

Evaluation of the Efficiency of *Opuntia ficus-indica* Cladode Cuttings for Vegetative Multiplication

Sondes STAMBOULI-ESSASSI¹, Rania HARRABI¹, Sadok BOUZID¹,
Fethia HARZALLAH-SKHIRI^{2*}

¹University of Tunis El Manar, Faculty of Sciences of Tunis, Bioresources, Biotechnology and Climate Change Laboratory, Manar II,
1060 Tunis, Tunisia; sondesessassi@topnet.tn; harrabirania@yahoo.fr; sadokbouzid@fst.rnu.tn

²University of Monastir, High Institute of Biotechnology of Monastir, Genetics, Biodiversity and Valorization of Bioresources Laboratory, Street Tahar Haddad,
5000 Monastir, Tunisia; fethiaprospis@yahoo.fr (*corresponding author)

Abstract

In Tunisia, *Opuntia ficus-indica* (L.) Mill., occupying many hundreds of thousand hectares, constitutes a future plant as a fruit tree, mainly due to its edible fruit and vegetal mass used as food. The continuously increasing demand for young plants for the extension of its cultivation requires the research of rapid, efficiency and economic methods ensuring conformity multiplication. With aim to a large production of plant material, a rapid *in situ* propagation method of the prickly pear cactus was developed. Varied portions of *Opuntia ficus-indica* cladodes harvested in spring or in autumn, planted horizontally or vertically were used in order to optimize rhizogenesis and secondary cladode initiation rates. Half, quarter and the tenth of cladode cuttings harvested and planted in spring vertically and in normal polarity show very interesting results concerning rhizogenesis and caulogenesis. Those portions of cladodes demonstrated the best results, vertically planted in normal polarity and 100% of rooting was observed on right ones. The number of roots was the highest on basal right tenth cuttings (80). Contrariwise, the percentage of secondary cladodes initiated was the highest on apical right cuttings (70 and 74%) and the longest roots were initiated on the two basal tenth cuttings, left and right ones (13 and 14 cm). This fragmentation represents a substantially gain of material and time especially for large cultivated surfaces of *Opuntia ficus-indica*. Reducing the cladode cutting size, do not reduce its rhizogenesis and caulogenesis potentialities.

Keywords: areole, *in situ* propagation, prickly pear cactus, Tunisia

Introduction

Interest in the genus *Opuntia* L. dates back to many thousands of years. Its origin and history were closely related to the ancient Mesoamerican civilizations and particularly to the Aztec culture (FAO, 2013). *Opuntia ficus-indica* (L.) Mill., prickly pear cactus or Barbary-fig cactus (Cactaceae, Opuntioideae), is a xerophytic plant, native to Mexico (Russell and Felker, 1987; Reyes-Agüero *et al.*, 2005). It grows in many regions of the world such as Africa, Australia and in the Mediterranean countries (Piga, 2004). It was well adapted to arid and semi-arid environments and tolerated poor soils (Barbera and Inglesse, 1993; Gallegos-Vázquez *et al.*, 2012). Its ability to adapt allows it to colonize marginal lands (Barbera and Inglesse, 1993). *Opuntia ficus-indica* has been long time ago domesticated throughout arid and semi-arid regions of the world and constituted until today an important crop in agricultural economy for many countries (Griffith, 2004).

In several countries, *Opuntia ficus-indica* was intensively cultivated in commercial plantations for a number of purposes. This cacti plant has formed one of the most valuable natural resources by its multiple benefits and uses for peasants and farmers. In fact, it is a source of fruit and of vegetables (pad and cladodes) for human consumption justified by its high nutritional value (Le Houérou, 1996; Haj Sadok *et al.*, 2008; Stintzing *et al.*, 2012), as well as a fodder for cattle and other animals especially when there is shortage of fresh forage due to drought (Scheinvar, 1995; Viguera and Portillo, 2001). The cactus pear sweet fleshy fruits called “tunas” or “figs” used since prehistoric times (Barbera and Inglesse, 1993), actually in great demand on local markets, are exported to several countries. Furthermore, traditionally “nopales” or cactus cladodes have been consumed in Mexico and United States, and actually more recently in European countries. *Opuntia ficus-indica* has also been grown as a host plant in Mexico for cochineal insects

breeding (FAO, 2002; Chavez-Moreno et al., 2009; 2011) used for the production of biological natural dyes (Nobel, 1994).

Opuntia species have the advantage of multiple uses in agro-industry (Saénz, 2006). Many industrial sectors use cactus pear fruits and their fleshy leaves as industrial raw materials for pharmaceutical and cosmetic industries, alcoholic drinks and food additives (Saénz et al., 2009; FAO, 2013). Moreover, plantations of prickly pear cactus could be a promising strategy for the protection of natural resources by protecting soils against erosion (Le Houérou, 1996; Mulas and Mulas, 2004). The plant was also used as a living fence in gardens and fields and it helps to combat desertification (Scheinvar, 1995; Khalafallah et al., 2007; Neffar et al., 2011; FAO, 2013), rehabilitation and conservation of steppe rangelands and degraded areas (Neffar et al., 2011).

In Tunisia, *Opuntia ficus-indica*, this permanent arborescent species, occupying many hundreds of thousand hectares, constitutes a future plant as a fruit tree, mainly due to its edible fruit and vegetal mass used as food. It is a part of the landscape in Tunisia with two spinescent and inermis varieties: *Opuntia ficus-indica* var. *spinescent* (spiny, spinous) and *Opuntia ficus-indica* var. *inermis* (Grant and Grant, 1979). In nature, intermediate forms can be encountered (Walali Loudyi and Skiredj, 2003). It covers an area of about 600 000 ha, distributed mainly in west-central regions on the plains of Kasserine (Thala region) (Nefzaoui and Ben Salem, 2002). This *Opuntia* species was cultivated in Tunisia as fruit tree on limited surfaces (Ben Salem et al., 2004). Actually there is a remarkable development of its culture considering its multiple uses. However, farmers prefer the culture of the variety *inermis* in the most favourable conditions hoisting it to a fruit tree status and to the production of a vegetal mass used as feed. The prickly pear grows spontaneously by natural propagation of cladodes with soil contact (Ramadan and Morsel, 2003). It has a mainly relative ease in vegetative propagation by pad or cladode cuttings providing genetically homogeneous clonal populations identical to the plant source (Escobar et al., 1986). Vegetative propagation, which is widely used, can be performed through the rooting of single or multiple cladodes, small portions of mature cladodes comprising two or more areoles, or by using fruits as propagules (Lazcano et al., 1999; Reyes-Agüero et al., 2006; Estrada-Luna et al., 2008). However, biological and morphogenetic studies of the prickly pear specifying the scientific aspects, correlations and tissues implemented in this vegetative propagation were limited. Micropropagation of *Opuntia ficus-indica* has been extensively studied and several protocols and strategies have been developed to propagate this species (Khalfallah et al., 2007; El Finti et al., 2012).

The continuously increasing demand for young plants for the extension of its cultivation requires the research of rapid, efficiency and economic methods ensuring conformity multiplication. The purpose of this study was to determine the relative importance of vegetative propagation through detached cladodes divided in portions more and more reduced and to choose the material that optimize the *in situ* cutting according to the seasons and the size of the used plant material.

Materials and Methods

Plant material

Cladodes used as plant material were cut at sub terminal junction with a sharp knife, on vigorous *Opuntia ficus-indica* var.

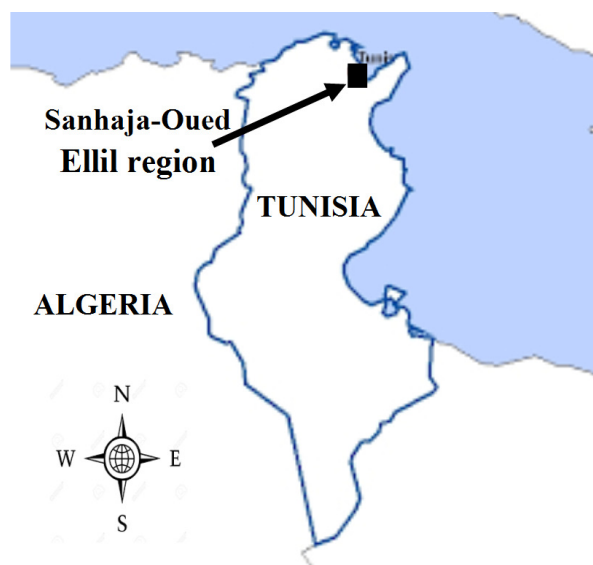


Fig. 1. Location of Sanhaja-Oued Ellil region on Tunisia map

inermis mother plants planted in a private field in Sanhaja-Oued Ellil region (36°48'N,10°6'E), located 20 km North-West of Tunis, Tunisia (Fig. 1). The environmental conditions were an average minimum temperature of 13 °C and an average maximum temperature of 23 °C per year. The pluvial precipitation was 462 mm per year. Cladodes were collected from several plants in the same sampling areas and were one year old and in a good physiological state. Cladodes damaged should be avoided. Each cladode sample was about 450 cm² of surface and weighted approximately 500 g.

Field experiment: Transplanting of entire and half cladode cuttings

Two field experiments, each one of 100 m² (25 m × 4 m) were carried out in the area of the Faculty of Sciences of Tunis; the culture soil was a vegetal soil (2/3) with fine sand (1/3). At the first field, cladodes or cuttings were harvested and planted in October (autumn). In the second, harvesting and planting were carried in March (spring). Each experiment field was divided into four lines spaced by 2.5 m. Fresh cladodes were transported to the laboratory and separated in two groups each one constituted by 105 cladodes. The entire and half-cladodes (longitudinally cut) (Figs. 2a and b) were allowed to air-dry in the shade for two weeks to promote cicatrisation. These practices increase transplanting success. In each field, 70 entire cladodes were transplanted in each of the first and the second line, and the 70 half ones in the third and the fourth lines (35 cladodes or halves of cladode by line). All cuttings were planted vertically, the basal part buried to 2/3 in moist soil to provide maximum surface contact.

Greenhouse experiment

a. Transplanting of the quarter of cladodes in pots under greenhouse shelters

Forty cladodes of *Opuntia ficus-indica* harvested in March from the same field of collection, were cut each one into 4 parts: ALe, AR, BLLe, BR (Fig. 2c), each one of about 113 cm². The 160 fourths of cladode cuttings, were allowed to air-dry in the shade

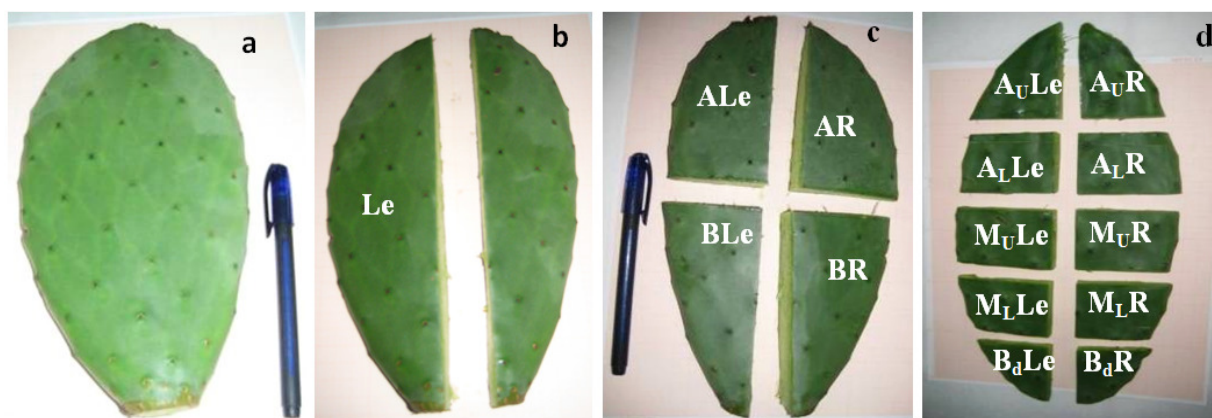


Fig. 2. Cuttings used in the study: entire (a), half (b), quarter (c), tenth (d) cutting. A: Apical, Le: left, R: right, B: Basal, AU: Apical upper, AL: Apical lower, Bd: Basal down, ML: Middle lower, MU: Middle upper

for two weeks and were planted vertically in 16 cm diameter and 2000 cm³ volume pots, filled with a mixture of peat (1/3), vegetal soil (1/3) and fine sand (1/3) and kept for 3 months under greenhouse conditions (Day/night air temperatures of 23 °C/18 °C). The cuttings were watered every 3 days with tap water.

b. Transplanting of the one-tenths of cladodes in pots under greenhouse shelters

Thirty cladodes, harvested in March, from the same field of collection, were divided each one into 10 parts (Fig. 2d). The apical (A) and median (M) zones were each one cut into 4 parts and the basal (B) zones into two parts. Thus, we obtain a total of 300 cuttings (10 cuttings per cladode, each one of approximately 45 cm²). The left cuttings were planted horizontally, and the right ones vertically, each one, in 16 cm diameter and 2000 cm³ volume pots, filled with the same soil mixture and placed at the same conditions as above. In both experiments, a randomised complete block design in four replications was used.

Growth analysis

The numbers of adventitious roots and of secondary cladodes initiated by cutting and the length of each root were reported at regular intervals of 3 days. The percentages of rooting and of secondary cladodes initiated were calculated after 3 months of planting.

Statistical analysis

Data collected from four independent experiments were subjected to analysis of variance (ANOVA) using SPSS 13.0 for Windows (SPSS Inc Chicago, IL, USA). The percentages of roots and of cladodes newly initiated were transformed using arcsin square-root ($\arcsin \sqrt{x}$) before ANOVA. Means were separated at the 5% significance level by a least significant difference test (Duncan's test).

Results and Discussion

In situ vegetative cuttings

a. Behaviour of the entire and half cladode cuttings

After 3 months of culture, the different transplanted cuttings were delicately dug up to analyze the extent of rhizogenesis and of axillary caulogenesis related to the season of harvest (autumn and

spring). The organogenesis responses of cladode cuttings were summarized in Table 1. Half cladode cuttings show very interesting results concerning rhizogenesis and caulogenesis. The number of roots initiated per cutting and the percentage of rooting were important and varied from 7.0 to 19.0 and from 81.4 to 98.5%, respectively. The most important percentage and the highest number of roots per cutting were obtained with entire cladodes harvested in spring. The percentage of rooting for *Opuntia ficus-indica* cuttings was affected with the season and also with the nature of the cutting. However, the number of roots was slightly more important on the entire cladodes than on halves of cladodes. The longest roots were obtained on entire cladodes transplanted in spring, followed by those transplanted in autumn, 17.0 and 15.0 cm, respectively. It is noted that initiated roots appear at the base of the entire and half-cladode but also at the surface of the portion buried, in contact with the soil (Figs. 3a and b). These adventitious roots, in all cuttings, derived from areolar meristems. The number of secondary cladodes (nopalitos) initiated on the cuttings varied from 1.6 to 8.5 and the percentage of those newly cladodes initiated was important and ranged from 5.2 to 33.3.

The highest number and percentage in secondary cladodes initiated were obtained in entire and half cuttings gathered in spring. Type of cladode had no significant effect ($p > 0.05$) on the percentage of secondary cladodes developed as reported by Gibson and Nobel (1986), but it was important to consider the number of secondary cladodes initiated per cutting. Cladodes newly initiated results from the differentiation of cladode areolar meristems: specialized buds (Gibson and Nobel, 1986; Bowers, 1996). These areoles are the homologous of the Dicotyledonous axillary buds (Boke, 1944) which are provided with dormant vegetative point protected by bristles or trichomes (Feugang *et al.*, 2006). The appearance of secondary new cladodes is mainly located at the apical zone (summit of the crown) of all cuttings but less frequently on the faces of cuttings (Figs. 3f-i).

b. Behaviour of the quarter-cladode cuttings in situ transplanting

After 3 months of culture, the obtained results are summarized in Table 2. The most important percentages of rooting were obtained with the right quarter cuttings, 97.5% for basal right (BR) and 87.5% for apical right (AR) quarter ones. No significant difference was observed in the percentages of rooting for apical left (ALe) and for basal left (BLe) quarter cladode cuttings, nevertheless percentages were less important (80.0%).

Table 1. Effect of season (autumn and spring) on the rhizogenesis and caulogenesis of the *in situ* *Opuntia ficus-indica* cuttings, entire and half ones

Season	Nature of cuttings	Number of roots initiated per cutting	% of rooting	Average length of root (cm)	Number of secondary cladodes initiated per cutting	% of secondary cladodes initiated
Autumn	Entire cladodes	10.0 ± 2.0b	91.4b	15.0 ± 2.4c	2.9 ± 1.1b	6.2a
	Half cladodes	7.0 ± 2.1a	81.4a	11.0 ± 1.8a	1.6 ± 1.1a	5.2a
Spring	Entire cladodes	19.0 ± 3.5c	98.5c	17.0 ± 2.3d	8.5 ± 1.8c	33.3b
	Half cladodes	10 ± 2.3b	87.1cb	13.0 ± 2.3b	8.4 ± 1.5c	32.7b

Note: Means ± SD with the same letter (s) in the same column are not significantly different ($p < 0.05$) (Duncan's multiple range test) (n = 35 cuttings per treatment).

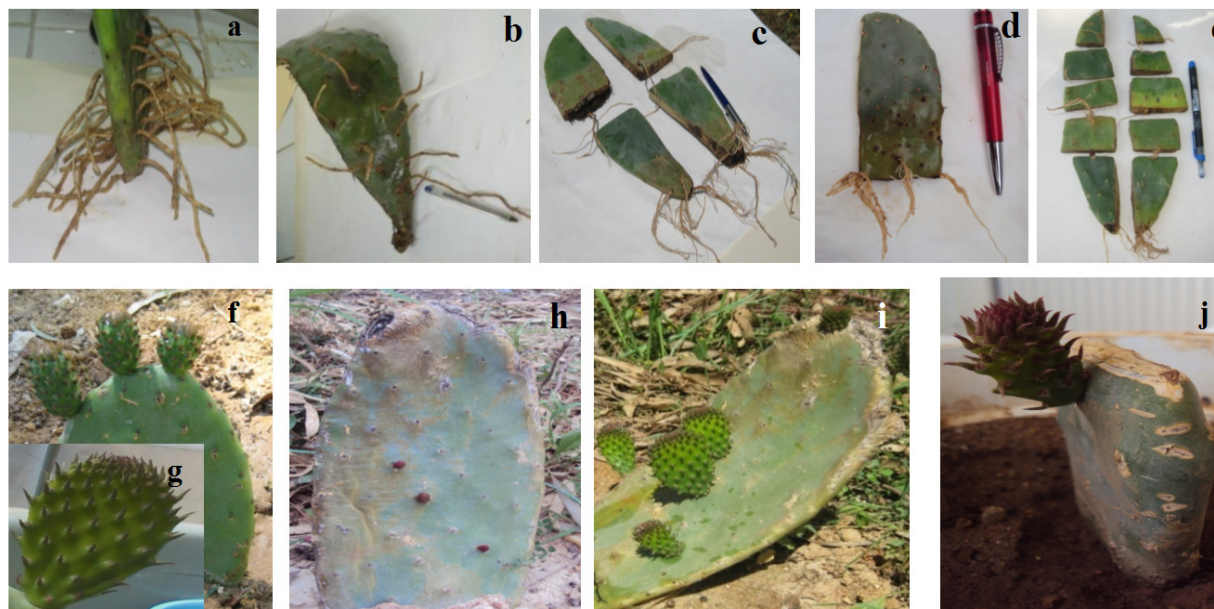


Fig. 3. Adventitious roots: entire (a), half (b), quarter (c and d), tenth cuttings (e). Secondary cladodes on cutting: entire cladode (f-i) and quarter (j) secondary cladodes

Table 2. Effect of the position of the spring quarter cladode cuttings on the percentages of rooting and of the secondary cladodes initiated, the average number of roots, the length of root per cutting and the average number of secondary cladodes initiated *in situ* from *Opuntia ficus-indica* cuttings

Spring quarter-cladode cuttings	Number of roots per cutting	% of rooting	Average length of roots (cm)	Number of secondary cladodes initiated per cutting	% of secondary cladodes initiated
ALe	3.9 ± 1.4a	80.0a	6.8 ± 1.2a	5.7 ± 1.3a	15.6a
AR	4.8 ± 1.3b	87.5b	8.8 ± 1.4c	8.4 ± 1.1b	17.1b
BLe	4.9 ± 1.3b	80.0a	7.8 ± 1.2b	5.4 ± 1.3a	17.9b
BR	5.8 ± 1.1c	97.5c	9.0 ± 1.1c	5.5 ± 1.3a	22.5c

Note: Means ± SD with the same letter (s) in the same column are not significantly different ($p < 0.05$) (Duncan's multiple range test) (n = 40 cuttings per treatment). A: Apical, L: left, R: right, B: Basal.

The longest adventitious roots were also obtained on the right quarter cuttings, 9.0 cm for BR ones and 8.8 cm for AR ones. On ALe and BLe quarter cuttings, the length of roots were less important (6.8 and 7.8 cm, respectively). The number of roots and of secondary cladodes initiated was not related to the position of the cladode cutting. The highest number of roots per cutting was obtained only on BR quarter cladode cuttings, contrariwise there was no difference between the AR and the BLe quarter cladode cuttings for this number, which stay interesting (4.8, 4.9).

There was no significant difference between the number of secondary cladodes initiated on basal cuttings (BLe and BR) and apical left (ALe) ones. Nevertheless, the highest number was reported on AR cuttings (8.4). The percentage of secondary cladodes developed varied from 15.6 to 22.5%, and the highest percentage was obtained with BR cuttings (22.5%). On the other side, on the ALe quarter cladodes, the initiated secondary cladodes percentage was the lowest (Fig. 3j). The results showed that the highest percentages and

numbers and the longest roots initiated were obtained on basal right (BR) cuttings. The initiation of adventitious roots from the areolar meristems, preferentially located in basal right cuttings could be correlated with the availability of high contents in auxins concentrated at the basal zone of cladodes (Figs. 3c and d). Polar basipetal auxin transport in higher plants is a directional and regulated process. In stems, auxin is transported and moves from the shoot apex toward the base (Lomax *et al.*, 1995; Bohn-Courseau, 2010).

c. Behaviour of the tenths of cladode cutting in situ transplanting

The 300 cuttings (30 cuttings for each tenth of cladode) planted horizontally for left cuttings and vertically for right ones were delicately dug up after three months of culture. The percentages of rooting and of the secondary cladodes initiated, the number of roots and of secondary cladodes initiated per cutting and the average length of roots, were reported in Table 3. The percentages of rooting

Table 3. Effect of the position of the spring tenth cladode cuttings on the percentages of rooting and of the secondary cladodes initiated, the average number of roots and the length of root per cutting initiated *in situ* from *Opuntia ficus-indica* cuttings

Spring tenth cladode cuttings	Number of roots per cutting	% of rooting	Length of roots (cm)	Number of secondary cladodes initiated per cutting	% of secondary cladodes initiated
A _u Le	32.0 ± 2.8e	70a	10.0 ± 1.0d	5.9 ± 1.0c	62c
A _u R	40.0 ± 2.3f	100e	8.0 ± 1.2c	9.1 ± 1.7a	70d
A _l Le	22.0 ± 2.2c	74a	10.0 ± 1.0d	6.0 ± 1.3c	64c
A _l R	10.0 ± 1.2a	100e	6.0 ± 1.3b	8.0 ± 1.3b	74d
M _u Le	20.0 ± 1.6b	83d	5.0 ± 1.3a	3.7 ± 1.1e	44a
M _u R	50.0 ± 2.3g	96c	5.0 ± 1.2a	5.3 ± 1.0c	42a
M _l Le	30.0 ± 2.0d	93b	6.0 ± 1.2b	3.4 ± 0.8e	65c
M _l R	20.0 ± 1.9b	100e	5.0 ± 0.9a	4.0 ± 1.0d	67c
B _a Le	55.0 ± 2.8h	93b	13.0 ± 1.4e	2.2 ± 1.0f	54b
B _a R	80.0 ± 3.2i	100e	14.0 ± 2.2f	3.0 ± 1.1e	60c

Note: Means ± SD with the same letter (s) in the same column are not significantly different ($p < 0.05$) (Duncan's multiple range test) ($n = 30$ cuttings per treatment). The left cuttings were planted horizontally; right ones vertically. A: Apical, Le: left, R: right, B: Basal, AU: Apical upper, AL: Apical lower, Bd: Basal down.

of the tenth of cladode cuttings (Fig. 3c) was the highest with all right portions of cladodes both apical (A_uR, A_lR) (100 and 100%), median (M_uR, M_lR) (96 and 100%) and basal (B_aR) (100%) ones. For all left cuttings the percentage of rooting was less important but reached an interesting percentage varying from 70 to 93%. The average number of roots on the apical and median upper right (A_uR, M_uR) cuttings was more important than on the same cuttings but left ones (A_uLe, and M_uLe) (40 against 32 and 50 against 20). The number of roots on the apical and median lower left (A_lLe, M_lLe) cuttings were less important but exceed those obtained on the same cuttings but right ones (A_lR, M_lR) (22 against 10 and 30 against 20).

The highest number of roots was obtained on the basal tenth cuttings and especially on the basal right ones (80 roots per cutting). The longest roots were initiated on the basal tenth cuttings, 13 cm for left ones and 14 cm for right ones. For the median cuttings, the lengths of roots were the lowest, whatever the original disposition on the cladode (5.0 to 6.0 cm). For the apical tenth cuttings, the lengths of roots were more important on left ones; 10.0 cm, against 8.0 and 6.0 cm for right cuttings. Some cuttings of this zone have initiated neoformed roots at the cicatrization basal section of the cutting. The percentages of secondary cladodes initiated were always more important on right cuttings (70, 74, 67 and 60%, respectively), with the exception for the median upper one (M_uR, 42%). The lowest percentages were recorded on median upper cuttings (M_uLe, M_uR) (42 and 44%). This essay allowed us to note, that the right cuttings planted vertically demonstrated best results in their aptitude for rhizogenesis and for the initiation of secondary cladodes then left ones planted horizontally. This could be related to the normal polarity of cuttings and to the distribution of auxins. Endogenous auxins may interact in the *in situ* cutting tissues resulting in a gradient of potential organogenic response from the shoot tip downward (Friml and Palme, 2002).

Results obtained with the tenth of cladode cuttings were more interest than those obtained with entire, quarter and half ones. Thus, after 3 months of culture of the different *Opuntia ficus-indica* cuttings (entire, 1/2, 1/4 and 1/10 cladodes) in experimental parcel and in pots under greenhouse, we can deduced that the improvement performance of this vegetative propagation method can be correlated with the reduction of the cutting size, confirming Barbera and Inglesse (1993) results. In our study we show that, in general, reducing the size of cladode cutting (1/4 and 1/10), do not reduce its rhizogenesis and caulogenesis potentialities. Padrón Pereira (2013) working on *Opuntia boldinghii* cactus vegetative propagation showed also that the small size and conditions of cladodes did not affect the growth. On the otherwise, Solano and Orihuela (2008)

reported that the use of half cladodes represents 50% saving in vegetative material in comparison with the traditional method. Generally, cuttings planted in normal polarity like respecting their position on mother plant gave more interesting results than those obtained with cuttings planted in horizontal polarity. Pending a precise structural analysis for the areolar complex, our study shows totipotency of these axillary meristematic polyvalent complexes, capable to differentiate in any specialized structure (root or secondary cladode) depending on their topographic position on the cladode in relation with internal morphogenetic correlations. These observations are in agreement with those mentioned by Nobel (1994). The basic meristematic units in *Opuntia* are the areoles (Gibson and Nobel, 1986). They are helically positioned on the cladodes (Sudzuki, 1995) and can develop either in branches, flowers or roots (Boke, 1980; Bowers, 1996). We note also that cladodes can initiate the rooting process soon after they come in contact with soil. We demonstrate that the rooting of cuttings can be induced at any period of the year, contrary to the axillary cladode initiation, which was more important in spring, mainly from areoles situated in the apical zone. For Nobel (1982), initiation of secondary cladodes can appear at any time of the year, or at two periods, September-November and February-April (Escobar *et al.*, 1986).

Conclusion

Over the years, farmers from North Africa and especially in Tunisia have developed asexual propagation methods for *Opuntia ficus-indica* using cladodes. This essay allowed us to note that half, quarter and the tenth of cladode cuttings planted in spring vertically and in normal polarity show very interesting results in these optimal conditions concerning rhizogenesis and caulogenesis. So, as a conclusion, the tenths of cladode cuttings despite their small size, have not lost their rooting potentiality and new cladodes regeneration. Then, reducing the cladode cutting size, do not reduce its rhizogenesis and caulogenesis potentialities. This fragmentation represents a substantial gain of material and time especially for large cultivated surfaces of *Opuntia ficus-indica*. With vegetative propagation, we can save a lot of time and money for commercial plant production. The main advantage of this propagation method is that the new plants contain the genetic material of the parent selected. Our results, allowed better understanding of certain morphogenesis aspects of this particular dicotyledonous. Deepening these results by cytological, physiological and molecular studies and recourse to biotechnological methods, will open wide perspectives for agro-economic and social valorisation of *Opuntia ficus-indica*.

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