Micropropagation of Cactus Pear (*Opuntia ficus indica*) by Organogenesis

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Abstract

The effects of different plant growth regulators (PGRs) on shoot development, adventitious bud induction, shoot bud multiplication, elongation and rooting were evaluated in cactus pear (*Opuntia ficus indica*). Shoot development from areoles was assessed on Murashige and Skoog (MS) medium containing different concentrations of 6-benzylaminopurine (BAP; 0-4 mg/l). Shoot segments were then cultured on the same initiation medium to induce adventitious buds. Adventitious shoot bud elongation and rooting were evaluated either on PGR-free MS medium, or on MS medium containing 0.5 mg/l of either 1-naphthalene acetic acid (NAA) or indole-3-butyric acid (IBA), and supplemented with different concentrations of gibberellic acid (GA_3; 0.5-1 mg/l). Shoot development from areoles was achieved in all BAP-containing media (82.6-100%). Adventitious bud induction was 100% in all BAP-containing media, while the highest average number of adventitious buds per explant (21.0) was observed in MS medium containing 3 mg/l BAP, with no significant difference with those supplemented with BAP at 2 and 2.5 mg/l (16.4-20 buds per explant). Increasing BAP concentration to 4 mg/l significantly decreased the average number of buds per explant (5.3). Shoot elongation and rooting were better on PGR-free MS medium, which gave an average shoot length of 3.4 cm, 100% rhizogenesis, an average number of roots per shoot of 11.8 and a root length of 2.6 cm. The regenerated shoots were successfully acclimatized, with a survival rate of 81.25%. The established protocol can be used for the large-scale propagation of cactus pear in Morocco.

Keywords: Adventitious buds, in vitro, multiplication, tissue culture.

Micropropagation du Cactus (*Opuntia ficus indica*) par Organogenèse

Résumé

Les effets de différentes substances de croissance sur le développement des pousses, l'induction des bourgeons adventifs, leur multiplication, élongation et enracinement ont été examinés chez le cactus (*Opuntia ficus indica*). Le développement des pousses à partir des aréoles a été évalué sur le milieu de Murashige et Skoog (MS) additionné de différentes concentrations de 6-benzylaminopurine (BAP; 0-4 mg/l). Par la suite, les fragments des pousses ont été transférés sur le même milieu d'initiation pour la formation des bourgeons adventifs. L'élongation et l'enracinement des bourgeons adventifs ont été testés sur le milieu MS sans hormones, et sur le milieu MS contenant 0,5 mg/l d'acide 1-naphtalène acétique (NAA) ou d'acide indole-3-butyrique (IBA), et additionné de différentes concentrations d'acide gibbérellique (GA_3; 0,5-1 mg/l). Le développement des pousses à partir des aréoles a été observé sur tous les milieux contenant la BAP (82,6-100%). De même, le pourcentage d'induction des bourgeons adventifs était de 100% dans tous les milieux additionnés de BAP. Par ailleurs, le nombre moyen le plus élevé de bourgeons adventifs par explant (21,0) a été observé sur le milieu MS additionné de 3 mg/l de BAP, sans différence significative avec les concentrations de 2 et 2,5 mg/l (16,4-20 bourgeons par explant). L'augmentation de la concentration de la BAP à 4 mg/l a significativement diminué le nombre moyen de bourgeons par explant (5,3). L'élongation et l'enracinement des bourgeons étaient meilleurs sur le milieu MS dépourvu d'hormones. En effet, sur ce milieu, la taille moyenne des bourgeons était de 3,4 cm, le pourcentage d'enracinement était de 100%, le nombre moyen de racines par bourgeon était de 11,8 alors que la longueur moyenne des racines était de 2,6 cm. Les pousses régénérées ont été acclimatées avec succès (81,25%). Le schéma de micropropgation développé peut être utilisé dans les programmes de multiplication massive du cactus au Maroc.

Mots-clés : Bourgeons adventifs, in vitro, multiplication, culture tissulaire.

تكاثر شجرة الصبار (*Opuntia ficus indica*) عن طريق تقنية التكوين العضوي

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ملخص

قمنا في هذا البحث بدراسة تأثير هرمونات نمو النباتات على نمو البتيلة، وكذلك على تكوين، تكاثر، استطالة وتجذير البراعم العرضية لشجرة الصبار (*Opuntia ficus indica*). تم تقييم نمو البتيلة على وسط موراشيج وسكوغ (MS) والذي تم تزويده بتركيزات مختلفة من هرمون 6-بنزيل أمينوبورين (BAP؛ 0-4 مجم/لتر). بعد ذلك قمنا بزراعة أجزاء البتيلة على نفس وسط النمو لتحفيز تكوين البراعم العرضية. تمت دراسة استطالة البراعم وتجذيرها على وسط MS يحتوي إما على 0.5 مجم/لتر من حمض 1-النفثالين أستيك (NAA) أو من حمض الإندول-3 بوتيريك (IBA)، إضافة إلى تركيزات مختلفة من حمض الجبريليك (GA_3؛ 0.5-1 ملغم/لتر). أظهرت نتائجنا نمو البتيلة على جميع الوسائط المحتوية على BAP (82.6-100٪)، بينما لم يلاحظ أي نمو على وسط MS الخالي من BAP. إضافة إلى ذلك، لوحظ تكوين البراعم العرضية في جميع الوسائط المحتوية على BAP (100٪)، كما لوحظ أعلى معدل تكاثر (21.0 برعم) على وسط MS المحتوي على 3 مجم/لتر من BAP، دون وجود فرق كبير مع تلك المحتوية علىBAP بتراكيز2 و2.5 ملغم/لتر (16.4-20 برعم)، في حين أدت زيادة تركيز BAP إلى 4 ملغم/لتر إلى انخفاض كبير في معدل تكاثر البراعم (5.3). أظهرت نتائجنا أيضا أن استطالة البراعم وتجذيرها تتم بشكل ممتاز على وسط MS الخالي من هرمونات النمو، حيث أنتج هذا الوسط أعلى معدل طول للبراعم (3.4 سم)، تكوين جذور بنسبة 100%، متوسط عدد جذور يعادل 11.8 لكل برعم وطول جذور متوسط بلغ 2.6 سم. تمت أقلمت النباتات المنتجة بنجاح، حيث أظهرت 81.25% منها نموا طبيعيا. يمكن استخدام هذا البروتوكول في برامج الإنتاج المكثف لأشجار الصبار في المغرب.

الكلمات المفتاحية: *براعم عرضية، داخل الأنابيب، تكاثر، زراعة الأنسجة.*

Introduction

Cactus pear (*Opuntia* spp.), also known as prickly pear, is a plant genus of the family Cactaceae (Gibson and Nobel, 1986). It is native to the tropical and subtropical regions of America, where its cultivation started thousands of years ago by the Mesoamerican people (Kiesling and Metzing, 2017; Nobel, 1994). Among all cactus pear species, *Opuntia ficus indica* (L.) Mill. (Miller, 1754) is the most economically important one (Caruso et al., 2010). *O. ficus indica* is a spineless species used for human consumption, as forage and for cosmetic and pharmaceutical purposes (Mazri, 2018; 2021). In addition, cactus pear plays an important ecological role in reducing the effects of desertification, soil degradation and erosion, slowing deforestation and preserving biodiversity (Nefzaoui et al., 2014; Paiva et al., 2016; Sáenz, 2013).

In Morocco, cactus pear is threatened by *Dactylopius opuntiae* (Cockerell, 1896) (Hemiptera:Dactylopiidae), also known as the cochineal scale insect and false carmine scale (De Lotto, 1974; Guerra and Kosztarab, 1992; Chávez-Moreno et al., 2009). Indeed, since its introduction in 2014, the cochineal caused several damages to cactus pear in many regions of the country (Bouharroud et al., 2016). As a way to rehabilitate cactus pear plantations in Morocco, researchers from the National Institute for Agronomic Research (INRA-Morocco) have identified and selected eight cactus pear varieties resistant to this cochineal (Sbaghi et al., 2018). Today, rapid and large-scale propagation of the selected cactus pear varieties have become a priority in Morocco. In fact, developing efficient propagation systems can significantly help in the rehabilitation of cactus pear plantations.

Cactus pear is generally propagated by cladodes (Mazri, 2018). However, the use of *in vitro* culture techniques can be very effective in accelerating the propagation of resistant cultivars. In fact, a technique such as organogenesis has already proven its efficiency as a powerful propagation method and efficient strategy to control pathogens and rehabilitate groves. For example, direct organogenesis is the main method used currently in Morocco for rapid and large-scale production of date palm cultivars resistant to *Fusarium oxysporum* f. sp. albedinis, and to rehabilitate groves affected by this fungus (Mazri and Meziani, 2013; Mazri et al., 2019). Direct organogenesis is the *in vitro* regeneration system by which adventitious buds are directly formed on explants (i.e., without callus phase) under appropriate culture conditions. It involves adventitious bud induction and proliferation, shoot elongation and rooting and plantlet acclimatization. Regarding *O. ficus indica*, some authors have already evaluated its propagation through organogenesis. However, significant impacts of the genotype, plant growth regulators (PGRs) and medium strength on shoot induction and multiplication, and root formation were revealed (Garcia-Saucedo et al., 2005; Khalafalla et al., 2007; Zoghlami et al., 2012). Therefore, it is important to develop and optimize regeneration systems for the best cactus pear cultivars.

The purpose of the present study was to evaluate the effects of PGRs on areole activation, adventitious bud induction, shoot elongation and rooting and plantlet acclimatization of cactus pear *O. ficus indica*. These experiments aimed to identify appropriate conditions for organogenesis of cactus pear. The developed regeneration system would be useful for the rapid and efficient propagation of cactus varieties resistant to *D. opuntiae*.

Materials and methods

Chemicals

All chemicals were purchased from Sigma-Aldrich (Steinheim, Germany) unless otherwise noted.

Plant material and surface sterilization

Sixteen-month-old cladodes of cactus pear (*O. ficus indica*) were collected in September 2019 from a mature tree located in the experimental station 'Saada' (31°37'23.246" N 8°8'58.988" W), Regional Center for Agronomic Research of Marrakech (CRRA-Marrakech, INRA). The cladodes were used immediately after harvest. They were thoroughly washed with tap water and then wiped with cotton containing 70% ethanol. Afterwards, the cladodes were cut into small segments of 1 cm diameter and 0.5 cm height, each containing one areole (i.e., one side with areole and the other side without areole), then surface sterilized under the laminar flow hood by immersion in 50% commercial bleach (2.5% calcium hypochlorite, ACE, Mohammedia, Morocco) for 15 min, followed by three 10-min rinses with sterile distilled water.

Effects of PGRs on shoot development from areoles

After surface sterilization, cactus pear explants were cultured on Murashige and Skoog (MS; Murashige and Skoog, 1962) medium supplemented with six concentrations (0; 2; 2.5; 3; 3.5 or 4 mg/l) of 6-benzylaminopurine (BAP). The explants were placed areole-side-up on culture medium. They were cultured for two months, with a transfer to fresh medium after the first month of culture. One explant was placed per jar (6.5 cm in diameter and 12 cm in height) containing 40 ml of culture medium, and for each BAP concentration, 23 jars were used. At the end of the initiation phase, the percentage of explants that developed shoots from areoles was calculated.

Effects of PGRs on adventitious bud induction and multiplication

In this experiment, the shoots developed from areoles were used as a source of explants. The shoots were cut into 0.5 cm^2 segments and cultured on the same induction medium, i.e., MS medium supplemented with different BAP concentrations (0; 2; 2.5; 3; 3.5 or 4 mg/l). Two explants were placed per jar, which was considered as one replicate, and 18 replicates were used per treatment. The explants were cultured for 6 months, with transfers to fresh medium at 1-month intervals. During the induction and multiplication of adventitious buds, the percentage of explants that induced adventitious buds was calculated after 2 months of culture, while the average number of adventitious buds per explant was calculated after 6 months of culture.

Effects of PGRs on shoot bud elongation and rooting

Adventitious buds of 0.5-0.8 cm in length, previously induced on MS medium containing 2.5 mg/l BAP, were transferred to different culture media to evaluate their effects on elongation and rooting. The shoots were cultured either on PGR-free MS medium, or on MS medium supplemented with different PGR combinations: 0.5 mg/l 1-naphthalene acetic acid (NAA) + 0.5 mg/l gibberellic acid (GA_3); 0.5 mg/l NAA+ 1

mg/l GA_3; 0.5 mg/l indole-3-butyric acid (IBA) + 0.5 mg/l GA_3; and 0.5 mg/l IBA + 1 mg/l GA_3. Four buds were placed per jar, which was considered as one replicate, and 14 jars were used per treatment. The shoots were kept in the elongation-rooting medium for 3 months, with transfers to fresh medium at 1-month intervals. At the end of the elongation-rooting phase, shoot length, and root number and length were recorded.

Culture conditions

All culture media were supplemented with 1 g/l polyvinylpyrrolidone (PVP), 50 g/l sucrose and gelled with 7 g/l agar. The pH of culture media was adjusted to 5.7 before autoclaving at 121 °C for 25 min. In all experiments, the cultures were maintained under a 16-h photoperiod at 25 $\pm$ 1°C.

Plantlet acclimatization

At the end of the elongation-rooting experiments, the plantlets developed under the optimal conditions were transferred to the greenhouse for acclimatization. The plantlets (n=32) were removed from culture media; the root system was carefully washed with tap water then they were planted in plastic pots (12 cm in diameter and 20 cm in height) containing 950 g of peat and gravel (1:1; w/w). The plantlets (1 to 3 plantlets per plastic pot; 25 plastic pots) were placed in a tunnel and covered with a transparent polyethylene bag for 2 weeks (98% relative humidity (RH) and 27 ºC). The polyethylene bag was then progressively removed over a period of 2 weeks to allow acclimatization to the greenhouse conditions (70% RH, 27 ºC). After 3 months in the greenhouse, the plantlet survival percentage was recorded.

Statistical analysis

Data on the percentage of explants developed shoots from areoles, induced adventitious buds, average number of adventitious buds, shoot length, root number and length, and survival rate of acclimatized plantlets were recorded. In all experiments, a completely randomized design was used. Percentage data were arcsine transformed before analysis. Data were submitted to analysis of variance (ANOVA) and means were compared by using the Student-Newman-Keuls (SNK) test at the 5% significance level. Data were analyzed by using SPSS v. 26 for windows.

Results and discussion

Effects of PGRs on shoot development from areoles

The highest percentage (100%) of shoot development from areoles was achieved by MS medium supplemented with 2.5, 3.5 and 4 mg/l BAP (Fig. 1). Statistical analysis showed no significant difference between these media and that supplemented with 3 mg/l BAP, which gave a percentage of shoot initiation of 91.3%. However, there was a significant difference with the medium containing 2 mg/L BAP (82.6%) and PGR-free MS medium. In this latter one, no shoot initiation was observed (Table 1).

Figure 1. Shoot development from areoles of cactus pear on MS medium supplemented with 2.5 mg/l BAP. The bar corresponds to 1 cm.

The promoting effect of BAP on shoot induction was observed in many other plant species (Singh and Tiwari, 2010; Thiyagarajan and Venkatachalam, 2012; Thomas and Shankar, 2009). For example, in *Gymnema sylvestre* (Retz.) R. Br. ex Sm. (Retzius, 1811) (Asclepiadaceae), the influence of different concentrations of BAP and kinetin was evaluated, and the highest frequency (65.56%) of shoot bud regeneration was found in the medium containing 1 mg/l BAP (Thiyagarajan and Venkatachalam 2013). Timofeeva et al. (2014) assessed the effects of different concentration of three cytokinins, namely BAP, thidiazuron (TDZ) and kinetin on bud initiation in *Laburnum anagyroides* Medik. (Medikus, 1787) (Fabaceae). Bud initiation occurred in all cytokinin-containing media. However, subsequent development was observed only in the medium containing 0.5 mg/l BAP.

Table 1. Effects of PGRs on shoot initiation, adventitious bud induction and multiplication in cactus pear (*O. ficus indica*)

Culture medium	Shoot initiation from Areoles (%)	Adventitious bud induction (%) from *in vitro* developed shoots	Average number of buds per explant
MS	0.0 ± 0.0 a	-	-
MS + 2 mg/l BAP	82.6 ± 38.7 b	100.0 ± 0 a	16.4 ± 9.9 bc
MS + 2.5 mg/l BAP	100.0 ± 0 c	100.0 ± 0 a	20.0 ± 7.4 c
MS + 3 mg/l BAP	91.3 ± 28.8 bc	100.0 ± 0 a	21.0 ± 8.5 c
MS + 3.5 mg/l BAP	100.0 ± 0 c	100.0 ± 0 a	12.6 ± 8.6 b
MS + 4 mg/l BAP	100.0 ± 0 c	100.0 ± 0 a	5.3 ± 5.1 a
F	91.468	-	11.051
p	0.000	-	0.000
DF for treatment factor	5	4	4
DF error	132	85	85

Data are means followed by standard deviations. Data in the same column followed by different letters are significantly different at the 5% level of SNK test. MS, Murashige and Skoog medium; BAP, 6-benzylaminopurine.

In cactus pear, Escobar et al. (1986) reported that BAP is necessary for shoot development from pre-existing buds of *Opuntia amyclaea* Tenore (Tenore, 1826), and recommended the concentration of 2.25 mg/l, which gave an organogenesis rate of 62-100%. Estrada-Luna et al. (2008) cultured isolated areoles of *Opuntia lanigera* Salm-Dyck (de Salm-Reifferscheidt-Dyck, 1850) on MS medium supplemented with 2.5 mg/l BAP for shoot induction. For *Opuntia ellisiana* Griffiths (Griffiths, 1910), Juarez and Passera (2002) recommended the combination of 2.25 mg/l BAP and 2 mg/l IBA, which resulted in 100% areole shooting. Regarding the species *O. ficus indica*, different PGR concentrations and combinations were recommended for shoot initiation, depending on the genotype. For genotypes Blanco sin Espina, Milpa Alta and Villa Nueva, Garcia-Saucedo et al. (2005) used the combination of 0.5 mg/l BAP and 0.5 mg/l GA_3. Zoghlami et al. (2012) cultured areole explants from *O. ficus indica* cv. Gialla on PGR-free MS medium, MS supplemented with 0.5 mg/l NAA, and MS supplemented with 0.5 mg/l BAP, and found that the medium containing BAP was the most appropriate for areole activation. The findings of the present study suggest the use of a concentration ranging from 2.5 to 4 mg/l of BAP for the *in vitro* induction of shoots from areoles of *O. ficus indica*.

Effects of PGRs on adventitious bud induction and multiplication

The findings of the present study revealed that all BAP-containing media promoted adventitious bud induction, with a high rate of 100% (Table 1). On the other hand, the results showed that the average number of shoot buds per explant varied significantly depending on BAP concentration (Table 1). The highest average number of shoot buds per explant (21.0) was observed in MS medium containing 3 mg/l BAP. This was followed by MS medium supplemented with 2.5 mg/l BAP (20.0 shoot buds per explant; Fig. 2) then by that containing 2 mg/l BAP (16.4 shoot buds per explant). Statistical analysis showed no significant difference between the media containing BAP at a concentration ranging from 2 to 3 mg/l. Increasing BAP concentration to 4 mg/l significantly decreased the average number of shoot buds per explant to 5.3.

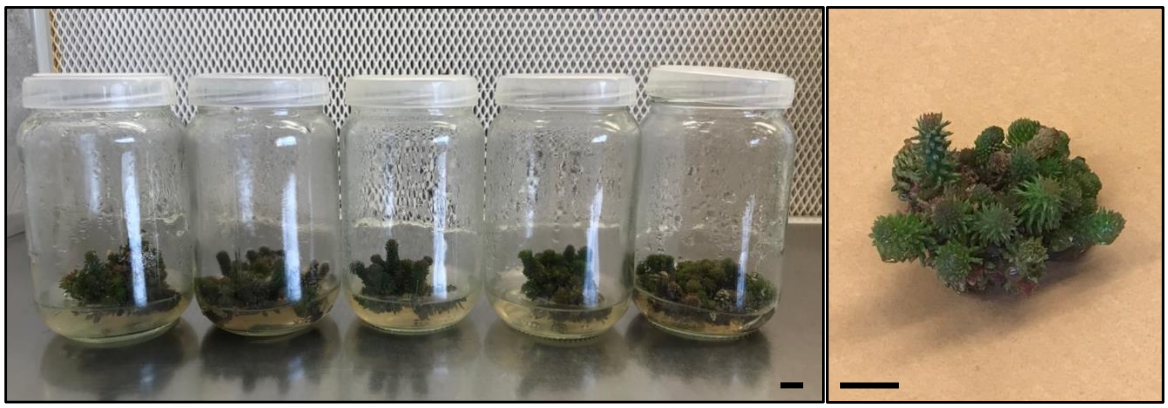

Figure 2. Adventitious shoot bud multiplication of cactus pear on MS medium supplemented with 2.5 mg/l BAP. Bars correspond to 1 cm.

The promoting effects of PGRs on adventitious bud induction is due to the fact that exogenous PGRs interact with the endogenous plant hormones, which results in cell division, differentiation and morphogenesis (Gaspar et al., 1996; Feher et al., 2003; Gaspar et al., 2003; Gaj, 2004). However, the PGR type and concentration required to induce morphogenic responses depend on the specie, genotype, explant type and culture conditions (Gaspar et al., 1996; Feher et al., 2003; Gaspar et al., 2003; Gaj, 2004). In cactus pear *O. ficus indica*, BAP (2-3 mg/l) seems to be the most appropriate PGR for adventitious bud induction (100%) and multiplication (16.4-21.0 buds per explant). According to Escobar et al. (1986), in *O. amyclaea*, the use of 2.25 mg/l BAP gave an average of 15 shoots per explant. Garcia-Saucedo et al. (2005) cultured shoot segments of *O. ficus indica* genotypes Blanco sin Espina, Milpa Alta and Villa Nueva on MS medium containing different BAP concentrations, and reported that the highest organogenesis rates (80-100%) and the highest number of buds per explant (4.2-13.8) occurred when BAP was used at the concentration of 0.11 mg/l.

Effects of PGRs on shoot bud elongation and rooting

Our findings revealed that PGR-free MS medium is the most appropriate for shoot elongation and rooting (Fig. 3). This medium gave an average shoot length of 3.4 cm. In media containing PGRs, the shoot length ranged from 1.0 to 1.6 cm, with no significant difference among them. In addition, in these media, new adventitious shoots were developed from the base of the primary ones.

Figure 3. Shoot elongation and rooting of cactus pear on PGR-free MS medium. Bars correspond to 1 cm.

Table 2. Effects of PGRs on shoot elongation and rooting in cactus pear (*O. ficus indica*)

Culture medium	Average shoot length (cm)	Adventitious root induction (%)	Average number of roots per explant	Average root length (cm)
MS	3.4 ± 1.5 a	100.0 ± 0.0 a	11.8 ± 4.4 a	2.6 ± 0.7 a
MS + 0.5 mg/l NAA + 0.5 mg/l GA_3	1.0 ± 0.5 b	100.0 ± 0.0 a	4.5 ± 2.4 bc	1.1 ± 0.6 b
MS + 0.5 mg/l NAA + 1 mg/l GA_3	1.1 ± 0.4 b	71.4 ± 46.8 b	2.4 ± 2.7 c	0.9 ± 0.8 b
MS + 0.5 mg/l IBA + 0.5 mg/l GA_3	1.5 ± 0.5 b	100.0 ± 0 a	5.9 ± 3.2 b	3.0 ± 1.4 a
MS + 0.5 mg/l IBA + 1 mg/l GA_3	1.6 ± 0.4 b	92.3 ± 27.7 a	3.7 ± 3.0 bc	2.7 ± 1.6 a
F	19.700	3.629	17.805	10.139
p	0.000	0.010	0.000	0.000
DF for treatment factor	4	4	4	4
DF error	64	64	64	64

Data are means followed by standard deviations. Data in the same column followed by different letters are significantly different at the 5% level of SNK test. MS, Murashige and Skoog medium; NAA, 1-naphthalene acetic acid; GA_3, Gibberellic acid; IBA, indole-3-butyric acid.

Regarding root induction and growth, the highest rooting percentage (100%) was observed in PGR-free MS medium, and in MS medium containing either the combination of 0.5 mg/l NAA and 0.5 mg/l GA_3, or that of 0.5 mg/l IBA and 0.5 mg/l GA_3 (Table 2). The highest average number of roots per shoot (11.8) was observed in PGR-free MS medium, while the highest average root length (3.0 cm) was observed in MS medium containing 0.5 mg/l IBA and 0.5 mg/l GA_3. Statistical analysis revealed significant differences among the different media evaluated. Our findings suggest the

use of PGR-free MS medium for shoot elongation and rooting. Escobar et al. (1986) evaluated the effects of different IBA concentrations on root induction from *in vitro* shoots of *O. amyclaea*, and reported the formation of 18 roots per shoot when the culture medium contained $5*10^{-5}$ M IBA. For *O. ellisiana*, Juarez and Passera (2002) suggested to culture the regenerated shoots on MS medium supplemented with 5-10.1 mg/l IBA, which gave 100% rooting. Garcia-Saucedo et al. (2005) compared rhizogenesis among three *O. ficus indica* genotypes (Blanco sin Espina, Milpa Alta and Villa Nueva) on different culture media, and reported that the highest average number of roots (24.2) occurred in cv. Blanco sin Espina on half-strength MS medium (MS/2) supplemented with 1.1 mg/l. In *O. ficus indica* cv. Gialla, Zoghlami et al. (2012) suggested the use of 1 mg/l IBA for *in vitro* root induction. Khalafalla et al. (2007) evaluated the effects of different auxins (NAA, indole-3-acetic acid (IAA) and IBA) on rhizogenesis of *O. ficus indica*, and noticed the highest rooting percentage (100%) on PGR-free medium and on MS medium supplemented with 0.5 mg/l IAA. On the other hand, the highest mean number of roots per shoot (15) was observed on the medium containing 0.5 mg/l IAA. Based on our findings, we suggest the use of PGR-free MS medium for shoot elongation and rooting of *O. ficus indica*.

Plantlet acclimatization was performed in a substrate composed of peat and gravel (Fig. 4). After 3 months in the greenhouse, the survival rate was 81.25%. Higher survival rates were reported in literature. For example, in *O. ficus indica*, Khalafalla et al. (2007) and Angulo-Bejarano and Paredes-López (2011) reported a survival rate of 100% during the acclimatization of organogenesis-derived plantlets. In *O. ellisiana*, Juarez and Passera (2002) also reported a 100% survival rate during acclimatization. These differences with our results might be explained by different factors such as the genotype/species, and/or the acclimatization process used.

Figure 4. Cactus pear plantlets after one month in the greenhouse.

Conclusions

An efficient regeneration system through organogenesis was developed for cactus pear (*O. ficus indica*). Areole shooting was successfully achieved on MS medium containing 2.5-4 mg/l BAP. Adventitious bud induction and multiplication were performed on MS medium supplemented with 2-3 mg/l BAP. Regarding shoot elongation and rooting, PGR-free MS medium was the best during this culture phase. The regenerated plantlets were successfully acclimatized to *ex vitro* conditions. The developed protocol will contribute to the rapid and large-scale propagation of cactus pear. Further studies will be carried out to assess the genetic conformity of regenerants, and the applicability of this protocol to cochineal-resistant varieties.

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