ACCUMULATION OF PHENOLIC COMPOUNDS IN *ARNICA MONTANA IN VITRO* PLANTLETS AFTER TREATMENT WITH METHYL JASMONATE

Maria Petrova[1]*, Maria Geneva[1], Kamelia Miladinova-Georgieva[1], Magdalena Sozoniuk[2], Kiril Mishev[1], Mariana Sichanova[1], Lyudmila Dimitrova[1], Margarita Dimitrova[1], Viktoria Ivanova[3], Antoaneta Trendafilova[3]

[1]Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Acad. G. Bonchev Street, Bldg. 21, 1113 Sofia, Bulgaria

[2]Institute of Plant Genetics, Breeding and Biotechnology, University of Life Sciences in Lublin, 20-950 Lublin, Poland

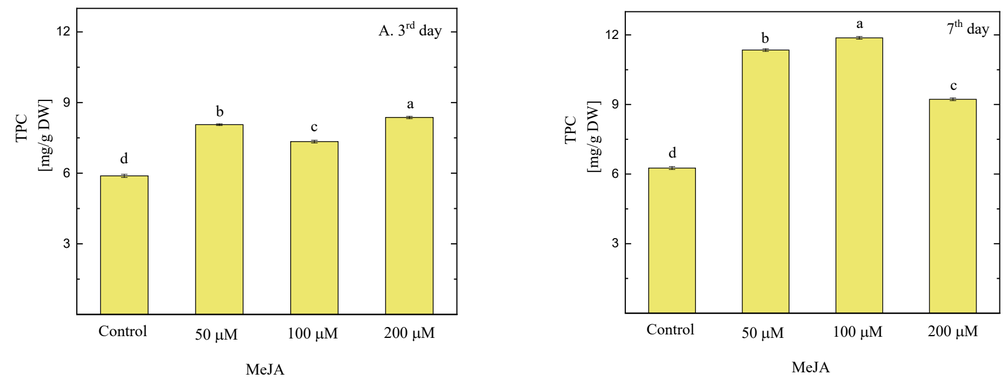
[3]Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Acad. G. Bonchev Street, Bldg. 9, 1113 Sofia, Bulgaria

*Presenting Author: marry_petrova@yahoo.com

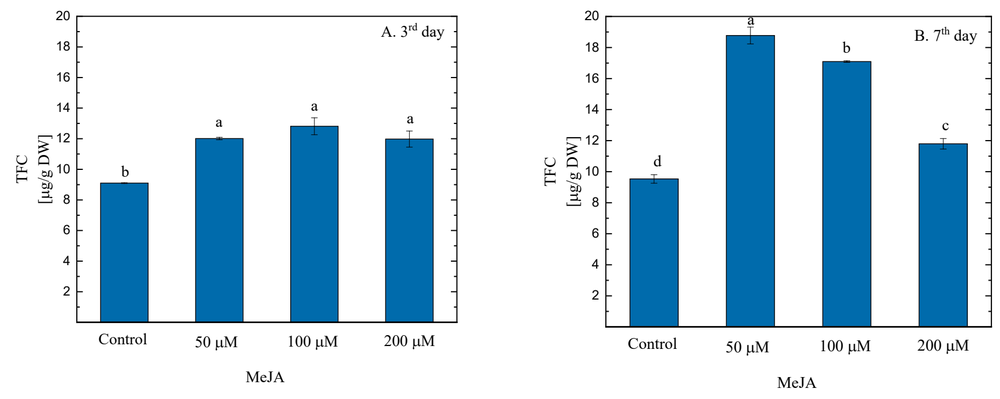
Arnica montana L. (mountain tobacco) is a valuable plant species, used for centuries to treat various diseases due to its anti-inflammatory properties. The plant is a rich source of numerous phytochemicals, including sesquiterpene lactones, helenalin, 11α, 13-dihydrohelenalin and their esters, phenols, flavonoids, and essential oils. *A. montana* is a European endemic species whose natural populations are threatened due to disturbances and poor management of the habitats in which the species occurs. Plant cell and tissue cultures can be an alternative tool for rapid and sustainable synthesis of bioactive compounds under precisely controlled conditions. Optimizing cultivation conditions without genetic manipulation reliably increases the synthesis of secondary metabolites. Methyl jasmonate (MeJA) is frequently used for the enhancement of secondary metabolites in *in vitro* culture.

This study aimed to assess the content of A. montana phenolic compounds after treatment with MeJA at concentrations of 50, 100, or 200 µM for 3 and 7 days.

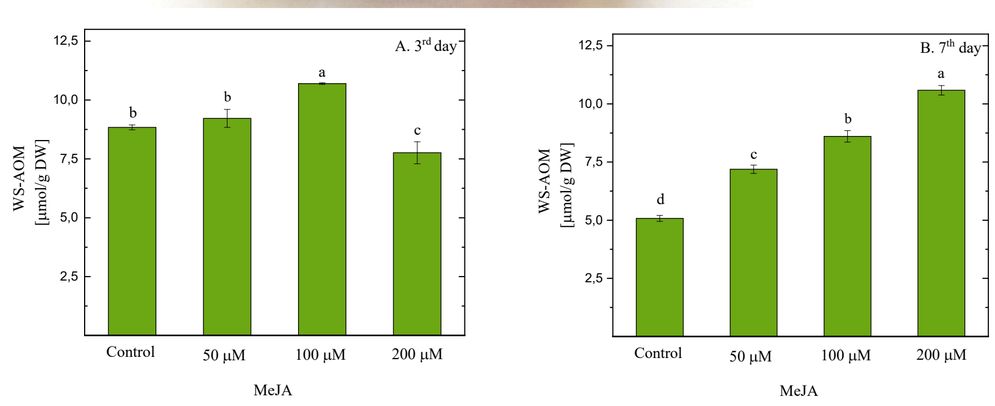
In vitro propagated shoots of *A. montana*



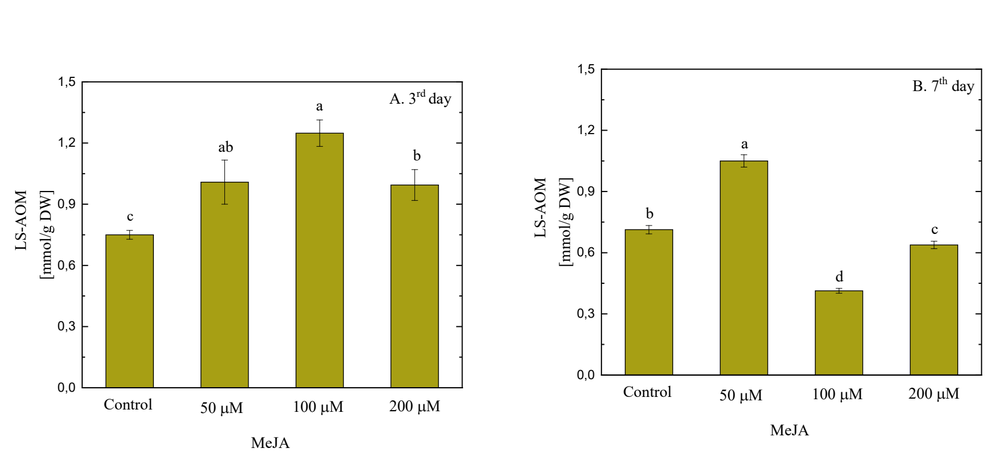
The total phenolic content in *in vitro* plants elicited with MeJA applied at different concentrations (0, 50, 100, and 200 µM) collected on 3rd (A) and 7th day (B) after treatment. Values are means ± SE, n = 20; different letters indicate significant differences assessed by the Fisher LSD test ($p \leq 0.05$) after performing ANOVA one-way analysis. We used the letter "a" for the highest data value and descended to the next for lower data values.



The total flavonoid content in *in vitro* plants elicited with methyl jasmonate applied at different concentrations (0, 50, 100, and 200 µM) collected on 3rd (A) and 7th day (B) after treatment.



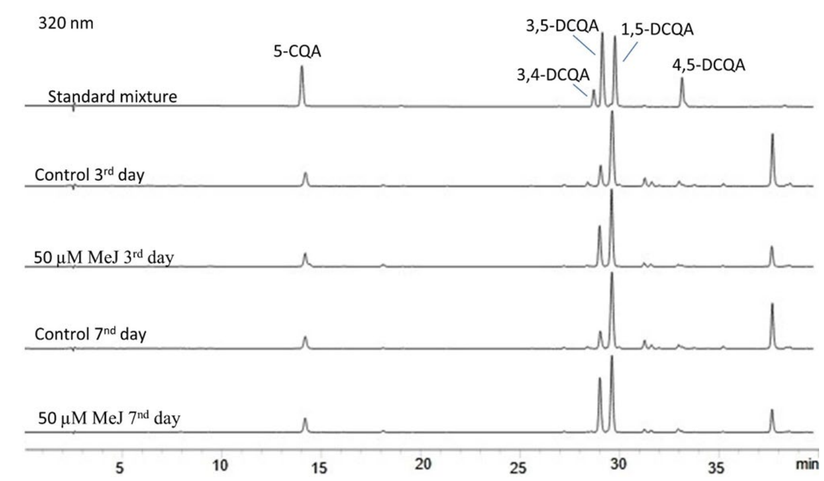
The water-soluble metabolites (WS-AOM) in *in vitro* plants elicited with methyl jasmonate applied at different concentrations (0, 50, 100, and 200 µM) collected on 3rd (A) and 7th day (B) after treatment.



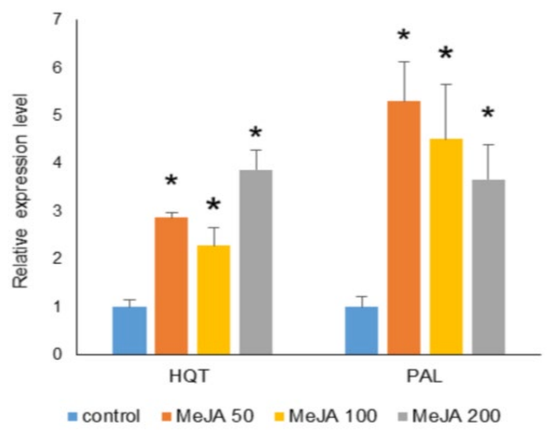
The lipid-soluble metabolites (LS-AOM) in *in vitro* plants elicited with methyl jasmonate applied at different concentrations (0, 50, 100, and 200 µM) collected on 3rd (A) and 7th day (B) after treatment.

Table: Content of caffeoylquinic acids [mg/g DW] in *Arnica montana* shoots elicited with different concentrations of MeJA

Treatment	5-CQA	3,4-DCQA	3,5-DCQA	1,5-DCQA	4,5-DCQA	Total
Control 3rd day	0.38±0.01d	0.06±0.01c	0.29±0.02d	1.43±0.01d	0.07±0.01c	2.24±0.01d
50 µM MeJA 3rd day	1.48±0.01a	0.09±00a	2.73±0.02a	5.39±0.03a	0.13±0.01a	9.82±0.01a
100 µM MeJA 3rd day	0.8±0.04b	0.07±00b	1.43±0.06b	3.07±0.03b	0.08±00b	5.45±0.02b
200 µM MeJA 3rd day	0.75±0.01c	0.05±00d	1.05±0.01c	2.61±0.02c	0.07±00c	4.53±0.01c
LSD	0.41	0.009	0.063	0.473	0.013	0.024
Control 7th day	0.53±0.01c	0.05±0.01b	0.38±0.01d	2.19±0.03c	0.09±0.01c	3.24±0.01d
50 µM MeJA 7th day	1.85±0.03a	0.06±0a	3.56±0.07b	6.16±0.11a	0.27±0.01a	12.43±0.04a
100 µM MeJA 7thday	1.83±0.04a	0.05±0b	4.05±0.1a	6.21±0.13a	0.23±0.03b	11.84±0.05b
200 µM MeJA7th day	1.75±0.03b	0.04±0c	2.93±0.09c	5.7±0.08b	0.2±0.02b	10.62±0.03c
LSD	0.05	0.0095	0.143	0.194	0.0364	0.0672



HPLC chromatogram at 320 nm of a standard mixture of CQAs, control, and sample treated with 50 µM MeJA



Expression profile of *HQT* encodes hydroxycinnamoyl-CoA quinate hydroxycinnamoyl transferase and *PAL* encodes phenylalanine ammonia lyase in *A. montana* under elicitation with MeJA.

Methyl jasmonate, applied for the first time to the species *A. montana*, effectively stimulates the synthesis of phenolics and flavonoids, the production of water- and lipid-soluble metabolites with antioxidant potential, and the accumulation of caffeoylquinic acids. *HQT* transcript level was mostly affected by 200 µM MeJA treatment (almost a 4-fold increase), while *PAL* was mostly induced by 50 µM MeJA (over a 5-fold increase). The observed upregulation of *PAL* and *HQT* caused by MeJA treatment was accompanied by increased accumulation of phenolic compounds in *in vitro* plants. The results obtained can serve as a basis for future, more in-depth studies of the mechanisms regulating the synthesis of phenolic compounds influenced by MeJA, along with the related changes in gene expression triggered by the elicitor.

Acknowledgements: This study was conducted with financial support from the Bulgarian National Science Fund, Ministry of Education and Science, Grant number КП-06-Н76/5 (05.12.2023).