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Micropropagation in vitro of *Opuntia Ficus-Indica* in south of Morocco

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Abstract

With aim of large production of plant material, a rapid micropropagation method of prickly pear cactus (*Opuntia ficus-indica*) was developed. Young cladode explants containing one areole were cultured on Murashige and Skoog (1962) medium (MS) with adenine sulfate (40 mg/1), monosodium phosphate (50 mg/l), sucrose (50 g/l), phytagel (0.3%) and benzyladenine (BA) at 22.2 µM. Best multiplication rates were obtained with BA at 0.5 mg/l than kinetin. To induce rooting, the prolif erated shoots were transferred to a medium containing half MS, sucrose (3%), phytagel (0.3%) and 0.5 mg/l of different auxins. In these culture conditions, 100% of plantlets rooted and highest number of root (21.05 and 16.2) was obtained, respectively wit h indole-3-butyric acid (IBA) and indole-3-acetic acid (IAA). The rooted shoots were easily acclimated and transferred to soil.

Keywords: Prickly pear, *Opuntia ficus-indica*, auxins, micropropagated plantlets.

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1. Introduction

Cactus pear (*Opuntia ficus indica* Mill.) also known as prickly pear is the most important plant species in the Cactaceae family. It is known as a multi-purpose plant since it can be used for human food (fruits and vegetables), forage, medicinal plants, and ornamental plants [1-4]. *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 [5-9]. 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. In recent years, the production of prickly-pear fruits increased as a result of an increase in producing area [10].

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 [5]. It is only used for scientific research [11]. 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 [12]. 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 propagation rates, reduced requirements for space, the production of healthy and pathogen-free plants [13-14].

A number of reports have been published describing the micropropagation of prickly pear [13-19]. However, there is no information concerning the micropropagation of Moroccan cultivar. As optimum growth regulator concentrations and combinations for in vitro shoot proliferation and rooting of prickly pear can vary, protocols suggested in the literature may not be suitable for every cultivar. Therefore, the main goal of this study was to develop an efficient protocol for in vitro propagation of Moroccan cultivar by in vitro culture of areoles. The objectives of this research were to: a) determine the medium composition and growth regulator concentration able to induce shooting; and b) evaluate the effects of different auxins on rooting plant.

2. Material and Methods

2.1. 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 Biology Department, Faculty of Sciences, University Ibn

Zohr, Agadir, Morocco during June 2009. The micropropagation studies were carried out with young cladodes obtained from this parcel.

Young cactus cladodes (Fig. 1A) (about 5–8 cm in length) were cut into pieces (Fig. 1B) 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 and cultured in a MS [20]. Medium, 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 BA at 22.2 µM for initiate culture and obtained the shoots for the proliferation phase. The pH of this medium was adjusted to 5.7 before sterilization at 121°C for 20 min. Four explants were planted per culture jar. The cultures were maintained for 6 weeks under continuous fluorescent light (54 $\mu molm^{-2} s^{-1}$) at 25 ± 1°C. These same incubation conditions were used in all subsequent experiments.

2.2. Shoot multiplication

Shoots developed in the initiation phase (2 cm in length) were separated and cultivated in the proliferation medium contained MS salts supplemented with different concentrations of BA (0.1; 0.5; 1 or 5 mg/l) or kinetin (0.1; 0.5; 1 or 5 mg/l) or in combination with NAA at 0.5 mg/l. These treatments were selected according to the references consulted and results of previous experiments [21-24]. We used 20 explants of each type per treatment (n = 20) and after 6 weeks of culture, number of shoots produced in each explant was determined. These experiments were conducted two or three independent times for each treatment.

2.3. Rooting of shoots

To stimulate root development, elongated shoots (2 à 6 cm) were separated and cultivated in the rooting media. The rooting technique consisted of exposing those shoots to different treatments: (1) MS/2, 30 g/1 sucrose, 0.3% phytagel, without growth regulators; (2) MS/2, 30 g/1 sucrose, 0.3% phytagel, with 0.5 mg/l IBA; (3) MS/2, 30 g/1 sucrose, 0.3% phytagel, with 0.5 mg/l IAA; (4) MS/2, 30 g/1 sucrose, 0.3% phytagel, with 0.5 mg/l NAA. These four 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 4 weeks after initiating the experiment.

2.4. Acclimatization of plants

Rooted plantlets were transferred to autoclaved soil and acclimatized with plastic covers for 4 weeks to prevent desiccation and to allow acclimatization, and then transferred to the greenhouse. Survival percentages were determined 12 weeks after transplantation.

2.5. Statistical analysis

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.

3. Results and Discussion

The effects of kinetin, BA and NAA concentrations on shoot multiplication are presented in Table 1. The highest number of shoots per explant was obtained with BA, with 1 and 5 mg/l, or combined with 0.5 mg/l NAA. The interaction of BA concentrations and NAA was not significant on shoot proliferation, and this result is similar to the previous findings [24]. They 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. Although, the number of shoots per explants increased with BA concentration, this also resulted in an increase of the size of the shoots produced.

Various cytokinins have been adopted both for culture initiation and shoot proliferation of the prickly pear [5, 24-26]. These results are consistent with previous findings [12, 23-24]. They found BA to be more effective than kinetin and 2iP (6-Dimethylaminopurine) in inducing shoot multiplication from *in vitro* culture of areoles of the prickly pear.

3.1. Root formation

For root formation, elongated shoots were transferred to different rooting media. Table 2 shows the effect of different auxins on number of root. In this study, 100% of the regenerated shoots were rooted after 4 weeks of culture in different treatment (Fig. 1C). This is confirmed in several publications: i) Khalafalla et al., 2007 [24] obtained highest percentage of root using IAA at 0.5 mg/l; *ii)* with 0.5 mg/l IBA or 0.5 mg/l IAA 21 species of Mexican cacti were rooted [27]; iii) satisfactory rooting occurred for three *Opuntia* genotypes treated with 0.5 mg/l IBA [19]. According to literature, rooting of several *Opuntia* species is favored in presence [28] or absence of auxins in culture media. Highest number (21.05) of roots per rooted plantlet was obtained using IBA at 0.5 mg/l [5, 12, 16-19].

3.2. Acclimatization of in vitro regenerated plants

In the present study, plants rooted in vitro were transferred to potting mixture and acclimatized initially for 4 weeks in culture room and then in glasshouse for 8 weeks (Fig. 1D). The survival frequency was enhanced to 100% and plantlets showed healthy and active growth. This result is similar to the reports for other micropropagated prickly pear cactus species [5, 12, 17, 26-29]. Hartmann *et al.* [28] 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.

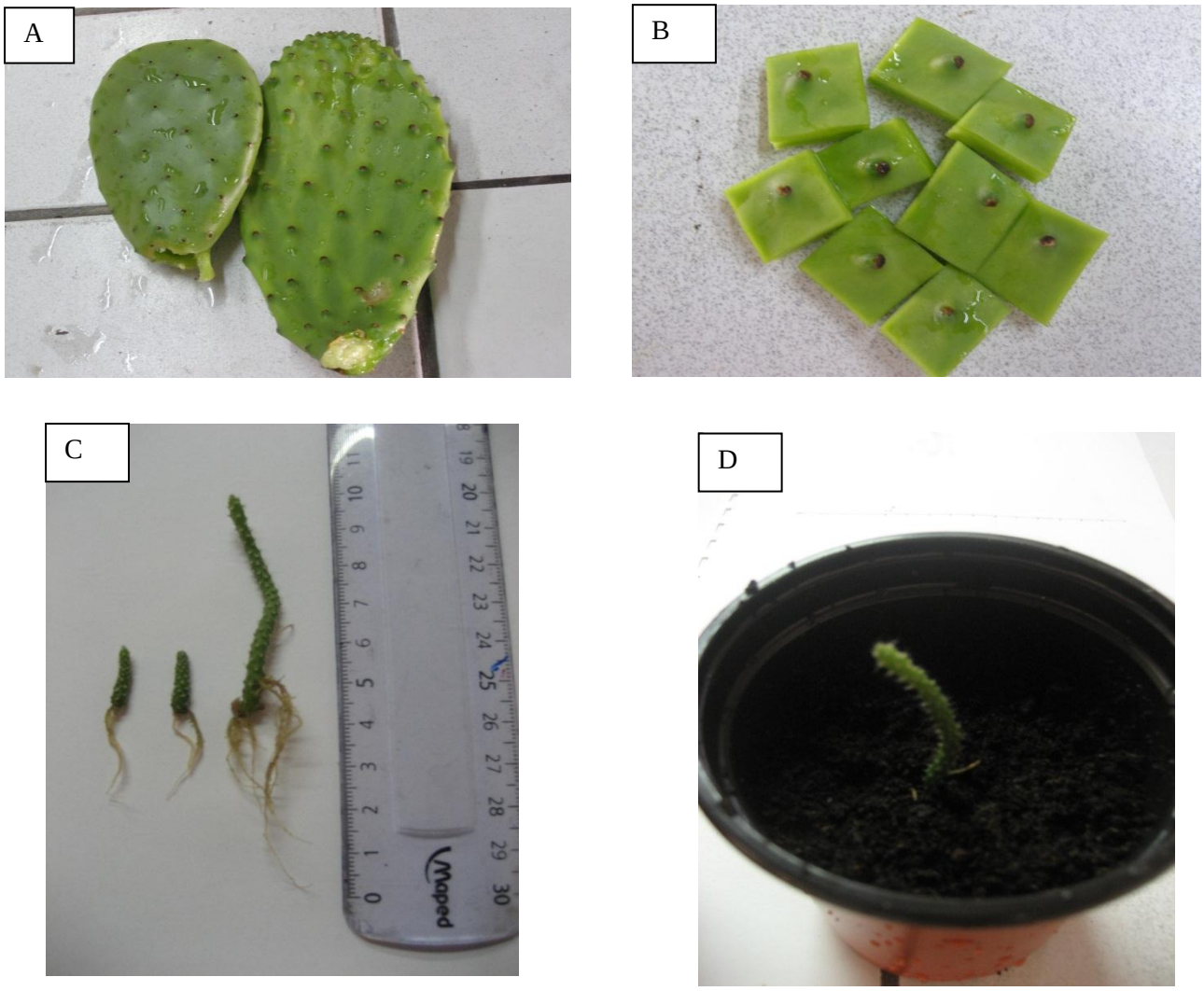

Fig. 1. In vitro micropropagation of cactus (*Opuntia ficus-indica*) (A-D). A: Young cladode cactus. B: Cladode explants containing one areole. C: Root induction on MS supplemented with IBA at 0.5 mg/l. D: Plant acclimatization in growth chamber.

Table 1. Effects of benzyladenine (BA) and kinetin (Kin) alone or in combination with 1-naphtalenacetic acid (NAA) on shoot number and length during proliferation subcultures of *Opuntia ficus-indica* on MS medium after 6 weeks of culture.

Growth regulator (mg/l)			Shoot number per explant	Shoot length (mm)
BA	Kin	NAA		
0,1	0	0	$2,7^{a}$	$9,39^{e}$
0,5	0	0	$3,65^{ab}$	$5,02^{c}$
1	0	0	$6,05^{ef}$	$4,21^{b}$
5	0	0	$15,25^{j}$	$4,1^{b}$
0	0,1	0	$3,65^{ab}$	$6,37^{d}$
0	0,5	0	$4,9^{cd}$	$5,07^{c}$
0	1	0	$8,25^{h}$	$3,97^{b}$
0	5	0	$3,2^{ab}$	$4,89^{c}$
0,1	0	0,5	3^{ab}	$8,87^{e}$
0,5	0	0,5	$4,1^{bc}$	$5,84^{d}$
1	0	0,5	$6,5^{fg}$	$3,98^{b}$
5	0	0,5	$13,5^{i}$	$3,29^{a}$
0	0,1	0,5	$3,9^{bc}$	$6,2^{d}$
0	0,5	0,5	$5,35^{de}$	$4,91^{c}$
0	1	0,5	$7,25^{g}$	$3,38^{a}$
0	5	0,5	$3,1^{ab}$	$4,01^{b}$

Means with same letter (s) in the same column are not significantly different according to Duncan's Test (P=0.05)

Table 2. Effect of auxins on rooting of *in vitro* derived shoots of *opuntia ficus-indica* after six weeks of culture on MS medium.

Treatment	% of rooting	Number of roots per shoot
free-hormone	100.0	$4,95^{a}$
IBA 0.5 mg/l	100.0	$21,05^{d}$
IAA 0.5 mg/l	100.0	$16,2^{c}$
NAA 0.5 mg/l	100.0	$9,3^{b}$

Means with same letter are not significantly different according to Duncan's Test (P=0.05).

4. Conclusion

In conclusion, 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. An efficient in vitro shoot proliferation system from areoles of Moroccan cultivar of cactus was developed. The results obtained shows that optimum shoot proliferation was obtained when explants were cultured in MS medium supplemented with 5 mg/l BA. A proliferation rate of 15 shoots per shoot explant every 6 weeks was obtained. One hundred percent of these microshoots regenerated roots after 4 weeks. The Micropropagated plants are under evaluation for their clonal fidelity and agronomic behavior.

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