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Investigation of the methyl jasmonate effect on the Taxol biosynthetic pathway through the expression of DBAT, BAPT, and TS genes

Investigação do efeito do metil jasmonato na via biossintética do Taxol através da expressão dos genes DBAT, BAPT e TS

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Abstract

Taxol is a valuable drug in the treatment of many cancers, which is extracted from plant sources, especially the Taxus plant. The restriction of plant resources has led to new solutions for increasing plant production of taxol. One of these solutions is the use of elicitors such as methyl jasmonate. In the present study, the effect of the elicitation by methyl jasmonate on the expression of genes involved in taxol biosynthesis was evaluated. For this purpose, taxus leaf and stem explants of *Taxus baccata* were elicited with concentrations of 0, 100, 250, and 500 µM methyl jasmonate for 48 and 72 hours, and then the expression of DBAT, BAPT, and TS genes in the different explants, concentrations, and treatment times were investigated using Real-time PCR method. The findings showed that the expression of the three genes under the elicitation of methyl jasmonate increased in the leaves and stems of the taxus plant, and their expression was higher in the leaves than in the stems. The effect of elicitation time was shown that increasing the elicitation time from 48 to 72 hours increases the expression of the three genes. Furthermore, the largest increase in expression for all three genes was observed at 250 µM methyl jasmonate. The results showed that methyl jasmonate can increase the production of taxol in *Taxus baccata* by increasing the expression of some genes involved in the taxol biosynthesis pathway.

Keywords: Bio-elicitor; Gene expression; Methyl jasmonate; Taxol; *Taxus baccata*.

Resumo

O taxol é um medicamento valioso no tratamento de muitos tipos de câncer, extraído de fontes vegetais, especialmente da planta Taxus. A restrição de recursos vegetais levou a novas soluções para aumentar a produção vegetal de taxol. Uma dessas soluções é o uso de eliciadores como o metil jasmonato. No presente estudo foi avaliado o efeito da elicitação por metil jasmonato na expressão de genes envolvidos na biossíntese de taxol. Para tanto, explantes de folha e caule de *Taxus baccata* foram induzidos com concentrações de 0, 100, 250 e 500 µM de metil jasmonato por 48 e 72 horas e, em seguida, a expressão dos genes DBAT, BAPT e TS nos diferentes explantes, concentrações e tempos de tratamento foram investigados usando o método de PCR em tempo real. Os resultados mostraram que a expressão dos três genes sob a elicitação de metil jasmonato aumentou nas folhas e caules da planta taxus, e sua expressão foi maior nas folhas do que nos caules. O efeito do tempo de elicitação mostrou que aumentar o tempo de elicitação de 48 para 72 horas aumenta a expressão dos três genes. Ademais, o maior

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aumento na expressão de todos os três genes foi observado em 250 µM de metil jasmonato. Os resultados mostraram que metil jasmonato pode aumentar a produção de taxol em *Taxus baccata* aumentando a expressão de alguns genes envolvidos na via de biossíntese de taxol.

Palavras-chave: Bio-eliciador; Expressão gênica; Metil jasmonato; Taxol; *Taxus baccata*.

INTRODUCTION

Plants or plant cells under laboratory conditions exhibit a physiological and morphological response to microbial, physical, or chemical agents called "elicitors". The use of elicitors, which is currently receiving the attention of researchers, is regarded as one of the most effective methods for enhancing the synthesis of secondary metabolites in medicinal plants. The accumulation of such metabolites frequently occurs in plants that are subject to stress such as various elicitors or signaling molecules (Huang et al., 2021; Jafarzadeh et al., 2020; Li et al., 2012; Lichota & Gwozdzinski, 2018; Al Khayri & Naik, 2016; Mutanda et al., 2021; Nims et al., 2006; Onrubia et al., 2013; Patel & Krishnamurthy, 2013). Jasmonates are among the important biological elicitors whose effect on stimulating the production of plant secondary metabolites has been reported (Al Khayri & Naik, 2016)

Normally, exogenous methyl jasmonate is used in plant cell culture to activate secondary metabolism, at the same time, studies on its effect on plant growth show that jasmonates have various biological activities such as inhibition of seed germination and growth, inhibition of pollen grains and prevention of root growth (Aslam et al., 2021). Signaling molecules such as salicylic acid (SA), jasmonic acid (JA), and methyl jasmonate (MeJA) are internal regulators that play key roles in plant growth and response to environmental stresses. These signaling molecules are involved in some signal transmission systems and lead to the induction of the activity of special enzymes that catalyze biosynthetic reactions related to the production of defense compounds such as polyphenols, alkaloids, and Pathogenesis-related proteins (often called PR proteins). The result of this process is the induction of defensive responses and the protection of the plant against attacks from pathogenic microbes (Balestrini et al., 2021). These signaling molecules, when used externally, also move along the length of the plant in a certain way and cause the expression of certain defense genes (Malik et al., 2020). Taxol is a compound of diterpene, the complex structure of which is frequently extracted from taxus (Heinig et al., 2013; Mutanda et al., 2021)

Currently, as the most important natural anticancer compound with a different mechanism from other similar medications in this area, it is used effectively worldwide to treat various types of cancer, including breast, uterus, skin, lungs, urinary tract, esophagus, and lymph nodes (Gunaydin et al., 2021; Heinig et al., 2013; Huang et al., 2021; Jafarzadeh et al., 2020; Li et al., 2012; Lichota & Gwozdzinski, 2018). At present, many efforts are being made to synthetic and semi-synthetic production of taxol, as well as its extraction in cell cultures, and each of these methods has specific problems. In view of the abundant use of this substance in medicine as well as the limitation of its production from plant sources, its biosynthesis mechanism should be thoroughly investigated (Fridlender et al., 2015; Sharifi-Rad et al., 2021)

Understanding the pattern and level of expression of the various genes and identifying the main genes involved in taxol biosynthesis may guide us in manipulating the taxol biosynthesis pathway in taxus (Qiao et al., 2020; Zhou et al., 2019). Furthermore, since the taxol biosynthesis pathway has not been fully identified, examination of the gene expression model and proteomic analysis may solve this problem (Shirazi et al., 2021; Takanashi et al., 2019). In this research, assuming the methyl jasmonate as an elicitor leads to an increase in taxol levels in taxus plants, the expression level of genes involved in the biosynthesis of taxols has been studied at methyl jasmonate elicitation.

MATERIAL AND METHODS

Plant material: A plant sample of the yew tree (*Taxus baccata*) was collected from the National Botanical Garden of Iran. A stock solution of methyl jasmonate with a concentration of 1 M was prepared in 70% ethanol and was sterilized using a 0.22 µM filter. Subsequently,

with a specific volume and concentrations of 0, 100, 250, and 500 mM, it was poured into sterile jars and 50 ml of water was added. The stems with leaves that were separated from the taxus tree were placed inside these jars and elicited by methyl jasmonate for 48 and 72 hours. Following elicitation, the samples were collected and dried. In this experiment, two stem and leaf explants were used to estimate the volume of taxol produced.

Evaluation of gene expression: The effect of using methyl jasmonate on the expression of genes encoding the enzymes involved in different steps of the taxane biosynthesis pathway including 10-deacetylbaccatin III-10-β-O-acetyltransferase (DBAT), baccatin III-13-O-(3-amino-3-phenyl propyl) transferase (BAPT) and taxadiene synthase (TS) were evaluated using Real-time PCR. The RNXTM-PLUS kit was used to extract RNA from the stem and leaf explants. To that end, the homogenized plant tissue was mixed with 2 ml of RNXTM-PLUS reagent and vortexed for 5 to 10 seconds. Following 5 minutes of incubation at room temperature, 200 µl of chloroform was added. They were properly mixed for 15 seconds, then incubated for 5 minutes at 4°C. Centrifugation was carried out at 10,000 rpm at a temperature of 4 ºC for 15 minutes and 1 ml of 75% ethanol was added to the remaining precipitate. After a short vortex, centrifugation was repeated at 7500 rpm and 4°C for 8 minutes, and the pellet was dissolved in 50 µl of DEPC water. The AddScript cDNA synthesis kit and the Oligo dT primer were used to synthesize cDNA. Finally, a Real-time PCR reaction using 2 µl of cDNA sample, 1 µl of each primer with a concentration of 10 pmol (Table 1), 12.5 µl of Cybergreen (Amplicon Bio, Korea), and 8.5 µl of nuclease-free water with a total volume of 25 µl was done. The temperature profile initiated with a 10-minute cycle at 95°C, followed by 40 cycles of 15 seconds at 95°C, 30 seconds at 54°C, and 30 seconds at 72°C. The comparative Ct method ($2^{-\Delta\Delta Ct}$) was used to study the relative changes in gene expression. 18SrRNA was considered as a housekeeping gene.

Table 1. The sequences of primers used for gene expression assay

Gene	Forward Primer	Reverse Primer	Amplicon length (bp)
BAPT	ATCCTGCAAAAGTGATTCGAG	ACTCCACTTCAAGTTCCCC	105
DBAT	GCCAGAAGACCCTTTATACCG	TTACTTTCTTCCCTAAGGCAT	147
TS	GGCAGATATAAATTTCACTCGAC	ATATTCGGGTTCAAATGTAGC	73
18SrRNA	GTGCACAAAATCCCGACTCT	GCGATCCGTCGAGTTATCAT	102

RESULTS

Evaluation of BAPT gene expression: Assessment of the BAPT gene expression under the elicitation of methyl jasmonate in the Taxus plant (Figure 1) demonstrated that the expression of this gene was higher in the leaf than in the stem. Moreover, by increasing the elicitation time from 48 hours to 72 hours, more up-regulation was observed. Methyl jasmonate concentration has also been effective in the expression of the gene, and up to 250 µM, the BAPT regulation was dose-dependent. However, at the 500 µM concentration, the gene expression level decreased relative to the 250 µM concentration.

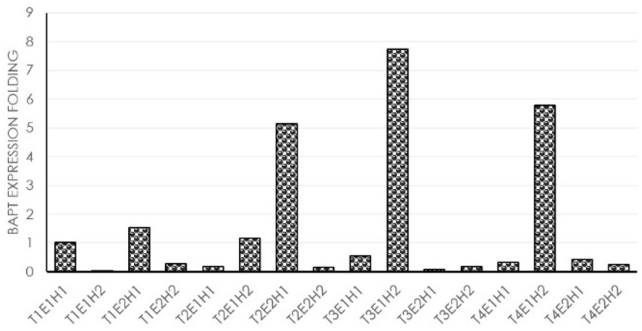

Figure 1. The folding of BAPT gene expression under treatment with methyl jasmonate at different concentrations, times, and explants (E1: leaf, E2: stem, H1: 48 h, H2: 72 h, T1: 0, T2: 100, T3: 250 and T4: 500 µM methyl jasmonate)

Evaluation of DBAT gene expression: DBAT gene expression was investigated in Taxus plants elicited by methyl jasmonate (Figure 2) and the results showed that the DBAT expression level was higher in the leaves than in the stems. The increase in DBAT expression was time-dependent and was higher in the 72-hour elicitation than in the 48-hour elicitation. Also, the highest level of expression of DBAT was observed at the concentration of 250 µM methyl jasmonate.

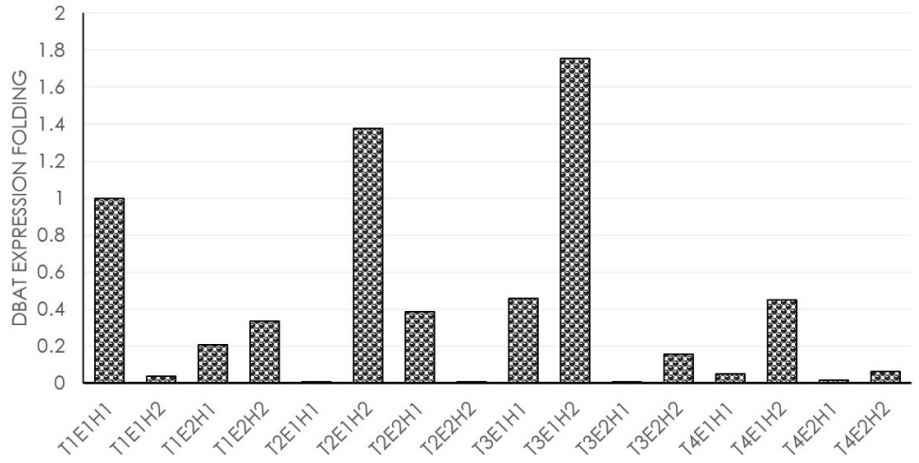

Figure 2. The folding of DBAT gene expression under treatment with methyl jasmonate at different concentrations, times, and explants (E1: leaf, E2: stem, H1: 48 h, H2: 72 h, T1: 0, T2: 100, T3: 250 and T4: 500 µM methyl jasmonate)

Evaluation of TS gene expression: TS gene expression was investigated in Taxus plants elicited with methyl jasmonate (Figure 3). The results showed that TS is more up-regulated in the leaves than in the stems. Also, the over-expression of TS was time-dependent and was higher in the 72-hour elicitation than in the 48-hour elicitation. The highest expression level of TS was observed at the concentration of 250 µM methyl jasmonate.

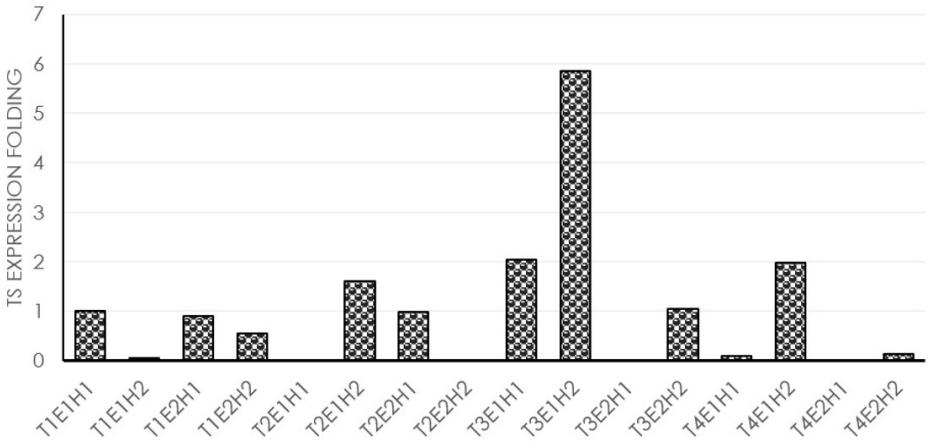

Figure 3. The folding of TS gene expression under treatment with methyl jasmonate at different concentrations, times, and explants (E1: leaf, E2: stem, H1: 48 h, H2: 72 h, T1: 0, T2: 100, T3: 250, and T4: 500 µM methyl jasmonate)

DISCUSSION

Overexpression of genes encoding enzymes involved in taxol biosynthesis is a powerful approach to increasing drug production. Other researchers have pointed to the role of methyl jasmonate as a biological elicitor and its effect on the expression of genes involved in the biosynthesis of secondary metabolites in various plants, which are mentioned below.

Li et al. (2012) analyzed the transcriptional profiles of *T. chinensis* cells 16 hours after MeJA treatment (T16) and mock-treated cells (T0) using RNA-seq to assess the transcriptional changes of Taxus cells in response to MeJA. It was found that 13469 genes were expressed differentially between the two-time points, including all JA biosynthesis pathway genes and genes related to Taxol. MeJA appears to stimulate a large number of genes involved in several related functional categories, such as plant hormone biosynthesis and phenylpropanoid biosynthesis. In addition, many genes encoding transcription factors were shown to respond to MeJA elicitation (Li et al., 2012).

Coronatine is a toxin produced by the pathogenic *Pseudomonas syringae* strain. This compound has recently received attention for its potential as a plant growth regulator and an inducer of secondary metabolism. To better understand the mechanism by which elicitors can affect taxol and related taxane biosynthesis, Onrubia et al. (2013) studied the effect of coronatine (Cor) and methyl jasmonate (MeJA) on Taxus cell culture. The results showed that the total taxane production in the cell suspension was significantly increased by both elicitors, from a maximum level of 8.14 mg/L in the control to 21.48 mg/L (day 12) with MeJA and 77.46 mg/L (day 16) with Cor. Expression analysis showed that tx, t13oh, t2oh, t7oh, DBAT, pam, bata, and dbtnbt genes were variably induced by the presence of elicitors. Notably, although taxane accumulation following induction with MeJA or Cor was quantitatively and qualitatively different, the gene expression induction patterns were similar, suggesting that both elicitors may have similar regulatory mechanisms to increase taxol production (Onrubia et al., 2013).

Using a selected *Taxus x Media* cell line cultured in a two-stage system, Sabater-Jara et al. (2014) studied the relationship between taxane production and transcript profiles of several genes involved in taxol metabolism in the presence of cyclodextrin and methyl jasmonate as elicitors. Gene expression was not clearly increased by the presence of cyclodextrins in the culture medium and was variably induced by methyl jasmonate, but when the culture was affected by both elicitors, a synergistic effect on transcript accumulation was observed. BAPT and DBTNBT genes, which encode the last two transferases involved in the taxol biosynthesis pathway, seem to show overexpression under the influence of both elicitors. Taxol biosynthesis was clearly increased by the combined action of methyl jasmonate and cyclodextrins, reaching production levels 55 times higher than in non-elicited cultures (Sabater-Jara et al., 2014).

In the current study, the expression of genes involved in the taxol biosynthetic pathway increased significantly in cells treated with methyl jasmonate and peaked after 72 hours of treatment. These findings are in accordance with the results obtained by Vongpaseuth & Roberts (2007), who showed that the expression of genes encoding enzymes involved in the first steps of taxol biosynthesis (GGPPS and TX) increased during the first 6 hours of MeJA elicitation and reached its peak two days later (Vongpaseuth & Roberts, 2007).

Nims et al. (2006) also reported that after methyl jasmonate elicitation (100 µM) in the *Taxus cuspidata* P991 cell line, taxol accumulates up to 3.3 mg/L within 7 days after elicitation (Nims et al., 2006). Therefore, elicitation time is an important factor in the expression level of genes involved in taxol biosynthesis.

In general, the results of this research demonstrated that a methyl jasmonate elicitor is an appropriate option for increasing the expression of taxol biosynthetic pathway genes in the *Taxus baccata*. Therefore, the use of elicitors to induce taxol production can be considered as a suitable solution in cell cultures and industrial production of taxol.

CONCLUSION

In this study, the expression of the DBAT, BAPT, and TS genes was assessed in the stem and leaf of the Taxus plant under elicitation with methyl jasmonate. The results showed that methyl jasmonate elicitation leads to overexpression of the genes in the leaves and stems of the Taxus plant and their expression was higher in the leaves than in the stems. The effect of elicitation time was also assessed and it was shown that increasing the elicitation time from 48 to 72 hours increases the expression of the genes. Another factor investigated was the

effect of different concentrations of methyl jasmonate, and the highest up-regulation for all the genes was observed at 250 µM concentration. The results showed that increased methyl jasmonate concentration does not necessarily lead to increased expression of the DBAT, BAPT, and TS genes. Generally, the highest level of gene expression was observed in the elicitation with a concentration of 250 µM and 72 hours and in the leaves of the *Taxus baccata*.

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