

Plants are unable to move, hence they lead a sedentary lifestyle. This does not mean, however, that they just give in to the influence of various environmental, frequently unfavorable, factors. The best evidence of the contrary is the enormous evolutionary success of the plant kingdom representatives, which include the largest and longest-living species on Earth. In the arsenal of the "secret weapons" that plants have at their disposal, low-molecular-weight organic compounds, called secondary metabolites or specialized metabolites, take an important place. They are stored mainly in vacuoles, in intercellular spaces or are secreted on the tissue surface. The specialized metabolites' composition in a given species is highly specific. Additionally, certain metabolites may be formed exclusively in specific cells, tissues or organs, and their concentration may vary depending on their function and the environmental conditions that plant needs to response to.

Specialized metabolites constitute an extremely diverse group of molecules with a wide range of biological activities. These activities include, among other things, direct communication between the plant and the environment, e.g. as repellents or indirectly, by mediating signaling pathways. The most important known functions of these compounds include protecting the plant against herbivores and parasites, protection against pathogenic microorganisms, attracting insects, but also inducing and stimulating beneficial plant-microbe interactions. Interestingly, some specialized metabolite biosynthetic pathways play a key role in the plant development. For example, in *Arabidopsis* (*Arabidopsis thaliana*) *MRN1* gene encodes a protein belonging to the oxidosqualene cyclase (OSC) family, involved in the biosynthesis of the triterpenoid marnerial. Knockout plants with unfunctional *MRN1* displayed delayed embryogenesis, late flowering as well as morphological disorders in the leaf development.

Based on the analyzes of the *Arabidopsis* reference genome, 13 OSC genes have been identified in this plant. Meanwhile, we recently discovered that one of them, *BARS1*, has been duplicated in a significant part of the *Arabidopsis* world's population. We detected both *BARS1* and its duplicated copy, which we named *BARS2*, in one third of over a thousand natural lines we tested. Currently these are the two least known genes in the *Arabidopsis* OSC family. We know that *BARS1* encodes a low-specificity enzyme – baruol synthase, implicated in the biosynthesis of a tetracyclic triterpenoid with an undetermined biological role. We demonstrated through the association studies that the presence of these gene duplication in plants is related to altered expression of neighboring genes and alteration in the root growth ratio in these plants. Additionally, plants with and without this duplication differ in the geographical distribution and the climatic conditions of their area of origin. We hypothesized that these differences may be adaptive. However, in order to thoroughly understand the molecular mechanisms underlying the phenotypic differences among the lines, in-depth analysis of both *BARS1* and *BARS2* as well as uncovering their function, is required.

The natural copy number variation of *BARS1/BARS2* genes offers an excellent model for functional studies. Therefore, in this project we aim to undertake an in-depth analysis of both genes at the genome, epigenome, transcriptome and metabolome levels, in lines differing by the *BARS2* presence or absence. We will also examine the genetic interactions and the redundancy level between the copies, through generating mutant lines with one or both genes disrupted as well as *BARS1*-overexpressing lines. Apart from that, we will perform a broad analysis of these genes' expression in various biotic and abiotic stress conditions as well as after plant hormone stimulations, in order to learn more about the biological processes involving *BARS1* and/or *BARS2*.

We expect that the versatile analysis of both genes will provide us with important clues, thus getting us closer to understand their biological function and allowing us to propose and study in the future the mechanisms involved in plant adaptation. Apart from that, our study will pave the way to a more efficient exploiting the intraspecific variation for the purpose of characterizing plant metabolic pathways. In the long term, it will contribute to the identification of new natural metabolites with biological activity and the application potential.