Unique changes in glucosinolate biosynthesis among species of Camelineae tribe of the Brassicaceae family

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Abstract

Glucosinolates are sulfur- and nitrogen- containing secondary metabolites commonly occurring in plants belonging to the Brassicaceae family. These compounds act as protection against attack of insects and pathogens. The two main classes of these metabolites are methionine-derived aliphatic glucosinolates (AG) and tryptophan-derived indolic glucosinolates (IG). Despite the well-documented presence of AG and IG in the investigated Brassicaceae species, some published results suggest that species belonging to one clade of Camelineae tribe may constitute an exception from this rule. The aim of this doctoral thesis was to determine, through systematic metabolomic, genomic and transcriptomic analyzes, glucosinolate biosynthetic capacity of selected species representing mentioned Camelineae clade, including *Capsella rubella*, *Capsella bursa-pastoris*, *Capsella grandiflora*, *Camelina sativa* and *Neslia paniculata*.

Performed untargeted metabolomic analyzes using liquid chromatography coupled with mass spectrometry showed that the tested species produce glucosinolates in inflorescences, siliques and roots, but not in leaves. We have also shown that IG are produced in these plants in much lower amounts than AG, and in addition strongly below the levels observed in the model plant *Arabidopsis thaliana*. Analyzes of the available genomic sequences enabled identification of orthologs of genes encoding enzymes involved in glucosinolate biosynthesis, while analyzes of their expression showed an organ-specific pattern correlating with the accumulation of glucosinolates. Identification and expression analysis of MYB transcription factor orthologs that control glucosinolate biosynthesis in *A. thaliana* suggested that the tested species lack a functional ortholog of MYB34 protein, which is involved in the regulation of gene expression during IG biosynthesis. This assumption was confirmed by the expression of *MYB34* gene from *C. rubella* in *A. thaliana*. Moreover, the increase in IG accumulation due to the expression of *MYB34* from *A. thaliana* in *C. rubella* demonstrated that loss of function by MYB34 is one of the molecular reasons of the reduced IG biosynthetic capacity in the tested Camelineae species.

Our metabolomic analyzes reveled also changes in AG and IG structural diversity in the tested species. These changes included preference for the accumulation of rarely occurring long-chain

AG and reduced capacity for modification of the IG indole core. Analyzes of gene orthologs encoding proteins involved in the extension of AG side chain and in IG modification have shown that the observed changes in glucosinolate diversity may result from the appearance of a new ortholog of methylthioalkylmalate synthase and from the backward evolution of monooxygenases belonging to the CYP81F family.