CHAPTER SIX

SUMMARY AND PERSPECTIVE

Environmental abiotic and biotic stresses are worldwide the primary limitations to crop production causing significant crop loss. However, understanding the physiological, biochemical and molecular responses of plants to stress and the identification of the regulatory pathways responsible for plant adaptation/tolerance to stress remains one of formidable challenges to plant science researchers around the world. Significant achievements have been recently made in understanding gene function during abiotic stress and a great number of transformed plants have been generated expressing a single or multiple genes with the aim to change metabolic pathways to obtain higher stress tolerance in plants. In contrast to pest-resistant transformed plants, where the resistance mechanism might rely on a single gene, such as the *Bt* gene (Sharma et al., 2000), the multi-loci nature for abiotic stress tolerance still renders it difficult to generate stress-tolerant plants (Bajaj *et al*., 1999; Iba, 2002; Wang *et al*., 2003). Several studies have previously shown that the introduction of a single gene into the plant genome has rarely resulted in the desirable phenotype of abiotic stress tolerance (Chen and Murata, 2002; Wang *et al*., 2003). This might be partly due that such gene is not expressed at a level required for stress protection.

At the onset of this PhD study, the the working hypothesis was to investigate whether the expression of a transgene in genetically engineered plant confer tolerance to frequently co-existing abiotic stresses, such as drought/heat, to which plants are often exposed under natural field conditions. For this, an exogenous rice cysteine proteinase inhibitor (OC-I) gene was expressed in transformed tobacco under the control of the constitutive 35S CaMV promoter. As a first new finding, this study has shown that exogenous OC-I

expression was not affected by drought, heat or a combination of both stresses. OC-I was also active *in vitro* providing evidence of maintenance of the structural integrity and function of the protein in a transformed plant under these stresses. Transformed plants were even able to accumulate active OC-I under stress exposure.

As a second new finding, this study has also shown that exogenous OC-I expression does not provide significant protection against the decline in plant performance due to drought/heat stress. Therefore, the working hypothesis that OC-I could provide stress protection in transformed tobacco could not be proved in this study. This is in contrast to other stress-tolerannce functional gene, such as compatible solutes (e.g. P5CS), HSPs and molecular chaperons (e.g. HVA1) or signal transduction and regulatory genes such as NPK1 and DREB1. These have been previously used to enhance abiotic stress tolerance in transformed plants including *Arabidopsis* and tobacco (Kasuga et al., 1999; Hong *et al*., 2000; Sivamani *et al*., 2000; Konstantinova *et al*., 2002; Shou *et al*., 2004).

As a third new finding, this study has shown that OC-I expression very likely affects gene expression. This result was found when OC-I expressing and non-expressing plants were compared and the cDNA-RDA technique was applied. One of the sequences identified to be affected by OC-I expression was the chlorophyll *a/b* binding protein gene of photosystem II. This gene was down-regulated in OC-I expressing plants in the presence and absence of heat stress. Differential expression of the chlorophyll *a/b* binding protein gene expression has been further found to be associated with lower chlorophyll content.

This might also be partially responsible for the difference found in plant performance between OC-I expressing and non-expressing plants.

As a fourth new finding, this study has provided first evidence that OCI-expression protects against protein degradation. Results of this study showed that expression and degradation of certain proteins was less affected when exogenous OC-I is expressed in a plant. OC-I possibly interacts with an endogenous proteinase(s) being responsible for protein degradation. However, this target proteinase(s) for OC-I in tobacco are currently still unknown.

As a fifth new finding, this study provided first information about such possible target proteinases. Two previously un-described papain-like cysteine proteinases could be isolated and characterized from green and senescent tobacco leaves. These proteinases might be a useful tool in future to obtain a more detailed understanding of possible OC-I cysteine proteinase interactions during plant development in a transformed plant. A study of this interaction might further help to also elucidate possible further benefits for plants expressing an exogenous proteinase inhibitor including the identification of proteins that are protected by OC-I expression from degradation.

Overall, this study has contributed to the advancement of science by providing advanced knowledge about involvement and stability of an exogenous phytocystatin in drought/heat stress, the effect of phytocystatin over-expression on gene regulation/expression and the identification of a first set of cysteine proteinases which might interact with a constitutively expressed exogenous phytocystatin.