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**Report of Anna Szilágyi**

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- Project title: Healthy flavonoid tomatoes
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**Brief outline of the scientific activity carried out in the host laboratory**

Main aims of the research visit

- a) to learn additional techniques in order to obtain more detailed information concerning the possible mechanisms behind the antioxidant response and generation of free radicals. These techniques included liquid chromatography and biochemical assays.
- b) to collect data on basil and other material at the same time as learning the new methods.
- c) at the end of the study, to be able to assess the questions remaining, and future areas of research.

Background

Certain flavonoids, a group of phenolic compounds widely occurring in the plant kingdom, have been shown to have antioxidant properties due to their role in scavenging free radicals and other oxidative species. A large number of epidemiological studies indicate that flavonoids present in the diet help to prevent certain human diseases, such as coronary heart disease and some forms of cancer through their antioxidant action.

Recently, several techniques and methods have been developed which can be used to manipulate the pathways of flavonoid synthesis in order to target specific compounds of interest. For example, genetic engineering and exposure to ultraviolet-B radiation have been used.

In the experiments carried out, basil (*Ocimum basilicum*), a popular herb in the human diet, was exposed to UV-B radiation, since it was previously found that the amount of phenolic compounds and antioxidant capacity increased in response to the supplemental UV-B radiation in this species. Flavonol biosynthesis was up-regulated in tomato (*Lycopersicum*

*esculentum*) in order to generate fruit with increased antioxidant capacity. This was done by transformation of tomato with e.g. the *Petunia chi-a* gene encoding chalcone isomerase. Resulting transgenic tomato lines produced an increase up to 78 fold in fruit peel flavonols. Analyses were done on both basil and tomato, the latter being one of the species used by the laboratory in Wageningen, and for which the methodology was already set up. This was very useful, since it gave me the opportunity of working with established methods and routines that were later also applied to basil.

#### Overview of methods used

Different techniques exist for evaluating and measuring antioxidant systems including both chemical and biophysical measurements. One of the methods for determining antioxidant capacity that I worked with, involved the application of free radicals, such as  $ABTS^{\bullet+}$ , as post-column reactants using an LC-PDA system equipped with an antioxidant detector.

The preformed radical cation 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate),  $ABTS^{\bullet+}$ , gives a strong blue colour, and was used with an on-line HPLC method for the detection of radical scavengers. The radical scavenging capacity was detected directly from the HPLC-separated analytes in a post-column reaction with the  $ABTS^{\bullet+}$ . The induced bleaching was detected as negative peaks simultaneously with the HPLC separation.

#### Realisation of the aims and future prospects

a) This on-line method of detection in HPLC eluates of analytes possessing radical scavenging activity was improved. This method was readjusted by making the system run for a long period of time that allowed us to analyse a series of samples. Testing and optimising the system at different pH (pH of the samples and the post-column reagent) and with several standards were carried out thoroughly prior to the real sample runs. Total antioxidant activity was measured by flow injection analysis.

b) Different tomato lines, control and UV-grown basil extracts, as well as raspberry and *Arabidopsis* leaf and stem extracts were analysed. Using two basil cultivars our preliminary results were confirmed according to which none of the main flavonoids could be found in the MeOH leaf extracts of this herb. The main compounds found were coumaric and caffeic acids. However, even the minor compounds, separated by HPLC, may possess radical quenching capacity, since the total antioxidant activity was found to be slightly higher in control basil compared to UV-grown plants. This decrease in the antioxidant capacity in UV-grown plants did not match with our other results, where experiments were carried out with fresh extracts. Therefore, we assume that this might be due to a rather fast deterioration of basil samples. One should also take into consideration, that other antioxidant compounds, besides certain phenolics, may be affected by the different experimental and treatment conditions, and this needs to be investigated. Further analyses of the other three plant materials were done after I had left PRI.

c) Further analysis of the phenolic compounds induced by UV-B radiation during growth are needed in order to answer some of the remaining questions; that is, a) which are these particular compounds, b) are they directly involved in the UV response, and c) which of the induced compounds can account for the change in antioxidant activity.

Apart from the valuable experience gained in the host laboratory, a further positive outcome is that the host, Dr Ric de Vos has strongly encouraged me to return to continue working in his laboratory for a longer time period that would include a complete study of the biochemistry of basil and the nature of antioxidants and ROS induced by UV-B radiation.