

## Additives Promote Adventitious Buds Induction from Anther Culture of Bitter Melon (*Momordica charantia* L.)

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**Abstract:** The effects of growth regulators, Thidiazuron (TDZ), silver nitrate ( $\text{AgNO}_3$ ), triacontanol and glutamate on adventitious buds induction from anther culture of bitter melon (*Momordica charantia* L.) were investigated. It was found that triacontanol was advantageous for buds development and adventitious buds differentiated from anther culture of bitter melon first time. On medium supplemented with glutamate  $0.1 \text{ mg L}^{-1}$ , protuberant structures differentiated on the surface of callus.  $\text{AgNO}_3$  and TDZ has no significant effect on promoting adventitious buds formation from anther culture of bitter melon.

**Key words:** Bitter melon (*Momordica charantia* L.), adventitious buds, additives, anther culture, TDZ, China

### INTRODUCTION

Bitter melon (*Momordica charantia* L.) is one of the most nutritional and medicinal plants belonging to family Cucurbitaceae. It contains high concentrations of antimicrobial properties and used as a traditional medicine for diabetes in India, China and Central America (Grover *et al.*, 2002; Yeh *et al.*, 2003; Sabahat and Perween, 2005). And reports found that this vegetable possesses effective components in preventing HIV (Lee-Huang *et al.*, 1990, 1995). Anther culture is a useful tool for the rapid generation of haploid plants for use in plant breeding programmes (Andrea *et al.*, 2001). Compared with conventional inbreeding, the *in vitro* androgenesis technique enables a faster generation of virtually fully homozygous lines (Aulinger *et al.*, 2003). However, there were few reports on anther culture in bitter melon and the results revealed that it was easy to induce callus but very difficult to differentiate buds (Tang *et al.*, 2009). The success of anther culture depends on numerous factors such as genotype, cold pretreatment, growth regulators and additives in medium. It is widely supposed that growth regulators and additives had a great effect on anther culture.

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; Li *et al.*, 2000; Zahoor and Faheem, 2009; Shagufta *et al.*, 2009). Additionally,  $\text{AgNO}_3$ , triacontanol and glutamate have been considered as promoters of adventitious bud induction in many species (Tantos *et al.*, 2001; Thiruvengadam *et al.*, 2006; Liu *et al.*, 2008). In the present investigation, factors that promote adventitious buds formation from anther culture of bitter melon were studied.

### MATERIALS AND METHODS

**Plant materials:** Anthers of balsam pear cv. Bixiu, Changbai, Dabai and Pangniu were the experimental materials in the present investigations. The mother plants were grown in the experimental plots using standard agronomic practices.

**Callus induction:** All operations were carried out in a laminar air-flow cabinet under aseptic conditions. The flower buds were pretreated for 24 h under  $4^\circ\text{C}$  condition then were dipped in 75% (v/v) alcohol for 30 sec, immersed in 0.1% (w/v) mercuric chloride solution with periodic agitation for 5 min and washed with sterile distilled water for 5 times. After filament removed, the intact anthers were inoculated on inducing medium. The medium consisted of MS mineral salts and vitamins (Murashige and Skoog, 1962), supplemented with 5% (w/v) sucrose, 0.65% (w/v) agar, 2,4-Dichlorophenoxyacetic acid (2,4-D)  $0.5 \text{ mg L}^{-1}$  and Benzyladenine (BA)  $2.0 \text{ mg L}^{-1}$ . The media were adjusted to pH 6 prior to addition of agar and sterilized at  $122^\circ\text{C}$  and 104 kPa pressure for 20 min. The anthers were cultured in a culture chamber at  $25^\circ\text{C}$  in the dark for 7 days and then at  $25^\circ\text{C}$  under 16 h daily illumination with 1500lx fluorescent light.

**Adventitious bud induction:** After 20 days, the newly formed callus were excised from the explants and transferred to subculture medium. Subculture medium consisted of MS mineral salts and vitamins, supplemented with different growth regulators: TDZ (0.05, 0.1, 0.5,  $1.0 \text{ mg L}^{-1}$ ) and 2,4-D (0.1, 0.5,  $1.0 \text{ mg L}^{-1}$ ); ZT (0.05, 0.1, 0.5,  $1.0 \text{ mg L}^{-1}$ ) and IBA (0.1, 0.5,  $1.0 \text{ mg L}^{-1}$ ); BA (0.5, 1.0, 2.0,  $4.0 \text{ mg L}^{-1}$ ) and NAA (0.1, 0.5,  $1.0 \text{ mg L}^{-1}$ ); KT (0.5, 1.0, 2.0,  $4.0 \text{ mg L}^{-1}$ ) and IAA (0.1, 0.5,  $1.0 \text{ mg L}^{-1}$ ). In order

to evaluate the effects of additives on adventitious bud induction, different concentrations of  $\text{AgNO}_3$  (1.0, 2.0, 4.0  $\text{mg L}^{-1}$ ), triacontanol (1.0, 2.0, 4.0  $\text{mg L}^{-1}$ ) and glutamine (0.1, 0.2, 0.4  $\text{mg L}^{-1}$ ) were supplemented in media separately. Subsequent subcultures were conducted every 20 days. Without special explanation, all the MS media contained 3% (w/v) sucrose, 0.65% (w/v) agar, TDZ 0.5  $\text{mg L}^{-1}$  and 2,4-D 0.1  $\text{mg L}^{-1}$  and the pH was adjusted to 6 before autoclaving.

Cultures were maintained in growth chambers at 25°C under 16 h daily illumination with 1500 lx fluorescent light.

## RESULTS AND DISCUSSION

**Different types of anther calluses:** Callus induced from anther could be morphologically divided into two types: One type was slight yellow, loosened callus (Fig. 1a). The other type was viridescence, granular, smooth callus (Fig. 1b).

**Effect of different growth regulators on adventitious bud induction:** Anther callus were transferred to medium supplemented with different concentrations and combinations of growth regulator, however there was no adventitious bud or embryoid formed. Part of slight yellow, loosened callus turned green and kept loosened (Fig. 2a) while the others turned dense and kept slight

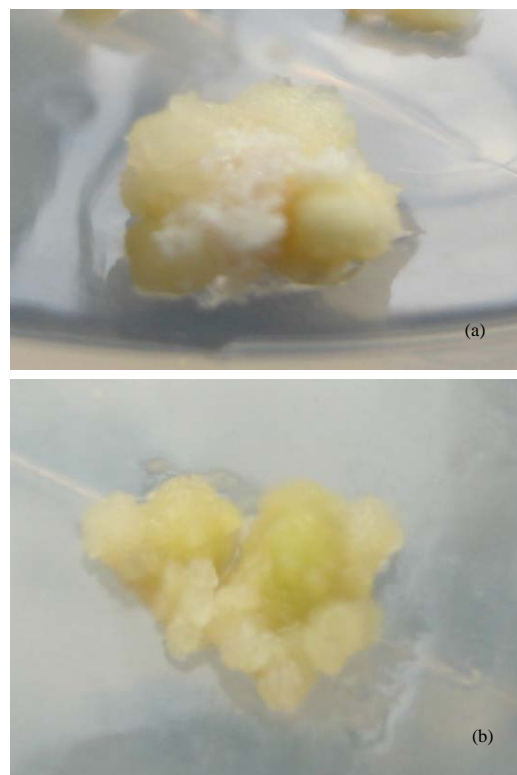


Fig. 1: Different types of anther calluses: a) Slight yellow, loosened callus; b) Viridescence, granular, smooth callus

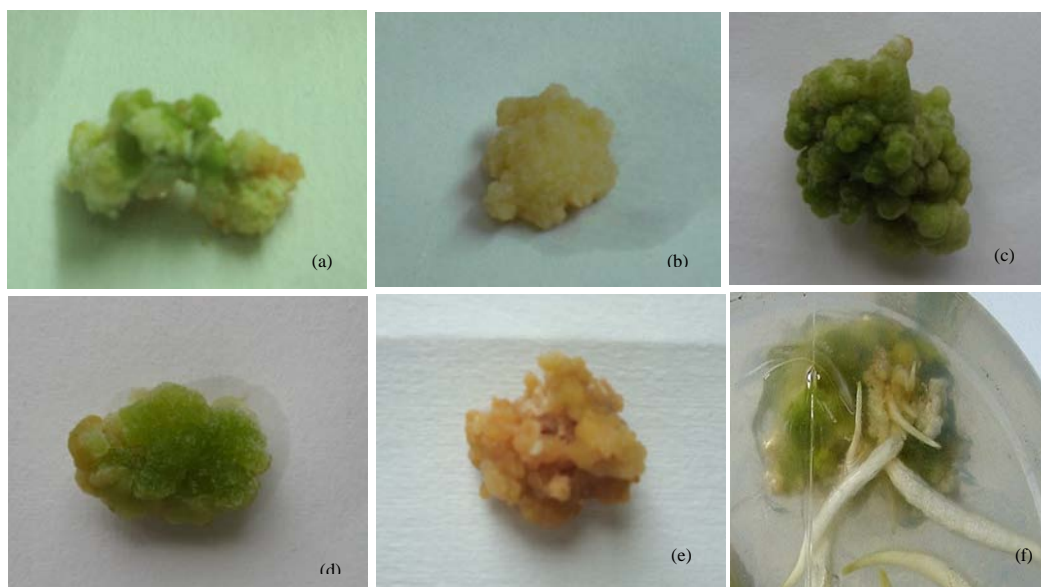


Fig. 2: Response of anther callus to differentiation media containing various hormones: a) Slight yellow, loosened callus turning green and keeping loosened; b) Slight yellow, loosened callus turning dense; c) Viridescence, granular, smooth callus turning dark green and dense; d) Viridescence, granular, smooth callus turning green and smooth; e) Callus browning; f) Callus differentiated roots

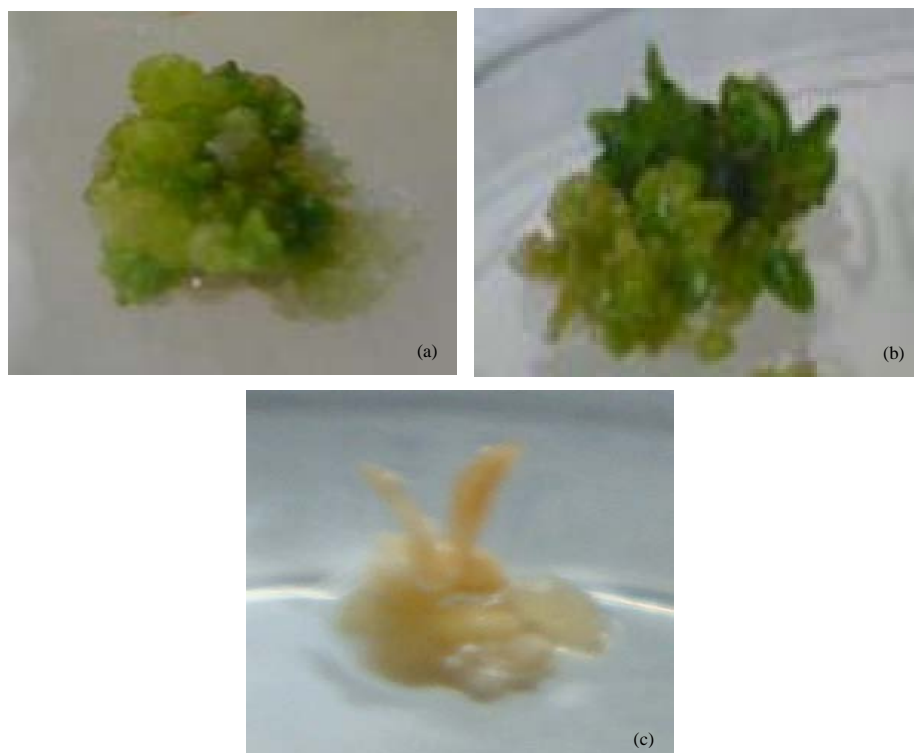


Fig. 3: Response of anther callus to differentiation media containing various additives: a) Point organization (growing point) on callus; b) Adventitious buds regenerated from callus and c) Protuberant structures differentiated on the surface of callus

yellow (Fig. 2b). On medium containing high concentration of KT, part of viridescence, granular, smooth callus swelled quickly, turned dark green and dense (Fig. 2c) while the others turned green and smooth (Fig. 2d). Callus transferred to subculture medium >3 times presented browning (Fig. 2e). On medium containing BA  $0.5 \text{ mg L}^{-1}$  and NAA  $1.0 \text{ mg L}^{-1}$ , some viridescence callus of Changbai differentiated roots (Fig. 2f).

#### Effect of different additives on adventitious bud induction:

There was no adventitious bud or embryoid formed from callus on medium supplemented with different concentrations of  $\text{AgNO}_3$ .

On medium containing triacontanol  $2.0 \text{ mg L}^{-1}$ , some small green points emerged on the surface of the viridescence callus of Bixiu (Fig. 3a), point organization grew and turned green, adventitious buds differentiated (Fig. 3b) but these turned brown and died after subcultured many times. On medium supplemented with glutamine  $0.1 \text{ mg L}^{-1}$ , protuberant structures differentiated on the surface of slight yellow callus (Fig. 3c) but there was no adventitious bud or embryoid formed, they turned brown and died in the end.

## CONCLUSION

In the present investigation, factors that promote adventitious buds formation from anther culture of bitter melon were studied.

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