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Chapter 3

Healthy and unhealthy plants: The effect of stress on the metabolism of Brassicaceae

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Abstract

Brassicaceae plants are one of the most popular vegetables consumed all over the world and considered to be a good source of bioactive phytochemicals. Additionally, *Brassica* species and varieties are increasingly becoming a research model in plant science, as a consequence of the importance of their primary and secondary metabolites. Plant interaction with environmental stress factors including animals and insects herbivory, pathogens, metal ions, light, among others, is known to lead to the activation of various defence mechanisms resulting in a qualitative and/or quantitative change in plant metabolite production. Pre-harvest and/or post-harvest conditions are also known to affect this, since plants produce signalling molecules (e.g. salicylic acid, jasmonic acid etc.), that cause a direct or indirect activation of metabolomic pathways. That ultimately affects the production of phytochemicals, such as carbohydrates (sucrose and glucose), amino acids, phenolics (phenylpropanoids and flavonoids) and glucosinolates. These phytochemicals have diverse applications due to their antimicrobial, antioxidant and anticarcinogenic properties, but on the other hand these compounds or their breakdown products can act as anti-nutritional factors in diet. In this review we report a wide range of stress induced metabolomic responses in *Brassica* plants commonly used for human consumption.

Keywords: Brassicaceae, glucosinolates, metabolomics, primary and secondary metabolites, plant stress response.

1 Introduction

Brassicaceae vegetables, commonly known as Crucifers, include different genera of cabbage, broccoli, cauliflower, Brussels sprouts, kale, etc. which are consumed all over the world.⁸⁹ Genetic resemblance of *Brassica* to *Arabidopsis* has made it an alternative model system in plant science,²⁸ and increased the value of *Arabidopsis* plant research. Brassicaceae vegetables are a good source of antioxidants because of their high phenolics and glucosinolate content.^{6, 27, 247} These compounds are generally considered to have a preventive role against cardiovascular diseases and different types of cancer^{6, 211, 248, 249} but on the other hand the antinutritional effects of polyphenols, glucosinolates, *S*-methylcysteine sulfoxide, tannins and erucic acid, from Brassicaceae vegetables have also been previously reported.^{250, 251}

Throughout the course of growth and development plants are ordinarily exposed to various environmental, biotic and abiotic factors,¹⁸ to which they respond with an activation of their defence system.²⁵² This results in a substantial and significant variation in the plant metabolome, both within and between the subspecies.^{27, 101, 253, 254} The inducing factors largely affect the primary and secondary metabolism of *Brassica* plants, resulting in the enhanced production of certain metabolites, e.g. amino acids, sugars, indoles, phenolics and glucosinolates^{6, 255-257} (**Figure 1**).

In particular some biotic and abiotic elicitors can result in an enhancement of the specific secondary metabolite production.¹⁹ Under these conditions a number of signal pathways can be pre-activated by salicylic acid (SA), jasmonic acid (JA), ethylene or abscisic acid pathways, which are generally involved in the defence responses.^{18, 19, 247} As an example, due to aforementioned factors, plant cells activate the chorismate pathway (**Figure 2**) that also results in changes in the plant phenolics.²⁵⁸⁻²⁶¹

Upon infestation by insects, plants can alter their resistance to pathogens in a complex manner (**Figure 1**).²⁴⁷ For example, in a case study the presence of *Pieris brassicae* caterpillars, feeding of the lower leaves of Brussels sprouts (*Brassica oleracea* var. *gemmifera*) triggers the release of volatiles from upper leaves.²⁶² In *Arabidopsis*, a Brassicaceae plant, a defence reaction used by the plant against the necrotrophic fungal pathogen *Alternaria brassicicola* was ineffective but proved to avoid damage by two bacterial leaf pathogens (*Xanthomonas campestris* pv *armoraciae* and *Pseudomonas syringae* pv *tomato*). In addition the resistance was locally and systemically effective against

turnip crinkle virus (TCV) and was associated with priming for SA dependent defence responses.²⁴⁷

In general the metabolic responses of plants vary according to the type of stress. These responses can be rather specific since the metabolomic pool of plant defence is composed of a variety of constitutive and induced metabolites.^{247, 263-265} The feature of the signalling is complex due to the simultaneous elicitation of several responses by the invading micro-organism.¹⁴⁵

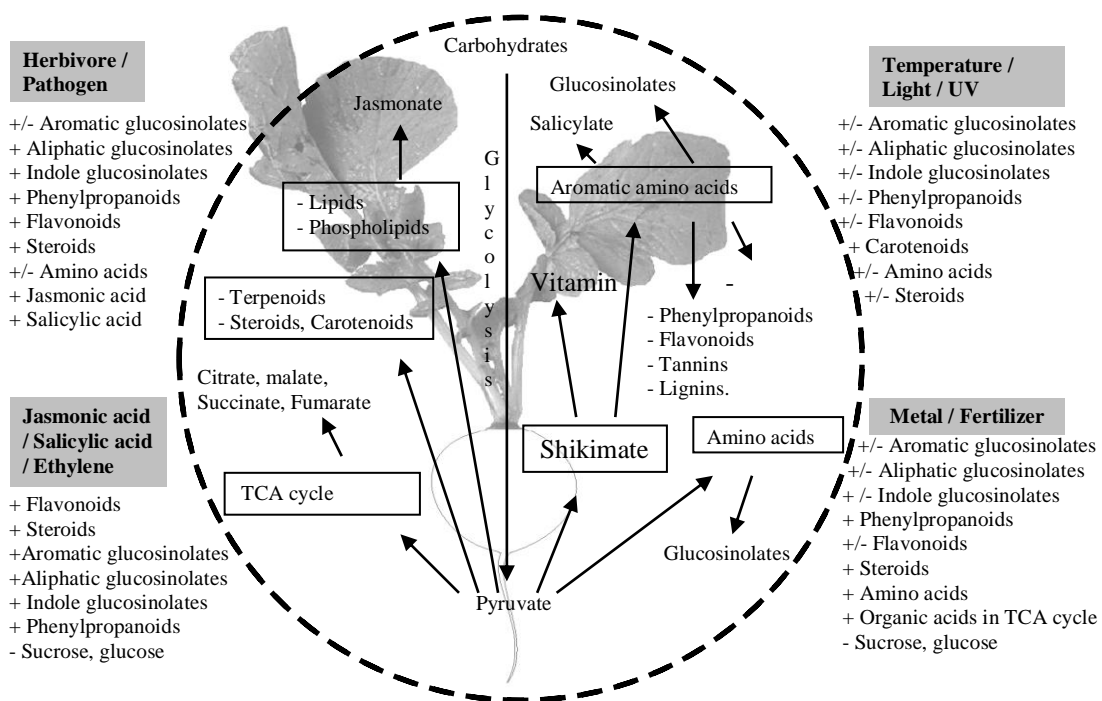


Figure 1 – Summary of the biosynthetic pathway and stress induced metabolite production. The basic metabolomic pathway is drawn in the circle and the stimuli and the compounds increased (+) or decreased (-) as a result of these are listed outside.

A multitrophic interaction (**Figure 3**) could also occur, as observed in the case of a specialist parasitic wasp of *Pieris rapae* caterpillars, *Cotesia rubecula*, which is attracted to *P. rapae* caterpillar infested *Arabidopsis* plants, due to volatiles.

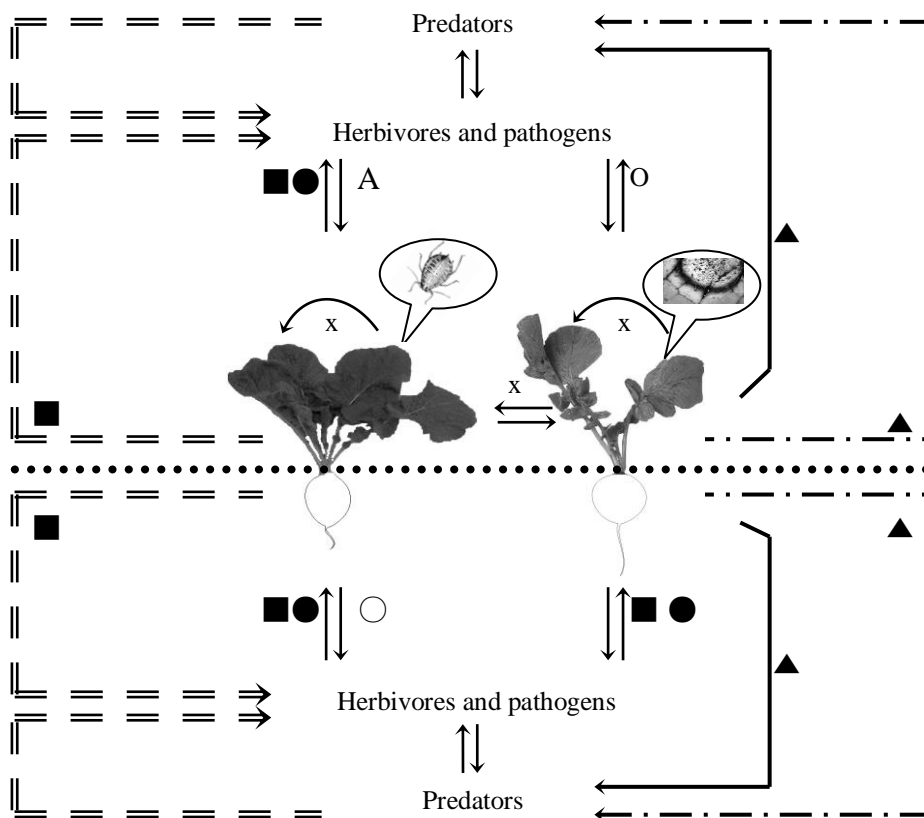


Figure 3 – Basic diagram of above and below ground stress related multitrophic interactions in *Brassica* spp. Direct interaction (—); attraction behaviour (---); repellence behaviour (≡≡≡); ground surface (●●●); (■) glucosinolates; (x) signalling molecules (jasmonic acid, salicylic acid and ethylene); (●) phenylpropanoids and flavonoids; (○) toxins and enzymes; (▲) terpenoids, nitriles and other leaf volatiles.

These volatiles are emitted as a defence against *P. rapae* attack and include metabolites from several major biosynthetic pathways, including terpenoids and green leaf volatiles.²⁶⁶ Genomic information of *Arabidopsis thaliana*, and the availability of characterized mutants and transgenes, can be exploited to address functional aspects of inducible

direct and indirect responses to stress.²⁶⁷ However, *Arabidopsis* is not used as a food source but have been used as a model plant. In these days, much research work is reported on the *Brassica* metabolomic alteration affected by environmental stress because *Brassica* has high genetic resemblance to *Arabidopsis* and even considered one of the most important food crops all over the world.

In this review we will focus on the interaction between the environmental factors and *Brassica* species, aiming at providing an overview of their reported metabolomic responses to pre-harvest or post-harvest stimuli.

2 Primary metabolites

2.1 Effect of biotic factors

Wild *Brassica* species in general have lower amino acid content than cultivated varieties due to diverse factors in nature,²⁶⁸ as the plants are exposed to different stress conditions. Response to stress is coordinated by signalling systems induced by herbivore and/or pathogen attack.²⁶⁹ It is evident, for example, that a decrease of the glucose, sucrose and amino acids levels are observed after methyl jasmonate (MeJA) elicitation of *Brassica rapa* leaves,⁴¹ but an increase in amino acid levels after their interaction with food born pathogenic bacteria.^{27, 270} Similarly, aphid infestation results in the increased production of primary metabolites including amino acids as well as some secondary metabolites.²⁶⁸

2.2 Effect of abiotic factors

Abiotic factors are also known to largely affect metabolite production. This was observed in different situations such as a characteristic linear increase of amino acids produced by drought stressed *Brassica napus* leaves, followed by a reduction in concentration upon rehydration of the plants.²⁷¹ Drought stress conditions have been proved to increase sugar contents in plants as in the case of cabbages, a high level of sugars was detected in water stressed seedlings when compared with control samples.²⁷²

Another abiotic factor, metal exposure, was seen to transiently increase photosynthetic pigments, proteins, free amino acids and sugar contents followed by a decrease when compared with controls.²⁷³ Wild type *Arabidopsis* plants exposed to cadmium stress were found to

generate oxidative damage, finally resulting in the significant loss of chlorophyll content.²⁷⁴ An increase in total free amino acids content in the leaves of *Brassica pekinensis* exposed to copper stress indicated that free amino acids play a role in the detoxification of the copper excess.²⁷⁵ The amount of low molecular weight organic acids, which are reported to be chelating agents, increased after metal stress in *Brassica*.²⁷⁶ Effective accumulation of metals (Cr, Fe, Zn, and Mn) also produced an increase of oil content up to 35% in *Brassica juncea* (cv. Rohini).²⁷³

Temperature also affects the metabolite content of plants. Carotenoids in Brassicaceae, including β -carotene, were found to be slightly decreased after thermal treatments.²⁷⁷ Fresh and processed leaves of kale, which are normally a good source of amino acids, were reported to lose 12 – 14 % of amino acid contents as compared with control, when cooked using traditional methods or when frozen.²⁷⁸ Boiling of kale, Brussels sprouts, broccoli and white cauliflower was also shown to cause a large decrease in the ascorbic acid content,^{277, 279} while exposure of broccoli (*Brassica oleracea* var. *italica*) to UV light or temperature of 7 – 13 °C caused an increase in its content.^{253, 280}

2.3 Effect of growth and storage

It is well known that the metabolomic profile of plants varies according to its growth stage as confirmed by the increase of ascorbic acid in three *Brassica* cultivars during the development of the inflorescence.²⁸¹ Even after harvesting the metabolomic changes still continue, e.g. broccoli undergoes major losses of sugars, organic acids, and proteins within the first 6 hours after harvest. This is followed by an increase in the free amino acid pools (especially glutamine and asparagine).^{128, 254} Loss of membrane fatty acids is also a feature of post-harvest broccoli senescence.^{254, 282} Storage of broccoli florets for 7 days in CO₂-containing atmosphere results in an increase in free amino acids and a decrease in protein amino acids, although the total amino acid content remained unchanged.²⁸³

3 Secondary metabolites

3.1 Glucosinolates

Glucosinolates are one of the most important groups of Brassicaceae metabolites derived from amino acid biosynthesis (e.g. methionine, tryptophan, phenylalanine etc.).^{256, 284, 285} The flavour and

odour of *Brassica* vegetables are typically related to their glucosinolate content.^{31, 286, 287} These are at least partly responsible for their benefits for human health including anti-carcinogenic, cholesterol-reducing, and other pharmacological effects^{6, 288-291} but on the other hand the antinutritional effects of glucosinolates are also reported.²⁵⁰

Glucosinolates are well known to be related to the plant defence response mechanisms, being induced after wounding and/or pathogen attack^{268, 292}, insect herbivory,^{255, 293, 294} exposure to salt stress,²⁹⁵ diverse environmental factors,^{231, 281} or by plant signalling molecules,^{296, 297} including the treatment with SA, JA and MeJA.^{298, 299} Following tissue damage endogenous plant enzymes (thioglucosidases or myrosinases) hydrolyze the glucosinolates to unstable aglycones, which rearrange to yield a variety of products including isothiocyanates, thiocyanates and nitriles. The nature of the products depends on the conditions of the hydrolysis and the particular glucosinolate.^{284, 299-301}

Even under drought stress the secondary metabolism continues,²⁹⁵ for example the total glucosinolate content of mature rapeseed is observed to increase following water deprivation.²⁶⁵

3.1.1 Effect of herbivory and pathogens

Induced defence responses are elicited when plants are exposed to different types of biotic stress such as attack by herbivores or pathogens.²⁴⁷ The activity of glucosinolates and their degraded products against various strains of microorganisms has been documented by many investigators,^{224, 302} being present in the leaves of *Brassica spp.* at concentrations that can prevent the development of pathogens. A report of the effect of the inoculation of *B. rapa* seedlings with the fungal pathogen *Alternaria brassicae*, described the catabolism of glucosinolates during the infection and subsequent release of isothiocyanates together with dimethyl disulphide, dimethyl trisulphide, and 4-oxoisphorone.³⁰³ Unusually a high concentration of 2-hydroxy-3-butenyl glucosinolate found in leaves of *B. oleracea* var *capita* cv *offenham compacta*. It may account for the greater susceptibility of this cabbage cultivar to *Brevicoryne brassicae* (L) (cabbage aphid), compared with other *B. oleracea* accessions examined. It was concluded that an increased production of 4-pentenylglucosinolate with a commensurate reduction in the 2-hydroxy-3-butenyl and 2-propenyl glucosinolates, could provide cultivated *Brassica* crops with some partial resistance to *Brevicoryne brassicae*.²⁶⁸

There is ample evidence that glucosinolate structures and levels influence host plant suitability for generalist and specialist herbivores.³⁰⁴ Monitoring of the total glucosinolate content in a resistant and a susceptible variety of cabbage (*B. campestris* ssp. *pekinensis*) during the development of club root disease, caused by *Plasmodiophora brassicae* revealed that their glucosinolates content differed significantly. The susceptible varieties showed a very high content of aliphatic glucosinolates while the resistant varieties showed high contents of aromatic glucosinolates.³⁰⁵

Levels of glucosinolates increase and their composition may be altered in response to herbivory and pathogen attack as shown in studies carried out on two wild *Brassica* species, *B. nigra* and *B. oleracea*, which were infested with larvae of the cabbage root fly, *Delia radicum*.^{269, 305} The systemic response in the leaves differed between plant species. While in the case of *B. nigra*, shoot glucosinolate levels steadily increased during the growth period, almost duplicating the original concentration after 14 days of infestation, *B. oleracea* plants did not show any significant changes in shoot glucosinolate levels as compared with a control group of plants.³⁰⁶

Increased glucosinolate accumulation, primarily of short-chain aliphatic methylsulfinyl glucosinolates, in response to insect feeding (generalist *Myzus persicae* and specialist *Brevicoryne brassicae*) was also observed in *A. thaliana*.²⁶⁹ Increased glucosinolate levels in *B. napus* and *Sinapis alba* reduced the extent of grazing by generalist herbivores but resulted in greater damage by the glucosinolate specialist beetle *Psylliodes chrysocephala* and butterfly *P. rapae*.³⁰⁷ Differences in susceptibility to herbivores among *S. alba*, *B. napus*, and *B. campestris* have been attributed to their glucosinolate content, particularly 4-hydroxybenzyl glucosinolate.^{308, 309} Mechanical wounding or feeding by the flea beetle (*Phyllotreta cruciferae*) was found to produce a three-fold increase in the concentration of indole glucosinolates in the cotyledons of one-week-old seedlings of the oilseed rape *B. napus*, *B. rapa* and *B. juncea*.³¹⁰ Similarly, another study reported that damage to the *B. napus* by *Psylliodes chrysocephala* induced systemic changes to the glucosinolate profile, most noticeably an increase in the concentration of indole glucosinolates.³¹¹

Aliphatic glucosinolate profiles have a significant impact on the development and performance of *Brevicoryne brassicae* and *Myzus persicae* on *A. thaliana*³¹², *B. napus*³¹³ and oilseed rape.³¹⁴ Conversely, no changes in the glucosinolate content of oilseed rape plants following

infection with Turnip Mosaic Virus were reported.³¹⁵ Decrease in glucosinolate content was observed in healthy and *Alternaria brassicae* inoculated seedlings of *B. rapa*.³⁰³

Indole glucosinolates degradation increased in resistant varieties after infestation with downy mildew possibly due to their conversion into other biochemical compounds involved in the resistance. These indole glucosinolates could be involved in a complex metabolomic process, in which they are not considered solely as metabolomic end-products but also as precursors of other molecules, such as phytoalexins or auxins, known for their involvement in the resistance to microorganisms.³¹⁶ The results of a study carried out on two susceptible and three resistant varieties of cauliflower plants (*Brassica oleracea* var. *botrytis*) infected with *Peronospora parasitica* to determine the correlation between glucosinolates and resistance to this microorganism showed that sinigrin content was higher in the resistant varieties than in susceptible ones and that if glucobrassicin decreased, methoxyglucobrassicin increased.³¹⁶ It is important to note that infection by a virulent bacterial pathogen was proved to induce the expression of genes responsible for indole glucosinolate biosynthesis.²⁸⁴

3.1.2 Effect of signalling molecules

It is evident that the presence of signalling molecules affects the glucosinolate profile of these species. In the case of *A. thaliana*, its responses to different stresses are coordinated by several interacting signalling systems including JA, SA and ethylene (ET) mediated pathways²⁶⁹ as shown in the infection with the fungus *Alternaria brassicicola*.³¹⁷ Plant growth-promoting bacteria produce this effect through several mechanisms, including the synthesis of indoleacetic acid (IAA).³¹⁸ It was also observed that in *Arabidopsis*, the rhizobacteria-induced systemic resistance is phenotypically similar to pathogen-induced systemic acquired resistance (SAR),²⁶ but functions independently of SA and requires responsiveness to JA and ethylene.³¹⁹

The concentration of gluconasturtiin was specifically increased by SA.³²⁰ *Brassica napus* plants exposed to MeJA accumulated indole glucosinolates in their leaves, the amount of which depended on the concentration of MeJA applied.³²¹ Moreover, an increase in glucosinolate levels, especially indole glucosinolates, was observed in *B. rapa* leaves after MeJA elicitation.⁴¹ The treatment of a resistant and a susceptible variety of Chinese cabbage (*B. compestris* ssp. *pekinensis*) with SA or JA, produced an increase in the total amount of glucosinolates, though the

response depended on the type of treatment applied. JA induced indole glucosinolates production only in the leaves, while SA induced indole glucosinolates in both leaves and roots of the cabbage.³⁰⁵ However, as a result of a negative cross-talk, SA has been observed to inhibit JA induced resistance of *Arabidopsis* to *Spodoptera exigua* by inhibiting production of glucosinolates by JA.²⁴⁷

Even more conclusive results were obtained from a study carried out on the glucosinolate content of leaves and cotyledons of *B. napus*, *B. rapa* and *B. juncea*, that revealed the content of glucobrassicin and 4-hydroxyglucobrassicin increased up to 20-fold after treatment of leaves and cotyledons with JA or MeJA.²⁹⁸ In contrast, treatment with abscisic acid resulted in low levels of indole glucosinolates in *B. napus*.³²²

3.1.3 Effect of fertilizers and salts

The accumulation of indole, aliphatic and aromatic glucosinolates could be enhanced by the presence of low nitrogen and high sulfur fertilizers. For example, the use of a sulfur fertilizer produced an increase in the level of glucosinolates, gluconapin, sinigrin and progoitrin.^{290, 323} Similarly, the total glucosinolate level was also observed to increase in response to sulfur availability in turnip rape (*B. rapa*)³²⁴ and kale (*B. oleracea* L. Acephala Group),⁷⁶ while three broccoli cultivars showed an increase in total glucosinolate content, only at the start of the inflorescence development followed by a rapid decrease depending on its fertilization with sulphur.²⁸¹ Another fertilizer, selenium, was observed to increase glucosinolates in general and sulforaphane in particular, when applied up to a certain doses, above which it decreased glucosinolate production.³²⁵ Submitting broccoli to salt stress increased their glucosinolate content, indicating the involvement of these compounds in its stress response.²⁹⁵ Some exceptions are also reported, as in the case of cadmium stress which produced no change in glucosinolate production in *B. rapa*.³

3.1.4 Effect of temperature and radiation

There is a relationship between temperature, radiation and glucosinolate content, though the synthesis of each individual glucosinolate is affected differently by each of these factors.^{253, 326} Glucosinolate concentration in canola increased when submitted to a temperature stress of 40 °C for 15 days during growth.³²⁷ A seasonal variation in aliphatic, indole and aromatic glucosinolate content was observed in three different varieties of *B. oleracea*.²²³ The same effect

was observed in broccoli, in which the aliphatic glucosinolates (especially glucoraphanin) content was observed to increase when kept at daily mean temperatures between 7–13 °C combined with the daily mean radiation of 10–13 mol/m²/day.²⁵³ The effect of radiation can depend on the species as in the case of *N. officinale* and *S. alba*, in which different aliphatic and indole glucosinolates had been identified. The concentration of these glucosinolates was significantly more affected by UV treatment in *N. officinale* than *S. alba*.³²⁸

3.1.5 Effect of post-harvest storage conditions

Bioavailability of glucosinolates and related isothiocyanates of *Brassica* vegetables is influenced by storage²⁸⁹ and processing, such as blanching and freezing, affecting the taste and aroma of final products.^{329, 330} Postharvest physical disruption of the plants (e.g. chewing, chopping, blending, juicing, cooking, freezing / thawing and high temperature) leads to the loss of cellular compartmentalization and subsequent contact of glucosinolates with myrosinase to be degraded to isothiocyanates,²³¹ nitriles, thiocyanates, epithionitriles and oxazolidines³³¹ (**Figure 4**).

When stored at ambient temperature (12 – 22 °C), there was no significant loss in glucosinolate content. The contents of individual and total glucosinolates decreased in *Brassica* vegetables (broccoli, Brussels sprouts, cauliflower and green cabbage) when stored in a domestic refrigerator (4 – 8 °C) for 7-days, though some of them, i.e., glucoiberin, glucoraphanin and glucoalyssin, suffered higher losses than sinigrin, gluconapin and progoitrin.²⁸⁹ The glucosinolates were the most affected constituents in rocket (*Eruca vesicaria* ssp. *sativa*) leaves as the content was reduced to 4 – 33%, when samples were stored in air while the decrease was 60 – 100% in low O₂ and high CO₂ conditions, after using sanitizers (chlorine, ozonated water, lactic acid, acidified sodium chlorite and peroxyacetic acid), in both cases.²⁸⁷

Significant loss of glucoerucin and glucoraphanin was observed in rocket during storage at 4 – 8 °C.³³² Storage of vegetables at –85 °C could cause significant loss of glucosinolates due to freeze–thaw fracture of plant cells, leading to enzymatic conversion of glucosinolates to isothiocyanates during thawing.²⁸⁹ Also a significant decline in glucosinolates content was observed during storage of *Brassica* vegetables at 4 °C and at 20 °C.³³³ On the other hand, indole and aliphatic glucosinolates in broccoli increased during storage period of 7 days at 7 – 13 °C.^{253, 334}

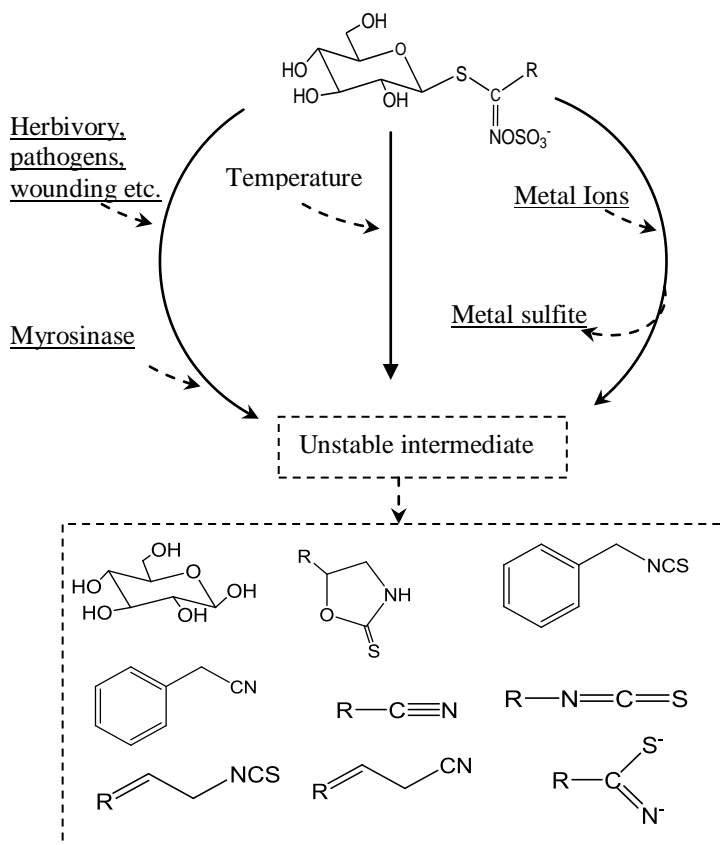


Figure 4 – Glucosinolate degradation.

3.2 Tryptophan derived phytoalexins

Phytoalexin synthesis, as a defence response of Brassicaceae plants, is induced by a number of molecular species, which can function as signal molecules including poly- and oligosaccharides, proteins, polypeptides, fatty acids,¹⁴⁵ and jasmonate among others.⁴¹ Brassicaceae phytoalexins are generally biogenetically derived from tryptophan but have also different chemical structures (**Figure 5**) as well as biological activities.³³⁵ Brassinin, a plant defence phytoalexin with antimicrobial activity, is produced by a variety of *Brassica* species in response to stress.^{336, 337} Camalexin, another phytoalexin, was found in highest concentrations in or around the dead cells where bacterial growth is restricted.³³⁸

3.2.1 Effect of herbivory and pathogens

The differing qualitative and/or quantitative profiles in phytoalexin production are associated to the resistance of different *Brassica* species against diverse fungal attacks. *Camelina sativa* and *Capsella bursa-pastoris* exhibited strong resistance to *Alternaria brassicae* and *Brassica campestris* ssp. *rapifera* was less resistant to *A. brassicae*³³⁹. Another species, *Arabis lyrata* produced camalexin during its interaction with two microorganisms, *Pseudomonas syringae* pv. *Maculicola* and *Cochliobolus carbonum*³⁴⁰ and the induction of two phytoalexins, wasalexin A and arvelexin (4-methoxyindolyl-3-acetonitrile) were observed after *Leptosphaeria maculans* attack on *Thlaspi arvense*³⁴¹. Further examples were observed in leaf and stem tissues of *B. napus* which accumulated two phytoalexins (methoxybrassinin and cyclobrassinin), following inoculation with *L. maculans*.³⁴² While *Alternaria brassicae* induced sinalexin production in *S. alba*.³⁴³ Three phytoalexins, indole-3-acetonitrile, arvelexin, and 1-methoxyspirobrassinin, were identified in *Erucastrum gallicum* leaves after infection by *Sclerotinia sclerotiorum*³⁴⁴ and *B. napus* ssp. *rapifera* produced isalexin, brassicanate A, and rutalexin, brassinin, 1-methoxybrassinin, spirobrassinin, brassicanal A and brassilexin were elicited with the phytopathogenic fungus *Rhizoctonia solani*.³⁴⁵

Canola and rapeseed (*B. rapa*) also accumulated diverse phytoalexins after inoculation with different strains of the biotroph *Albugo candida*.²⁶³ It is important to note that most of the phytoalexins exhibit antifungal activity against the economically important pathogenic fungi *Leptosphaeria maculans*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum*.³⁴⁶ However, some phytopathogenic fungi may have enzymes that can detoxify the phytoanticipins or phytoalexins produced by their host.^{337, 347}

3.2.2 Effect of abiotic factors

It has been proved that both biotic and abiotic elicitation can provoke a response in many *Brassica* species consisting in the production of diverse phytoalexins (**Figure 5**). This effect was observed in the leaves of the oilseed, canola and rapeseed (*B. rapa*) which accumulated the phytoalexins spirobrassinin, cyclobrassinin, rutalexin, rapalexin A and B, brassinin, brassilexin and brassianal C, apart from the phytoanticipins, indolyl-3-acetonitrile, caulilexin C, and arvelexin, after spraying with copper chloride.^{263, 341}

In Brassicaceae, CuCl_2 has shown to be an effective inducer of phytoalexin production^{343, 348} as in the case of *Thlaspi arvense* in which the production of two phytoalexins, wasalexin A and arvelexin (4-methoxyindolyl-3-acetonitrile) was detected after exposure to this abiotic elicitor.³⁴¹ Another report described the concentration of spirobrassinin to be 4 fold of that observed in compatible interactions, with the highest concentration found 2 days after spraying with copper chloride.²⁶³

The effect of another type of abiotic stress, UV light, was described for cauliflower (*Brassica oleracea* var. *botrytis*) florets in which the production of several phytoalexins, i.e., isalexin, (S)-spirobrassinin, 1-methoxybrassinin, brassicanal C, caulilexins A, B and C was enhanced.³⁴⁶ UV light also induced isalexin, brassicanate A, and rutalexin, brassinin, 1-methoxybrassinin, spirobrassinin, brassicanal A and brassilexin synthesis in *B. napus* ssp. *Rapifera*.³⁴⁵

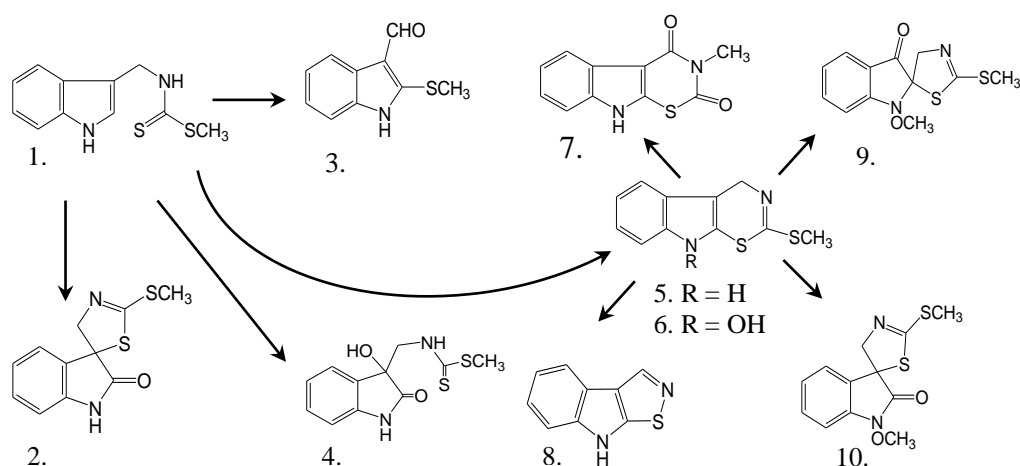


Figure 5 – Biosynthetic pathways of *Brassica* phytoalexins: Brassinin (1), spirobrassinin (2), brassicanal A (3), dioxibrassinin (4), cyclobrassinin (5), sinalbin (6), rutalexin (7), brassilexin (8), erucalexin (9), (*R*)-1-methoxyspirobrassinin (10).

3.3 Phenolics

Brassicaceae vegetables are consumed both raw and processed.³⁴⁹ The content of polyphenols can be influenced by various factors such as the variety, climatic conditions, biotic and abiotic stress caused by the preharvest and postharvest conditions.^{73, 89, 350, 351}

3.3.1 Effect of herbivory and pathogens

It has been proposed that phenolics play an antioxidative role in the plant defence system as a backup to the primary ascorbate-dependent detoxification system.¹³ This is supported by observations such as the rapid accumulation of phenolics detected in a significant proportion of the *Arabidopsis* cells undergoing a hypersensitive response.³³⁸ In the case of microbial infection, the set of metabolites detected differed according to the type of microorganism involved, probably reflecting the chemical environment of the invaded tissue and the mechanism of action of the attacking organism.^{27, 352} For example, metabolomic changes of *Brassica*, induced by different foodborne bacteria such as *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhimurium* and *Shigella flexneri*, were found to vary according to the bacterial species, though in all cases an increase in phenolic compounds including sinapoyl-malate, caffeoyl-malate was observed.²⁷ In the case of insect herbivory, aphid feeding involves phenolics in the formation of salivary sheaths around the penetration sites.³⁵³

Increases in phenolic biosynthesis gene expression or enzyme activity and accumulation of the products of these enzymes, are commonly associated with JA treatment or herbivory in many plants.³¹⁷ This was observed in MeJA treated *B. rapa* leaves, in which 5-hydroxyferuloyl malate, caffeoyl malate, coumaroyl malate, feruloyl malate, and sinapoyl malate were identified.^{41, 354}

3.3.2 Effect of fertilizers

The production of phenolic metabolites responds to changes in nutrient availability in a highly complex manner.^{161, 350} Nitrogen stress triggers the gene expression of flavonoid pathway enzymes and nitrate availability was shown to directly affect the enzyme activity in the phenylpropanoid pathway.³⁵⁰ Sulfur fertilization increased the average phenolic contents from 96 to 111 mg/100 g of gallic acid, of fresh weight in *B. rapa*¹⁶¹ and this quantitative change was confirmed by other studies. The patterns of phenolics did not change qualitatively in leaves of *B. oleracea* var. *costata* grown using different agronomical practices.³⁵⁰ The most representative flavonols in *B. rapa* subsp. *Sylvestris* are kaempferol and quercetin derivatives but myricetin was present only in trace amounts which was reduced rather than increased by sulfur fertilization unlike the total phenolics content. This response indicates that the total phenolics pool may be shifted towards the accumulation of different compounds based on precursor availability, presence of enzymes activators (or co-

factors) and/or other effectors, such as sulfur availability.¹⁶¹ A great increase of anthocyanins in response to cadmium stress was observed in *Brassica juncea*.³⁵⁵

3.3.3 Effect of growth stage

Age also proved to affect the phenolic pattern in these plants as in the case of young *B. oleracea* var. *costata* leaves in which 15 phenolics were found. Five of aforementioned phenolics were kaempferol derivatives and 10 were cinnamic acid derivatives, while *p*-coumaroyl-3-*O*-quinic acid and 13 kaempferol derivatives were detected in old leaves. Only two kaempferol derivatives were found common in both types of leaves.³⁵⁰ Three broccoli cultivars exhibited an increase in phenolic compounds coinciding with the inflorescence development.²⁸¹

3.3.4 Effect of temperature and radiation

Flavonoids play an important role in plants as flower pigments and when formed as metabolites in the response to biotic or abiotic stress.³⁵⁶ Temperature and radiation seem to act as a trigger for biosynthetic pathways.²⁵³ As was also shown by the detection of higher concentrations of phenolic compounds when exposure of plants to sunlight is increased.³⁵⁷ Similarly, UV-B radiation was observed to induce an enhanced production of soluble phenolics in red cabbage³⁵⁸ and an increase in the flavonoid content of *S. alba* and *Nasturtium officinale*, where especially in the case of *S. alba* the increase of quercetin was found to be 10 fold higher than in *N. officinale*.³²⁸ Another study carried out on *B. napus* showed that irradiation with UV-B rays produced a 70–150% increase in the overall amount of flavonoids, four of which were identified as quercetin and kaempferol glucosides.³⁵⁹ Another study confirmed UV-B induced accumulation of quercetin glycosides and correlated them to plant UV-B tolerance.³⁶⁰ UV light (8 kJ m⁻²) treated samples of minimally processed broccoli showed higher phenolic contents than untreated (control) plants.²⁸⁰

Post-harvest temperature conditions have also proved to influence the metabolite profile in plants. Contents of lutein in broccoli increased, when kept at daily mean temperatures between 7–13 °C.²⁵³ Boiling of kale, Brussels sprouts, broccoli and white cauliflower was observed to cause a large decrease in its antioxidant activity, due to the loss of polyphenols.^{277, 279} Freezing has been found to be one of the most effective methods of preserving the nutritive constituents of raw Brassicaceae vegetables.²⁷⁷ It leads to a greater preservation of

polyphenol levels, which were even observed to increase during this process as part of the plant response to stress.²⁷⁹ Also, a higher antioxidant activity was observed in broccoli plants, kept at 4 °C for 21 days as compared with control samples.²⁸⁰ In the case of rocket leaves storage, the stability of quercetin derivatives differed, the glycosides showing more stability than the corresponding acylated glycosides.²⁸⁷ Another study revealed a drastic reduction of the total anthocyanin content of cauliflower and the formation of isomers with all thermal pre-treatments except microwave heating.³⁶¹

3.4 Steroids

Brassinosteroids (BRs) are a group of naturally occurring plant steroidal compounds in *Brassica* having a wide range of biological activities and the ability to confer tolerance to *Brassica* plants against a wide spectrum of biotic and abiotic stresses³⁶². These stress factors include, low and high temperatures, drought, high saline concentrations, pathogen attack^{362, 363} and exposure to heavy metals.³⁶⁴ Sterols have been recently recognized not only as precursors of brassinosteroids (**Figure 6**) and membrane constituents, but also as modulators of plant development.³⁶⁵

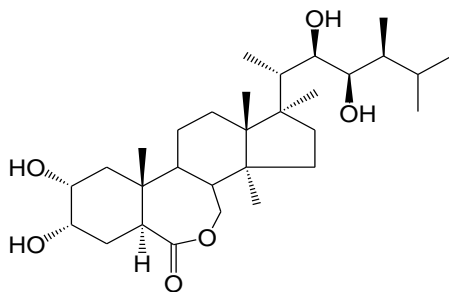


Figure 6 – Brassinosteroid.

Also, there is evidence of cross-talk between BRs and abscisic acid, JA and ethylene.³⁶² Treatment with 24-epibrassinolide, a brassinosteroid, increases tolerance to several environmental stresses such as basic thermo-tolerance in *B. napus*,³⁶⁶ or to drought and cold stress in the case of seedlings of both *A. thaliana* and *B. napus*, aside from helping them to overcome salt-stress-induced inhibition of seed germination.³⁶³ The expression of the *B. napus* steroid sulfotransferase genes was found to be induced by SA, suggesting that in addition to

increased accumulation of an antimicrobial protein, plants respond to pathogen infection by modulating steroid-dependent growth and developmental processes.³⁶⁷

Brassinosteroids also proved to be able to protect the membrane integrity of the radish seedling from Cd-induced oxidative stress, minimizing the impact of reactive oxygen species by increasing antioxidant enzyme activity, a possible secondary defence mechanism against oxidative stresses.³⁶⁸

4 Conclusion

Brassica species are a rich source of health affecting compounds and are widely considered as a food and model for plant science research, in diverse fields (**Figure 3**). During growth, plants are exposed to various biotic (herbivory, fungal, bacterial and/or viral infection) and abiotic (metals, UV, temperature) stresses. It leads to gene expression and biochemical changes, which finally results in an enhancement of the synthesis of primary and secondary metabolites. In this process, a number of signal pathways will be activated as *Brassica* defence responses, including SA, JA, ethylene and abscisic acid pathways¹⁸ (**Figure 1**). On the other hand SA, JA and ethylene dependent defence pathways may also affect each other.

The systemic induced responses of different *Brassica* species led to hypothesize that plants might use a far more complex defensive strategy than a common set of biosynthetic pathways. The metabolomic changes can be quite specific, since the pool of plant defence-related compounds is composed of a variety of constitutive and induced metabolites. The set of *Brassica* metabolites observed, after infection with different microorganisms differs. It probably reflects the diverse chemical composition and mechanism of action of the invading organism, which can at the same time activate gene expression and block specific sites of a metabolomic pathway, or even metabolize the plant defence compounds.

As plants in the field are often exposed to more than one form of stress, there is selection pressure for them to evolve coordinated rather than conflicting defence mechanisms.²⁴⁷ It would be interesting to understand plant-defence mechanism, when multiple stress factors are present.

5 Future Perspectives

The mechanisms by which metabolites help the plant to resist stress remain largely unclear. While there is an increasing interest in above ground interactions, there is a strong need to study underground mechanisms and interactions of plants as well, because they have not been adequately addressed yet. The complementation of the ecological approach, with the understanding of the molecular basis of plant defence strategies, employed against different attackers, should be useful to fully comprehend the extent of the integration of these mechanisms.

The study of multitrophic interactions is also an interesting area for research. This involves comparing the genomic, proteomic and metabolomic situation of the plant after being attacked by different organisms, either individually or in combination²⁷. Further investigation of the systemic induced emission of volatiles, observed in different species, would also contribute to shed light on the ecological significance and regulatory mechanism, behind these defensive responses²⁶². 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. Additionally, since *Brassica* plants are considered to be important food, it is essential to fully understand, how different environmental factors triggering mechanisms and pathways affect their metabolomic profile, since these will ultimately affect the plant's quality and its functional properties. It also attributes to taste and aroma as well as levels of health affecting compounds, which will influence consumer acceptability.