6. Summary

Within the course of this PhD thesis twodimensional gelelectrophoresis and MALDI-TOF-MS based methods have been evaluated and developed to fulfil the aim of large scale plant proteomics.

Particular attention was paid to the optimisation of proteinextraction, proteinseparation and proteinstaining. In this context a new fractionation based proteinextraction method which gave rise to an 300 % increased display of proteinspots on 2-DE gels could be established. On the basis of the developed techniques we were able to establish a set of 2-DE standardpatterns from 8 different *Arabidopsis thaliana* tissues.

Apart from the optimisation of 2-DE techniques an improved, robust and automated MALDI sample preparation system could be established. The set up of these methods allows the analysis and handling of more than 1000 proteinspots from 2-DE gels per day.

In a following step the combination of the 2-DE-and the MALDI protocols will be employed for the large scale identification of a vast portion of the *Arabidopsis thaliana* proteome.

As a primary step it was possible in a set of proof of principle experiments to identify 681 proteinspots from two different *Arabidopsis thaliana* leaf fraction 2-DE gels. Further we identified 352 proteinspots from an *Arabidopsis thaliana* silique 2-DE gel. In total the number of these preliminary identified proteins exceeds by far the number of previous published 2-DE proteomic data from *Arabidopsis thaliana.*

In a second proof of principle experiment it was possible to show the increased separation capabilities of 2-DE gels. In this example the identification of differentially expressed proteins from water starved cucumber plants was achieved. This experiment clearly showed the need of two-dimensional separation of proteins from complex mixtures to display differentially expressed proteins, since one-dimensional protein separation is not sufficient to fulfil this task.

In a last example the combination of tissue prefractionation techniques with 2-DE and MALDI-MS was used to identify an *Arabidopsis thaliana* subproteome. In this case the purification of cytosolic 80S ribosomal proteins was achieved by sucrose gradient density centrifugation of *Arabidopsis thaliana* leaf tissue. In this experiment it was possible to identify a large part (70 %) of the expected protein components of plants cytosolic ribosome from a single gel in a single round of identification. In total a number of 224 proteinspots could be identified from this ribosomal sample.