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An approach to the molecular mechanism of methyl jasmonate and vanadyl sulphate elicitation in *Taxus baccata* cell cultures: The role of *txs* and *bapt* gene expression

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ABSTRACT

Before the biotechnological production of the anticancer compound taxol can be improved, the mechanism that regulates its biosynthetic pathway needs to be further understood. In this paper we have studied the effect of methyl jasmonate (MeJ) and vanadyl sulphate (VS) on the taxane pattern and transcript profile of two key genes involved in taxol biosynthesis, *txs* and *bapt*, in a selected *Taxus baccata* cell line. Our results showed that MeJ significantly activated both taxol and baccatin III production (4- and 3.6-fold, respectively), whereas VS only enhanced taxol production (also 4-fold). At the same time, MeJ treatment clearly increased the expression of the *txs* and *bapt* genes but the presence of VS in the medium only increased *bapt* gene expression.

The results suggest that the elicitation conditions assayed are good strategies to improve taxol production, and that the elicitors have different and probably interfering mechanisms of action in taxol biosynthesis. To our knowledge, this is the first time that a relationship between the expression of the *txs* and *bapt* genes and the taxane profile has been shown in *T. baccata* cell cultures.

1. Introduction

The biotechnological production of the anticancer agent taxol has become a commercial reality for various companies, but the low productivity of *Taxus* cell cultures remains a problem to be solved [1]. Most attempts to increase the biotechnological production of taxanes, recently summarised [2], have been based on empirical procedures that take into account in-put factors such as culture conditions, immobilization techniques, elicitor treatment, and feeding experiments, and out-put factors such as biomass and taxane production, with few considering what is happening at the molecular level in the targeted *Taxus* cells.

The most successful treatments to enhance taxol production involve supplementing the culture medium with methyl jasmonate (MeJ) in various growth conditions and working with several cell lines derived from different *Taxus* species [3–13]. However, the effect of the elicitor at a molecular level has been scarcely studied [14] and consequently the control of the taxol biosynthetic pathway remains unclear. The only study to investigate transcriptional regulation of taxol biosynthesis in MeJ-elicited *Taxus* cells [15] reports an up-regulation of genes encoding early pathway enzymes and a far lower level of terminal enzyme transcripts (Fig. 1). We have previously reported that the taxane pattern in *Taxus* × *media* cell cultures [16] is altered by elicitors such as MeJ and arachidonic acid (AA), noting for the first time that vanadyl sulphate has similar effects.

It has also been shown that the response of cell cultures to elicitation depends not only on factors related to the elicitor itself (type, concentration, duration of elicitation, etc.) but also on the species, cell line and state of development of the culture. In the taxol biosynthetic process, different elicitors seem to act at different metabolic steps or transcriptional levels [15–17] but this needs to be confirmed by further studies.

Although the taxol biosynthetic pathway is not yet completely elucidated, it is known that the first committed step is the cyclization of geranylgeranyl diphosphate (GGPP) to the taxa-(4,5),(11,12)-diene. The reaction is catalyzed by taxadiene synthase (TXS), a monomeric protein of 79 kDa, purified and characterized by Hezari et al. [18] and its cDNA cloned by Wildung and Croteau [19]. Afterwards, oxygen and acyl groups are added to the taxane core by oxygenation at multiple positions mediated by cytochrome P450 mono-oxygenases. After the formation of a hypothetical polyhydroxylated precursor, benzoylation and acetylation lead to baccatin III (see Fig. 1). Another essential step in

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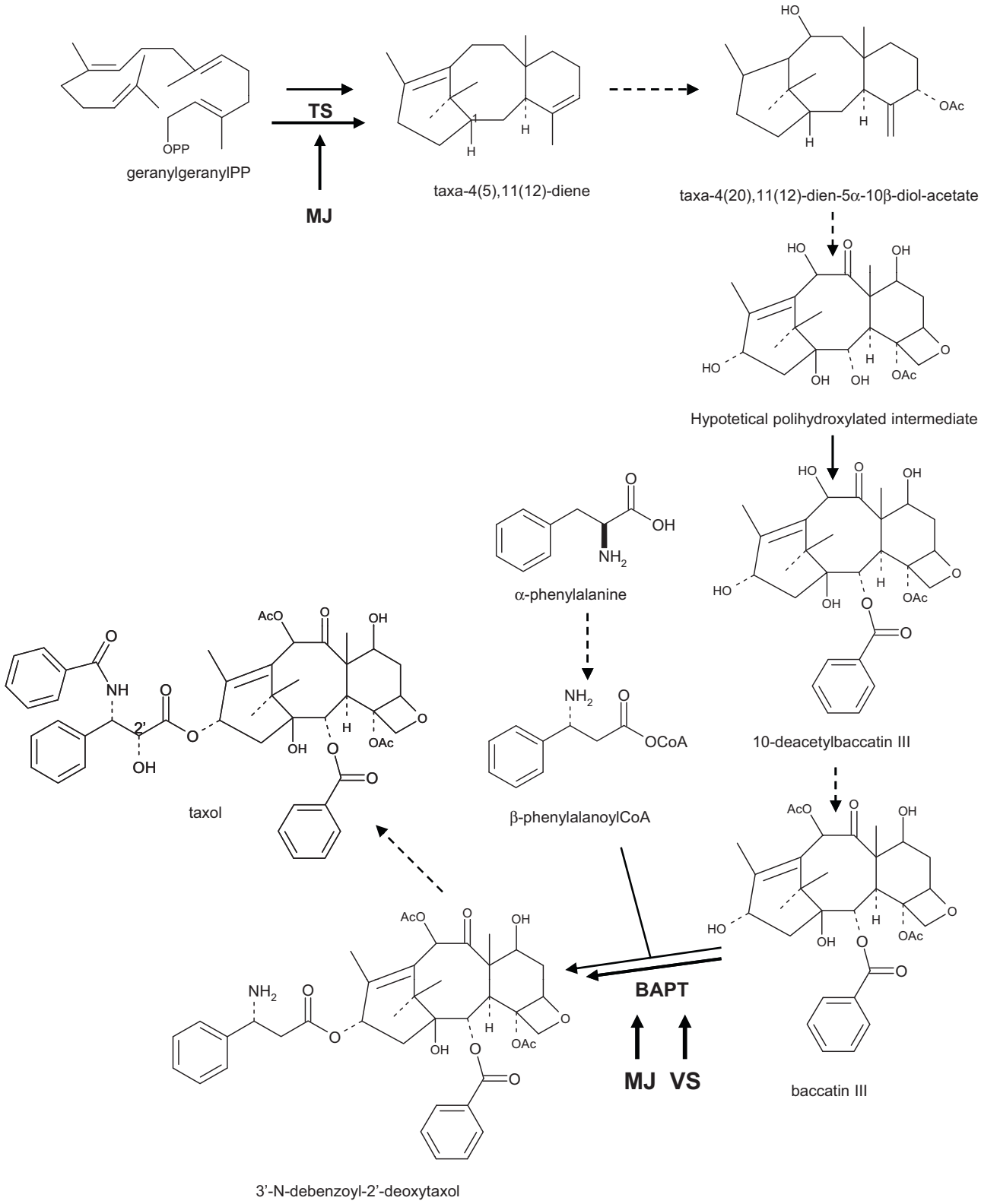

Fig. 1. The positive action of the elicitors MeJ and VS on taxol biosynthesis.

the taxol biosynthesis is the esterification of baccatin III with the β-phenylalanoyl-CoA side chain derived from β-phenyalanine, a reaction catalyzed by C-13-phenylpropanoyl-CoA transferase (BAPT). Subsequent hydroxylation and a new benzoylation yield the final compound, taxol (see Fig. 1) [20].

The main novelties of this manuscript are that, for the first time, the effects of the elicitor vanadyl sulphate as well as the combination of methyl jasmonate and vanadyl sulphate on *Taxus baccata* cells are reported. It is an important novelty to know how vanadyl sulphate affects the expression of two genes codifying