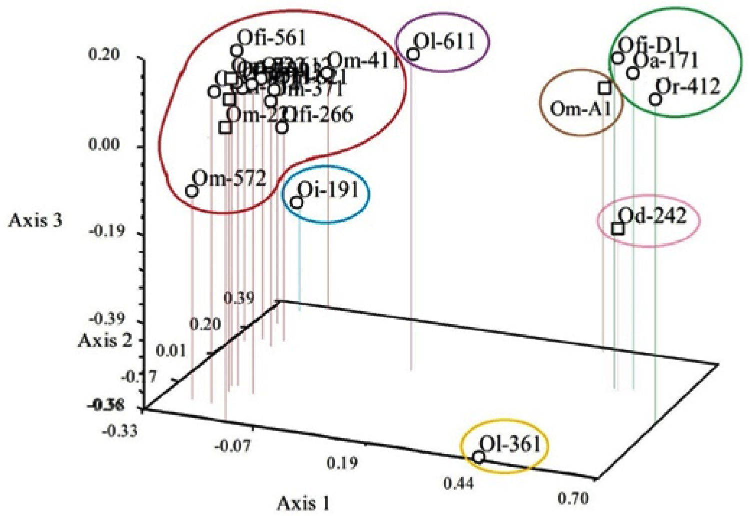

Fig. 6: 3D graphical distribution based on principal components analysis (PCA) showing the distribution of the 22 Opuntia spp. accessions.

phylogenetic relationships of 15 *Opuntia* species from Argentina, Bolivia, Brazil, Paraguay, and Uruguay[33]. Our findings are in contrast with those of Valadez-Moctezuma et al. (2014)[39], who indicated that the assessment of genetic diversity with RAPD markers was relatively more effective than with ISSR markers. In our study, the total number of polymorphic bands (64) and the PIC values (0.62-0.97) of ISSR markers were different from those found by Valadez-Moctezuma et al. (2014)[39], in which the number of polymorphic bands was 70 and the PIC varied from 0.21 to 0.27. This difference in polymorphic bands number and PIC values could be explained by the different ISSR primers employed (different sequences), the difference among accessions and the high distance between the geographical sites (Mexico and Morocco). Regarding the species *Opuntia ficus indica*, Zoghlami et al. (2007)[44] used 8 RAPD primers (over 22 screened) to assess the genetic variability within 36 Tunisian accessions, and a considerable genetic diversity was detected. In Morocco, the genetic diversity within *O. ficus indica* accessions was evaluated using RAPD markers[18], and the effect of the geographical origin was revealed. In our case, findings showed high genetic similarity among the studied accessions of *O. ficus indica*, even though they were collected from different regions of Morocco.

Conclusion

This paper is the first report to assess the genetic diversity within and among *Opuntia* species in Morocco using morphological descriptors and molecular markers. A low level of genetic diversity was observed among the accessions analyzed and a high similarity between *O. ficus indica* and *O. megacantha* accessions was highlighted. Our findings show also that ISSR markers are very efficient and informative in *Opuntia* species. Our results should be taken into consideration for plant breeding and genetic resource conservation programs. Further investigations are currently underway in order to determine the biochemical characteristics of cladode, flower, fruit and juice of these species, and to specify their molecular families to highlight the most appropriate use of each species.

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