Abstrakt v angličtině (English abstract)

Tobacco male gametophyte has a strongly dehydrated cytoplasm and represents a metabolically inactive stage. Upon cytoplasm rehydration, pollen grain becomes metabolically active and after the activation is finished, the pollen tube growth through a selected pollen aperture starts. The rehydration together with metabolic activation are accompanied by the regulation of translation and post-translational modifications (mainly phosphorylation) of the existing proteins. In this Ph.D. thesis, there were identified phosphopeptides from tobacco *(Nicotiana tabacum)* mature pollen, pollen activated *in vitro* 5 min and pollen activated *in vitro* 30 min. The total proteins from the above male gametophyte stages were extracted. The protein extract was trypsinized and the acquired peptide mixture was enriched by MOAC (metal oxide/hydroxide affinity chromatography) with titanium dioxide matrix. The enriched fraction was subjected to liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS).

Totally, there were identified 471 phosphopeptides, carrying 432 exactly localized phosphorylation sites. The acquired peptide identifications were mapped to 301 phosphoproteins that were placed into 13 functional categories, dominant of which were transcription, protein synthesis, protein destination and storage, and signal transduction. Notable part of phosphorylated peptides were shown to be regulated during pollen activation; 209 regulated phosphorylated peptides were listed into seven groups based on their regulatory trends, majority of which were identified exclusively in mature pollen. Moreover, there were found five phosphorylation motifs with a central phosphoserine and one phosphothreonine motif, which were predicted to be recognized by several kinase families. The members of these kinase families were then found in tobacco pollen proteome and pollen tube secretome.

Collectively, this Ph.D. thesis represents the first phosphoproteomic study of any angiosperm (Angiospermae) activated pollen, and notably broadens the identified part of tobacco *(Nicotiana tabacum)* male gametophyte phosphoproteome.

Key words: male gametophyte, pollen activation, titanium dioxide, phosphopeptide enrichment, phosphoproteomics, kinase, phosphorylation motif