Metabolic changes in Arabidopsis thaliana plants overexperssing chalcone synthase

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Chapter 1

General Introduction

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Crop plants are major sources of natural products used as pharmaceuticals, agrochemicals, flavors, fragrances, food, and pesticides [Balandrin and Klocke, 1988]. An in-depth understanding of the plant's metabolism is helpful for the improvement of their growth and yield [Carrari *et al.,* 2003]. As we know, green plants produce simple sugars by combining CO_2 and H_2O with energy from the sun by photosynthesis. Plants use sugars to make primary compounds such as starch, pectin, cellulose, fat, amino acids, proteins and nucleic acids for nutrition and construction of the plant structure. They also produce compounds which seem to have no explicit use for the plants and these are usually termed secondary metabolites. Secondary metabolites are defined as compounds that play a role in the interaction of the cell/organism with its environment to ensure the survival of the organism in its ecosystem [Verpoorte, 2000]. There are many secondary metabolites present in plants and they are classed in groups such as alkaloids, terpenoids, flavonoids, essential oils, phenolics and others.

Metabolic engineering of plants promises to create new opportunities in agriculture, environmental applications, production of chemicals, and even medicine. Metabolic engineering is referred to as the directed improvement of cellular properties through the modification of specific biochemical reactions or the introduction of new ones, with the use of recombinant DNA technology [Stephanopoulos, 1999]. It is generally referred to as "*the targeted and purposeful alteration of metabolic pathways found in an organism in order to better understand and utilize cellular pathways for chemical transformation, energy transduction, and supramolecular assembly*" [Lessard, 1996]. Plant biotechnology and transgenic plants are based on the latest technologies and current research on the engineering, synthesis, utilization, and control of primary and secondary plant metabolism. In terms of DNA techniques, several approaches have been used for

the modification of the host cell to achieve the desired goal. These include: inhibiting gene expression or inhibiting encoded enzyme itself to eliminate a competitive pathway or a toxic product; amplification of a gene or group of genes to improve the synthesis of existing products [Shimada *et al.*, 1998; Cameron *et al.*, 1998] the expression of a heterologous enzyme(s) to extend the substrate range [Panke *et al.*, 1998], to produce novel products [Stassi *et al.*, 1998], to provide pathways for the degradation of toxic compounds [Keasling *et al.*, 1998; Xu *et al.*, 1996], or to design a more environmentally resistant plant [Smirnoff, 1998].

Among the secondary metabolite groups in plants, flavonoids are the most common group of polyphenolic plant secondary metabolites. In plants, flavonoids play an important role in biological processes. Besides their function as pigments in flowers and fruits to attract pollinators and seed dispersers, flavonoids are involved in UV-scavenging, fertility and disease resistance [Winkel, 2001]. Since flavonoids are present in a wide range of fruits and vegetables, flavonoids form an integral part of the human diet. Currently there is broad interest in the effects of dietary polyphenols on human health. In addition to the potent antioxidant activity of many of these compounds *in vitro*, an inverse correlation between the intake of certain polyphenols and the risk of cardiovascular diseases, cancer and other age related diseases has been observed in epidemiological studies [Harborne *et al.,* 2000]. Enhancing flavonoid biosynthesis in chosen crops may provide new raw materials that have the potential to be used in food designed for specific benefits to human health.

Characterization of flavonoid biosynthesis at the genetic level has been done in *Arabidopsis,* maize, snapdragon, parsley and petunia [Christie *et al.,* 1996; Feldbrügge *et al.,* 1997; Wade *et al.,* 2001; Koes *et al.,*1989; Junghans *et al.,*1993]. Since *Arabidopsis thaliana* was the first plant to have its entire nuclear genome sequenced, it has become the most important model system for plant biology. *Arabidopsis* is particularly useful in the characterization of the flavonoid biosynthetic pathway due to the relative simplicity of the genetics for the pathway's enzymes and with exception of flavonol synthase, all the major enzymes of the flavonoid biosynthesis pathway in *Arabidopsis* are encoded by single-copy genes [Winkel, 2001]. In all plants, the precursor of the first flavonoid molecule is naringenin chalcone. Naringenin chalcone is synthesized by the first enzyme of the flavonoid biosynthesis, chalcone synthase (CHS).

Modifications by specific suites of downstream enzymes this intermediate goes into a variety of end products.

Many analytical methods e.g. gas chromatography (GC)/mass spectrometry (MS), high-performance liquid chromatography (HPLC)/MS, capillary electrophoresis (CE)/MS, and nuclear magnetic resonance spectroscopy (NMR) have been used for identification of metabolites in crude plant extracts. There is no single technique that allows a comprehensive detection of all metabolites but in principle ^{1}H-NMR can detect any metabolite containing hydrogen. Thus the ^{1}H-NMR spectra of biological fluids or tissue extracts are a rich source of qualitative and quantitative information on the compounds present, covering compounds of all chemical classes. NMR is therefore considered as an important technique that can contribute to metabolic profiling of an organism.

Furthermore, integration of metabolomic data with other -omic data is performed to identify the gene/protein functions and eventually leading to metabolic and cellular simulation *in silico*. For this purpose, data processing and analysis methods have to be applied. For example multivariate data analysis such as principal component analysis (PCA) and hierarchical cluster analysis (HCA) are carried out for data mining and can be used for identification of biomarkers for the response of the plants to certain forms of stress, comparison of plants for identifying resistance related compounds, chemotaxonomy and for quality control of food and botanicals.

Many studies related to CHS or using CHS mutant plants have been published [Mol *et al.,* 1983; Saslowsky *et al.,* 2001; Le Gall *et al.,* 2005] but so far most studies are restricted to the molecular level and information on metabolic changes is still lacking. Introduction of *CHS* in *Arabidopsis thaliana* would be the way to study the effect of overexpression of this gene on the metabolome of the plants and the flavonoid biosynthesis pathway.

Aim of thesis

The aim of the present study was to investigate the effect of chalcone synthase (*CHS*) overexpresion in *Arabidopsis thaliana* on primary and secondary metabolism.

Outline of the thesis

This thesis starts with a review of the function of CHS in plants and especially in plant resistance (**Chapter 2**). **Chapter 3** deals with the work on *Agrobacterium*-mediated transformation of heterologous chalcone synthase in *Arabidopsis thaliana* Col. 0. The effect of overexpression of *CHS* on the transcriptional level is discribed in this chapter. The activity of the CHS enzyme in the transgenic plants is reported in **Chapter 4.** In **Chapter 5** metabolic profiling of *Arabidopsis thaliana* using nuclear magnetic resonance spectroscopy (NMR) is described. In this chapter the primary and secondary metabolites of *Arabidopsis thaliana* Col. 0 which can be detected by NMR are reported. **Chapter 6** reports the metabolic profiling of CHS transgenic *Arabidopsis.* Metabolomic changes upon UV-A/blue light treatment of *Arabidopsis thaliana* were investigated (**Chapter 7). Chapter 8** deals with the study of the effect of the non-pesticide chemical, Benzo(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester (BTH) on the *Arabidopsis* metabolome. Finally, the general summary and discussion of thesis are given in **Chapter 9**.