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仙人掌的微繁殖

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摘 要：成功建立了仙人掌离体快繁的实验体系，并且对影响微繁殖的一些因素，诸如激素组合、外植体的物理状态、大量元素的含量等进行了研究。结果表明：BA 对仙人掌芽增殖具明显作用，MS+BA 5.0 mg/L+IBA 0.1 mg/L 为最适增殖培养基；接种方式实验表明劈接优于整棵。钙、镁离子浓度对试管苗生长没有影响，但影响生根数及根长；NAA 抑制根的伸长，但一定浓度可促进生根。总体而言，最适的生根培养基为 1/2 MS。同时发现块接比单芽接具有优势。试管苗在形态上出现一些变异。实验结果对仙人掌科其它植物的快速繁殖具有参考意义。

关键词：仙人掌；离体培养；增殖；茎段；纵劈；茎节

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Micropropagation of *Opuntia dillenii* (Ker-Gawl.) Haw.

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Abstract: A protocol is described for rapid in vitro propagation of the valuable chylocaula *Opuntia dillenii* (Ker-Gawl.) Haw. *in vitro*. The influence of various combinations of growth regulators, the physical stage of explants and the concentration of the macroelements were also evaluated. It was found that bud variation was dependent on BA supply, the synergistic combination of 5.0 mg/L and 0.1 mg/L IBA induced the optimum frequency(5.3 buds/stem segment). Stem segment with longitudinal split was prior to whole segment on bud induction. The concentration of Ca^{2+}, Mg^{2+} affected root number and length, but had no effects on plantlet height and growth. Though root length of plantlets declined with increased NAA concentration, 0.2 mg/L NAA promoted root number. Considering together, the best rooting medium was half-strength MS. It was observed that sprout tuber was superior to single nodal plate on bud number as well as bud growth. Some morphological variations exhibited in the transferred test-tube plants. This micropropagation procedure may provide the basis for improvement of this chylocaula and was beneficial to the tissue culture of other plants of Cactaceae.

Key words: *Opuntia dillenii*; *in vitro*; micropropagation; stem segment; longitudinal split; nodal plate

Opuntia dillenii (Ker-Gawl.) Haw. is a perennial chylocaula of the Cactaceae, *Opuntia* genus with planate green nodal plate, obovate to elliptic, with clumped arbusculate. It is distributed in Florida of America, the West Indies, Mexico and South-America. And there are also some natural or half-wild species grow in China, Australia and India. Cactus(*O. dillenii*) can be propagated by seeds or

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by stem cuttings. It usually used in grafting as stock of *Zygocacuts truncatus* (Hqw.)K. Schum. or *Schlumbergera bridgesii* (Yu *et al*, 1993).

The stems, flowers and fruits of cactus are commonly used as forages, foods and medicines in ancient China. It had already regarded as a traditional Chinese medicinal material listed in "Traditional Chinese Medicinal Dictionary" (Jiangsu New Medicinal Academy, 1986).

Recent reports indicated the analgesic and anti-inflammatory properties of *O. dillenii* (Park *et al*, 1998; Loro *et al*, 1999). Besides, the composition (Chen *et al*, 1998; Elkossori *et al*, 1998) and chemical constituents (Qiu *et al*, 2000) of Cactus was investigated. Further, Cactus pear fruit may become a new source for a natural food addictive (Saenz *et al*, 1998; Zhang *et al*, 1992; Fu *et al*, 1993). And exploitation perspective of Cactus has been proposed (Wang *et al*, 2001).

We only found very few previous reports about in vitro culture techniques of Cactaceae: Chen *et al* (1999) only reported the grafting of *O. dillenii*, but didn't referred to it's micropropagation; Cai *et al* (2000) just published propagation of *Echinocactus grusonii* very simply. Escobar *et al* (1986), Mohamed *et al* (1995), Chen *et al* (2001) respectively reported the tissue culture of *O. amyclaea*, *O. ficus-indica*, *O. milpa alta* detailed, which referred to the proper medium, transplanted conditions and position of explants.

In the resent paper, we report a rapid propagation through stem segments of wild plants of *O. dillenii* followed by successful establishment of regenerated plants in pear and vermiculite. This system will rapidly provide many plants for investigations of the efficacy and potential commercial application.

1 Materials and methods

1.1 Plant materials

New sprouted nodal plate.

1.2 Methods

1.2.1 Surface sterilization and inoculation

Juvenile nodal plates were excised from maternal plants. The explants were washed thoroughly for 30 min under running tap water, followed by surface sterilized by dipping in 70% ethanol for 30 s, then immersed in 0.1% $HgCl_2$ for 9 min, followed by 5 rinses with sterile distilled water. Exposed ends of each explant were given a fresh cut before they were cultured in MS medium.

1.2.2 Culture medium and conditions

The culture medium used for the present work was basal medium with 3% (w/v) sucrose and 0.8% (w/v) agar. The media were further augmented with different concentrations of hormones. The pH of the media was adjusted to 5.8. The following media were used:

MS_0: MS (Control); MS_1: MS with 2.0 mg/L BA, 0.1 mg/L IBA; MS_2: MS with 5.0 mg/L BA, 0.1 mg/L IBA; MS_3: MS with 8.0 mg/L BA, 0.1 mg/L IBA; MS_4: MS with 10.0 mg/L BA, 0.1 mg/L IBA.

CM_0: half-strength MS (1/2 MS) with 59.86 mg/L Ca^{2+}, 18.01 mg/L Mg^{2+}; CM_1: 1/2 MS with strengthened ($1\times$) Ca^{2+} (119.71 mg/L) and Mg^{2+} (36.03 mg/L); CM_2: 1/2 MS with strengthened ($2\times$) Ca^{2+} (179.57 mg/L) and Mg^{2+} (54.04 mg/L).

R_0: 1/2 MS (Control); R_1: 1/2 MS with 0.1 mg/L NAA; R_2: 1/2 MS with 0.2 mg/L NAA; R_3: 1/2 MS with 0.5 mg/L NAA.

The cultures were maintained at 25±2 ℃ with 12-h day light at an intensity of 60 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (National MZD 30 w).

1.2.3 Multiplication of nodal plate cultures

Nodal plates (1.3cm height) from MS medium were cultured on propagational media ($M_0 - M_4$) to evaluate the effect of combination of hormones. Subsequent subcultures were at 30-day intervals. Nodal plates sprouted in M_2 medium were used in the following experiments.

1.2.4 Comparison of inoculation manner

Nodal plates were subcultured in two manners: one inoculated with the whole nodal plate;

the other nodal plates split longitudinally, then placed the longitudinal section exposed to the medium.

1.2.5 Effect of Ca^{2+} and Mg^{2+}

Nodal plates were subcultured on media (CM_0 —CM_2) to evaluate the effect of Ca^{2+} and Mg^{2+} on growth of shoots.

1.2.6 Induction of rooting

Nodal plates were subcultured in rooting media (R_0—R_3) to evaluate the influence of NAA concentrations.

Rooted plants were washed in tap-water and then transplanted into peat + vermiculite (1 : 1) pots, covered for 10 days with storage bags to avoid dehydration. Then acclimatized plants were transferred to ambient conditions.

Observations were recorded on the number of buds per explant, number of roots per shoot, root length, shoot height and plantlet fresh weight after 30 days; rooting data was recorded after 10 days.

2 Results

2.1 Shoot regeneration

Shoot regeneration from *O. dillenii* nodal plate explants cultured on MS basal and MS medium supplemented with various concentrations of BA in combination with IBA is summarized in Table 1.

Table 1 Effects of combination of hormones on propagation of *O. dillenii*

Media type	Number of shoots tested	Number of buds/shoot	Small buds	Morphological character
M_0	16	0	—	Single, high
M_1	12	3.4	+++	Strong, clump
M_2	16	5.3	++++	Strong, clump
M_3	16	0.8	++	Small
M_4	12	0.3	+	glomerate

Note: buds (>3 mm) are assessed by counting; "—" means no bud; "+" means the quantity of buds (<3 mm).

The results presented in Table 1, show that the number of buds produced increased as the BA concentration increased from 0 to 5.0 mg/L. However, explants on 8.0 mg/L and 10.0 mg/L BA produced few, small and glomerate buds, indicating an upper limitation BA concentration for the micropropagation of *O. dillenii* (Plate I:1). However, it must be pointed out that shoots which sprouted first were obtained on M_2 medium 18 days after induction, the first sprouting was delayed on M_1 medium on the 22nd d, and took place after 30 days on M_3, M_4 media. In additional, the higher concentration of BA was found to promote callus formation.

2.2 Inoculation manner

This experiment (Table 2) showed that buds produced from split explants were about twice as much as that of whole explants (Plate I:2).

Table 2 Effects of inoculation fashion on propagation of *O. dillenii*

Inoculation manner	Number of explants	Number of buds/explants
Whole	24	2.5±1.6
Split	12×2	4.8±2.4

Note: Means data followed are significantly different using test of significance ($t > t_{0.05}$).

2.3 Growth of shoots

The effects of Ca^{2+} and Mg^{2+} on the growth of *O. dillenii* are shown in Table 3.

Table 3 Effects of concentration of Ca^{2+} and Mg^{2+} on growth of *O. dillenii*

Media type	Number of shoots tested	Number of roots /shoot	Average length of root (cm)	Average height of shoot (cm)	Average fresh weight of plantlet (g)
CM_1	24	49	2.41	1.89	0.093 3
CM_2	24	20	1.26	1.66	0.079 8
CM_3	24	27	2.8	1.53	0.117 1

The shoots cultivated on CM_1 produced more roots than those cultivated on other media. The mean length of the roots produced on CM_1 and CM_3 media was significantly higher than that obtained on CM_2. But there's not an evident effect of treatments on shoot height and plantlet fresh weight. The above results showed that Ca^{2+} and Mg^{2+} affect the induction and elongation of roots. The present study reveals that the relatively low concentration of Ca^{2+} and Mg^{2+} of MS is beneficial

for root induction and growth of *O. dillenii* (Plate I:3).

2.4 Rooting

From 85 shoots used to evaluate the rooting, all the plantlets were obtained giving an average rooting of 100% after 10 days' cultivation.

With regards to the rooting media, length of roots declined with increasing NAA concentration. The highest observed number of roots was obtained using 0.2 mg/L NAA, indicating a little promotion of NAA on root induction(Fig. 1). Considering together, the number of roots per shoot and root length, the appropriate and economical rooting medium is 1/2 MS (Plate I:4).

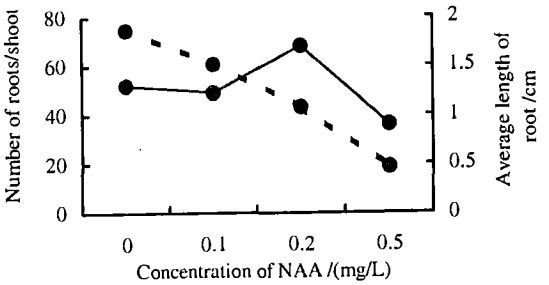

Fig. 1 Effects of concentration of NAA on rooting of *O. dillenii*

—●—Roots/Shoot; ···●···Average length of root

3 Discussion

From these results it appears that it is possible to micropropagate cactus with satisfactory results. Among the growth regulators utilized to induce shoot proliferation, MS with 5.0 mg/L BA, 0.1 mg/L IBA, seems to give the best results. This result was different from the papers of other researchers (Escober *et al*, 1986; Mohamed *et al*, 1995; Chen *et al*, 2001), which may caused by different species of Cactaceae.

The required level of cytokinin in the medium seems to be higher than other plants. But the variation tendency of bud sprouting is as same as previous reports(Zhou *et al*, 2000; Zhang *et al*, 1996). Explants on high concentration of BA commonly produced glomerate and malformed shoots(Zhou *et al*, 2000; Zhang *et al*, 1996; Fracaro & Echeverriga-ray, 2001). All the nodal plates induced in the present experiment are cylindrical which lost their natural flattened form. Newly developed nodal plates from the acclimatized plantlets gradually displayed the flattened tendency (Plate I:5). This result can be attributed to the high level of BA, which is considered as an important factor on cell division during plant tissue culture. Because the effect of exophytohormone, cells of nodal divided in all directions so as to form a cylindrical shape. The influence of BA *in vitro* decreased as the plantlets transferred to ambient conditions. So the newly sprouted nodal plates returned to normal. Similar variation was not reported in other papers (Chen *et al*, 1999; Cai *et al*, 2000; Escobar *et al*, 1986; Mohamed *et al*, 1995; Chen *et al*, 2001).

In the current study, the physical condition of the tissue through explanting significantly affected the micropropagation of *O. dillenii*. Split explants induced more buds can be attributed to the large contact surfaces to the medium, which can absorb more nutrients and hormones. It was also reported in other plants(Zhou *et al*, 1999a, b).

An interesting phenomenon was also observed in the present study when sprout tuber and single nodal plate were cultured in the M_2 medium with the same conditions. The buds induced from sprout tuber grew vigorously and rapidly, but those induced from single nodal plate grew shortly and slowly (Plate I:6). This finding can be attributed to the build-up effect(Yan, 1991). Each explant diffused metabolites into the surrounding medium during cultural periods. The levels of such substance would affect the growth and proliferation of cultures, which are reached in high density of explants populations, but not in low density populations where the metabolites diffuse out into a relatively large volume of culture medium. This phenomenon was found in single cell cultivation firstly, something like the paper raft nurse culture.

General speaking, medium with auxin is more

advantageous to the rooting of shoots. But the present study reveals the inhibited effect of NAA on rooting.

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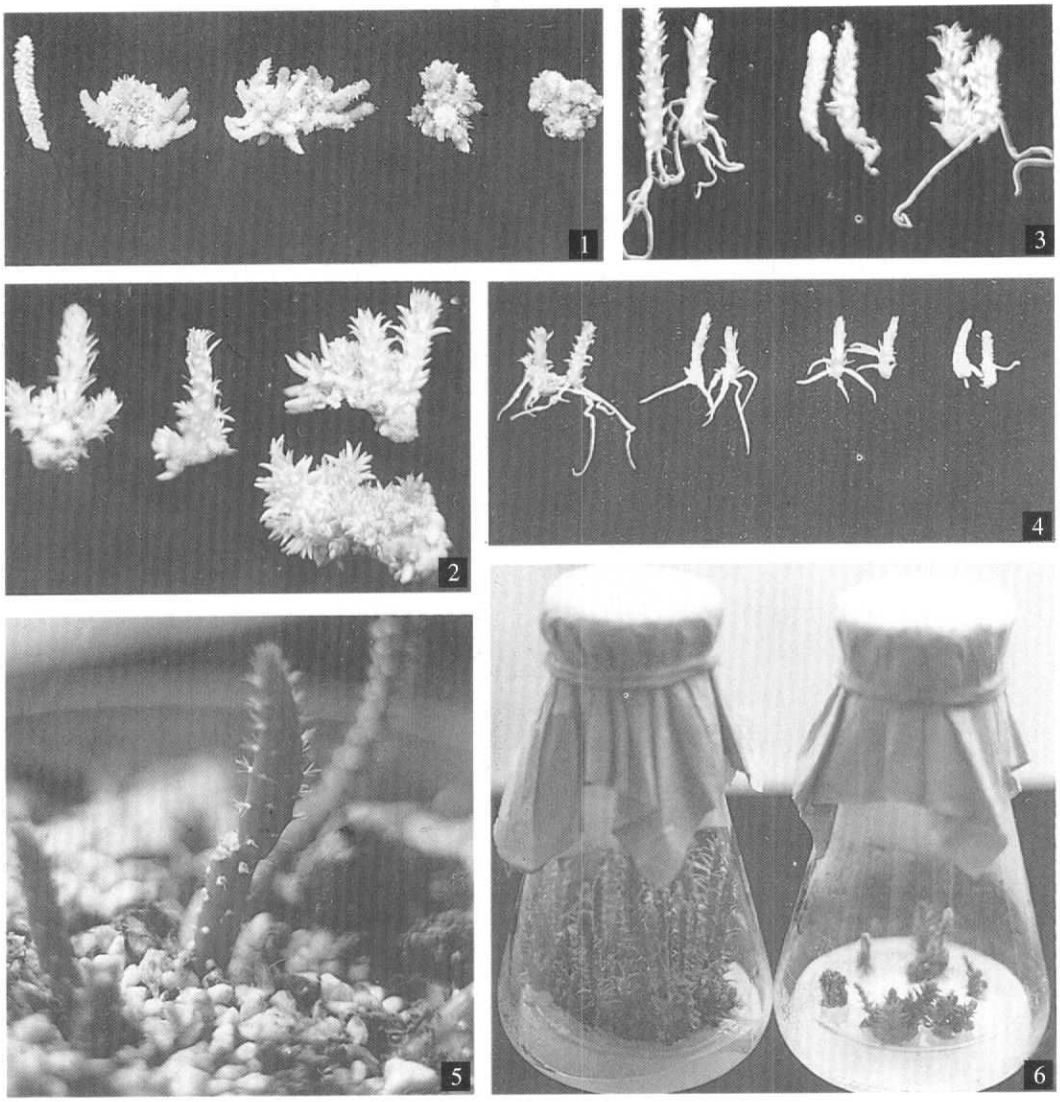

1. Effects of combination of hormones on propagation of *O. dillenii* (from left to right: M_0, M_1, M_2, M_3, M_4); 2. Effects of inoculation manners on propagation of *O. dillenii* (left: whole; right: split); 3. Effects of concentration of Ca^{2+} and Mg^{2+} on growth of *O. dillenii* (from left to right: CM_0, CM_1, CM_2); 4. Effects of concentration of NAA on rooting of *O. dillenii* (from left to right: R_0, R_1, R_2, R_3); 5. Newly developed nodal plate from the acclimatized plantlet; 6. Effects of sprout tuber and single nodal plate on propagation of *O. dillenii* (left: sprout tuber; right: single nodal plate).