Stress response and health affecting compounds in Brassicaceae

Jahangir, M.

Chapter 9

General discussion, conclusion and future perspectives

Muhammad Jahangir

Division of Pharmacognosy, Section Metabolomics, Institute of Biology, Leiden University, Leiden, The Netherlands

General Discussion and Conclusion of the Thesis

Brassica vegetables are a rich source of health affecting compounds and are widely used as food, moreover they are a model for plant science research. These vegetables represent a major part of the human diet all over the world providing nutritionally significant constituents, such as phenolic compounds, vitamins, fibres, soluble sugars, minerals, fats, and carotenoids.

The metabolome changes during plant growth, which represents the changes in metabolomic fluxes through different pathways. The present study shows the importance of plant age as a factor for nutritional value of vegetables for human consumption and suggests that young plants are a better source of nutrients as compared with old plants.

During growth, plants are exposed to various biotic (e.g. herbivory, fungal, bacterial and/or viral infection) and abiotic (e.g. metals, UV, temperature, drought) stresses. The metabolomic changes can be quite specific, since the plant defence-related compounds are composed of a variety of constitutive and induced metabolites. The set of *Brassica* metabolites differs after infection with different microorganisms. Plant response to bacterial stress depends on the type of invading bacteria. It probably reflects the diverse chemical composition and mechanism of action of the invading organism, which can at the same time may activate gene expression and block specific steps of a metabolomic pathway in the plant, or even metabolize the plant defence compounds.

Under metal stress, plants produce primary and secondary metabolites which increase with increasing metal concentrations up to a certain point, beyond which primary and secondary metabolite concentration decreases. The increase of phenolic compounds is dependent on both the type of metal and its concentration. Apparently the response to metal stress is observed in the both leaves and roots, which could be due to metal tolerance mechanisms or metal accumulation in the whole plant. This response and accumulation of metals is more dependent on type of metal rather than metal concentration.

Postharvest storage temperature is also crucial for the metabolomic variation in vegetables. By decreasing the temperature the rate of metabolomic variation decreases. As compared with room temperature, lower metabolomic variation happens in the vegetables stored at 4 ^{o}C, but least metabolomic changes are observed in the samples stored at –20 ^{o}C and –80 ^{o}C, although due to freezing injury higher amount of phenolics are observed, but with further increasing the time this stabilizes. The storage temperature of 4 ^{o}C is comparatively better for consumption purpose, but for experimental purpose it is advantageous to keep the vegetable samples at –80 ^{o}C.

As aforementioned, in nature plants have multitrophic interactions during growth and developmental processes.[255] The power of metabolomics analytical methods is the analysis of wide spectra of compounds resulting in a huge data set in an unbiased and comprehensive manner.[445] These enormous metabolomic data sets can be assessed by multivariate analysis, usually stating with an unsupervised method such as principal component analysis (PCA). To understand the specificity of the interactions of the plant and its environment a large amount of data on to *Brassica* was obtained concerning the effects of the defence signal compounds, such as jasmonic acid, salicylic acid, and furthermore of infection with pathogenic and non pathogenic fungi, as well as human pathogenic bacteria and metals. Effects were measured at different developmental stages of the plant. Finally also the effect of storage for different periods and temperatures was evaluated. An overlap of different treatments was observed that needs to be studied in more detail.

To try to make a clear overall picture, as a next step data from all experiments described in this thesis for leaves (**Figure 1**) and roots (**Figure 2**), covering a period of 4 years were subjected to PCA analysis. A clear discrimination in the PCA score plot is observed for the plant species. But also the metabolomic changes observed for the different treatments of the same species are visible in these PCA score plots. For the loading plot it was concluded that serine, glucose and sucrose are higher in the plants grown in hydroponic conditions and infected by food born bacteria (in accordance with the analysis made in **chapter 5**), while glucosinolates and phenylpropanoids were found in higher amounts in radish stored at different storage temperatures for different time period (in accordance with the analysis made in **chapter 7**). When focusing on stored radish, the initial storage samples were found to be higher in glucosinolate and phenylpropanoids. GABA and alanine are found to be as discriminating metabolites for *B. rapa* samples treated with metals (conform analysis in **chapter 6**).

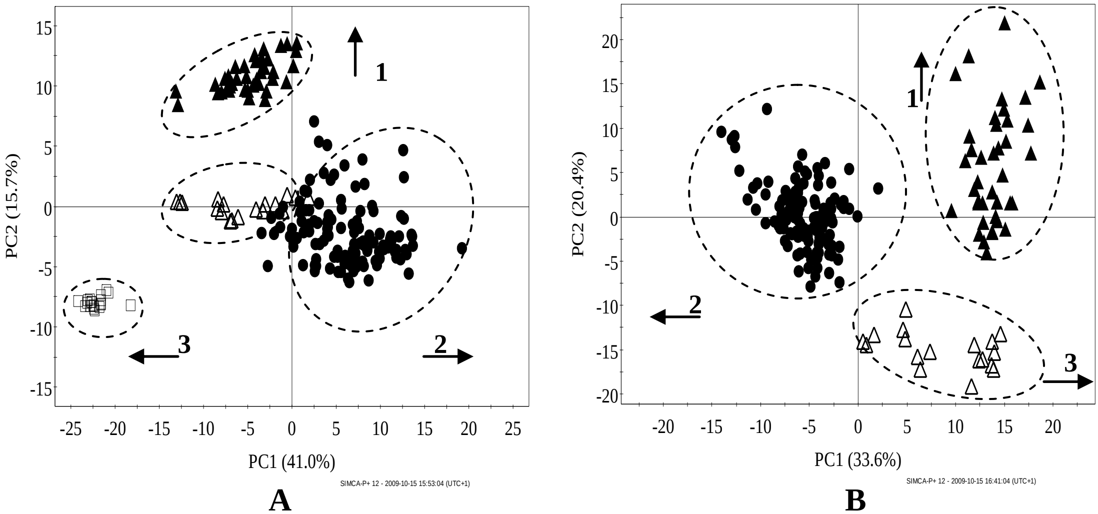

Figure 1: Score plot (PC1 vs PC2) of PCA for leaves (A) and roots (B). *Brassica rapa* stressed with metals (as in **chapter 6**) (▲), Effect of post harvest storage time and temperature on *Raphanus sativus* (as in **chapter 7**) (●), Change in *Brassica rapa* and *Raphanus sativus* metabolome at different developmental stages (as in **chapter 4**) (Δ), Effect of food born bacteria on *Brassica rapa* in hydroponic conditions (as in **chapter 5**) (□). **1** = GABA, alanine, acetate, threonie ; **2** = feruloyl malate, 5-hydroxyferuloyl malate, sinapoyl malate, coumaroyl malate, and caffeoyl malate, kaempferol, quercetin, gallic and malic acid, fumaric acid, valine, glutamine, glutamate, glucobrassicin, gluconapolefrin, progoitrin and neoglucobrassicin; **3** = Serine, glucose, sucrose.

The samples for different developmental stage (conform **chapter 4**) were grouped together near post harvest stored radish. The discrimination of these samples from post harvest stored radish and metal affected plants is due to the higher amount of sucrose, serine and glucose. A similar result is obtained for root samples of all experiments, except that hydroponically grown plants are not included, as in that case we could not generate root samples. Partial least square-discriminant analysis (PLSDA) as a supervised method of analysis was used to analyse the same data. The grouping was made on the classes based on experimental conditions. A similar result was obtained as with the PCA analysis (**Figure 1**). From the results it is obvious that the different treatments applied did result in different responses of the plant, though in part they do overlap. Plants apparently have specific responses to different forms of stress. Signal compounds like methyljasmonate and salicylate, also have overlap with these responses. The good news of this final overall analysis is that a metabolomics approach does allow datamining in results obtained in different experiments done over the years and results in the conclusion that plants differ in specific responses to different forms of stress. The "bad" news of this discovery is that to learn for understanding the regulation of plant defence is even more complex than originally anticipated.

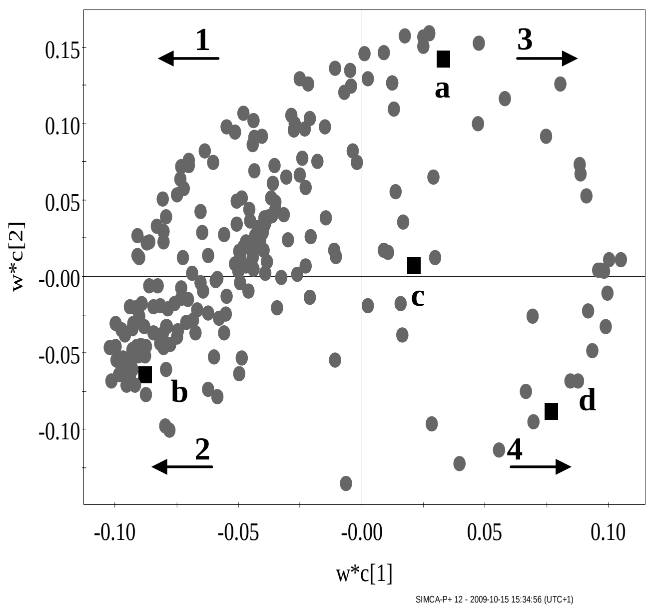

Figure 1-A: Loading plot (PC1 vs PC2) of PLSDA for leaves (A) and roots (B). *Brassica rapa* stressed with metals (as in **chapter 6**) (a), Effect of post harvest storage time and temperature on *Raphanus sativus* (as in **chapter 7**) (b), Change in *Brassica rapa* and *Raphanus sativus* metabolome at different developmental stages (as in **chapter 4**) (c), Effect of food born bacteria on *Brassica rapa* in hydroponic conditions (as in **chapter 5**) (d). **1** = GABA, alanine, acetate ; **2** = feruloyl malate, 5-hydroxyferuloyl malate, sinapoyl malate, coumaroyl malate, and caffeoyl malate, kaempferol, quercetin, gallic and malic acid, fumaric acid, valine, threonie, glutamine, glutamate, glucobrassicin, gluconapolefrin, progoitrin and neoglucobrassicin; **3** = α-glucose; **4** = β-glucose, sucrose, serine.

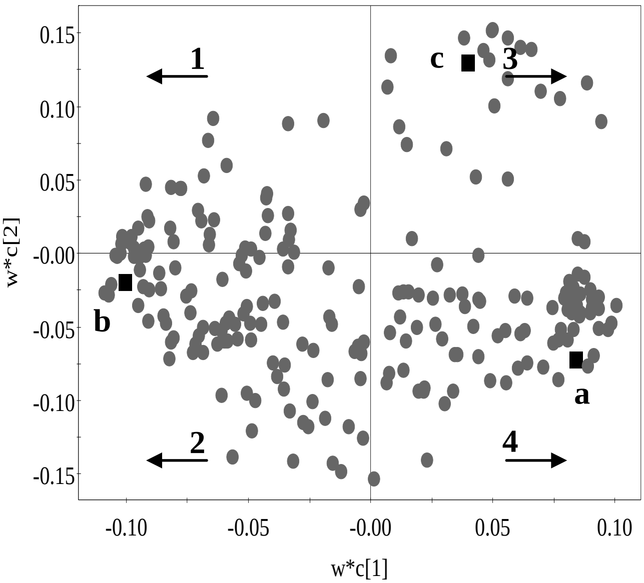

Figure 1-B: Loading plot (PC1 vs PC2) of PLSDA for leaves (A) and roots (B). *Brassica rapa* stressed with metals (as in **chapter 6**) (a), Effect of post harvest storage time and temperature on *Raphanus sativus* (as in **chapter 7**) (b), Change in *Brassica rapa* and *Raphanus sativus* metabolome at different developmental stages (as in **chapter 4**) (c), Effect of food born bacteria on *Brassica rapa* in hydroponic conditions (as in **chapter 5**) (d). **1** = GABA, alanine, acetate ; **2** = feruloyl malate, 5-hydroxyferuloyl malate, sinapoyl malate, coumaroyl malate, and caffeoyl malate, kaempferol, quercetin, gallic and malic acid, fumaric acid, valine, threonie, glutamine, glutamate, glucobrassicin, and neoglucobrassicin, glucose; **3** = Serine; **4** = sucrose, gluconapolefrin, progoitrin.

The presence of health promoting constituents, such as phenolics, glucosinolates, amino acids, vitamins, sugars, fibre etc. makes Brassicaceae plants healthy food. The combined presence of antioxidant, anticancer and antimicrobial compounds seems to distinguish *Brassica* vegetables from many other vegetables, however, the active compounds are present only at low levels and certainly not at a level that could make these vegetables a medicinal plant. Rather one should see the presence of the various biologically active compounds as proof for the health sustaining, or supporting character of *Brassica* vegetables.

Metabolomics aims at both the qualitative and quantitative analysis of all metabolites, and but no single method can analyse all the metabolites in a single run. In this scenario we have to select a good method by keeping resolution, reproducibility, sensitivity, sample preparation and handling into account.[37]

Although HPLC and MS are highly sensitive analytical techniques and used for plant metabolomic studies but in my view NMR is an excellent tool to have a macroscopic view on the plant metabolome due to its high resolution, reproducibility, ease of sample preparation and sample handling. Thus the use of NMR is of great interest in functional genomics and systems biology studies of biological processes. For the identification of metabolites the use of various 2D NMR methods can overcome the problem of signals overlapping in ^1H NMR.[37, 39]

In order to be able to draw conclusions from a systems biology study using metabolomics, it is in particular important to use both the analytical method and the data analysis in an unbiased holistic manner.[445] NMR analysis coupled with multivariate data analysis techniques are well known recently for metabolomic studies in plants.[388] As NMR is used to study a wide range of diverse group of metabolites to analyse in a single run, it is advantageous to use multivariate data analysis tools for NMR data processing and analysis.

Future Perspectives

Clear evidence for health promotion by phytochemicals and/or their breakdown products is still missing, so further research work is needed by using in vivo experiment. A major constraint is the question of the required dose to have optimal effect. This is a matter of content of the active compounds in the vegetables, the amount eaten, and the actual amount absorbed in the body. All are quite variable and consequently food will never become a medicine, at best health supporting items in our diet. *Brassica* plants can be biofortified and further research work can be carried out in this aspect, but metabolic variation during biofortification should monitored constantly during this process.

Studies on phytonutrients and health, taking sensory factors and food preferences into account, constitute an important area of research. Through selective breeding or genetic improvement these compounds may be enhanced in plants.

However environmental effects will cause changes in the metabolome, particularly multitrophic interactions of *Brassica*, under stress conditions are still unclear. Further research work is needed to study the above ground and below ground multitrophic interactions of plants in connection with phytonutrients.

An increased insight into plant-microbe interaction may allow the detection of contaminants, in this case, pathogenic bacteria in vegetables and fruits, thus helping to ensure safety for human consumption. In plant–microbe interactions, it is very important to analyze the microbial volatiles that play a role for immediate activation of the defence mechanisms in plant tissues.

After post-harvest storage at freezing temperatures, the physical quality of radish was not acceptable anymore and thawing caused vast metabolomic changes. Proper drying method should be used to deal with such expected metabolomic changes. Further research is needed to study the production and degradation of primary and secondary metabolites during storage, with relation to their breakdown products.

As an important group of health affecting compounds in Brassicaceae, the glucosinolate content as response of plant to external stimuli should be evaluated in more detail, in relation to the response of plant to external stimuli.

Brassica phytochemicals, especially glucosinolates are well known for biological activities, including anticancer and antioxidant activities. But their actual function or the mechanism has not yet been fully elucidated. Glucosinolates are known for their anti-nutritional affects, and also certain compounds like phenolic acids, tannins etc. may so form anti-nutritional complexes. A better understanding of molecular and cellular mechanism for the phytochemicals and their breakdown products will be helpful for considering these compounds as health affecting compounds, in positive or negative manner, particularly in connection with obesities.

Undoubtedly, all these efforts should contribute to provide the means of controlling these different defence systems, leading to the development of more resistant plant varieties ultimately providing higher yields along with higher nutritional value.