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Salicylic Acid Increases Transcription of *hyp-1* Gene in Common St. John's Wort

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ABSTRACT: *Hypericum perforatum* is one of the most important medicinal plants which is used in traditional medicine. Considering the importance of *hyp-1* gene function in hypericin biosynthesis we investigated the effect of different concentration of SA on the expression of this gene. In this way, calli from leaf explants were treated with three concentrations of SA and the activity of *hyp-1* gene was evaluated using RT-PCR, 24, 48 and 72 hours after treatment. In control samples there was no variation in gene expression. In the samples treated with 100µM SA, a decrease in gene expression was observed after 24, 48 and 72 hours. The same trend was seen with 250µM SA. The maximum *hyp-1* gene expression was witnessed in 24 hours after treatment while the minimum was in 72 hours. In 24, 48 and 72 evaluated times, increasing the concentration of SA resulted in higher expression of *hyp-1* gene.

Key words: Gene expression, *hyp-1* gene, *Hypericum perforatum*, Salicylic Acid.

INTRODUCTION

Secondary metabolites have various physiological functions in a plant life cycle and are considered as important resources for pharmaceuticals (Kosuth *et al*, 2007). In recent years, studies on plant pharmaceuticals are increasingly conducted to evaluate the effects of these compounds on diseases (Bourgaud *et al*, 2001). Common St. John's wort, *Hypericum perforatum*, has two important metabolites among others, hypericin and hyperforin, that have significant influences on some neurophysiological diseases. The genes involved in the biosynthesis pathways of these compounds have been revealed (Verpoorte and Memelink, 2002). The relationship of some of these genes such as HpPKS1, HpPKS2 and *hyp-1* with the hypericin and hyperforin content has been demonstrated (Karppinen, 2010).

Chemosynthesis of hypericin and hyperforin is not economic and hence plants are the only preferred source of these compounds. There are two options for producing plant material to be used for extraction of secondary metabolites: cultivation in the field and *in vitro* culture. Field production of this plant is vulnerable to environmental clues such as pollutants, fungi, viruses and pests (Murch *et al*, 2000). However, *in vitro* culture is carried out in a controlled environment and is not struggling with those issues (Don-Palmer and Keller, 2010).

There are different methods to increase the yield of secondary metabolites *in vitro* cultures. Among them, application of elicitors is one of the most efficient approaches. Salicylic acid functions as a plant hormone and has been shown to have stimulative effects on these metabolites. It can contribute in signal transduction processes in plants to activate defense responses (Muller *et al*, 1993). Based on our knowledge, there no report on the influence of salicylic acid on the activity of *hyp-1* gene in *Hypericum perforatum*. This research aimed to evaluate the effects of different concentrations of this elicitor on *hyp-1* in callus tissues in time course.

MATERIALS AND METHODS

Hypericum perforatum cv. New Stem seeds were obtained from Richters Herbs Inc., Canada. 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 µMm-2s-1 for 6 weeks. Leaf explants from *in vitro* grown seeds were cut into 3-4 mm stripes and put on the MS medium solidified with agar complemented with 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 kept on the same medium containing different concentrations of salicylic acid (0, 100 and 250 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.

For RNA extraction, total RNA extraction kit (Jena Bioscience, Germany) was used. Treatment with DNase I was performed to remove possible DNA contamination. Quality and quantity of RNA were evaluated with electrophoresis and spectrophotometry.

For cDNA synthesis, 1µg RNA was treated with MMuLVRevert Aid (Fermentas, Poland) based on the company protocol using Oligo-dT18 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 at 1°C intervals. Analysis of qRT-PCR data based on two biological and three technical replications was performed through the method 2?Ct (Wang *et al*, 2015).

RESULTS

After application of three concentrations of salicylic acid on calli, a significant change was observed in the accumulation of *hyp-1* transcripts. In control samples, the variability of gene expression in different sampling times was not discernible and there was no change even 72 h after treating the samples with the elicitor (Fig. 1).

In the samples treated with 100 µM salicylic acid, hype-1 gene expression altered substantially after 24, 48 and 72 h. This alternation was a declining trend in time course and the expression index experienced 0.82 fold diminishing after 48h comparing to 24h expression amount and after 72 h, it was even 0.69 less than that in 48 h (Fig. 2).

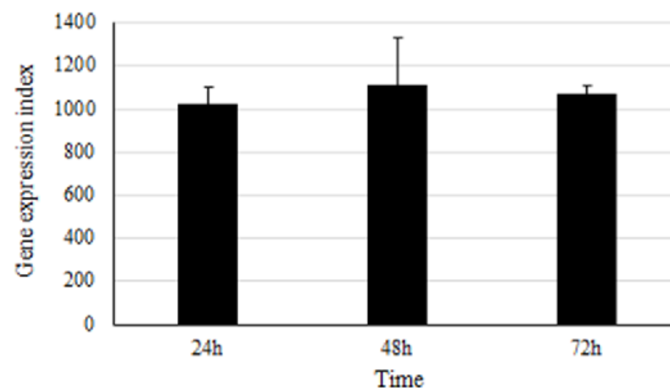

Fig. 1. The expression pattern of *hyp-1* gene in control samples.

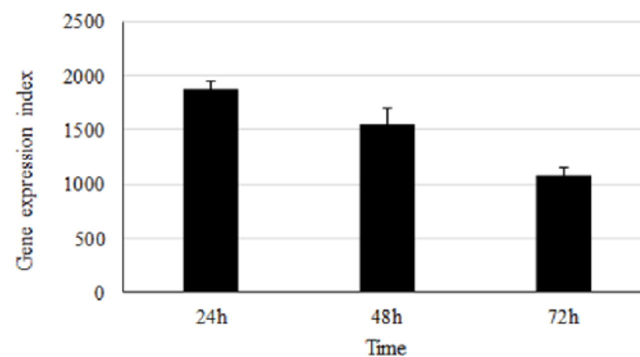

Fig. 2. The *hyp-1* gene expression in different sampling times after treating with 100 µM salicylic acid.

Treatment of the calli with 250 µM salicylic acid had a similar result in the expression of *hyp-1* gene in time course. The expression indices for 24, 48 and 72 h sampling times were respectively 2281, 2013 and 1753 (Fig. 3).

As it has been illustrated in Fig. 4, 24 h after treatment of the calli, the transcript accumulation is rising with increasing the concentration of salicylic acid, so that the highest expression index (2281) was witnessed in the highest exerted concentration (250 µM).

In the sampling time 48 h after exposure to salicylic acid, the *hyp-1* gene induction rate had an increasing slope notably from 0 to 250 µM. The gene expression index changed from 1109 in control calli to 2013 in 250 µM (Fig. 5).

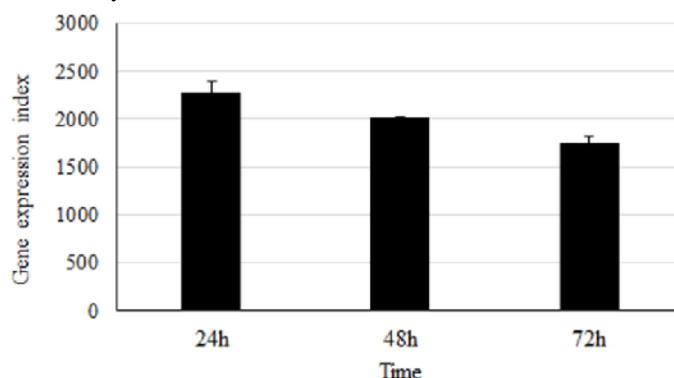

Fig. 3. The expression indices of *hyp-1* gene in 250 µM salicylic acid treated callus explants in three sampling times.

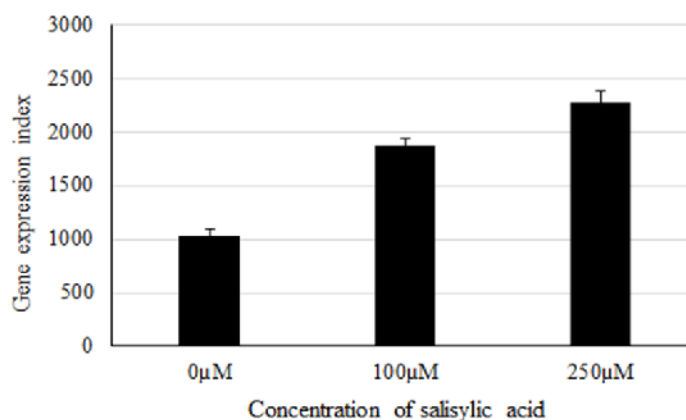

Fig. 4. The comparison of *hyp-1* gene activity 24 h after treatment of calli with different concentrations of salicylic acid.

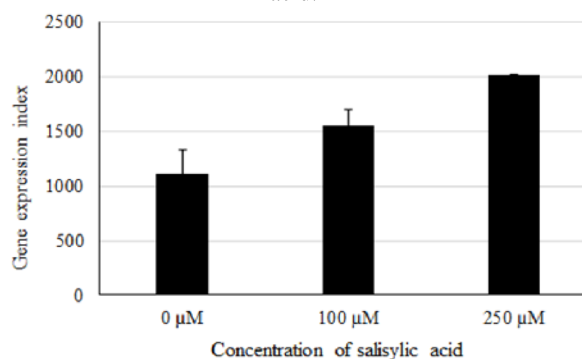

Fig. 5. Transcript accumulation rate of *hyp-1* gene 48 h after exposure to different salicylic acid concentrations.

Fig. 6 demonstrates a dramatic upsurge of *hyp-1* gene induction 72 h after treatment with 250 µM salicylic acid which reached to 1753. There was no noteworthy change in the other two concentrations of this elicitor. It can be concluded that the expression of *hyp-1* in the *in vitro* grown calli of *Hypericum perforatum* is remarkably dependent on the concentration of salicylic acid and the time duration of the exposure of the explants to this compound. The most striking influence was recorded in the 250 µM concentration after 24 h.

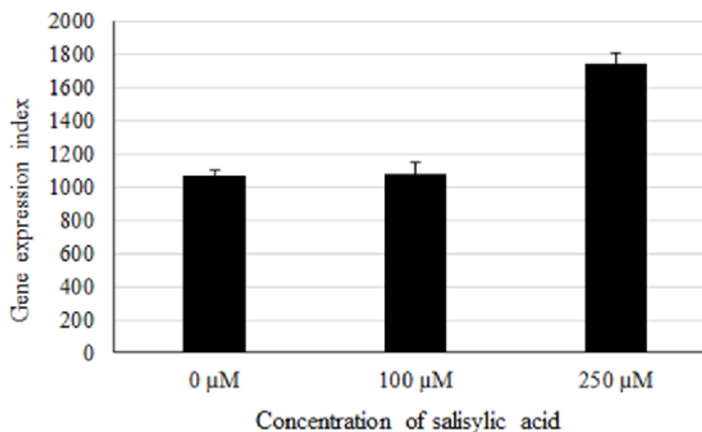

Fig. 6. The *hyp-1* gene expression in calli 72 h after treating with 0, 100 and 250 µM salicylic acid.

DISCUSSION

The results of the present research show that *hyp-1* gene is active in all calli of treated and control samples. This can be a proof for the previous results reporting a trace amount of hypericin production in of *in vitro* grown St. John's wort callus tissues. However, the shortage of this research was the lack of measuring hypericin content in the treated and control callus explants. If we would be able to compare the results of the *hyp-1* gene expression reported here and hypericin content, then this research would contribute more in the elucidation of the mechanism of salicylic acid effect on increasing hypericin content. The only probable explanation of this effect can be the elicitor role in turning on the defense system of the plant and thereafter the activation of secondary metabolite production. This research also shows that exposure time and concentration of the elicitor are two determining factors in the extent of its influence on metabolite production (Horvath *et al*, 2007).

The interesting part of the results of this study was the decrease observed in the expression of *hyp-1* gene after 72 h which can be related to the gradual inactivation of salicylic acid during time may be due to reacting with other molecules (Raskin, I. 1992).

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