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# Using various biotic and abiotic elicitors in hazelnut cell suspension cultures to investigate the expression of the 3-N-Debenzoyl-2-DeoxyTaxoln-Benzoyltransferase gene in the Paclitaxel biosynthesis pathway

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## *Abstract*

### Objective

Paclitaxel (PC) is a naturally occurring chemotherapeutic medication used to treat various malignancies. Hazelnut (*Corylus avellana* L.) is currently a readily available and affordable Paclitaxel source. In this study, the potential effects of some biotic and abiotic elicitors were investigated on expression levels of the DBTNBT gene (3-N-debenzoyl-2-deoxytaxolN-benzoyltransferase) in cell suspension cultures of *C. avellana*. The DBTNB gene is one of the key genes in the downstream biosynthesis pathway of Paclitaxel.

### Materials and methods

Hazelnut yellow friable calli, taken from and often subcultured in MS media, supplemented with 2,4-D (2 mg/L) and BAP (0.2 mg/L), was suspended in liquid MS of the same composition. The elicitors methyl jasmonate (MeJA) (0, 100 and 200 (µL)), silver nitrate (0, 15 and 30 (mg/L)), or fungus extract (0, 25, 50, and 100 (mg/L)) were applied to the hazelnut cell cultures for 48 hours during the middle growth phase.

**Results**

The present study demonstrated the inductive effects of the elicitors on the expression of the DBTNBT gene. Compared to the control samples, in the *C. avellana* cell suspension culture, DBTNBT gene expression was affected positively by MeJA, and the most increase in DBTNBT gene expression (17.6-fold) was obtained from the treatment of 200 µL MeJA. The rate of gene expression rose considerably, up to 14 times greater than that of the control, when the concentration of AgNO3 was increased to 30 mg/L. Fungal extract affected DBTNBT gene expression; a suspension culture of *C. avellana* cells treated with 50 mg/L fungal extracts of *C. globosum* revealed a 4.75-fold increase in DBTNBT gene expression relative to the reference. However, 100 mg/L of *C. globosum* extracts reduced gene expression compared to the control.

**Conclusions**

MeJA had the highest degree of DBTNBT gene expression of any elicitor therapy used in this investigation, however, all applied elicitation treatments were able to successfully increase the DBTNBT gene expression in hazelnut cell suspension cultures.

**Keywords**: *Corylus avellana*, DBTNBT, gene expression, paclitaxel, qRT–PCR

**Abbreviations**: 2,4-D=2,4-dichlorobenzaldehyde, BAP=benzyl amino purine, PC=paclitaxel.

**Paper Type:** Research Paper.

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## Introduction

Plants are the early food origin for humans and animals. The effective secondary metabolites are also used to treat most diseases. The use of medicinal plants as natural sources of medicine has increased. Compared with chemical medicines, herbal medicines have high value and importance in providing health and hygiene, and despite the progress of science and technology,

many medicines are based on natural compounds extracted from plants and the use of it has been increasing over the past few years (Li & Weng, 2017). Herbal medicines have been made in recent decades via the use of secondary metabolites, which are naturally formed in plants under stress and other situations. Plants can create secondary metabolites by *in vitro* cultures, and cell growth and metabolic processes can be effectively controlled to market purer products (Atanasov et al., 2015). Based on their biosynthetic pathway, secondary metabolites can be classified to three groups: phenols, nitrogenous compounds, and terpenes. Terpenes are among the most significant secondary metabolites due to their therapeutic qualities (Cox-Georgian et al., 2019). Based on the chemical structure, 4000 distinct terpenes have been found; paclitaxel, which possesses anticancer therapeutic characteristics, is one of the most significant of these molecules (Chopra et al., 2021). Paclitaxel (PC), also known as Taxol, is a triterpenoid with a complex biochemical structure (Eum et al., 2011). It was first isolated and extracted from the bark of several Taxaceae family yew species in 1971, and it was marketed under the name PC in 1989 (Rezaei et al. 2011b). The production and storage of these complex substances, in large quantities, through chemical methods are usually very difficult or impossible. PCs along with other related compounds, such as baccatin III, are produced in the cells of yew and hazelnut as well as some fungi and endophyte bacteria of these two plants (Gallego et al., 2015; Isah, 2015). Hoffman et al. reported PC and taxanes in the branches, stems, and leaves of the hazelnut plant *C. avellana* L. for the first time (Hoffman & Shahidi 2009). Studies have indicated that the generation of taxanes, particularly PCs, can be achieved with hazelnut cell culture as a viable substitute source (Hoffman & Shahidi 2009; Ghanati et al. 2011; Rahpeyma et al. 2017; Salehi et al. 2020; Goktepe-Atilgan et al. 2023). Hazelnut (*C. avellana*) is native to Eurasia and is a member of the Coryluideae subfamily, the Betulaceae family, and the *Corylus* genus. (Yang et al., 2023). To date, 25 different species have been identified around the world, and in Iran, the native hazelnuts are *C. avellana* L. The availability and abundance of hazelnut plants, as well as their ability to grow under laboratory conditions, callus induction, and maintenance conditions, make them superior options for producing PCs compared with yew trees (Isah, 2019). The possibility of commercial production and development of these metabolites under laboratory conditions through metabolite engineering and molecular and racial modification of medicinal plants is made possible by the identification of the genes encoding the biosynthetic pathways of secondary metabolites and their physiological functions in plants. While elicitation and environmental optimization have provided some success in increasing PC accumulation *in vitro*, understanding the metabolism of PC at the molecular level is essential for process optimization. Through the use of direct and indirect molecular techniques, a better understanding of PC biosynthesis has been gained, although knowledge of other aspects of the global metabolism of paclitaxel, such as its regulation, transport, and degradation, is lacking

(Vongpaseuth & Roberts, 2007; Wilson & Roberts, 2012). Considering the importance of PC as an important anticancer medicinal substance, it is very important to understand the biosynthetic pathways complicated and investigate the genes involved in the production of PC in hazelnuts to increase its amount and also to engineer the metabolites that produce it. DBTNBT, or 3-N-debenzoyl-2-deoxyTaxol N-benzoyl, is the final gene in the PC biosynthesis pathway and one of its other significant genes (Walker et al., 2002; Zhang et al., 2023). It catalyzes the following reaction:

N-debenzoyl-(3'-RS)-Taxol + benzoyl-CoA → Taxol + coenzyme A + H+

This enzyme is a member of the transferase enzyme family. By transferring aminoacyl groups, acetyltransferase contributes to the production of diterpenoids. Given the commercial and medicinal importance of PC, understanding and researching its biosynthesis pathways will be crucial for advancing the field and addressing its constraints. For this purpose, in this research, for the first time, we investigated the expression of one of the key genes of the PC biosynthetic pathway (DBTNBT) in hazelnut cell suspension culture using both live and nonliving elicitors, which is an effective step toward a more accurate understanding of its role in PC production.

## Materials and methods

**Plant materials and callus formation:** This study employed the cotyledons of *C. avellana* L., a plant species native to Iran's Eshkevarat-Rudsar area, which is known as the wonderland of Gilan. The technique (Shirazi et al. 2020), which is briefly explained below, was applied for callus induction. MS basic culture medium (Murashige & Skoog, 1962) was applied, supplemented with 30 g/L sucrose, 7 g/L agar (pH 8.5), BAP (0.2 mg/L), and 2,4-D (2 mg/L). After the hazelnut seeds were peeled, they were soaked for 20 minutes in running water with a small amount of dishwashing liquid. Then, we followed this procedure in the following sequences: immersion for 20 minutes in a 5% sodium hypochlorite solution; three rounds of sterile distilled water washing; using 70% alcohol for two minutes; and finally, three washes in sterile distilled water. The isolated cotyledon pieces were grown on the presterilized, ready-made culture media inside jam jars. The cultures were kept at 25 ˚C in the dark. After 21 days, the calli had formed.

**Cell suspension preparation:** In hazelnut, a cell suspension culture was established with 5 g of yellow friable callus per 30 mL of MS liquid culture media with the same hormonal composition and 100 mg/L citric acid, 100 mg/L ascorbic acid (sterilized with a 0.22 filter), 30 g/L sucrose, pH 5.8, and kept at 25 ˚C in darkness on rotating shakers at 110 rpm. The suspensions were subcultured every 10 days with fresh liquid media (Shirazi et al. 2020) until the cells reached homogeneity.



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**Elicitation of the cell suspension culture:** Seven-day-old suspensions were subjected to elicitor treatments to investigate the changes in the desired gene expression. The elicitors in this study, included silver nitrate at concentrations of 15 and 30 mg/L, MeJA at concentrations of 100 and 200 µM, and *Chaetomium globosum* extract at concentrations of 25 mg/L, 50 mg/L, and 100 mg/L.

**To prepare the *C. globosum* extract:** *C. globosum* is a hazelnut endophytic fungi that enhanced paclitaxel production in *C. avellana* cell suspension culture (Salehi et al., 2019). It was obtained from the National Center of Genetic and Biological Resources of Iran. The preparation of the fungal extract followed the earlier description provided by Salehi et al. (2019). In brief, the fungal isolates' agar plugs with their mycelia were grown separately in PDB media and incubated for six days at 25 °C at a shaker speed of 110 rpm. After the broth was filtered out, the mycelia were repeatedly cleaned with double-distilled water. Following thorough crushing in liquid nitrogen, the fungal mycelia were soaked in water (3 mg/mL), mixed well, and incubated for 60 minutes at 90 °C while being continuously shaken. For fifteen minutes, the suspension was centrifuged at 10,000×g. Following centrifugation, the supernatant was collected, passed through cellulose acetate syringe filters with a 0.22 µm pore size, and labelled the cell extract (CE). Molecular analysis of the expression of the DBTNBT gene via RNA extraction and cDNA synthesis. Hazelnut RNA was extracted via a Denazist total RNA isolation kit.

**Molecular investigations of DBTNBT gene expression-RNA extraction and cDNA synthesis:** Total RNA was extracted from the isolated cells from cell suspension cultures of hazelnuts subjected to either elicitation or control treatment via a Denazist Asia kit (Tehran, Iran) in accordance with the manufacturer's instructions. The quantity of RNA extracted was calculated through a NanoDrop device, and its quality was determined through 1.5% agarose gel electrophoresis. The A260/A280 and A260/A230 ratios were determined applying spectrophotometry at 260 nm, 280 nm, and 230 nm to clarified RNA concentration and purity; optimum values approximately 2.0 and 2.0-2.2, respectively. The extracted samples were stored in a -80 °C freezer for the purpose of gene expression analysis. An Easy cDNA Synthesis Kit from Pars Tous Company (Mashhad, Iran) was used to generate cDNA according to the provided guidelines.

**RT–PCR analysis:** RT–qPCR primers were designed from the sequence of the 3'-N-debenzoyl-2'-deoxyTaxol N-benzoyl transferase gene (DBTNBT) (accession number: MF421526) and the ACTIN housekeeping gene by means of Oligo 7 software, as showed in Table 1. To carry out RT–PCR reaction in a 20 µL volume: 1 µL of cDNA template, 1 µL of each forward and reverse primer (final concentration: 600 nM), and 10 µL of PCR Master Mix (2x Taq PCR Master Mix Kit ) were applied based on a cycling protocol containing 1 cycle of 95 °C for

4 mins, 40 cycles of 95 °C for 1 min, 57 °C for 1 min, 72 °C for 1 min, and finally 1 cycle of 72 °C for 5 mins. To carry out RT–qPCR, 10 µL of SYBR Green Real-Time PCR Master Mix (Pars Tous Biotech Company) was applied in a reaction containing 2 µL of cDNA (1 µg) 6 µL of PCR grade D. H2O, and 1 µL of each forward or reverse primer. Amplifications were performed in a MiniOpticon™ Real-Time PCR device (Bio-Rad, USA) under the following steps: 94 °C for 15 min, 35 cycles of 94 °C for 30 s, 50–60 °C for 30 s, 72 °C for 30 s, and a final cycle of 75 °C for 10 min. Real-time PCR data were analyzed applying REST software and SPSS with Duncan's test. The graphs were generated via the Microsoft Excel software.

**Table 1. The sequences of the primers**

| Gene name | primers 5'_3' | product (nt) |
|---|---|---|
| ACTIN Forward | GCCCTTGACTATGAGCAGGA | 114 |
| ACTIN Reverse | GCAACGGAATCTCTCAGCTC | |
| DBTNBT Forward | TTCTCTTGCAGCAACCCTAC | 111 |
| DBTNBT Reverse | TTCCTTCTCAGGCAAAGCTAC | |

**Results**

**Setting up of cell suspension cultures and elicitor induction:** Hazelnut seeds grown on MS media developed a callus after 21 days (Figure 1). The homogenous hazelnut cell suspension from multiple subcultures was used for elicitor treatment. The elicitor was added to the cell suspension on day seven when the cells reached the logarithmic growth phase.

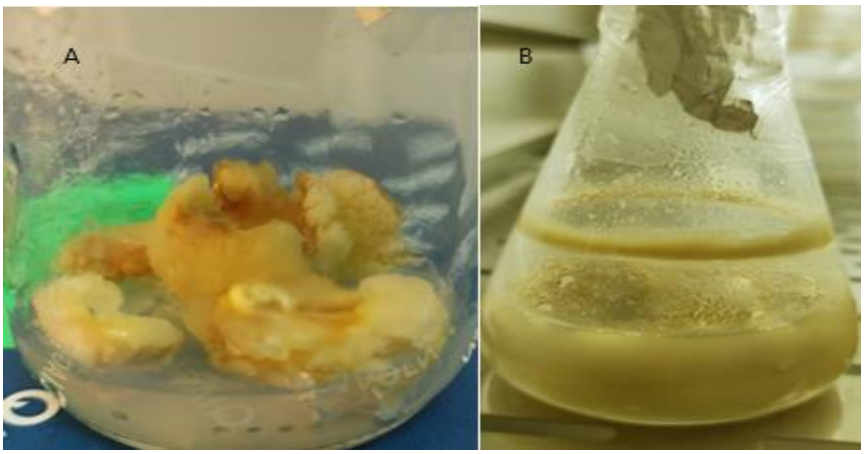


**Figure 1. A) Callus induction from hazelnut cotyledons after 21 days. B) Cultivated cells in suspension before elicitor application.**

**RNA extraction and cDNA synthesis:** Sufficient amounts of high-quality RNA were extracted from cell masses cultured in suspension. Monitoring of the 18S rRNA and 28S rRNA



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bands on a 1% agarose gel revealed good quality and accuracy of the extracted RNA. The use of the NanoDrop device for RNA quality control revealed that all the extracted RNAs had ratios of 1.8–2.07 and 2–2.17 for 260/280 and 230/260, respectively, and that the RNA concentrations ranged from a minimum of 740.3 ng/µL to a maximum of 2380 ng/µL. RT–PCR was conducted with the DBTNBT gene primers to verify the accuracy of the synthesized cDNA, and it was discovered that the DBTNBT gene was amplified as a single band of 111 bp without an additional band (Figure 2).

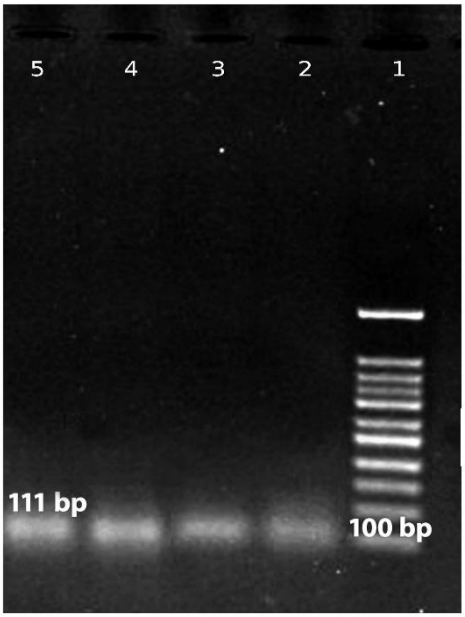


**Figure 2. Agarose gel electrophoresis of the products of RT–PCR amplification of the DBTNBT gene. 1: Ladder 100 bp, 2-5: Samples**

**Effects of fungal extracts, silver nitrate, and MeJA on DBTNBT gene expression:** The proper level of DBTNBT gene expression was monitored and ascertained through real-time PCR. A single peak in the melting curve signifies the assay's correctness and the target gene's specific amplification, whereas the standard curve's increasing trend in cycles 28–40 suggests an acceptable cDNA concentration (Figure 3). REST software was used to analyze the changes in DBTNBT gene expression, and SPSS software was used to compare the mean values with Duncan's test, which revealed that the *C. globosum* extract was significantly effective. The concentration of 50 mg/L of fungal extract had the highest DBTNBT gene expression, which was 4.75 times greater than that of the control, out of the three concentrations (25, 50, and 100 (mg/L)) (Figure 4). The results also revealed that the expression of this gene was negatively impacted or decreased when the concentration of the fungal extract was increased above 50 mg/L. The results

of the effect of treatment with different concentrations of silver nitrate on changes in DBTNBT gene expression revealed that this elicitor had a substantial effect on the expression of this gene, and the highest expression level of this gene was observed at the concentration of 30 mg/L, which was a 14-fold increase compared with that of the control (Figure 5). The analysis of the data regarding the impact of 0, 100, and 200 µM MeJA as an elicitor on DBTNBT expression revealed the changes in DBTNBT gene expression at different concentrations of MeJA. At the concentrations of 100 µM and 200 µM, the highest expression level was associated with 200 µM, which was 17.6-fold greater than that of the control (Figure 6).

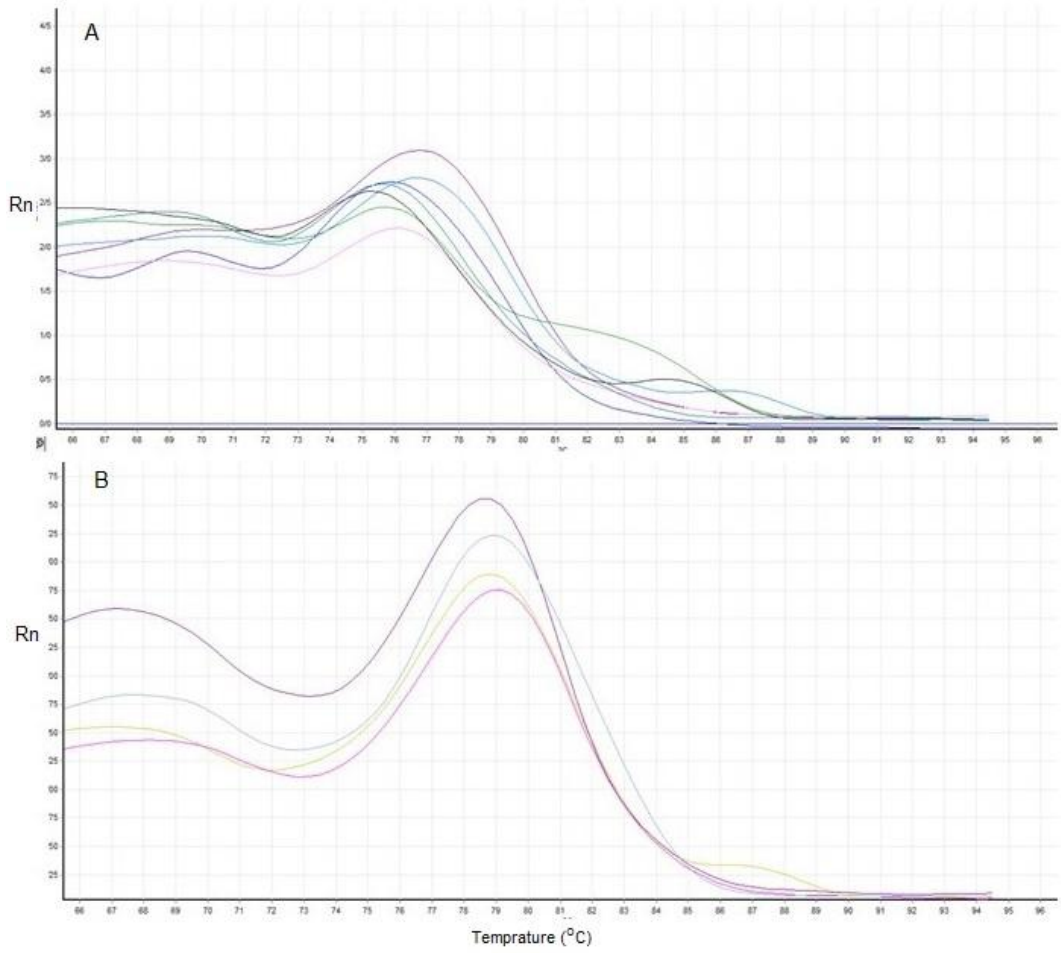


**Figure 3. RT–qPCR melting curve for A) the ACTIN gene with an annealing temperature of 60 °C and B) the DBTNBT gene with an annealing temperature of 56 °C**

**Discussion**

Stimulating metabolic pathways in cultured plant cells and tissues to enhance the production of target molecules is a biotechnological strategy expected to significantly improve the yield of secondary metabolites (Tatsis & O'Connor 2016). In 1983, both biological and non-biological



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elicitors were applied to *T. brevifolia* cell suspension to activate genes related to the biosynthetic pathway of PC production. Non-biological elicitors such as vanadyl sulfate, silver nitrate, and cobalt chloride have been shown to increase PC production in yew cell cultures. Additionally, the use of MeJA, salicylic acid, and fungal elicitors generally increases the output of PCs in yew (Onrubia et al. 2010). Most parts of the PC biosynthetic pathway have been identified in yew, and the expression levels of most genes associated with this pathway have been studied and determined. However, this pathway is still unknown in hazelnuts, with the exception of a few genes involved at the beginning of the pathway.

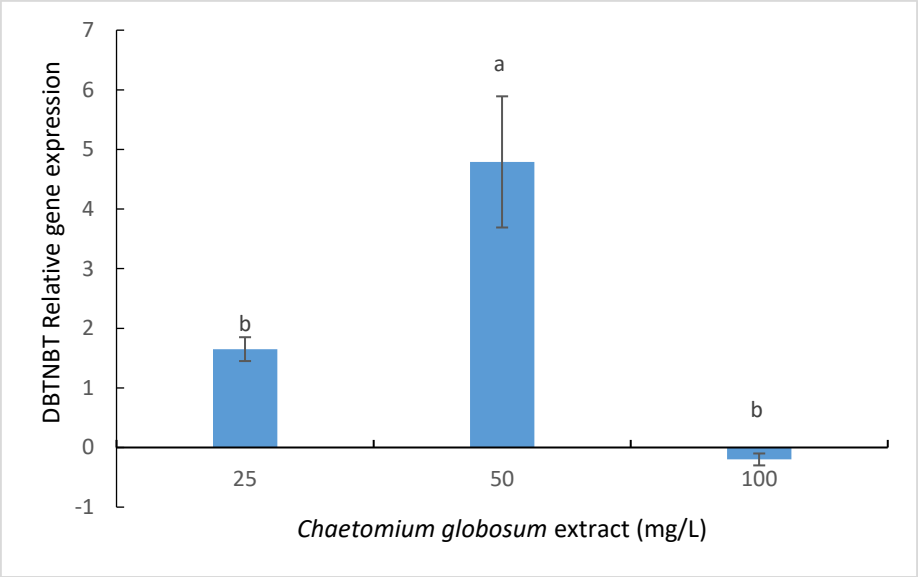


**Figure 4. Relative changes in DBTNBT gene expression at different concentrations of *Chaetomium globosum***

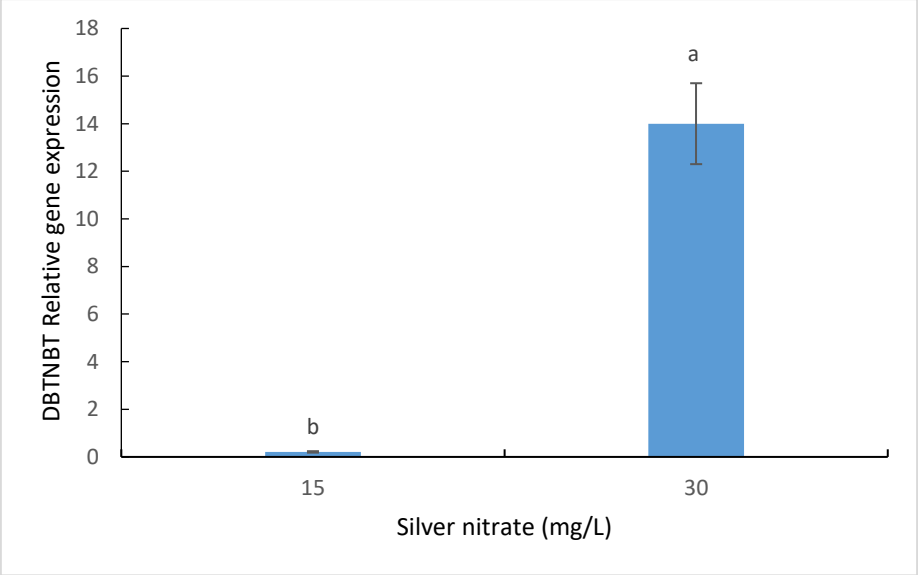




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**Figure 5. Relative changes in DBTNBT gene expression in response to different concentrations of silver nitrate (mg/L)**

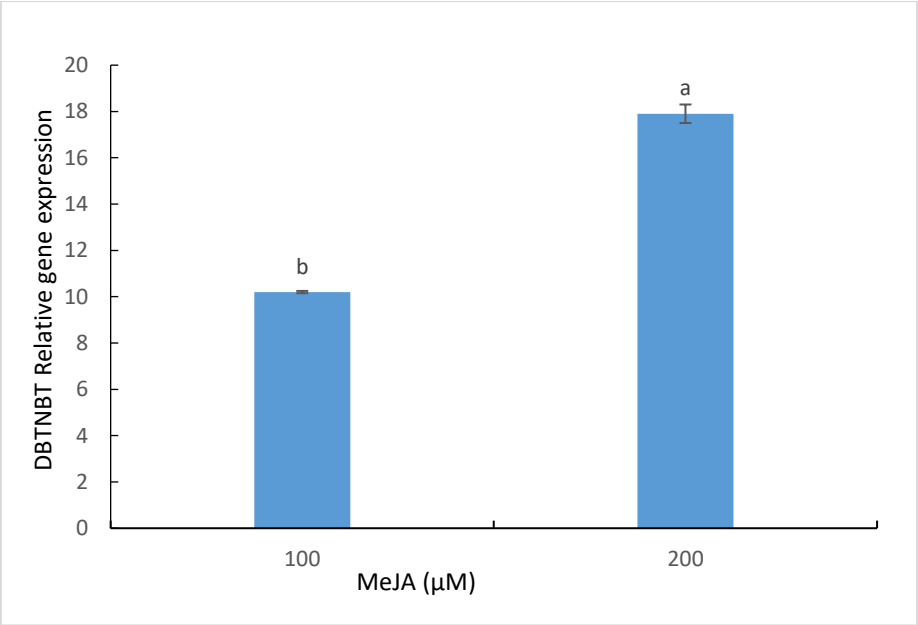


**Figure 6. Relative changes in DBTNBT gene expression with different concentrations of MeJA**

To date, only 2 specific genes, taxadiene-5α-ol-O-acetyltransferase (TDAT) and 3-N-debenzoyl-2-deoxyTaxol-N-benzoyltransferase (DBTNBT), have been identified and isolated from the PC biosynthetic pathway. In this study, alterations in DBTNBT gene expression were examined via hazelnut cell suspension cultures stimulated with live and other living elicitors. The results of this study show that MeJA has a MeJA at a concentration of 200 µM increased DBTNBT gene expression in hazelnut cells by up to 17.6-fold. MeJA increases the expression of the GGPPS gene, which catalyzes the first step of the upstream PC biosynthetic pathway, in Taxus and hazelnut cells (Hefner et al. 1998; Wang & Zhong 2002; Maheshwari et al. 2008). Studies on the expression of key genes of the PC biosynthetic pathway in *Taxus* spp. have shown that MeJA increases the expression of all of these genes, both upstream and downstream of the pathway (Lenka et al. 2015). To be more precise, treatment with MeJA upregulated the expression of upstream genes such as GGPPS, TASY, TXS, and T5aH, and DBBT, BAPT, and DBAT genes downstream (Nims et al. 2006; Onrubia et al. 2010). The use of high doses of MeJA in hazelnuts was more effective at increasing the expression of another early gene in the PC biosynthesis pathway, TDAT (Raeispour Shirazi et al. 2021). In hazelnut suspension culture, the production of taxanes, including PCs, increased after 60 days in response to the influence of MeJA and chitosan (Rezaei et al. 2011a). Furthermore, PC production was increased 16-fold, compared with

that of controls, in *T. baccata* cell culture after MeJA treatment and treatment with several other agents (Khosroushahi et al. 2006). Ethylene is a naturally occurring plant inhibitor that decreases taxon production in cultured yew cells; however, ethylene inhibitors, such as cobalt chloride and silver nitrate, can increase PC production in yew cell cultures (Zhang & Wu 2003). Zhang and Wu (2003) reported that the addition of 20 mM cobalt chloride and 30 mM silver nitrate to cultures of *T. yunnanensis* increased the production of PC. The amount of PC in the cells of *T. baccata* was significantly increased by the addition of large amounts of silver and cobalt nitrate (Khosroushahi et al., 2006). Silver nitrate effectively affects the relationship between two products, bacatin III and PC in cell suspension cultures of *Taxus* spp. (Yuan et al. 2002). In this study, silver nitrate significantly affected the expression of the DBTNBT gene and increased the expression of this gene up to 14-fold. Fungal elicitors are reported to be the most surprising strategy to increase secondary metabolites in plant cell cultures (Stierle et al. 1993; Wang et al. 2012; Tashackori et al. 2018; Salehi et al. 2020). The different and distinct responses of plant cells to fungal elicitors are related to the unique interactions between plants and fungi (Somssich & Hahlbrock 1998). Plant cells contain information in their plasma membrane that identifies fungal pathogens and activates defense systems (Zhao et al. 2005). The identification of irritating substances by receptors in plant cells is the first step in activating the defense system. The specific structure of the receptor guarantees the particular recognition of certain stimuli (Barrett & Heil 2012). Medicinal plants also have specific binding sites on their surface that, upon detection of fungal elicitors, stimulate a defense system and increase the production of secondary metabolites (Zhai et al. 2017). The use of fungal elicitors to increase PC production in *Taxus* ssp. has a long history dating back to the early 1990s (Christen et al. 1991; Strobel et al. 1992). In fact, fungal stimulation is considered one of the most effective methods for enhancing PC production in *Taxus* ssp. cells (Wang et al. 2001; Lan et al. 2003; Zhang et al. 2011). The effects of coculture of *C. avellana* cells with the *Epicoccum nigrum* strain YEF2, an endophytic fungus that produces PC, and the effects of inducers derived from this fungal strain on the production of PC were investigated as a beneficial approach to improve PC production. The results revealed that endophytes, the cooperation of *C. avellana* and *E. nigrum* cells, the amount of fungal inoculum, and the establishment time of the cooperation play important roles in the production of PCs. To achieve maximum production of PCs, the interaction time is crucial (Salehi et al. 2018). At suboptimal fungal concentrations, it appears that not all binding sites are fully saturated with fungi, leading to increased paclitaxel production. In addition, concentrations above the optimal limit can impair the production of paclitaxel. Therefore, the effects of fungal elicitors on paclitaxel production depend on the concentration of the inducer (Salehi et al. 2019). Our results showed that among the three concentrations tested, 5% was the most effective in DBTNBT gene

expression, and a decrease in DBTNBT gene expression was observed at concentrations greater than 5% and concentrations less than 5%, which is similar to Salehi's results concerning PC production in hazelnuts.

**Conclusions:** With the aim of understanding the mechanism of action of elicitors on the expression of key genes of the PC biosynthetic pathway in hazelnut plants, in this study, we investigated, for the first time, the expression of the DBTNBT gene under the influence of a series of elicitors and reported that treatment with silver nitrate at a concentration of 30 mg/L significantly increased the expression of the DBTNBT gene. In addition, the highest DBTNBT gene expression was achieved after treatment with MeJA at a concentration of 200 μM. The analysis of gene expression data revealed that among the 3 different concentrations of *C. globosum* fungal extract (100, 50, and 25 mg/L), 50 mg/L had the highest gene expression (4.75-fold) compared with that of the control.

## Author contributions

The project was designed and supervised by S.A.R. R.B.S performed all the experiments. M.M. cosupervised the research and analyzed the data. S.A.R. analyzed the data and wrote the manuscript. All the authors discussed the results and contributed to the final manuscript

## Data availability statement

The datasets generated and analyzed through the current investigation are available from the corresponding author upon reasonable request.

## Acknowledgements

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## Conflict of interest

The authors declare that there are no conflicts of interest.

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# استفاده از الیسیتورهای مختلف زنده و غیر زنده در کشت سوسپانسیون سلول فندق جهت بررسی تغییرات در بیان یکی از ژن‌های کلیدی در مسیر بیوسنتز تاکسول، N-۳ - Debenzoyl-2-Deoxytaxoln-Benzoyltransferase (DBTNBT)

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## چکیده

**هدف:** پاکلیتاکسل (تاکسول) که داروی طبیعی موثر شیمی درمانی است، برای درمان طیف وسیعی از سرطان ها استفاده می شود. در حال حاضر، فندق (*Corylus avellana* L.) را می توان به عنوان یک منبع ارزان و در دسترس برای تهیه تاکسول دانست. این مطالعه اثرات احتمالی الیسیتورهای مختلف را بر سطوح بیان ژن DBTNBT (3-N-debenzoyl-2-deoxytaxolN-benzoyltransferase) در کشت های سوسپانسیون سلولی فندق بررسی کرد. ژن DBTNBT یک ژن کلیدی درگیر در مسیر بیوسنتز تاکسول است.

**مواد و روش‌ها:** کشت سوسپانسیون سلولی فندق در MS مایع همراه با ۲ میلی‌گرم در لیتر ۲,۴ D-و ۰/۲ میلی‌گرم در لیتر BAP انجام شد. کشت های سلولی فندق با عصاره قارچی *Cheatomium globosum* در غلظت های ۲۵، ۵۰ و ۱۰۰ میلی‌گرم در لیتر ، متیل جاسمونات (MeJA) (۰، ۱۰۰ و ۲۰۰ میکرولیتر) و نیترات نقره (۰، ۱۵ و ۳۰ میلی‌گرم در لیتر) تیمار شدند.

**نتایج:** نتایج حاکی از اثرات مثبت الیسیتورها بر بیان ژن DBTNBT بود. به طوری که بالاترین سطح بیان ژن DBTNBT در کشت سوسپانسیون سلولی فندق تیمار شده با عصاره قارچی *C. globosum* در غلظت ۵۰ میلی‌گرم در لیتر مشاهده شد و سطح

بیان ژن DBTNBT در این غلظت ۴/۷۵ برابر نسبت به شاهد افزایش یافت. به طور مشابه، در کشت سوسپانسیون سلولی فندق با متیل جاسمونات، بیان ژن DBTNBT در غلظت ۲۰۰ میکرولیتر ۱۷/۶ برابر در مقایسه با نمونه های شاهد افزایش یافت. علاوه بر این، هنگامی که غلظت $AgNO_3$ دو برابر گردید و به ۳۰ میلی گرم در لیتر رسید، میزان بیان ژن ۱۴ برابر نسبت به تیمار شاهد افزایش یافت.

**نتیجه گیری:** یافته های این مطالعه نشان می دهد بالاترین میزان بیان ژن DBTNBT مربوط به تیمار MeJA است، با وجود اینکه تمام تیمارهای بکار رفته قادر به افزایش موفقیت آمیز بیان ژن DBTNBT در کشت سوسپانسیون سلولی فندق بودند.

**کلمات کلیدی:** پاکلیتاکسول، تغییرات بیان ژن، *Corylus avellana*، qRT-PCR

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