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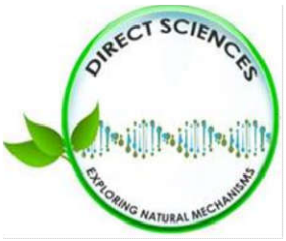


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# Metabolomics as a Supplementary Tool for the Advanced Plant Research Excursions

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**Abstract:** Recently, a tremendous growth has been observed in Biotechnology research, demonstrating itself as a tool to improve plant yield (i.e. for crops, ornamental and medicinal plants) in quantitative and qualitative aspect. The targets of biotechnology research are hard to achieve until the metabolomic pathways are not explored for their up/down regulatory behaviors during any biotechnological adaptation. The exploration of such metabolomic flux of cells or tissues, a series of snapshots for the target biological material leads towards the metabolomics based system biology research. This strategy is of immense importance for identification of genes involved in metabolomic pathways of plants, altered through biotechnological excursions. Our group demonstrates the application of these tools in the research of various plants, including tobacco and *Brassica*. In this view, multiple chromatographic and spectroscopic techniques i.e. GC/MS, NMR, HPLC etc. coupled with multivariate data analysis are being used as supplementary tools in biotechnology research. Specifically about *Brassica* metabolome multiple experiments were conducted to explore the alteration in metabolomics flux through triggering biological system by external stimulus. In present work, a metadata of effect of multiple stress factors (including bacterial stress, metal stress, cold storage developmental stages and varietal characterization) is studied through multivariate data analysis. The results reveals that the metabolomics alterations are stimulus specific.

**Key words:** Metabolomics, Systems Biology, Stress Biology

## Introduction

*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,

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soluble sugars, minerals, fats, and carotenoids.

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 metabolome changes during plant growth represents the changes in metabolomic fluxes through different pathways.These changes can be quite specific, since the plant defence-related compounds are composed of a variety of constitutive and induced metabolites.

Metabolomics aims at both the qualitative and quantitative analysis of all metabolites, and but no single method can analyze 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(Verpoorte, Choi, and Kim, 2007).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.(Dowlatabadi et al., 2009; Verpoorte et al., 2007).

As aforementioned, in nature plants have multitrophic interactions during growth and developmental processes (Gols et al., 2007). 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 (Tikunov et al., 2005). 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. In present work, previous reported data of our group was used with the application of Principal component analysis (PCA) and Partial least square-discriminant analysis (PLSDA) to explore the effect of multiple stress factors and their specificity to alter metabolomics flux.

## Materials and Methods

### Metal application

The metal application, processing and data results of previously reports were used (Jahangir et al., 2008).

### Storage condition

Various storage conditions, processing and data results of previously reports were used (Jahangir et al., 2014).

### Bacterial stress

Multiple bacterial stress factors were used, with data processing and the results of previously reports were used (Jahangir et al.,2008).

### Growth stages

The data of growth stages was used from the previously studies (Jahangir et al., 2014).

### Statistical analysis

Principal component analysis (PCA) and Partial least square-discriminant

analysis (PLSDA) was carried out bythe SIMCA-P+ software (v. 12.0.1, Umetrics, Umeå,Sweden) as reported previously (Jahangir et al., 2014) by using unit variance (UV).

**Results and Discussion**

It is demonstrated that the plant age is an important factor for nutritional value of vegetables for human consumption, and our study suggests that young plants are a better source of nutrients as compared with old plants(Jahangir et al., 2014). The set of *Brassica* metabolites also differs after infection with different microorganisms, where it is clearly reveled that 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 defense compounds(Jahangir et al., 2008).

In another study, primary and secondary metabolites are accumulated in plants in response to metal ion stress, where upto a certain level of metallic ions these metabolites are increased, while beyond such specific concentration a decline in these metabolites is observed. Such responses of plants (in roots and/or shoots) are specific to the type and concentration of metallic ions (Jahangir et al., 2008).

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 oC, but least metabolomic changes are observed in the samples stored at –20 $^{o}$C and –80 $^{o}$C, although due to freezing injury 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 (Jahangir et al., 2014).

To try to make a clear overall picture, as a next step data from all experiments described above for leaves (Figure 1) and roots (Figure 2), covering a period of 4 years were subjected to PCA analysis (Jahangir, 2010). 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, while glucosinolates and phenylpropanoids were found in higher amounts in radish stored at different storage temperatures for different time period. 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.

The samples for different developmental stage were grouped together near post harvest stored radish. The discrimination of these samples from postharvest 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.

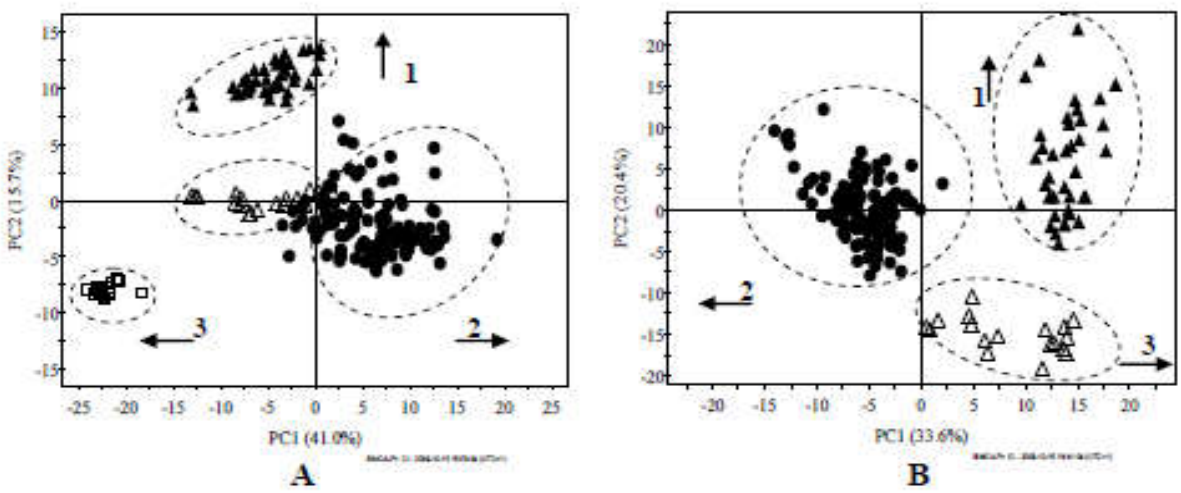


**Figure 1:** Score plot (PC1 vs PC2) of PCA for leaves (A) and roots (B). *Brassicarapa*stressed with metals (▲), Effect of post harvest storage time and temperature on *Raphanussativus* (●), Change in *Brassicarapa*and *Raphanussativus*metabolome at different developmental stages (Δ), Effect of food born bacteria on *Brassicarapa*in hydroponic conditions (□). **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.

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 methyl-jasmonate and salicylate, also have overlap with these responses. The good news of this final overall analysis is that a metabolomics approach does allow data mining in results obtained in different experiments done over the years.

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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.

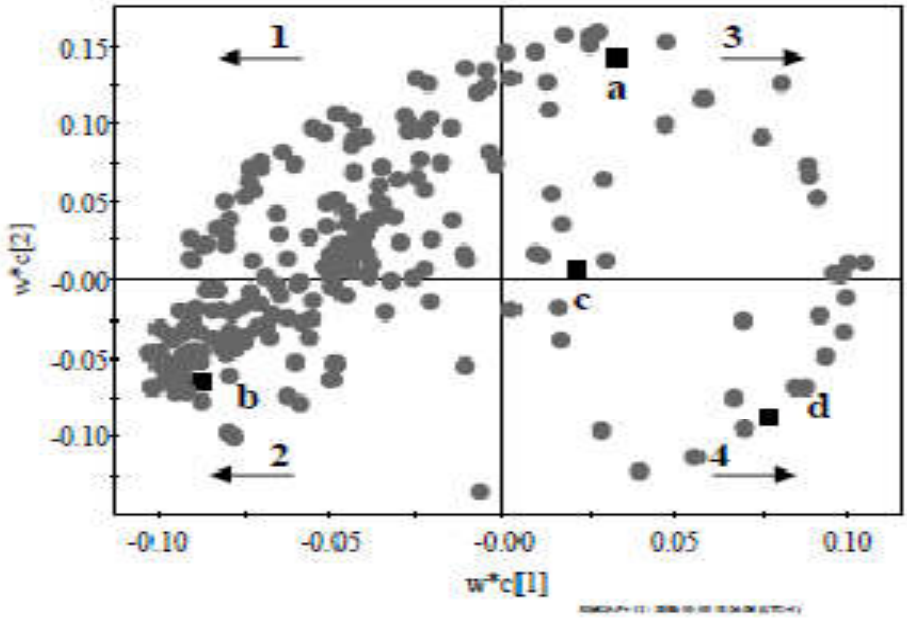


**Figure 1-A:** Loading plot (PC1 vs PC2) of PLSDA for leaves (A) and roots (B). *Brassicarapa*stressed with metals (a), Effect of post harvest storage time and temperature on *Raphanussativus*(b), Change in *Brassicarapa*and *Raphanussativus*metabolome at different developmental stages (c), Effect of food born bacteria on *Brassicarapa*in hydroponic conditions (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** = □-glucose; **4** = □-glucose, sucrose, serine.

The presence of health promoting constituents, such as phenolics, glucosinolates, amino acids, vitamins, sugars, fiber etc. makes *Brassica*ceae 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.

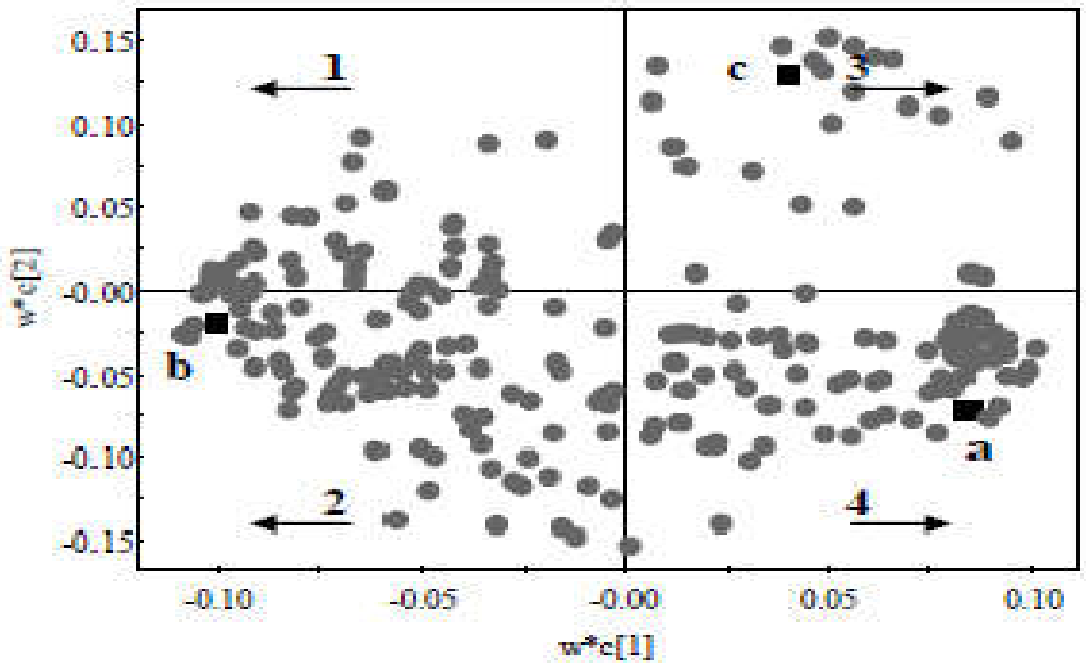


**Figure 1-B:** Loading plot (PC1 vs PC2) of PLSDA for leaves (A) and roots (B). *Brassicarapa*stressed with metals (a), Effect of post harvest storage time and temperature on *Raphanussativus*(b), Change in *Brassicarapa*and *Raphanussativus*metabolome at different developmental stages (c), Effect of food born bacteria on *Brassicarapa*in hydroponic conditions (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.

## Conclusion

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(Tikunov et al., 2005). NMR analysis coupled with multivariate data analysis techniques are well known recently for metabolomic studies in plants(Soylu, 2006). As NMR is used to study a wide range of diverse group of metabolites to analyze in a single run, it is advantageous to use multivariate data analysis tools for NMR data processing and analysis.

Undoubtedly, metabolomics contributes to a major extent for the exploration of plant systems, leading to the development of more resistant plant varieties ultimately providing higher yields along with higher nutritional value.

## Acknowledgement

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**References**

Dowlatabadi, R., Weljie, A. M., Thorpe, T. A., Yeung, E. C. and Vogel, H. J. 2009. Metabolic footprinting study of white spruce somatic embryogenesis using NMR spectroscopy. Plant Physiol Biochem. 47(5):343-350.

Gols, R., Raaijmakers, C., Van Dam, N., Dicke, M., Bukovinszky, T. and Harvey, J. 2007. Temporal changes affect plant chemistry and tritrophic interactions. Basic Applied Ecol. 8(5): 421-433.

Jahangir, M. 2010. Stress response and health affecting compounds in Brassicaceae: Department of Pharmacognosy and Metabolomics, Institute of Biology (IBL), Faculty of Science, Leiden University.

Jahangir, M., Abdel-Farid, I. B., Choi, Y. H., and Verpoorte, R. 2008. Metal ion-inducing metabolite accumulation in Brassica rapa. J. Plant Physiol. 165(14): 1429-1437.

Jahangir, M., Abdel-Farid, I. B., de Vos, C., Jonker, H. H., Choi, Y. H., and Verpoorte, R. 2014. Metabolomic variation of Brassica rapa var. rapa (var. raapstelen) and Raphanus sativus L. at different developmental stages. Pak. J. Bot. 46(4): 1445-1452.

Jahangir, M., Abdel-Farid, I. B., Mahmood, Z., Jamil, M., Rey, P.-J., Choi, Y. H. and Verpoorte, R. 2014. Metabolomic Approach: Postharvest Storage Stability of Red Radish (Raphanus sativus L.). J. Chemical Society Pak. 36(5).

Jahangir, M., Kim, H. K., Choi, Y. H. and Verpoorte, R. 2008. Metabolomic response of Brassica rapa submitted to pre-harvest bacterial contamination. Food Chem. 107(1), 362-368.

Soylu, S. 2006. Accumulation of cell-wall bound phenolic compounds and phytoalexin in Arabidopsis thaliana leaves following inoculation with pathovars of Pseudomonas syringae. Plant Sci. 170(5): 942-952.

Tikunov, Y., Lommen, A., de Vos, C. R., Verhoeven, H. A., Bino, R. J., Hall, R. D. and Bovy, A. G. 2005. A novel approach for nontargeted data analysis for metabolomics. Large-scale profiling of tomato fruit volatiles. Plant Physiol. 139(3): 1125-1137.

Verpoorte, R., Choi, Y. and Kim, H. 2007. NMR-based metabolomics at work in phytochemistry. Phytochem. Rev. 6(1): 3-14.