

Additives Promote Adventitious Buds Induction from Stem Segments of Bitter Melon (*Momordica charantia* L.)

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Abstract

The effects of thidiazuron (TDZ), silver nitrate (AgNO_3) and triacontanol on adventitious buds induction from stems of balsam pear (*Momordica charantia* L.) were investigated. It was found that TDZ was necessary for bud development and the higher concentration of it could induce adventitious buds efficiently, while 0.1 mg/L was the best concentration to induce adventitious buds considered effect and cost. Bud formation was significantly affected by AgNO_3 and triacontanol. Best results were obtained at concentration of 2.0 mg/L separately.

Keywords: Bitter melon (*Momordica charantia* L.), Adventitious buds, Additives

1. Introduction

Bitter melon (*Momordica charantia* L.) is an annual tendril herbage plant of balsam pear genus of Cucurbitaceae family, which is a kind of important and valuable vegetable crop and medicinal plant (Tang *et al.*, 2009). It is difficult and taking too much time to breed using normal method, while tissue culture is an efficient method for plant breeding and can shorten breeding period. However there were few reports on tissue culture in bitter melon and the results revealed that it was easy to induce callus and roots but very difficult to differentiate buds (Yang *et al.*, 2002; Song *et al.*, 2004). In the former study, TDZ was shown to provide sufficient stimulus for induction of shoot regeneration in a variety of plant species. (Murthy *et al.*, 1998; Zahoor *et al.*, 2009; Shagufta *et al.*, 2009). Additionally, AgNO_3 and triacontanol have been considered as promoters of adventitious bud induction in many species (Tantos *et al.*, 2001; Liu *et al.*, 2008).

In the present investigation, factors that promote adventitious buds formation from stems of balsam pear were studied. The aim of the paper is to provide the theoretical and technical basis for rapid propagation and breeding.

2. Materials and methods

2.1 Plant materials

Stems about 2mm in diameter of balsam pear cv. Bixiu were the experimental materials in the present investigations. The mother plants were grown in the experimental plots using standard agronomic practices.

2.2 Callus induction

Stems harvested from field were rinsed with a little detergent, and washed in running water for 1h. Then they were surface sterilized with 75% (v/v) alcohol for 1 minute, and immersed in 0.1% (w/v) mercuric chloride

solution with periodic agitation for 6 minutes in a laminar air-flow cabinet under aseptic conditions. After surface-sterilized, stems were finally washed 5 times with sterile distilled water. Then stems divided in 5-mm-long were inoculated on MS medium (Murashige & Skoog, 1962), supplemented with 3% (w/v) sucrose, 0.6% (w/v) agar, IBA 1.0mg/ L and BA 2.0 mg/L. The pH was adjusted to 5.8.

2.3 Adventitious bud induction

After 20 days the newly formed callus were separated from the explants and transferred to subculture medium. Subculture medium consisted of MS mineral salts and vitamins, IBA 0.2 mg/ L and different concentrations of TDZ (Table 1). In order to evaluate the effects of additives on adventitious bud induction, different concentrations of AgNO₃ and triacontanol were supplemented in media separately. Subsequent subcultures were conducted every 20 days. Without special explanation, all the MS media contained 3% (w/v) sucrose, 0.6% (w/v) agar, IBA 0.2 mg/ L and TDZ 0.01mg/L, and the pH was adjusted to 5.8 before autoclaving. After 40 days of culture, the data of adventitious buds were recorded.

Differentiation rate = number of callus induced adventitious buds / number of callus × 100%.

Average number of buds per callus = number of adventitious buds / number of callus induced adventitious buds

2.4 Culture conditions

Cultures were maintained in growth chambers at 28°C in the dark for 7 days, and then at 28°C under 16 h daily illumination with 1500 lx fluorescent light.

3. Result and discussion

3.1 Effect of TDZ on adventitious bud induction

The effect of TDZ providing sufficient stimulus for induction of shoot regeneration has been reported for many plant species (Li *et al.*, 2000). Statistical analysis showed that the differentiation rate of balsam pear was influenced significantly by TDZ (Table 1). Poor result was obtained at TDZ free medium with no adventitious bud formation. With the increasing of TDZ concentration from 0.01 mg/L up to 0.5 mg/L, callus exhibited the higher differentiation rate. The results showed that TDZ was necessary for bud development and the higher concentration of it could induce adventitious buds efficiently. It was notable that TDZ concentration up to 0.5 mg/L, the bud differentiation rate was the highest as the same as result obtained at 0.1 mg/L TDZ, however the average number of buds per callus was relative low, and the buds prolonged more than proliferated (Fig. 1). When concentration of TDZ was 0.1mg/L, the adventitious bud induction rate reached the highest and callus gave the most dense clumps of buds (Fig. 2). Moreover, TDZ was relatively costly, so 0.1 mg/L was the suitable concentration concluded in the present study.

<Table 1>

3.2 Effect of AgNO₃ on adventitious bud induction

Analysis of variance of the adventitious bud induction rate indicated that bud formation was significantly affected by AgNO₃ and its concentration. According to the data in table 2, the highest inductivity of buds and average number of buds per callus were obtained when media added AgNO₃ 2.0 mg/L or 4.0 mg/L, which was similar with the result of canola (Hazrat *et al.*, 2007). In previous studies, it was widely supposed that too high concentration of AgNO₃ had negative effect on bud formation and resulted in decreased regenerative ability even death (Zhou & Zhuang, 2007; Liu *et al.*, 2007). According to this experiment, with the concentration of AgNO₃ up to 8.0 mg/L, the callus was prone to necrosis (Fig. 3). It can be concluded that high concentration of AgNO₃ was toxic for cultures, while relatively low concentration was optimal.

<Table 2>

3.3 Effect of triacontanol on adventitious bud induction

Effect of triacontanol on adventitious bud induction was statistically significant (Table 3). Apparently, in medium with low concentration of triacontanol few buds similar with triacontanol free medium were obtained. And it was advantageous to differentiate buds when medium added with triacontanol 2.0 mg/L or 4.0 mg/L, which was in agreement with the result of watermelon (Wang & Wei, 2007). The existence of triacontanol even at low concentration was efficacious for average number of buds per callus. When callus cultured in medium supplemented with triacontanol 4.0 mg/L, it occurred not only adventitious buds but also few leaves (Fig. 4). It was speculated that formation of buds and leaves desired different contents of triacontanol respectively.

<Table 3>

<Figure 1-4>

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Table 1. Effect of TDZ on adventitious bud induction

TDZ concentration (mg/L)	Average number of buds per callus	Differentiation rate (%)
0	0d	0d
0.01	1.23c	18.06c
0.05	1.95b	24.68b
0.1	2.96a	36.49a
0.5	1.32c	37.33a

Means having the same letter in the columns were not significantly different according to Duncan's multiple range test at P=0.05 (The same was the following tables).

Table 2. Effect of AgNO₃ on adventitious bud induction

AgNO ₃ concentration (mg/L)	Average number of buds per callus	Differentiation rate (%)
0	1.10b	13.70b
2.0	1.96a	36.36a
4.0	2.16a	33.33a
8.0	1.21b	18.67b

Table 3. Effect of triacontanol on adventitious bud induction

Triacontanol concentration (mg/L)	Average number of buds per callus	Differentiation rate (%)
0	1.20b	12.82b
1.0	2.09a	14.86b
2.0	2.13a	30.67a
4.0	1.95a	33.33a



Figure 1. Buds prolonged more than proliferated



Figure 2. Callus gave the most dense clumps of buds



Figure 3. Callus prone to necrosis



Figure 4. Callus formed adventitious buds and leaves