Journal of Advances in Biology & Biotechnology
3(2): 58-67, 2015; Article no.JABB.2015.032
ISSN: 2394-1081

SCIENCEDOMAIN *international*
www.sciencedomain.org

SCIENCEDOMAIN

Investigation of the Effects of the Basal Medium, Auxin and Antioxidants on the Induction and Maintenance of Callus and Taxol Production in Yew (*Taxus baccata*)

Sahar Bagheri Toulabi[1], Ahmad Moieni[1*], Faezeh Ghanati[2] and Fereshte Emami[1]

[1]Department of Plant Breeding and Biotechnology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran.
[2]Department of Plant Biology, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran.

Author's contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JABB/2015/13900
Editor(s):
(1) Chung-Jen Chiang, Department of medical laboratory science and biotechnology, China medical university, Taiwan.
Reviewers:
(1) Anonymous, Turkey.
(2) Anonymous, China.
(3) Anonymous, China.
(4) K. Yaprak Kantoglu, Turkish Atomic Energy Authority Saraykoy Nuclear Research and Training Center, Ankara, Turkey.
(5) Anonymous, India.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=929&id=39&aid=8404

Original Research Article

Received 8th September 2014
Accepted 2nd February 2015
Published 12th March 2015

ABSTRACT

Production of Taxol, the most promising chemotherapuetic drug, depends on *Taxus* spp. cell culture. Since there are numerous difficulties in the *in vitro* cell culture of this recalcitrant genus, this study was conducted in order to establish an efficient protocol for callus induction and maintenance of it. Callus cultures from *T. baccata* young stems were established in B5 and MS media supplemented with different concentrations of 2,4-D and NAA. The effects of basal culture media, type and concentration of auxin were assessed on callus growth. The callus morphology (compactness and color) also was compared using non-parametric test (Mann–Whitney U test). The results showed that the best basal medium for callus induction and growth was in B5, and the highest callus growth in B5 medium obtained when NAA was supplied as auxin. Two mg/L NAA was

**Corresponding author: E-mail: moieni_a@modares.ac.ir;*

the optimum auxin concentration among different applied levels of this hormone. The results also revealed that incubation of the calli in the dark conditions in the presence of 0.1 g/L of citric acid and ascorbic acid significantly reduced the phenolic secretion from calli, compared to those grown in light or those which were treated with 0.05 g/L of these antioxidants. Following experiments confirmed that stem of *T. baccata* was the best explant for callus induction and growth. In addition, the morphological study described various callus types and various colors formed during callus induction in the different conditions. The data related to this part confirmed that B5 medium and NAA provided the best tissue type (friable) and color (yellowish-green) of the calli. In the optimized medium, specific yield of Taxol was about 70 µg/g DW (Dry Weight). This amount of Taxol production is noticeable in *Taxus* spp. calli, compared with available literature on the production of Taxol by *T. baccata* cells.

Keywords: Medium; callus; plant growth regulator; taxol; Taxus baccata.

ABBREVIATIONS

FW: fresh weight, DW: dry weight, 2,4-D: 2,4-Dichlorophenoxyacetic acid, NAA: α-Naphthaleneacetic acid, PGRs: plant growth regulators, RGR: relative growth rate.

1. INTRODUCTION

Taxol (generic name: paclitaxel), is a diterpenamid which is widely used in the treatment of breast and ovarian cancer and AIDS-related Kaposi's sarcoma [1,2]. Today Taxol is the most promising chemotherapuetic drug, and shows promise against other cancers [3,4]. Taxol has been extracted from yew trees [5,6]. Due to the very low yield of purification of Taxol from the yew trees (0.004–0.01% dry weight basis), and slow growth of few natural resources [5,7], researchers have been led to find cost effective and sustainable alternative approaches to afford sufficient quantities of Taxol for the growing demand for chemotherapeutic uses and clinical researches.

In vitro synthesis of Taxol has been accomplished, but given the complexity of chemical structure and numerous steps in the synthesis procedure as well as very low yield of Taxol [5,8]; it is not a commercially feasible method for Taxol production [9]. The other approaches were examined for Taxol production such as fungal fermentation [10-12], semi-synthesis starting from intermediates which isolated from yew needles, [13,14], and cloning of the genes involved in Taxol biosynthesis [15]. Among other methods, plant cell and tissue culture seems to provide a sustainable and rich source of Taxol [16-18] due to the homogeneous culture of plant cells, elimination of the need for intensive labor for cultivation of greenhouse or field-grown plants, and safer production platform in a closed bioreactor system [19]. Accordingly, the first step to obtaining a *Taxus* sp. cell suspension is to establish fast-growing friable callus cultures [20]. Regarding the fact that *Taxus* species are recalcitrant in the culture medium, several studies have been conducted to manipulate the culture medium for callus induction. Most of these researches have been focused on assessing the effect of basal macro- and micro- nutrients of the media [16,21]. A few studies however, have been compared the effect of type or relative amounts of plant growth regulators on the induction of callus in the yew explants, neither the morphology of calli has been considered widely or statistically.

One major obstacle in *Taxus* spp. cell culture and maintenance of its callus is the secretion of phenolice compounds in the medium that obstruct callus growth and lead to browning and finally death of the cells. Application of antioxidant compounds has been suggested as a solution by scholars [22,23].

In the present research, we tried to set up an efficient protocol for establishment of sustainable calli from *Taxus baccata.* so that could be used in production of Taxol. The effects of two different basal media (B5 and MS), different types of growth regulators (NAA or 2,4-D), at different concentrations on induction of callus in different explants of *Taxus baccata* were studied. The effects of light and certain antioxidants on prevention of browning and improvement of callus maintenance were investigated as well.

2. MATERIALS AND METHODS

2.1 Plant Material

Explants were collected from a mature *Taxus baccata* grown at the Botanical garden of

University College of Agriculture and Natural Resources (35 47′, 51º 10′ at an altitude of 1321 m), University of Tehran, located in Karaj, Alborz Province, Iran, in June, July, and August 2012. Since the plant materials were collected from one tree during a year, the impact of season and genotype were ignored. Young stems and needles (leaves) of tree were selected as source of explants. The plant materials were washed with detergent (liquid soap), rinsed with tap water for 30 minutes, and subsequently were surface sterilized by washing with 70% ethanol for 15 seconds, and Na-hypochlorite for 10 minutes and rinsing with sterile distilled water in intervals. Segments of 1-2 cm from needles and stems were cultured on the media in laminar air flow conditions.

2.2 Culture Media and Plant Growth Regulators

The first experiment was conducted to evaluate the effects of basal nutrients of B5 [24] and MS [25] media and type of auxin (NAA and 2,4-D) on callus formation. Both media were supplemented with 3% sucrose and 8 g/L agar agar and were containing 0, 0.5, 1, 2, 4, 6, 8, and 10 mg /L of NAA or 2,4-D. Basal media of both MS and B5 without plant growth regulators were used as controls.

The pH of all media was adjusted to 5.7 with either KOH or HCl prior to autoclaving for 20 min at 121ºC. All cultures were incubated in darkness at 25±2ºC until the calli were emerged (20 days). These conditions were kept constant while the calli were growing.

2.3 Effect of Culture Media on the Morphology of Calli

Concerning the fact that morphology is an important factor in the mass production of calli, certain morphological features i.g., color and compactness of the calli which produced in different media were compared. Accordingly, the calli were ranked in 5 groups (Tables 1 and 2) in terms of their compactness (Fig. 1) and color (Fig. 2).

2.4 Effects of Antioxidants and Light on Maintenance of Callus

Based on the results of previous steps, a B5 basal medium containing 2 mg/L NAA was selected as the best medium for induction of callus. The effects of antioxidants (citric acid and ascorbic acid) and light (25 µmol m^{-2} s^{-1}) on secretion of phenolics from the calli were evaluated in this medium (B5) according to a design shown in Table 3. All cultures were incubated at 25±2ºC during the induction and growth of calli.

Table 1. Ranking of different callus types in *T. baccata* calli

Tissue type	Number
Dry	1
Wateri	2
Cotton form	3
Friable – cotton form	4
Friable	5

Table 2. Ranking of *T. baccata* calli according to their colors

Color	Number
Dark brown	1
White	2
Brown	3
Yellowish – brown	4
Yellowish - green	5

2.5 Taxol Extraction and Analysis

Taxol extraction and analysis were conducted according to the procedure reported previously [26,27]. Callus samples were collected after 6 weeks of culture and dried at 25ºC. The extraction of Taxol performed employing methanol and dichloromethane. Dried cells (ca. 50 mg) were powdered and dissolved in 4 mL methanol and ultrasonicated for 20 min. The mixture was centrifuged at 4000 rpm for 15 min. The supernatant was collected and evaporated at 25ºC to dryness under vacuum by a rotary evaporator.

To eliminate polar impurities, the residue was re-dissolved in a mixture of dichloromethane and water (1:1). The mixture was centrifuged at 4000 rpm for 15 min and dichloromethane phase was separated. This process was repeated two times and the dichloromethane phase evaporated and re-dissolved in acetonitril before analysis by HPLC (Waters, model code: 6CE). The HPLC system equipped with a reverse-phase C18 column (OSD – UG -5, 4.6 mm × 250 mm × 10 µm) and UV detector. Taxol was eluted using a gradient of acetonitrile: water (60: 40) and was detected at 227 nm. Quantification of Taxol was performed with an external standard of it (Sigma, USA).

Table 3. The integrated treatments of antioxidants and light on *T. baccata* calli

Treatment	Ascorbic acid (g/L)	Citric acid (g/L)	Light condition	
			Light	Dark
1	0.05	0.05		+
2	0.1	0.1		+
3	0.05	0.05	+	
4	0.1	0.1	+	

Fig. 1. Different types of tissue in *T. baccata* calli after 5 weeks of culture
1: friable, 2: friable – cotton form, 3: cotton form, 4: wateri, 5: dry

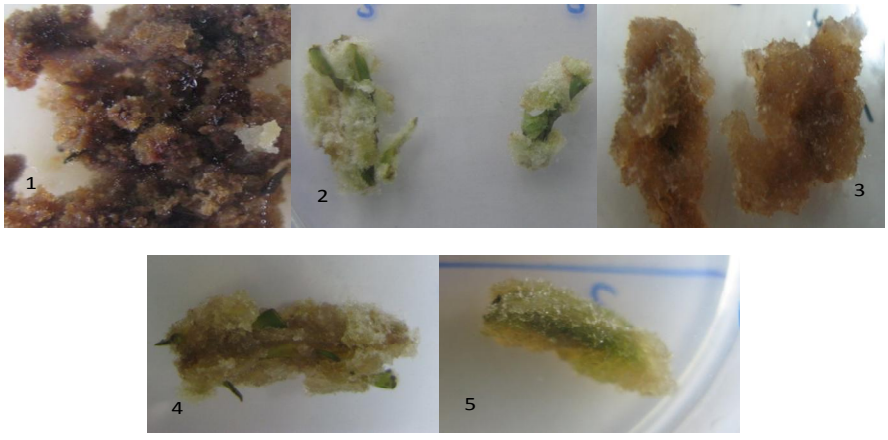

Fig. 2. Different colors observed in *T. baccata* calli after 5 weeks of culture
1: dark – brown, 2: white, 3: brown, 4: yellowish-brown, 5: yellowish-green

2.6 Measurement of Callus Growth

Growth of the calli was monitored by measuring initial and final fresh weights. Relative growth rate (RGR) was calculated as: FW2- FW1/ FW1 where FW1 and FW2 were the initial and final fresh weights of the calli after 6 weeks of treatments.

2.7 Statistical Analysis

The factorial design based on completely random design (CRD) was used to examine the effects of

culture media, plant growth regulators and hormone concentrations.

The experiments were repeated at least three times with five replications. Each replicate (Petri dish, 60 × 15 mm) was composed of 3 explants. Duncan's multiple range tests and student's test were used for comparison among the means.

The nonparametric Kruskal–Wallis test and Mann–Whitney test were used to investigate the effects of culture media, plant growth regulator and hormone concentration on type of callus tissue and its color, considering a $p \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1 Effects of Culture Media, Plant Growth Regulators and Hormone Concentration on Callus Induction

The calli were emerged 1 week after culture from different segments of *T. baccata* on hormone containing B5 and MS media, while there was no callus formation in the hormone free control ones (Figs. 3 and 4). Relative growth rates of the calli are compared in Table 4. As shown in Table 4, the highest callus growth obtained when B5 medium was used, compared with MS medium. Although some researchers have evaluated the effect of modified MS and B5 media, to the best of our knowledge, there is no report on comparison the effect of basal MS and B5 media on callus production. Consequently, we selected B5 medium with more reliability for callus induction and production.

Application of different kinds of auxin in aforesaid media showed that callus formation and growth varied significantly depending on the type of auxin (Table 4). The data showed that NAA was significantly effective not only in induction of callus but also in improvement of its growth, compared to 2,4-D. Consistent with our results, Mihaljevic et al. [14] showed that NAA was the best hormone to promote callus production by *T. baccata* explants. However, it should be noted that for induction of callus in other species of *Taxus* spp., other kinds of auxin may be more appropriate [28]. In order to investigate the wider range of hormone concentration, we examined seven different levels of NAA. Among applied concentrations of NAA, the best one for promotion of the growth of calli was 2 mg/L (Table 5).

From preliminary studies it was shown that applied explants (stem and needle (leaf) were different in response to the medium and induction of callus (Data not shown). Therefore, the differential response of stem and needles explants was evaluated in B5 medium containing 2 mg/L NAA. The results significantly confirmed again that stem segments of *T. baccata* were more appropriate for induction of callus and promotion of callus growth, in comparison with needle segments (Figs. 4 and 5). This is in accordance with Baebler et al. [28] report.

Table 4. Effects of culture medium and type of auxin on relative growth rate (RGR) of callus in *Taxus baccata* stem explants

Medium	Auxin	RGR
MS	-	0.32±0.01
B5	-	0.44±0.01*
	2,4-D	0.31±0.01
	NAA	0.45±0.01 *

Data are presented as the means±SE with n = 3. Asterisk refers to significant differences at p≤0.05, according to the Student's t-test

Fig. 3. Stem explants cultured on different media after 6 weeks. (a): explants in hormone-free basal media, (b): explants in hormone containing media (B5 medium containing NAA 2 mg/L)

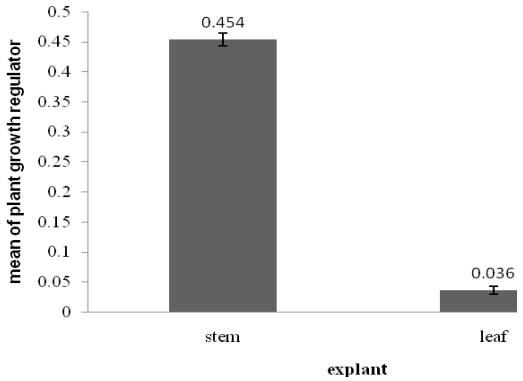

Fig. 4. Effect of explant type of *T. baccata* on callus growth (B5 medium containing NAA 2 mg/L). The values represent the mean±SE

Table 5. Effect of different concentrations of NAA on relative growth rate (RGR) of *Taxus baccata* calli

NAA (mg/L)	RGR of *Taxus baccata* calli
0.5	0.31±0.1 [c]
1	0.39±0.1 [b]
2	0.45±0.1 [a]
4	0.40±0.1 [b]
6	0.37±0.1 [b]
8	0.38±0.2 [b]
10	0.39±0.2 [b]

Data are presented as the means ± SE with n = 3. Means with the same letter are not significantly different, according to Duncan's multiple range test (P<0.05)

3.2 Effect of Culture Media on the Morphology of Calli

In spite of numerous studies on the establishment of callus from *Taxus* spp., the morphological features of *Taxus* spp. calli have not been vastly investigated. Therefore, considering the importance of callus appearance in its selection for mass production, the effects of basal medium, type and concentration of auxin were assessed on color as well as tissue type of calli of *T. baccata*.

In our ranking, the best tissue type of callus and its color were numbered 5. The most frequent friable calli (accounted for 5) were emerged in the medium containing NAA, compared to those 2,4-D containing media (Fig. 6). This confirmed again that NAA was more appropriate for induction of best callus in *T. baccata*, as resulted from the first experiment too. The effect of basal medium on type of tissue of *T. baccata* calli was not significant, according to Mann–Whitney U test (Fig. 6).

The effect of different hormone concentrations on type of tissue of *T. baccata* calli was not significant; according to Kruskal–Wallis H test.

Mann–Whitney U tests showed significant effect of basal medium on the color of the calli. As shown in Fig. 7, the most frequent calli with the best color (yellowish-green, no.5) were observed in B5 medium.

Mann–Whitney U test and Kruskal–Wallis H test showed no significant differences in callus color in medium containing different auxins at various concentrations.

3.3 Effects of Antioxidants and Light on Callus Maintenance

Secretion of phenolics from explants and calli and browning of the calli has been a common obstacle to which all researchers who deal with *Taxus* spp. culture have been challenging. Bai et al. [29] reported that light irradiation stimulated browning of calli and remarkably hindered their growth. Application of antioxidants in *Taxus* spp. culture has been suggested [30,31]. In the present research browning of *T. baccata* started after 2 weeks of culture. We evaluated the effects of light / dark and combination of citric acid and ascorbic acid on *Taxus* spp. calli. These antioxidants were applied in concentrations of 0.05 and 0.1 g/L, in B5 medium supplemented with 2 mg/L NAA. The results showed that incubation of the calli in dark conditions in the presence of 0.1 g/L of citric acid and ascorbic acid significantly reduced the phenolic secretion from calli, compared to those grown in light or treated with 0.05 g/L of antioxidants.

3.4 Taxol Analysis

Taxol was detected in *T. baccata* calli (Fig. 8). Specific yield of Taxol in our optimized medium (B5 medium containing 2 mg/L NAA, 0.1 g/L ascorbic acid and 0.1 g/L citric acid, in the darkness) was about 70 µg/g DW which was noticeably high, compared to those reported for other *Taxus* species [5], or even for *T. baccata* calli [4].

Overall, to overcome the problem of induction and growth of *Taxus baccata* callus, this report presents a reproducible and effective method for suitable production of *T. baccata* callus, in which the optimized medium produced a quality callus and improved the yield of Taxol, and could be used, for cell suspension culture and Taxol production.

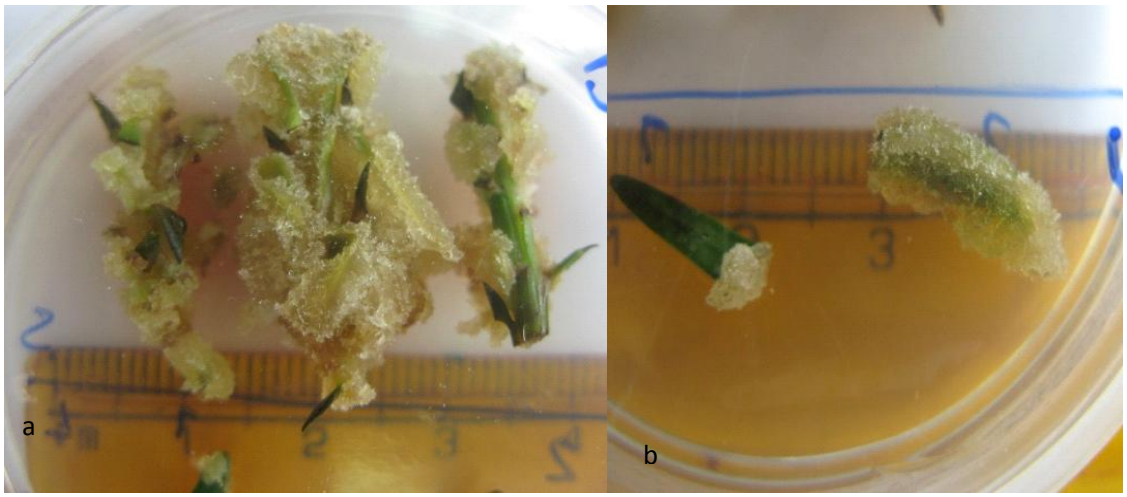

Fig. 5. Differential response of *T. baccata* explants to B5 medium supplemented with 2 mg/L NAA in induction of callus after 6 weeks (a: stem, b: needle)

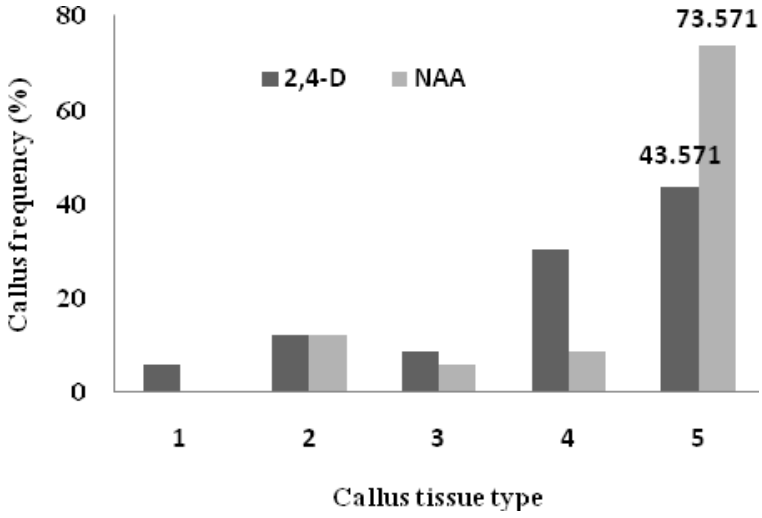

Fig. 6. Comparison the effects of type of auxin (NAA and 2,4-D) on the callus morphology from stem

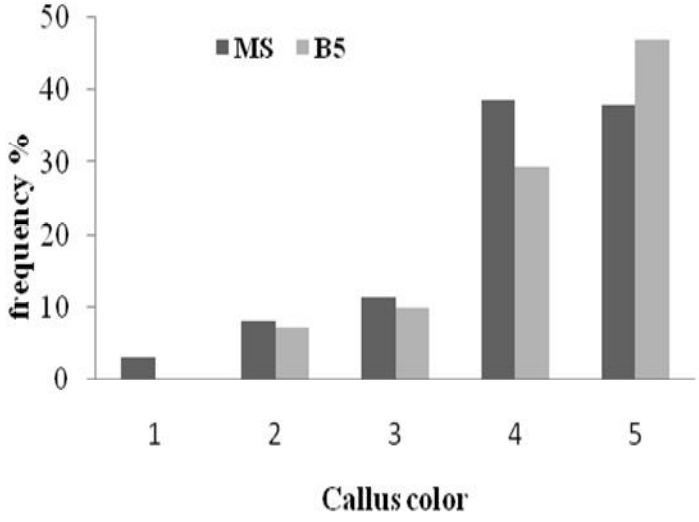

Fig. 7. Comparison of callus color of *Taxus baccata* obtained from stem in different basal media

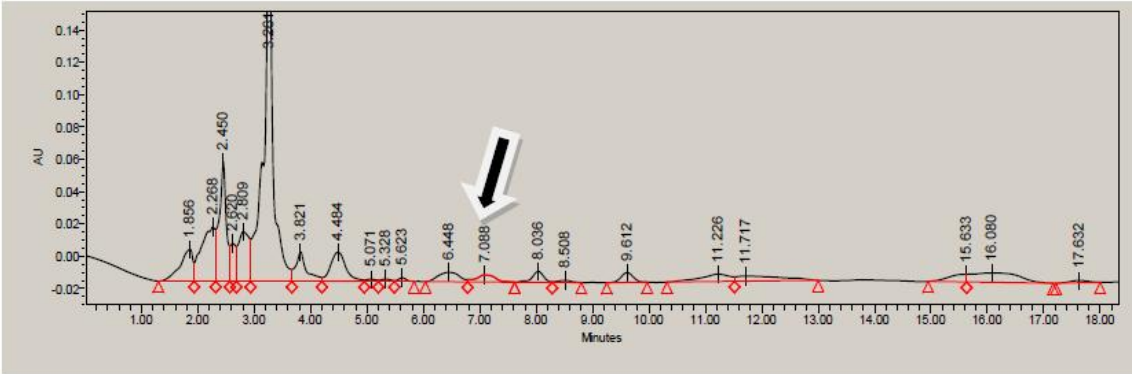

Fig. 8. Chromatogram of HPLC showing the presence of Taxol at RT of 7.088 min

4. CONCLUSION

In conclusion, an efficient protocol for callus induction and maintenance of *Taxus baccata* was developed. In this regard, effects of culture media, plant growth regulators and hormone concentration on callus induction B5 medium containing 2 mg/L NAA was optimized. Also, the results showed that B5 medium and NAA provided the best tissue type (friable) and color (yellowish-green) of the calli. Finally, the results revealed that dark conditions in the presence of 0.1 g/L of citric acid and ascorbic acid has significantly reduced the phenolic secretion from calli.

Despite the above-mentioned conclusions, as Cusido et al. [20] asserted the optimization of conditions for callus induction and growth is tedious process, as the response to callus induction and growth varies according to different factors. Therefore, our results on *T. baccata* can be improved by other factors in future research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Expo´ sito O, Bonfill M, Onrubia M, Jane A, Moyano E, Cusido RM, Palazon J, Pinol MT. Effect of Taxol feeding on Taxol and related taxane production in *Taxus baccata*

suspension cultures. N. Biotechnol. 2009;00:1-8.
2. Zhang CH, Fevereiro PS, He G, Chen Z. Enhanced paclitaxel productivity and release capacity of *Taxus chinensis* cell suspension cultures adapted to chitosan. Plant Sci. 2007;172:158–163.
3. Mousavi B, Parsaeimehr A, Irvani N. Influences of growth regulators on callus induction, ephedrine and pseudoephedrine contents and chemical analysis of mature embryo of *Ephedra strobilacea* Bunge. AAB Bioflux. 2011;3(1):39-45.
4. Woo DDL, Miao SYP, Pelayo JC, Woolf AS. Taxol inhibits progression of congenital polycystic kidney disease. Nature. 1994;368:750–753.
5. Witherup KM, Look SA, Stasko MW, Ghiorzi TJ, Cragg GM. Taxus spp. needles contain amounts of Taxol comparable to the bark of *Taxus brevifolia*: Analysis and isolation. Nat prod. 1990;53:1249-1255.
6. Yuan YJ, Li C, Hu ZD, Wu JC. A double oxidative burst for Taxol production in suspension cultures of *Taxus chinensis* var. mairei induced by oligosaccharide from *Fusarium oxysprum*. Enzyme Microb Tech.2002;30:774–778.
7. Wheeler NC, Jech K, Masters S, Brobst SW, Alvarado AB, Hoover AJ, Snader K M. Effects of genetic, epigenetic, and environmental factors on Taxol content in *Taxus brevifolia* and relates species. Nat prod. 1992;55:432–440.
8. Holton RA, Somosa C, Kim HB, Liang F, Biediger J, Boatman PD, Shindu M, Smith CC, Kim S, Nadizadeh H, Suzuki Y, Tao C, Vu P, Tang S, Zhang P, Murthi KK, Gentile LN, Liu JH. First total synthesis of Taxol. J. Am. Chem. Soc. 1994;116:1597–1600.
9. Hoffmana A, Shahidi F Paclitaxel and other taxanes in hazelnut. J. Functional Food. 2009;1:33-37.
10. Li YC, Tao WY. Interactions of Taxol-producing endophytic fungus with its host (*Taxus* spp.) during Taxol accumulation. Cell Biol. Int. Rep. 2009;33:106-112.
11. Strobel G, Yang XS, Sears J, Kramer R, Sidhu RS, Hess WM. Taxol from Pestalotiopsis microspora, an endophytic fungus of *Taxus wallachiana*. Microbiol. 1996;142:435-440.
12. Wang JF, Li GL, Lu HY, Zheng ZH, Huang YJ, Su WJ. Taxol from *Tubercularia* sp. TF5, an endophytic fungus of *Taxus mairei*. FEMS Microbiol Lett. 2000;193:249e53.
13. Baloglu E, Kingston DG. A new semisynthesis of paclitaxel from baccatin III. Nat. Prod. 1999;62:1068–71.
14. Mihaljevic S, Bjedovc I, Kovac M, Leljak D, Jelaska S. Effect of Explant Source and Growth Regulators on in vitro Callus Growth of *Taxus baccata* L. Washingtonii. Biotechnol. 2002;40(4):299–303.
15. Jennewein S, Croteau R. Taxol: biosynthesis, molecular genetics, and biotechnological applications. Appl Microbiol Biotechnol, 2001;57:13-9.
16. Fett-Neto AG, Melanson SJ, Sakata K, DiCosmo F. Improved growth and Taxol yield in developing calli of *Taxus cuspidata* by medium composition modification. Bio/Technology. 1993;11:731–734.
17. Lan WZ, Yu LJ, Li MY, Qin WM. Cell death unlikely contributes to Taxol production in fungal elicitor-induced cell suspension cultures of *Taxus Chinensis*. Biotechnol. Lett. 2003;25:47–49.
18. Zhang CH, Wu JY. Ethylene inhibitors enhance elicitor-induced paclitaxel production in suspension cultures of *Taxus* spp. Cells. Enzyme Microb Tech. 2003;32:71–77.
19. Huang TK, Mcdonald KA. Bioreactor systems for *In vitro* production of foreign proteins using plant cell cultures, Biotechnolo Adv. 2012;30:398–409.
20. Cusido, R. M. Onrubia, M. A. Sabater-Jara, B., Moyano,.E. Mercedes Bonfill, Goossens AM, Pedreño A, Palazon JA. Rational approach to improving the biotechnological production of taxanes in plant cell cultures of *Taxus* spp., Biotechnol Adv. 2014;32(6):1157–1167.
21. Jaziri M, Zhiri A, Guo YW, Dupon JP, Shimonura K, Hamada H, Van Haelen M, Homes J. *Taxus* sp. cell, tissue and organ cultures as alternative sources for taxoids production: A literature survey. Plant Cell Tissue Organ Cult. 1996;46:59-75.
22. Gibson DM, Ketchum REB, Vance NC, Christen AA. Initiation and growth of cell lines of *Taxus brevifolia*. Plant Cell Rep. 1993;12:479-482.
23. Maheshwari P, Garg S, Kumar A. Taxoids: Biosynthesis and *In vitro* production. Biotechnol. Mol. Biol. Rev. 2008;3(4):71-87.
24. Gamborg OL, Miller RA, Ojima K. Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell Res. 1968; 50:151-158.

25. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Plant Physiology. 1962;15:473-495.
26. Khosroushahi AY, Valizadeh M, Ghasempour Khosrowshahi AM, Naghdibadi H, Dapour NR. Omidi Y. Improved taxol production by combination of inducing factors in suspension cell culture of *Taxus baccata*. Cell Biol. Int. 2006;30:262-269.
27. Tapia N, Zamilpa A, Bon fill M, Ventura E, Cruz-Vega D, Del Villar A. Effect of the culture medium and biotic stimulation on taxane production in *Taxus globosa* Schltdl *In vitro* cultures. Acta Physiol Plant. 2013;35:3447–3455.
28. Baebler S, Hren M, CamLoh M, Ravnikar M, Bohanec B, Plaper I, Ucman R, Zel J. Establishment of cell suspension culture of Yew (Taxus media rehd) and assessment of their genomic stability. In vitro Cell. Dev. Biol. 2005;41:338–343.
29. Bai J, Ito N, Sakai J, Kitabatake M, Fujisawa H, Bai L, Dai J, Zhang S, Hirose K, Tomida A, Tsuruo T, Ando, M. Taxoids and Abietanes from Callus Cultures of *Taxus cuspidata*. Nat. Prod. 2005;68:497-501.
30. Zhang CH, Mei XG, Liu L, Yu LJ. Enhanced paclitaxel production induced by the combination of elicitors in cell suspension cultures of *Taxus chinensis*. Biotechnol Lett. 2000;22:1561–1564.
31. Furmanowa M, Oledzka H, Sykłowska-Baranek K, J´ozefowicz J, Gieracka S. Increased taxane accumulation in callus cultures of *Taxus cuspidata* and Taxus media by some elicitors and precursors. Biotechnol. Lett. 2007;22:1449–1452.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=929&id=39&aid=8404