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# The influence of jasmonic acid on induction of *HpPKS1* gene in *Hypericum perforatum* L.

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**ABSTRACT**

Given the importance of *HpPKS1* gene in the biosynthesis of hyperforin in St. John's wort, the effects of different concentrations of jasmonic acid on the expression of this gene was studied in this research. In an MS based medium free of elicitors, the *HpPKS1* gene expression in the time periods of 24, 48 and 72 hours after culture was respectively 1283, 1322 and 1390 that shows a relatively stable situation. In the samples treated with 100 µM jasmonic acid, *HpPKS1* gene expression index after 24, 48 and 72 hours of culture, was almost doubled compared to the elicitor-free samples which clearly indicates a growing trend of gene expression over time. A similar trend was observed 48 hours after treatment. As the concentrations increased from zero to 500 µM jasmonic acid, the relative amounts of gene expression, were respectively 1322, 3077 and 5320. The results observed at 72 hours also showed a similar trend so that zero and 500 µM concentrations of jasmonic acid caused respectively 1390 and 6759, the minimum and maximum amount of *HpPKS1* gene expression in cultures. Based on the above results, it can be concluded that with increasing concentration of jasmonic acid and extending the duration of this treatment in callus culture of St. John's wort *HpPKS1* gene expression index increased.

**Key words:** Gene expression, *HpPKS1*, Hyperforin, *Hypericum perforatum*, jasmonic acid.

## INTRODUCTION

*Hypericum perforatum* is one of important medicinal plants being exploited increasingly in traditional medicine and involves a wide range of secondary metabolites. The quality of *Hypericum* products can be affected by different factors such as pollutants, fungi, bacteria, viruses and insects that are influential in the content of medicinal compounds of the plant (Murch *et al*. 2000). Therefore, it seems necessary to use some alternative methods for producing purer and more confident compounds of this plant. Among these methods are *in vitro* tissue culture and application of different biotic and abiotic elicitors. In nature, plants respond to the invasion of pathogens, insects, and other stresses with activating defense mechanisms including induction of secondary

metabolites such as phytoalexins, hypersensitivity reactions as well as structural defensive barriers (Vascosuelo *et al*. 2007). Elicitors act eventually on the genes encoding enzymes that work in the secondary metabolites pathways. In other words, inducers are considered as signal molecules that activate the signal transduction network and lead to the expression of the biosynthesis-related genes (Gadzovska *et al*. 2002). Jasmonic acid is one of these inducing elicitors that its efficiency in increasing secondary metabolites production has been proved (Raoa and Ravishankar, 2002).

The study of the expression pattern of the genes is an essential tool for understanding how a living organism responds to its environment. Plants are able to react to their environment fast or slowly through changing the expression of their genes (Hazen *et al*. 2003). Based on the literature, the expression of *HpPKS1* genes is related to the concentration of hyperforin in *Hypericum* plants. Transcript accumulation of *HpPKS1* and hyperforin content in floral buds are higher compared to leaves internal and external parts, stem and especially roots. But, in general this gene is more active in the shoots than roots. Because this gene and its final products, hyperforin and hypericin, are involved in repelling herbivores and insects (Beerhues, 2006). However, there is no report, based on our knowledge, on the effect of elicitors on the expression of this gene. In the present research, we are addressing the effectiveness of jasmonic acid as an elicitor in the induction of *HpPKS1* gene in *in vitro* callus tissues.

## 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 surface-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 containing 1 mg/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 2-3 mm 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 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 DNase I was done. Quality and quantity of RNA were evaluated with electrophoresis and spectrophotometry.

**Gene expression study:** For cDNA synthesis, 1 µg RNA was treated with MMuLV RevertAid (Fermentas, Poland) based on the company protocol using Oligo-$dT_{18}$ primers. To study the expression of *HpPKS1* gene as the target gene, its specific primers (forward: 5'-TGTACGTCTCATCCAGTGAGC-3' and reverse: 5'-ACACCACCGTAACAGCCTAAG-3') and gene specific primers of the GAPDH gene as the internal control (forward: 5'-ATGGACCATCAAGCAAGGACTG-3' and reverse: 5'-GAAGGCCATTCCAGTCAACTTC-3') 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 1°C temperature shift. 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).

## RESULTS AND DISCUSSION

The calli were treated with three concentrations of jasmonic acid and samples were taken in three times after treatment. After analyzing the expression level of *HpPKS1* gene compared to the internal control gene, *GAPDH*, the results showed that in the elicitor-free medium the *HpPKS1* gene expression indices after 24, 48 and 72 h was 1283, 1322 and 1390, respectively, without any significant difference. This show that whenever there is no elicitor, the amount of gene transcripts is not changing (figure 1).

While, in the treated samples with 100 µM jasmonic acid, the expression indices of the target gene were 1856, 3077 and 5059 after 24, 48 and 72 h, respectively, which shows an increasing trend in the induction of *HpPKS1* gene with lengthening sampling time (figure 2). The same situation was observed in the calli treated with 500 µM of this elicitor (figure 3).

The maximum amount of gene activity was recorded at 72 h after treatment and the minimum was at 24 h after. Jasmonic acid could act in these treated calli as an efficient elicitor and induced the activation of the *HpPKS1* gene. With delaying the sampling time from 24 h to 72 h after treatment, a discernable increase in the expression of *HpPKS1* gene was witnessed. In both concentrations of 100 and 500 µM, the highest amount of transcript accumulation was seen after 72 h. However, the higher the concentration of the elicitor, the more the activity of the gene.

On the other hand, at all three sampling times, with increasing the concentration of jasmonic acid, we recorded more expression of the *HpPKS1* gene.

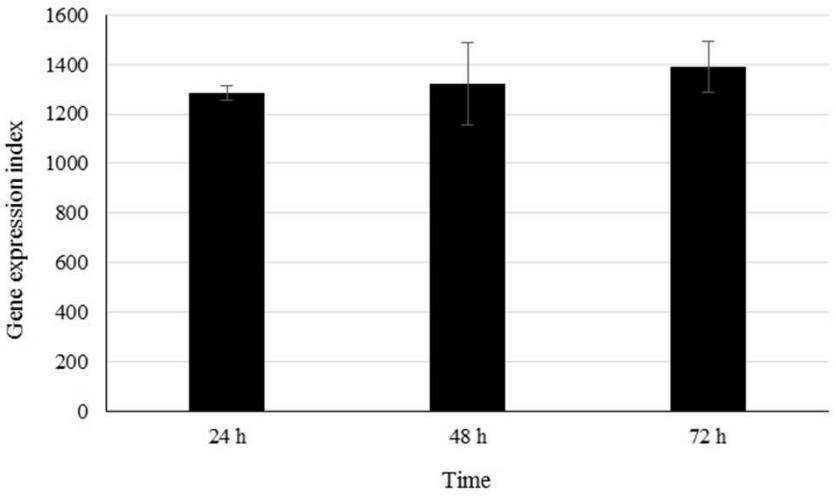


**Fig. 1.** The expression level of *HpPKS1* gene in control calli in time course.

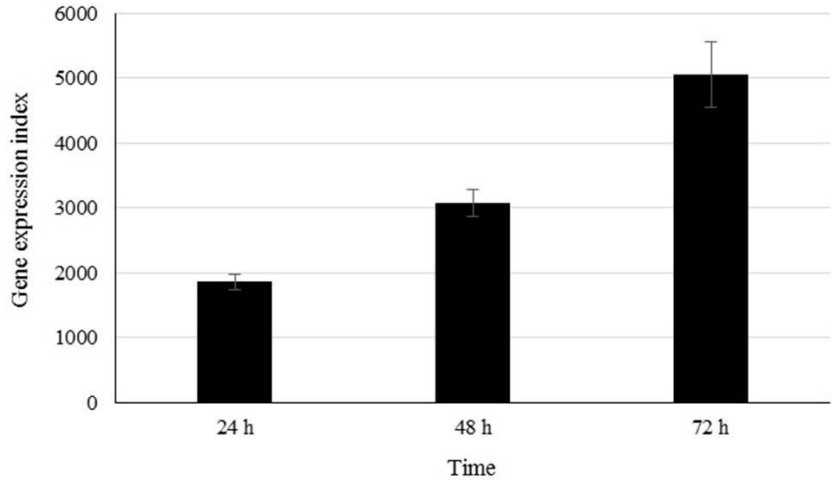


**Fig. 2.** The comparison of *HpPKS1* gene expression under 100 µM jasmonic acid treatment in different times.

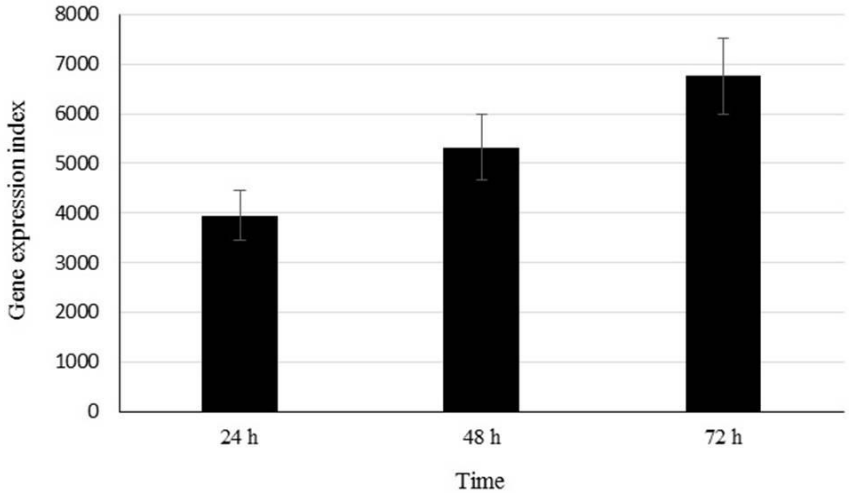


**Fig. 3.** The expression pattern of *HpPKS1* gene in response to 500 µM jasmonic acid in different times in the calli of *Hypericum perforatum* L.

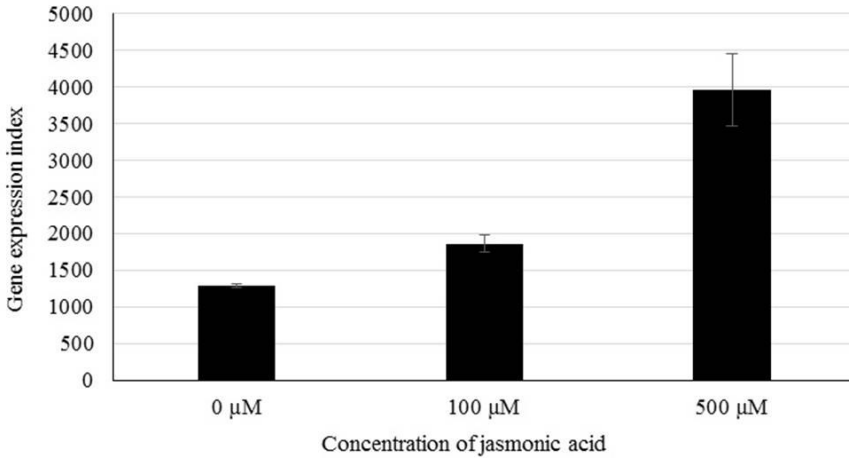


**Fig. 4.** The expression indices of *HpPKS1* gene 24 h after treatment with different concentration of jasmonic acid.

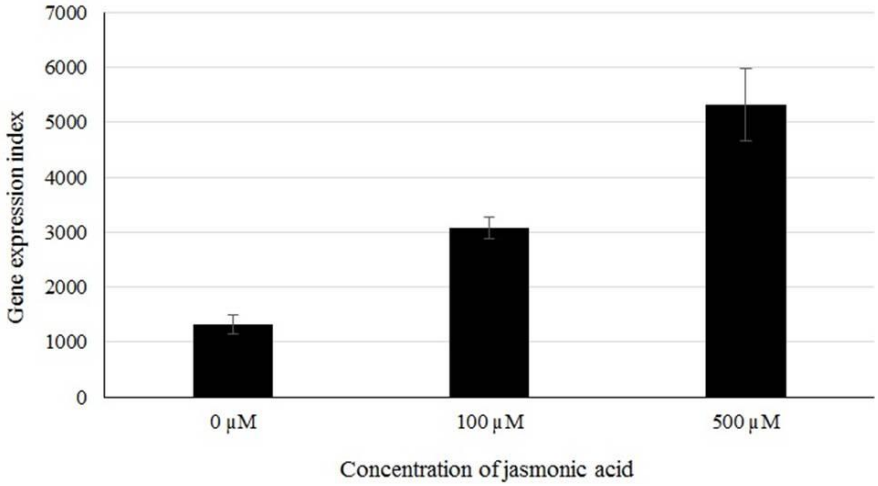


**Fig.5.** The comparison of gene expression levels 48 h after treating *Hypericum perforatum* calli with different concentration of jasmonic acid.

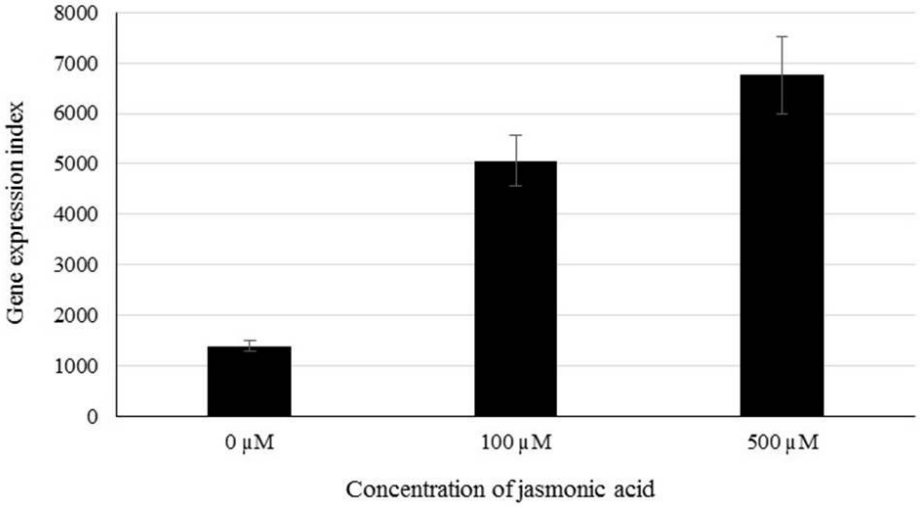


**Fig. 6.** The amount of *HpPKS1* transcript accumulation after 72 h in *Hypericum perforatum* calli treated with different jasmonic acid concentrations.

The figures 4, 5 and 6 illustrate this trend. In figure 4, it can be seen that the expression index 24 h after treatment was 1283, 1858 and 3955 for the concentrations of 0, 100 and 500 µM of jasmonic acid.

Similarly, after 48 h, when the concentration of jasmonic acid increased from 0 to 500 µM, the expression indices of the gene was recorded as 1322, 3077 and 5320, accordingly (figure 5). The same situation was resulted after 72 h. The concentrations of 0 and 500 µM of jasmonic

acid caused the expression indices of 1390, 6759 for *HpPKS1* gene.

Jasmonic acid is a signal molecule that increases the resistance of the plant against pathogens and herbivores through stimulating the production of secondary metabolites. Studies show that jasmonic acid and its derivative methyl jasmonate can cause the accumulation of these the secondary metabolites (Gadzovska *et al*. 2007). *HpPKS1* has proved to be an important gene in hyperforin biosynthesis pathway and its level of expression is in accordance with the amount of hyperforin produced in the plant. The role of hyperforin in plant is yet to be revealed, however based on the fact that its higher amounts has been extracted from the upper parts of the plant, its protective role in plant against invasive organism has been postulated (Karppinen, 2010).

The present research confirms that jasmonic acid can induce the expression of *HpPKS1* gene in a concentration and time dependent manner. Nevertheless, the optimum amount of jasmonic acid for inducing more activity of the *HpPKS1* gene is yet to be evaluated.

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