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甜瓜品种 GT-1 植株再生体系的建立

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摘 要:为了建立高效稳定的甜瓜作物的植株再生体系,研究了甜瓜品种 GT-1 在 16 种培养基上的植株再生情况。结果显示:将从 5 d 龄甜瓜无菌苗上切取的子叶外植体接种在 MS+6-BA1 mg/L 的不定芽诱导培养基上,黑暗条件培养 3 d,再转至光下继续培养,诱导出高效不定芽丛。再经过在 MS+6-BA0.05 mg/L 伸长培养基上培养后,将无根不定苗转到不含激素的 MS 培养基上进行生根诱导,获得完整植株。通过这种培养程序,获得了高达 100%的不定芽诱导率,平均每个外植体上诱导出 14 株健壮植株。这项研究为甜瓜的遗传转化打下良好的基础。

关键词:甜瓜;植株再生;不定芽
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Establishment of plant regeneration system for *Cucumis melon* cv. GT-1

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Abstract: In order to establish a stable and efficient plant regeneration system for *Cucumis melon*, plant regeneration of *C. melon* cv. GT-1 was studied on 16 culture mediums. The cotyledon explants excised from 5-day-old germfree seedlings were cultured on the MS medium containing 1.0 mg/L 6-BA in darkness for 3 days, and then further cultured under light condition for induction of adventitious buds. Bud clusters thus generated were transferred onto the MS medium supplemented with 0.05 mg/L 6-BA. The rootless elongated shoots were then transferred to hormone-free MS medium, on which roots were induced and complete plants were obtained. Through the protocol, an adventitious bud induction rate of 100% was obtained and an average of 14 healthy complete plantlets were generated from each explant. The study laid a good foundation for gene transformation of *C. melon*.

Key Words: *Cucumis melon*; Plant regeneration; Adventitious bud

Cucumis melon is an important cash crop and has been cultivated in China for more than 3000 years. The fruit is prone to softening and rot during transportation and storage. Fruit quality loss during transportation and storage results in huge economic loss and greatly restricts the development of *Cucumis melon* industry.

With the development of biotechnology, the problem may be solved by transferring an antisense ACC oxidase I (ACO1) gene to *C. melon*. However, establishment of a plant regeneration system is a prerequisite for the application of gene transfer techniques. There are some investigations involving tissue culture of *C. melon*[1-10]. Two facts encourage us to conduct a similar study of plant regeneration here. One is that the ability of regeneration is highly genotype-depended and different cultivars may differ significantly in regeneration rate. The other is the low induction rate in the previous studies.

The primary aim of this study is to develop a high frequency regeneration system for *C. melon* cv. GT-1.

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1 Materials and Methods

1.1 Materials

Seeds of *C. melon* cv. GT-1 were provided by Gansu Academy of Agricultural Science.

1.2 Methods

The seeds were shelled and surface-sterilized in 70% ethanol for 15 s, followed by a treatment with 0.1% mercuric chloride for 7 min. After being rinsed with sterile distilled water for 4 times, they were dried on sterile filter paper, inoculated on the hormone-free MS medium and cultured under 25℃ and a 16/8 h (light/dark) photoperiod with a light intensity of 3000 lx during light period in a growth chamber. The MS medium was supplemented with 3% sucrose and solidified with 0.7% agar. The pH of the medium was adjusted to 7.0 with 1 mol/L NaOH before autoclaving for 25 min at 121℃.

Cotyledons were excised from 5-day-old germfree seedlings with a cut 1 mm away from the hypocotyl. The excised cotyledons were then cut transversely through the center into two pieces. The adaxial halves were inoculated on 16 bud induction mediums with different hormone combinations listed in Table 1.

Table 1 Adventitious bud induction mediums tested

Code	Culture medium	Code	Culture medium
11	MS+0.1 mg/L 6-BA	31	MS+ 1.0 mg/L 6-BA
12	MS+0.1 mg/L 6-BA+0.1 mg/L IAA	32	MS+1.0 mg/L 6-BA+0.1 mg/L IAA
13	MS+0.1 mg/L 6-BA +0.5 mg/L IAA	33	MS+1.0 mg/L 6-BA+0.5 mg/L IAA
14	MS+0.1 mg/L 6-BA +1.0 mg/L IAA	34	MS+1.0 mg/L 6-BA+1.0 mg/L IAA
21	MS+0.5 mg/L 6-BA	41	MS+2.0 mg/L 6-BA
22	MS+0.5 mg/L 6-BA +0.1 mg/L IAA	42	MS+2.0 mg/L 6-BA+0.1 mg/L IAA
23	MS+0.5 mg/L 6-BA +0.5 mg/L IAA	43	MS+2.0 mg/L 6-BA+0.5 mg/L IAA
24	MS+0. 5 mg/L 6-BA +1.0 mg/L IAA	44	MS+2.0 mg/L 6-BA+1.0 mg/L IAA

The explants on the bud induction mediums were incubated in darkness at 26℃ for 3 days and further cultured at the same temperature under a 16/8 h (light/dark) photoperiod with a light intensity of 3000 lx during light period. The germfree seedlings with cotyledons cut off were also cultured on the MS medium under the same temperature and photoperiod conditions. Clusters of adventitious buds induced on the bud induction mediums were excised and transferred to the MS medium supplemented with 0.05 mg/L 6 -BA for shoot growth. When the shoots grew to 1-2 cm in height, they were transferred to hormone-free MS medium for root induction. Callus induction rate and adventitious bud induction rate, defined as the percentage of explants with callus and percentage of explants with adventitious buds among the all the non-contaminated explants, respectively, were determined after 30 days of bud induction culture Shoot regeneration index, the number of shoots per explant, was calculated as the number of shoots divided by explant number after 30 days of culture on hormone-free MS medium.

2 Results

2.1 Leave growth in the germfree seedlings without cotyledons

5-day-old seedlings with cotyledons excised were cultured on the MS mediums. After 3 days, leaves grew on the shoots of the seedlings (Plate-A). The result showed that the growing points of the germfree seedlings remained on the shoots. Therefore none of the bud clusters on the cotyledon explants in our experiment resulted from the apical buds of the seedlings. The bud clusters were all adventitious and induced on mediums.

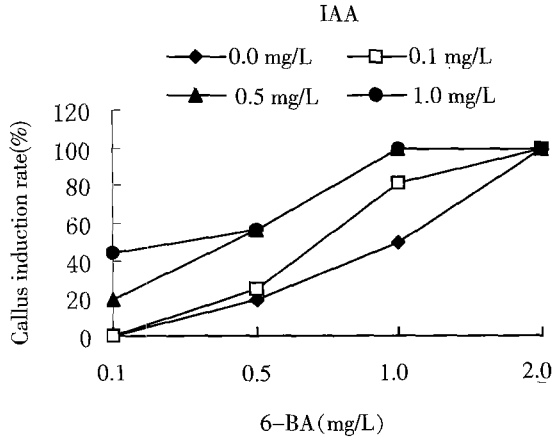

Fig. 1 Callus induction rates after 30 days of culture on the adventitious bud induction mediums with the presence of various plant growth regulators

2.2 The effects of treatments of 6-BA and IAA on callus induction rate

The experiment showed that 6-BA and IAA had significant effects on callus induction rate, which varied between 0 and 100% (Fig.1).

With the increase in the concentration of 6-BA, the callus induction rate tended to increase. The four hormone combinations with 6-BA at 2 mg/L resulted in a 100% callus induction rate The four mediums containing 0.1 mg/L 6-BA gave poor callus induction. Two of them had zero callus induction. Callus formation also showed an increasing tendency with the increase in IAA concentration. The highest concentration of IAA at 1.0 mg/L in this experiment resulted in the highest callus induction rate, while the lowest IAA at 0.1 mg/L produced the lowest callus induction. IAA could greatly improve the effect of 6-BA at low concentrations (0.1 mg/L, 0.5 mg/L and 1 mg/L), while its influence was limited when 6-BA was at a high concentration (2 mg/L). The experiment also showed that the higher ratio of IAA and 6-BA stimulated the growth of roots. Roots were induced on two tested mediums (Mediums 13 and 14, see Table 1) with a high IAA to 6-BA ratio, which was 5 and 10, respectively (Plate-B).

2.3 The effects of 6-BA and IAA on adventitious bud induction rate

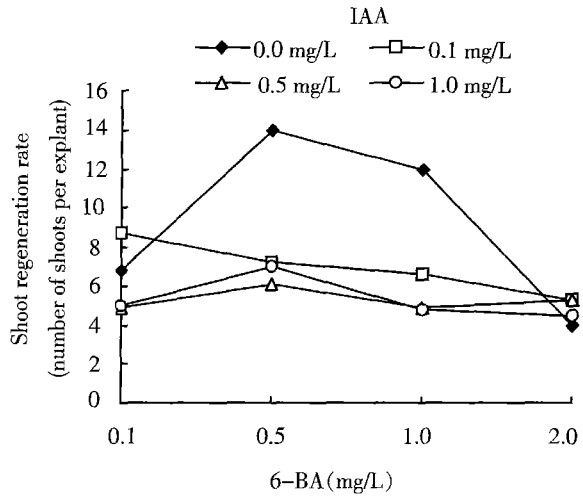

Fig. 2 Shoot regeneration indexes after 30 days of culture on hormone-free medium transferred from bud induction mediums with different concentrations of plant hormones

The results suggested that 6-BA was necessary to induce adventitious buds. The induction rate of adventitious bud varied between 50 and 100 % (Table 2). But the effect of plant hormones on the induction rate of adventitious bud did not showed a trendency as clear as that on the callus. Mediums 21 and 31 produced the highest induction rate of adventitious bud, which was 100%.

Table 2 Callus and bud induction from the explants cultured on different bud induction mediums

Medium Code	Number of explants	Number of explants with callus	Callus induction rates (%)	Number of explants with Adventitious buds	Adventitious buds induction rates (%)	Shoot regeneration index	Notes
11	64	0	0 H	48	75 E	6.76 F	Explants on mediums 11 and 12
12	64	0	0 H	56	88 C	8.76 C	responded slowly.1 explants on
13	64	12	19 G	44	69 F	4.82 K	Medium 13 and 3 on medium 14
14	64	28	44 E	40	63 G	5.00 J	rooted
21	64	12	19 G	64	100 A	14.00 A	Buds and callus coexisted and the
22	64	16	25 F	44	69 F	8.54 D	callus was loose and white
23	64	36	56 C	60	94 B	6.10 H	
24	64	36	56 C	60	94 B	7.04 E	
31	64	32	50 D	64	100 A	12.00 B	Buds and callus coexisted and
32	64	52	81 B	56	88 C	6.56 G	some buds were covered by a large
33	64	64	100 A	44	69 F	4.82 K	loose and brown callus and hardly
34	64	64	100 A	32	50 H	4.76 L	visible
41	64	64	100 A	32	50 H	4.00 N	Large number of brown callus
42	64	64	100 A	52	81 D	5.28 I	covered the buds. A few buds
43	64	64	100 A	48	75 E	5.26 I	on medium 43 and almost all
44	64	64	100 A	48	75 E	4.50 M	buds on medium 44 were vitrified

Note: The interaction of 6-BA and IAA on the callus induction, adventitious buds induction and the shoot regeneration index were statistically significant ($P<0.01$) (The significance analysis was based on Duncan's new multiple range method).

The adventitious bud clusters were transferred to MS medium supplemented with 0.05 mg/L 6-BA for shoot elongation. The elongated shoots were then transferred to the rooting medium and the complete plants were obtained (Plate-D~F). The number of the plants obtained from different bud induction mediums differed greatly. Medium 21 produce the highest shoot regeneration index, followed by Medium 31, while Medium 41

resulted in the lowest shoot regeneration index. Their shoot regeneration index was 14.00, 12.00 and 4.00, respectively (Fig.2).

In general, moderate concentrations of 6-BA (0.5 mg/L and 1.0 mg/L) and a lower concentration of IAA resulted in good adventitious bud induction and shoot regeneration. Too low or too high concentrations of 6-BA caused poor adventitious bud induction and shoot regeneration. In our experiment, the explants responded quickly to high concentrations of 6-BA, however too high concentration of 6-BA (≧2 mg/L) induced vigorous callus growth and restrained the induction of adventitious bud. The large amount of loose callus almost covered the whole explants and the adventitious buds could hardly be seen on these mediums. On the contrary, too low level of 6-BA (≦0.1 mg/L) produced slow response of the explants, which grew more slowly than those in the other mediums. For example, some explants on the Medium 11 had no response while others were produced many buds after 30 days of culture. Therefore, moderate concentrations of plant hormone were beneficial to the shoot regeneration.

2.4 The effects of explants on the induction rate of adventitious bud

As mentioned in 2.2 and 2.3, the explants responded differently on different medium due to the different concentrations of plant hormones. Explants were an important factor that significantly affected the induction rate of adventitious bud. In the same medium, even in the same culture vessel, different explants responded differently (Plate-C). Some of the explants developed many adventitious buds; some grew into callus; some produced roots;, and others had no response to the treatment. The explants showed in Plate-C were cultured on the MS medium supplemented with 0.1 mg/L 6-BA and 1 mg/L IAA. Similar phenomenon was also observed in the other mediums. This might be caused by several factors, including different endogenous hormone contents and different nutrient levels in the explants. Hence it is necessary to select plant materials for the experiment to get more reliable results.

3 Discussions

Dark culture is a necessary part of plant regeneration system for genetic transformation, because dark culture increases induction rate of adventitious buds[6-7] and dark condition is needed for the co-culture of the explants and engineering bacteria during the genetic transformation. In our experiment, dark culture for 3 days before transferring the culture to light conditions resulted in a high frequency of adventitious bud and shoot induction, which agreed with the previous studies[6-7].

DIRKS et al[8] investigated the effects of different plant hormone combinations on adventitious bud induction in 5 cultivars and found 1 mg/L 6-BA was the optimum for the genotypes tested[8]. GONSALVES et al[9] obtained an adventitious bud induction rate of 18%~89% in additional 5 genotypes. Moreover, he suggested that bud induction mediums with 1 mg/L 6-BA seemed to be applicable to all genotypes of *C. melon*[9]. In our experiment, the explants cultured on the medium supplemented with 0.5 mg/L or 1.0 mg/L 6-BA resulted in the highest induction rate of adventitious bud, which is generally similar to their results. Our experiment also showed that concentrations of 6-BA at 0.5 and 1.0 mg/L induced more adventitious buds, while too high concentrations of 6-BA (e.g. 2 mg/L) stimulated the production of callus. Our results were in agreement with those reported by Lu[10]. Ma suggested it was necessary to add a low concentration of auxins and a high concentration of cytokinins in order to increase adventitious bud induction [11]. In our experiment, Mediums 21 and 31 without IAA led to the highest adventitious bud induction rate. The more IAA added, the more callus induced and the less adventitious buds formed.

Studies have suggested that explant is an important factor of the plant tissue culture of *C. melon*[11-12]. Our experiment (see 2.4) further proved the suggestion that explants on the same medium may respond differently. The result revealed that it was important to select identical experiment material.

4 Conclusions

An important precondition for genetic transformation is to establish a perfect plant regeneration system. Our study results showed that moderate concentrations of 6-BA and lower concentration of IAA could be ben-

eficial to the shoot regeneration of *C. melon* cv. GT-1. MS medium supplemented with 0.5 mg/L 6-BA or 1.0 mg/L 6-BA were the optimal for adventitious buds induction. Induction rate on both mediums reached 100%. In addition, the best medium for shoot regeneration was the MS containing 1.0 mg/L 6-BA, on which 14 healthy complete plants were obtained from each explant. (The plates seen on inside back cover)

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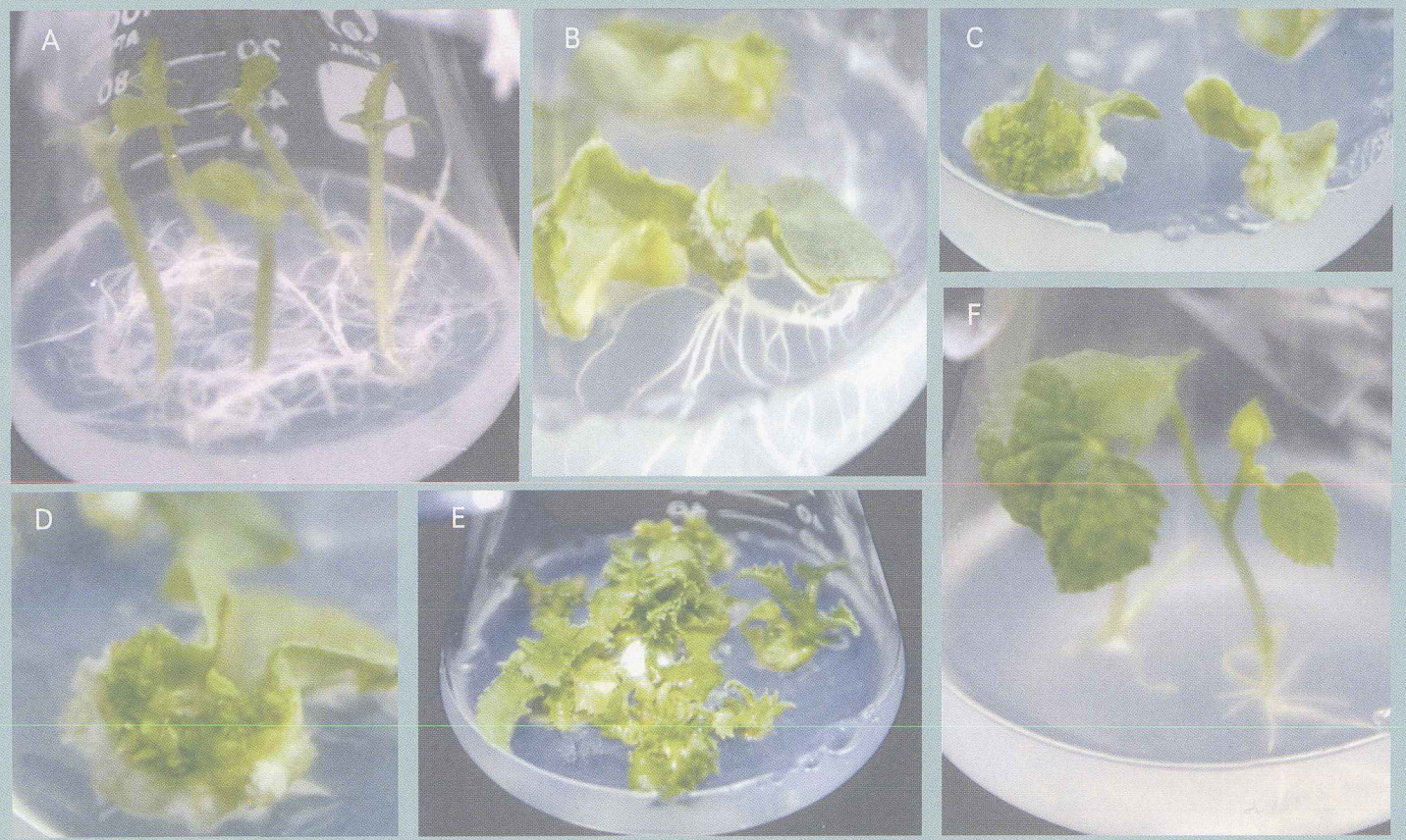

Explanation of plates

A. The germfree seedlings from which the explants were prepared (the leaves grew from the seedlings without cotyledon after 3 days when they were cultured on the previous mediums); B. The explants on which induced root (the high ratio of IAA and 6—BA could induce roots on the differentiation mediums); C. Different response of the expants on the exact same medium (In the same medium, even if in the exact same culture vessel, different explants responded differently. Some of the explants grew high frequency adventitious buds; others had no response to the treatment); D. The adventitious buds induced on the adventitious bud induction mediums medium (the growth of adventitious buds inducted on the differentiation medium 21 after 30 days when the explants were cultured on the mediums); E. The adventitious buds on the elongation medium (the growth of adventitious buds transferred to the elongation medium); F. The complete plant on the hormone—free MS medium (the adventitious buds on the elongation medium were transferred to the rooting medium when they were grew to 1～2 cm, and rooted, thus complete plants were obtained)