Conclusions

My thesis was devoted to the investigation of a complex regulatory network of transcription factors in the *Arabidopsis thaliana* male gametophyte. In addition to the abovementioned objective, the second aim of the thesis was the creation of a web-based transcriptome database and data mining tool characterized by an innovative concept of presentation and evaluation of the data from DNA chips.

The results of my thesis can be summarized as follows:

1. We created a database called Arabidopsis Gene Family Profiler (arabidopsisGFP) (http://agfp.ueb.cas.cz/) which was made available on-line. This database was developed in order to provide a new tool for presentation of gene expression data (transcriptome), allowing the user to display the data in a quick and intuitive way using the innovative and when launched also unique concept of a virtual plant. The database employs a progressive idea "from simple to complex“, but also provides the user with more traditional data presenting options. The aGFP database can serve as a reference manual for the expression of individual genes and gene families in *Arabidopsis thaliana* under physiological conditions at different phases of the life cycle and in individual organs and tissues or cell types.

2. A chapter reviewing available servers for deposition of the transcriptome data and on-line tools for their analysis is a part of the submitted PhD thesis. Substantial part of the text deals with the expression data originating from plants, primarily from *Arabidopsis thaliana*. Our review is intended to help the user to gain quick overview of the available tools and databases and to summarize the potential and advantages as well as disadvantages of their use. By this approach, we wish to make easier access for a common user to the complex transcripromic datasets in user friendly environment. The next result of my PhD thesis is the analysis of the regulatory network of late transcription factors which regulate development of the male gametophyte after the second pollen mitosis (PM II). *In-silico* identification and selection of the transcription factors *w*as followed by genotypic and phenotypic evaluation of mature pollen grains from individual T-DNA lines with knock-out function of several transcription factors. The total number 37 of the analyzed T-DNA lines is 37. They represent 21 early transcription factors from several gene families (C2H2 zinc finger, bZIP, MADS-box protein family, etc.). I have found and described several transcription factors in which the insertion mutation seriously affected the development and/or structure of the pollen grain (e.g. aborted pollen grains, changes in position and spatial arrangement of the male germ unit (MGU), increased number of pollen grains with one or two nuclei). The following genes from various metabolic and signaling pathways have been identified as potential candidates for pollen-specific developmental regulators: At2g40620 (bZIP protein), At3g10470 (C2H2 zinc finger protein), At3g57390 (MADS-box protein), At4g05330 (C2H2 zinc finger protein), At5g54680 (bHLH protein) and At1g35490 (bZIP protein).

3. The next result presented in the PhD thesis is a published paper on functional characterization of the *atbZIP34* mutant. My contribution to the project consisted in the phenotypic analysis of the mature pollen grains and subsequent evaluation of the analysis. *atbZIP34* mutant was discovered during analysis of the transcription factors network in the male gametophyte of *Arabidopsis thaliana*. Its mature pollen grains showed number of abnormalities observed in both fluorescence and electron microscopy, for example, defects in the shape and form of exine or reduced endomembrane system. Based on further results of my colleague, Ms Antonia Gibalova, the first author of the respective publication, and on results of other co-authors , the proposed role of the

AtbZIP34 factor is in the regulation of exine formation and its involvement in the regulation of the lipid metabolism and/or subcellular transport. Further analysis of the *AtbZIP34* transcription factor is the aim of the PhD thesis of Ms Antonia Gibalova.

4. My previous analysis of the transcription factors (see paragraph 3) discovered the interesting T-DNA line which showed high ratio of dead pollen grains and other phenotypic abnormalities when compared to the wild type pollen of *Arabidopsis thaliana*. The identified line was SAIL_1168_C11 which carries insertion in the At1g70790 gene. My initial idea that it was a C2H2 transcription factor, confirmed by available transcription factor databases, was not later confirmed by further *in-silico* analysis. With the help of bioinformatic tools I only discovered the C2 domain, which currently rules out the possibility that the gene functions as a transcription regulatory unit. My next experiments which are not the subject of this submitted PhD thesis will be focused on disproving the presence or finding a new DNA binding domain. The result of this part of

the PhD thesis is functional characterization of the At1g70790 gene which we call, based on the increased number of dead pollen grains, *DEPOLL* (DEath POLLen grains). The result of my research is a finding that the protein product of the *DEPOLL* gene is responsible for proper formation of the intine and/or functional coupling of the plasma membrane and the pollen grain wall. The consequent analysis of the expression data from the *depoll* pollen grains on the Affymetrix ATH1 array revealed several groups of genes with confirmed or expected relationship to the metabolism of the pollen grain wall (lipid transfer protein (LTP), polygalacturonase, invertase/pectin methylesterase inhibitor proteins, xyloglucan endotransglucosylase/hydrolase gene family). Examination of these genes represents further potential for the discovery of specific components which are essential for proper formation of the pollen grain intine including its functional coupling with the plasma membrane. Last but not least, my next step will be uncovering of the specific function of the DEPOLL protein which, with high probability, functions as a transcription inhibitor of many genes which are related to the formation of the pollen grain wall and its involvement in specific metabolic and/or signaling pathways.