Abstract

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Title of Doctoral thesis: Transport mechanisms of secondary metabolites across membranes of plant cells

An attention was focused on the metabolism of isoflavones in recent years, including their transport within plants and cultures *in vitro*. A research is focused on the transport mechanism identification across cell or vacuolar membrane. There are localized different transporters as or the transport of some metabolites can take place through membrane vesicules. The release of five isoflavones (genistin, genistein, biochanin A, daidzein and formononetin) into nutrient medium was observed in suspension culture of *Genista tinctoria* L and *Trifolium pratense* L. These cultures were treated with NH_4VO_3 solutions (1 a 10 μM concentration) when isoflavon content in medium was higher after 24 hours. This increased volume of isoflavones was supressed by different transport mechanism inhibitors. The transport of isoflavones in *T. pratense* was influenced by ABC inhibitors from MRP subfamily, but genistein concentration in medium was lower after treatment with MDR subfamily inhibitors. Brefeldin A, which blocks vesicular transport, also decreased concentration of isoflavones in nutrient medium. The transport mechanism of the observed isoflavones in *G. tinctoria* was not exactly described due to the results in nutrient medium. There was also lack of statistically significant increase of isoflavones amount in dry mass after inhibitors treatment. The volume of secondary metabolites was effected by different inhibitors, when genistin concentration in medium was decreased by NH_4Cl application, which has impact on proton gradient and corresponding transporters. Genistin absorption was also observed in isolated vacuoles. Its transport into vacuoles was blocked by proton pumps inhibitors, which maintain proton gradient, thus the transport probably took place through MATE proteins. There was also measured higher concentration of genistein in supernatant and vacuoles in the samples. This aglycon was released from added genistin and its absorption by vacuoles was dependent on proton pump inhibitor.