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Environmental and Experimental Botany 67 (2009) 23–33

Contents lists available at ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

Review

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

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ARTICLE INFO

Article history:
Received 18 January 2009
Received in revised form 31 May 2009
Accepted 13 June 2009

Keywords:
Brassicaceae
Phenylpropanoids
Flavonoids
Glucosinolates
Metabolic response
Plant stress response

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 defense 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 signaling molecules (e.g. salicylic acid, jasmonic acid, etc.) that cause a direct or indirect activation of metabolic 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 anti-carcinogenic 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 the stress-induced metabolic responses in the *Brassica* plants commonly used for human consumption.

Contents

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doi:10.1016/j.envexpbot.2009.06.007

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 (Podsedek, 2007). Genetic resemblance of *Brassica* to *Arabidopsis* has made it an alternative model system in plant science (Abdel-Farid et al., 2007), and increased the value of *Arabidopsis* plant research. Brassicaceae vegetables are a good source of antioxidants because of their high phenolics and glucosinolate content (Moreno et al., 2006; Bruce and Pickett, 2007; Jahangir et al., 2008). These compounds are generally considered to have a preventive role against cardiovascular diseases and different types of cancer (Byers and Perry, 1992; Verhoeven et al., 1997; Kushad et al., 1999; Moreno et al., 2006) but on the other hand the anti-nutritional effects of polyphenols, glucosinolates, *S*-methylcysteine sulphoxide, tannins and erucic acid, from Brassicaceae vegetables have also been previously reported (Griffiths et al., 1998; Lotito and Frei, 2006).

Throughout the course of growth and development plants are ordinarily exposed to various genetic, environmental, biotic and abiotic factors (Zhao et al., 2007), to which they respond with an activation of their defense system (Hayat et al., 2007). This results in a substantial and significant variation in the plant metabolome, both within and between the subspecies (Eason et al., 2007; Schonhof et al., 2007; Singh et al., 2007; Jahangir et al., 2008). 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 (Fig. 1) (Moreno et al., 2006; Bellostas et al., 2007; Gols et al., 2007; Petersen et al., 2007). In particular some biotic and abiotic elicitors can result in an enhancement of the specific secondary metabolite production (Sudha and Ravishankar, 2002). Under these conditions a number of signal pathways can be pre-activated by salicylic acid (SA), jasmonic acid (JA), ethylene and abscisic acid pathways, which are generally involved in the defense responses (Sudha and Ravishankar, 2002; Bruce and Pickett, 2007; Zhao et al., 2007). As an example, due to aforementioned factors, plant cells activate the chorismate pathway (Fig. 2) that also results to change in plant metabolome (Shomerilan et al., 1991; Rathinasabapathi, 2000; Qasim et al., 2003; Martinez-Ballesta et al., 2004).

Upon infestation by insects, plants can alter their resistance to pathogens in a complex manner (Fig. 1) (Bruce and Pickett, 2007). For example, in a case study the presence of *Pieris brassicae* caterpillars, feeding off the lower leaves of Brussels sprouts (*Brassica oleracea* var. *gemmifera*) triggers the release of volatiles from upper leaves (Mattiacci et al., 2001). In *Arabidopsis*, a Brassicaceae plant, a defense 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 defense responses (Bruce and Pickett, 2007).

In general the metabolic responses of plants vary according to the type of stress. These responses can be rather specific since the metabolic pool of plant defense is composed of a variety of constitutive and induced metabolites (Bouchereau et al., 1996; Chinnusamy et al., 2004; Bruce and Pickett, 2007; Pedras et al., 2008). The feature of the signaling is complex due to the simultaneous elicitation of several responses by the invading microorganism (Smith, 1996). A multitrophic interaction (Fig. 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. These volatiles are emitted as a defense against *P. rapae* attack and contain metabolites from several major biosynthetic pathways, including terpenoids and green leaf volatiles (Van Poecke et al., 2001). 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 (Dicke et al., 2003). However, *Arabidopsis* is not used as a food source but have been used as a model plant. In these days, many research works have been reported on the *Brassica* metabolic 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 metabolic 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 different factors (Cole, 1997), as the plants are exposed to different stress conditions. Response to stress is coordinated by signaling systems induced by herbivore and/or pathogen attack (Mewis et al., 2005). It is evident, for example, that the decrease of the glucose, sucrose and amino acids levels are observed after methyl jasmonate (MeJA) elicitation of *Brassica rapa* leaves (Liang et al., 2006a), or the increase in amino acid levels after their interaction with food born pathogenic bacteria (Brandl, 2006; Jahangir et al., 2008). Similarly, aphid infestation results in the increased production of primary metabolites including amino acids as well as some secondary metabolites (Cole, 1997).

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 (Good and Zaplachinski, 1994). Water 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 to control samples (Sasaki et al., 1998).

Another abiotic factor, metal exposure, was seen to initially increase photosynthetic pigments, proteins, free amino acids and sugar contents followed by a decrease compared to controls (Singh and Sinha, 2005). Wild type *Arabidopsis* plants exposed to cadmium stress were found to generate oxidative damage, finally resulting in

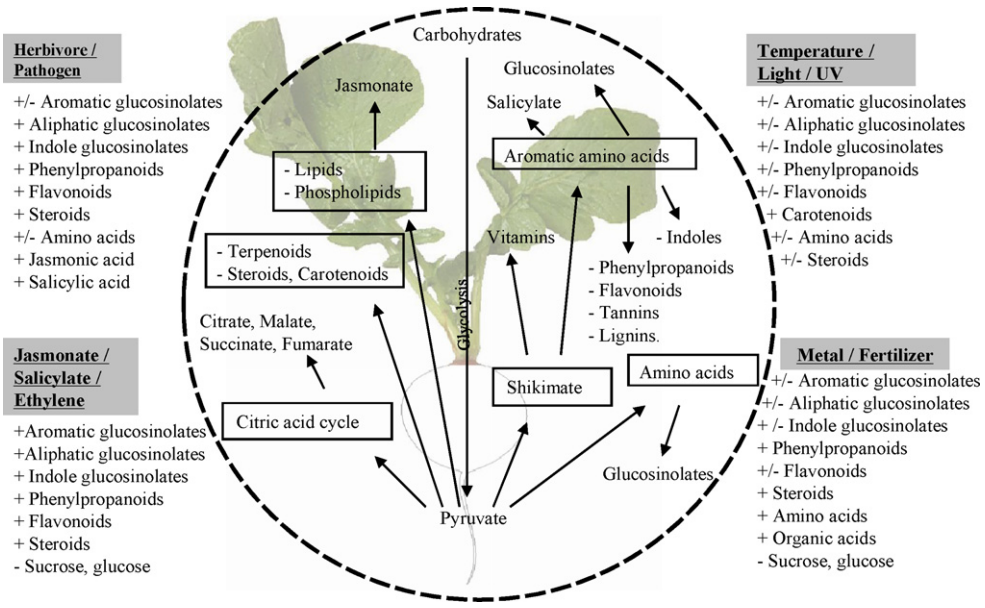

Fig. 1. Summary of the biosynthetic pathway and stress-induced metabolite production. The basic metabolic pathway is drawn in the circle and the stimuli and the compounds increased (+) or decreased (−) as a result of these are listed outside.

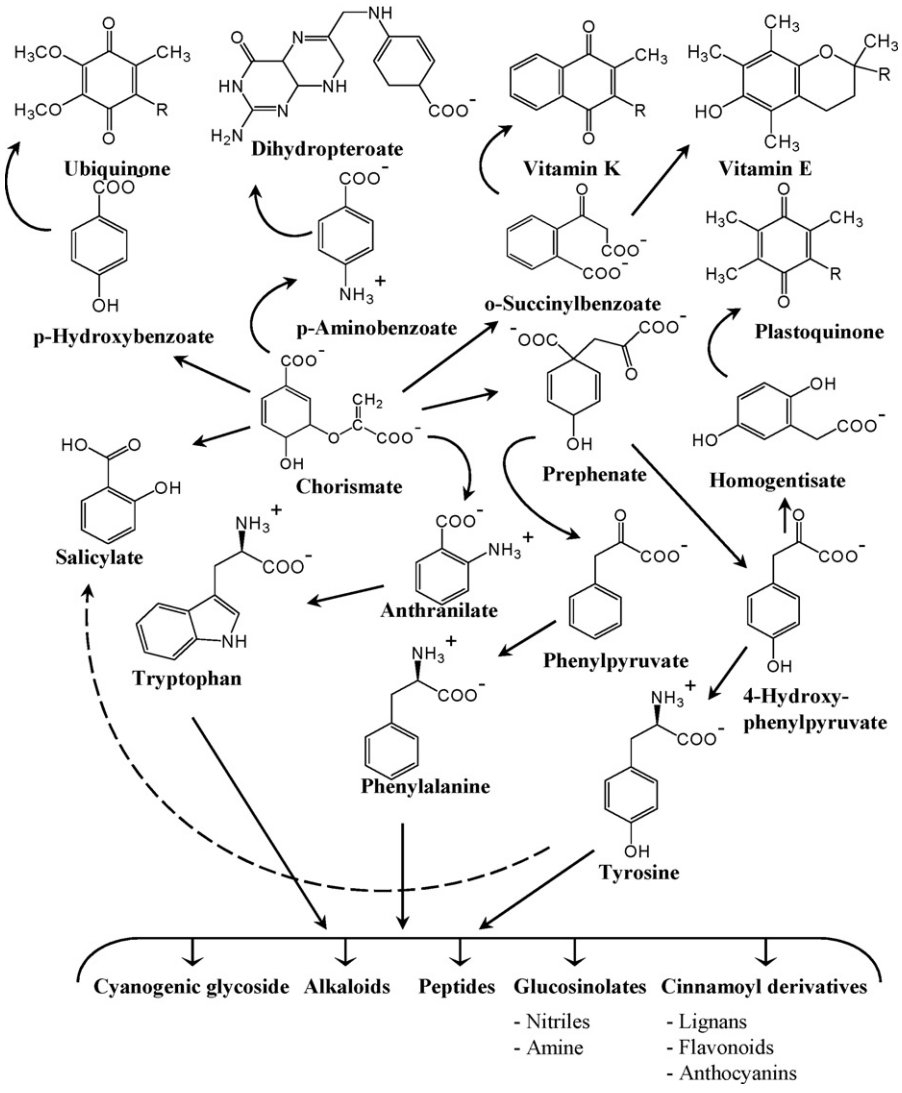

Fig. 2. Essential plant metabolites derived from chorismate.

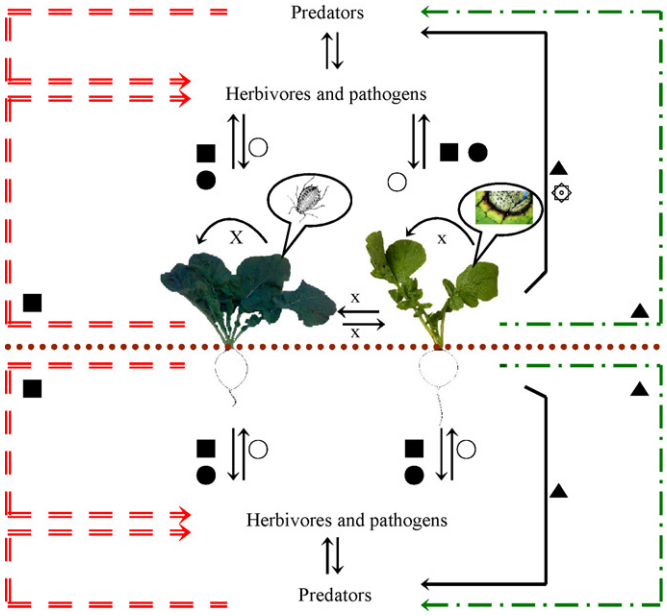

Fig. 3. Basic diagram of above and below ground stress related multitrophic interactions in *Brassica*. Direct interaction (—); attraction behavior (— · —); repellence behavior (==); ground surface (•••); (■) glucosinolates; (×) signaling molecules (jasmonic acid, salicylic acid and ethylene); (●) phenylpropanoids and flavonoids; (○) toxins and enzymes; (▲) terpenoids, nitriles and other leaf volatiles.

the significant loss of chlorophyll content (Zawoznik et al., 2007). An increase in total free amino acids content in the leaves of *Brassica pekinensis* exposed to copper stress indicated that free amino acids plays a role in the detoxification of the copper excess (Xiong et al., 2006). The amount of low molecular weight organic acids, which are reported to be chelating agents, increased after metal stress in *Brassica* (Seth et al., 2008). Effective accumulation of metals (Cr, Fe, Zn, and Mn) also produced an increase of oil content up to 35% in *Brassica juncea* (cv. Rohini) (Singh and Sinha, 2005).

Temperature also affects the metabolite content of plants. Carotenoids in Brassicaceae, including β-carotene, were found to be slightly decreased after thermal treatments (Gebczynski and Lisiewska, 2006). Fresh and processed leaves of kale, which are normally a good source of amino acids, were reported to contain between 76 and 78% of amino acid content as compared to control, when cooked using traditional methods or when frozen (Lisiewska et al., 2008). Boiling of kale, Brussels sprouts, broccoli and white cauliflower also proved to cause a large decrease in the ascorbic acid content (Gebczynski and Lisiewska, 2006; Sikora et al., 2008), while exposure of broccoli (*B. oleracea* var. *italica*) to UV light and 7–13 °C temperature caused an increase in its content (Lemoine et al., 2007; Schonhof et al., 2007).

2.3. Effect of growth and storage

It is a well known fact that the metabolic profile of plants vary according to its growth stage as confirmed by the increase of ascorbic acid in three cultivars during the development of the inflorescence (Vallejo et al., 2003a). However, after harvesting the metabolic changes still continue, e.g. broccoli undergoes major losses of sugars, organic acids, and proteins within the first 6 h of harvest. This is followed by an increase in the free amino acid pools (especially glutamine and asparagine) (King and Morris, 1994; Eason et al., 2007). Loss of membrane fatty acids is also a feature of post-harvest broccoli senescence (Page et al., 2001; Eason et al., 2007). Storage of broccoli florets for 7 days in CO_2-containing atmosphere results in an increase in non-protein amino acids and a decrease in protein amino acids, although the total amino acid content remained unchanged (Hansen et al., 2001).

3. Secondary metabolites

3.1. Glucosinolates

Glucosinolates are one of the important Brassicaceae metabolites derived from amino acid biosynthesis (Stoewsand, 1995; Halkier and Du, 1997; Chen and Andreasson, 2001; Bellostas et al., 2007; Podsedek, 2007). The flavour and odour of *Brassica* vegetables are typically related to their glucosinolate content (Martinez-Sanchez et al., 2006; Jones et al., 2006; Padilla et al., 2007). These are at least partly responsible for their benefits for human health including anti-carcinogenic, cholesterol-reducing, and other pharmacological effects (Van Poppel et al., 1999; Moreno et al., 2006; Cieslik et al., 2007; Song and Thornalley, 2007) but on the other hand the anti-nutritional effects of glucosinolates are also reported (Griffiths et al., 1998).

Glucosinolates are well known to be related to the plant defense response mechanisms, being induced after wounding and/or pathogen attack (Doughty et al., 1991; Cole, 1997), insect herbivory (Gols et al., 2007; Martin and Muller, 2007; Burow et al., 2008), exposure to salt stress (Lopez-Berenguer et al., 2008), diverse environmental factors (Rosa et al., 1997; Vallejo et al., 2003a), or by plant signaling molecules (Kliebenstein et al., 2002; Mikkelsen et al., 2003), including the treatment with SA, JA and MeJA (Bodnaryk, 1994; Mithen, 2001). Following tissue damage, glucosinolates undergo hydrolysis catalyzed by the enzyme myrosinase to produce a complex array of products, which include volatile isothiocyanates in *Brassica* (Mithen, 1992). Specifically, 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 (Fenwick et al., 1983; Manici et al., 2000; Chen and Andreasson, 2001; Mithen, 2001). It was observed that under environmental stress such as drought, secondary metabolism was not restricted (Lopez-Berenguer et al., 2008) and similarly, the total glucosinolate content of mature rapeseed was observed to increase following water deprivation (Bouchereau et al., 1996).

3.1.1. Effect of herbivory and pathogens

Induced defense responses are elicited when plants are exposed to different types of biotic stress such as attack by herbivores or pathogens (Bruce and Pickett, 2007). The activity of glucosinolates and their products against various strains of microorganisms has been documented by many investigators (Wittstock and Halkier, 2002; Sisti et al., 2003), 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 (Doughty et al., 1996). Unusually a high concentration of 2-hydroxy-3-butenyl glucosinolate found in leaves of *B. oleracea* var. *capita* cv *offenham compacta*. It may accounts for the greater susceptibility of this cabbage cultivar to *Brevicoryne brassicae* (L) (cabbage aphid), compared to other *B. oleracea* accessions examined. It concludes 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 *B. brassicae* (Cole, 1997).

There is ample evidence that glucosinolate structures and levels influence host plant suitability for generalist and specialist herbivores (Agrawal and Kurashige, 2003). Monitoring of the total glucosinolate content in a resistant and a susceptible variety of cabbage (*B. compestris* ssp. *pekinesis*) 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 (Ludwig-Muller et al., 1997).

Levels of glucosinolate substrates increase and their composition may be altered in response to herbivory and pathogen attack in several Brassicaceae species (Ludwig-Muller et al., 1997; Mewis et al., 2005). This was evident in a study carried out on two wild *Brassica* species, *B. nigra* and *B. oleracea*, which were infested with larvae of the cabbage root fly, *Delia radicum*. 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 significant changes in shoot glucosinolate levels as compared to a control group of plants (Van Dam and Raaijmakers, 2006). Increased glucosinolate accumulation, primarily of short-chain aliphatic methylsulfinyl glucosinolates, in response to insect feeding (generalist *Myzus persicae* and specialist *B. brassicae*) was also observed in *A. thaliana* (Mewis et al., 2005). 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* (Giamoustaris and Mithen, 1995). Differences in susceptibility to herbivores among *S. alba*, *B. napus*, and *B. campestris* have been attributed to their glucosinolate content, particularly 4-hydroxybenzyl glucosinolate (Bodnaryk, 1991; Hopkins et al., 1998). Mechanical wounding or feeding by the flea beetle (*Phyllotreta cruciferae*) was found to produce a 3-fold increase in the concentration of indole glucosinolates in the cotyledons of 1-week-old seedlings of the oilseed rape *B. napus*, *B. rapa* and *B. juncea* (Bodnaryk, 1992). Similarly, another study reported that damage to the *B. napus* by *P. chrysocephala* induced systemic changes to the glucosinolate profile, most noticeably an increase in the concentration of indole glucosinolates (Bartlet et al., 1999).

Aliphatic glucosinolate profiles have a significant impact on the development and performance of *B. brassicae* and *M. persicae* on *A. thaliana* (Mithen et al., 1995), *B. napus* (Magrath et al., 1993) and oilseed rape (Giamoustaris et al., 1994). Conversely, no changes in the glucosinolate content of oilseed rape plants following infection with Turnip Mosaic Virus were reported (Spak et al., 1993). Decrease in glucosinolate content was observed in healthy and *A. brassicae* inoculated seedlings of *B. rapa* (Doughty et al., 1996). 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 metabolic process, in which they are not considered solely as metabolic end-products but also as precursors of molecules, such as phytoalexins or auxins, known for their involvement in the resistance to microorganisms (Menard et al., 1999). The results of a study carried out on two susceptible and three resistant varieties of cauliflower plants (*B. 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 glucobrassicin decreased, while methoxyglucobrassicin increased (Menard et al., 1999). 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 (Chen and Andreasson, 2001).

3.1.2. Effect of signaling molecules

It is evident that the presence of signaling molecules clearly affects the glucosinolate profile of these species. In the case of *A. thaliana*, its responses to different stresses are coordinated by several interacting signaling systems including JA, SA and ethylene (ET) mediated pathways (Mewis et al., 2005) as in the case of fungus *A. brassicicola* infection (Moran and Thompson, 2001). Plant growth-promoting bacteria produce this effect through several mechanisms, including the synthesis of indole acetic acid (IAA) (Farwell et al., 2007). It was also observed that in *Arabidopsis*, the rhizobacteria-induced systemic resistance is phenotypically similar to pathogen-induced systemic acquired resistance (SAR) (Van Loon et al., 1998), but functions independently of SA and requires responsiveness to JA and ethylene (Leon-Kloosterziel et al., 2005). The concentration of gluconasturtin was specifically increased by SA (Kiddle et al., 1994). *B. napus* plants exposed to MeJA accumulated indole glucosinolates in their leaves, the amount of which depended on the concentration of MeJA applied (Doughty et al., 1995). Moreover, an increase in glucosinolate levels, especially indole glucosinolates, was observed in *B. rapa* leaves after MeJA elicitation (Liang et al., 2006a). The treatment of a resistant and a susceptible variety of Chinese cabbage (*B. compestris* ssp. *pekinesis*) 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 (Ludwig-Muller et al., 1997). 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 (Bruce and Pickett, 2007). Even more conclusive were the results of 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 (Bodnaryk, 1994). In contrast, treatment with abscisic acid resulted in low levels of indole glucosinolates in *B. napus* (Mollers et al., 1999).

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 sulphur fertilizers. For example the use of a sulphur fertilizer produced an increase in the glucosinolates, gluconapin, sinigrin and progoitrin (Kaur et al., 1990). Similarly, the total glucosinolate level was also observed to increase in response to sulphur availability in turnip rape (*B. rapa*) (Kim et al., 2002) and kale (*B. oleracea* L. Acephala Group) (Kopsell et al., 2003), while three broccoli cultivars showed an increase in total glucosinolate content at the start of the inflorescence development followed by a rapid decrease depending on its fertilization with sulphur (Vallejo et al., 2003a). 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 (Robbins et al., 2005). Submitting broccoli to salt stress increased their glucosinolate content, indicating the involvement of these compounds in its stress response (Lopez-Berenguer et al., 2008). Some exceptions are also reported, as in the case of cadmium stress which produced no change in glucosinolate production in *B. rapa* (Siemens et al., 2002).

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 (Schonhof et al., 2007; Volden et al., 2008). Glucosinolate concentration in canola increased when submitted to a temperature stress of 40 °C for 15 days during growth (Aksouh et al., 2001). A sea-

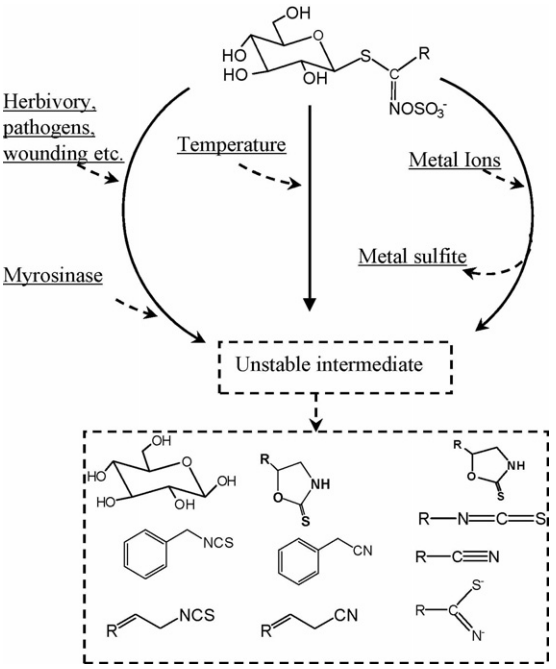

Fig. 4. Glucosinolate degradation.

sonal variation in aliphatic, indole and aromatic glucosinolate content was observed in three different varieties of *B. oleracea* (Cartea et al., 2008). 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 and 13 °C combined with the daily mean radiation of 10–13 mol m^{-2} day^{-1} (Schonhof et al., 2007). The effect of radiation can depend on the species as in the case of *Nasturtium 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* (Reifenrath and Muller, 2007).

3.1.5. Effect of post-harvest storage conditions

Bioavailability of glucosinolates and related isothiocyanates of *Brassica* vegetables is influenced by storage (Song and Thornalley, 2007) and processing, such as blanching and freezing, affecting the taste and aroma of final products (Schöne et al., 1994; McNaughton and Marks, 2003). Post-harvest 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 form isothiocyanates (Rosa et al., 1997), nitriles, thiocyanates, epithionitriles and oxazolidines (Fig. 4) (Bones and Rossiter, 2006). 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, Brussel sprouts, cauliflower and green cabbage), when stored at 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 (Song and Thornalley, 2007). The glucosinolates were the most affected constituents in rocket (*Eruca vesicaria* ssp. *sativa*) leaves as the content was reduced between 4 and 33%, when samples were stored in air while the decrease was between 60 and 100% in low O_2 and high CO_2 conditions (Martinez-Sanchez et al., 2006). Significant loss of glucoerucin and glucoraphanin was observed in rocket during storage at 4–8 °C (Force et al., 2007). 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 (Song and Thornalley, 2007). Also a significant decline in glucosinolates content was observed during storage of *Brassica* vegetables at 4 and at 20 °C (Winkler et al., 2007). On the other hand, indole and aliphatic glucosinolates in broccoli increased during storage period of 7 days at 7–13 °C (Hansen et al., 1995; Schonhof et al., 2007).

3.2. Tryptophan derived phytoalexins

Phytoalexin synthesis, as a defense 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 (Smith, 1996), and jasmonate among others (Liang et al., 2006a). Brassicaceae phytoalexins are generally biogenetically derived from tryptophan but have rather different chemical structures (Fig. 5) as well as biological activities (Pedras et al., 2003b). Brassinin, a plant defense phytoalexin with antimicrobial activity, is produced by a variety of *Brassica* species in response to stress (Pedras and Ahiahonu, 2005; Pedras et al., 2007). Camalexin, another phytoalexin, was found in highest concentrations in or around the dead cells where bacterial growth is restricted (Soylu, 2006).

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 *A. brassicae* but *B. campestris* ssp. *rapifera* was less susceptible to *A. brassicae* (Conn et al., 1988). Another species, *Arabis lyrata* produced camalexin during its interaction with two microorganisms, *P. syringae* pv. *Maculicola* and *Cochliobolus carbonum* (Zook et al., 1998) and the induction of two phytoalexins, wasalexin A and arvelexin (4-methoxyindolyl-3-acetonitrile) were observed after *Leptosphaeria maculans* attack on *Thlaspi arvense* (Pedras et al., 2003a). Further examples were observed in leaf and stem tissues of *B. napus* which accumulated two phytoalexins, methoxybrassinin and cyclobrassinin, following inoculation with *L. maculans* (Dahiya and Rimmer, 1988). While *A. brassicae* induced sinalexin production in *S. alba* (Pedras and Smith, 1997). Three phytoalexins, indole-3-acetonitrile, arvelexin, and 1-methoxyspirobrassinin, were identified in *Erucastrum gallicum* leaves after infection by *Sclerotinia sclerotiorum* (Pedras and Ahiahonu, 2004) and *B. napus* ssp. *rapifera* produced isalexin, brassicanate A, and rutalexin, brassinin, 1-methoxybrassinin, spirobrassinin, brassicanal A and brassilexin when elicited with the phytopathogenic fungus *Rhizoctonia solani* (Pedras et al., 2004). Canola and rapeseed (*B. rapa*) also accumulated diverse phytoalexins after inoculation with different strains of the biotroph *Albugo candida* (Pedras et al., 2008). It is important to note that most of the phytoalexins exhibit antifungal activity against the economically important pathogenic fungi *L. maculans*, *R. solani* and *S. sclerotiorum* (Pedras et al., 2006). However, some phytopathogenic fungi may have enzymes that can detoxify the phytoanticipins or phytoalexins produced by their host (Van Etten et al., 1995; Pedras and Ahiahonu, 2005).

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 (Fig. 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 (Pedras et al., 2003a,

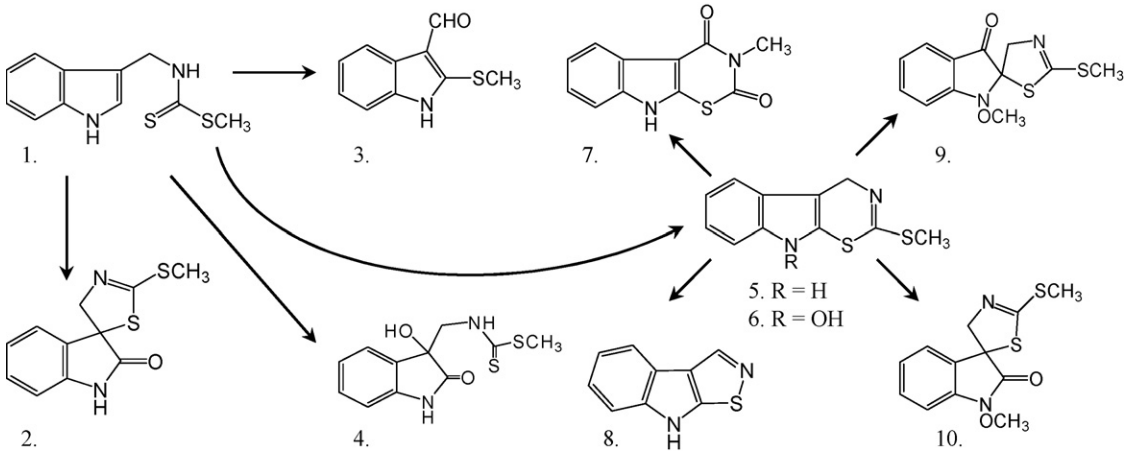

Fig. 5. Biosynthetic pathways of *Brassica* phytoalexins: brassinin (1), spirobrassinin (2), brassicanal A (3), dioxibrassinin (4), cyclobrassinin (5), sinalbin (6), rutalexin (7), brassilexin (8), erucalexin (9) and (*R*)-1-methoxyspirobrassinin (10).

2008). In Brassicaceae, $CuCl_2$ has shown to be an effective inducer of phytoalexin production (Rouxel et al., 1991; Pedras and Smith, 1997) as in the case of *T. arvense* in which the production of two phytoalexins, wasalexin A and arvelexin (4-methoxyindolyl-3-acetonitrile) was detected after exposure to this abiotic elicitor (Pedras et al., 2003a). 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 (Pedras et al., 2008). The effect of another type of abiotic stress, UV light, was described for cauliflower (*B. oleracea* var. *botrytis*) florets in which the production of several phytoalexins, i.e., isalexin, (*S*)-spirobrassinin, 1-methoxybrassitin, brassicanal C, caulilexins A, B and C was enhanced (Pedras et al., 2006). UV light also induced isalexin, brassicanate A, and rutalexin, brassinin, 1-methoxybrassinin, spirobrassinin, brassicanal A and brassilexin synthesis in *B. napus* ssp. *Rapifera* (Fig. 5, Pedras et al., 2004).

3.3. Phenolics

Brassicaceae vegetables are consumed both raw and processed (Kusznierewicz et al., 2008). The content of polyphenols can be influenced by various factors such as the variety, climatic conditions and biotic and abiotic stress caused by the pre-harvest and post-harvest conditions (Dixon and Paiva, 1995; Grace and Logan, 2000; Podsedek, 2007; Sousa et al., 2008).

3.3.1. Effect of herbivory and pathogens

It has been proposed that phenolics play an antioxidative role in the plant defense system as a backup to the primary ascorbate-dependent detoxification system (Sakihama et al., 2002). 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 (Soylu, 2006). In the case of microorganism 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 (Vereecke et al., 1997; Jahangir et al., 2008). For example, metabolic changes of *Brassica*, induced by different food borne 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 (Jahangir et al., 2008). In the case of insect herbivory, aphid feeding involves phenolics in the formation of salivary sheaths around the penetration sites (Miles, 1999). 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 (Moran and Thompson, 2001). This was observed in MeJA treated *B. rapa* leaves in which five phenylpropanoids conjugated with malate were identified as 5-hydroxyferuloyl-, caffeoyl-, coumaroyl-, feruloyl-, and sinapoyl-malate together with a high increase of hydroxycinnamates and phenylpropanoids levels (Liang et al., 2006a,b).

3.3.2. Effect of fertilizers

The production of phenolic metabolites responds to changes in nutrient availability in a highly complex manner (De Pascale et al., 2007; Sousa et al., 2008). 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 (Sousa et al., 2008). Sulphur fertilization increased the average phenol content from 96 to 111 mg of gallic acid 100 g^{-1} of fresh weight in *B. rapa* (De Pascale et al., 2007) and this quantitative change was confirmed by other studies (Vallejo et al., 2003b). The patterns of phenolics did not change qualitatively in leaves of *B. oleracea* var. *costata* grown using different agronomical practices (Sousa et al., 2008). The most representative flavonols in *B. rapa* subsp. *sylvestris* are kaempferol and quercetin derivates but myricetin was present only in trace amounts which was reduced rather than increased by sulphur fertilization unlike the total phenol content. This response indicates that the total phenol 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 sulphur availability (De Pascale et al., 2007). A great increase of anthocyanins in response to cadmium stress was observed in *B. juncea* (Mobin and Khan, 2007).

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 3-*p*-coumaroylquinic acid and 13 kaempferol derivatives were detected in old leaves. Only two kaempferol derivatives were found common in both types of leaves (Sousa et al., 2008). Three broccoli cultivars exhibited an increase in phenolic compounds coinciding with the inflorescence development (Vallejo et al., 2003a).

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 (Gidda and Varin, 2006). Temperature and radiation seem to act as a trigger for biosynthetic pathways (Schonhof et al., 2007). Evidence of this is provided by the detection of higher concentrations of phenolic compounds when exposure of plants to sunlight is increased (Vallejo et al., 2003b). Similarly, UV-B radiation was observed to induce an enhanced production of soluble phenolics in red cabbage (Gitz et al., 1998) and an increase in the flavonoid content of *S. alba* and *N. officinale*, where especially in the case of *S. alba* the increase of quercetin was found to be 10-fold higher than in *N. officinale* (Reifenrath and Muller, 2007). 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 (Olsson et al., 1998). Another study confirmed UV-B induced accumulation of quercetin glycosides and correlated them to plant UV-B tolerance (Wilson et al., 2001). UV light (8 kJ m^{-2}) treated samples of minimally processed broccoli showed higher phenolic contents than untreated (control) plants (Lemoine et al., 2007).

Post-harvest temperature conditions have also proved to influence metabolite profile in plants. Contents of lutein in broccoli increased, when kept at daily mean temperatures between 7 and 13 °C (Schonhof et al., 2007). 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 (Gebczynski and Lisiewska, 2006; Sikora et al., 2008). Freezing has been found to be one of the most effective methods of preserving the nutritive constituents of raw Brassicaceae vegetables (Gebczynski and Lisiewska, 2006). 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 (Sikora et al., 2008). Also, a higher antioxidant activity was observed in broccoli plants, kept at 4 °C for 21 days as compared to control samples (Lemoine et al., 2007). In the case of rocket leaves storage, the stability of quercetin derivatives differed, the glycosides showing more stability than the corresponding acylated glycosides (Martinez-Sanchez et al., 2006). 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 (Lo Scalzo et al., 2008).

3.4. Steroids

Brassinosteroids (BRs) are a group of naturally occurring plant steroidal compounds in *Brassica* having a wide range of biological activities and ability to confer tolerance to *Brassica* plants against a wide spectrum of biotic and abiotic stresses (Krishna, 2003). These stress factors includes, low and high temperatures, drought, high saline concentrations, pathogen attack (Krishna, 2003; Kagale et al., 2007) and exposure to heavy metals (Janeczko et al., 2005). Sterols have been recently recognized not only as precursors of brassinosteroids (Fig. 6) and membrane constituents, but also as modulators of plant development (Fujioka and Yokota, 2003).

Also, there is evidence of cross-talk between BRs and abscisic acid, JA and ethylene (Krishna, 2003). Treatment with 24-epibrassinolide, a brassinosteroid, increases tolerance to several environmental stresses such as basic thermo-tolerance in *B. napus* (Dhaubhadel et al., 1999), 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 (Kagale et al., 2007). 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 (Rouleau et al., 1999). 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 defensive mechanism against oxidative stresses (Anuradha and Rao, 2007).

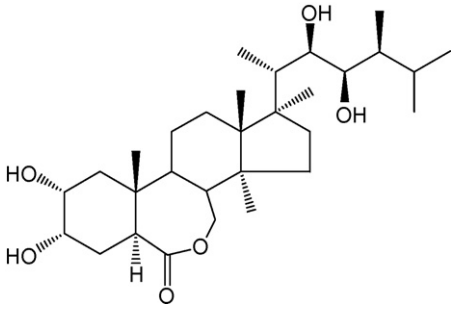

Fig. 6. Brassinosteroid.

4. Conclusion

Brassica species are a rich source of health affecting compounds and are widely considered as a staple food and model for plant science research, in diverse fields (Fig. 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* defense responses, including SA, JA, ethylene and abscisic acid pathways (Fig. 1) (Zhao et al., 2007). On the other hand salicylic acid, jasmonic acid and ethylene-dependent defense pathways may also affect each other.

The systemically 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 metabolic changes can be quite specific, since the pool of plant defense-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 metabolic pathway, or even metabolize the plant defense compounds.

As plants are naturally often exposed to more than one form of stress, there is selection pressure for them to evolve coordinated rather than conflicting defense mechanisms (Bruce and Pickett, 2007). It is likely to understand, that the timing and the order in which different organisms attack and influence defense responses, when multiple stress factors are present.

5. Future prospects

The mechanisms by which metabolites induce stress tolerance 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, which have not been adequately addressed yet. The complementation of the ecological approach, with the understanding of the molecular basis of plant defense 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 combination (Jahangir et al., 2008). Further investigation of the systemically induced volatiles emission, observed in different species, would also contribute to shed some light on the ecological significance and regulatory mechanisms, behind these defensive responses (Mattiacci et al., 2001). Undoubtedly, all these efforts should contribute to provide the means of controlling these different defense systems, leading to the development of more resistant plant varieties. Additionally, since *Brassica* plants are considered to be important staple food, it is essential to fully understand, how different environmental factors triggering mechanisms and pathways affect their metabolic profile, since these will ultimately affect its quality, functional properties, and attributes such as taste and aroma, which will influence consumer acceptability.

References

Abdel-Farid, I.B., Kim, H.K., Choi, Y.H., Verpoorte, R., 2007. Metabolic characterization of *Brassica rapa* leaves by NMR spectroscopy. J. Agric. Food Chem. 55, 7936–7943.

Agrawal, A.A., Kurashige, N.S., 2003. A role for isothiocyanates in plant resistance against the specialist herbivore *Pieris rapae*. J. Chem. Ecol. 29, 1403–1415.

Aksouh, N.M., Jacobs, B.C., Stoddard, F.L., Mailer, R.J., 2001. Response of canola to different heat stresses. Aust. J. Agric. Res. 52, 817–824.

Anuradha, S., Rao, S.S.R., 2007. The effect of brassinosteroids on radish (*Raphanus sativus* L.) seedlings growing under cadmium stress. Plant Soil Environ. 53, 465–472.

Bartlet, E., Kiddle, G., Williams, I., Wallsgrove, R., 1999. Wound-induced increases in the glucosinolate content of oilseed rape and their effect on subsequent herbivory by a crucifer specialist. Entomol. Exp. Appl. 91, 163–167.

Bellostas, N., Sorensen, A.D., Sorensen, J.C., Sorensen, H., 2007. Genetic variation and metabolism of glucosinolates. Adv. Bot. Res. 45, 369–415.

Bodnaryk, R.P., 1991. Developmental profile of sinalbin (*p*-hydroxybenzyl glucosinolate) in mustard seedlings, *Sinapis alba* L., and its relationship to insect resistance. J. Chem. Ecol. 17, 1543–1556.

Bodnaryk, R.P., 1992. Effects of wounding on glucosinolates in the cotyledons of oilseed rape and mustard. Phytochemistry 31, 2671–2677.

Bodnaryk, R.P., 1994. Potent effect of jasmonates on indole glucosinolates in oilseed rape and mustard. Phytochemistry 35, 301–305.

Bones, A.M., Rossiter, J.T., 2006. The enzymic and chemically induced decomposition of glucosinolates. Phytochemistry 67, 1053–1067.

Bouchereau, A., Clossais-Besnard, N., Bensaoud, A., Leport, L., Renard, M., 1996. Water stress effects on rapeseed quality. Eur. J. Agron. 5, 19–30.

Brandl, M.T., 2006. Fitness of human enteric pathogens on plants and implications for food safety. Annu. Rev. Phytopathol. 44, 367–392.

Bruce, T.J., Pickett, J.A., 2007. Plant defence signalling induced by biotic attacks. Curr. Opin. Plant Biol. 10, 387–392.

Burow, M., Zhang, Z.Y., Ober, J.A., Lambrix, V.M., Wittstock, U., Gershenzon, J., Kliebenstein, D.J., 2008. ESP and ESM1 mediate indol-3-acetonitrile production from indol-3-ylmethyl glucosinolate in *Arabidopsis*. Phytochemistry 69, 663–671.

Byers, T., Perry, G., 1992. Dietary carotenes, vitamin C, and vitamin E as protective antioxidants in human cancers. Annu. Rev. Nutr. 12, 139–159.

Cartea, M.E., Velasco, P., Obregon, S., Padilla, G., De Haro, A., 2008. Seasonal variation in glucosinolate content in *Brassica oleracea* crops grown in northwestern Spain. Phytochemistry 69, 403–410.

Chen, S., Andreasson, E., 2001. Update on glucosinolate metabolism and transport. Plant Physiol. Biochem. 39, 743–758.

Chinnusamy, V., Schumaker, K., Zhu, J.K., 2004. Molecular genetic perspectives on cross-talk and specificity in abiotic stress signalling in plants. J. Exp. Bot. 55, 225–236.

Cieslik, E., Leszczynska, T., Filipiak-Florkiewicz, A., Sikora, E., Pisulewski, P.M., 2007. Effects of some technological processes on glucosinolate contents in cruciferous vegetables. Food Chem. 105, 976–981.

Cole, R.A., 1997. The relative importance of glucosinolates and amino acids to the development of two aphid pests *Brevicoryne brassicae* and *Myzus persicae* on wild and cultivated *Brassica* species. Entomol. Exp. Appl. 85, 121–133.

Conn, K.L., Tewari, J.P., Dahiya, J.S., 1988. Resistance to *Alternaria brassicae* and phytoalexin elicitation in rapeseed and other crucifers. Plant Sci. 56, 21–25.

Dahiya, J.S., Rimmer, S.R., 1988. Phytoalexin accumulation in tissues of *Brassica napus* inoculated with *Leptosphaeria maculans*. Phytochemistry 27, 3105–3107.

De Pascale, S., Maggio, A., Pernice, R., Fogliano, V., Barbieri, G., 2007. Sulphur fertilization may improve the nutritional value of *Brassica rapa* L. subsp. *sylvestris*. Eur. J. Agron. 26, 418–424.

Dhaubhadel, S., Chaudhary, S., Dobinson, K.F., Krishna, P., 1999. Treatment with 24-epibrassinolide, a brassinosteroid, increases the basic thermotolerance of *Brassica napus* and tomato seedlings. Plant Mol. Biol. 40, 333–342.

Dicke, M., van Poecke, R.M.P., De Boer, J.G., 2003. Inducible indirect defence of plants: from mechanisms to ecological functions. Basic Appl. Ecol. 4, 27–42.

Dixon, R.A., Paiva, N.L., 1995. Stress-induced phenylpropanoid metabolism. Plant Cell 7, 1085–1097.

Doughty, K.J., Blight, M.M., Bock, C.H., Fieldsend, J.K., Pickett, J.A., 1996. Release of alkenyl isothiocyanates and other volatiles from *Brassica rapa* seedlings during infection by *Alternaria brassicae*. Phytochemistry 43, 371–374.

Doughty, K.J., Kiddle, G.A., Pye, B.J., Wallsgrove, R.M., Pickett, J.A., 1995. Selective induction of glucosinolates in oilseed rape leaves by methyl jasmonate. Phytochemistry 38, 347–350.

Doughty, K.J., Porter, A.J.R., Morton, A.M., Kiddle, G., Bock, C.H., Wallsgrove, R., 1991. Variation in the glucosinolate content of oilseed rape (*Brassica napus* L.) leaves. II. Response to infection by *Alternaria brassicae* (Berk.) Sacc. Ann. Appl. Biol. 118, 469–477.

Eason, J.R., Ryan, D., Page, B., Watson, L., Coupe, S.A., 2007. Harvested broccoli (*Brassica oleracea*) responds to high carbon dioxide and low oxygen atmosphere by inducing stress-response genes. Postharvest Biol. Technol. 43, 358–365.

Farwell, A.J., Vesely, S., Nero, V., Rodriguez, H., McCormack, K., Shah, S., Dixon, D.G., Glick, B.R., 2007. Tolerance of transgenic canola plants (*Brassica napus*) amended with plant growth-promoting bacteria to flooding stress at a metal-contaminated field site. Environ. Pollut. 147, 540–545.

Fenwick, G.R., Heaney, R.K., Mullin, W.J., 1983. Glucosinolates and their breakdown products in food and food plants. Crit. Rev. Food Sci. Nutr. 18, 123–201.

Force, L.E., O'Hare, T.J., Wong, L.S., Irving, D.E., 2007. Impact of cold storage on glucosinolate levels in seed-sprouts of broccoli, rocket, white radish and kohl-rabi. Postharvest Biol. Technol. 44, 175–178.

Fujioka, S., Yokota, T., 2003. Biosynthesis and metabolism of brassinosteroids. Annu. Rev. Plant Biol. 54, 137–164.

Gebczynski, P., Lisiewska, Z., 2006. Comparison of the level of selected antioxidative compounds in frozen broccoli produced using traditional and modified methods. Innov. Food Sci. Emerg. Technol. 7, 239–245.

Giamoustaris, A., Magrath, R., Mithen, R., 1994. Modifying the aliphatic glucosinolate content of oilseed rape and its effect upon pest and pathogen interactions. Aspects Appl. Biol. 39, 125–132.

Giamoustaris, A., Mithen, R., 1995. The effect of modifying the glucosinolate content of leaves of oilseed rape (*Brassica napus* ssp. Oleifera) on its interaction with specialist and generalist pests. Ann. Appl. Biol. 126, 347–363.

Gidda, S.K., Varin, L., 2006. Biochemical and molecular characterization of flavonoid 7-sulfotransferase from *Arabidopsis thaliana*. Plant Physiol. Biochem. 44, 628–636.

Gitz, D.C., Liu, L., McClure, J.W., 1998. Phenolic metabolism, growth, and UV-B tolerance in phenylalanine ammonia-lyase-inhibited red cabbage seedlings. Phytochemistry 49, 377–386.

Gols, R., Raaijmakers, C.E., van Dam, N.M., Dicke, M., Bukovinszky, T., Harvey, J.A., 2007. Temporal changes affect plant chemistry and tritrophic interactions. Basic Appl. Ecol. 8, 421–433.

Good, A.G., Zaplachinski, S.T., 1994. The effects of drought stress on free amino-acid accumulation and protein-synthesis in *Brassica napus*. Physiol. Plant. 90, 9–14.

Grace, S.C., Logan, B.A., 2000. Energy dissipation and radical scavenging by the plant phenylpropanoid pathway. Philos. Trans. R. Soc. Lond. B 355, 1499–1510.

Griffiths, D.W., Birch, A.N.E., Hillman, J.R., 1998. Antinutritional compounds in the Brassicaceae: analysis, biosynthesis, chemistry and dietary effects. J. Hortic. Sci. Biotechnol. 73, 1–18.

Halkier, B.A., Du, L.C., 1997. The biosynthesis of glucosinolates. Trends Plant Sci. 2, 425–431.

Hansen, M., Moller, P., Sorensen, H., de Trejo, M.C., 1995. Glucosinolates in broccoli stored under controlled-atmosphere. J. Am. Soc. Hortic. Sci. 120, 1069–1074.

Hansen, M.E., Sorensen, H., Cantwell, M., 2001. Changes in acetaldehyde, ethanol and amino acid concentrations in broccoli florets during air and controlled atmosphere storage. Postharvest Biol. Technol. 22, 227–237.

Hayat, S., Ali, B., Hasan, S.A., Ahmad, A., 2007. Brassinosteroid enhanced the level of antioxidants under cadmium stress in *Brassica juncea*. Environ. Exp. Bot. 60, 33–41.

Hopkins, R.J., Ekbom, B., Henkow, L., 1998. Glucosinolate content and susceptibility for insect attack of three populations of *Sinapis alba*. J. Chem. Ecol. 24, 1203–1216.

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

Janeczko, A., Koscielniak, J., Pilipowicz, M., Szarek-Lukaszewska, G., Skoczowski, A., 2005. Protection of winter rape photosystem 2 by 24-epibrassinolide under cadmium stress. Photosynthetica 43, 293–298.

Jones, R.B., Faragher, J.D., Winkler, S., 2006. A review of the influence of postharvest treatments on quality and glucosinolate content in broccoli (*Brassica oleracea* var. *italica*) heads. Postharvest Biol. Technol. 41, 1–8.

Kagale, S., Divi, U.K., Krochko, J.E., Keller, W.A., Krishna, P., 2007. Brassinosteroid confers tolerance in *Arabidopsis thaliana* and *Brassica napus* to a range of abiotic stresses. Planta 225, 353–364.

Kaur, S., Gupta, S.K., Sukhija, P.S., Munshi, S.K., 1990. Accumulation of glucosinolates in developing mustard (*Brassica juncea* L.) seeds in response to sulfur application. Plant Sci. 66, 181–184.

Kiddle, G.A., Doughty, K.J., Wallsgrove, R.M., 1994. Salicylic acid-induced accumulation of glucosinolates in oilseed rape (*Brassica napus* L.) leaves. J. Exp. Bot. 45, 1343–1346.

Kim, S.J., Matsuo, T., Watanabe, M., Watanabe, Y., 2002. Effect of nitrogen and sulphur application on the glucosinolate content in vegetable turnip rape (*Brassica rapa* L.). Soil Sci. Plant Nutr. 48, 43–49.

King, G.A., Morris, S.C., 1994. Early copositional changes during postharvest senescence of broccoli. J. Am. Soc. Hortic. Sci. 119, 1000–1005.

Kliebenstein, D.J., Figuth, A., Mitchell-Olds, T., 2002. Genetic architecture of plastic methyl jasmonate responses in *Arabidopsis thaliana*. Genetics 161, 1685–1696.

Kopsell, D.E., Kopsell, D.A., Randle, W.A., Coolong, T.W., Sams, C.E., Curran-Celentano, J., 2003. Kale carotenoids remain stable while flavor compounds respond to changes in sulfur fertility. J. Agric. Food Chem. 51, 5319–5325.
Krishna, P., 2003. Brassinosteroid-mediated stress responses. J. Plant Growth Regul. 22, 289–297.
Kushad, M.M., Brown, A.F., Kurilich, A.C., Juvik, J.A., Klein, B.P., Wallig, M.A., Jeffery, E.H., 1999. Variation of glucosinolates in vegetable crops of *Brassica oleracea*. J. Agric. Food Chem. 47, 1541–1548.
Kusznierewicz, B., Smiechowska, A., Bartoszek, A., Namiesnik, J., 2008. The effect of heating and fermenting on antioxidant properties of white cabbage. Food Chem. 108, 853–861.
Lemoine, M.L., Civello, P.M., Martinez, G.A., Chaves, A.R., 2007. Influence of postharvest UV-C treatment on refrigerated storage of minimally processed broccoli (*Brassica oleracea* var. *italica*). J. Sci. Food Agric. 87, 1132–1139.
Leon-Kloosterziel, K.M., Verhagen, B.W.M., Keurentjes, J.J.B., VanPelt, J.A., Rep, M., VanLoon, L.C., Pieterse, C.M.J., 2005. Colonization of the *Arabidopsis rhizosphere* by fluorescent *Pseudomonas* spp. activates a root-specific, ethylene-responsive PR-5 gene in the vascular bundle. Plant Mol. Biol. 57, 731–748.
Liang, Y.S., Choi, Y.H., Kim, H.K., Linthorst, H.J.M., Verpoorte, R., 2006a. Metabolomic analysis of methyl jasmonate treated *Brassica rapa* leaves by two-dimensional NMR spectroscopy. Phytochemistry 67, 2503–2511.
Liang, Y.S., Kim, H.K., Lefeber, A.W.M., Erkelens, C., Choi, Y.H., Verpoorte, R., 2006b. Identification of phenylpropanoids in methyl jasmonate treated *Brassica rapa* leaves using 2-dimensional nuclear magnetic resonance spectroscopy. J. Chromatogr. A 1112, 148–155.
Lisiewska, Z., Kmiecik, W., Korus, A., 2008. The amino acid composition of kale (*Brassica oleracea* L. var. acephala), fresh and after culinary and technological processing. Food Chem. 108, 642–648.
Lo Scalzo, R., Genna, A., Branca, F., Chedin, M., Chassaigne, H., 2008. Anthocyanin composition of cauliflower (*Brassica oleracea* L. var. *botrytis*) and cabbage (*B. oleracea* L. var. *capitata*) and its stability in relation to thermal treatments. Food Chem. 107, 136–144.
Lopez-Berenguer, C., Martinez-Ballesta, M.C., Garcia-Viguera, C., Carvajal, M., 2008. Leaf water balance mediated by aquaporins under salt stress and associated glucosinolate synthesis in broccoli. Plant Sci. 174, 321–328.
Lotito, S.B., Frei, B., 2006. Consumption of flavonoid-rich foods and increased plasma antioxidant capacity in humans: cause, consequence, or epiphenomenon? Free Radic. Biol. Med. 41, 1727–1746.
Ludwig-Muller, J., Schubert, B., Pieper, K., Ihmig, S., Hilgenberg, W., 1997. Glucosinolate content in susceptible and resistant Chinese cabbage varieties during development of clubroot disease. Phytochemistry 44, 407–414.
Magrath, R., Herron, C., Giamoustaris, A., Mithen, R., 1993. The inheritance of aliphatic glucosinolates in *Brassica napus*. Plant Breed. 111, 55–72.
Manici, L.M., Lazzeri, L., Baruzzi, G., Leoni, O., Galletti, S., Palmieri, S., 2000. Suppressive activity of some glucosinolate enzyme degradation products on *Pythium irregulare* and *Rhizoctonia solani* in sterile soil. Pest Manag. Sci. 56, 921–926.
Martin, N., Muller, C., 2007. Induction of plant responses by a sequestering insect: relationship of glucosinolate concentration and myrosinase activity. Basic Appl. Ecol. 8, 13–25.
Martinez-Ballesta, M.C., Martinez, V., Carvajal, M., 2004. Osmotic adjustment, water relations and gas exchange in pepper plants grown under NaCl or KCl. Environ. Exp. Bot. 52, 161–174.
Martinez-Sanchez, A., Allende, A., Bennett, R.N., Ferreres, F., Gil, M.I., 2006. Microbial, nutritional and sensory quality of rocket leaves as affected by different sanitizers. Postharvest Biol. Technol. 42, 86–97.
Mattiacci, L., Rocca, B.A., Scascighini, N., D'Alessandro, M., Hern, A., Dorn, S., 2001. Systemically induced plant volatiles emitted at the time of "danger". J. Chem. Ecol. 27, 2233–2252.
McNaughton, S.A., Marks, G.C., 2003. Development of a food composition database for the estimation of dietary intakes of glucosinolates, the biologically active constituents of cruciferous vegetables. Br. J. Nutr. 90, 687–697.
Menard, R., Larue, J.P., Silue, D., Thouvenot, D., 1999. Glucosinolates in cauliflower as biochemical markers for resistance against downy mildew. Phytochemistry 52, 29–35.
Mewis, I., Appel, H.M., Hom, A., Raina, R., Schultz, J.C., 2005. Major signaling pathways modulate *Arabidopsis* glucosinolate accumulation and response to both phloem-feeding and chewing insects. Plant Physiol. 138, 1149–1162.
Mikkelsen, M.D., Petersen, B.L., Glawischnig, E., Jensen, A.B., Andreasson, E., Halkier, B.A., 2003. Modulation of CYP79 genes and glucosinolate profiles in *Arabidopsis* by defense signaling pathways. Plant Physiol. 131, 298–308.
Miles, P.W., 1999. Aphid saliva. Biol. Rev. 74, 41–85.
Mithen, R., 1992. Leaf glucosinolate profiles and their relationship to pest and disease resistance in oil seed rape. Euphytica 63, 71–83.
Mithen, R., 2001. Glucosinolates—biochemistry, genetics and biological activity. Plant Growth Regul. 34, 91–103.
Mithen, R., Clarke, J., Lister, C., Dean, C., 1995. Genetics of aliphatic glucosinolates. III. Side chain structure of aliphatic glucosinolates in *Arabidopsis thaliana*. Heredity 74, 210–215.
Mobin, M., Khan, N.A., 2007. Photosynthetic activity, pigment composition and antioxidative response of two mustard (*Brassica juncea*) cultivars differing in photosynthetic capacity subjected to cadmium stress. J. Plant Physiol. 164, 601–610.
Mollers, C., Nehlin, L., Glimelius, K., Iqbal, M.C.M., 1999. Influence of in vitro culture conditions on glucosinolate composition of microspore-derived embryos of *Brassica napus*. Physiol. Plant. 107, 441–446.
Moran, P.J., Thompson, G.A., 2001. Molecular responses to aphid feeding in *Arabidopsis* in relation to plant defense pathways. Plant Physiol. 125, 1074–1085.
Moreno, D.A., Carvajal, M., Lopez-Berenguer, C., Garcia-Viguera, C., 2006. Chemical and biological characterisation of nutraceutical compounds of broccoli. J. Pharm. Biomed. Anal. 41, 1508–1522.
Olsson, L.C., Veit, M., Weissenbock, G., Bornman, J.F., 1998. Differential flavonoid response to enhanced UV-B radiation in *Brassica napus*. Phytochemistry 49, 1021–1028.
Padilla, G., Cartea, M.E., Velasco, P., De Haro, A., Ordas, A., 2007. Variation of glucosinolates in vegetable crops of *Brassica rapa*. Phytochemistry 68, 536–545.
Page, T., Griffiths, G., Buchanan-Wollaston, V., 2001. Molecular and biochemical characterization of postharvest senescence in broccoli. Plant Physiol. 125, 718–727.
Pedras, A.S.C., Jha, M., Minic, Z., Okeola, O.G., 2007. Isosteric probes provide structural requirements essential for detoxification of the phytoalexin brassinin by the fungal pathogen *Leptosphaeria maculans*. Bioorg. Med. Chem. 15, 6054–6061.
Pedras, M.S.C., Ahiahonu, P.W.K., 2004. Phytotoxin production and phytoalexin elicitation by the phytopathogenic fungus *Sclerotinia sclerotiorum*. J. Chem. Ecol. 30, 2163–2179.
Pedras, M.S.C., Ahiahonu, P.W.K., 2005. Metabolism and detoxification of phytoalexins and analogs by phytopathogenic fungi. Phytochemistry 66, 391–411.
Pedras, M.S.C., Chumala, P.B., Suchy, M., 2003a. Phytoalexins from *Thlaspi arvense*, a wild crucifer resistant to virulent *Leptosphaeria maculans*: structures, syntheses and antifungal activity. Phytochemistry 64, 949–956.
Pedras, M.S.C., Jha, M., Ahiahonu, P.W.K., 2003b. The synthesis and biosynthesis of phytoalexins produced by cruciferous plants. Curr. Org. Chem. 7, 1635–1647.
Pedras, M.S.C., Montaut, S., Suchy, M., 2004. Phytoalexins from the crucifer rutabaga: structures, syntheses, biosyntheses, and antifungal activity. J. Org. Chem. 69, 4471–4476.
Pedras, M.S.C., Sarwar, M.G., Suchy, M., Adio, A.M., 2006. The phytoalexins from cauliflower, caulilexins A, B and C: isolation, structure determination, syntheses and antifungal activity. Phytochemistry 67, 1503–1509.
Pedras, M.S.C., Smith, K.C., 1997. Sinalexin, a phytoalexin from white mustard elicited by destruxin B and *Alternaria brassicae*. Phytochemistry 46, 833–837.
Pedras, M.S.C., Zheng, Q.A., Gadagi, R.S., Rimmer, S.R., 2008. Phytoalexins and polar metabolites from the oilseeds canola and rapeseed: differential metabolic responses to the biotroph *Albugo candida* and to abiotic stress. Phytochemistry 69, 894–910.
Petersen, I.L., Hansen, H.C.B., Ravn, H.W., Sorensen, J.C., Sorensen, H., 2007. Metabolic effects in rapeseed (*Brassica napus* L.) seedlings after root exposure to glyphosate. Pest. Biochem. Physiol. 89, 220–229.
Podsedek, A., 2007. Natural antioxidants and antioxidant capacity of *Brassica* vegetables: a review. LWT-Food Sci. Technol. 40, 1–11.
Qasim, M., Ashraf, M., Ashraf, M.Y., Rehman, S.U., Rha, E.S., 2003. Salt-induced changes in two canola cultivars differing in salt tolerance. Biol. Plant. 46, 629–632.
Rathinasabapathi, B., 2000. Metabolic engineering for stress tolerance: installing osmoprotectant synthesis pathways. Ann. Bot. 86, 709–716.
Reifenrath, K., Muller, C., 2007. Species-specific and leaf-age dependent effects of ultraviolet radiation on two Brassicaceae. Phytochemistry 68, 875–885.
Robbins, R.J., Keck, A.S., Banuelos, G., Finley, J.W., 2005. Cultivation conditions and selenium fertilization alter the phenolic profile, glucosinolate, and sulforaphane content of broccoli. J. Med. Food 8, 204–214.
Rosa, E.A.S., Heaney, R.K., Fenwick, G.R., Portas, C.A.M., 1997. Glucosinolates in crop plants. Hortic. Rev. 19, 99–215.
Rouleau, M., Marsolais, F., Richard, M., Nicolle, L., Voigt, B., Adam, G., Varin, L., 1999. Inactivation of brassinosteroid biological activity by a salicylate-inducible steroid sulfotransferase from *Brassica napus*. J. Biol. Chem. 274, 20925–20930.
Rouxel, T., Kollmann, A., Boulidard, L., Mithen, R., 1991. Abiotic elicitation of indole phytoalexins and resistance to *Leptosphaeria maculans* within *Brassicae*. Planta 184, 271–278.
Sakihama, Y., Cohen, M.F., Grace, S.C., Yamasaki, H., 2002. Plant phenolic antioxidant and prooxidant activities: phenolics-induced oxidative damage mediated by metals in plants. Toxicology 177, 67–80.
Sasaki, H., Ichimura, K., Okada, K., Oda, M., 1998. Freezing tolerance and soluble sugar contents affected by water stress during cold-acclimation and de-acclimation in cabbage seedlings. Sci. Hortic. 76, 161–169.
Schöne, F., Kirchheim, U., Schumann, W., 1994. Glucosinolate degradation by rapeseed myrosinase and effect on rapeseed acceptability by growing pigs. Anim. Feed Sci. Technol. 48, 229–235.
Schonhof, I., Klaring, H.P., Krumbein, A., Claussen, W., Schreiner, M., 2007. Effect of temperature increase under low radiation conditions on phytochemicals and ascorbic acid in greenhouse grown broccoli. Agric. Ecosyst. Environ. 119, 103–111.
Seth, C.S., Chaturvedi, P.K., Misra, V., 2008. The role of phytochelatins and antioxidants in tolerance to Cd accumulation in *Brassica juncea* L. Ecotox. Environ. Safe. 71, 76–85.
Shomerilan, A., Jones, G.P., Paleg, L.G., 1991. In vitro thermal and salt stability of pyruvate-kinase are increased by proline analogs and trigonelline. Aust. J. Plant Physiol. 18, 279–286.
Siemens, D.H., Garner, S.H., Mitchell-Olds, T., Callaway, R.M., 2002. Cost of defense in the context of plant competition: *Brassica rapa* may grow and defend. Ecology 83, 505–517.
Sikora, E., Cieslik, E., Leszczynska, T., Filipiak-Florkiewicz, A., Pisulewski, P.M., 2008. The antioxidant activity of selected cruciferous vegetables subjected to aquathermal processing. Food Chem. 107, 55–59.

Singh, J., Upadhyay, A.K., Prasad, K., Bahadur, A., Rai, M., 2007. Variability of carotenes, vitamin C, E and phenolics in Brassica vegetables. J. Food Compos. Anal. 20, 106–112.
Singh, S., Sinha, S., 2005. Accumulation of metals and its effects in *Brassica juncea* (L.) Czern. (cv. Rohini) grown on various amendments of tannery waste. Ecotox. Environ. Safe. 62, 118–127.
Sisti, M., Amagliani, G., Brandi, G., 2003. Antifungal activity of *Brassica oleracea* var. *botrytis* fresh aqueous juice. Fitoterapia 74, 453–458.
Smith, C.J., 1996. Accumulation of phytoalexins: defence mechanism and stimulus response system. New Phytol. 132, 1–45.
Song, L.J., Thornalley, P.J., 2007. Effect of storage, processing and cooking on glucosinolate content of *Brassica* vegetables. Food Chem. Toxicol. 45, 216–224.
Sousa, C., Pereira, D.M., Pereira, J.A., Bento, A., Rodrigues, M.A., Dopico-Garcia, S., Valentao, P., Lopes, G., Ferreres, F., Seabra, R.M., Andrade, P.B., 2008. Multivariate analysis of tronchuda cabbage (*Brassica oleracea* L. var. *costata* DC) phenolics: influence of fertilizers. J. Agric. Food Chem. 56, 2231–2239.
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, 942–952.
Spak, J., Lewis, J., Fenwick, G.R., 1993. Changes in the glucosinolate content of oilseed rape plants following infection with Turnip Mosaic-Virus. Physiol. Mol. Plant Pathol. 43, 437–442.
Stoewsand, G.S., 1995. Bioactive organosulfur phytochemicals in *Brassica oleracea* vegetables—a review. Food Chem. Toxicol. 33, 537–543.
Sudha, G., Ravishankar, G.A., 2002. Involvement and interaction of various signaling compounds on the plant metabolic events during defense response, resistance to stress factors, formation of secondary metabolites and their molecular aspects. Plant Cell Tiss. Org. Cult. 71, 181–212.
Vallejo, F., Garcia-Viguera, C., Tomas-Barberan, F.A., 2003a. Changes in broccoli (*Brassica oleracea* L. var. *italica*) health-promoting compounds with inflorescence development. J. Agric. Food Chem. 51, 3776–3782.
Vallejo, F., Tomas-Barberan, F.A., Garcia-Viguera, C., 2003b. Effect of climatic and sulphur fertilisation conditions, on phenolic compounds and vitamin C, in the inflorescences of eight broccoli cultivars. Eur. Food Res. Technol. 216, 395–401.
Van Etten, H.D., Sandrock, R.W., Wasmann, C.C., Soby, S.D., McCluskey, K., Wang, P., 1995. Detoxification of phytoanticipins and phytoalexins by phytopathogenic fungi. Can. J. Bot., S518–S525.
Van Dam, N.M., Raaijmakers, C.E., 2006. Local and systemic induced responses to cabbage root fly larvae (*Delia radicum*) in *Brassica nigra* and *B. oleracea*. Chemoecology 16, 17–24.
Van Loon, L.C., Bakker, P., Pieterse, C.M.J., 1998. Systemic resistance induced by rhizosphere bacteria. Annu. Rev. Phytopathol. 36, 453–483.
Van Poecke, R.M.P., Posthumus, M.A., Dicke, M., 2001. Herbivore-induced volatile production by *Arabidopsis thaliana* leads to attraction of the parasitoid *Cotesia rubecula*: chemical, behavioral, and gene-expression analysis. J. Chem. Ecol. 27, 1911–1928.
Van Poppel, G., Verhoeven, D.T.H., Verhagen, H., Goldbohm, R.A., 1999. In: Zappia, V. (Ed.), Brassica Vegetables and Cancer Prevention—Epidemiology and Mechanisms. Kluwer Academic/Plenum Publ., pp. 159–168.
Vereecke, D., Messens, E., Klarskov, K., De Bruyn, A., Van Montagu, M., Goethals, K., 1997. Patterns of phenolic compounds in leafy galls of tobacco. Planta 201, 342–348.
Verhoeven, D.T.H., Verhagen, H., Goldbohm, R.A., vandenBrandt, P.A., van Poppel, G., 1997. A review of mechanisms underlying anticarcinogenicity by Brassica vegetables. Chem. Biol. Interact. 103, 79–129.
Volden, J., Borge, G.I.A., Bengtsson, G.B., Hansen, M., Thygesen, I.E., Wicklund, T., 2008. Effect of thermal treatment on glucosinolates and antioxidant-related parameters in red cabbage (*Brassica oleracea* L. ssp. *capitata* f. rubra). Food Chem. 109, 595–605.
Wilson, K.E., Thompson, J.E., Huner, N.P.A., Greenberg, B.M., 2001. Effects of ultraviolet-A exposure on ultraviolet-B-induced accumulation of specific flavonoids in *Brassica napus*. Photochem. Photobiol. 73, 678–684.
Winkler, S., Faragher, J., Franz, P., Imsic, M., Jones, R., 2007. Glucoraphanin and flavonoid levels remain stable during simulated transport and marketing of broccoli (*Brassica oleracea* var. *italica*) heads. Postharvest Biol. Technol. 43, 89–94.
Wittstock, U., Halkier, B.A., 2002. Glucosinolate research in the *Arabidopsis* era. Trends Plant Sci. 7, 263–270.
Xiong, Z.T., Liu, C., Geng, B., 2006. Phytotoxic effects of copper on nitrogen metabolism and plant growth in *Brassica pekinensis* Rupr. Ecotox. Environ. Safe. 64, 273–280.
Zawoznik, M.S., Groppa, M.D., Tomaro, M.L., Benavides, M.P., 2007. Endogenous salicylic acid potentiates cadmium-induced oxidative stress in *Arabidopsis thaliana*. Plant Sci. 173, 190–197.
Zhao, T.J., Liu, Y., Yan, Y.B., Feng, F., Liu, W.Q., Zhou, H.M., 2007. Identification of the amino acids crucial for the activities of drought responsive element binding factors (DREBs) of *Brassica napus*. FEBS Lett. 581, 3044–3050.
Zook, M., Leege, L., Jacobson, D., Hammerschmidt, R., 1998. Camalexin accumulation in *Arabis lyrata*. Phytochemistry 49, 2287–2289.