



Stress Response: Alteration in Brassicaceae Metabolome

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Abstract: As staple food, Brassicaceae has global recognition and acceptance for human and animal consumption. The crops in Brassicaceae are a good source of compounds effecting health and are generally used as food, moreover they are a model for plant science research. The constituents those are important nutritionally includes, phenolic compounds, vitamins, fibers, soluble sugars, minerals, fats, and carotenoids. During growth, various biotic (e.g. herbivory, fungal, bacterial and/or viral infection) and abiotic (e.g. metals, UV, temperature, drought) stresses affects the plants. The metabolomic changes can be reasonably specific, as the plant defense-associated compounds consists of a variety of constitutive and induced metabolites. In nature plants have multitrophic interactions during growth and developmental processes. 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. 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 defense indication compounds, for example salicylic acid, jasmonic acid and furthermore of infection with pathogenic and non pathogenic fungi, as well as human pathogenic bacteria and metals. Effects were measured at various developmental stages of the plant. Finally also the consequence of storage for various periods and temperatures was evaluated. An overlap of different treatments was observed that needs to be studied in more detail. The metabolomics changes during plant growth, characterizes the changes in metabolomic fluxes through diverse pathways.

The current review shows the significance of plant stress factors as a feature for variance in nutritional value of vegetables for human utilization

Keywords: Brassicaceae; plant stress; metabolomics; systems biology

Introduction

Vegetables are always considered as health promoting food. Among vegetables Brassicaceae are well recognized all over the world containing compounds considered good for health. A huge number of data is existing for compounds considered good for health in these vegetables. The health promoting influence of these compounds (Jahangir et al., 2009b) are due to a variety of phytochemicals which includes primary (amino acids, organic acid and carbohydrates) and as well as secondary metabolites (including glucosinolates and phenolics), along with vitamins and minerals. Different biosynthetic pathways inter connects these metabolites and are influenced by various external stimuli. Plant metabolic responses in term of metabolite production and activation of metabolomic pathways are stress specific (Jahangir et al., 2009a). Certain internal or external factors play an important role for the alteration of metabolite profile of vegetables, thus nutritional value for human is changed. These factors are associated to the plant reaction to external stress factors (Jahangir et al., 2008a; Jahangir et al., 2008b), helps the plant to go on. The main theme of this review to give an overview of recent trend of NMR metabolomics as a tool of system biology. Where study was carried out to evaluate the phytocimical stats of Brassica under stress conditions by analyzing as a holistic approach.

General Overview of Stress Induced Metabolomic Response of Brassicaceae

A considerable quantitative metabolomic variation among different species of Brassica is reported, which shows a nutritional difference of these species. In developmental and growth stages, the plant has to manage with various stress factors including attack of animals, pathogens, insects, and/or metal, temperature, UV and

drought stress. These internal physiological growth or external stimuli regulation is responsible to guide the inactivation or improvement of various metabolic pathways which results in qualitative and/or quantitative changes in metabolite plant production. At the same time plants are exposed to multiple stresses in nature, so a natural selection pressure is there for them to evolve coordinated rather than disagreeing defense mechanisms (Bruce and Pickett, 2007).

Plants at the time of stress enhances secondary metabolite production is which has been observed, this change is particular in conditions of the type of interaction. It may be depending on the nature of stimuli and/or invading microorganism, that might in particular activate or suppress gene expression and persuade or block specific sites of a metabolomic pathway, or even result in catabolism of the plant defense compounds (Jahangir et al., 2009a).

Metabolomic Assessment of *Brassica rapa* (var. raapstelen) and *Raphanus sativus* L.

Brassica rapa (var. raapstelen) and *Raphanus sativus* (red radish) are known to be an established model systems in recent plant research. Metabolomic data can mislead researchers if all the factors that are affecting the plant growth are not taken into consideration. The concentration of secondary as well as primary metabolites also depends on the plant age (Abdel-Farid et al., 2007). Metabolomic difference has been assessed during the three developmental stages. A two way analytical Amino acids, organic acids, chlorophyll, tocopherols, ascorbic acid, carotenoids, sucrose, glucosinolates and phenylpropanoids are obviously the discriminating metabolites in this study. In plant species, the metabolomic variation is equally both spatial and temporal, and various trends under identical conditions

may be observed for the species. With respect to increase in plant age previously reported (Abdel-Farid et al., 2007) these results confirm the change in plant metabolomic pool.

Metabolomic response of Brassica to preharvest bacterial contamination

Different metabolomic pathways are induced by cell defense response leading to altered production of certain primary and/or secondary metabolites in Brassica. These plant self-protective responses (Vaquero et al., 2007), are activated upon recognition of signal molecules from pathogens (Mehdy, 1994) or the stimuli from the chemical environment (Vereecke et al., 1997).

Relations of plants to plant pathogens has been studied extensively, this is different in case of interaction with human pathogens, which may spread in the environment, mainly in land irrigated directly as well as indirectly by drainage / sewage water. In this situation the possibility of the interaction of these microbes with vegetables cannot be excluded, although visual symptoms for this interaction cannot be observed. In order to investigate Brassica interaction with characteristically food borne bacteria such as *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhimurium* and *Shigella flexneri*, PCA in combination with NMR and PLS-DA treatment of the data was applied to *B. rapa* which had been subjected to these pathogens (Jahangir et al., 2008b). They observed that by doing PCA analysis, a microbial class (gram-positive and gram-negative) dependent response of *B. rapa* was easily seen and even when compared visually in case of differentially treated plant samples. In plants those were under stress by bacteria a higher production of secondary metabolites was observed. Higher production of GABA, specifically is notable in *Bacillus* infested plants, while in

general higher amount of phenolics were observed in bacterial infected plants as compared with control and blank. The bacteria thus induces a systemic resistance. Generally threonine and α -amino butyric acid (GABA) were the discriminating metabolites in gram-positive bacteria treated plants, in comparison with those treated with gram-negative bacteria that exhibited a significant increase in sinapoyl malate, caffeoyl malate and histidine on the other hand (Jahangir et al., 2008b). Certain volatiles are produced in case of microorganisms that play a pivotal role in SAR, particularly in case of *Bacillus* spp. it is obvious from the study so far done that 2,3-butandiol, produced by these bacteria plays an vital role in systemic induced resistance of plants. Such microbial volatiles may trigger certain biochemical changes in primary and secondary metabolism of plants, causing elicitation of plant defense (Ryu et al., 2004).

Metabolomic Response to Metal Stress

The environment has been made threatening by rapid industrial development as well as by extreme changes in atmospheric conditions around the globe made the for plant development because of contaminated air and soil. In these situations, plants may have to deal with high concentration of metal ions, consequential in changes in plant metabolism (Guo et al., 2007). Due to this metabolomic alteration results in the activation of multiple metabolomic ways, by changing the quantity and activity of key enzymes of various metabolomic pathways (Guo et al., 2007). It seems that this reaction of plant to stress is experienced in the both roots and leaves (Memon et al., 2001).

The plants grown in higher levels of metal ionic circumstances increase the production of amino acids, phenolics and glucosinolates. Among these phenolics and

amino acids are reported to have a metal chelating effect (Xiong et al., 2006) indicates that the observed increase in phenylpropanoids and amino acids might be a detoxification reaction of the plant. though, high metal concentrations (500 mmol) produced a decrease in primary and secondary metabolites as compared with reasonable concentrations (50 and 100 mM) which may have an adverse effect of metal ions on secondary as well as primary metabolism and due to the toxic effect of metals at high concentrations (Jahangir et al., 2008a).

Metabolomic Response to Post Harvest Temperature and Storage Time

All over the world Brassicaceae vegetables are consumed enormously. The presence of a range of phytochemicals (amino acids, glucosinolates, flavonoids, vitamins and mineral nutrients), is the main reason for importance to human and animals as a food source vegetables. A systems biology approach was applied to study the response of Brassicaceae metabolites to preharvest and postharvest factors. It is observed that various environmental, storage and/or growth factors induce metabolic responses in terms of changes of primary and/or secondary plant metabolites profiles. The aforementioned factors cause changes in plant metabolites finally leading to change in quality.

Vegetables pass through many stages from field to cooking pan. The preharvest growth circumstances are well known to extensively affect the plant metabolome, but the storage conditions after harvest also play a vital role for the nutritional character of vegetables. The degradation of these products may be due to the decline of secondary metabolites. This phenomenon is dependent upon the temperature and storage time (Tomas-Barberan and Espin, 2001; Bednarek et al., 2005). Low temperature is

known as an important factor in postharvest storage of Brassica vegetables, either with the purpose of consumption or for purposes of experiments.

Fungal Infection and Metabolomic Alteration in *Brassica rapa*

After characterization of the *B. rapa* metabolome and differentiation between cultivars and developmental stages, the next step is to test the effect of the fungal infection on the *B. rapa* metabolome. The metabolic interactions between three cultivars of *B. rapa* (Raapstelen, Herfstraap and Oleifera) and three pathogenic fungi (*Leptosphaeria maculans*, *Aspergillus niger*, and *Fusarium oxysporum*) were evaluated using NMR spectroscopy combined with multivariate data analysis. Phenylpropanoids (sinapoyl- and feruloyl malate), flavonoids (kaempferolglucosides) and aliphatic glucosinolates (progoitrin) together with fumaric acid were found highly associated with the fungal infection. Subsequently, the fungal species-specific inducing metabolites were evaluated. *Fusarium oxysporum* infected plants induced more strongly than the other fungi the accumulation of phenylpropanoids (sinapoyl-, feruloyl- and 5-hydroxyferuloyl malate), flavonoids (kaempferol and quercetinglucosides), progoitrin and fumaric acid. The results showed also that the response to infection is dependent not only on infecting fungus but also on the host plant (Abdel-Farid et al., 2009; Abdel-Farid et al., 2010).

Although 2D-NMR increased considerably the number of recognized metabolites ahead of that from 1D-NMR, the overlapping of the glucosinolates signals hampered the identification of all individual glucosinolates in the plant extract. So after evaluation of the effect of fungal infection on Brassica metabolome and profiling of the fungal and cultivar-species specific metabolites by NMR, after infection of two

cultivars HPLC was used to profile the individual glucosinolate contents (Herfstraap and Oleifera) with two fungal pathogens (*L. maculans* and *F. oxysporum*) especially that by NMR these two cultivars showed significant change in glucosinolates (Abdel-Farid et al., 2010). Infection enhanced the accumulation of total, aliphatic and indoleglucosinolates in Herfstraap infected with *L. maculans* or *F. oxysporum* and indoleglucosinolates in Oleifera infected with *F. oxysporum*. If we make comparison with *L. maculans* the pathogen *F. oxysporum* induced accumulation of higher levels of individual aliphatic glucosinolates (gluconapin, progoitrin, glucobrassicinapin and gluconapoleiferin) and 4-hydroxyglucobrassicin in Herfstraap and higher level of glucobrassicin and 4-hydroxyglucobrassicin in Oleifera. These higher glucosinolate levels in Herfstraap than in Oleifera, both infected with fungi, together with the HPLC data on healthy plants (Abdel-Farid et al., 2010) may be connected with Herfstraap being a resistant cultivar and Oleifera a susceptible one. The results of HPLC analysis of Herfstraap and Oleifera infected with *L. maculans* and *F. oxysporum* are in line with NMR results of infected Brassica with different fungi as discussed (Abdel-Farid et al., 2010). The response of different cultivars and varieties of Brassica species to biotic stress was found to differ in glucosinolate profiles (Doughty et al., 1991; Menard et al., 1999). The response of each cultivar of Brassica was obviously different depending on the infecting fungus. To get a clear picture of metabolic changes in the fungal infected Brassica, another experiment has been done, in which only one Brassica cultivar (Raapstelen) was infected with one species of fungi (*Leptosphaeria maculans*) and harvested in two different time points. This study allows us to obtain some information on the dynamics of

metabolomic changes after infection. The infection of *B. rapa* with *L. maculans* reduced the accumulation of indoleglucosinolates and enhanced the accumulation of phenylpropanoids, flavonoids (quercetin) and IAA. Plants exposed to stress such as being infected by pathogens undergo many distinct changes in the metabolites originating from phenylpropanoid pathways (Dixon and Paiva, 1995). Phenylpropanoids biosynthetic pathways are among the most frequently observed metabolic activities that are transcriptionally induced upon infection of plants with pathogens. The role of hydroxycinnamic acid derivatives in plant defense responses to biotic or abiotic stresses was demonstrated. However, infection of *B. rapa* with *Agrobacterium tumefaciens* suppressed the accumulation of flavonoids (kaempferol and quercetin) and phenylpropanoids (sinapoyl- and coumaroyl malate) in comparison to the controls (Simoh et al., 2009; Simoh et al., 2010) showing that the defense response is specific for the infecting organism.

The cinnamic acid derivatives are also precursors of flavonoids. Chinese cabbage leaves response to the fungal pathogen *Alternaria brassicae* by accumulation of anthocyanins. Epidermal constitutive anthocyanin production is an indicator of resistance to bacterial blight *Xanthomonas campestris* in cotton leaves (Kangatharalingam et al., 2002). In Brassicaceae indolic metabolism is very complex and includes various functionally separate classes of compounds, including the phytohormone auxin (IAA) and indoleglucosinolates. IAA showed a significant increase in infected plants with *L. maculans*. IAA not only acts as an vital hormone of plant contributing to a variety of developmental processes (Weiler et al., 1993) but might also work as a metabolite that can act as a chemical agent in plant

defense against a wide variety of plant associated bacteria in the rhizosphere. The increased levels of IAA in *B. rapa* after *L. maculans* infection may thus be due to the fact that IAA plays a role in the defense response of Brassica. That the level of IAA increased significantly after MJ treatment (Liang et al., 2006a; Liang et al., 2006b) and JA treatment supports this. A significant decrease was observed in the dominant indoleglucosinolates (glucobrassicin and neoglucobrassicin) in Raapstelen infected with *L. maculans*. At the same time a significant increase was found in all detected phenylpropanoids, flavonoids and IAA suggesting that channeling of precursors may be altered in infected plants (Abdel-Farid et al., 2009).

Alteration of *Brassica rapa* Metabolome after JA and SA Treatment

Not only fungal infection influence the Brassica metabolome but as well as some other factors change the metabolome of *B. rapa*. In plant resistance to fungal infection the signal compounds JA and SA have a task in signal transduction pathways. A wide range of primary and secondary metabolites discriminate JA and SA treated plants from control. The secondary metabolites are derived from the chorismate pathways (phenylpropanoids, flavonoids, IAA, indole and benzoyl glucosinolates).

Methyl jasmonate induced coumaroyl conjugates in barley leaves, phenylpropanoids (sinapoyl-, caffeoyl-, feruloyl- and coumaroyl malate) in *B. rapa* (Liang et al., 2006a; Liang et al., 2006b), flavonoids (kaempferol and quercetinglucosides), indoleglucosinolates in *Arabidopsis* and Brassica species (Mikkelsen et al., 2003; Liang et al., 2006a), benzoyl glucosinolates in *B. napus* and aliphatic glucosinolates in *B. napus* and *B. rapa* (Doughty et al., 1995).

SA induced flavonoids and anthocyanins in *B. oleracea* (Cole, 1997), indoleglucosinolates (neoglucobrassicin and 4-methoxy-glucobrassicin), aliphatic glucosinolates (gluconapin) and benzoyl glucosinolates (gluconasturtiin) in *Brassica campestris*ssp, *Pekinensis* (Ludwig-Muller et al., 1997).

NMR Spectroscopy and Metabolomic Regulation in *Brassica rapa* Infected with Fungi and Treated with JA and SA Treatment

Infected and treated plants showed changes in manifold of primary and secondary metabolites. Infected plants are characterized by higher levels of amino acids, phenylpropanoids, quercetin, indoleacetic acid (IAA), 4-methoxy-glucobrassicin and benzoyl glucosinolates than in jasmonic acid (JA) and salicylic acid (SA) treated plants. Jasmonic acid and salicylic acid treated plants showed higher levels of fumaric acid, kaempferol, aliphatic and indoleglucosinolates than fungal infected plants. Apart from infection with fungi signal compounds act prominently and more powerfully on the glucosinolates content (Liang et al., 2006a). Whereas fungal infection acts prominently on phenylpropanoids, quercetin and IAA if compared with signal molecules treated plants. Infection of *B. rapa* with fungi or treatment with signal molecules consequence in decreased sucrose levels with simultaneous accumulation of several amino and organic acids. This pattern indicates altered carbohydrate metabolism following infection or treatment with signal compounds (Abdel-Farid et al., 2009). A decreased level of sucrose was also noticed during the infection of *B. rapa* with food-borne pathogenic bacteria (Jahangir et al., 2008b), *B. rapa* infected with *Agrobacterium tumefaciens* (Simoh et al.,

2009), *B. rapa* treated with methyl jasmonate (Liang et al., 2006a).

Characterization of *Brassica rapa* Cultivars by NMR Spectroscopy

Developmental stages greatly influence the pattern of synthesis and accumulation of secondary metabolites (Abdel-Farid et al., 2007). Brassica has many other secondary metabolites in addition to glucosinolates such as flavonoids and phenylpropanoids (Jahangir et al., 2009b). As primary metabolites are very important as they take part directly or indirectly in the biosynthesis of secondary metabolites, the distribution of primary and secondary metabolites in different developmental stages of some cultivars of *B. rapa* shows the picture of the whole of metabolites in the plant (Stoewsand, 1995; Mattoli et al., 2010; Kortensniemi et al., 2014). Nuclear magnetic resonance (NMR) spectroscopy is used to measure the metabolome of *B. rapa* and multivariate data analysis to differentiate between different cultivars and developmental stages. The high concentration of phenylpropanoids and glucosinolates in six-week old plants reflects their role in constitutive defense. The build-up of these secondary metabolites which are derived from amino acids and organic acids may explain the decrease of the latter observed in six week-old plants (Abdel-Farid et al., 2007). Obviously ageing is an important factor determining the level of various secondary metabolites. This should be taken into account in doing studies of these plants under different forms of stress.

Conclusion and Future Perspectives

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. Without any doubt, all these hard work should have a say to provide the means of controlling these various defense systems, leading to the development of more resistant plant varieties ultimately providing higher yields along with higher nutritional value.

Researches on health and phytonutrients, gaining 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 underground multitrophic connections of plants in relation 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 defense 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. 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 be monitored constantly during this process.

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