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The Effect of 6-Benzylaminopurine on hyp-1gene in *Hypericum perforatum* L.

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ABSTRACT: Considering the importance of hyp-1 gene function in hypericin biosynthesis in *Hypericum perforatum*, we studied the effect of various concentrations of benzylaminopurine (BAP) on the expression of this gene. In this way, calli from leaf explants were treated with three concentrations of BAP and the activity of hyp-1 gene was evaluated via Real Time PCR, 24, 48 and 72 hours after treatment. In control samples there was no variation in gene expression. In the samples treated with 0.5 mg/l BAP, 72 hours after treatment, the expression of hyp-1 gene was significantly increased. In the samples treated with 2 mg/l BAP, a 3-fold increase was resulted in the expression of hyp-1 gene in all sampling times in comparison with control. In this project, the minimum hyp-1 gene expression was after 24 hours in control while the maximum was witnessed in 72 hours after treatment with 2 mg/l BAP.

Key words: BAP, Gene expression, hyp-1 gene, Hypericin, *Hypericum perforatum*,

INTRODUCTION

St. John's wort (*Hypericum perforatum* L.) is an important medicinal plant due to having secondary metabolites like hypericin, pseudohypercin and hyperforin (Figuerio *et al*, 2010). These and other metabolites, despite having many physiological functions in plant cells during its lifecycle, serve as the best sources for phytopharmaceaticals (Kosuth *et al*, 2007).

Staggering amount of work has been performed to produce more metabolites from in and ex vitroplant materials (Vardapetyan *et al*. 2006). To increase the efficiency of metabolite production in cell cultures, culture condition improvement and application of elicitors have been used (Kirakosyan *et al*, 2001). These conditions include culture medium salts, vitamins, amount of sugar, temperature, light intensity and quality, organic compounds, hormones and so forth.

At the moment, the majority of medicinal plants genome is known and the communications among enzymes, internal and external signals and other molecules is yet to be cleared. Understanding the exact mechanism of secondary metabolite biosynthesis and the genes involved is necessary (Deepak *et al*, 2007). There is some literature on the effect of biotic and abiotic factors on hypericin production (Chawla, 2002). However, there is no report, based on our knowledge, about the efficiency of plant growth regulators on the production of hypericin and its biosynthesis pathway genes. In the present research, we are evaluating the influence of 6-benzylaminopurine (BAP) on the expression of the last gene of the pathway (hyp-1) in in vitro grown callus tissues.

MATERIALS AND METHODS

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 medium (Murashige, and Skoog, 1962) 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 these in vitro plants were cut into 3-4 mm stripes and put on the MS medium solidified with agar and 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 BAP (0, 0.5 and 2 mg/l) 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.

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.

For cDNA synthesis, 1 µg RNA was treated with MMuLVRevertAid (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 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°Ct (Wang *et al*, 2015).

RESULTS AND DISCUSSION

Results showed that in BAP free medium, the amount of gene expression was almost constant in different exposure time durations (Fig. 1). Nevertheless, there was a little amount of expression in the calli even without treating with the elicitor.

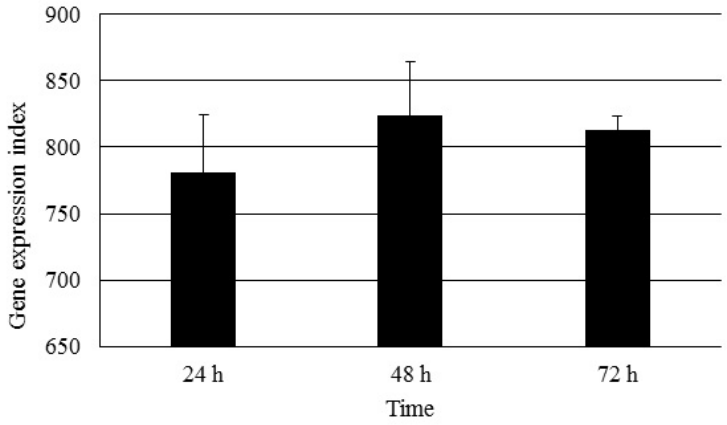

Fig. 1. The comparison of hyp-1 gene expression in the control samples in time course.

In the callus tissues exposed to 0.5 mg/L BAP, although no significant change was observed in the expression of hyp-1 gene after 24 and 48 hours, there was a momentous surge in its expression after 72 hours (Fig. 2). In the calli exposed to 2 mg/l of BAP, from 24 h after, there was a sharp rise in the expression of hyp-1 gene at 48 and 72 h after the exposure, while the expression index did not change from 48 h to 72 h after (Fig. 3). The expression of the gene even after 24 h was 2-fold greater than the control, however. This shows a positive effect of BAP on inducing the hyp-1 gene as early as 24 h after exposure. The amount of increase in the expression indices after 48 and 72h was discernible and reached more than 3-fold in comparison to the control.

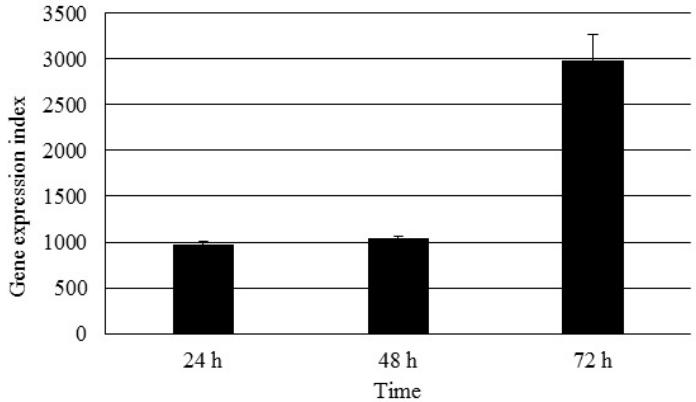

Fig. 2. The expression of hyp-1gene in the calli exposed to 0.5 mg/l BAP in time course.

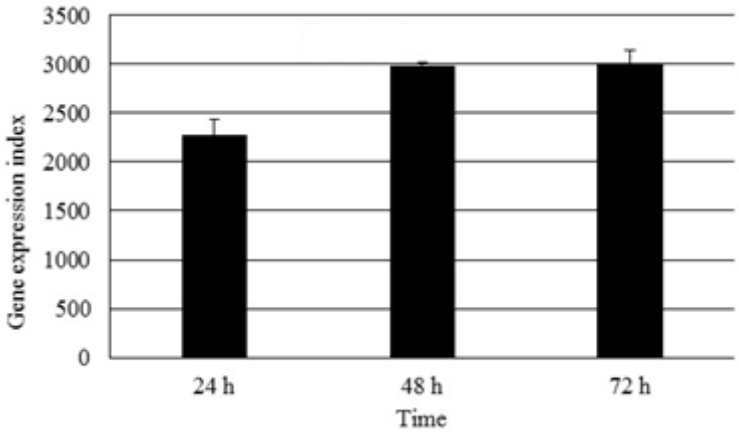

Fig. 3. The expression indices for the hyp-1 gene in 2 mg/l BAP concentrations.

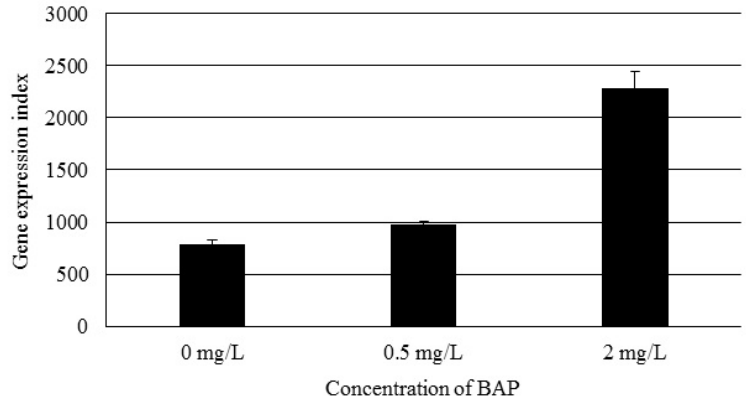

Fig. 4. The hyp-1 gene activity 24 h after exposure to different concentrations of BAP.

The effect of the dose of BAP used in this experiment was considerable. After 24 h, the lower amount of BAP and the zero amount as well, did not increase noticeably the gene expression; nonetheless, 2 mg/l dose of BAP could increase up to 2-fold the hyp-1 expression (Fig. 4). The same trend was witnessed 48 h after exposure with a 3-fold upsurge in the highest dose of BAP (Fig. 5).

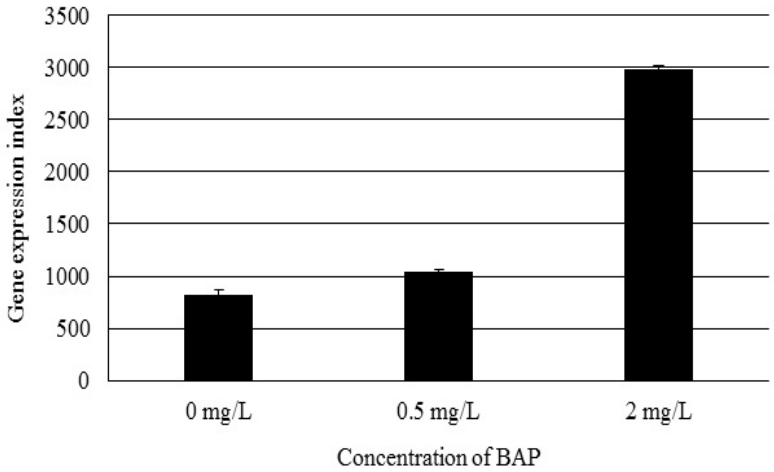

Fig. 5. Transcript accumulation in the calli exposed to different BAP dosages 48 h after treatment.

While the time passed, the effect of different doses of BAP was not so noteworthy. After 72 h, the expression of hyp-1 gene was almost the same in 0.5 and 2 mg/l doses but 3-fold greater than that in the control, 0 mg/l (Fig. 6). Comparing figures 5 and 6, it is clear that the expression of hyp-1 gene in the 2 mg/l dose of BAP has its maximum in this research earlier than 0.5 mg/l dosage. The least amount of expression was observed in 0 mg/l BAP and after 24 h. All these results show that time and dose, both seem to be effective on the expression of hyp-1 gene in calli under BAP exposure.

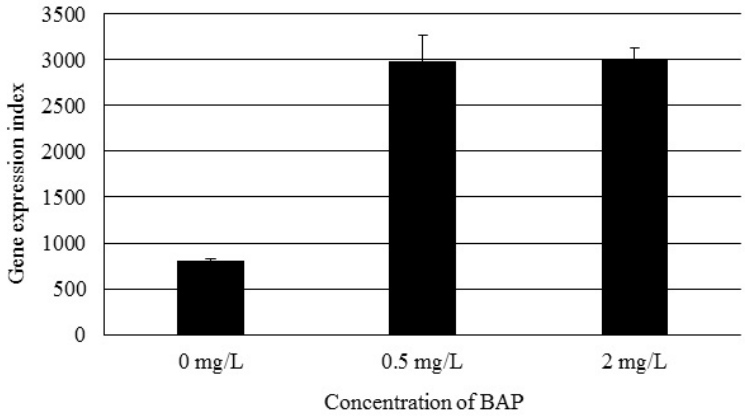

Fig. 6. The amount of mRNA level in callus tissues 72 h after exposure to BAP.

There are different reports on the production of naphthodianthrones in callus and cell cultures. Some studies put this forward that biosynthesis of bioactive compounds such as hypericin and hyperforin is not possible in undifferentiated tissue like callus and cell cultures and differentiation is a necessity for a cell to be able to synthesize these metabolites (Pasqua *et al*, 2003; Dias *et al*, 1999). Contrarily, some researchers have reported the existence of naphthodianthrones in calli (Gadzovska *et al*, 2005). There are evidences that the only compounds produced in undifferentiated callus cultures of *H. perforatum* are xanthones (Ferrari *et al*, 2002). On the other hand, a research team has announced that they have been able to produce hypericin in cell suspension culture (Kirakosyan and Kaufman, 2002). However, other studies showed that the amount of hypericin in callus is considerably lower than that in the whole plant (Rani *et al*, 2001). Approving this, there is another document reporting that the highest amount of hypericin has been observed in shoots and plantlets of *H. perforatuim*, while this amount was so little in calli (Santarem and Astaria, 2003).

Production of hypericin in cell masses and clumps has been possible in darkness (Bais *et al*, 2002). Elicitors have frequently been demonstrated efficient in the induction of in vitro production of hypericin in meristem cultures (Sirvent and Gibson, 2002). These contrasting results can be due to different conditions of tissue culture, age of the callus, light quality and quantity, genotype and phytohormones used in the media (Gadzovska *et al*, 2005).

In conclusion, we could show that time and concentration of BAP, a plant growth regulator, are both determining factors in the effectiveness of BAP in increasing hyp-1 gene expression and possibly hypericin production. Mutual analysis of the gene expression and secondary metabolite content is duly recommended.

REFERENCES

Bais, H.P., Walker, T.S., McGrew, H.J. & Vivanco, J.M. (2002). Factors affecting the growth of cell suspension cultures of *Hypericum perforatum* L. (St. John´s wort) and production of hypericin. *In Vitro Cellular & Developmental Biology - Plant*. **38**: 58-65.

Chawla, H.S. (2002). Introduction to plant biotechnology. 2nd Ed. Science publishers Inc, USA.

Deepak, S.A., Kottapalli, K.R., Rakwal, R., Oros, G., Rangappa, K.S., Iwahash, H., Masuo, Y. &Agrawal, G.K. (2007). Real-time PCR: Revolutionizing detection and expression analysis of genes. *Current Genomics.* **8**(4): 234-235.

Dias, A.C.P., Seabra, R.M., Andrade, P.B. & Ferreira, M. (1999). The development and evaluation of HPLC-DAD method for the analysis of the phenolic fractions from in vivo and in vitro biomass of *Hypericum* species. *Journal of Liquid Chromatography and Related Technology*. **22**: 215-227.

Ferrari, F., Pasqua, G., Monacelli, B. &Botta, B. (2002).Xanthones from calli of *Hypericum perforatum*. *Natural Product Research*. **19**: 171-176.

Figuerio, A.A., Correa, C.M., Astaria, L.V., Santarem, E.R. (2010). Long-term maintenance of in vitro cultures affects growth and secondary metabolism of St. John´s wort. *Ciencia Rural*. **40**: 2115-2121.

Gadzovska, S., Maury, S., Ounnar, S., Righezza, M., Kascakova, S., Refregiers, M., Spasenoski, M., Joseph, C. & Hagege, D. (2005). Identification and quantification of hypericin and pseudohypericin in different *Hypericum perforatum* L. in vitro cultures. *Plant Physiology Biochemistry*. **43**: 591-601.

Kirakosyan, A., Hayashi, H., Inoue, K., Charchoglyan, A., Vardapetyan, H. & Yammoto, H. (2001). The effect of cork pieces on pseudohypericin production in cells of *Hypericum perforatum* L. Shoots. *Russian Journal of Plant Physiology*. **48**: 816-819.

Kirakosyan, A. & Kaufman, P. (2002). Natural products in the New Millennium: Prospects and Industrial Applications. Kluwer Academic Publications, Netherlands, p. 375-389.

Kosuth, J., Katkovcinova, Z., Olexova, P. & Cellarova, E. (2007).Expression of the hyp-1 gene in early stages of development of *Hypericum perforatum* L. *Plant Cell Reports*. **26**: 211-217.

Pasqua, G., Avato, P., Monacelli, B., Santamaria, A.R. & Argentieri, M.P. (2003). Metabolites in cell suspension cultures, calli and in vitro regenerated organs of *Hypericum perforatum* cv. Topas. *Plant Science*. **165**: 977-982.

Rani, N.S., Balaji, K. & Veeresham, C. (2001). Production of hypericin from tissue culture of *Hypericum perforatum*. *Indian Journal of Pharmaceutical Sciences.* **63**(5): 431-433.

Santarem, E.R. & Astaria, L.V. (2003). Multiple shoot formation in *Hypericum perforatum* L. and hypericin production. *Brazilian Journal of Plant Physiology*. **15**: 43-47.

Sirvent, T. & Gibson, D. (2002). Induction of hypercins and hyperforin in *Hypericum perforatum* L. in response to biotic and chemical elicitors. *Physiological and Molecular Plant Pathology*. **60**: 311-320.

Vardapetyan, H.R., Oganesyan, A.A., Kabasakalyan, E.E. & Tiratsuyan, S.G. (2006). The influence of some elicitors on growth and morphogenesis of *Hypericum perforatum* L. callus cultures. *Russian Journal of Developmental Biology*. **37**: 350-353.

Wang, Y., Feng, G., Wang, J., Zhou, Y., Liu, Y., Shi, Y. & Li, Z. (2015). Differential effects of tumor necrosis factor-α on matrix metalloproteinase-2 expression in human myometrial and uterine leiomyoma smooth muscle cells. *Human Reproduction*. **30**(1): 61-70.