AcademicPres

Available online at www.notulaebiologicae.ro

Print ISSN 2067-3205; Electronic 2067-3264

Not Sci Biol, 2013, 5(1):39-44

Notulae Scientia Biologicae

In Vitro Propagation of Three Moroccan Prickly Pear Cactus *Opuntia* and Plant Establishment in Soil

Aissam EL FINTI, Rachida EL BOULLANI, Naima AIT AABD, Fouad MSANDA, Mohammed A. SERGHINI, Abdelhamid EL MOUSADIK

University Ibn Zohr, Science Faculty, Laboratory of Biotechnologies and Natural Resources Valorization, B.P. 8106, Agadir, Morocco; elmousadik@yahoo.fr

Abstract

Opuntia is one of the most widespread cacti, primarily due to their edible fruit and vegetable mass used as feed. The high demand for young plants of *Opuntia* made it necessary to find a rapid method of multiplication of the cactus, the safest method consisting *in vitro* micropropagation of species belonging to this genus. With aim of large production of plant material, a propagation system of three important prickly pear cactus cultivar (*Opuntia ficus-indica*) in Morocco was developed. Segments of healthy young cladode (containing one areole) were cultivated in Murashige and Skoog medium (MS) containing adenine sulfate (40 mg/1), monosodium phosphate (50 mg/l), sucrose (50 g/l), phytagel (0.3%) and benzyladenine (BA) at 22.2 µM, to start the process of micropropagation. In vitro-developed shoots from areoles were used as secondary explants to induce shoot development in the MS medium with 5 mg/l of BA. All of the three studied cultivars showed an important multiplication rate in this medium. 'Sidi Ifni M' ('Moussa') cultivar shows the greatest number of shoots followed by 'Sidi Ifni A' ('Aissa') and 'Delahia' 17.26, 14.12 and 12.13 respectively. Rooting of *in vitro*-generated shoots was achieved most efficiently on half-strength MS basal medium supplemented with 0.5 mg/l of indole-3-butyric acid (IBA) or IAA. Rooting frequencies were in the range from 95 to 100% and the highest mean number of root (19.1) was obtained with IBA for 'Delahia' cultivar. All micropropagated plants were transferred to greenhouse and all of them survived acclimatization process and showed good overall growth.

Keywords: benzyladenine, micropropagated plantlets, Moroccan cultivar, *Opuntia ficus-indica*, prickly pear

Introduction

Opuntia spp. belong to the *Cactaceae* family and are native to Central America. The most economically important species is *Opuntia ficus indica*, cultivated both for fruits and cladodes (Nobel, 2002). The genus includes other important edible species that occur worldwide as either wild or cultivated species in many arid or sub-arid areas (e.g., the Mediterranean region) (Nobel, 2002, Nobel *et al.*, 2002). *Opuntia ficus indica* is a xerophytic, succulent spiny or spineless type, CAM (Crassulacean Acid Metabolism) plant. Prickly pear is the most cultivated edible cactus crop in the world and is widely distributed in Mexico and the South American continent. It is also grown in many other regions of the world such as Africa, the Mediterranean area, Australia and south-western USA (Mohamed-Yasseen *et al.,* 1995a; Piga, 2004).

Cactus pear is known as a multi-purpose plant since it can be used for human food (fruits and vegetables), fodder, medicinal and ornamental plants (Casas and Barbera, 2002; Rodriguez-Felix, 2002). This plant is recognized as a good indicator of noxious (Nobel, 1994). The low productivity of the native fodder crops hampers animal nutrition during the dry season; in arid and sub-arid regions of Morocco *Opuntia* clones are an alternative for cattle and goat feed, as a valuable forage resource.

In Morocco, prickly pears have been grown for many years, especially in arid areas. The *Opuntia* plants are grown not only for fruit production but also as defensive hedges or for erosion control in reclaimed areas. Recently, the production of prickly-pear fruits increased as a result of an increase in producing area (Boujghagh and Chajia, 2001).

Generally, prickly pear cactus species can be sexual and asexually propagated. Seed propagation, presents three main problems: genetic segregation, a long juvenile stage and the slow growth of seedlings compared to asexually propagated material (Mohamed-Yasseen *et al.*, 1995a). It is only used for scientific research (Rojas-Aréchiga and Vásquez-Yanes, 2000). 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 (Estrada-Luna *et al.*, 2008). All these methodologies require large spaces for propagation and present a low propagation rate. Therefore, the micropropagation is a feasible alternative option for the rapid multiplication and maintenance of germplasm, because it provides high

Received 18 November 2012; accepted 03 February 2013

propagation rates, reduced requirements for space, the production of healthy and pathogen-free plants (Johnson and Emino, 1979; Smith *et al.*, 1991).

Many types of cactus were micropropagated by axillary shoot multiplication. Depending on the genus, the explants tested for tissue culture were different: terminal shoots of seedlings, lateral or vertical sections of plants or cladodes and simples areoles. A number of reports have been published describing the efficient and rapid multiplication of different prickly pear species by "*in vitro*" micropropragation (Escobar *et al.*, 1986; García-Saucedo *et al.*, 2005; Johnson and Emino, 1979; Rubluo *et al.*, 1996; Smith *et al.*, 1991), however, a general protocol is not available yet, because most plant responses to tissue culture are highly dependent on the genotype and some important modifications and adjustments might be performed when a new species or cultivar is considered for tissue culture (Estrada-Luna *et al.*, 2008), especially to optimize the overall environmental culture conditions, type of media, concentration and combination of plant hormones, etc. during the shoot proliferation stage. Rooting and plantlet acclimatization conditions might also be studied since they may limit the success of micropropagation (Hartmann *et al.*, 1997). As regards Moroccan cultivars, recently El Finti *et al.* (2012) developed a rapid micropropagation method of prickly pear cactus. They described optimum growth regulator concentrations and combinations for in vitro shoot proliferation and rooting.

Therefore, the objectives of this research were to evaluate *in vitro* protocol developed by El Finti *et al.* (2012) on three important Moroccan prickly pears cultivars, by *in vitro* culture of areoles and determinate the response of these three cultivars towards propagation *in vitro* process. The selected cultivars, for this study, were 'Sidi Ifni M', 'Sidi Ifni A' and 'Delahia'.

Material and methods

Plant material and initiation of culture

The plant of cactus were collected from trees of different areas of Morocco and cultivated on the parcel of the Faculty of Sciences University Ibn Zohr, Agadir, Morocco (latitude: 30.406026; longitude: 9.544408). The micropropagation studies were carried out with young cladodes obtained from this parcel.

Young cactus cladodes (about 5-8 cm in length) were cut into segments and surface disinfected by washing under running tap water and laundry bleach during 20 min. Under laminar flow hood in sterile conditions, the cladodes were soaked in ethanol 70% for 5 min, followed by immersion in 2% sodium hypochlorite plus Tween-20 for 25 min, and then rinsed five times with sterile distilled water. Finally, disinfected explants were cut to 1 cm^2 pieces each containing one areole (Fig. 1A) and cultured in a MS basal medium (Murashige and Skoog, 1962) (Fig. 1B), supplemented with 50 g/l sucrose, 50 mg/l monosodium phosphate, 40 mg/l adenine sulfate and 0.3% phytagel. This medium was supplemented with N6-Benzyladenine at 22.2 µM for initiate culture by inducing areole activation and shoots growth which will be used in the proliferation phase as described by El Finti *et al.* (2012) and Estrada-Luna *et al.* (2002).

In each of shoot initiation, proliferation and rooting treatments, all the used culture media were adjusted to pH 5.7±0.1 with NaOH 1N before sterilization at 121°C for 20 minutes. Four explants were placed vertically in 300 ml capacity glass culture jars containing 30 ml medium. Cultures were maintained at 25±1°C under fluorescent lamps with light intensity of 54 $\mu mol.m^{-2}s^{-1}$ at culture level and 16 hours photoperiod. These same incubation conditions were used in all subsequent experiments (proliferation and rooting treatments).

Shoot multiplication

Shoots developed in the initiation phase (2 cm in length) were separated and cultivated in basal Murashige and Skoog (1962) medium contained 5% sucrose, 50

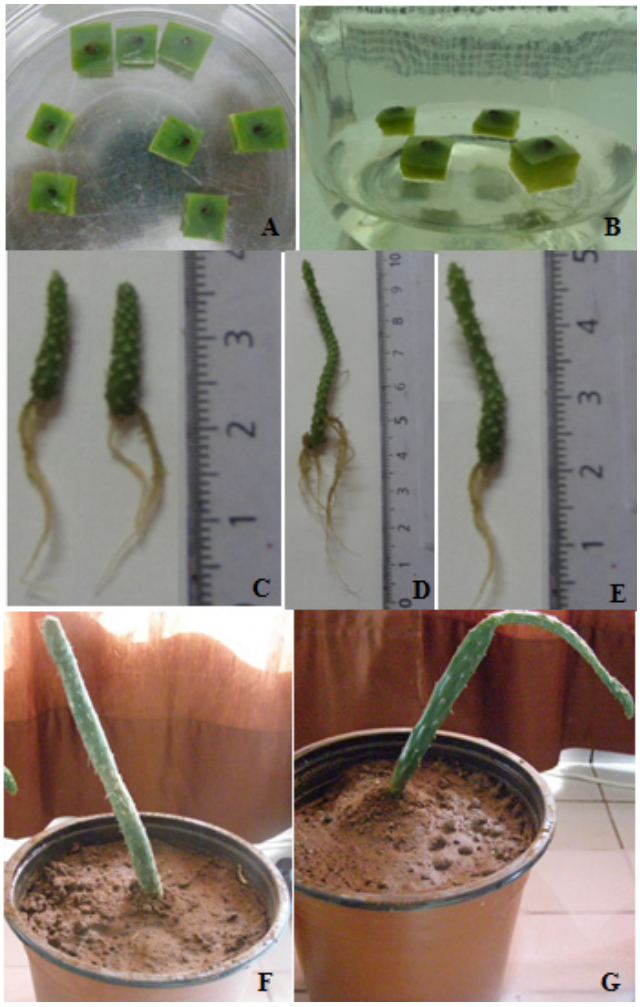

Fig. 1. *In vitro* micropropagation of cactus (*Opuntia ficus-indica*) (A-G). A: Cladode explants containing one areole; B: Explants in initiation medium; C-D-E: Root induction on MS supplemented with IBA at 0.5 mg/l, F-G: Plant acclimatization in greenhouse

mg/l monosodium phosphate, 40 mg/l adenine and 0.3% phytagel, for shoots proliferation. This medium was supplemented with 5 mg/l of N6-Benzyladenine (BA) alone or in combination with 0.5 mg/l NAA. These treatments were selected according to the references consulted and results of previous experiments (El Finti *et al.*, 2012; Khalafalla *et al.*, 2007; Martínez-Váquez and Rubluo, 1989). We used 20 explants of each treatment (n = 20) and after six weeks of culture, number of shoots produced in each explant and total length (mm) were determined. These experiments were conducted three independent times for each treatment.

Rooting of shoots

To stimulate root development, elongated shoots (5 to 8 mm) were separated and cultivated in the rooting media. The rooting technique consisted of exposing those shoots to two treatments: (1) MS/2, 30 g/1 sucrose, 0.3% phytagel, with 0.5 mg/l IBA; (2) MS/2, 30 g/1 sucrose, 0.3% phytagel, with 0.5 mg/l IAA. These two experiments were repeated at three times to confirm the results. For this rooting experiment, 20 shoots were used per treatment and the number of roots developed per explant in each treatment was evaluated six weeks after initiating the experiment.

At the end of the sixth week of culturing, the produced rooted shoots (vitroplants) were submitted to the acclimatization trial.

Acclimatization of plants

In the acclimatization trial, the rooted plantlets (vitroplants) resulting from rooting treatments were transferred to autoclaved soil and acclimatized with plastic covers for four weeks to prevent desiccation and to allow acclimatization, and then transferred to the greenhouse. Survival percentages were determined twelve weeks after transplantation.

Statistical analysis

During the proliferation phase the cumulative number, of the new-formed shoots per explant, were counted after six weeks of culture. At the end of rooting treatment, the rooting response (%); number of roots formed per shoot and length of roots in cm were recorded. For acclimatization trial, the survival percentage of the acclimatizated vitroplants was calculated after twelve weeks from acclimatization procedure. Analysis of variance (ANOVA) was performed to test the significance of the difference between treatments. When significant differences were found ($p \leq 0.05$), a multiple comparison test of means (Duncan's test) was calculated.

Results and discussion

The initiation medium used to culture isolated areoles was able to break dormancy in axillary buds and start new shoot development, which after growing about 2 cm in length (six weeks after culture) were used as secondary explants in proliferation experiments. It is interesting to note that only one shoot was obtained per explant at this stage.

Multiplication rate

For the proliferation stage, the effects of N6-Benzyladenine alone or in combination with NAA on shoot multiplication are presented in Tab. 1. In general, all of the three studied cultivars showed an important multiplication rate in both mediums tested. The analysis of variance (ANOVA) detected significant effects between the two treatments applied to the three cultivars. In general, data showed that the addition of BA to the media, during the proliferation phase, resulted in increased shoot number per explant in comparison to the BA combined with NAA (Tab. 1). Response to the both treatment was different in the three cultivars and the best number of shoots formed was observed in cultivars 'Sidi-Ifni M' followed by 'Sidi-Ifni A' and 'Delahia' (Tab. 1).

Shoot proliferation responses varied according to the type of cytokinin and concentration added to the culture medium Estrada-Luna *et al.* (2008). Various cytokinins have been adopted both for culture initiation and shoot proliferation of the prickly pear (Juarez and Passera, 2002; Khalafalla *et al.*, 2007; Mohamed-Yasseen *et al.*, 1995a). Our results are consistent with those of De Medeiros *et al.* (2006), Estrada-Luna *et al.* (2007) (2008) and Khalafalla *et al.* (2007), who found BA to be more effective than kinetin and 2iP (6-Dimethylaminopurine) in inducing shoot multiplication from *in vitro* culture of areoles of cactus. The previous results of El Finti *et al.* (2012), on moroccan cultivars, showed that 5 mg/l BA was the best concentration to induce the formation of a large number of shoots.

Several naturally occurring and synthetic cytokinins have been evaluated to induce shoot proliferation in many plant species. However, it seems that the responses are particular or depend on the type and concentration of cytokinin, the target tissue and some environmental conditions (Hartmann *et al.*, 1997). The use of the BA in cacti micropropagation has been reported previously (Escobar *et al.*, 1986; Hubstenberger *et al.*, 1992) and it is very active and efficient in other plant species (Hartmann *et al.*, 1997). The addition of this compound on culture media was enough to induce shoot proliferation in both induction and proliferation stages particularly in *O. amyclaea, O. ficus-indica, O. streptacantha, O. robusta, O. leucotricha*, and *O. cochinera* (Escobar-Araya *et al.*, 1986; Estrada-Luna, 1988; Juárez and Passera, 2002).

The addition of 0.5 mg/l of NAA to the proliferation medium decrease significantly the shoot number for all cultivars (Tab. 1). The decrease was important in the case of 'Sidi-Ifni M' cultivar. This demonstrates that the interaction between these two hormones wasn't perfect for our cultivars. Recently, El Finti *et al.* (2012) using Moroccan cactus, reported that the highest number of shoots per explant was obtained with 5 mg/l of BA alone, or combined with 0.5 mg/l NAA. In the same sense, Khalafalla *et al.*

(2007) reported that NAA at 0.5 mg/l in combination with different BA concentrations did not significantly affect the number of shoots per explant in prickly pear. However media with cytokinins alone have proven to be more efficient for most *Opuntia* species (Escobar-Araya *et al.*, 1986; Havel and Kolar, 1983; Johnson and Emino, 1979; Juárez and Passera, 2002).

Size of new shoots formed was lower for BA alone or in combination with NAA; this small size is related to the high concentration of BA. This observation has already been reported by El Finti *et al.* (2012). They confirmed that increasing the number of shoots per explant with increasing concentration of BA, resulted in a decrease in the size of shoots produced. Also, Amin and Jaiswal (1987) and Mohamed-Yasseen *et al.* (1995a) reported that high cytokinin levels have depressed shoot growth from guava and tuna respectively.

Cultivation of explants vertically in the medium was effective for shoots development. In this regard, Estrada-Luna *et al.* (2008) found that explants vertically oriented produced more shoots than the ones cultured in a horizontal position.

Regarding the growth regulator, our data showed that concentrations of 5 mg/l generated shoots a high number than the other treatment (Tab. 1). This concentration is relatively high considering the ones used in other prickly pear cactus species (Juárez and Passera, 2002; Llamoca-Zárate *et al.*, 1999; Mohamed-Yasseen *et al.*, 1995a), particularly for *Opuntia amyclaea* in which a low concentration (2.25 mg/l) of BA produced one of the best results obtained for any cacti averaging the development of 15 shoots per explant (Escobar-Araya *et al.*, 1986). On the other hand, this concentration might be low compared to the high concentrations (10 mg/l BA) used in *Opuntia polyacantha* to activate axillary meristems (Mauseth and Halperin, 1975). This confirms the fact that each cacti species, even within the same genus, responds differently to growth regulators, hence *in vitro* proliferation systems must be developed for each one of them specifically (Hubstenberger *et al.*, 1992).

Root formation

For root formation, elongated shoots were transferred to different rooting media. Tab. 2 shows the effect of different auxins on percentage, number and length of roots.

a. Rooting response

In this study, 95 to 100% of the regenerated shoots were rooted after six weeks of culture in different treatments (Tab. 2). This is confirmed in several publications: 1) Khalafalla *et al.* (2007) obtained highest percentage of roots using IAA at 0.5 mg/l; 2) with 0.5 mg/l IBA or 0.5 mg/l IAA, 21 species of Mexican cacti were rooted (Pérez-Molphe-Balch *et al.*, 1998); 3) satisfactory rooting occurred for three *Opuntia* genotypes treated with 0.5 mg/l IBA (García-Saucedo *et al.*, 2005); 4) 100% of shoots rooted after six weeks of culture for a moroccan cacti (El Finti *et al.*, 2012). According to literature, rooting of several *Opuntia* species is favored in presence (Hartmann *et al.*, 1997) or absence of auxins in culture media.

b. Number of roots

All shoots produced roots in *in vitro* rooting media (Tab. 2) by four weeks with a mean greater than eleven roots per rooted shoot. The number of roots formed per rooted plantlet was significantly affected by the different auxins used in this investigation (Tab. 2). The highest number of roots/explant (19.1) was exhibited for shoots cultured on half strength MS medium provided with 0.5 mg/l IBA (Fig. 1C-D-E), while the minute one (11.22

Tab. 1. Effects of benzyladenine (BA) alone or combined with 1-naphtalenacetic acid (NAA) on shoot number and length during proliferation stage of three *Opuntia ficus-indica* cultivars on MS medium after six weeks of culture

Cultivar	*In vitro* proliferation treatment	Shoot number per explant	Shoot length (mm)
'Sidi-Ifni A'	BA 5 mg/l	$14{,}12^{b} \pm 1{,}33$	$5{,}10^{d} \pm 0{,}81$
	BA 5 mg/l; NAA 0,5 mg/l	$12{,}40^{c} \pm 1{,}1$	$4{,}26^{e} \pm 0{,}96$
'Sidi-Ifn M'	BA 5 mg/l	$17{,}26^{a} \pm 1{,}17$	$7{,}40^{a} \pm 0{,}92$
	BA 5 mg/l; NAA 0,5 mg/l	$14{,}24^{b} \pm 0{,}91$	$6{,}76^{b} \pm 1{,}06$
'Delahia'	BA 5 mg/l	$12{,}13^{d} \pm 1{,}05$	$5{,}63^{c} \pm 1{,}06$
	BA 5 mg/l; NAA 0,5 mg/l	$11{,}59^{e} \pm 1{,}09$	$4{,}94^{d} \pm 0{,}64$

Note: Means with same letter (s) in the same column are not significantly different according to Duncan's test ($p \leq 0.05$)

Tab. 2. Effect of auxins on rooting of *in vitro* derived shoots of three *Opuntia ficus-indica* cultivars after six weeks of culture on MS medium

Cultivar	*In vitro* rooting treatment	% of rooting	Number of root per shoot	Length of root (cm)
'Sidi-Ifni A'	IAA 0.5 mg/l	97	$11{,}22^{f} \pm 1{,}41$	$2{,}19^{f} \pm 1{,}01$
	IBA 0.5 mg/l	95	$15{,}33^{c} \pm 1{,}26$	$3{,}21^{d} \pm 0{,}91$
'Sidi-Ifni M'	IAA 0.5 mg/l	98	$12{,}50^{e} \pm 1{,}30$	$3{,}16^{e} \pm 1{,}11$
	IBA 0.5 mg/l	100	$16{,}03^{b} \pm 1{,}02$	$5{,}30^{b} \pm 1{,}21$
'Delahia'	IAA 0.5 mg/l	95	$13{,}68^{d} \pm 1{,}61$	$4{,}52^{c} \pm 0{,}52$
	IBA 0.5 mg/l	100	$19{,}10^{a} \pm 2{,}43$	$6{,}93^{a} \pm 0{,}44$

Note: Means with same letter (s) in the same column are not significantly different according to Duncan's test ($p \leq 0.05$)

roots/explant) was recorded for shoots grew on half strength MS-medium added 0.5 mg/l IAA. Although the number of roots produced by IAA is lower than the IBA, this number remains important in comparison with the results of other studies where the medium was free hormones (Sawsan *et al.,* 2005).

These results affirmed that using IBA allowed shoots to form roots at greatest number (Escobar-Araya *et al.*, 1986; Estrada-Luna, 1988; García-Saucedo *et al.*, 2005; Mohamed-Yasseen *et al.*, 1995a). Furthermore, García-Saucedo *et al.* (2005) indicated that IBA interacted significantly with the culture medium and the materials, having a strong influence for plantlet rooting.

c. Length of roots

The results in Tab. 2 indicated that all tested medium in this investigation allowed the root to grow up in the length ranged between 2.19 to 6.93 cm with a significant difference between these values. Thus, length of root was significantly different between the treatments and the best size was achieved with 0.5 mg/l of IBA (6.93 cm in 'Delahia' cultivar). The root length formed was affected by the type of auxin added to the culture medium, which was tested.

Acclimatization of in vitro regenerated plants

The success of transplantation of *in vitro* regenerated plants depends on a number of factors such as the rooting system, plant vigor, duration, and conditions of acclimatization etc. In the present study, plants rooted *in vitro* were transferred to potting mixture and acclimatized initially for four weeks in culture room and then in glasshouse for eight weeks. The survival frequency was enhanced to 100% and plantlets showed healthy and active growth (Fig. 1F and G). This result is similar to the reports for other micropropagated prickly pear cactus species (Ault and Blackmon, 1987; Clayton *et al.*, 1990; El Finti *et al.*, 2012; Estrada-Luna, 1988; Juárez and Passera, 2002; Mohamed-Yasseen *et al.*, 1995a). Hartmann *et al.* (1997) suggest that after micropropagation this group of plants has functional roots, adequate cuticle, and the ability of controlling stomata functioning that reduces transplant shock produced by excessive water loss during acclimatization, which is important to maintain a continuous growth.

Conclusions

The methodology described in the present study is highly suitable for the micropropagation of cactus. The existing protocols for this species were not carried out on moroccan cultivars, and an efficient *in vitro* shoot proliferation system from areoles was developed. The results shows that optimum shoot proliferation was obtained when explants were cultured in MS medium supplemented with 5 mg/l BA. A proliferation rate of 17 shoots per shoot explant every six weeks was obtained. One hundred percent of these microshoots regenerated roots after six weeks. Our findings are particularly important because they demonstrate effective, feasible protocols for large-scale *in vitro* clonal micropropagation of this specie.

References

Amin M, Jaiswal VS (1987). Rapid clonal propagation of guava through *in vitro* proliferation of nodal explants of mature trees. Plant Cell Tiss Org Cult 9:235-244

Ault JR, Blackmon WJ (1987). *In vitro* propagation of *Ferocactus acanthodes* (*Cactaceae*). Hort Sci 22:126-127.

Boujghagh M, Chajia L (2001). Le cactus: outil de gestion de la sécheresse dans le sud ouest marocain. Terre et Vie 52:1-7.

Bravo-Hollis H, Sanchez-Mejorada H (1991). Las Cactaceas de Mexico. Univ Nacional Autonoma de Mexico, Vol 3. 643 p.

Casas A, Barbera G (2002). Mesoamerican domestication and diffusion, 143-162 p. In: Nobel PS (Ed.). Cacti: Biology and Uses, University of California, Berkeley, California, USA.

Clayton PW, Hubstenberger JF, Phillips GC (1990). Micropropagation of members of the *Cactaceae* subtribe *Cactinae*. J Am Soc Hortic Sci 115(2):337-343.

De Medeiros LA, De Ribeiro RC, S Gallo LA, De Oliveira ET, Dematte MESP (2006). *In vitro* propagation of *Notocactus magnificus*. Plant Cell Tiss Org Cult 84:165-169.

El Finti A, El Boullani R, El Ayadi F, Ait Aabd N, El Mousadik A (2012). Micropropagation *in vitro* of *Opuntia Ficus-Indica* in south of Morocco. IJCBS 1:6-10.

Escobar-Araya HA, Villalobos AVM, Villegas MA (1986). *Opuntia* micropropagation by axillary proliferation. Plant Cell Tiss Org Cult 7:269-277.

Estrada-Luna AA, Martínez-Hernández JJ, Torres-Torres ME, Chablé-Moreno F (2007). Micropropagation of an ornamental prickly-pear cactus (*Opuntia lanigera* Salm-Dyck) and effects of GA3 on plant growth after transplantation. SNA Research Conference 52:261-263.

Estrada-Luna AA, Martínez-Hernández JJ, Torres-Torres ME, Chablé-Moreno F (2008). *In vitro* micropropagation of the ornamental prickly pear cactus *Opuntia lanigera* Salm-Dyck and effects of sprayed GA3 after transplantation to ex vitro conditions. Sci Hortic 117:378-385.

Estrada-Luna AA (1988). Producción de brotes e injertación *in vitro* de seis especies de nopal (*Opuntia* spp.) originarias del Altiplano Potosino-Zacatecano. Tesis de MC. Colegio de Postgraduados, Montecillo, Edo, De México, México, 160 p.

Estrada-Luna AA, López-Peralta C, Cárdenas-Soriano E (2002). *In vitro* micrografting and histology of the graft union formation of selected species of prickly pear cactus (*Opuntia* spp.). Sci Hortic 92:317-327.

Feng F, Li HB, Lu QF, Xie JY (2000). Tissue culture of *Aloe* spp. J Southwest AgricUniv 22(2):157-159.

García-Saucedo P, Valdez-Morales M, Valverde ME, Cruz-Hernández A, Paredes-López O (2005). Plant regeneration of three Opuntia genotypes used as human food. Plant Cell Tiss Org Cult 80:215-219.

Hartmann HT, Kester DE, Davies Jr FT, Geneve RL (1997). Instructors Manual for Plant Propagation-Principles and Practices (6th ed). Prentice-Hall, Englewood Cliffs, NJ, 151 p.

Havel L, Kolar Z (1983). Microexplant isolation from *Cactaceae*. Plant Cell Tiss Org Cult 2:349-353.

Hubstenberger JF, Clayton PW, Phillips GC (1992). Micropropagation of cacti (*Cactaceae*), 49-68 p. In: Biotechnology in Agriculture and Forestry. High tech and micropropagation IV. Vol. 20. Ed: Bajaj YPS Springer-Verlag, Berlin International Horticultural Congress.

Infante R (1992). *In vitro* axillary shoot proliferation and somatic embryogenesis of yellow pitaya *Mediocactus coccineus* (Salm-Dyck). Plant Cell Tiss Org Cult 31:155-159.

Inglese P, Basile F, Schirra M (2002). Cactus pear fruit production, 163-183 p. In Nobel PS (Ed.). Cacti: Biology and Uses, University of California Press, California, USA.

Johnson JL, Emino ER (1979). Tissue culture propagation in the *Cactaceae*. Cactus Succul J 51:275-277.

Juárez MA, Passera CB (2002). *In vitro* propagation of *Opuntia ellisiana* Griff. And acclimatizationto field conditions. Biocell 26 (3):319-324.

Khalafalla MM, Abdellatef E, Mohameed Ahmed MM, Osman MG (2007). Micropropagation of cactus (*Opuntia ficus-indica*) as strategic tool to combat desertification in arid and semi arid regions. Int J Sustain Crop Prod 2(4):1-8.

Kluge M, Ting IP (1978). Crassulacean acid metabolism: an ecological analysis, Ecological Studies series, vol. 30. Springer-Verlag, Berlin, Germany, 629-655 p.

Llamoca-Zárate RM, Aguiar LF, Landsmann J, Campos FAP (1999). Whole plant regeneration from the shoot apical meristem of *Opuntia ficus-indica* Mill. (*Cactaceae*). J Appl Bot 73:83-85.

Machado MF, Prioli AJ (1996). Micropropagation of *Cereus peruvianus* Mill. (*Cactaceae*) by areole activation 32(3):199-203.

Martínez-Váquez O, Rubluo A (1989). *In vitro* mass propagation of the nearextinct Mammillaria san-angelensis Sñchez-Mejorada. J Hortic Sci 64:99-105.

Mata-Rosas M, Monroy de la Rosa MA, Goldammer KM, Chávez-Avila VM (2001). Micropropagation of *Turbinicarpus laui*, an endemic and endangered species. In Vitro Cell Dev Biol Plant 37:400-404.

Mauseth JD, Halperin W (1975). Hormonal control of organogenesis in *Opuntia polyacantha* (*Cactaceae*). Am J Bot 62(8):869-877.

Mauseth JD (1979). A new method for the propagation of cacti: sterile culture of axillary buds. Cactus Succul J 51:186-187.

Mizrahi Y, Nerd A, Nobel PS (1997). Cacti as crops. Hort Rev 18:291-319.

Mohamed-Yasseen Y, Barringer SA, Splittstoesser WE, Schnell J (1995)a. Rapid propagation of tuna (*Opuntia ficus-indica*) and plant establishment in soil. Plant Cell Tiss Org Cult 42:117-119.

Mohamed-Yasseen Y, Barringer SA, Splittstoesser WE (1995b). Micropropagation of the endangered succulent *Stapelia semota* by axillary proliferation. Cactus Succul J 67(6):366-368.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco cultures. Physiol Plant 15:473-497.

Nobel PS (2002). Cacti: Biology and Uses. University of California, Berkeley, California, USA, 262-265 p.

Nobel PS, Barrera E, De Beilman DW, Doherty JH, Zutta BR (2002). Temperature limitations for cultivation of edible cacti in California, Madroño 49:228-236 p.

Nobel JL (1995). Changes in water potential and its component during shoot formation in cacti callus. Physiol Plant 45:92-97.

Nobel PS (1994). Remarkable Agaves and Cacti. Oxford University Press, New York.

Pérez-Molphe-Balch E, Pérez-Reyes ME, Villalobos-Amador E, Meza-Rangel E, Morones-Ruiz LR, Lizalde-Viramontes H (1998). Micropropagation of 21 species of Mexican cacti by axillary proliferation. In Vitro Cell Dev Biol Plant 34:131-135.

Piga A (2004). Cactus pear: A fruits of nutraceutical and functional importance. J Profess Assoc Cactus Develop 6:9-22.

Pimienta-Barrios E, Munoz-Urias A (1995). Domestication of *Opuntia* and cultivated varieties, 58-63 p. In: Barbera G, Inglese P, Pimienta-Barrios E (Eds.). Agro-ecology, cultivation and uses of cactus pear. FAO International Technical Cooperation Network on Cactus Pear.

Polisetty R, Patil P, Deveshwar JJ, Khetarpal S, Chandra R (1996). Rooting and establishment of *in vitro* grown shoot tip explants of chickpea (*Cicer arietinum* L.). Indian J Exp Biol 34:806-809.

Rodriguez-Felix A (2002). Postharvest physiology and technology of cactus pear fruits and cactus leaves. Acta Hort 581:191-199.

Rojas-Aréchiga M, Vásquez-Yanes C (2000). Cactus seed germination: a review. J Arid Environ 44:85-104.

Rubluo A, Reyes J, Rodriguez-Garay B, Pimienta-Barrios E, Brunner I (1996). Métodos de propagación biotecnológicos y convencionales en cactáceas para zonas áridas, In: Técnicas Convencionales y Biotecnológicas para la Propagación de Plantas de Zonas Áridas, (Izquierdo J, Palomino G (eds). Santiago) Chile, Vol 9 pp. 345.

Russell CE, Felker P (1987). The prickly-pears (*Opuntia* spp., *Cactaceae*): A source of human and animal food in semi-arid regions. Economic Bot 41(3):433-445.

Sakr SS, El-Khateeb MA, Abd-El-Kareim AH (1999). *In vitro* production of *Yucca elephantips*. Bull Fac Agric, Univ Cairo 50(2):265-282.

Sawsan SS, Abou-Dahab TA, Youssef EMA (2005). *In vitro* propagation of cactus (*Cereus peruvianus* L.). Arab J Biotech 8(1)169-176.

Smith RH, Burdick JP, Anthony J, Reilley AA (1991). *In vitro* propagation of *Coryphantha macromeris*. Hort Sci 26(3):315.

Youssef EMS (1994). Effect of cytokinins and reported cub cultures on *in vitro* micropropagation potentiality of *Acacia salicina* Lindll. Tissue culture Lab.; Timber Trees Res Dept, Inst, Agric Res Cen, Giza (1):30-43.

Youssef-Aboueash EMA (1997). Some studies on the vegetative propagation of *Populus alba* L. and *Tamarix articulata* Vobl. M.Sc. Thesis Fac Agric, Cairo Univ 90 p.