



# Could evolutionary factors affect endogenous cytokinin pools in *Hypericum* species *in vitro*?

IEB CAS

Kalina Danova<sup>1\*</sup>, Vaclav Motyka<sup>2</sup>, Petre Dobrev<sup>2</sup>

<sup>1</sup>Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., bl. 9, 1113 Sofia, BULGARIA, [k\\_danova@abv.bg](mailto:k_danova@abv.bg)

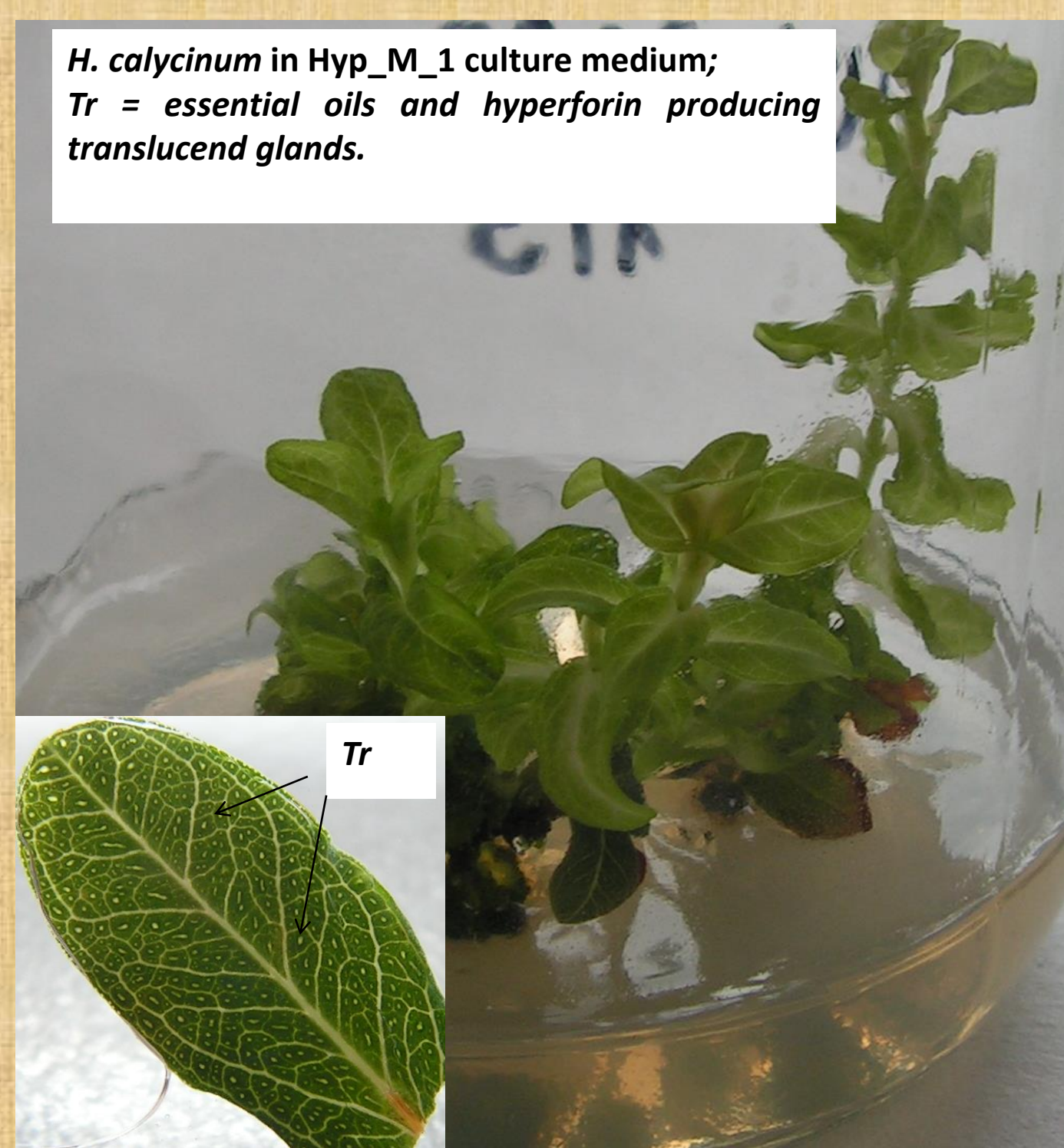
<sup>2</sup>Institute of Experimental Botany, Czech Academy of Sciences, Prague, Czech Republic, [motyka@ueb.cas.cz](mailto:motyka@ueb.cas.cz)

The *Hypericum* genus comprises over 450 species distributed worldwide and classified into 36 sections. The most widely studied representative of the genus is *H. perforatum*, which has been applied in medicinal practice since the 1st century A.D. up to modern times. The species has been utilized for a wide array of pharmacological properties such as antimicrobial, antiviral, anti-inflammatory, wound-healing and anticancer, amongst many others.

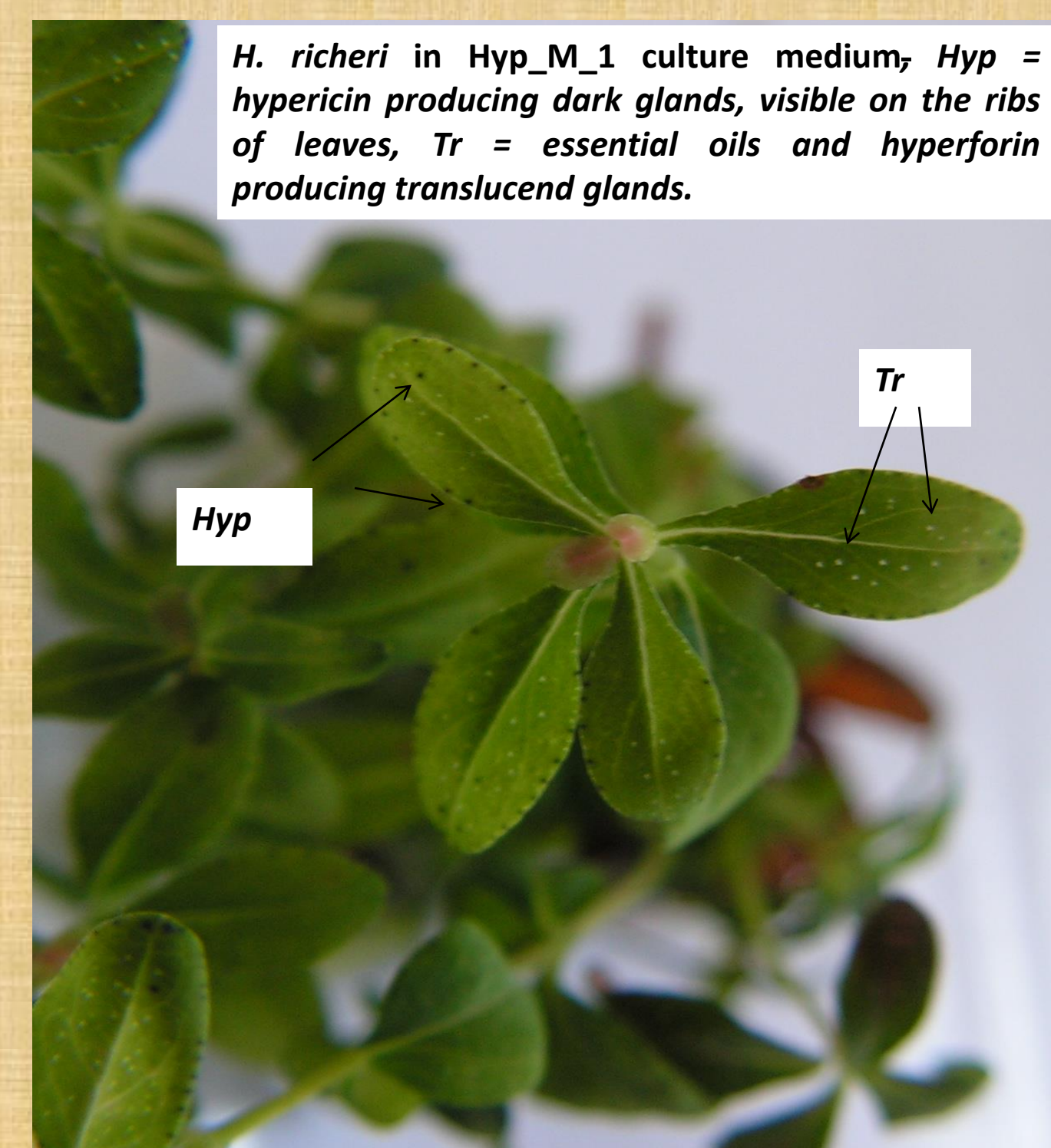
Research has shown that the production levels of condensed naphthodianthrones hypericin and pseudohypericin are related to the evolutionary development of the species in accordance with their sections' distribution. The flora of Bulgaria comprises 22 species of the genus, distributed within 11 sections. Of them, one species is Bulgarian (reported now as extinct), and five are Balkan endemics [1 and references cited within].



*H. calycinum* in Hyp\_M\_1 culture medium; Tr = essential oils and hyperforin producing translucent glands.



*H. richeri* in Hyp\_M\_1 culture medium; Hyp = hypericin producing dark glands, visible on the ribs of leaves, Tr = essential oils and hyperforin producing translucent glands.



## Material and methods

- For the purpose of multiplication stimulation, cytokinin (CK) *N*<sup>6</sup>-benzyladenine (BA) and auxin indole-3-butyric (IBA) acid were exogenously applied in different combinations (Table 1).
- The contents of endogenous phytohormones were quantified using high-performance liquid chromatography electrospray tandem-mass spectrometry (HPLC-ESI-MS/MS) [2].
- Data of the content of total endogenous CKs were studied and their pools and ratios between *cis*- and *trans*-zeatin types were compared.

In the present work, shoot cultures of hypericin non-producing *H. calycinum* L. (of the primitive *Ascyrea* section), hypericin producing *H. perforatum* L. and *H. tetrapterum* Fries (both section *Hypericum*) and *H. richeri* Vill. (section *Drosocarpium* - the most advanced in our study) were developed.

Table 1. Plant growth regulators supplementations to *in vitro* grown *Hypericum* shoot cultures

Medium abbreviation	BA [mg/l]	IBA [mg/l]
HypM_0	-	-
Hyp_M_1	0.2	-
Hyp_M_2	0.2	0.1
Hyp_M_3	0.1	0.2

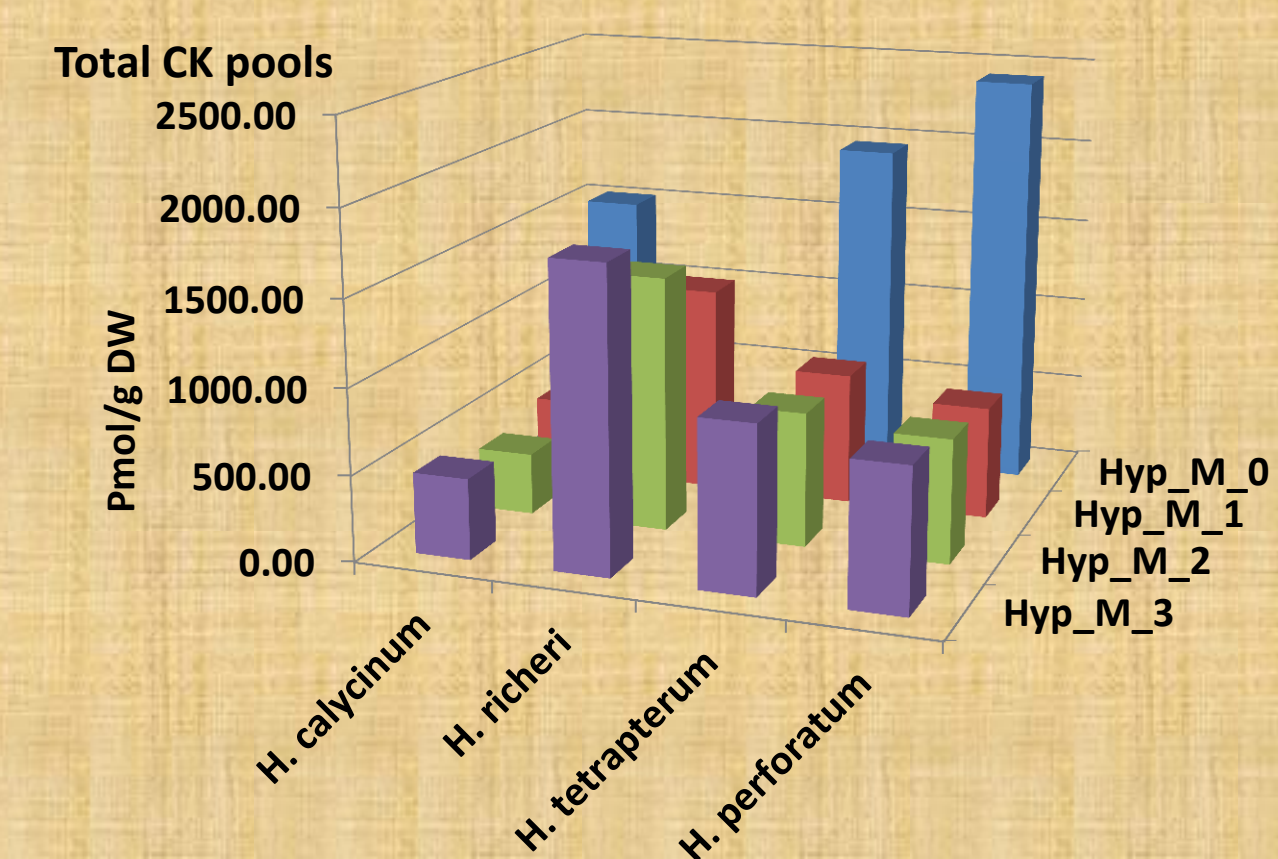


Figure 1. Total cytokinin pools established for the four culture media supplementations for the four *Hypericum* species

## Results

While the most primitive *H. calycinum* showed the lowest levels of total CKs, as well as of *trans*- and *cis*-zeatins, the highest total CK pools were recorded for the most evolutionary developed *H. richeri*. The analysis of data regarding the *trans*- and *cis*-zeatin types, however, showed an interesting interplay when comparing amongst the three hypericin producing species. Thus, while the highest levels of *trans*-zeatin types were detected for the most evolutionary developed hypericin producing *H. richeri*, on the contrary *cis*-zeatins dominated in *H. tetrapterum*.

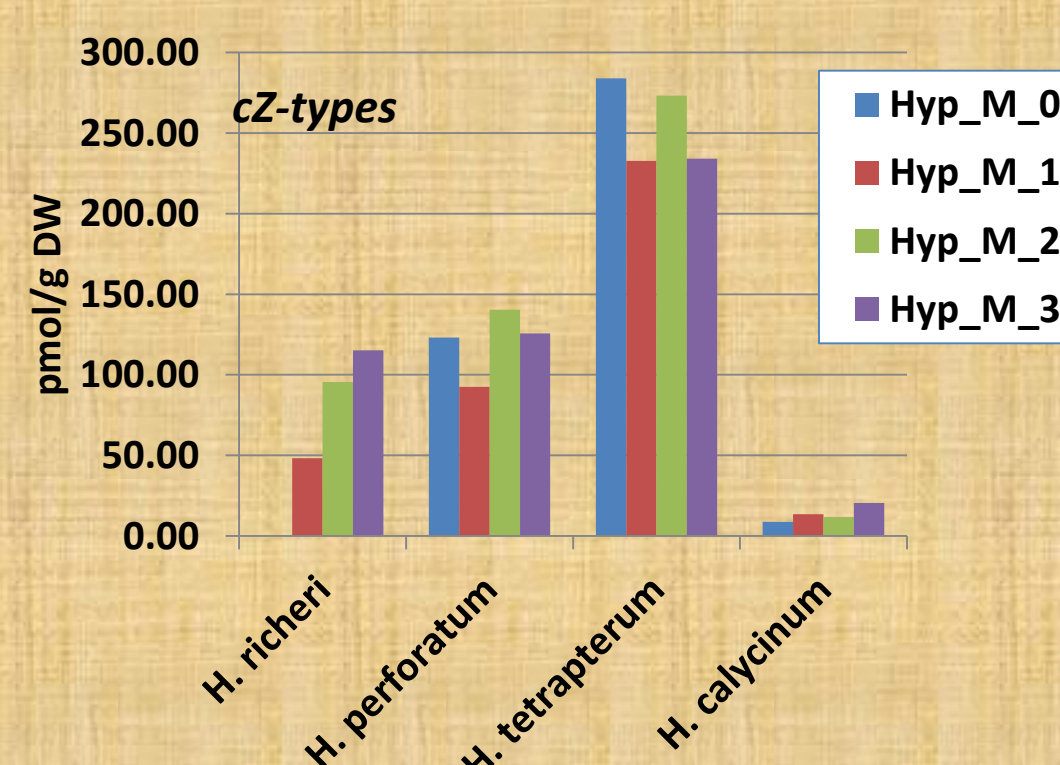


Figure 2. *Cis*-zeatin types CK pools established for the four culture media supplementations for the four *Hypericum* species

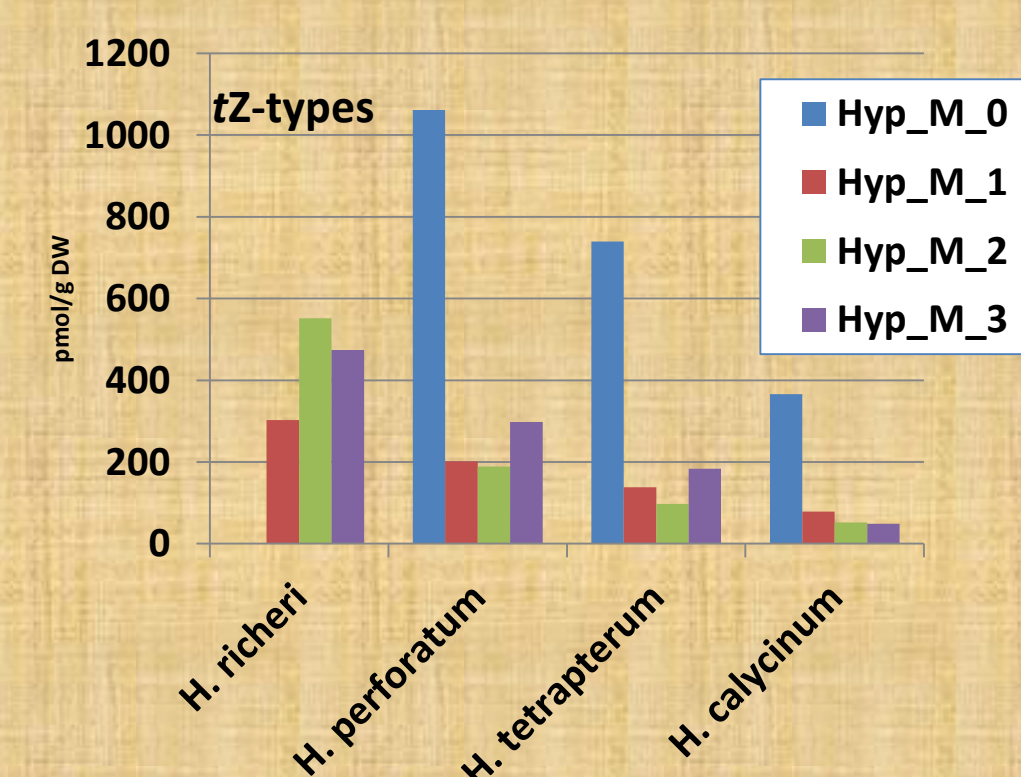


Figure 3. *Trans*-zeatin types CK pools established for the four culture media supplementations for the four *Hypericum* species

## References

- Danova K (2015) Potential of the Balkan Flora as a Source of Prospective *Hypericum* Genotypes for the Conventional and Biotechnological Delivery of Phytopharmaceuticals. Chapter 2 in: *Hypericum: Botanical Sources, Medical Properties and Health Effects*. Howard R. Davis (Ed), Series Plant Science Research and Practices, Nova Science Publishers, USA, ISBN: 978-1-63482-701-0
- Danova, K, Motyka, V, Todorova, M, Trendafilova, A, Krumova, S, Dobrev, P, Andreeva, T, Oreshkova, T, Taneva, S, Evstatieva, L. Effect of cytokinin and auxin treatments on morphogenesis, terpenoid biosynthesis, photosystem structural organization, and endogenous isoprenoid cytokinin profile in *Artemisia alba* Turra *in vitro*. *Journal of Plant Growth Regulation*, 37, 2, Springer, 2018, DOI:10.1007/s00344-017-9738-y, 403-418.

## Acknowledgements:

We are thankful to grants KP-06-H39/6, National Scientific Fund, Bulgaria and 19-12262S, the Czech Science Foundation, Czech Republic.

## Conclusion

Results were indicative of the relations between evolutionary level and endogenous CK production. Thus, it might be hypothesized that both complexity of evolutionary development, as well as hypericin production capacity, might be in close interplay with parameters of physiological adaptation in the plant organism such as endogenous CKs production.