



## Improvement of cephalomanine production in *Taxus chinensis* cells by a combination of sucrose and methyl jasmonate

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### Abstract

Cell suspension cultures of *Taxus chinensis*, supplemented with 25 g sucrose l<sup>-1</sup>, produced 11 mg cephalomanine l<sup>-1</sup>, 21 g biomass l<sup>-1</sup> and 19 nkat geranylgeranyl diphosphate (GGPP) synthase activity g protein<sup>-1</sup>. Supplementation of the cultures with 100 μM methyl jasmonate (MJA) produced 17 mg cephalomanine l<sup>-1</sup>, 6 g biomass l<sup>-1</sup> and 78 nkat GGPP synthase activity g protein<sup>-1</sup>. Addition of sucrose and MJA together produced 24 mg cephalomanine l<sup>-1</sup>, 18 g biomass l<sup>-1</sup> and 55 nkat GGPP synthase activity g protein<sup>-1</sup>.

### Introduction

Cephalomanine, although having less anticancer activity than taxol, can be used as a starting material for the semi-synthesis and modification of taxane drugs (Pandey *et al.* 1998). The most promising route for the large-scale production of taxanes seems to be to use plant cell culture to produce the parent molecule that is then chemically converted into the desired taxane drugs. Elicitation of plant cells by methyl jasmonate (MJA) can increase the production of taxanes (Ketchum *et al.* 1999, Laskaris *et al.* 1999b, Yukimune *et al.* 2000). A high sucrose concentration also improves production of taxanes (Kim *et al.* 1995, Wang *et al.* 1999). So far there has been no reports on the effect of a combined treatment of MJA elicitation and sucrose supplementation on cephalomanine production in *Taxus* cell suspension cultures. In this report, enhanced cephalomanine production was obtained by using this combination. Further, the roles of MJA elicitation and sucrose supplementation in enhancing cephalomanine production were elucidated by analyzing the changes of cell growth and geranylgeranyl diphosphate (GGPP) synthase activity.

### Materials and methods

#### *Plant materials and culture conditions*

*Taxus chinensis* cell lines, isolated from *Taxus chinensis* zygote embryos, were grown in modified MS medium as previously described (Zhang *et al.* 2000). Ten g fresh wt cells were inoculated into a 250 ml Erlenmeyer flask containing 100 ml liquid modified MS media, 25 g sucrose l<sup>-1</sup>, or 100 μM MJA, or a combination of 25 g sucrose l<sup>-1</sup> and 100 μM MJA was added into the 9 day old cultures. The flasks were shaken at 120 ± 5 rpm at 25 ± 1 °C.

#### *Cell growth and cephalomanine determination*

Cell growth was determined by dry wt after the cells had been lyophilized. Cephalomanine extraction and analysis were the same as taxol extraction and analysis (Zhang *et al.* 2000). Cephalomanine production in the sample was the combination of cephalomanine in cells and medium. Cephalomanine standard was from National Cancer Institution (USA).

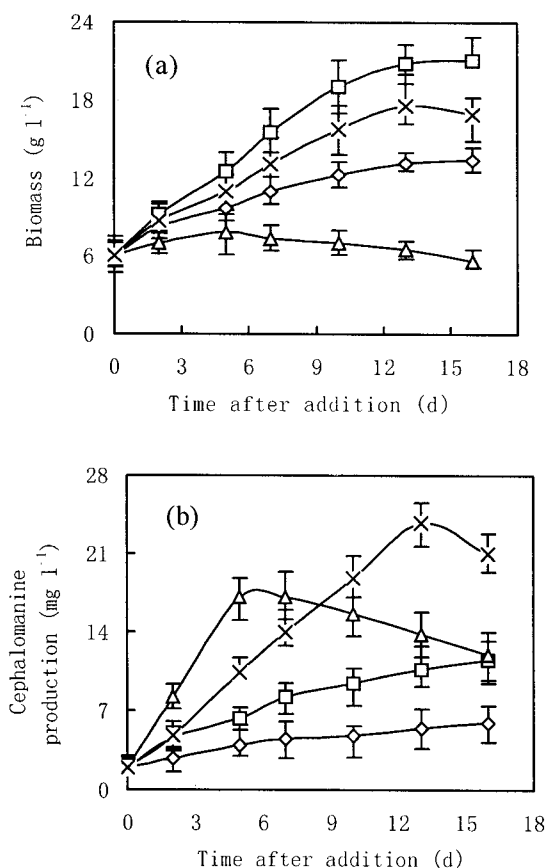


Fig. 1. Time course of cell growth (a) and cephalomanine production (b) in cell suspension cultures of *Taxus chinensis* treated with 25 g l<sup>-1</sup> sucrose (□), or 100 μM methyl jasmonate (△), or 25 g sucrose l<sup>-1</sup> and 100 μM methyl jasmonate (X), or neither sucrose nor methyl jasmonate (◇). Sucrose and methyl jasmonate were added on the 9th day. Values are means of triplicate results and error bars represent standard errors.

#### Assay for GGPP synthase activity

GGPP synthase activity assay and protein extraction were according to Laskaris *et al.* (1999a). Protein concentration was determined following the method of Bradford.

## Results

#### Effects of sucrose and MJA treatment on cell growth and cephalomanine production

The effects of sucrose and MJA on cell growth and cephalomanine production in cell suspension cultures of *Taxus chinensis* are shown in Figure 1. The maximum response was with the addition of sucrose and

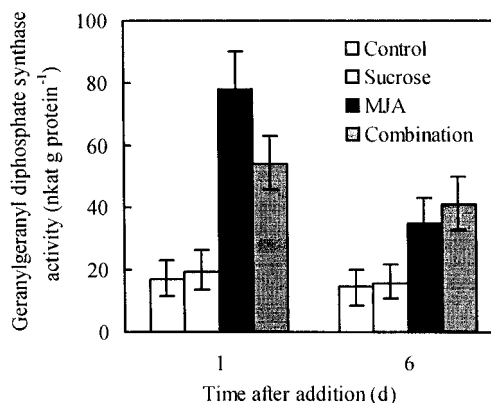


Fig. 2. Effects of 25 g sucrose l<sup>-1</sup> supplementation and 100 μM methyl jasmonate on geranylgeranyl diphosphate synthase activity in cell suspension cultures of *Taxus chinensis*. Sucrose and methyl jasmonate were added on the 9th day. Notes: control, neither sucrose nor methyl jasmonate; sucrose, 25 g sucrose l<sup>-1</sup>; MJA, 100 μM methyl jasmonate; combination: addition of 25 g sucrose l<sup>-1</sup> and 100 μM methyl jasmonate together. Values are means of triplicate results and error bars represent standard errors.

MJA together which gained a biomass of 18 g l<sup>-1</sup> and cephalomanine at 24 mg l<sup>-1</sup>.

#### Effects of sucrose and MJA treatment on GGPP synthase activity

GGPP synthase activity in *Taxus baccata* cell suspension cultures is rapidly induced by methyl jasmonate (Laskaris *et al.* 1999b). Measurements of this activity in the present cell cultures (Figure 2) showed that GGPP synthase activity was highest when sucrose and MJA were added together; the response after 1 day of 55 nkat g protein<sup>-1</sup> was higher than after 6 days.

## Discussion

The manipulation of the sucrose concentration in culture medium is very important for biomass accumulation and taxol production (Kim *et al.* 1995, Wang *et al.* 1999). Our results demonstrated that sucrose supplementation enhanced the growth of *Taxus* cells and slightly improved cephalomanine production (Figure 1). MJA treatment, however, depressed cell growth but enhanced cephalomanine production. Inhibition of *Taxus* cell growth and enhancement of taxane induced by MJA had also been observed in other reports (Ketchum *et al.* 1999, Yukimune *et al.* 2000). Our results also demonstrated that the combined treatment produced more biomass than that of MJA elicitation

and higher cephalomanine production than that of sucrose supplementation (Figure 1).

Laskaris *et al.* (1999a,b) reported that there was a close relationship between taxanes production and GGPP synthase activity. MJA or the addition of sucrose and MJA together increased the GGPP synthase activity while sucrose supplementation did not. Enhancement of cephalomanine production thus appears to be due to the synergetic result of biomass accumulation induced by sucrose supplementation and the activation of taxane biosynthesis induced by MJA elicitation.

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