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Study of the cladodes phenotypic diversity of 36 accessions of cactus in the arid region of Tafilalet

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Abstract. The prickly pear (*Opuntia ficus indica* Mill.) could be one of the most important forage crop species in the arid southeast region of Morocco. In this work, we report the analysis of cladodes traits belonging to 36 accessions of prickly pear (28 accessions of *Opuntia ficus indica* form inermis and 8 accessions of *Opuntia ficus indica* form amycleae) from different regions of Morocco and grown in the experimental station of Errachidia. Twenty-three phenotypic characters of cladodes were analyzed using analysis of variance and multivariate analysis (PCA). All attributes were significantly different within and between the two forms of cacti. The shape of the cactus and the geographical origin affected the characteristics of the cladodes. Comparison of our results with those obtained for the same accessions grown in the station Melk Zehar located in Agadir (southwest Morocco) revealed significant differences in favor of the Agadir environment. This study allowed us to group the characters of the cladodes into three classes for the two cactus forms.

Keywords. Opuntia – Cladodes – Phenotypic variability – Accessions.

Étude de la diversité phénotypique des cladodes de 36 accessions de cactus dans la région aride de Tafilalet

***Résumé**. Le figuier de Barbarie (*Opuntia ficus indica *Mill.) pourrait être l'une des espèces fourragères alternatives importantes du point de vue agro-économique dans la région aride du sud-est du Maroc. Dans ce travail, nous rapportons l'analyse des raquettes de 36 accessions de figuier de Barbarie (28 accessions d'*Opuntia ficus *indica forme inermis et 8 accessions d'*Opuntia ficus *indica forme amycleae) provenant de différentes régions du Maroc et cultivées dans le domaine expérimental d'Errachidia. Vingt-trois caractères phénotypiques de la raquette ont été analysés en utilisant l'analyse de la variance et l'analyse multivarié (ACP). Tous les paramètres se sont révélés significativement différents au sein et entre les deux formes de cactus. La forme du cactus et l'origine géographique ont affecté les caractéristiques de la raquette. La comparaison de nos résultats avec ceux obtenus pour les mêmes accessions d'Opuntia cultivées dans le domaine de Melk Zehar d'Agadir (sud-ouest Morocco) ont révélé des différences notables en faveur de l'environnement d'Agadir. Cette étude nous a permis de grouper les caractères de la raquette en trois classes et ce pour les deux fromes de cactus.*

***Mots-clés.** Cactus – Raquette – Variabilité phénotypique – Accessions.*

I – Introduction

Cactus species are native to the Americas. *Opuntia ficus indica* Mill. is mainly grown in dry, arid and semi-arid countries, including Morocco, Tunisia, Algeria, South Africa, Italy, Brazil, Argentina, Chile and Mexico (Felker and Inglese 2003). The Cactus was introduced in Morocco around the 16th century. Its area has constantly increased from 50,000 ha in 1998 to more than 140,000 ha (Arba *et al*., 2000; 2009).

Several successful genotype breeding programs are carried out around the world, including Mexico, United States, Italy, India, South Africa and Argentina (Reyes-Aguero *et al*., 2005; Zoghlami *et al*., 2007; Peña-Valdivia ert al. 2008; De Wit *et al*., 2010; Soni *et al.,* 2015; Nefzaoui, 2016; Samah *et al*., 2016; Adli *et al*., 2017; Mondragón-Jacobo and Chessa, 2017) .

Moroccan national genetic diversity of this species is apparent with different color varieties and harvest periods that vary from one variety to another and from one locality to another. The main varieties distinguished by the farmers are: Aissa, Moussa, Dellahia, Al Akria, Achter, Draibina, Acherfie and Mles. In addition, most of the published information on the biodiversity of cacti grown in Morocco focuses on these varieties (Boujghagh, 2011 ; El Finti *et al*., 2013 ; Bendhifi *et al*., 2015 ; El Kharrassi *et al*., 2016 ; Mabrouk *et al*., 2016).

Therefore, given the important role of the genus *Opuntia* in the ecology of arid and semi-arid regions of Morocco and its valuable diversity, we conducted this experiment that consisted in evaluating the morphological characteristics of the cladodes of 36 accessions of *Opuntia* and identifying the main features that contribute to their discrimination.

II – Materials and methods

The field trial was conducted at the Errachidia experimental station (31°55 N; 4°26.9; 1060 m a.s.l.). It was planted during March 2011. The soil of the test plot is sandy-loamy. The average annual rainfall is 139 mm and summer temperatures can reach 50ºC. The winter is very cold and the minimum temperature can drop to –7°C (29-years data from the Errachidia experimental station).

The plant material studied was the genus *Opuntia* sp. which includes two species: *Opuntia ficus indica* Mill form inermis and *Opuntia ficus indica* Mill form amycleae (syn: *Opuntia Megacantha* Slam Dyk). Thirty three cladodes used in this study came from the Melk Zhar experimental station of Agadir. These accessions were collected from different regions of Morocco (Table 1). The other three accessions came from the Errachidia region. These accessions are quite representative of the diversity of the prickly pear in Morocco. Irrigation was provided by drip irrigation, according to the season of year and rainfall (generally six irrigations per year were ensured). Each ecotype was repeated 4 times. The density was 1250 plants / ha (2 x4 m). The experiment aimed to compare cladodes characters of 36 cactus accessions. The experimental design was a Complete Random Block design with 4 replications. The experimental unit consisted of a single shrub per accession.

Twenty-two quantitative morphological characteristics of cladodes were evaluated in this study (Table 2).The identification of suitable characters is an essential element for the selection of species, accessions, genotypes and / or cultivars. For this work we used the list of cactus descriptors previously developed by the International Technical Cooperation Network on Cactus and approved by Biodiversity International (Chessa and Nieddu, 1997). The evaluation was also performed in accordance with some *Opuntia* descriptors previously used by Reyes-Aguero *et al*. (2005). The characteristics studied were also recorded according to the test guidelines for cactus and xoconostle of the International Union for the Protection of New Varieties of Plants (UPOV, 2004).

For each accession, six healthy, with no malformations and non-lignified cladodes of two or three years old per accession were selected. These cladodes were harvested during the month of March. All the morphometric parameters were measured by a graduated scale. The thickness of the cladode, the length of the spines were measured with digital calipers. The surface and the perimeter were calculated by assimilating the cladode to an ellipse. The dry weight of cladode was obtained at 65°C in the oven during three days. The number of areoles per cladode was measured on a surface of 25 cm² on the central part of both sides of the cladode. The other attributes were quantified .The qualitative characteristics have been described and noted.

Table 1. Code and origin of the different accessions of cactus collected in Morrocco

Code	Collection site	Altitude (m)	Code	Collection site	Altitude (m)	Code	Collection site	Altitude (m)
18	Maader Tiznit	252	135	Aït Ayache (Khenifera)	1550	183	El-Jadida 2	20
29	Aït Boufouln S. Ifni	1040	137	Laassri (Khenifera)	520	T1	Er-Rachidia 1	1048
33	Tlata Isboya Sidi Ifni 1	305	141	Tighboula (Ksiba)	715	T2	Er-Rachidia 2	1042
36	Tlata Isboya S. Ifni 2	305	142	Kesbat Tadla	500	T3	Er-Rachidia 3	1055
70	Aghroud Agadir	10	145	Ouaouizeght (Beni Mellal)	900	45	Ouled Berhil Taroudant	490
77	Sebt Guerdane Taroudant	185	147	Ouled Ayach (Beni Mellal)	275	62	Taroudannt	238
83	Aït laaza Taroudant	275	149	Ouled Cheikh	750	93	Cherarda Oulad Taïma 1	128
105	Had Dra Essaouira	200	160	Ain Lahcen Titouan 2	240	96	Cherarda O. Taïma 2	128
114	Barraket Lamine Safi	147	161	Ain Lahcen Titouan 3	270	118	Jamaat Shaïm Safi	167
130	Assaka Ouarzazate	1380	174	Ouled Moussa (Bouznika)	365	131	Taliouine Taroudant	1200
132	Skoura Ouarzazate	1220	181	Azemour	28	157	Ain Lahcen Titouan 1	210
134	Rich (Midelt)	1320	182	El-Jadida 1	20	184	Sidi Ismail (El-Jadida)	150

Table 2. Attributes evaluated for the cladodes (Cladode descriptors)

CHR (AB) : Cladode height (cm)	D : Distance CE	NLAR : Number of lines of areolas
CD (CD) : Cladode diameter (cm)	CTH : Cladode thickness (cm)	NLARt : Number of lateral areoles
LDC : Lower diameter of the cladode (cm)	LLS : Length of the largest spine	FWC : Fresh weight of the cladode (g)
SI : Shape index AB/CD	LSS : Length of the smallest spine	DMC : Dry matter of the cladode (g)
UW (AE) : Upper width (cm)	NTH : Number of thorns	SC: Surface of the cladode (cm²)
LW (BE) : Lower width (cm)	DAR-D : Density of areoles on 25cm²	PC: Perimeter of the cladode (cm)
TWD : Top widest distance (cm)	NAR : Number of areoles in the center	
WDB : Widest distance in the bottom (cm)	TNAR/GI : Total number of areolas / cladode	

For quantitative traits, means ± standard deviation and coefficients of variation were calculated. The attributes measured were first subjected to variance analysis and the means compared with the Student Newman and Keuls (TSK) test at the 5% error threshold based on the smallest significant amplitude. Principal Component Analysis (PCA) was used to assess the degree of similarity between the tested attributes of Opuntia and to understand the relationships between them. We used the Xlstat 2014 software for statistical analysis.

III – Results and discussion

1. Analysis of the variability of the cladode shape

The analysis of the variance showed that all the measured parameters were significant according to the Student Newman and Keuls (TSK) test.

2. Comparison of cladode parameters between the Agadir and Errachidia stations

The cactus develops slightly different characteristics depending on the environment. Indeed, fourteen accessions of the 31 grown in the Errachidia station have developed a cladode mean lenght

bigger than that of those grown in Agadir station. Likewise for the maximum width, but for only 12 accessions that have developed a larger average diameter. The shape index is relatively similar between the two stations. In the same way, half of the accessions at Errachidia have developed a greater thickness compared to those of Agadir station. We should note that the thickness depends on the irrigation and the period of measurements. Nevertheless, there is a big difference in the number of spines and the length of the largest spine between the Agadir and Errachidia stations. The presence or absence of thorns is controversial. Indeed, accessions without thorns and growing in a stressful environment (heat, drought) could become spiny. Rebman and Pinkava (2001) reported that, in growing habitat, the presence of spines, the number of spines per areola, and the number of areolas may differ drastically between regions.

3. PCA Analysis of the main morphometric parameters of the cladode

The analysis of cladode characters by the scatter plot representation (Fig.1) of the PCA indicates variability and similarity between several accessions in this study. This variation in cladode characteristics certainly influenced the growth and the performance of the accessions.

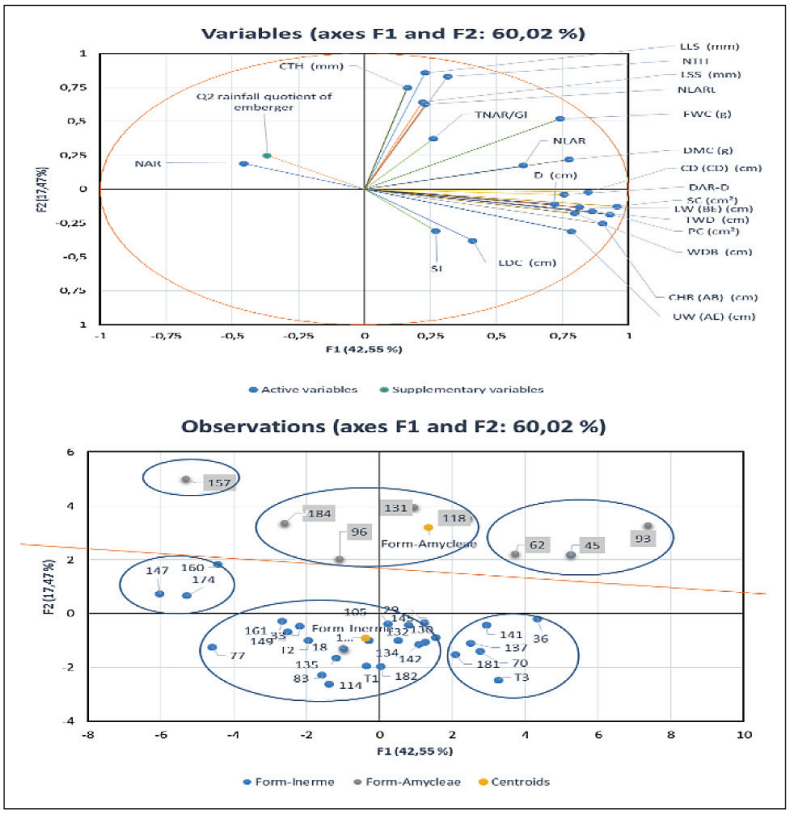

Fig. 1. Correlation circle (left side) and PCA analysis of cladodes parameters.

The first 3 components represent 70.98% of the variation. The first component represent 42.55% of variation and is defined by : CHR (AB), CD (CD), UW (AE), LW (BE), TWD, WDB, D, NAR, NLAR, FWC, DMC, SC and PC. The second component explains 17.5% of the total variation and is correlated to: CTH, LLS, LSS, NTH, NLARt and the shape of the cactus. The Third component (11%)

explain meanly the Shape index (SI). This result is satisfactory compared to Peña-Valdivia *et al* (2008) who reported that the first three CPs explained 46% of the total variability.

The biplot (1-2) showed the existence of different groups of accessions from the two forms (inermis and amycleae) (figure 1). The difference found between the inermis accessions and the amycleae ones would be due to the characters of the spines of the amycleae that are correlated with axis 2 (above the orange line). The same results were found by Colunga *et al*. (1986) who separated inermis and amycleae accessions from a group of 55 Opuntias including cultivated and spontaneous cultivars. Similarly, Peña-Valdivia *et al*., (2008) showed that the 46 Opuntia accessions from Mexico were separated into two groups according to the presence or absence of spines on the cladodes. In our case, the accessions on the right have the largest size, while those on the left are smaller and have important number of areola. Therefore, three groups can be distinguished in both forms. It seems that morphological characters are negatively correlated with Emberger's quotient Q2, which means that provenances with high Q2 will have smaller cladodes and vice versa. In this graph (Fig. 1) some parameters like Shape index (SI) are not explained (explained in the third component). Some characters are useless (not explained by the tree first component) in this study and further investigations should be done to reduce the number of descriptors.

IV – Conclusion

This evaluation of the cladode phenotypic diversity of prickly pear is important for establishing future breeding programs and for the *in situ* conservation of this germoplasm. Three classes or groups of cultivars of cactus have been observed among the two forms of cactus (six in total). These groups of prickly pear accessions have different characteristics (meanly size and thorns) represent a heritage of great importance. This paper explains only the variability of cladode shape, other reports will be focused on the fruit characteristics and the production.

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