Stress response and health affecting compounds in Brassicaceae

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Chapter 1

General introduction and outline of the thesis

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General Introduction

The nutritional health and well-being of humans are entirely dependent on plant based food, as plants are critical components of the dietary food chain. Plants are a good source of health-promoting chemicals, providing almost all essential mineral and organic nutrients to humans either directly, or indirectly.[1]

Brassica vegetables including *Raphanus sativus* (radish)[2] and *Brassica rapa*[3] are consumed all over the world and are an important part of human diet.[4] As *Brassica* vegetables are rich sources of phytochemicals including amino acids,[5] glucosinolates, flavonoids, vitamins and mineral nutrients.[6] The variability in these bioactive food components depends on preharvest growth conditions and post harvest storage and/or processing conditions.[7] The effects on health obviously also depends on the amount of consumption, and the food processing culture of the community.[8]

Brassica secondary metabolites play an important role in its food quality. Besides the minerals and vitamins, plants can provide an array of interesting phytochemicals in the diet.[1] A part from appearance and flavour of vegetables, these metabolites are also known for their biological activities[9-11] including antioxidant,[12-14] anticancer, anti-inflammatory[15] and antiviral activities.[16] Some of these phytochemicals may function solely as aforementioned activities, but many of these metabolites may activate adaptive cellular stress-response pathways, including neuron hormesis.[17] On the other hand these compounds can also act as pro-oxidants under certain conditions.[13]

During the growth period *Brassica* plants are frequently subjected to various biotic and abiotic stress factors.[18] In this case secondary metabolites are useful products for plants, as stress response.[19] The contamination of soils and water with metals has created a major environmental problem, resulting in major production losses and hazardous health problems.[20, 21] At the same time plants have also been used for remediation of metals contaminated soil.[22] In this case the degree of accumulation of toxic metals (Cd, Cu, Zn, Mn, and Fe etc.) depends on the type of metal and its concentration.[23, 24] This results in the intensification of reactive oxygen species (ROS) generation, leading to the oxidative stress in plant cells.[20, 21]

In the mean time environmental microorganisms play a critical role in the interaction of the plant with the soil. These microorganisms are also responsible for diverse metabolomic functions that affect soil and plant health.[25] In this scenario the non-pathogenic bacteria can induce a similar systemic resistance in plants like pathogenic bacteria,[26] causing an alteration in plant metabolome, depending on the type of bacteria interacting with the plant.[27]

Preharvest growth stage and post harvest low temperature storage are also critical for the plant metabolome. Differences in plant metabolome at different developmental stages might be due to changes in channelling of precursors.[28] While at low temperatures by the disruption of cell membranes and cell walls, phytochemicals are released that may lead to changes in the metabolites pool.[29] Understanding the plant tolerance to 0^{o}C or below 0^{o}C temperatures and the relation to plant metabolite production is extremely complex.[30] But the most important postharvest condition necessary for maintaining vegetable quality is low temperature, as it maintains cellular integrity,[31] while extending the shelf life, as low temperature reduces the post harvest quality deterioration.[32]

In response to these stress factors plants produce a diverse array of primary and secondary metabolites.[19, 21] Especially amino acids, phenolics and glucosinolates play an important role in plant defence, either directly or indirectly.[13, 33]

Generation of reliable metabolite profiling data for the investigation of the role of metabolites is necessary.[34] As it is impossible to measure all metabolomic changes simultaneously,[30] systems biology as a holistic approach can be used to examine different biological processes, operating as an integrated system and visualize how individual metabolomic pathways are interconnected to each other.[35] Depending on selectivity and sensitivity different analytical approaches can be used. It includes nuclear magnetic resonance (NMR), thin layer chromatography (TLC), high performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC/MS) and liquid chromatography-mass spectrometry (LC/MS).[35] Among all aforementioned techniques, NMR spectroscopy is considered as, non-destructive, highly reproducible, and easy for sample handling. It allows the analysis of a large group of compounds in a single run, and is the most suited quantitative and qualitative metabolomic technique.[36, 37] It is used for high-throughput screening, metabolite fingerprinting, metabolite profiling and can also be used to investigate the operation of plant metabolomic networks.[38, 39] Multivariate data analysis techniques coupled with aforementioned analytical techniques can be used to exploit a large data set for understanding and interpreting the information in an integrative manner[40] as plotting the data in the space defined by the two or three principal components, provides a rapid mean of visualizing similarities or differences in the data set.[35] In this case Principal Component Analysis (PCA) is one of the oldest and most widely used multivariate techniques.[35, 41] To make a clear discrimination in a data set partial least square-discriminant analysis (PLS-DA) as a supervised data analysis tool can also be applied[27] to study the plant response to various stress factors.

Aim of the thesis

The aim of the present study was to investigate the effect of preharvest and postharvest factors on the plant's primary and secondary metabolite production by using a systems biology approach.

Outline of the thesis

The thesis begins with a review of literature describing the phytochemicals in Brassicaceae and their role as health affecting compounds (**Chapter 2**) showing the importance of Brassicaceae vegetables for human diet. The next chapter is a literature survey focusing on plant metabolome changes during the various growth stages and by different stimuli. Ultimately this leads to the changes in health affecting compounds in Brassicaceae (**Chapter 3**). Nutritional value of plants at different developmental stages was also evaluated in this project. Quantitative analysis was conducted for amino acids, organic acids and sugars by NMR, and for vitamins and glucosinolates by HPLC. NMR metabolomic analysis as a non-targeted approach coupled with multivariate data analysis was also used (**Chapter 4**). The effect of non plant pathogenic bacteria (**Chapter 5**) and different metal ions (**Chapter 6**) as preharvest stress factors was evaluated, showing the change in primary and secondary metabolite production. Metabolomic changes under low temperature storage conditions are also reported in this thesis (**Chapter 7**). Extracts of several *Brassica* varieties were assayed for different biological activities including, acetylcholine esterase inhibition, antimicrobial activity, as well as CB1 and adenosine receptor binding activity (**Chapter 8**). Finally the future perspectives for further research work in related areas are discussed (**Chapter 9**).