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**Research Article**

# OPTIMIZATION OF CALLUS INDUCTION AND SUBCULTURE CONDITIONS OF *TAXUS CHINENSIS*

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**ABSTRACT**

*Taxus chinensis* (Pilger) Rehd.var. mairei (Lemee et Levl.) Cheng et L. K. Fu is an evergreen plant, which belongs to the Taxaceae family. These slow growing gymnosperms were harvested in large amounts to provide taxol for clinical trials. Due to the relative scarcity of the natural resources and the low yield of Paclitaxel (taxol), it is very necessary to produce alternate sources of taxol by callus cultures. In this work, we established conditions for callus induction and subculture of *T. chinensis*. The experimental results showed that B5 medium supplemented with 1 mg/L 2,4-dichlorophenoxy acetic acid (2,4-D), 30 g/L sucrose and 7.5 g/L agar (pH 5.5) was effective for inducing callus and the better explants for callus induction was petiole. When the callus was sub cultured on seven different media and tamed several times, it could grow fast and anti-browning on the B5 medium containing 1 mg/L α-naphthaleneacetic acid (NAA), 0.5 mg/L 2, 4-D, 0.05 mg/L kinetin (KT), 0.05 g/L ascorbic acid (Vc), 0.1 g/L activated carbon (Ac), 30 g/L sucrose and 7.5 g/L agar (pH 5.75).

**Keywords**: *Taxus chinensis*, induction, callus optimization, anti-browning

## INTRODUCTION

*Taxus chinensis* is a slow growing evergreen tree distributed in China. Paclitaxel (Taxol) is a complex diterpenoid secondary product of the genus *Taxus* that was approved for treatment against ovarian and breast cancers and shows promise against other cancers[1]. Due to the relative scarcity of the few natural resources and the low yield of taxol, the supply of taxol is restricted for the clinical trials and treatment. Interest in alternative methods for taxol production has been intensifying. Given the fact that the cases of malignancies are increasingly growing, the demand to such a potent anticancer medicament is also enhancing[2]. Callus culture provides a convenient system for studying the biosynthesis of taxol. It may be a viable alternative for taxol product[3,4]. However, there were some pitfalls that may dramatically affect the bioprocess engineering of taxol through the culture systems. For example, one of the most interfering phenomena with this approach is the production of the copious phenolic compounds, which results in a particular phenomenon called "tissue browning". In fact, it is considered as a serious impediment during mass production of natural secondary metabolites via bio-processing since the tissue browning event involves many toxic compounds through the phenolization process eventually resulting in the necrosis of cells[2,5,6]. In this investigation, The conditions of *T. chinensis* callus induced were optimized by using two basic media and two explants, and we selected the anti-browning subculture medium among Murashige and Skoog (MS)[7] and modified B5[8,9] media supplemented with varying concentrations of plant growth regulators (PGRs) and antioxidant. The information provides a support for the further research of *T. chinensis*.

## MATERIALS AND METHODS

### Plant material

The stems and petioles were collected from *T. chinensis* in The South China Botanical Garden, Guangzhou, China.

### Preparation of tissue culture media

The callus was induced and subcultured on MS and B5 media with properties summarized in Table 1 and Table 2. Callus were cultured in a 100-mL Erlenmeyer flask containing 40 mL of liquid medium supplemented with sucrose at 30 g/L and agar at 7.5 g/L, respectively. The pH of the media combinations was adjusted and they were autoclaved at 121°C for 20 min.

### Callus induction assays

The explants were washed thoroughly under running tap water for 30 min, surface-sterilized with 75 % (v/v) ethanol for 1 min followed by rinsing with sterile distilled water, deep- sterilized with 0.1 % (w/v) aqueous solution of mercuric chloride for 6-15 min, and washed 5 times in sterilized water. Stem and petiole were aseptically cut into pieces (1 × 0.5 cm) and segments (1.5 cm), respectively, and cultured for inducing of primary callus at 25 ± 1°C in the dark. The Callus color, type and frequency of induction were evaluated after 40 days of culture (without transfer). The induction rate of callus formation was calculated as the ratio of the number of explants producing callus to the total number of explants plated on callus induction medium[10].

### Callus subculture

Firstly, calli were cultured in conical flasks in the dark at 25 ± 1°C, and subcultured every four weeks. After several subcultures on the callus-induction medium with explants, vigorously growing and loose callus cultures were selected for subculture on MS and B5 media in the present of varying concentrations of PGRs, ABT rooting powder, ascorbic acid (Vc) and activated carbon (Ac).

## RESULTS AND DISCUSSION

### Callus induction

The callus culture was induced from young stems and petioles of *T. chinensis*. The effects of culture media and explants on callus induction were shown in Table 2 and 3, respectively. Statistical

analyses indicated that B medium was the better medium for callus induction, displaying an average induction frequency of 85 %, which was double more than A medium. Table 3 demonstrated that petiole was better explants source for callus induction, compared with stem. The initial callus were aqua and white (Figure 1a-b). However, the callus browning was emerged after 50 days culture (Figure 1c-d).

**Table 1: Composition of media used for callus induction**

| Formula | Basal media | -D (mg/L) | Sucrose (g/L) | Medium pH |
|---|---|---|---|---|
| A | MS | 1 | 30 | 5.75 |
| B | B5 | 1 | 30 | 5.5 |

**Table 2: Composition of media used for callus subculture**

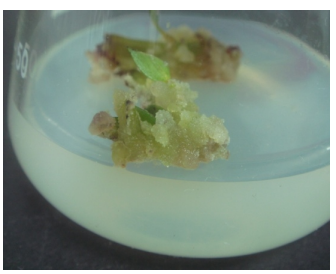
| Basal media | 2,4-D (mg/L) | NAA (mg/L) | KT (mg/L) | Vc (g/L) | ABT (g/L) | Ac (g/L) | Medium pH |
|---|---|---|---|---|---|---|---|
| MS | 0.1 | - | - | - | 0.1 | - | 5.6 |
| MS | 0.1 | - | - | 0.1 | 0.1 | - | 5.6 |
| MS | 0.1 | - | - | - | 0.1 | - | 5.75 |
| B5 | 0.1 | - | - | 0.05 | 0.1 | 0.1 | 5.6 |
| B5 | 0.1 | - | - | 0.1 | 0.1 | 0.1 | 5.6 |
| B5 | 0.5 | 1 | 0.5 | 0.05 | | 0.1 | 5.75 |
| B5 | 0.5 | 1 | | 0.05 | | 0.1 | 5.75 |

**Table 3: Effects of induction culture media on callus formation**

| Media | Explants | Calli induced | Inoculum amount | Induction Frequency (%) | Average (%) |
|---|---|---|---|---|---|
| A | Stem | 16 | 40 | 40 | 41.3 |
| | Petiole | 17 | 40 | 42.5 | |
| B | Stem | 30 | 40 | 75 | 85 |
| | Petiole | 38 | 40 | 95 | |

**Table 4: Effects of different organs on callus induction**

| Explants | Media | Calli induced | Inoculum amount | Induction frequency (%) | Average (%) |
|---|---|---|---|---|---|
| Stem | A | 16 | 40 | 40 | 57.5 |
| | B | 30 | 40 | 75 | |
| Petiole | A | 17 | 40 | 42.5 | 68.8 |
| | B | 38 | 40 | 95 | |



**(a)**

**(b)**

**(c)**

**(d)**

**Figure 1: Callus induction from *T. chinensis* (a) Callus induction from petiole of 25 d (b) Callus induction from stem of 25 d (c) Callus induction from petiole of 50 d (d) Callus induction from stem of 50 d**

**(a)** **(b)**

**Figure 2: Callus subculture on B5 medium with (a) 0.5 mg/L KT (b) 0.05 mg/L KT**

**Callus subculture**

To prevent the browning became the dominant factor in the successful callus induced and subculture. The phenomenon of callus browning could be inhibited by appropriate concentration of Vc and Ac[11] (Zheng *et al*., 2011). Our experimental results demonstrated that B5 medium was more suitable for callus subculture than MS medium. And the callus growth in the B5 medium of adding 0.05 mg/L KT is better than 0.5 mg/L KT (Figure 2 a-b). In addition, the phenomenon of browning had not eliminated because of the adding of ABT rooting powder, which was not agree with Ye[12]. However, the experiments indicated that the browning was relaxed by adding 0.05 g/L Vc and 0.1 g/L Ac. After several times cultured, calli were white and loose in the B5 medium containing 1 mg/L NAA, 0.5 mg/L 2,4-D, 0.05 mg/L KT, 0.05 g/L Vc, 0.1 g/L Ac, 30 g/L sucrose and 7.5 g/L agar (pH 5.75) (Figure 2b).

## CONCLUSION

The callus induced and subculture is the initial steps in the taxol biosynthesis regulation in cell cultures of *T. chinensis*. The characteristics of slow growing in gymnosperm make it more difficult[13]. In the present study, we optimized the explants and media of callus induction, and we have described conditions which controlled the callus culture browning successfully.

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