In vivo and *in vitro* characterization of plant enzymes potentially involved in the flavonoid and coumarin biosynthetic pathway

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Flavonoids and coumarins belong to phenylpropanoids - a diverse group of plant secondary metabolites which widely occur in plant kingdom. They not only play various functions in plants, but also have found important applications in human life. One of the most interesting functions of phenylpropanoids, is their role in plant responses to environmental stresses. The phenylpropanoid biosynthetic pathway is intensively studied since years, but still many steps are not fully elucidated.

The QTL and GWAS studies performed previously by our research group, led us to select a set of candidate genes possibly having impact on the phenylopropanoid and coumarin composition within plants (1). The model plant *Arabidopsis thaliana* used in this study is perfectly suitable for this kind of research, thanks to its features - mainly the great choice of mutant lines and its tremendous environmental variability.

The main goal of my study is to perform functional analysis of selected candidate genes. The analysis started with the phenotypic characterization of suitable *Arabidopsis* mutant lines and accessions. All plants were grown in different environmental conditions (soil cultivation, hydroponics, *in vitro* cultures) with and without the addition of chosen biotic and abiotic stresses. The stress factors used were chosen beforehand during broad *in silico* analyses, in particular gene expression databases. Next step of characterisation will be heterologous expression in bacterial system and in *Nicotiana benthamiana* by transient transformation. After obtaining purified proteins overproduced in *E.coli* Rosetta 2, *in vitro* enzymatic activity will be estimated. In case of *N. benthamiana* transformed plant, secondary metabolites will be extracted and targeted metabolic profiling will be performed.

References:

(1) Siwinska, Joanna, et al. "Identification of QTLs affecting scopolin and scopoletin biosynthesis in *Arabidopsis thaliana*." BMC plant biology 14.1 (2014): 280.

Acknowledgements:

This work was supported by the NCN grant UMO-2014/15/B/NZ2/01073.