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Effect of methyl jasmonate, salicylic acid and yeast extract on secondary metabolites production in *Arnica montana* tissue cultures

Magdalena Sozoniuk[1], Maria Petrova[2], Maria Geneva[2], Antoaneta Trendafilova[3], Kiril Mishev[2], Kamelia Miladinova-Georgieva[2], Viktoria Ivanova[3]

[1] Institute of Plant Genetics, Breeding and Biotechnology, University of Life Sciences in Lublin, Akademicka Street 15, 20-950 Lublin, Poland;
[2] Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Acad. G. Bonchev Street, Bldg. 21, 1113 Sofia, Bulgaria;
[3] Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Acad. G. Bonchev Street, Bldg. 9, 1113 Sofia, Bulgaria

E-mail: magdalena.sozoniuk@up.lublin.pl

INTRODUCTION

Arnica montana is a pharmacologically important species known for its accumulation of sesquiterpene lactones (STLs), which represent the principal bioactive constituents responsible for its therapeutic properties. The STLs are secondary metabolites derived from the mevalonate pathway and play a central role in plant defense. The main STLs in *A. montana* are pseudoguaianolide-type sesquiterpene lactones, specifically helenalin (H) and 11α,13-dihydrohelenalin (DH). The biosynthesis of secondary metabolites in plants can be enhanced by elicitation, a strategy employing biotic or abiotic signals to activate defense-related metabolic pathways. Among the most studied elicitors are methyl jasmonate (MeJA), salicylic acid (SA), and yeast extract (YE), each known to activate different signaling cascades. MeJA is closely linked to jasmonate-mediated stress responses, SA is central to systemic acquired resistance, and YE provides complex biotic signals. Understanding their comparative effects is crucial for optimizing secondary metabolite production in plant systems and improving their biotechnological applications. The aim of this study was to explore the influence of these elicitors on STL accumulation in *A. montana* tissue cultures and provide insights into the regulation of their production in this species.

MATERIAL AND METHODS

The source material for setting up the experiment comprised *A. montana* L. seeds obtained from the experimental plots of the Department of Industrial and Medicinal Plants of the University of Life Sciences in Lublin (Poland). Shoot cultures were induced from sterile germinated seeds. Nodal segments (1 cm in length) were isolated from two-month-old in vitro cultures and were utilized as explants for elicitor treatment studies. Following concentrations of elicitors were tested: for MeJA and SA - 50, 100 or 200 μM, for YE - 50, 100 and 200 mg/l. Yeast extract (YE) and salicylic acid (SA) were medium components for the whole period of the cultivation (5 weeks). For the trials with methyl jasmonate, four-week-old shoots grown on control MSB0.5 medium were transferred for 3 and 7 days to MSB0.5 medium supplemented with MeJA.

Qualitative sesquiterpene lactones analysis in *A. montana* shoots was performed via HPLC-DAD-ESI-MS using Shimadzu LC-2040C 3D Nexera-i and a Shimadzu LCMS 2020 (single quadrupole). Quantitative HPLC analysis was performed on a Shimadzu Nexera-I LC2040C 3D Plus liquid chromatograph equipped with PDA detector.

The following genes were selected for RT-qPCR analysis: *FDS*, *GAO*, and *GAS* (main genes involved in sesquiterpene lactones biosynthesis), as well as *HMGCR*, *IDI*, *DXS*, and *DXR* (genes involved in the biosynthesis of terpene precursors from MVA and MEP/DOX biosynthetic pathways).The RNA extraction was performed with the use of TRIzol-based procedure. The RT-qPCR reactions were performed with PowerUp™ SYBR™ Green Master Mix (Applied Biosystems™) on QuantStudio 3 System.

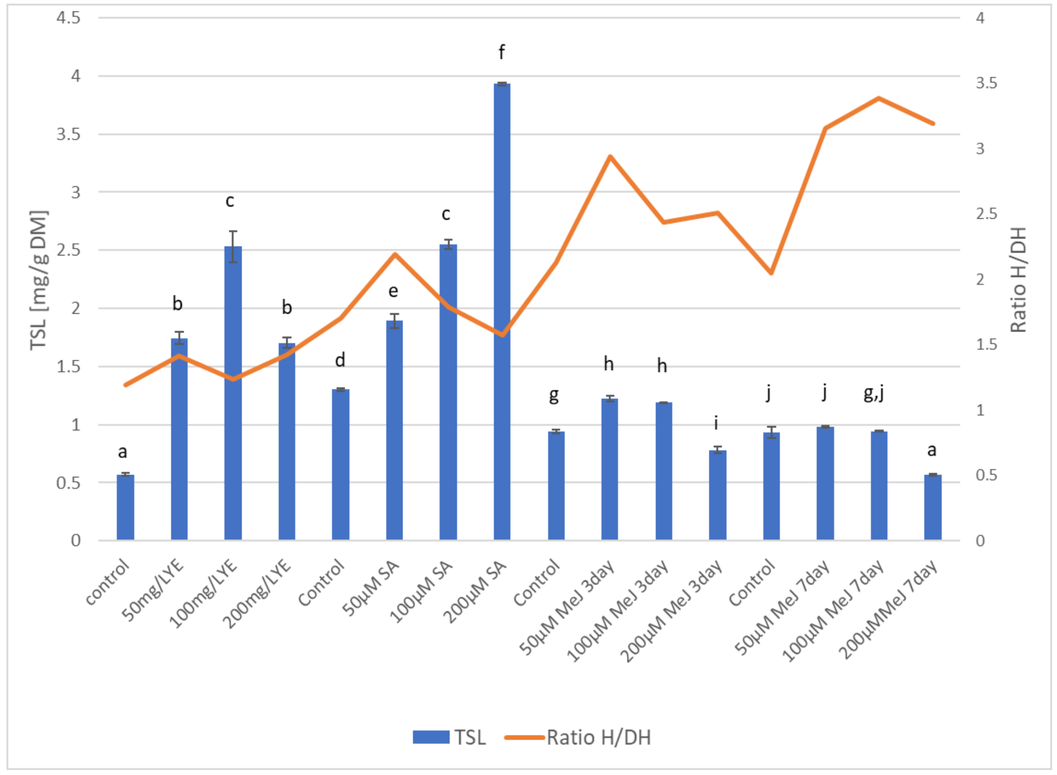

Fig. 1. Total SL content in *in vitro* micropropagated *A. montana* shoots and ratio H/DH (ratio of detected Helenalin- and Dihydrohelenalin-type lactones). Values are means ±SD. Different letters indicate significant difference ($p \leq 0.05$, t-test).

RESULTS AND CONCLUSIONS

Yeast extract and salicylic acid exerted a stronger influence on sesquiterpene lactone biosynthesis compared to methyl jasmonate. Among the tested conditions, treatment with 100 mg/L yeast extract produced the most pronounced effect, leading to an almost 4.5-fold increase in STL content in in vitro shoots relative to untreated controls. Salicylic acid also enhanced sesquiterpene lactone production in *in vitro* shoots. STL content increased with rising SA concentration, reaching the highest level at 200 μM, which corresponded to a nearly 3-fold increase compared to the control. In shoots treated with methyl jasmonate, a slight increase in sesquiterpene lactone (STL) content was observed on day 3 at 50 μM and 100 μM, while 200 μM resulted in a decrease compared to the control. By day 7, STL levels had declined in all treated samples.

The transcript level analysis showed distinct expression patterns of the genes involved in sesquiterpene lactone biosynthesis upon elicitor treatments. In general, a similar pattern of expression was observed in both YE- and SA-treated shoots. They displayed strong upregulation of key genes (*GAS* and *GAO*) involved in the final steps of STLs biosynthesis. Moreover, YE elicitation resulted in increased transcription of *FDS*, which provides the universal C15 precursor for sesquiterpenes. On the other hand, SA-treatment led to induction of *HMGCR*, which is the key rate-limiting enzyme in the mevalonate (MVA) pathway. The shoots treated with MeJA exhibited the least pronounced changes in gene expression. None of the treatments had an effect on the expression of genes associated with MEP/DOXP pathway (*DXR* and *DXS*) that were analyzed. Gene expression data support the quantitative HPLC findings and provide insight into the regulation of secondary metabolism in *Arnica* in response to different elicitor treatments.

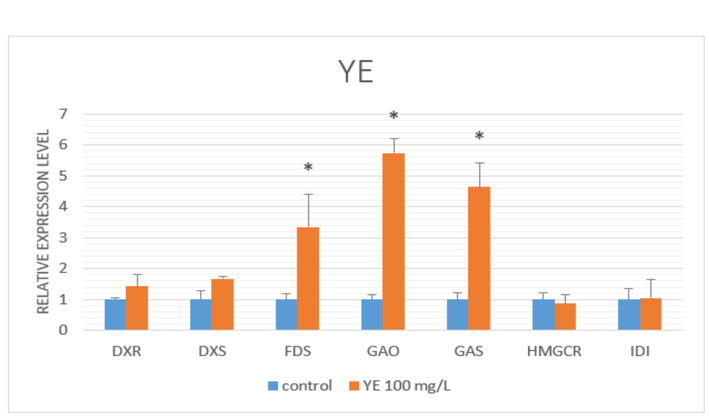

Fig. 2. The relative expression profiles of genes-of-interest under elicitation conditions, where highest levels of STLs were found. Data represents mean ± SD (n=3), asterisk represents significant difference ($P < 0.05$, student's t-test). Abbreviations:
DXR (1-deoxy-D-xylulose-5-phosphate reductoisomerase, EC 1.1.1.267),
DXS (1-deoxy-D-xylulose-5-phosphate synthase, EC 2.2.1.7),
FDS (farnesyl diphosphate synthase, EC 2.5.1.92),
GAO (germacrene A oxidase, EC 1.14.14.95),
GAS (germacrene-A synthase, EC 4.2.3.23),
HMGCR (hydroxymethylglutaryl-CoA reductase, EC 1.1.1.34),
IDI (isopentenyl-diphosphate Delta-isomerase, EC 5.3.3.2).

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