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Research Article
Assessment and Identification of Cactus (*Opuntia* spp.) Ecotypes Grown in a Semi-arid Mediterranean Region

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Abstract

Background and Objective: The cactus, *Opuntia* species represent a focal point in crop production that is used as a source of food, forage and soil conservation purposes. So, the main objective of this study was to study the genetic relationships between different *Opuntia* ecotypes, their adaptation to the Tunisian climatic conditions by changing their morphological traits and also to identify the most useful traits for discrimination among them. **Materials and Methods:** The genetic diversity among 45 ecotypes of *Opuntia* species, collected from 9 different regions of the world was investigated. This collection was assessed using the descriptors UPOV, The International Union for the Protection of New Varieties of Plants. **Results:** The obtained data were analyzed by using principal component analysis (ACP) and 3 principal component (PC) axes accounted for 58.26% of the total cumulative variation. Average linkage cluster analysis was also performed and 5 main clusters were identified. The Tunisian *Opuntia ficus-indica* (Pr28) formed a separate group and displayed a distinct branching pattern indicating the native Tunisian originality. **Conclusion:** These results prove that this collection of *Opuntia* ecotypes must be kept as valuable genetic resources to enrich the *Opuntia* gene pool.

Key words: Descriptors, clusters, ecotypes, *Opuntia* species, variability

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The cactus (*Opuntia* species) has a large area of distribution according to their wide ability to withstand arid and semi-arid conditions. These species can grow in less fertile soil. The genus *Opuntia* is economically the most important as a great number of species that produces edible fruits, like as *Opuntia ficus-indica* (L.) Mill., *O. megacantha* Salm-Dyck, *O. stricta* Haw., *O. dilennei* (Ker Gawl.) Haw. *O. schummannii* Weber, *O. robusta* Wendl. and *O. albicarpa* Scheinvar[1]. *Opuntia* species is dicotyledonous angiosperm, belongs to the family of Cactaceae which is made up of a group of approximately 1,600 species in 130 genera subdivided in the three subfamilies Pereskioideae, Opuntioideae and Cactoideae[2]. *Opuntia* species flowers are frequently hermaphrodite, but cross-pollination occurs most often[3-5]. Bees are the major pollinators of the prickly pears[1]. The Cactaceae are one of the most interesting families due to their extensive set of peculiar adaptations to water scarcity, which allow them to be perennial and evergreen despite the sometimes extreme dry conditions of their environment[6].

Tunisia is one of the most important genetic sources for *Opuntia* species in the world and provides an important source of variation for plant breeding. However, as it's the case with other species used in fruit production, our country has few species of its own native *Opuntia* species.

Opuntia species covers an area of about 600,000 ha in Tunisia, distributed mainly in west-central regions on the plains of Kasserine[7]. This species was cultivated in Tunisia as fruit tree on limited surfaces[8]. The species has a typical physiology with the photosynthetic process called Crassulacean Acid Metabolism (CAM); stomata close during the day in order to maintain hydration of tissues. The importance of *Opuntia* genus as a forage plant in the 19th century was the outcome of the need to feed livestock in the arid zones, where the dry seasons are very long. *Opuntia* is an excellent feed for livestock[9].

The molecular techniques are very effective for the characterization of plant genetic resources, however, the morphological characterization should always be considered as a useful tool for the use in collections and description studies[10]. *Opuntia* genus identification and descriptor evaluation currently is a priority for all scientists involved in economic and agronomic use of this plant as a crop.

The descriptor list developed by Chessa and Nieddu[11] is very helpful in identifying the many forms and varieties within the commercial fruit types on the basis of external morphological characters, such as overall plant architecture, pad morphology, spine characters and fruit characters. Lastly, the isozyme work of Chessa *et al.*[12] and the RAPD data of Wang *et al.*[13] have begun to identify unique proteins and DNA fragments to clones that are nearly identical morphologically[14].

Description of the morphological characteristics is the usual methodology accepted from a legal point of view for patenting and registration of varieties[15].

The aim of this study was to investigate genotypic variation among 45 ecotypes (11 species of *Opuntia*) selected from 9 areas cross the world using morphologic descriptor to increase fruit production and improve cattle feeding.

MATERIALS AND METHODS

Plant materials and site description: The study included 45 ecotypes collected from 9 different geographic origins in the world and which represent 8 species of *Opuntia* genus (Table 1). These are planted in the experimental site of National Institute of Research in Rural Engineering, Water and Forests (INRGREF, Tunisia). All the ecotypes have undergone the same culture edaphoclimatic conditions.

The study area is located in Kairouan (Hendi Zitoun), the central region of Tunisia (Lower semi-arid bioclimate, Latitude is 35°40'41", Longitude is 10°05'46"and altitude is 68 m a.s.l.). Annual rainfall in the area averages is 163 mm, the mean annual temperature is 21.47°C.

Evaluation methods: During harvest season of 2017 and 2018, 20 fruits from each ecotype randomly collected and 10, 1 year cladodes per each ecotype sampled to be evaluated according to the *Opuntia* descriptor established by the International Union for the Protection of New Varieties of Plants (UPOV[16]). The 45 ecotypes evaluated based on 5 qualitative traits and 16 quantitative traits. The study was carried out at forest ecology laboratory from Jan, 2017-October, 2018.

The following cladode and fruit quantitative traits were measured using a digital caliper: Cladode length (Lo_Ra), cladode width (Lar_Ra), cladode thickness (ER_Ra) the mean distance between areoles (Me_Ra), fruit length (Lo_Fr) and fruit diameter (Da-Fr). However, cladode fresh weight (PF_Ra), cladode dry weight (PS_Ra), water reserve (Te_Ea), fruit flesh weight (Po_Ch), fruit peel weight (Po_Pe), fruit fresh weight (PF_Fr) and weight of seeds (Po_Pp) were measured using an electronic balance. The average number of areoles (Nb_Ar) in each cladode and number of seeds in each fruit (Nb_Pe) were also counted and taken into consideration.

Table 1: *Opuntia* ecotypes surveyed their codes and their origins

Species	Origin	Used codes	National codes
Opuntia ficus-indica	Tunisia	Pr1	69239
Opuntia ficus-indica	Ethiopia	Pr2	73064
Opuntia ficus-indica	Tunisia	Pr3	74071
Opuntia ficus-indica	South Africa	Pr4	73056
Opuntia ficus-indica	Morocco	Pr5	74115
Opuntia ficus-indica	Italy	Pr6	73053
Opuntia laevis SP3	New Mexico	Pr7	74110
Opuntia laevis SP4	New Mexico	Pr8	74111
Opuntia laevis SP5	New Mexico	Pr9	74112
Opuntia ficus-indica	Algeria	Pr10	69200
Opuntia crassa	Mexico	Pr11	69205
Opuntia ficus-indica	Tunisia	Pr12	68247
Opuntia ficus-indica	Tunisia	Pr13	69208
Opuntia ficus-indica	Tunisia	Pr14	69244
Opuntia ficus-indica	Tunisia	Pr15	69243
Opuntia ficus-indica	Tunisia	Pr16	69242
Opuntia ficus-indica	Tunisia	Pr17	69240
Opuntia ficus-indica	Morocco	Pr18	74082
Opuntia ficus-indica	Morocco	Pr19	74083
Opuntia ficus-indica	Morocco	Pr20	74081
Opuntia helvetica	Tunisia	Pr21	69249
Opuntia ficus-indica	Tunisia	Pr22	67028
Opuntia ficus-indica	Tunisia	Pr23	74076
Opuntia ficus-indica	Tunisia	Pr24	74077
Opuntia ficus-indica	Morocco	Pr25	74080
Opuntia ficus-indica	Morocco	Pr26	74078
Opuntia ficus-indica	Tunisia	Pr27	73052
Opuntia ficus-indica	Sicily	Pr28	69237
Opuntia maxima	Algeria	Pr29	69212
Opuntia ficus-indica	Algeria	Pr30	70604
Opuntia ficus-indica	Tunisia	Pr31	73058
Opuntia ficus-indica	Mexico	Pr32	73049
Opuntia ficus-indica	Algeria	Pr33	69198
Opuntia maxima	Algeria	Pr34	69199
Opuntia crassa	Algeria	Pr35	69219
Opuntia ficus-indica	Algeria	Pr36	69220
Olearia tomentosa	Algeria	Pr37	69210
Opuntia ficus-indica	Tunisia	Pr38	69245
Opuntia ficus-indica	Tunisia	Pr39	69246
Opuntia ficus-indica	Tunisia	Pr40	69248
Opuntia ficus-indica	Italy	Pr41	69233
Opuntia ficus-indica	Italy	Pr42	69234
Opuntia ficus-indica	Tunisia	Pr43	69201
Opuntia ficus-indica	Tunisia	Pr44	69202
Opuntia ficus-indica	Tunisia	Pr45	69203

Fruit were collected at full maturity. Maturity was determined on the basis of the color characteristics of each ecotype. pH values of juice (pH_Fr) were measured using pH meter. Total Soluble solids of fruit (Ts_Fr) were determined with the refractometrical method using a Zeiss hand refractometer. Fruit flesh firmness (Fe_Fr) was recorded by a digital penetrometer (Turoni, Forlì, Italy) on two opposite cheeks.

Moreover, a series of qualitative fruit and cladode parameters were evaluated using the *Opuntia* descriptor (UPOV[16]), fruit shape in longitudinal section (Fo_Fr), depression of receptacle scar (Ci_Fr), length of stalk (LPF), juice color (Co_Ju) and shape of the cladode (Fo_Ra).

Statistical analysis: Analysis of morphological traits were carried out using SAS software package (SAS Institute Inc., Cary, NC, USA Version 9.2). The mean values for each parameter of a given genotype were used to perform statistical analysis of morphological traits. 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. The PCA was performed using SPSS software statistics, the dendrogram was constructed using the Unweighted Pair Group Method with Arithmetic means (UPGMA).

RESULTS

Quantitative characteristics of fruits and cladodes: The cladode size parameters showed noteworthy difference among the elevens species of *Opuntia* (Table 2). The analysis of variance showed that most parameters were significant ($p\leq 0.01$) between the 45 ecotypes.

Cladode width encompassed an extensive range between the largest *O. ficus-indica* (Pr45) with 28.68 cm and the smallest one (Pr43) with 9.6 cm, while, Cladode length ranged between 45.02 and 21.44 cm for, respectively these two latter. Cladode thickness varied between 7.29 and 25.74 mm for *O. ficus-indica* (Pr2) from Ethiopia and *O. Crassa* (Pr35) from Algeria respectively. The two *O. ficus-indica* (Pr15 and Pr16) exhibited the highest cladode fresh weight. However, the *O. ficus-indica* (Pr43) which is also Tunisian origin registered the light cladode fresh weight. The *O. ficus-indica* (Pr44) registered the heavy cladode dray weight (124 g). Nevertheless, *O. ficus-indica* (Pr2) is the lightest. The Mexicain *O. crassa* (Pr11) and the Tunisian *O. ficus-indica* (Pr45) have a low number of areole (1.5 areoles/cladode). Water reserve varied widely between 71.9 and 94.14 g for Pr11 and Pr23, respectively. The highest value for the mean distance between areoles was determined in the Tunisian *O. ficus-indica* (Pr45) with an average of 6.32 cm and the lowest also in the Tunisian one (Pr44) with an average of 2.22 cm (Fig. 1).

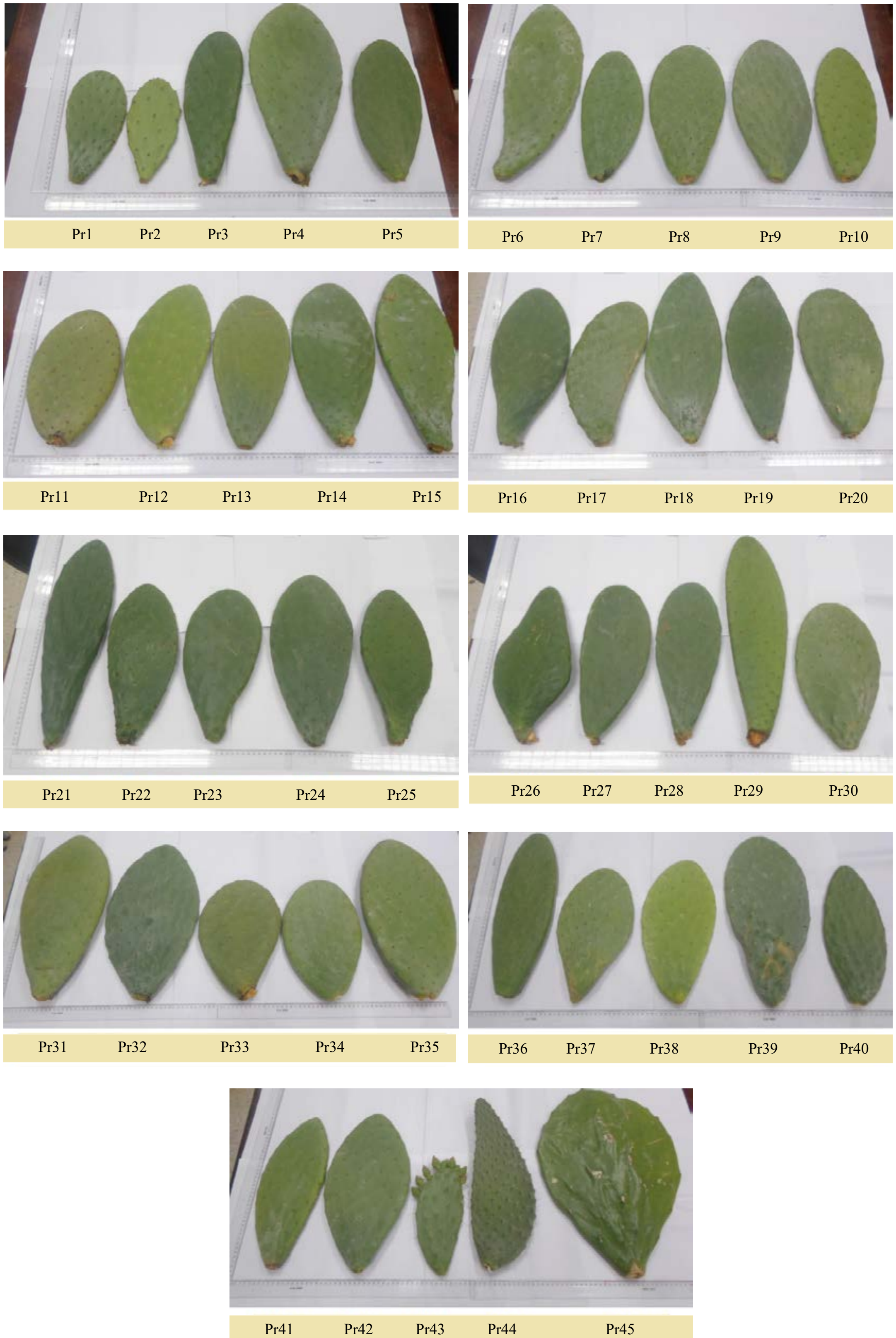

Fig. 1: Cladodes of 45 studied ecotypes

Table 2: Cladode quantitative traits of 45 *Opuntia* ecotypes

Ecotypes	PF_Ra (kg)	Lo_Ra (cm)	Lar_Ra (cm)	Ep_Ra (mm)	Nb_Ar	PS_Ra (g)	Me_Ra (cm)	Re_Ea (%)
Pr1	0.46±0.1^{f-k}	36.80±4.4^{af}	16.22±1.1^{c-j}	9.25±2.08^{ij}	2.26±0.1^{d-j}	65.0±10.8^{bc}	3.70±0.2^{b-g}	88.74±1.7^{ab}
Pr2	0.18±0.0^{jk}	27.96±2.9^{f-i}	11.58±1.1^{kl}	7.29±1.7^{j}	2.42±0.1^{c-i}	20.0±4.6^{e}	4.30±0.6^{bc}	84.34±5.4^{ab}
Pr3	0.62±0.1^{b-j}	37.48±5.8^{a-f}	16.08±2.3^{c-k}	14.16±1.84^{c-j}	2.37±0.5^{d-i}	57.6±2.6^{bc}	3.62±0.4^{b-g}	85.94±2.4^{ab}
Pr4	0.85±0.12^{a-h}	36.06±3.1^{a-g}	17.42±2.2^{b-g}	17.48±5.0^{a-i}	2.09±0.3^{e-k}	63.4±14.9^{bc}	3.44±0.1^{c-g}	91.13±0.8^{ab}
Pr5	0.99±0.2^{a-f}	37.70±4.1^{a-f}	17.72±0.9^{b-f}	19.22±3.35^{a-h}	2.12±0.1^{e-k}	73.0±10.22^{b}	3.66±0.3^{b-g}	86.53±4.8^{ab}
Pr6	0.78±0.3^{a-h}	35.58±2.9^{a-g}	16.10±2.5^{c-k}	17.78±7.65^{a-i}	2.25±0.4^{d-j}	64.2±4.0^{bc}	3.24±0.4^{c-h}	87.08±2.5^{ab}
Pr7	0.88±0.3^{a-h}	41.48±4.5^{a-c}	13.66±2.0^{e-l}	18.39±5.1^{a-i}	3.07±0.4^{ab}	71.4±15^{b}	3.40±0.8^{c-g}	84.57±2.6^{ab}
Pr8	0.87±0.2^{a-h}	29.80±4.6^{d-h}	16.80±1.7^{b-h}	23.88±4.1^{ab}	1.76±0.1^{i-k}	68.0±9.0^{bc}	3.36±0.4^{c-g}	84.68±3.4^{ab}
Pr9	0.84±0.0^{a-h}	32.66±4.2^{b-h}	18.40±2.4^{b-e}	18.59±3.6^{a-i}	1.78±0.2^{i-k}	82.0±11.4^{b}	3.18±0.8^{c-h}	86.47±4.1^{ab}
Pr10	0.80±0.2^{a-h}	34.02±2.7^{b-g}	17.08±1.7^{b-h}	18.29±3.4^{a-i}	2.00±0.1^{e-k}	61.4±5.5^{bc}	3.72±0.2^{b-g}	85.82±2.0^{ab}
Pr11	0.64±0.1^{b-j}	26.84±3.5^{h-i}	17.98±2.4^{b-f}	20.18±6.7^{a-g}	1.50±0.1^{k}	72.6±9.0^{b}	2.96±0.1^{d-h}	71.91±16^{c}
Pr12	1.16±0.3^{a-c}	42.04±6.1^{ab}	20.92±1.98^{b}	17.50±2.4^{a-i}	2.00±0.2^{e-k}	71.6±8.4^{b}	3.98±0.4^{b-e}	85.07±1.7^{ab}
Pr13	1.05±0.3^{a-e}	37.84±6.1^{a-f}	17.44±2.0^{b-g}	21.90±4.2^{a-e}	2.16±0.1^{e-k}	69.6±15.1^{bc}	3.70±0.2^{b-g}	87.78±1.8^{ab}
Pr14	1.15±0.2^{a-c}	36.74±2.8^{a-f}	18.30±1.1^{b-e}	22.95±4.2^{a-d}	2.00±0.1^{e-k}	72.0±6.6^{b}	3.70±0.5^{b-g}	86.57±2.1^{ab}
Pr15	1.33±0.3^{a}	37.68±4.3^{a-f}	19.50±2.2^{bc}	23.34±7.0^{a-c}	1.93±0.19^{g-k}	68.0±12.5^{bc}	3.56±0.5^{b-g}	82.18±11.8^{b}
Pr16	1.32±0.2^{a}	41.50±4.3^{a-c}	18.74±1.8^{b-d}	23.79±5.7^{ab}	2.21±0.06^{d-j}	72.8±5.7^{b}	3.84±0.3^{b-f}	88.77±1.5^{ab}
Pr17	0.50±0.1^{e-k}	34.10±2.7^{b-g}	13.38±1.4^{e-l}	14.78±3.3^{b-j}	2.56±0.2^{b-h}	76.2±10.2^{b}	2.92±0.2^{d-h}	87.75±5.4^{ab}
Pr18	0.64±0.1^{b-j}	34.52±4.7^{b-g}	14.94±2.2^{c-k}	15.36±2.5^{b-j}	2.33±0.3^{d-i}	61.4±9.7^{bc}	3.54±0.3^{b-g}	90.81±0.9^{ab}
Pr19	0.78±0.0^{a-h}	35.78±3.0^{a-g}	16.68±0.4^{b-i}	17.04±1.3^{a-i}	2.14±0.1^{e-k}	61.4±6.8^{bc}	3.74±0.1^{b-g}	89.09±3.6^{ab}
Pr20	0.87±0.3^{a-h}	37.06±7.2^{a-f}	18.44±2.3^{b-e}	15.19±2.7^{b-j}	2.00±0.2^{e-k}	70.0±7.3^{b}	3.72±0.5^{b-g}	92.02±2.1^{ab}
Pr21	0.68±0.8^{b-j}	38.30±2.3^{a-f}	15.24±0.8^{c-k}	15.21±1.7^{b-j}	2.51±0.1^{b-h}	69.4±8.9^{bc}	3.44±0.3^{c-g}	94.07±0.7^{a}
Pr22	0.57±0.2^{d-k}	40.74±2.4^{a-c}	14.40±2.0^{d-k}	15.10±2.05^{b-j}	2.85±0.2^{a-d}	76.4±8.8^{b}	2.90±0.4^{e-h}	91.61±4.9^{ab}
Pr23	0.56±0.2^{d-k}	29.22±5.6^{d-h}	14.54±0.9^{d-k}	19.58±5.2^{a-g}	2.02±0.4^{e-k}	58.2±17.4^{bc}	3.20±0.4^{c-h}	94.14±2.8^{a}
Pr24	0.62±0.1^{b-j}	35.96±3.8^{a-g}	15.98±1.9^{c-k}	16.74±3.1^{a-i}	2.27±0.3^{d-j}	77.0±4.6^{b}	3.58±0.5^{b-g}	90.43±3.1^{ab}
Pr25	0.47±0.2^{f-k}	31.18±5.9^{c-h}	12.18±2.6^{i-l}	16.76±3.2^{a-i}	2.59±0.3^{b-h}	72.2±14.1^{b}	2.54±0.2^{gh}	88.05±3.5^{ab}
Pr26	0.31±0.08^{h-k}	30.00±3.2^{d-h}	11.98±2.2^{j-l}	12.54±3.3^{e-j}	2.54±0.3^{b-h}	72.6±12.0^{b}	2.66±0.6^{f-h}	86.00±10.4^{ab}
Pr27	0.90±0.2^{a-g}	37.28±3.6^{a-f}	17.50±2.7^{b-g}	19.34±1.6^{a-h}	2.15±0.2^{e-k}	66.4±9.4^{bc}	3.74±0.4^{b-g}	88.56±1.5^{ab}
Pr28	0.72±0.1^{b-i}	37.14±6.7^{a-f}	17.90±1.0^{b-f}	15.14±2.7^{b-j}	2.06±0.3^{e-k}	73.0±14.3^{b}	3.72±0.1^{b-g}	89.79±2.7^{ab}
Pr29	0.71±0.1^{b-i}	35.48±5.3^{a-g}	13.38±1.2^{e-l}	20.11±3.4^{a-g}	2.64±0.2^{a-g}	58.2±14.2^{bc}	3.6±0.6^{b-g}	87.16±4.1^{ab}
Pr30	1.08±0.2^{a-d}	37.16±3.7^{a-f}	17.80±1.8^{b-f}	23.03±5.2^{a-d}	2.10±0.2^{e-k}	71.4±8.8^{b}	3.62±0.3^{b-g}	87.61±3.8^{ab}
Pr31	1.16±0.3^{a-c}	35.90±4.1^{a-g}	17.60±1.6^{b-g}	24.08±6.1^{ab}	2.05±0.2^{e-k}	74.2±7.5^{b}	3.78±0.2^{b-f}	87.37±4.6^{ab}
Pr32	1.01±0.2^{a-f}	36.82±3.7^{a-f}	18.04±0.8^{b-f}	21.01±2.27^{a-f}	2.03±0.1^{e-k}	68.6±10.3^{bc}	3.80±0.2^{b-f}	84.68±5.3^{ab}
Pr33	1.14±0.1^{a-d}	38.38±2.6^{a-e}	19.60±1.7^{bc}	20.44±5.46^{a-g}	1.96±0.09^{f-k}	72.4±6.2^{b}	4.28±0.3^{bc}	88.34±2.1^{ab}
Pr34	0.63±0.1^{b-j}	38.06±1.8^{a-f}	12.66±1.6^{h-l}	17.64±3.9^{a-i}	3.04±0.3^{a-c}	63.0±5.7^{bc}	4.10±0.7^{b-e}	88.72±3.5^{ab}
Pr35	0.82±0.2^{a-h}	28.26±1.8^{e-i}	14.92±1.8^{c-k}	25.74±4.2^{a}	1.90±0.1^{h-k}	67.8±11.5^{bc}	3.18±0.2^{c-h}	88.53±3.4^{ab}
Pr36	0.63±0.2^{b-j}	36.74±5.4^{a-f}	13.50±1.5^{e-l}	18.42±3.1^{a-i}	2.71±0.2^{a-e}	74.2±9.8^{b}	3.30±0.5^{c-g}	89.25±1.6^{ab}
Pr37	0.92±0.3^{a-g}	36.08±3.8^{a-g}	17.90±1.9^{b-f}	19.05±2.2^{a-h}	2.01±0.06^{e-k}	72.0±5.9^{b}	4.00±0.5^{b-e}	89.58±3.5^{ab}
Pr38	1.14±0.3^{a-d}	41.26±5.3^{a-c}	16.98±3.8^{b-h}	23.85±1.7^{ab}	2.54±0.7^{b-h}	75.6±15^{b}	3.82±0.4^{b-f}	90.95±1.1^{ab}
Pr39	0.58±0.1^{b-j}	37.56±3.5^{a-f}	14.24±2.3^{d-k}	13.42±1.7^{d-j}	2.66±0.2^{a-f}	74.4±16.9^{b}	3.36±0.6^{c-g}	91.77±1.4^{ab}
Pr40	0.23±0.06^{i-k}	25.04±3.3^{hi}	13.30±0.6^{e-l}	10.91±3.5^{g-j}	1.87±0.1^{h-k}	27.2±2.2^{de}	4.10±1.1^{b}	86.58±3.6^{ab}
Pr41	0.69±0.2^{b-j}	41.24±5.6^{a-c}	16.04±1.9^{c-k}	13.29±4.5^{e-j}	2.59±0.4^{b-h}	78.2±11.1^{b}	3.54±0.7^{b-g}	87.02±3.8^{ab}
Pr42	0.85±0.36^{a-h}	39.08±4.04^{a-d}	18.80±3.8^{bcd}	13.61±6.3^{d-j}	2.13±0.4^{e-k}	75.2±1.92^{b}	4.16±0.7^{b-d}	87.93±4.9^{ab}
Pr43	0.15±0.04^{k}	21.44±2.01^{i}	9.60±0.9^{l}	9.99±4.9^{h-j}	2.24±0.2^{d-j}	42.0±4.6^{cd}	3.14±0.3^{c-h}	86.24±4.1^{ab}
Pr44	0.42±0.03^{g-k}	41.10±3.9^{a-c}	12.92±1.4^{g-l}	9.49±0.99^{ij}	3.20±0.4^{a}	124.0±45.3^{a}	2.22±0.1^{h}	88.78±3.96^{ab}
Pr45	1.26±0.4^{ab}	45.02±3.7^{a}	28.68±3.2^{a}	12.18±2.8^{f-j}	1.57±0.9^{k}	57.2±4.1^{bc}	6.32±0.9^{a}	87.73±2.4^{ab}

Different small letters in the same column indicate significantly different values within ecotypes at $\alpha \leq 0.05$, -: Set of successive letters between the two mentioned letters

The heaviest fruit was found in *O. ficus-indica* 'Pr28' from Sicily with an average of 125.8 g. In contrast, the mixture of species such as Pr1, Pr35, Pr37, Pr43 and Pr44 had significantly the lightest fruits (Table 3). They do not differ significantly in properties, but do differ significantly in relationship with all other genotypes.

The seeds weight of the studied *Opuntia* ecotypes ranged from 3.78-0.47 g. The lightest value was recorded for Pr45 and Pr35 and the lightest one for Pr33. Seeds number varied widely between 290.5 (at Pr38) and 23.66 (at Pr35). Weight of peel ranged between 85.99 g in Pr9 and 7.13 in Pr35, while the weight of flesh fruit varied from 62.42-2.62 g being the highest in Pr28. This latter genotype showed the heaviest fruit fresh weight. However the Pr1 showed the lightest fruit fresh weight. The Pr37, Pr40, Pr43 and Pr44 did not differ significantly from the Pr1. With regard to the firmness at fruit maturity, the Pr37 was firm. Nevertheless, Pr2 and Pr43 have the soft fruit with an average of 8.72 and 8.82N, respectively.

Table 3: Fruit quantitative traits of 45 *Opuntia* ecotypes

Ecotypes	PF_Fr (g)	Fe_Fr (N)	Po_Ch (g)	Po_Pe (g)	Nb_Pe	Pe_Pp (g)
Pr1	8.26±0.8^{n}	16.46±3.1^{a-c}	2.97±0.2^{s}	5.29±0.6^{q}	58.33±3.7^{no}	1.10±0.1^{de}
Pr2	39.31±0.8^{m}	8.72±0.9^{c}	11.70±3.1^{o-r}	27.60±2.9^{k-q}	101.66±3.7^{lm}	1.47±0.7^{b-e}
Pr3	78.29±7.4^{e-g}	11.76±0.9^{a-c}	42.73±2.9^{bc}	35.56±4.6^{h-m}	159.33±9.8^{h-j}	2.06±1.1^{a-e}
Pr4	67.38±0.7^{f-k}	16.07±2.1^{a-c}	19.75±1.2^{i-o}	47.63±0.9^{f-k}	199.66±1.5^{c-h}	1.90±0.0^{a-e}
Pr5	44.92±1.8^{lm}	16.07±0.1^{a-c}	11.66±1.3^{o-r}	33.26±2.7^{i-n}	72.66±3.05^{mn}	1.12±0.1^{de}
Pr6	54.79±4.1^{i-m}	16.46±0.9^{a-c}	43.99±2.6^{b}	10.80±4.0^{o-q}	219.00±9.5^{b-e}	2.63±0.2^{a-e}
Pr7	107.48±3.1^{bc}	12.15±3.7^{a-c}	23.93±2.3^{f-k}	83.55±1.3^{ab}	249.00±4.5^{b}	2.52±1.0^{a-e}
Pr8	76.77±5.0^{e-h}	14.70±5.4^{a-c}	24.77±6.9^{f-j}	52.00±3.7^{e-j}	184.33±15.0^{e-j}	2.29±0.6^{a-e}
Pr9	107.67±6.6^{bc}	12.25±1.3^{a-c}	21.69±1.2^{h-m}	85.98±7.7^{a}	228.00±5.1^{b-d}	2.36±0.8^{a-e}
Pr10	110.89±6.6^{b}	16.17±1.2^{a-c}	39.59±1.0^{b-d}	71.29±7.1^{a-d}	220.66±11.$^{b-e}$	2.97±0.2^{a-d}
Pr11	59.60±7.5^{g-m}	13.81±2.0^{a-c}	22.18±2.5^{h-m}	37.41±7.9^{h-l}	37.33±3.0^{op}	1.01±0.1^{de}
Pr12	66.93±10.6^{f-k}	9.80±2.8^{bc}	28.69±1.4^{e-i}	38.23±11.3^{h-l}	117.66±8.5^{kl}	2.05±0.5^{a-e}
Pr13	56.30±7.6^{i-m}	13.23±2.1^{a-c}	21.95±2.4^{h-m}	34.34±5.7^{h-m}	191.00±16.0^{d-i}	2.43±0.8^{a-e}
Pr14	107.36±15.4^{bc}	12.93±3.1^{a-c}	31.40±4.4^{d-h}	75.95±11.2^{a-c}	208.00±7.9^{c-f}	3.46±0.9^{a-c}
Pr15	76.68±8.6^{e-h}	11.56±1.0^{a-c}	31.95±4.6^{d-g}	44.73±13.0^{g-k}	166.66±6.8^{g-j}	2.23±0.6^{a-e}
Pr16	92.26±10.9^{c-e}	13.03±5.9^{a-c}	38.41±4.7^{b-d}	53.85±6.3^{d-i}	233.33±22.0^{bc}	3.56±1.5^{ab}
Pr17	52.23±6.2^{i-m}	13.32±0.4^{a-c}	21.16±1.3^{i-n}	31.06±7.6^{j-n}	170.00±10^{f-j}	2.06±0.1^{a-e}
Pr18	57.59±5.8^{h-m}	11.07±2.0^{a-c}	13.28±0.9^{m-q}	44.30±6.7^{g-k}	165.00±14.1^{h-j}	1.63±0.6^{b-e}
Pr19	47.27±1.9^{k-m}	11.76±2.7^{a-c}	16.64±2.9^{j-p}	30.63±3.1^{j-o}	156.00±4.3^{ij}	1.61±0.4^{b-e}
Pr20	47.46±5.0^{k-m}	14.60±0.9^{a-c}	11.05±0.5^{o-s}	36.40±5.3^{h-l}	102.00±2.6^{lm}	1.71±0.4^{a-e}
Pr21	45.04±5.1^{lm}	12.64±0.9^{a-c}	14.66±1.6^{k-q}	30.38±4.9^{j-o}	121.33±4.9^{kl}	1.31±0.3^{c-e}
Pr22	98.46±7.9^{b-d}	13.62±0.0^{abc}	33.29±8.3^{d-f}	65.17±6.9^{cd-f}	114.33±7.2^{kl}	1.67±0.4^{a-e}
Pr23	65.40±5.2^{f-l}	14.01±2.9^{a-c}	35.08±6.5^{c-e}	30.32±1.3^{j-o}	198.00±4^{c-i}	2.15±0.0^{a-e}
Pr24	95.94±7.3^{b-d}	11.76±1.1^{a-c}	41.60±2.1^{bc}	54.35±9.5^{d-i}	113.00±7.2^{kl}	1.97±0.3^{a-e}
Pr25	39.78±7.1^{m}	12.44±3.3^{a-c}	24.49±1.0^{f-j}	15.29±6.9^{m-q}	120.00±6^{kl}	1.50±0.5^{b-e}
Pr26	93.35±5.5^{b-e}	11.36±1.4^{a-c}	24.54±2.57^{f-j}	68.81±3.1^{b-e}	120.33±3.5^{kl}	2.24±0.5^{a-e}
Pr27	50.52±2.8^{i-m}	10.58±0.3^{a-c}	29.37±6.7^{d-i}	21.14±3.9^{l-q}	194.33±2.5^{d-i}	2.45±0.4^{a-e}
Pr28	125.83±15.4^{a}	12.05±1.1^{a-c}	62.48±2.4^{a}	63.35±12.9^{c-g}	206.00±9.6^{c-g}	3.49±1.2^{ab}
Pr29	52.63±1.8^{i-m}	15.87±0.9^{a-c}	16.95±0.3^{j-p}	35.68±1.5^{h-m}	164.00±43.9^{h-j}	2.22±0.8^{a-e}
Pr30	65.99±14.7^{f-l}	13.23±2.3^{a-c}	23.21±2.8^{g-l}	42.77±15.6^{h-l}	161.33±37.1^{h-j}	2.35±0.3^{a-e}
Pr31	61.34±1.7^{g-l}	10.97±1.5^{a-c}	13.72±2.4^{l-q}	74.61±3.3^{f-k}	172.33±41.6^{f-j}	3.04±1.6^{a-d}
Pr32	63.12±6.2^{g-l}	11.46±1.4^{a-c}	26.39±2.6^{e-j}	36.73±6.2^{hi-l}	115.33±22.1^{kl}	2.44±0.7^{a-e}
Pr33	84.88±2.2^{d-f}	11.36±4.6^{a-c}	29.36±0.4^{e-i}	55.51±1.8^{d-h}	251.00±12.5^{b}	3.79±0.5^{a}
Pr34	40.38±1.2^{m}	11.27±3.6^{a-c}	9.76±1.9^{p-s}	30.62±1^{j-o}	161.66±22.6^{h-j}	1.20±0.8^{de}
Pr35	11.60±0.8^{n}	10.38±0.9^{a-c}	4.47±0.8^{rs}	7.13±1.5^{q}	23.66±3^{p}	0.58±0.0^{e}
Pr36	49.82±13.1^{j-m}	16.46±1.4^{a-c}	28.72±9.6^{e-i}	21.09±20.9^{l-q}	159.66±10^{h-j}	2.55±0.5^{a-e}
Pr37	8.42±1.5^{n}	18.81±2.8^{a}	3.20±0.4^{s}	5.21±1.4^{q}	91.33±3.7^{lm}	0.95±0.1^{de}
Pr38	51.93±5.7^{i-m}	17.15±2.4^{a-c}	22.23±1.4^{h-m}	29.7±7.1^{k-o}	290.00±10^{a}	2.18±0.9^{a-e}
Pr39	68.30±7.0^{f-k}	13.91±2.1^{a-c}	21.99±0.3^{h-m}	46.31±7.3^{g-k}	188.33±2.5^{e-i}	2.00±0.2^{a-e}
Pr40	20.51±2.2^{n}	17.44±1.8^{b}	7.00±0.7^{q-s}	13.5±1.7^{n-q}	177.00±9.8^{f-j}	2.26±0.2^{a-e}
Pr41	49.64±13.4^{j-m}	13.72±2.5^{a-c}	17.97±4.7^{j-o}	31.66±10.6^{j-n}	159.66±12.6^{h-j}	1.28±0.1^{de}
Pr42	71.08±6.1^{f-i}	12.15±1.4^{a-c}	42.12±2.2^{bc}	28.96±5.7^{k-o}	145.33±23.3^{jk}	1.45±1.0^{b-e}
Pr43	22.43±1.6^{n}	8.82±0.9^{c}	12.48±2.5^{o-r}	9.94±4.1^{pq}	54.66±2.0^{no}	1.85±0.5^{a-e}
Pr44	7.59±0.7^{n}	16.64±3.1^{a-c}	2.62±0.6^{s}	4.96±0.2^{n-q}	56.33±4.0^{no}	1.07±0.1^{de}
Pr45	69.49±11.2^{f-j}	15.87±5.2^{a-c}	32.24±4.6^{d-g}	37.25±6.8^{h-l}	83.33±10.9^{l-n}	0.47±0.2^{e}

Different small letters in the same column indicate significantly different values within cultivars at $\alpha\leq0.05$, -: Set of successive letters between the two mentioned letters

Fruit diameter was between 50.31 and 20.56 mm, the lowest values were recorded by (Pr1, Pr35, Pr37, Pr43 and Pr44). Fruit length varied from 9.46 cm for the Tunisian *O. ficus-indica* (Pr45) to 3.29 cm for the Algerian *O. crassa* (Pr35). Based on fruit size properties, *O. ficus-indica* such as Pr1, Pr3 and Pr44 were the *Opuntia* genotypes with the smallest fruits, while Pr28 was the genotype with the largest ones (Fig. 2).

Total Soluble solids (Ts_Fr) are between 15.33 °Brix for the Morrocain *O. ficus-indica* (Pr25) and 4.8 °Brix for the Algerian *O. tomentosa* (Pr37). The *O. ficus-indica* such as Pr31, Pr42 and Pr45 did not differ significantly from Pr37 (Fig. 3). The pH of fruit juice varied between 5.4 in *O. laevis* (Pr7) to 2.96 equally for *O. crassa* (Pr35) and *O. ficus-indica* (Pr45) (Fig. 4).

Qualitative characteristics of fruits and cladodes: The fruit trait such as length of receptacle scar (Ci_Fr) showed noteworthy differences among genotypes. This parameter varied between slightly to strongly depressed. It can be seen that the main differences among the 8 studied species were related to the juice color (Fig. 5)

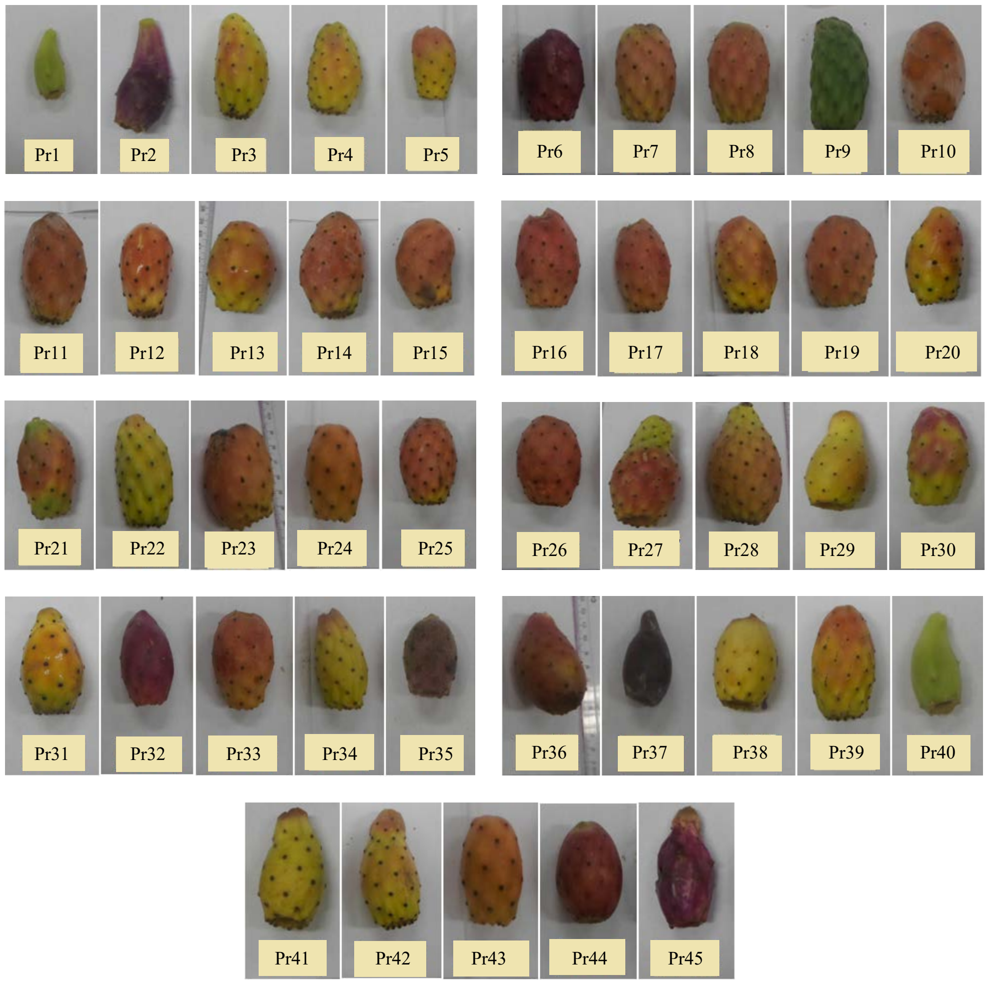

Fig. 2: Fruits of 45 studied ecotypes

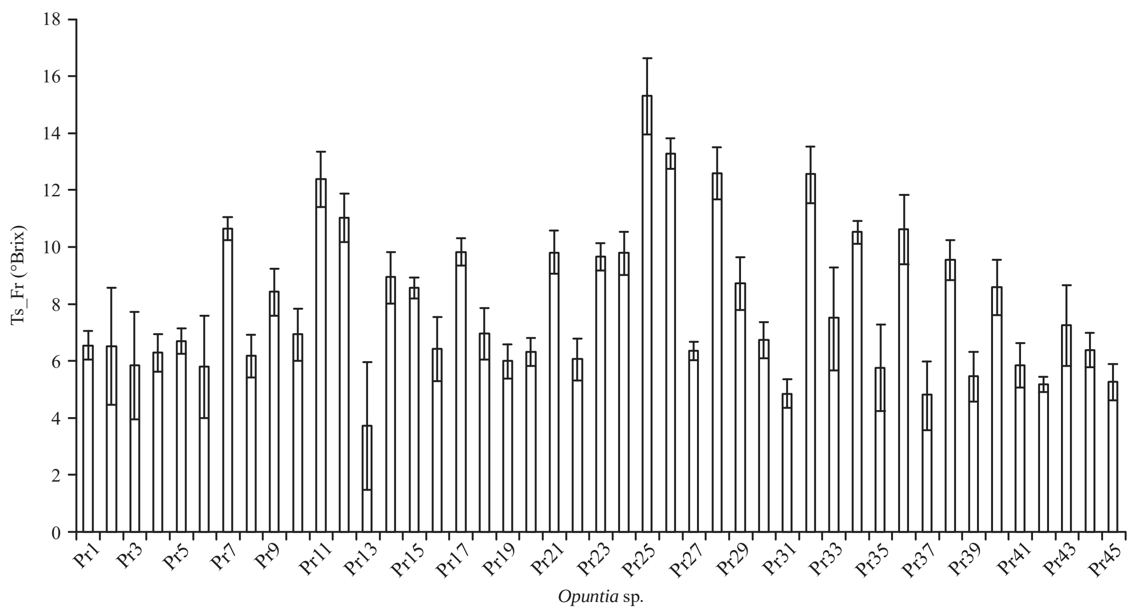

Fig. 3: Total soluble solids for fruit juice of 45 *Opuntia* ecotypes

Table 4: Qualitative traits of 45 *Opuntia* ecotypes

Ecotypes	LPF	Ci_Fr	Co_Ju	Fo_ Fr	Fo_Ra
Pr1	Medium	Absent or slightly depressed	Yellow	Medium elliptic	Broad elliptic
Pr2	Long	Strongly depressed	Purple	Narrow elliptic	Broad elliptic
Pr3	Medium	Strongly depressed	Yellow	Medium elliptic	Narrow obovate
Pr4	Short	Absent or slightly depressed	Orange	Narrow elliptic	Broad obovate
Pr5	Short	Strongly depressed	Orange	Narrow elliptic	Broad elliptic
Pr6	Short	Moderately depressed	Orange	Narrow elliptic	Narrow obovate
Pr7	Short	Strongly depressed	Orange	Oblong	Broad elliptic
Pr8	Short	Strongly depressed	Orange	Oblong	Broad elliptic
Pr9	Medium	Strongly depressed	Purple	Oblong	Broad obovate
Pr10	Short	Strongly depressed	Purple	Oblong	Broad elliptic
Pr11	Short	Strongly depressed	Red	Medium elliptic	Broad obovate
Pr12	Short	Strongly depressed	Yellow	Oblong	Broad elliptic
Pr13	Short	Strongly depressed	Orange	Oblong	Broad elliptic
Pr14	Short	Strongly depressed	Orange	Oblong	Broad elliptic
Pr15	Short	Strongly depressed	Orange	Medium elliptic	Rhombic
Pr16	Short	Strongly depressed	Orange	Oblong	Broad elliptic
Pr17	Short	Strongly depressed	Orange	Narrow elliptic	Narrow obovate
Pr18	Short	Strongly depressed	Orange	Obloblongong	Rhombic
Pr19	Short	Absent or slightly depressed	Orange	Oblong	Rhombic
Pr20	Short	Strongly depressed	Orange	Narrow elliptic	Broad elliptic
Pr21	Short	Strongly depressed	Orange	Medium elliptic	Narrow elliptic
Pr22	Medium	Strongly depressed	Pink	narrow elliptic	Broad elliptic
Pr23	Medium	Moderately depressed	Orange	Narrow elliptic	Broad elliptic
Pr24	Short	Strongly depressed	Orange	Narrow elliptic	Broad elliptic
Pr25	Short	Moderately depressed	Orange	Oblong	Broad elliptic
Pr26	Short	Moderately depressed	Orange	Medium elliptic	Rhombic
Pr27	Short	Strongly depressed	Orange	Oblong	Broad elliptic
Pr28	Long	Absent or slightly depressed	Red	Oblong	Narrow obovate
Pr29	Long	Strongly depressed	Yellow	Medium elliptic	Medium elliptic
Pr30	Long	Strongly depressed	Purple	Oblong	Broad obovate
Pr31	Medium	Strongly depressed	Yellow	Medium elliptic	Broad elliptic
Pr32	Short	Strongly depressed	Purple	Medium elliptic	Broad elliptic
Pr33	Short	Strongly depressed	Orange	Oblong	Broad elliptic
Pr34	Short	Strongly depressed	Yellow	Medium elliptic	Broad elliptic
Pr35	Short	Strongly depressed	Red	Obovate	Broad elliptic
Pr36	Medium	Moderately depressed	Pink	Obovate	Medium elliptic
Pr37	Long	Absent or slightly depressed	Purple	Narrow elliptic	Rhombic
Pr38	Long	Moderately depressed	Yellow	Narrow elliptic	Broad elliptic
Pr39	Short	Moderately depressed	Orange	Oblong	Broad elliptic
Pr40	Long	Strongly depressed	Yellow	Medium elliptic	Rhombic
Pr41	Long	Moderately depressed	Yellow	Narrow elliptic	Narrow elliptic
Pr42	Long	Strongly depressed	Orange	Obovate	Narrow elliptic
Pr43	Short	Absent or slightly depressed	Orange	Medium elliptic	Narrow obovate
Pr44	Medium	Moderately depressed	Purple	Oblong	Rhombic
Pr45	Short	Absent or slightly depressed	Purple	Narrow elliptic	Broadobovate

and fruit shape (Fig. 2). Generally, the *Opuntia* species had fruits with broad elliptic shape and orange to purple juice color (Table 4). Regarding the shape of cladode (FR), a noteworthy degree of variability among genotypes was observed (Fig. 1). The shape of cladode varied from medium-narrow elliptic to oblong (Table 4).

Correlation among variables: Simple correlation coefficient analysis showed the existence of significant positive and negative correlations among quantitative characteristics (Table 5).

Principal component analysis (PCA) was used to examine the variation of *Opuntia* species based on quantitative traits.

The first three axes accounted for 58.62% of the variability among 45 ecotypes (Table 6). The first PC axis (PC1) accounted for 5.74% of the variation, whereas the second (PC2) and the third axes (PC3) accounted for 2.67 and 2.13%, respectively. The first one was mainly related to cladode thickness, cladode fresh weight, fruit fresh weight, fruit diameter, seeds number, seeds weight, peel weight, flesh weight, fruit length, total soluble solid and pH of fruit juice.

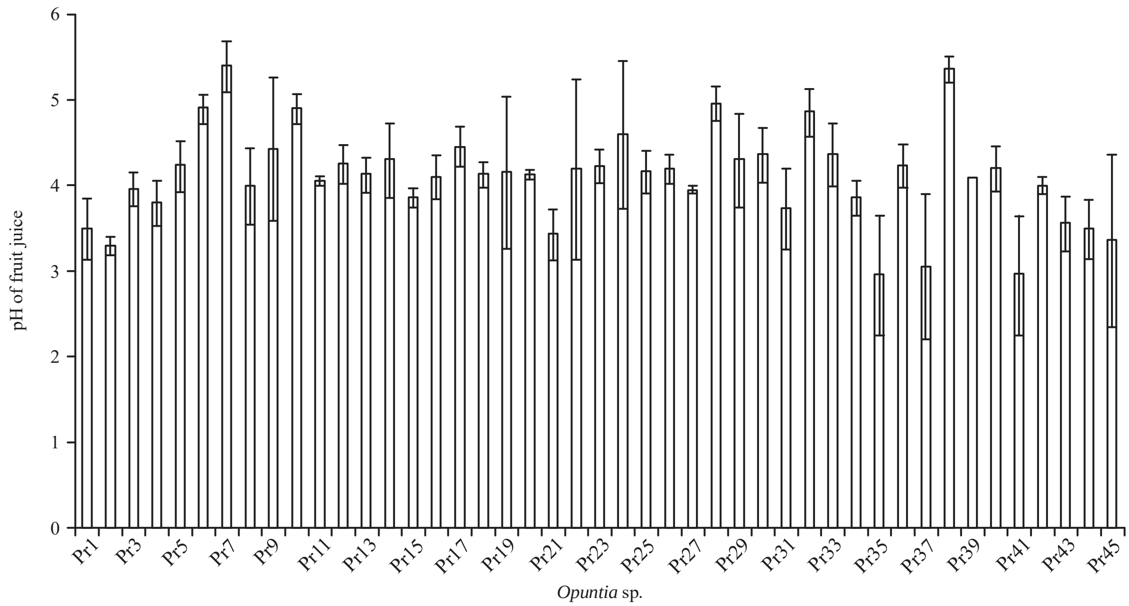

Fig. 4: pH of the 45 *Opuntia* ecotypes fruit juice

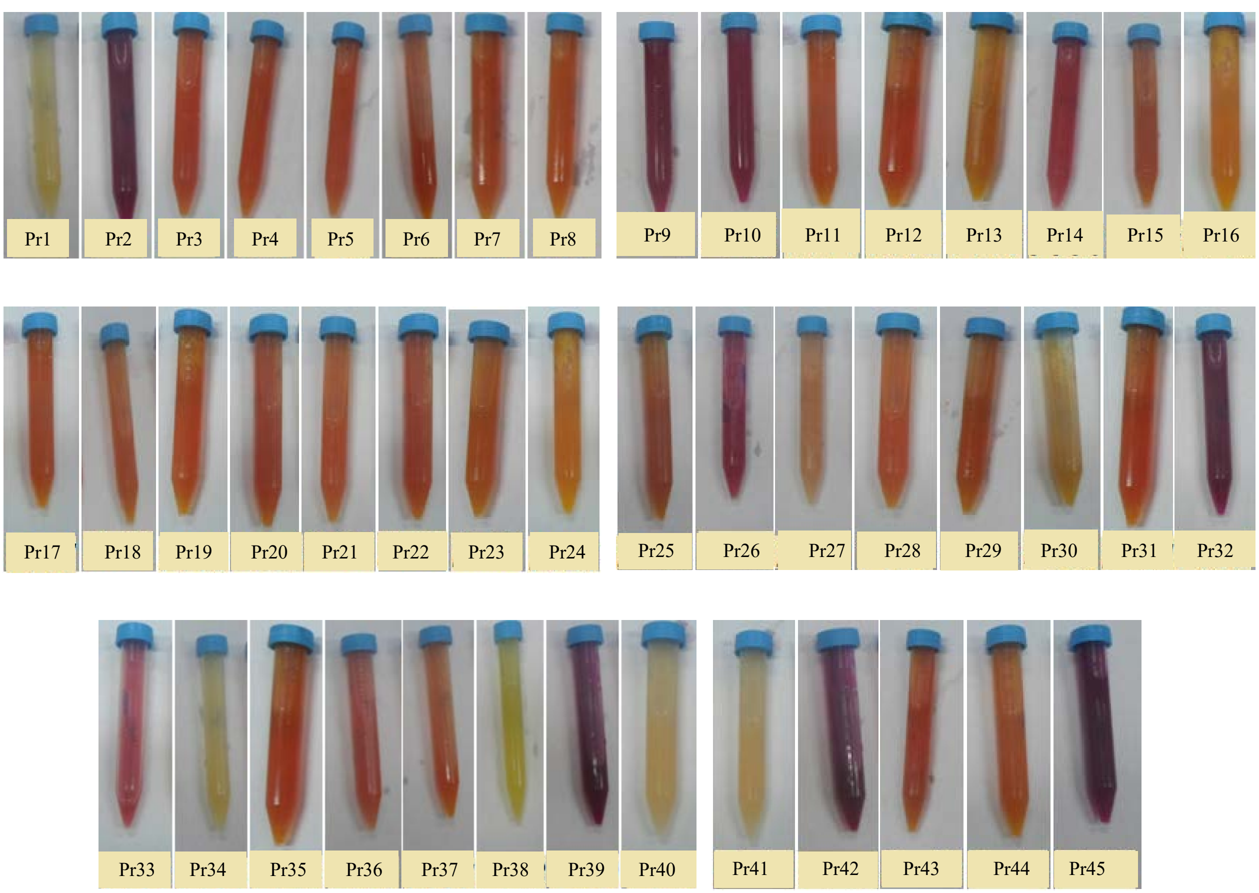

Fig. 5: Juice color of the studied *Opuntia* ecotypes

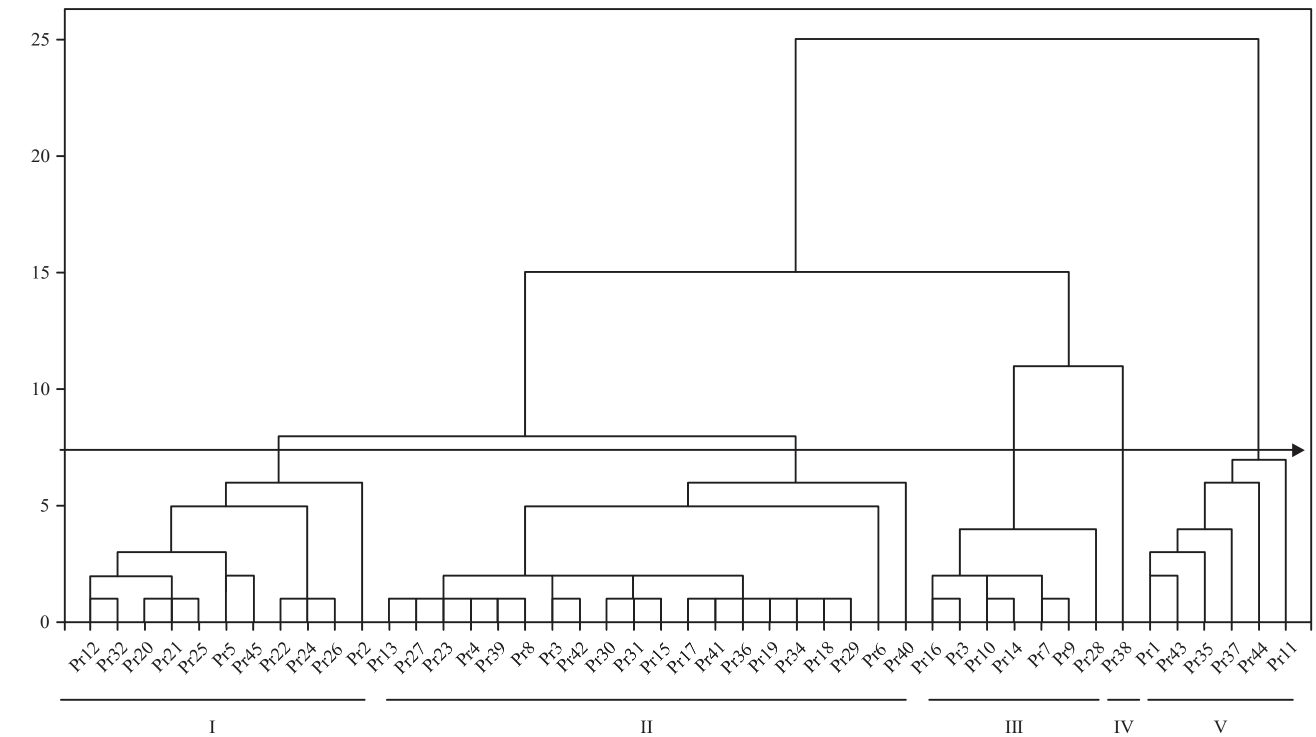

Fig. 6: Dendrogram for cluster analysis showing the 23 morphometric parameters relationships between the 45 ecotypes studied in hendi Zitoun (central region of Tunisia)
→: Cutting level

Table 5. Correlation among quantitative traits

Traits	PF_Ra	Lo_Ra	Lar_Ra	Ep_Ra	Nb_Ar	PS_Ra	Re_Ea	PF_Fr	Lo_Fr	D_Fr	Po_Ch	Po_Pe	Po_Pp	Nb_Pe	Ph_Fr
Lo_Ra	0.6**	1													
Lar_Ra	0.79**	0.51**	1												
Ep_Ra	0.72**	Ns	0.32*	1											
Nb_Ar	-0.39**	Ns	-0.65**	-0.31*	1										
PS_Ra	Ns	0.51**	Ns	Ns	Ns	1									
Re_Ea	Ns	Ns	Ns	Ns	0.32**	Ns	1								
PF_Fr	Ns	Ns	Ns	Ns	Ns	Ns	Ns	1							
Lo_Fr	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	1						
D_Fr	0.48**	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	1					
Po_Ch	0.26*	Ns	0.32*	Ns	Ns	Ns	Ns	0.72**	0.62**	0.77**	1				
Po_Pe	0.31*	Ns	Ns	0.38**	Ns	Ns	Ns	0.59**	0.48**	0.63**	0.54**	1			
Po_Pp	0.3*	Ns	Ns	Ns	Ns	Ns	Ns	0.89**	0.55**	0.77**	0.46**	0.54**	1		
Nb_Pe	0.35*	Ns	Ns	0.36*	Ns	Ns	Ns	0.52**	0.43**	0.62**	0.45**	0.74**	0.5**	1	
Ph_Fr	Ns	Ns	Ns	Ns	0.61**	Ns	Ns	0.52**	0.36*	0.62**	0.52**	0.6**	0.49**	Ns	1
Ts_Fr	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	0.61**	0.44**

*Significant at $\alpha \leq 0.05$, **Significant at $\alpha \leq 0.01$, Ns: Not significant

The second principal component (PC2) was highly correlated to width of cladode and mean distance between areoles. The third component (PC3) was determined by cladode length, cladode dry weight, areole number per cladode, cladode water reserve and fruit firmness. The PCA showed that 18 phenotypic traits are the discriminating characters for the 45 *Opuntia* genotypes in Tunisia.

The different *Opuntia* ecotypes are identified, based on the similarity of their morphological characteristics and their hierarchical clustering are shown in Fig. 6. The populations were grouped into five clusters by cluster analysis. These 5 groups can be considered as distinct germplasm pools. Genotypes in the same group have the greatest similarity.

Table 6: Principal component analysis (PCA) for the 45 ecotypes studied in Hindi Zitoun central region of Tunisia using quantitative traits

	PC axes		
Traits	PC1	PC2	PC3
Eigenvalue	5.750	2.670	2.130
Explained proportion of variance (%)	31.890	14.860	11.860
Cumulative proportion of variance	31.890	46.760	58.260
PF_Ra	0.612	0.474	0.174
Lo_Ra	0.396	0.487	0.592
Lar_Ra	0.452	0.766	0.124
Ep_Ra	0.470	0.116	0.116
Nb_Ar	-0.152	-0.400	0.621
PS_Ra	0.122	0.006	0.761
Me-Ra	0.215	0.689	-0.338
Re_Ea	-0.063	0.170	0.516
PF_Fr	0.853	-0.188	-0.191
Lo_Fr	0.734	0.096	-0.172
D_Fr	0.952	-0.072	-0.047
Fe_Fr	-0.159	0.334	0.479
Po_Ch	0.789	-0.077	-0.079
Po_Pp	0.787	-0.185	-0.036
Nb_Pe	0.710	-0.125	0.202
Po_Pe	0.656	-0.354	0.060
Ph_Fr	0.544	-0.440	0.168
Ts_Fr	0.162	-0.643	-0.040

DISCUSSION

The *Opuntia* ecotypes collected from Central Region of Tunisia and assessed by using morphological traits, showed a wide variation. The cladode dimension varied independently on the species which is in contradiction of the results found by Feugang *et al.*[17]. The *O. ficus-indica* (Pr45) with the greatest cladode length and width and *O. crassa* (Pr35) with the highest thickness will be selected in the objective to increase fruit production and improve cattle feeding in the arid and semi-arid zones of the country, which largely depend on *Opuntia* plant during droughts.

The cladodes with the highest dimensions (length, width, thickness, fresh weight and water content) are responsible for a large amount of mucilage which seems to be the most important limiting factor for new consumers using as forage. *Opuntia* species can provide a continuous, valuable supply of fresh fodder during the dry season, given its succulent non-deciduous vegetative structure, a feature rarely found in other forage species.

The presence of spines on the cladode is a serious impediment to widespread utilization of *Opuntia* genus. The Mexicain *O. crassa* (Pr11) and the Tunisian *O. ficus-indica* (Pr45) possess the low number of areole per cladode (1.5 areoles/cladode) and can be classified as spineless species. However, the Tunisian *O. ficus-indica* (Pr44) was the spiny species and had the greatest number of areoles/cladode. This latter, is more aggressive and better adapted to spread.

Spineless *Opuntia* species are thought to be the result of domestication and hybridization to facilitate their use as forage and human consummation. Zimmer[18] reported that there is evidence that only spineless forms were introduced to South Africa more than 250 years ago and they reverted back to the original spiny form over a period of nearly 200 years. These clues suggest the existence of recessive genes associated with spininess and confirm the ability of the species of *Opuntia* to reproduce from seed.

The *O. ficus-indica* (Pr28) from Sicily recorded the highest fruit fresh weight (125.83 g) which explain the adaptation of this one to arid Tunisian climate. Nevertheless, the Tunisian *O. ficus-indica* (Pr1, Pr40, Pr43 and Pr44) and the two Algerian species *O. crassa* (Pr35) and *O. tomentosa* (Pr37) registered the lowest fruit fresh weight (Table 3). This large difference in fruit fresh weight can be under various factors such as the richness of the soil in mineral elements, climate conditions and genetic potential of the species. Reyes-Aguero and Valiente-Banuet[4] showed that fruit weight in *Opuntia* is affected by the order of production of the flower bud and the number of fruits on the cladode. Thus, floral buds that sprout earlier usually become heavier fruits. Furthermore, the heaviest fruits are obtained from cladodes with only six fruits[19,20].

The Tunisian *O. ficus-indica* (Pr38) contains 290 seeds/fruit. However, the *O. crassa* (Pr 35) contain the few number (23.66 seeds/fruit).The *O. maxima* registered an average of 164-161 seeds/fruit which is in accordance with these found by Vila and Gimeno[21]. Several authors have reported a great variation in the number of seeds, from 1-5 to more than 2000 seeds per fruit[4,18]. This variation is observed within and/or between species depending on factors such as the age and size of the plant and the number of flowers/plant[17]. The presence of a great number of normal seeds in the *Opuntia* fruit is considered an obstacle for broadening its commercialization[22].

Flesh firmness, skin color and total soluble solids are the main parameters to assess the fruit maturity at harvesting. The *O. ficus-indica* (Pr38) is the firmest fruit (18.81 N) in comparison to the Ethiopian one (Pr2) with an average of 8.72 N. The *O. ficus-indica* (Pr38) characterized by high firmness can be stored for a long time. Firmness is a relevant property for consumer acceptability and quality control[23]. This fruit consists of a thick peel, covered with small thin spines, enclosing a sweet juicy pulp intermixed with many hard seeds. So, Firmness can be related to the thickness of peel. Result found by El-Gharras *et al.*[24] in Morocco showed that the fruit juice of *Opuntia* genotypes contains about 11-16% total soluble solids which is higher than the values of fruit juice of this collection. A total soluble solid does not depend on environmental conditions while fruit size increases with moisture availability during fruit maturation[25]. In general, it may be concluded that the knowledge of pH and total soluble solids in fruit may prove to be a powerful tool in evaluating both fruit maturity and quality.

The difference detected in morphological parameters can be the result of genetic variability inherent to *Opuntia* species. This is confirmed by the studies that have been conducted by Arba[26], where he noted that the difference in vegetative and fruit production in the *Opuntia* explains that it has significant genetic diversity, which varies from one variety to another and from one locality to another.

Great variability was detected in this study with juice color. A natural dye would be extracted to be used as commercial food colorant.

Therefore diversity in morphological traits among genotypes may be due to climatic or genetic differences. The morphological traits are probably affected by genetic and ecological factors. Indeed, it has been reported that altitude, soil drainage, temperature and precipitation affect the development and morphology of *Opuntia* plants[27].

This gene pool is useful in breeding program to produce other genotypes when combining the best morphological traits of the fruit and cladode with resistance to climatic and edaphic factors. It is necessary to know where the sterility barriers to hybridization occur within the currently species, such as *O. ficus-indica*, *O. maxima*, *O. Crassa*, *O. laevis*, *O. tomentosa* and *O. helvetica*.

Opuntia ficus-indica (Pr28) has the longest stalk and the heaviest fresh fruit. However, the *O. tomentosa* (Pr37) has the longest stalk and the lightest fresh fruit weight. This finding can confirm the absence of link between fruit weight and stalk length.

Simple correlation coefficient analysis showed the existence of significant positive and negative correlations among characteristics (Table 5). The *Opuntia* fruit traits such as (D-Fr), (Po-Ch), (Po-Pe), (Po-Pe) and Nb-Pe revealed a strong dependence on cladode fresh weight (PF-Ra) with ($r = 0.48$, $r = 0.26$, $r = 0.31$, $r = 0.30$ and $r = 0.35$), respectively. This latter was strongly correlated (P 0.01) with fruit diameter ($r = 0.48$) and less with weight of fruit flesh ($r = 0.26$), seeds weight ($r = 0.31$), peel weight $r = 0.3$) and seed number ($r = 0.35$). Areole number/cladode was also affected negatively by fresh cladode weight ($r = -0.39$) and cladode width and thickness with ($r=-0.65$) and ($r = -0.31$), respectively. The majority variables related to fruit size were correlated positively with cladode fresh weight at $p = 0.01$ (Table 5), indicating role of cladode to increase fruit size, this can be attributed to the photosynthetic rate of the cladode.

Hierarchical cluster analysis (Fig. 6) was carried out based on quantitative traits, allowed the assessment of similarity or dissimilarity and clarified inter and intra-specific relationships in studied *Opuntia* germplasm. There was diversity among genotypes, which were separated into 5 groups (Fig. 6). The first cluster (I) consisted of 11 *O. ficus-indica* from Tunisia (Pr12, Pr22, Pr24 and Pr45), Morocco (Pr5, Pr20 and Pr25), Mexico (Pr32) and Ethiopia (Pr2) and one *O. halvetica* from Tunisia (Pr21).This latter genotype, Pr25 and Pr20 were found to be very similar despite their belonging to two different species.

The second cluster (II) contained the majority of *O. ficus-indica* genotypes, *O. maxima* and *O. laevis*. *O. maxima* (P34 and Pr29) are distinguishable particularly by the low quantitative traits related to fruit and cladode. A series of homonyms were detected. Indeed, the *O. ficus-indica* species (Pr13, Pr27, Pr23, Pr4 and Pr3) and the *O. laevis* (Pr 8) were the closest, as, both species were characterized by high fruit firmness and water reserve. The *O. ficus-indica* (Pr 3 and Pr42) were probably homonyms and the *O. ficus-indica* (Pr31, Pr30 and Pr15) also can be considered as homonyms. The highest similarity was observed between the five *O. ficus-indica* genotypes (Pr17, Pr41, Pr36, Pr19 and Pr18) and the two *O. maxima* species (Pr34 and Pr29) identified as being synonymous with the smallest fruit. The two *O. ficus-indica* (Pr6) from Italy and Pr40 from Tunisia were easily distinguished from other genotypes. Then it's only supposed that DNA fingerprinting could help to explain the morphological similarities among the closely linked genotype.

The third cluster (III) was divided in two sub-clusters, the first one with three similar couples (Pr16, Pr33), (Pr10, Pr14) and (Pr7, Pr9) and likely homonyms and the second with the Italian *O. ficus-indica* (Pr28).The Tunisian *O. ficus-indica* (Pr38) formed a separate group (IV) and displayed a distinct branching pattern indicates the native Tunisian originality.

The fifth group (V) contained three *Opuntia* species with high distribution which was divided into two sub clusters. The first contained one Mexican *O. crassa* (Pr11) and the second contained a mixture of different species from different geographical origin such as Pr1, Pr35, Pr37, Pr43 and Pr44. We distinguish that *O. ficus-indica* (Pr1) and (Pr43) from Tunisia were very closely related and they probably belong to the same genotypes with the smallest quantitative traits (Cladodes and fruits). However, *O. carassa* (Pr11) and (Pr35)

From Mexico and Algeria respectively and *O. tomentosa* (Pr37) from Algeria showed few differences. This shows the adaptation of the introduced species and the modification of the morphological characters according to the Tunisian climatic conditions. While, Erre *et al*.[27] found that soil and land characteristics were not correlated with morphometric parameters. It should also be noted that *Opuntia* ecotypes are distributed among the different groups independently of their locality (Fig. 6).

Dendrogram showed high variation between *Opuntia* genotypes indicating that studied germplasm can be considered in breeding programs as a good gene pool for different traits.

CONCLUSION

The analysis of morphologic traits can allow the investigation of the level of genetic diversity among *Opuntia* ecotypes from different regions of the world. Results of this study will be useful for conserving and managing genetic resources in Tunisia. These results showed large variations in the morphologic and pomological properties of 45 *Opuntia* ecotypes. However, this information may not be enough to characterize the cactus (*Opuntia* species) collected in the central area of Tunisia. Therefore, it could be necessary to characterize all the species based on biochemical and molecular techniques.

SIGNIFICANCE STATEMENT

This study discover several morpho-chemical characteristics of *Opuntia* species like natural dye, number of seeds, °Brix··· that can be beneficial for human health. This study will help the researcher to uncover the critical areas of commercial exploration in agricultural activity and this collection of 45 ecotypes can be considered in breeding programs as a good gene pool for different traits that many researchers were not able to explore. Thus a new theory on drawing up a descriptor peculiar to the genus *Opuntia* may be arrived at.

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