

Full Length Research Paper

The influence of endogenous hormones on the formation of buds from stems of bitter melon (*Momordica charantia* L.)

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Stems of bitter melon (*Momordica charantia* L.) cv. Dabai were used to establish *in vitro* cultures. The endogenous hormone concentrations (indoleacetic acid [IAA], abscisic acid [ABA], gibberellins 3 [GA₃], zeatin [ZT]) of the calluses were determined by means of high pressure liquid chromatography (HPLC). The endogenous ZT was higher in the stem calluses that had formed buds, and there was a higher IAA/ZT ratio and GA₃/ZT ratio in the calluses having no capacity for buds formation. The results showed that addition of plant growth regulator influences endogenous hormone status and it will be helpful for *in vitro* propagation of bitter melon.

Key words: Endogenous phytohormones, high pressure liquid chromatography, *in vitro* culture, bitter melon (*Momordica charantia* L.).

INTRODUCTION

Bitter melon (*Momordica charantia* L.) is an annual tendril herbage plant belonging to family Cucurbitaceae. It is an important and valuable vegetable because it contains high concentrations of ascorbic acid and iron (Behera et al., 2008). Bitter melon also has antimicrobial properties and is 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). Some researches has concentrated on stem culture for fast propagation in bitter melon, and it was found that it was easy to induce callus and very difficult to differentiate buds (Tang et al., 1997; Tang et al., 1999; Sultana and Bari Miah, 2003).

The endogenous hormone levels have been regarded as critical for bud formation and even for plant regeneration in *in vitro* culture for many plant species

(Ansarali et al., 2009; Imtiaz et al., 2009). To the best of our knowledge, there has been no report on endogenous hormones of stems calluses during *in vitro* culture in bitter melon. A better understanding of the relationship between endogenous hormone concentrations in the calluses and the bud formation competence will be helpful for *in vitro* propagation of bitter melon.

This study measured and analyzed the correlation between the endogenous hormone status of stem calluses and their ability to form buds.

MATERIALS AND METHODS

Tender stems of about 2 mm in diameter were collected from bitter melon. The donor plants were grown in the experimental plots. Stems were surface-sterilized with 75% (v/v) alcohol for 2 min, then immersed in 0.1% (w/v) mercuric chloride solution with periodic agitation for 8 min, and finally washed five times with sterile distilled water. Stems that were 5-mm-long were inoculated on MS medium (Murashige and Skoog, 1962), containing 2,4-D 1.0 mg/L and BA 2.0 mg/L. After 30 days, the newly formed callus were separated from the explants and transferred to subculture medium. Subculture medium consisted of MS mineral salts and vitamins, BA (0.5, 1.0, 2.0 and 4.0 mg/L) in combination with 2,4-D (0.5 and 1.0 mg/L). Subsequent subcultures were done every 30 days. All culture media were supplemented with 3% (w/v) sucrose, 0.7% (w/v) agar, and the pH was adjusted to 5.8 before autoclaving. Cultures were

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Abbreviations: ABA, Abscisic acid; B, calluses that had formed buds; BA, benzyladenine; 2,4-D, 2,4-dichlorophenoxyacetic acid; FW, fresh weight; GA₃, gibberellins 3; HPLC, high pressure liquid chromatography; IAA, indole acetic acid; MS, Murashige and Skoog (1962); NB, calluses that had no bud formation capacity; ZT, zeatin.

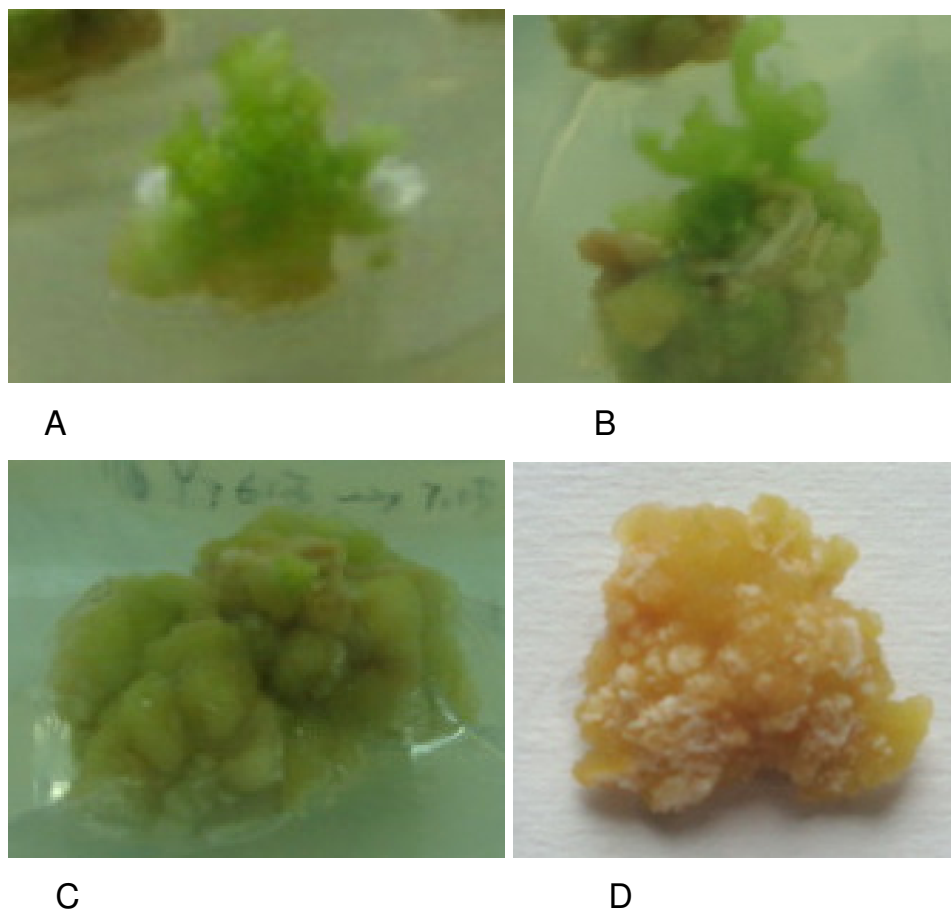


Figure 1. Response of stem calluses of bitter melon. (A) Callus with green protuberances; (B) Buds regenerated from protuberances; (C) Callus without sign of organization; (D) Soft, yellow and translucent callus.

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.

Hormone concentration of calluses

After 60 days of culture under the aforementioned maintenance conditions, samplings were harvested for evaluation of the differences of the endogenous hormone concentrations in the calluses by HPLC (Varian Pro Star 240, made in the USA).

The determination of indole acetic acid (IAA), abscisic acid (ABA), gibberellins 3 (GA₃) and zeatin (ZT) was done on the same sample. Samples of the stem calluses were surface dried and cleaned with a paper towel, immediately weighed and frozen in liquid nitrogen and stored at -70°C. Samples (approximately 1 g fresh weight [FW]) were ground in liquid nitrogen, homogenised and then extracted overnight with 30 ml 80% cold aqueous methanol (< 0°C) in darkness at 4°C. The extract was centrifuged at 5000 r/min and 4°C for 15 min and the supernatant was collected. Then fresh cold methanol was poured into the remnant, extracted three times using the aforementioned methods. The total methanolic extract was dried in rotary evaporator and dissolved in 10 ml methanol. IAA, ABA, GA₃ and ZT were measured by the injection of the extract into a reverse-phase HPLC, with a methanol gradient in 0.6% acetic acid (Chen and Yang, 2005).

Statistical analysis

A randomized complete block design was used for the experiments. For callus induction, 6 explants per conical flask were inoculated in 100 ml flasks containing 30 ml of nutrient medium each, with 30 replicates per treatment. For differentiation, each treatment was applied to 30 calluses (5 calluses per conical flask and 6 replicates per treatment). Endogenous hormonal concentrations were determined in at least three biological replications. Significance between means was tested by Duncan's multiple range test (Duncan, 1955).

RESULTS

Comparative analysis of callus response

After 7 days of culture, the stem explants expanded and showed evidence of swelling at the cut edge. Callus enlarged in size along the time of culture. After culture for 30 days, 78.3% stems had induced callus. After transferred to the subculture medium, the calluses in MS medium containing BA 2.0 mg/L and 2, 4-D 0.5 mg/L proliferated and showed few green protuberances (Figure 1A). At the second subculture, buds emerged from the

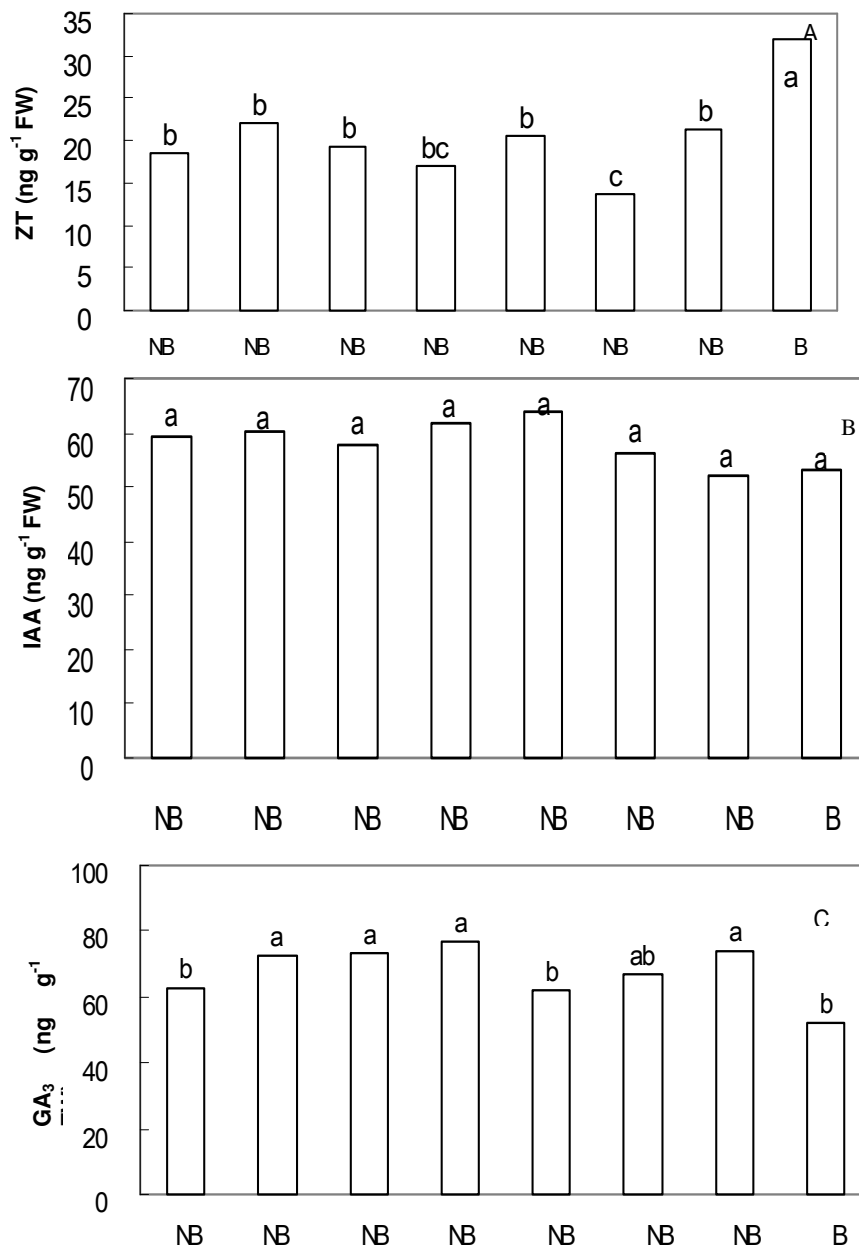


Figure 2. Endogenous concentrations of ZT (A), IAA (B), GA₃ (C), ABA (D), IAA/ZT ratio (E) and GA₃/ZT ratio (F) in the stem callus that had formed buds and calluses that had no bud formation capacity of bitter melon. Significant differences (<math><0.05</math>) between the concentrations and ratios of stem calluses were marked with distinct letters.

surface of these protuberances (Figure 1B). On the contrary, the callus subcultured in other types of media had no bud formation and most of them turned into soft, yellow and translucent (Figure 1C and D), and the bud formation rate was only 7.9%.

Hormone concentration of callus

In the subsequent hormone analysis, stem calluses

differentiated no buds in seven types of media and callus that differentiated buds in only one media were collected. The endogenous hormone concentrations in the calluses of the eight categories are presented in Figure 2. Higher concentrations of ZT were found in the B calluses as compared to the concentrations measured in the others. No statistically significant differences were found in the IAA concentrations between the different callus categories. Stem B calluses contained significant lower concentrations of ABA than others, and were slightly lower

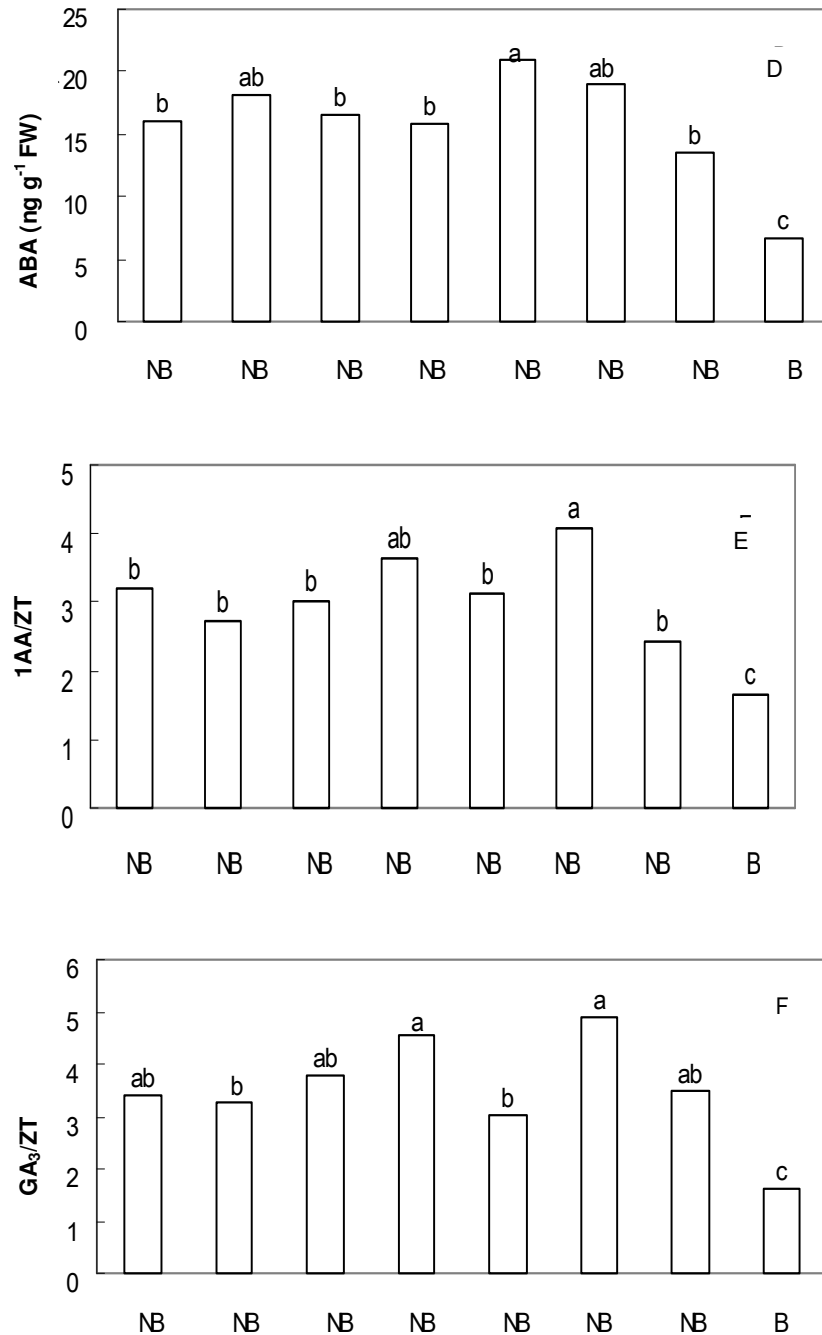


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with regards to GA₃. For the IAA/ZT ratio, it was drastically lower in the B calluses than in the NB calluses, and the same phenomenon was observed with respect to GA₃/ZT ratio.

DISCUSSION

In this study, higher concentrations of ZT were found in the B calluses of stem, while higher IAA/ZT and GA₃/ZT

ratios were found in the NB callus. Significantly higher ZT concentrations were found in the calluses that formed buds, and inclusion of ZT in this study was consistent with earlier reports (Yoshimatsu and Shimomura, 1994; Sarul et al., 1995). In former studies, it was found that high concentrations of ABA were typical to adventitious bud-formation (Song and Gao, 2006; Guo and Gai, 1997; Liu et al., 2007). Our results differ from theirs, since we found that concentrations of ABA were lower in the stem B calluses than in the stem NB calluses. This may be because

different genotypes were evaluated in the studies. In this study, lower levels of IAA/ ZT ratio in the stem calluses seemed to be associated with the presence of bud formation, which is in agreement with the results of studies on other plants (Zaffari et al., 2000; Wang et al., 2005). With respect to the GA₃ in stem calluses, the high concentration may suppress adventitious bud formation (Ye and Wang, 1997; Luo et al., 1998). A similar result was also obtained in this study and lower GA₃/ZT ratio in stem calluses was considered as an important factor for buds differentiation.

To the best of our knowledge, this is the first work where endogenous hormone concentrations of stem calluses in bitter melon is analysed.

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