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# Jasmonic acid influence on the activity of *hyp-1* gene in St. John's wort (*Hypericum perforatum*)

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**Key words:** Gene expression, *hyp-1*, Jasmonic acid, St. John's wort

**Abstract**

Considering the importance of *hyp-1* gene function in hypericin biosynthesis in *Hypericum perforatum*, we studied the effect of various concentrations of jasmonic acid on the expression of this gene through qRT-PCR method. In this way, calli from leaf samples of St. John's wort *in vitro* grown plants were treated with three concentrations of jasmonic acid (JA) and the activity of *hyp-1* gene was evaluated via real-time PCR, 24, 48 and 72 hours after treatment. The results showed that in control samples there was no variation in gene expression. The samples treated with 100 µM JA, a time course increase in gene expression was observed. The same trend was seen with 500 µM JA. Accordingly, the maximum *hyp-1* gene expression was witnessed in 72 hours after treatment, while the minimum was in 24 hours. In all evaluated times, increasing the concentration of JA resulted in higher expression of *hyp-1* gene. Moreover, the expression of *hyp-1* 24 hours after increased up to about 1, 1.5 and 3 fold in the 0, 100 and 500 µM JA treatments, respectively. A similar trend was observed after 48 h, and the expression of the gene went higher up to 4 fold in 500 µM JA. After 72 h, a 5.5 fold increase was recorded in the maximum concentration of JA. It can be concluded that with increasing the concentration of JA as well as in time course, a significant increment could be seen in the expression of *hyp-1* gene.

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## Introduction

Various species of *Hypericum* have been used in traditional medicine for curing wounds, inflammation, urinary tract problems, stress and so forth (Filandrinos *et al*., 2006). One of the species, *Hypericum perforatum* because of having medicinally important metabolites such as hypericin and hyperforin, has attracted much of interest between ordinary people as well as scientists (Sirvent and Gibson, 2002). It has been revealed that these compounds can act as inhibitors of the enzymes like monoamine oxidase and catechol-O-methyl transferase. These inhibitors alleviate depression symptoms through increasing the concentration of neuronal transmitters in the central nervous system (Bais *et al*., 2002). Hypercin has also an antiviral activity which affects the enzymes like protein kinase, mono-amine oxidase, telomerase, dopamine-beta-hydroxylase, reverse transcriptase and cytochrome 450.

Chemosynthesis of Hypericin is difficult and not economic. So, the only source of this compound is plant material. In this way, *in vitro* grown plant and tissues can be a good alternative to extract these metabolites (Vardapetyan *et al*., 2006). Utilization of elicitors for stimulating metabolite production pathways in these plants, addition of precursors and optimization of *in vitro* condition are the ways to increase efficiency of *in vitro* metabolite production. Elicitors can of biological or non-biological origin (Zhao *et al*., 2005). These eliciting agents can act through activating genes and enzymes working in the related biosynthetic pathways (Gadzovska *et al*., 2012).

Jasmonic acid is among those compounds that has been used frequently as an elicitor. This organic compound is an endogenous plant hormone that increases plant defense against pathogens and herbivores and stimulates many metabolic pathways. Studies have shown that jasmonic acid and its derivative methyl jasmonate application can lead to the accumulation of secondary metabolites in plants (Gadzovska *et al*., 2007). Hypericin production has also increased using jasmonic acid as an elicitor (Xu *et al*., 2005).

At the moment, the knowledge about medicinal plants genomes and their function is little. Although the manner by which enzymes, signal transducers and other molecules interact remains unknown, yet it is possible to design some experiments to reveal some these interactions. This research is dealing with the effect of one of these molecules, jasmonic acid, on the expression of *hyp-1* gene, which plays a great role in the hypericin production pathway in St. John's wort.

## Materials and methods

*Plant materials*

The seeds of *Hypericum perforatum* cv. New Stem were purchased from Richters Herbs Inc., Canada. In order to get sterile plantlets, the seeds were sterilized and cultured on MS (Murashige and Skoog, 1962) medium and after vernalization for a week, kept in 16/8 photoperiod, 25/22 ± 3°C day/night temperature and light intensity of 70 $\mu Mm^{-2}s^{-1}$ for 6 weeks. Leaf explants from these *in vitro* plants were cut into 3-4 mm stripes and put on the MS medium solidified with agar and containing 1mg/L BAP and 0.5 mg/L IAA in darkness at 25°C. After four weeks, grown calli were cultured in the same condition. Resulted calli were cut into 2-3mm pieces and were treated on the same medium containing different concentrations of jasmonic acid (0, 100 and 500 µM) and kept in darkness at 25°C. For RNA extraction, calli were sampled after 24, 48 and 72 h after treatment, frozen in liquid nitrogen and maintained at -80°C.

*RNA extraction*

Total RNA extraction kit (Jena Bioscience, Germany) was used for RNA extraction based on the kit manual. To remove possible DNA contamination, treatment with DNaseI was done. Quality and quantity of RNA were evaluated with electrophoresis and spectrophotometry.

*Gene expression study*

For cDNA synthesis, 1µg RNA was treated with MMuLV Revert Aid (Fermentas, Poland) based on the company protocol using Oligo-$dT_{18}$ primers. To study the expression of *hyp-1* gene, its specific primers

(forward: CAGGCTGTTTAAGGCATTGGTC and reverse: GGGATGTCCATCAACGAAAGTG) as the target gene and GAPDH gene specific primers (forward: ATGGACCATCAAGCAAGGACTG and reverse: GAAGGCCATTCCAGTCAACTTC) as the internal control gene were used. For qRT-PCR, a Real-Time PCR machine (StepOne, ABI, USA) was used. PCR steps were as one cycle of 94°C for 10 min and 45 cycles of 94°C for 15 s and 60°C for 45 s. Melting curve analysis was carried out from 60°C to 95°C with read intervals of 1 min. Analysis of qRT-PCR data based on two biological and three technical replications was performed through the method $2^{\Delta Ct}$ (Wang *et al*., 2015).

**Result and discussion**

As it can be seen in Fig. 1, in the elicitor free medium, the expression level of *hyp-1* is not changing in the three sampling times. This means that without elicitor there is not any significant change in the activity of this gene during three days. However, in the treated samples with 100 µM jasmonic acid, the expression of the gene shows an increasing pattern in the time course (Fig. 2.). A similar trend was observed in the samples treated with 500 µM jasmonic acid (Fig. 3.). The maximum gene activity was recorded 72 hours after treatment and the minimum of that 24 h after.

Comparing these three figures, it can be concluded that jasmonic acid was effective as an elicitor to stimulate the expression of *hyp-1*. In addition, with increasing time duration after treatment, the gene activity increases as well, so that in both concentrations of jasmonic acid, the highest amount of *hyp-1* activity was witnessed in 72 h after exposure.

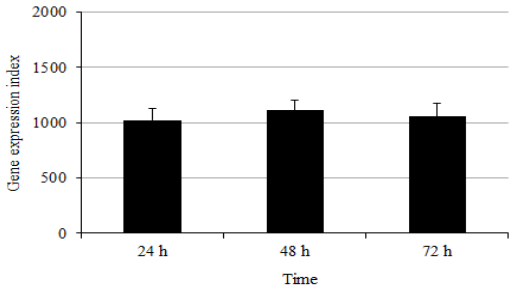


**Fig.1.** The expression pattern of *hyp-1* gene in control samples.

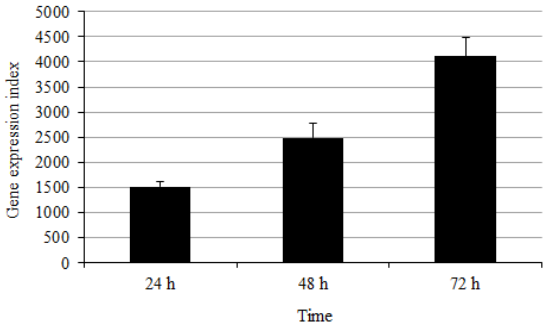


**Fig.2.** The expression indices of *hyp-1* gene in 100 µM jasmonic acid treated callus explants in three sampling times.

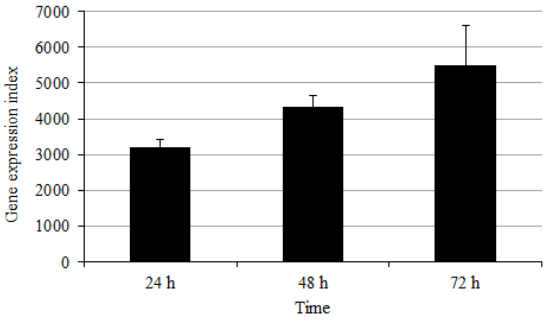


**Fig. 3.** The *hyp-1* gene expression in different sampling times after treating with 500 µM jasmonic acid.

The interesting result was the effect of the concentration of jasmonic acid on the gene expression. The results in the fig. 2 and 3 shows that in all sampling times, 500 µM elicitor could have more influence on the gene expression in comparison to the lower concentration (100 µM).

In fig. 4., it is illustrated that the relative expression of *hyp-1* gene, 24 h after exposure in the two concentrations of jasmonic acid (100 and 500 µM) has been raised in comparison to the control 1.5 and 3 times, respectively. The same trend was observed 48 h after exposure, so that there was a 4-fold increase in the expression of *hyp-1* gene in 500 µM samples compared to the control (fig. 5.).

The effects 72 h after treatment had similar consequences (fig. 6.). The highest amount of jasmonic acid used in this research could increase the gene activity up to 5.5-fold compared to the control.

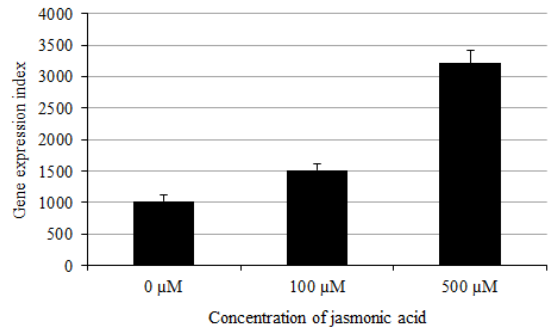


**Fig. 4.** The *hyp-1* gene expression in calli 24 h after treating with 0, 100 and 500 µM jasmonic acid.

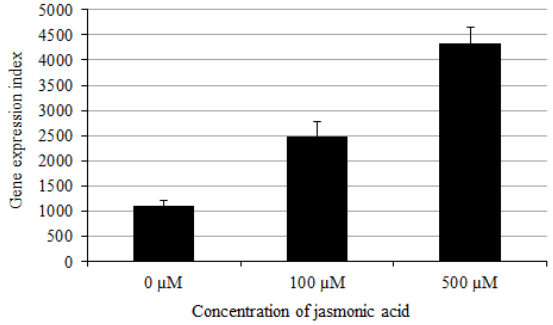


**Fig. 5.** The comparison of *hyp-1* gene activity 48 h after treatment of calli with different concentrations of jasmonic acid.

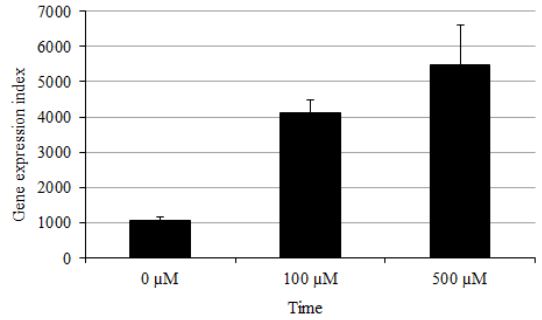


**Fig. 6.** Transcript accumulation rate of *hyp-1* gene 72 h after exposure to different jasmonic acid concentrations.

There are many studies on the production of hypericin and increasing its rate in *Hypericum* genus (Gadzovska *et al*., 2005). Most of these studies show that the capacity of hypericin production in tissue culture is related to the degree of cell differentiation (Vardapetyan *et al*., 2006). Nevertheless, in the case of naphthodianthrones, there are some contradictory reports in the literature. Some groups declare that biosynthesis of bioactive compounds is not possible in undifferentiated cell suspension cultures and calli and that differentiation is necessary for their biosynthesis (Pasqua *et al*., 2003). Dias *et al* (1998) reported that there is no hypericin in calli of St. John's wort *in vitro* plants. Ferrari *et al* (2002) described that xanthones are the only compounds produced in St. John's wort undifferentiated calli in darkness. Kartning and Gobel (1996) observed a significant difference in the maount of hypericin in calli and shoots of this plant. However, the results of Santarem and Astarita (2003) showed a trace amount of hypericin in callus of St. John's wort. Bais *et al* (2002) informed about the possibility of hypericin production in cell masses of St. John's wort. In darkness. Gadzovska *et al* (2007) also presented the plausibility of hypericin and pseudo-hypericin through application of exogenous jasmonic acid.

The question unanswered here is how the biosynthetic pathway of hypericin is being affected in different conditions. It is clear that jasmonic acid acts as a secondary signal transduction molecule form cell to cell through activating phenylalanineammonia-lyase, glutathione s-transferase and chalcone synthases and causes the production of defense related molecules such as tanins and phytoalexins (Zhao *et al*., 2005). The results of this research can put an answer to this question.

The increase on the expression of the *hyp-1* gene in the present research can be an explanation for the results of Gadzovska *et al* (2007) that observed an increase in the production of hypericin in response to jasmonic acid. Nevertheless, how this effect occurs and how jasmonic acid can promote the expression of *hyp-1* gene is yet to be revealed. The roles of other possible paralogs of *hyp-1* in *Hypericum perforatum* that may have complementary roles in the production of hypericin as well as other mediator genes are yet to be elucidated. Furthermore, we could not measure the trace amounts of hypericin in the treated callus cultures to compare them with the gene expression results which can be served as a shortcoming of this research and need to be done in the future.

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