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Plant-derived sulfur containing natural products produced as a response to biotic and abiotic stresses: A review of their structural diversity and medicinal importance

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Summary

Plant-derived sulfur-containing secondary metabolites constitute a small group of low-molecular weight natural products, which play a vital role in plant-pest interactions in numerous plant families and represent major defense molecules in the Asteraceae, Alliaceae, and Brassicaceae families.

In this review we highlight the crucial role of environmental stress factors in the production of S-containing secondary metabolites. Furthermore, we describe a serendipitous variety of plant-derived sulfur-containing natural products produced or induced under biotic and abiotic stress and their structural diversity, promising pharmacological properties for use by humans, and beneficial effects for plants. Specifically, cruciferous phytoalexins are known as elicit plant defense molecules. Glucosinolates are candidates for tumor-preventive effects. Cysteine sulfoxides found in garlic are considered as profound antimicrobial agents. In this review, we discuss types of S bonds in the molecules and their relevance for the medicinal effect as well as the biological activities of sulfur-containing secondary metabolites and possible future avenues.

Keywords: Phytoalexins, glucosinolates, isothiocyanates, allicin, bioactivity, sulphur

Introduction

Sulfur is found in important metabolic molecules such as common sulfur-containing amino acids (methionine and cysteine), glutathione, proteins and sulpholipids (Abdallah et al., 2010), as well as glucosinolates and allyl Cys sulfoxides (Saito, 2004). Subsequently, these molecules play important roles in the plant lifecycle and the protection of plants from different environmental stresses and pathogens (Lee et al., 2011). Additionally, protein synthesis is reduced under sulfur deficiency conditions, in addition to the accumulation of soluble inorganic and organic nitrogenous compounds, e.g., asparagine (Mortensen et al., 1992). The symptoms of sulfur deficiency resemble that of nitrogen deficiency but are visible in younger leaves, as described by Schnug and Haneklaus (2005). Moreover, without an adequate sulfur supply, plants cannot make efficient use of nitrogen and other nutrients and do not reach their full growth potential, in addition to an increased susceptibility to diseases (Rausch and Wachter, 2005). Therefore, sulfur nutrition is known to have a potential effect on plant health (Bloem et al., 2007), which can be maintained by a significant homeostasis between nitrogen and sulfur, as indicated by Gerendás et al. (2008a) and Maathuis (2009). For instance, several experimental studies have suggested that glucosinolate concentrations are influenced by higher sulfur fertility levels (Kopsell et al., 2003; Aries et al., 2006; Malhi et al., 2007; Schonhof et al., 2007).

Plants regulate the use of available sulfur, which is required for plant growth and development, in addition to resistance to stress (Hawkesford, 2012). For synthesis of cysteine, a key component and a direct/indirect precursor of thiol-containing peptides, including glutathione (GSH), phytochelatins (PCs), and metallothioneins (MTs) (Davidian and Kopriva 2010), inorganic sulfate is taken up by the roots from the rhizosphere and activated to adenosine 5'-phosphosulfate (APS) by ATP sulfurylase. Consequently, APS reductase catalyzed the reduction of APS into sulfite, which is reduced to sulfide and ultimately incorporated into *O*-acetylserine to form cysteine (reviewed by Kopriva et al., 2012). On the other hand, APS is phosphorylated by APS kinase to obtain 3-phosphoadenosine 5-phosphosulfate (PAPS), which participates in the synthesis of other S-containing tryptophan-derived (indolic) and methionine-derived (aliphatic) natural products including glucosinolates and phytoalexines (Frerigmann and Gigolashvili, 2014). The sulfur assimilation pathways including enzymes involved in cysteine biosynthesis, and an overview of cysteine-rich peptides and S-containing secondary metabolites biosynthesis are shown in Fig. 1.

Both primary and secondary S-containing compounds play an important role in plant health and regulatory mechanism under stresses (Rausch and Wachter, 2005). In this regard, glutathione (GSH) is considered as the most important antioxidant and protector of plants under oxidative stress and the major non-protein sulfur source in plants (Bloem et al., 2007; Haneklaus et al., 2007, 2009; Rennenberg and Herschbach, 2012). It is very important to note that over the past decade, the important roles of secondary S-containing compounds in oxidative stress signaling and responses have received more attention (Chan et al., 2019). For instance, Del Carmen Martínez-Ballesta et al. (2013) found that both foliar and root glucosinolate pool was increased in several Brassica species under abiotic stresses including drought and salt stress. Moreover, the hydrolysis products of glucosinolates might be implicated in the oxidative stress responses (Chan et al., 2019).

The regulation of primary and secondary sulfur metabolism has been investigated by using genomic, biochemical, and cellular studies. In this context, Mugford et al. (2011) indicated that over-expression of APS reductase did not affect glucosinolate levels but increased the accumulation of thiols. Consequently, both glucosinolate and thiols levels were not affected in mutants lacking the APR2 isoform of this enzyme.

Until now, the biosynthesis of S-containing secondary metabolites, the regulatory mechanisms involved in their production in response to biotic and abiotic stress, is still not fully understood.

The main objective of this review is to emphasize the importance of environmental stress factors in the induction of S-containing secondary metabolites. This could be used as a tool to manipulate the biosynthesis and induction of novel S-containing natural products with potential biological activities.

In this review, we discuss plant-derived S-containing natural products isolated or induced under biotic and abiotic stresses, in addition to their medicinal significance.

Plant-derived sulfur containing natural products produced under biotic and abiotic stresses

Sulfur-containing phytoalexins from Brassicaceae

Naturally occurring cruciferous phytoalexins are only produced in

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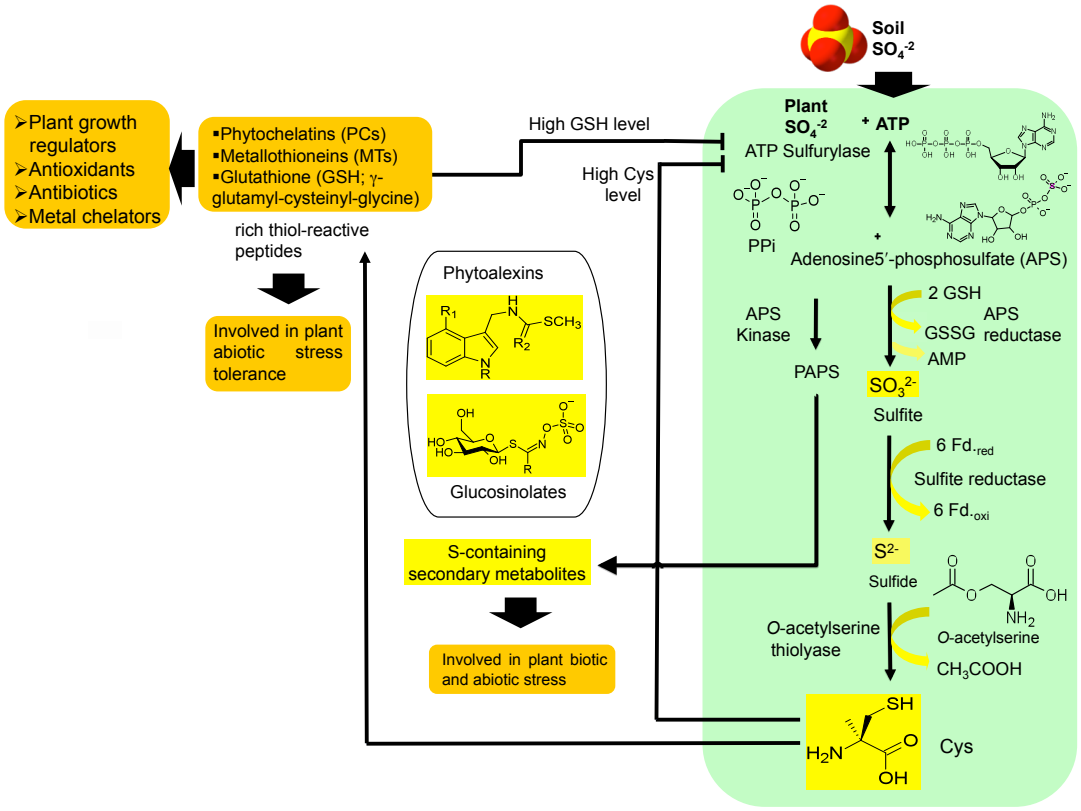


Fig. 1.: Sulfur assimilation pathway. The initial step of sulfur assimilation pathway is catalyzed by ATP-sulfurylase. Role of ATP-sulfurylase in plant abiotic and biotic stress tolerance through different rich thiol-reactive peptides including (Cys, GSH, and MTs) and S-containing secondary metabolites is listed. Positive and negative regulation of ATP-sulfurylase is indicated by arrows and blunt ends, respectively (Redrawn from SAITO 2004; HIRAI and SAITO 2008).

small quantities in damaged tissue and as complex mixtures inside plant tissue, and following exposure to pathogenic microbes. Therefore, their isolation requires multiple chromatographic steps owing to their chemical stability under extraction as well as isolation steps, which afford small quantities. Approximately 44 phytoalexins are known and their structures have mostly been confirmed by chemical synthesis. Brassinin (**1**), 1-methoxybrassinin (**3**), and cyclobrassinin (**6**), were obtained from the inoculation of Chinese cabbage heads *Brassica campestris* with the bacterium *Pseudomonas cichorii*. Additionally, ultraviolet irradiation or inoculation with the bacterium *Erwinia carotovora* enhanced the production of these molecules. Their structures were identified by spectroscopic analysis and confirmed by synthesis (TAKASUGI et al., 1986). Three brassinin-related stress metabolites, named brassitin (**2**), 1-methoxyspirobrassinol (**22**), and (2R,3R)-1-methoxyspirobrassinol methyl ether (**23**), were obtained from the Japanese radish "Daikon" after inoculation with *Pseudomonas cichorii*. The occurrence of 1-methoxyspirobrassinol (**22**), and 1-methoxyspirobrassinol methyl ether (**23**) suggests the involvement of oxidized intermediates in the biosynthesis from brassinin to spirobrassinin (MONDE et al., 1995). 4-Methoxybrassinin (**4**) was obtained from the inoculation of white cabbage heads *B. oleracea* with *Pseudomonas cichorii* (MONDE et al., 1990). 1-Methoxybrassitin (**5**) was obtained from the Chinese cabbage *Brassica campestris* (Cruciferae) in addition to brassinin (**1**), 1-methoxybrassinin (**3**), cyclobrassinin (**6**) (TAKASUGI et al., 1988). Cyclobrassinin sulfoxide (**7**) was found in elicited leaves of *Brassica juncea* (DEVYS et al., 1990). Sinalbin A (**8**) was produced by white mustard *Sinapis alba* as a result of treatment with biotic and abiotic elicitors. Additionally, sinalbin B (**9**) was found in extracts from elicited plants, and not in non-elicited controls. The structures of compounds **8** and **9** were confirmed by chemical synthesis (PEDRAS and ZAHARIA, 2000). Phytoalexins 4-methoxycyclobrassinin (**10**), rutalexin (**11**), dehydrocyclobrassinin (**12**), 4-methoxydehydrocyclobrassinin (**13**), spirobrassinin (**20**), brassicanate A (**27**), brassicanal A (**28**), caulilexin A (**30**), brassilexin (**31**), in addition to 1-methoxybrassinin (**3**) and 4-methoxybrassinin (**4**), were detected in the nonpolar extract of roots of canola (*Brassica napus*) infected with phytopathogenic *Plasmodiophora brassicae* (clubroot). Quantitative analysis of the compounds was performed by using HPLC (DAD and LC-ESI-MS) analysis (PEDRAS et al., 2008). Surprisingly, the concentration of many of the induced compounds collected from the infected roots of canola was significantly increased after several weeks (Tab. 1). Cabbage tissue *Brassica oleracea* inoculated with *Pseudomonas cichorii* delivered 1-methoxybrassenins A and B (**14** and **15**) (MONDE et al., 1991). Brassicanal C (**29**) was produced in florets of cauliflower (*Brassica oleracea*) under abiotic conditions (UV light), along with isalexin, spirobrassinin (**20**), 1-methoxybrassitin (**5**), and caulilexins A (**30**) and B (PEDRAS et al., 2006a). Wasalexins A (**16**) and B (**17**) were obtained from the foliar tissue of wasabi under elicitation with *P. lingam*, *P. wasabiae* or $CuCl_2$ (PEDRAS et al., 1999). UV irradiation of chopped stem tubers of kohlrabi (*Brassica oleracea*) and subsequent incubation for 4 days resulted in the isolation of (R)-1-methoxy-spirobrassinin (**21**) along with cyclobrassinone, and spirobrassinin (**20**). Additionally, the production of these indole phytoalexins was also enhanced by elicitation with $CuCl_2$ (GROSS et al., 1994). However, the authors did not determine the yields of compounds **20** and **21**, which were induced in response to UV light and $CuCl_2$ conditions. Erucalexin (**24**) was found in leaves of dog mustard *Erucastrum gallicum* in response to biotic stress such as that elicited with *S. sclerotiorum* and abiotic stress elicited with $CuCl_2$ (PEDRAS et al., 2006). UV radiation, NaCl irrigation, or $CuCl_2$ spray induced the production of wasalexins A (**16**) and B (**17**) in Thellun-

giella halophila; biswasalexins A1 (**18**) and A2 (**19**) were obtained from head-to-tail photodimerization of wasalexin A (**16**) (PEDRAS et al., 2009). Sinalexin (**32**) was found in white mustard (*Sinapis alba*) under biotic or abiotic stress (PEDRAS and SMITH, 1997). Brussalexin A (**33**) was produced in Brussels sprouts irradiated under UV light (PEDRAS et al., 2007a). Furthermore, camalexin (**34**) and 6-methoxycamalexin (**35**) were produced by *Camelina sativa* leaves as a response to fungus *Altenmia brassme* elicitation (BROWNE et al., 1991). 1-Methyl-camalexin (**36**) was obtained from the leaves of *Capsella bursa-pastoris* elicited by *Alternaria brassicae* (JIMENEZ et al., 1997). Regarding compounds **34**, **35** and **36**, which were obtained from different plant species in very low quantities, the authors did not mention exactly how much compounds were isolated in total from the crude material. Hence, quantitative data is very important to understand how much of the compounds was induced in how many days after inoculation.

Canola plants *Brassica rapa* delivered rapalexins A (**37**) and B (**38**) as a response to biotic stress when infected with the oomycete *Albugo candida* and abiotic stress by UV (PEDRAS et al., 2007b). Isolated or induced S-containing natural products under biotic and abiotic stress, their plant sources and yield data are listed in Tab. 1.

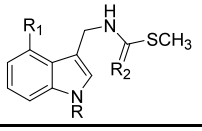
From the findings mentioned above, it is very important to note that the elicited compounds have different incubation periods, which are required for maximum production. Consequently, the induced compounds can be detected within hours up to weeks depending on type of stress and the particular species as reviewed by (PEDRAS et al., 2011). For instance, brassicanal C (**29**) was detected after 48 hours and reached its maximum production after approximately three days upon elicitation with UV light (PEDRAS et al., 2006a). However, the yield was relatively lower after 5 days. Rapalexins A (**37**) and B (**38**) were induced after 8 and 5 days, respectively, and reached their maximum production after 9 and 8 days, respectively. The yield of both compounds was not increased after 10 days (PEDRAS et al., 2007b). Although the phytoalexins above have been identified by mass spectrometry, NMR, IR, and UV spectroscopy, HPLC-DAD-MS represent the most reliable technique to identify and quantify them in plant extracts hence most of these compounds were produced in low quantities (PEDRAS et al., 2006).

Organosulfur compounds obtained from garlic

Cysteine sulfoxides found in garlic (*Allium sativum* L. family Liliaceae) and other *Allium* species are synthesized by the enzyme alliinase in crushed plant material (KUSTERER and KEUSGEN 2010). Allicin (**40**), a typical cysteine sulfoxide of garlic, is synthesized from non-proteinogenic amino acid alliin (**39**), as a first primary product (KUSTERER and KEUSGEN 2010). Rearrangement of compound **40** delivers diallyl sulfides (such as compounds **41** and **44**), dithiins (**45** and **46**), ajoene (**42**) (AMAGASE et al., 2001). Furthermore, thiacremonone (**47**) was isolated from garlic (KIM et al., 2012). Importantly, allicin (**40**) is known as a defence compound with a wide range of pharmacological properties (BORLINGHAUS et al., 2014). For instance, several garlic preparations have been produced to prevent stroke and arteriosclerosis. In this regard, allicin (**40**) and other organosulfur compounds were suggested to be the main metabolites responsible for the entire activity (KREST and KEUSGEN 1999).

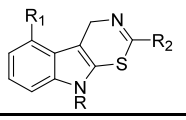
Glucosinolates

Glucosinolates are known as sulfur-containing molecules present in cruciferous vegetables including *Arabidopsis thaliana* and *Brassica* crop species (AHUJA et al., 2016). More than 200 different naturally occurring glucosinolates have been identified (KOPRIVOVA and KOPRIVA 2016). The general structure of glucosinolates is shown in Fig. 6; more than 200 side-groups (R) were found in the literature (FRANCO et al., 2016). Different side-groups (R) of some glucosinolates are shown in Fig. 7. Glucosinolates form the essential component of the dual glucosinolate-myrosinase system, which consists of glucosinolates and their hydrolytic enzymes, myrosinases, which catalyze the breakdown of glucosinolate into various bioactive compounds such as isothiocyanates because of tissue disruption or insect attack (BARTH et al., 2006; PITANN et al., 2017). In addition to isothiocyanates, thiocyanates and nitriles are considered as important breakdown products of glucosinolates, which played a pivotal role in plant defense against various pathogenic microbes and herbivores (HUSEBY et al., 2013).



Compound	Name	R	R_1	R_2
1	Brassinin	H	H	S
2	Brassitin	H	H	O
3	1-Methoxybrassinin	OCH_3	H	S
4	4-Methoxybrassinin	H	OCH_3	S
5	1-Methoxybrassitin	OCH_3	H	O

Fig. 2: Brassinin (**1**), brassitin (**2**), 1-methoxybrassinin (**3**), 4-methoxybrassinin (**4**) and 1-methoxybrassitin (**5**)



Compound	Name	R	R_1	R_2
6	Cyclobrassinin	H	H	SCH_3
7	Cyclobrassinin sulfoxide	H	H	$S(O)CH_3$
8	Sinalbin A	OCH_3	H	$S(O)CH_3$
9	Sinalbin B	OCH_3	H	SCH_3
10	4-Methoxycyclobrassinin	H	OCH_3	SCH_3

Fig. 3: Cyclobrassinin (**6**), cyclobrassinin sulfoxide (**7**), sinalbins A and B (**8** and **9**) and 4-methoxycyclobrassinin (**10**)

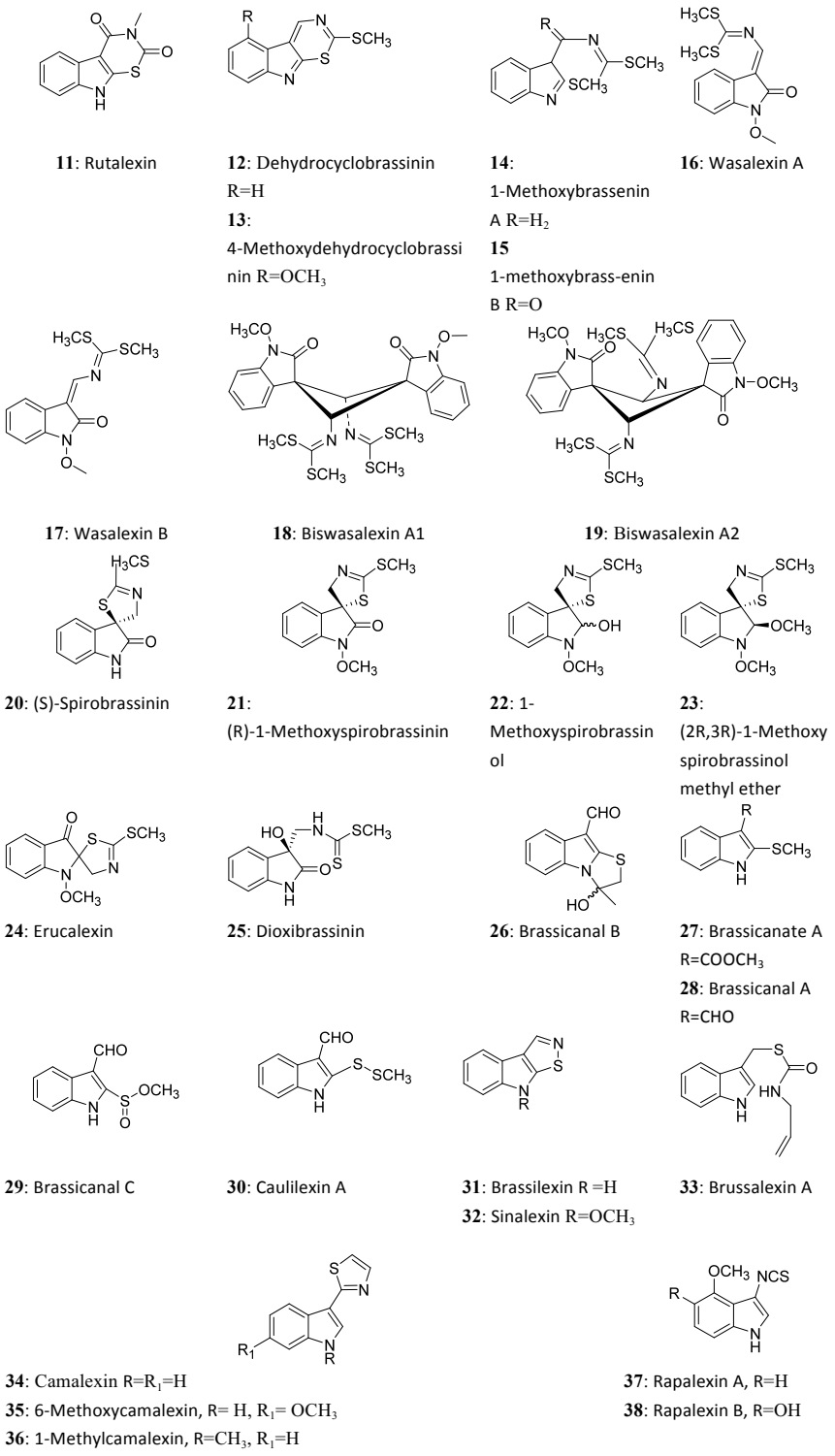
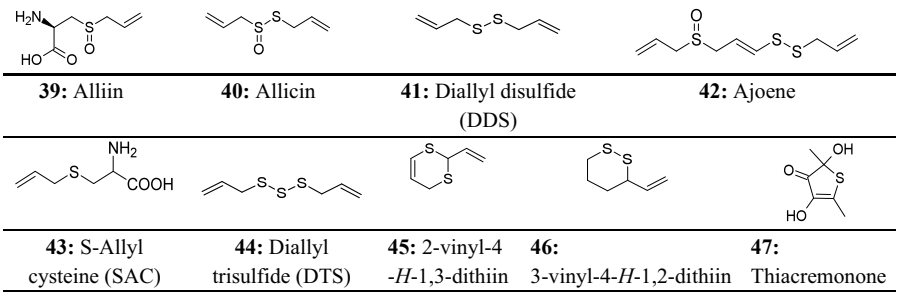


Fig. 4: Structures of sulfur-containing phytoalexins (**11**-**38**)

Sinigrin (**48**) is hydrolyzed by myrosinase upon mechanical injuries of the plant tissue, which produces compounds named allyl isothiocyanate, ally thiocyanate, allyl cyanide, and 1-cyano-2,3-epithiopropane (GERENDÁS et al., 2009; SALADINO et al., 2016). The biosynthesis of glucosinolates can be manipulated by several environmental stress factors including light, nutrients, fungal infection, wounding, and insect damage (GERENDÁS et al., 2008b; RADOJCIC REDOVNIKOVIC et al., 2008). For instance, KIM et al. (2018) indicated that kale grown under treatments with NaCl, Na_2SeO_3, or both would enhance the biosynthesis of glucosinolates including sinigrin (**48**) as well as isothiocyanates. In a recent study, GEILFUS et al. (2016) found that the total glucosinolates content in Chinese cabbage (*Brassica rapa* L. ssp. Pekinensis) increased with higher N and S treatment. However, the ratios among individual glucosinolates remained unchanged. Additionally, the authors observed that the addition of 0.3 g sulfur per pot significantly enhanced the whole shoot biomass compared with the 0 g sulfur control (GEILFUS et al., 2016). A previous report indicated that the infection of Chinese cabbage (*Brassica*



39: Alliin	**40:** Allicin	**41:** Diallyl disulfide (DDS)	**42:** Ajoene

43: S-Allyl cysteine (SAC)	**44:** Diallyl trisulfide (DTS)	**45:** 2-vinyl-4 -*H*-1,3-dithiin	**46:** 3-vinyl-4-*H*-1,2-dithiin	**47:** Thiacremonone

Fig. 5: Structures of organosulfur compounds found in garlic (**39**-**47**)

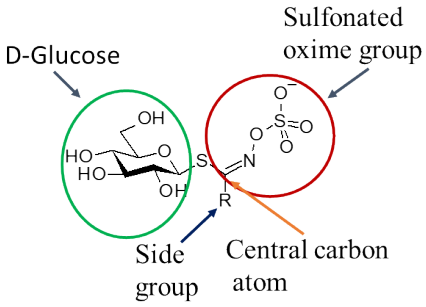


Fig. 6: General structure of glucosinolates

campestris sp. pekinensis) with *Plasmodiophora brassicae* changed the levels of various glucosinolates. Additionally, significant differences in the glucosinolate content was observed in susceptible and tolerant varieties (Ludwig-Müller et al., 1997). Subsequently, the authors reported that tolerant varieties induced the production of aromatic glucosinolates between 14 and 30 days following the infection, whereas susceptible varieties enhanced the biosynthesis of aliphatic and indole glucosinolates. Other reports indicate that the level of indole glucosinolates increased in oilseed rape and Chinese cabbage in response to *Alternaria brassicae* infection (Doughty et al., 1991; Rostás et al., 2002). Furthermore, the biosynthesis of indole glucosinolate was significantly increased by elicitation of *Arabidopsis* with *Erwinia carotovora* (Brader et al., 2001). A recent study indicated that additional sulfur supply in the nutrient medium enhanced the content of aliphatic glucosinolates in *Eruca sativa* (Kastell et al., 2018).

Moreira-Rodríguez et al. (2017) studied the effect of UVA and UVB light and methyl jasmonate on the biosynthesis of glucosinolates and other metabolites in broccoli sprouts. The results revealed that treatments with UVA + methyl jasmonate and UVB + methyl jasmonate induced the level of total glucosinolate by ~154% and ~148%, respectively. Methyl jasmonate (MJ) stimulated the biosynthesis of indole glucosinolates such as neoglucobrassicin (**61**) (~538%). Furthermore, UVB increased the level of aliphatic and indole glucosinolates, including glucoraphanin (**51**) (~78%) and 4-methoxy-glucobrassicin (**60**) (~177%) (Moreira-Rodríguez et al., 2017). Isolated or induced compounds under stress, their plant sources and yield data are listed in Tab. 1.

Types of S bonds in the molecules and their relevance for the medicinal effect

Structural diversity of plant-derived sulfur containing natural products is not only means the potential chemical structures (**1-53**) for drug development, however the more interesting feature of these different compounds, which might be responsible for the selective and specific biological activities. Different sulfur bonds in the molecules may have relevance for the medicinal effect of S-containing natural products. For instance, disulfide bonds have gained significant attention in various fields including pharmaceutical, biochemical and biotechnological fields. They have important properties such as the capability to break into a reduced form of glutathione in a thiol-disulfide exchange reaction, they are stabile in human body, and have no physiological toxicity (Wang et al., 2016). Disulfide bonds participate in the biological activity of several S-containing secondary metabolites, as reported by Feng et al. (2016), who indicated that the disulfide bond is important for the antimicrobial activity of ajoene (**42**). Moreover, thiosulfinates including ajoene (**42**) demonstrated antimicrobial potential owing to the presence of a disulfide bond, which reacts with the thiol groups of cellular proteins. Because ajoene (**42**) contains a sulfinyl group, which has been found to be responsible for the antibacterial activity of allicin (**40**), its antimicrobial activity has been attributed to the presence of a disulfide bond and the sulfinyl (Feng et al., 2016). Moreover, allicin (**40**) is known as a reactive sulfur species (Gruhlke and Slusarenko, 2012) with oxidizing characteristics. For instance, it can oxidize thiols in cells, including glutathione and cysteine residues in proteins, through disulfide bond formation. Consequently, redox-stimulated structural changes in proteins result in a net change of loss or gain function, which is known for the plant protein NPR1, a key protein in pathogen-triggered immunity (Tada et al., 2008).

Pedras et al. (2006a) reported that among phytoalexins elicited in floret of cauliflower under abiotic stress (UV light), caulilexin A (**30**), which has a disulfide bridge, demonstrated a complete growth inhibition of *Rhizoctonia solani* at 5.0×10^{-4} M and *Sclerotinia sclerotiorum* at 1.0×10^{-4} M. Moreover, compound (**30**) exhibited complete growth inhibition at 1.0×10^{-8} M in a TLC bioassay against *Cladosporium cucumerinum*, whereas all other tested phytoalexins inhibited the mycelial growth at 1.0×10^{-6} M, which is 100 times higher concentration.

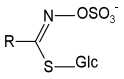
Moreover, *Allium* species, which contain a vinyldithiinsin group with exo and endo double bonds in a ring system, have demonstrated remarkable bioactivities. For instance, 3,4-dihydro-3-vinyl-1,2-dithiin (**46**) was reported to have higher antioxidative activity for human LDL than aliphatic dialk(en)yl disulfides and trisulfides. This can be attributed to the conjugation of a double bond to a nonbonding electron on the sulfur in a ring system, as reported by Higuchi et al. (2003)

Biological activities of plant-derived sulfur containing metabolites produced under biotic and abiotic stresses

Antimicrobial activity

In principle plants have three main approaches to fight pathogenic microbes including strengthening the cell wall, inhibition of microbial enzymes by apoplastic defense and synthesis of toxic natural products such as phytoalexins (Bloem et al., 2015).

Phytoalexins **1**-**38** have shown promising antifungal activities against a wide range of plant pathogenic fungi in addition to antibac-



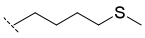
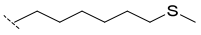
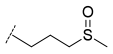
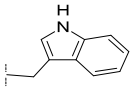
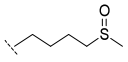
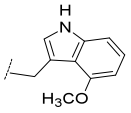
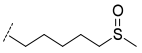
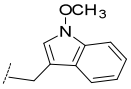
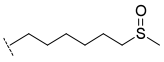
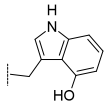
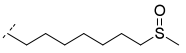
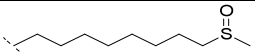
No.	Name	R	No.	Name	R
48	Sinigrin		**57**	Glucoerucin	
49	Gluconapin		**58**	Glucosquerellin	
50	Glucoiberin		**59**	Glucobrassicin	
51	Glucoraphanin		**60**	4-Methoxygluco-brassicin	
52	Glucoalyssin		**61**	Neoglucobrassicin	
53	Glucohesperin		**62**	4-Hydroxygluco-brassicin	
54	Glucoibarin		**63**	Glucotropaeolin	
55	Glucohirsutin		**64**	Gluconastrutiin	
56	Glucoibervirin				

Fig. 7: Different side groups (R) of some glucosinolates (**48**-**64**)

terial activity (reviewed by Pedras and Yaya, 2010). For instance, the phytoalexins sinalbins A (**8**) and B (**9**) demonstrated antifungal activity against plant pathogenic fungi *Leptosphaeria maculans*. At a concentration of 5×10^{-4} M, sinalbin A (**8**) exhibited complete inhibition of spore germination (ED_{50} 2×10^{-4} M at 48 h) for the duration of the assay, which was 7 days. Additionally, Sinalbin B (**9**) showed moderate activity (ED_{50} 7×10^{-4} M at 48 h) at a similar concentration (5×10^{-4} M), which was approximately 30% germination inhibition in comparison to controls, after 48 h (Pedras and Zaharia, 2000).

The antimicrobial potential of *Allium* species and their organosulfur compounds including allicin (**40**) thiosulphinates, and their transformation products has been previously reported (Samadi and Keusgen, 2013). For instance, the minimal concentration of diallyl disulfide (**41**) and allicin (**40**) to inhibit the growth of *Escherichia coli* and *Staphylococcus aureus* was 6.15 and 0.17 mM, respectively, which was 35× more than allicin (**40**) alone (Koch and Lawson, 1996). Additionally, pure allicin (**40**) extracted from garlic showed remarkable antibacterial activity towards *Bacillus* spp., *Salmonella typhimurium*, and *Vibrio cholerae* with complete growth inhibition at a concentration of 80 µM (Borlinghaus et al., 2014).

Strehlow et al. (2016) reported that sample solutions contain 3 mg/mL racemic alliin and 0.2 mg/mL alliinase did not exhibit antibacterial activity, on the other side the combination of racemic alliin and alliinase, which might be contain 0.75 mg/mL allicin (**40**) demonstrated inhibitory activity against *E. coli*. It is very important to mention the substantial achievement of Strehlow et al. (2016), who studied the stabilization of both alliin (**39**) and alliinase in lactose microspheres obtained by previously described spray-drying formulation. The authors indicated for the first time that allicin (**40**) synthesized *in situ* could be readily used for the treatment of pulmonary microbial infections (Strehlow et al., 2016).

A previous study indicated that Several studies have indicated the antibacterial potential of glucosinolates and their hydrolysis products, in addition to their activity against molds and yeasts (Saladino et al., 2016). Sinigrin (**48**) had weak antimcrobial activity, whereas its hydrolysis products exhibited a promising effect on the inhibition of microbial growth (Brabban and Edwards, 1995; Pitann et al., 2017).

Anticancer potential

Indole phytoalexins are known to have anti-cancer, chemopreventive, and antiproliferative activity (Chripkova et al., 2016). Furthermore, many phytoalexins such as brassinin (**1**), cyclobrassinin (**6**), and spirobrassinin (**20**), exhibit a cytotoxic effect (Sabol et al., 2000). Additionally, 1-methoxybrassinin (**3**) (Pilatova et al., 2005), 1-methoxyspirobrassinol (**22**), and 1-methoxyspirobrassinol methyl ether (**23**) have demonstrated antiproliferative activity (Monde et al., 2005). Diallyl sulfides, such as diallyl disulfide (**41**) and diallyl trisulfide (**44**) obtained from garlic, have anticancer effects (Lai et al., 2012). The cytotoxicity of compound **44** showed great effects on the production of reactive oxygen species (ROS); compound **44** is known as key mediator in the apoptotic signaling pathway and induces the ROS-dependent caspase pathway in U937 leukemia cells (Choi and Park, 2012). Compound (**44**) was found to activate apoptosis against a diverse group of human cancer cell lines *in vitro* in addition to providing better protection towards tumor growth in animal models *in vivo* such as colorectal cancer (Yu et al., 2012). S-allylcysteine (**43**) exhibited an *in vitro* cancer chemopreventive effect. It was suggested to be an interesting therapeutic agent for prostate cancer (Liu

Tab. 1: Plant species, investigated plant part, type of stress, isolated or induced compounds of interest and yield data

Plant species	Investigated plant part	Kind of stress	Isolated or induced compounds	Yield data	References
Brassica campestris L. ssp. *Pekinensis*	Cabbage heads	Biotic and abiotic	Brassinin (**1**), 1-methoxybrassinin (**3**), and cyclobrassinin (**6**),	Brassinin (**1**) (8 mg), 1-methoxybrassinin (**3**) (39 mg), and cyclobrassinin (**6**) (20 mg)	(TAKASUGI et al., 1986)
Raphanus sativus var. hortensis	Roots	Biotic	Brassitin (**2**), 1-methoxyspirobrassinol (**22**), and (2R,3R)-1-methoxyspirobrassinol methyl ether (**23**)	Brassitin (**2**) (6 mg), 1-methoxyspirobrassinol (**22**) (6 mg), and (2R,3R)-1-methoxyspirobrassinol methyl ether (**23**) (16 mg)	(MONDE et al., 1995)
B. oleracea L. var. capitata	Cabbage heads	Biotic	4-Methoxybrassinin (**4**)	Compound **4** (6 mg)	(MONDE et al., 1990)
Brassica campestris L. ssp. pekinensis	Cabbage heads	Biotic	1-Methoxybrassitin (**5**)	Compound **5** (15.9 mg)	(TAKASUGI et al., 1988)
Brassica juncea	leaves	Biotic	Cyclobrassinin sulfoxide (**7**)	Not determined	(DEVYS et al., 1990)
Sinapis alba	Leaf and stem tissues	Biotic and abiotic	Sinalbins A (**8**) and B (**9**)	Sinalbin A (**8**) (3.5 mg) and sinalbin B (**9**) (5.5 mg)	(PEDRAS and ZAHARIA, 2000)
Brassica napus cv. Westar	Infected roots	Biotic	4-Methoxycyclobrassinin (**10**), rutalexin (**11**), dehydrocyclo-brassinin (**12**), 4-methoxy-dehydrocyclobrassinin (**13**), spirobrassinin (**20**), brassilexin (**31**)	The concentration of compound **10** (32 nmol/g fresh weight). Compound **11** (8.5 and 9.6 nmol/g fresh weight) in roots collected four and five to six weeks after inoculation, respectively. Compound **12** and **13** were induced after five weeks in the infected roots but not in the control roots. The concentration of compounds **20** and **31** was 21-26 and 5-7 nmol/g fresh weight, respectively. They were detected only in the infected roots collected after five and six weeks.	(PEDRAS et al., 2008)
Brassica oleracea var. *Capitata*	Cabbage tissue	Biotic	1-Methoxybrassenins A (**14**) and B (**15**)	Compound **14** (6.1 mg) and **15** (120 mg)	(MONDE et al., 1991)
Brassica oleracea var. *botrytis*	Florets	Abiotic	Brassicanal C (**29**)	The concentration of compound **29** was 0.22±0.05 µmol/100 g fresh floret tissue, 0.49±0.09 µmol/100 g fresh floret tissue, 0.43±0.09 µmol/100 g fresh floret tissue, and 0.30±0.09 µmol/100 g fresh floret tissue after 48, 72, 96 and 120 hours, respectively.	(PEDRAS et al., 2006a)
Wasabia japonica, syn. *Eutrema wasabi*	Foliar tissue	Biotic and abiotic	Wasalexins A (**16**) and B (**17**), and biswasalexins A1 (**18**) and A2 (**19**)	Wasalexins A (**16**) and B (**17**) were produced in relatively low amounts under NaCl conditions. Compound **18** (60 nmol/g fresh weight) and **19** (15 nmol/g fresh weight) in UV-irradiated plants. The yield was decreased for **18** and **19** in plants sprayed with $CuCl_2$, in which the concentration of compound **18** was approximately 10 nmol/g fresh weight and **19** was not detected	(PEDRAS et al., 2009)
Brassica oleracea var. gongylodes	Stem tubers	Abiotic	(R)-1-methoxy-spirobrassinin (**21**)	not determined	(GROSS et al., 1994)
Erucastrum gallicum	Leaves	Biotic and abiotic	Erucalexin (**24**)	The yield of compound **24** was 2.2 mg. The isolation steps were repeated four times to obtain a sufficient amount for structure elucidation and bioactivity. (12 mg was isolated in total from 200 plants and 6 g of extract).	(PEDRAS et al., 2006)
Sinapis alba	Leaves	Biotic or abiotic	Sinalexin (**32**)	1.4 mg	(PEDRAS and SMITH, 1997)
B. oleracea var. *gemmifera*	Sprouts	Abiotic	Brussalexin A (**33**)	2 mg of compound **33** was obtained from 3.9 kg of fresh tissue.	(PEDRAS et al., 2007a)
Camelina sativa	Leaves	Biotic	Camalexin (**34**) and 6-methoxycamalexin (**35**)	Not determined	(BROWNE et al., 1991)
Capsella bursa-pastoris	Leaves	Biotic	1-Methyl-camalexin (**36**)	Not determined	(JIMENEZ et al., 1997)
Brassica rapa		Biotic	Rapalexins A (**37**) and B (**38**)	For rapalexin A (**37**), the 0.4-06, 0.7-1.1, and 0.7-0.9 nmol g^{-1} fresh leaves were produced after 8, 9, 10 days, respectively from inoculation. However, the for compound **38**, 2.2-3.3, 4.1-8.1, 4.3-8.3. 5.3-14.7, 3.9-9.7, and 7.5-9.1 nmol g^{-1} fresh leaves were produced after 5, 6, 7, 8, 9, and 10 days, respectively from inoculation. Compound **37** was only detected after 8 days	(PEDRAS et al., 2007b)
Brassica oleracea	Roots	Abiotic	Sinigrin (**48**)	The concentration increased by the addition of Na_2SeO_3 alone or in combination with NaCl.	(KIM et al., 2018)
Brassica oleracea var. *italica*	Broccoli sprouts	Abiotic	Glucoraphanin (**51**)	UVB increased the content to 23.6±2.1 mmol/kg dry weight	(MOREIRA-RODRÍGUEZ et al., 2017)
Brassica oleracea var. *italica*	Broccoli sprouts	Abiotic	4-Methoxy-glucobrassicin (**60**)	UVB increased the content to 12.7±0.5 mmol/kg dry weight	(MOREIRA-RODRÍGUEZ et al., 2017)
Brassica oleracea var. *italica*	Broccoli sprouts	Abiotic	Neoglucobrassicin (**61**)	Increased synergistically by 96.4±1.5 and 92.8±6 mmol/kg dry weight under UVA+ MJ and UVB+ MJ treatment, respectively.	(MOREIRA-RODRÍGUEZ et al., 2017)

et al., 2012). Moreover, allicin (**40**), has been reported to be a promising candidate as a tumor suppressor in human colon cancers (Bar-Chen et al., 2010). More and above, several studies have indicated that Brassicaceae species possess chemoprevention towards different types of cancers in humans owing to their high glucosinolates content (Merah, 2015).

Anti-inflammatory activity

In a recent study, garlic, allicin (**40**), and the commonly used drug praziquantel were used to treat six groups of mice infected with *Schistosoma mansoni cercariae*. The results showed that the treatment decreased the worm burden in addition to the reduction of serum concentrations of liver fibrosis markers and proinflammatory cytokines. Nonetheless, praziquantel exhibited the most inhibitory activity for the reduction of the number of worms (Metwally et al., 2018). The study of Lee et al. (2012) indicated that two isomers (Z and E) of ajoene (**42**) and their oxidized sulfonyl derivatives demonstrated anti-inflammatory activity by suppressing the production of nitric oxide and prostaglandin E2 (PGE2) in addition to the expression of the pro-inflammatory cytokines including tumor necrosis factor α (TNFα), interleukin-1 β (IL-1β), and interleukin-6 in lipopolysaccharide (LPS)-stimulated macrophages.

The study of Vo et al., (2013) indicated that aromatic glucosinolates had a moderate anti-inflammatory effect. The authors indicated that because naturally occurring glucosinolates in plant material were present as a mixture of various aromatic and aliphatic compounds, these metabolites might act synergistically during consumption of a normal diet. They also suggested that these compounds can be hydrolyzed by the enzyme myrosinase, which is present in various brassica species. The hydrolysis products include isothiocyanates and glucoraphanin (**51**) and sulforaphane in the case of the aliphatic metabolite. Fahey et al. (1997) suggested that these metabolites have better bioactivity than the entire glucosinolates.

Neuroprotective properties

Among glucosinolates, glucoraphanin (**51**), sulforaphane, and isothiocyanates were found to be the most fascinating compounds as modulators of various systems associated with the pathogenic mechanism of various neurological diseases including oxidative stress, apoptosis, and inflammation (Venditti and Bianco, 2018). Additionally, garlic compounds demonstrated a neuroprotection effect, which was attributed to their antioxidant capacity, modulation of apoptosis mediators and reduction of the formation of amyloid protein (Venditti and Bianco, 2018).

Plant protection

Phytoalexins are serendipidious plant metabolites, owing to their amazing role in protecting plants against a wide range of microbial pathogenic microorganisms. Elevated levels of such defensive natural products in plants might increase their resistance to disease (Pedras and Yaya, 2010; Bloem et al., 2015).

Curtis et al. (2004) studied the antifungal activity of allicin (**40**) in fresh garlic juice by using a plate-diffusion method using spore-seeded agar and found that allicin (40) demonstrated strong *in vitro* activity against many plant-pathogenic fungi such as *Magnaporthe grisea*, *Botrytis cinerea, Plectospherella cucumerina*, and *Alternaria brassicicola*.

Furthermore, nematicidal activities of diallyl disulfide (**41**), and diallyl trisulfide (**44**) against the pine wood nematode (*Bursaphelenchus xylophilus*) have been reported; diallyl trisulfide (**44**) exhibited more than 10-fold lower LC_{50} than diallyl disulfide (**41**) (Cetintas and Yarba, 2010). Distilled garlic oil significantly inhibited root galling after inoculation of tomato roots with root-knot nematode (*Meloidogyne incognita*) (Danquah et al., 2011). The insecticidal and acute toxicity effect of diallyl disulfide (**41**) and diallyl trisulfide (**44**), against *Callosobruchus Chinensis* (found in bean weevil) were studied; diallyl trisulfide (**44**) possessed stronger toxicity than diallyl disulfide (**41**) and crude garlic oil. It has been reported that diallyl trisulfide (**44**) demonstrated promising effect against rice weevil *Sitophilus oryzae*, red flour beetle (*Tribolium castaneum*), and the maize weevil (*Sitophilus zeamais*) (Huang et al., 2000; Mikhaiel, 2011; Koul, 2004). Diallyl trisulfide (**44**) was the strongest fumigant ingredient in garlic oil and showed higher activity than diallyl disulfide (**41**) against the pine wood nematode (*Bursaphelenchus xylophilus*) (Koul, 2004; Park et al., 2006; Nowsad et al., 2009; Mikhaiel, 2011). Several garlic-based products are on the market today for use in different agricultural and horticultural practices. However, many of these products have not been approved by the FDA (US Food and Drug Administration) (Anwar et al., 2017).

Glucosinolates hydrolysis products have demonstrated potential antimicrobial activity against different plant pathogenic microorganisms (Bloem et al., 2015; Hanschen et al., 2018). Accordingly, they could be used as alternatives to commercial chemicals to control phytopathogenic microorganisms. Glucosinolates are mainly present in young leaves, seeds, and siliques; additionally, their intermediates are found in leaves, stems, and roots (Agneta et al., 2014). Aghajanzadehdivaei (2015) indicated, that the higher content of indole glucosinolates and subsequently their hydrolysis products, which were detected in roots, could be attributed to their better stability in the soil than in air. Furthermore, Ludwig-Mueller et al. (1999) reported that these metabolites might limit the development of root disease, which has been raised by *Plasmodiophora brassicae*. Volatile molecules obtained from macerated Brassicae root tissue decreased the fungal infection of wheat, *Gaeumannomyces graminis* (Angus et al., 1994). Rhizospheric strains of *Fusarium* possessed a potential effect on *Lepidium sativum* against *Pythium ultimum*. Subsequently, these strains enhanced the production of benzyl isothiocyanate, and of its precursor glucotropaeolin in the roots of Brassicaceae plants. Moreover, the assemblage of isothiocyanate in roots enhanced the resistance of *L. sativum* against *Pythium ultimum* (Ishimoto et al., 2004). Moreover, cauliflower plants (*Brassica oleracea* var. botrytis) were infected by *Peronospora parasitica* to evaluate the relationship between glucosinolates and resistance against downy mildew. The authors reported that a higher amount of sinigrin (**48**) was detected in resistant varieties in comparison to susceptible ones (Ménard et al., 1999).

Different biological activities of plant-derived S-containing secondary metabolites were emphasized to alert researchers of the importance of biotic and abiotic stress factors as a potential tool to produce and enhance the synthesis of S-containing secondary metabolites with remarkable bioactivities. As reported in several studies, it is clear that sulfated phytoalexins and glucosinolates constitute an important part of the plant defense repertoire owing to their fascinating antimicrobial activity, which is induced by stress.

Less research has been performed on *in vivo* studies of bioactive phytoalexins, glucosinolates and their hydrolysis products, as most of the reviewed bioassay studies have been performed *in vitro*. Accordingly, *in vivo* experiments in animal models are very important to confirm the investigated antimicrobial, anticancer, anti-inflammatory, antiviral, antioxidant and neuroprotective activities of the mentioned S-containing compounds.

Owing to the increase in drug resistance against many pathogenic fungi and bacteria around the world, there is a need to improve testing methods to identify bioactive S-containing natural products against clinically pathogenic microbes. *In vitro* bioassays are very necessary to gain valuable insights into S-containing natural products susceptibility testing.

Conclusions

Sulfur-containing natural products have fascinating biological activities and an important role in response to biotic and abiotic stress. Phytoalexins are an example of a defense system produced by plants against pests and pathogens. Garlic and its compounds possess health-promoting properties, as reported in many studies. The physiologically active compounds present in garlic exhibit potential pharmacological benefits. Moreover, glucosinolates hydrolysis products have a biocidal effect.

Currently, sulfur research has occupied the forefront of interest in plant science and involves various plant species. Specifically, sulfur assimilation pathway enzymes including ATP-sulfurylase are major targets of recent plant nutrition research, which could offer big benefits including improved productivity and quality of crops in addition to their resistance to multiple stress conditions. Moreover, an in-depth investigation of the regulation of sulfur metabolism will allow a better understanding on how deeply sulfur metabolism is involved in the biosynthesis of plant primary and secondary metabolites. The examples reported in this review suggest that the use of a sulfur stress or other environmental biotic or abiotic stresses could be another tool to manipulate the biosynthetic machinery of sulfur-containing natural products to induce new and bioactive compounds with interesting bioactivities for human and plant health.

Author Contributions

MAA and KHM conceptualized the idea, MAA wrote, and edited the manuscript and KHM provided input during preparation, edited, and submitted the manuscript.

The authors declare no conflict of interest.

References

Abdallah, M., Dubousset, L., Meuriot, F., Etienne, P., Avice, J.-C., Ourry, A., 2010: Effect of mineral sulphur availability on nitrogen and sulphur uptake and remobilization during the vegetative growth of *Brassica napus* L. J. Exp. Bot. 61, 2635-2646. DOI: 10.1093/jxb/erq096

Aghajanzadehdivaei, T., 2015: Sulfur metabolism, glucosinolates and fungal resistance in Brassica. Doctoral thesis. University of Groningen, 172, Netherlands.

Agneta, R., Lelario, F., De Maria, S., Mollers, C., Bufo, S.A., Rivelli, A.R., 2014: Glucosinolate profile and distribution among plant tissue and phonological stages of field-grown horseradish. Phytochemistry 106, 178-187. DOI: 10.1016/j.phytochem.2014.06.019

Ahuja, I., de Vos, R.C.H., Rohloff, J., Stoopen, G.M., Halle, K.K., Ahmad, S.J.N., Hoang, L., Hall, R.D., Bones, A.M., 2016: Arabidopsis myrosinases link the glucosinolate-myrosinase system and the cuticle. Sci. Rep 6, 38990. DOI: 10.1038/srep38990

Amagase, H., Petesch, B.L., Matsuura, H., Kasuga, S., Itakura, Y., 2001: Intake of garlic and its bioactive components. J. Nutr. 131, 955-962. DOI: 10.1093/jn/131.3.955S

Angus, J.F., Gardner, P.A., Kirkegaard, J.A., Desmarchelier, J.M., 1994: Biofumigation: isothiocyanates released from Brassica roots inhibit growth of take-all fungus. Plant Soil 162, 107-112. DOI: 10.1007/BF01416095

Anwar, A., Gould, E., Tinson, R., Groom, M., Hamilton, C.J., 2017: Think yellow and keep green – Role of sulfanes from garlic in agriculture. Antioxidants (Basel). 6, 3. DOI: 10.3390/antiox6010003

Aries, A., Rosa, E., Carvalho, R., 2006: Effect of nitrogen and sulfur fertilization on glucosinolates in the leaves and roots of broccoli sprouts (*Brassica oleracea* var Italica). J. Sci. Food Agric. 86, 1512-1516. DOI: 10.1002/jsfa.2535

Bar-Chen, W., Golan, T., Peri, I., Ludmer, Z., Schwartz, B., 2010: Allicin purified from fresh garlic cloves induces apoptosis in colon cancer cells via Nrf2. Nutr. Cancer 62, 947-957. DOI: 10.1080/01635581.2010.509837

Barth, C., Jander, G., 2006: Arabidopsis myrosinases TGG1 and TGG2 have redundant function in glucosinolate breakdown and insect defense. Plant J. 46, 549-562. DOI: 10.1111/j.1365-313X.2006.02716.x

Bloem, E., Haneklaus, S., Salac, I., Wickenhäuser, P., Schnug, E., 2007: Facts and fiction about sulphur metabolism in relation to plant-pathogen interactions. Plant Biol. 9, 596-607. DOI: 10.1055/s-2007-965420

Bloem, E., Haneklaus, S., Schnug, E., 2015: Milestones in plant sulfur research on sulfur-induced-resistance (SIR) in Europe. Front. Plant Sci. 5. DOI: 10.3389/fpls.2014.00779

Borlinghaus, J., Albrecht, F., Gruhlke, M.C., Nwachukwu, I.D., Slusarenko, A.J., 2014: Allicin: chemistry and biological properties. Molecules 19, 12591-12618. DOI: 10.3390/molecules190812591

Brabban, A.D., Edwards, C., 1995: The effects of glucosinolates and their hydrolysis products on microbial growth. J. Appl. Bacteriol. 79, 171-177.

Brader, G., Tas, E., Palva, E.T., 2001: Jasmonic-dependent induction of indole glucosinolates in Arabidopsis by culture filtrates of the nonspecific pathogen *Erwinia carotovora*. Plant Physiol. 126, 844-860.

Browne, L.M., Conn, K.L., Ayer, W.A., Tewari, J.P., 1991: The camalexins: new phytoalexins produced in the leaves of *Camelina sativa* (Cruciferae). Tetrahedron 47, 3909-3914. DOI: 10.1016/S0040-4020(01)86431-0

Cetintas, R., Yarba, M.M., 2010: Nematicidal effects of five plant essential oils on the southern root-knot nematode, Meloidogyn. incognita race 2. J. Anim. Vet. Adv. 9, 222-225. DOI: 10.3923/javaa.2010.222.225

Chan, K.X., Phua, S.Y., Van Breusegem, F., 2019: Secondary sulfur metabolism in cellular signalling and oxidative stress responses. J. Exp. Bot. pii: erz119. DOI: 10.1093/jxb/erz119

Choi, Y.H., Park, H.S., 2012: Apoptosis induction of U937 human leukemia cells by diallyl trisulfide induces through generation of reactive oxygen species. J. Biomed. Sci. 19, 50. DOI: 10.1186/1423-0127-19-50

Chripkova, M., Zigo, F., Mojzis, J., 2016: Antiproliferative effect of indole phytoalexins. Molecules. 21, 1626. DOI: 10.3390/molecules21121626

Curtis, H., Noll, U., Störmann, J., Slusarenko, A.J. 2004: Broad-spectrum activity of the volatile phytoanticipin allicin in extracts of garlic (*Allium sativum* L.) against plant pathogenic bacteria, fungi and Oomycetes. Physiol. Mol. Plant Pathol. 65, 79-89. DOI: 10.1016/j.pmpp.2004.11.006

Danquah,W.B., Back, M.A., Grove, I.G., Haydock, P.P.J., 2011: In vitro nematicidal activity of a garlic extract and salicylaldehyde on the potato cyst nematode, Globodera pallida. Nematology 13, 869-885. DOI: 10.1163/138855411X560959

Davidian, J.-C., Kopriva, S., 2010: Regulation of sulfate uptake and assimilation – the same or not the same? Mol. Plant, 3, 314-325. DOI: 10.1093/mp/ssq001. Epub 2010 Feb 5

Del Carmen Martínez-Ballesta, M., Moreno, D.A., Carvajal, M. 2013: The physiological importance of glucosinolates on plant response to abiotic stress in Brassica. Int. J. Mol. Sci. 14, 11607-11625. DOI: 10.3390/ijms140611607

Devys, M., Barbier, M., Loiselet, I., Rouxel, T., Sarniguet, A., Kollmann, A., Bousquet, J., 1990: Cyclobrassinin sulphoxide, a sulphur-containing phytoalexin from *Brassica juncea*. Phytochemistry 29, 1087-1088. DOI: 10.1016/0031-9422(90)85408-8

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. DOI: 10.1111/j.1744-7348.1991.tb05648.x

Fahey, J.W., Zhang, Y., Talalay, P., 1997: Broccoli sprouts: An exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. Proc. Natl. Acad. Sci. USA, 94, 10367-10372. DOI: 10.1073/pnas.94.19.10367

Franco, P., Spinozzi, S., Pagnotta, E., Lazzeri, L., Ugolini, L., Camborata, C., Roda, A., 2016: Development of a liquid chromatography – electrospray ionization – tandem mass spectrometry method for the simultaneous analysis of intact glucosinolates and isothiocyanates in

Brassicaceae seeds and functional foods. J. Chromatogr. A 1428, 154-161. DOI: 10.1016/j.chroma.2015.09.001

Frerigmann, H., Gigolashvili, T., 2014: MYB34, MYB51, and MYB122 distinctly regulate indolic glucosinolate biosynthesis in *Arabidopsis thaliana*. Mol. Plant 7, 814-828. DOI: 10.1093/mp/ssu004

Geilfus, C.-M., Hasler, K., Witzel, K., Gerendás, J., Mühling, K.H., 2016: Interactive effects of genotype and N/S-supply on glucosinolates and glucosinolate breakdown products in Chinese cabbage (*Brassica rapa* L. ssp. pekinensis). J. Appl. Bot. Food Qual. 89, 279-286. DOI: 10.5073/JABFQ.2016.089.036

Gerendás, J., Breuning, S., Stahl, T., Mersch-Sundermann, V., Mühling, K.H., 2008a: Isothiocyanate concentration in Kohlrabi (*Brassica oleracea* L. gongylodes) plants as influenced by sulphur and nitrogen supply. J. Agric. Food Chem. 56, 8334-8342. DOI: 10.1021/jf800399x

Gerendás, J., Podestát, J., Stahl, T., Kübler, K., Brückner, H., Mersch-Sundermann, V., Mühling, K.H., 2009: Interactive effects of sulphur and nitrogen supply on the concentration of sinigrin and allyl-isothiocyanate in Indian mustard (*Brassica juncea* L.). J. Agric. Food Chem. 57, 3837-3844. DOI: 10.1021/jf803636h

Gerendás, J., Sailer, M., Fendrich, M.L., Stahl, T., Mersch-Sundermann, V., Mühling, K.H., 2008b: Influence of sulphur and nitrogen supply on growth, nutrient status and concentration of benzyl-isothiocyanate in cress (*Lepidium sativum* L.). J. Sci. Food Agric. 88, 2576-2580. DOI: 10.1002/jsfa.3374

Gross, D., Porzel, A., Schmidt, J., 1994: Phytoalexin Emit Indolstruktur aus Kohlrabi (*Brassica oleracea* var. gongylodes). Z. Naturforsch. C 49c, 281-285. DOI: 10.1515/znc-1994-5-601

Gruhlke, M.C.H., Slusarenko, A.J., 2012: The biology of reactive sulfur species (RSS). Plant Physiol. Biochem. 2012, 59, 98-107. DOI: 10.1016/j.plaphy.2012.03.016

Haneklaus, S., Bloem, E., Schnug, E., 2007: Sulfur and Plant Disease. In: Datnoff, L.E., Elmer, W.H., Huber, D.M. (eds.), Mineral Nutrition and Plant Disease, 101-118. Minnesota, MN: APS Press; St. Paul.

Haneklaus, S., Bloem, E., Schnug, E., 2009: Plant disease control by nutrient management: sulphur. In: Walters, D. (ed.), Disease control in crops – biological and environmentally friendly approaches, 221-236. Chichester: WileyBlackwell.

Hanschen, F.S., Pfitzmann, M., Witzel, K., Stützel, H., Schreiner, M., Zrenner, R., 2018: Differences in the enzymatic hydrolysis of glucosinolates increase the defense metabolite diversity in 19 *Arabidopsis thaliana* accessions. Plant Physiol. Biochem. 124, 126-135. DOI: 10.1016/j.plaphy.2018.01.009

Hawkesford, M.J., 2012: Sulfate uptake and assimilation – Whole plant regulation. In: De Kok, L. et al. (eds.), Sulfur metabolism in plants. Proceedings of the International Plant Sulfur Workshop, Vol 1. Springer, Dordrecht.

Higuchi, O., Tateshita, K., Nishimura, H., 2003: Antioxidative activity of sulfur-containing compounds in Allium species for human low-density lipoprotein (LDL) oxidation in vitro. J. Agric. Food Chem. 51, 7208-7214. DOI: 10.1021/jf034294u

Hirai, M.Y., Saito, K., 2008: Analysis of systemic sulfur metabolism in plants using integrated '-omics' strategies. Mol. Biosyst. 4, 967-973. DOI: 10.1039/b802911n

Huang, Y., Chen, S.X., Ho, S.H., 2000: Bioactivities of methyl allyl disulfide and diallyl trisulfide from essential oil of garlic to two species of stored-product pests, *Sitophilus zeamais* (Coleoptera: Curculionidae) and *Tribolium castaneum* (Coleoptera: Tenebrionidae). J. Econ. Entomol. 93, 537-543. DOI: 10.1603/0022-0493-93.2.537

Huseby, S., Koprivova, A., Lee, B.R., Saha, S., Mithen, R., Wold, A.B., Bengtsson, G.B., Kopriva, S., 2013: Diurnal and light regulation of sulphur assimilation and glucosinolate biosynthesis in Arabidopsis. J. Exp. Bot. 64, 1039-1048. DOI: 10.1093/jxb/ers378. Epub 2013 Jan 10

Ishimoto, H., Fukushi, Y., Tahara, S., 2004: Non-pathogenic Fusarium strains protect the seedlings of *Lepidium sativum* from *Pythium ultimum*. Soil Biol. Biochem. 36, 409-414. DOI: 10.1016/j.soilbio.2003.10.016

Jimenez, L.D., Ayer, W.A., Tewari, J.P., 1997: Phytoalexins produced in the leaves of Capsella bursa-pastoris (shepherd's purse). Phytoprotection 78, 99-103. DOI: 10.7202/706124ar

Kastell, A., Schreiner, M., Knorr, D., Ulrichs, C., Mewis, I., 2018: Influence of nutrient supply and elicitors on glucosinolate production in E. sativa hairy root cultures. Plant Cell, Tissue and Organ Culture (PCTOC) 132, 561-572. DOI: 10.1007/s11240-017-1355-8

Kim, E.J., Lee, D.H., Kim, H.J., Lee, S.J., Ban, J.O., Cho, M.C., Jeong, H.S., Yang, Y., Hong, J.T., Yoon, D.Y., 2012: Thiacremonone, a sulfur compound isolated from garlic, attenuates lipid accumulation partially mediated via AMPK activation in 3T3-L1 adipocytes. J. Nutr. Biochem. 23, 1552-1558. DOI: 10.1016/j.jnutbio.2011.10.008

Kim, S.Y., Park, J.E., Kim, E.O., Lim, S.J., Nam, E.J., Yun, J.H., Yoo, G., Oh, S.R., Kim, H.S., Nho, C.W., 2018: Exposure of kale root to NaCl and Na_2SeO_3 increases isothiocyanate levels and Nrf2 signalling without reducing plant root growth. Sci. Rep. 8, 3999. DOI: 10.1038/s41598-018-22411-9

Koch, H.P., Lawson, L.D., 1996: Garlic: The Science and Therapeutic Application of *Allium sativum* L. and Related Species; Williams & Wilkins: Baltimore, MD, USA, 1996.

Kopriva, S., Mugford, S.G., Baraniecka, P., Lee, B.-R., Matthewman, C.A., Koprivova, A., 2012: Control of sulfur partitioning between primary and secondary metabolism in Arabidopsis. Front. Plant Sci. 3, 163. DOI: 10.3389/fpls.2012.00163

Koprivova, A., Kopriva, S., 2016: Sulfation pathways in plants. Chem. Biol. Interact. 259, 23-30. DOI: 10.1016/j.cbi.2016.05.021

Kopsell, D.E., Kopsell, D.A., Randle, W.M., 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. DOI: 10.1021/jf034098n

Koul, O., 2004: Biological activity of volatile di-n-propyl disulfide from seeds of neem, Azadirachta indica (Meliaceae), to two species of stored grain pests, *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst). J. Econ. Entomol. 97, 1142-1147.

Krest, I., Keusgen, M., 1999: Stabilization and pharmaceutical use of alliinase. Pharmazie 54, 289-293.

Kusterer, J., Keusgen, M., 2010: Cysteine sulfoxides and volatile sulfur compounds from Allium tripedale. J. Agric. Food Chem. 58, 1129-1137. DOI: 10.1021/jf903581f

Lai, K.C., Kuo, C.L., Ho, H.C., Yang, J.S., Ma, C.Y., Lu, H.F., Huang, H.Y., Chueh, F.S., Yu, C.C., Chung, J.G., 2012: Diallyl sulfide, diallyl disulfide and diallyl trisulfide affect drug resistant gene expression in colo 205 human colon cancer cells in vitro and in vivo. Phytomedicine 19, 625-630. DOI: 10.1016/j.phymed.2012.02.004

Lee, B.-R., Koprivova, A., Kopriva, S., 2011: The key enzyme of sulfate assimilation, adenosine 5′-phosphosulfate reductase, is regulated by HY5 in Arabidopsis. Plant J. 67, 1042-1054. DOI: 10.1111/j.1365-313X.2011.04656.x

Lee, D.Y., Li, H., Lim, H.J., Lee, H.J., Jeon, R., Ryu, J.H., 2012: Anti-inflammatory activity of sulfur-containing compounds from garlic. J. Med. Food 15, 992-999. DOI: 10.1089/jmf.2012.2275

Liu, Z., Li, M., Chen, K., Yang, J., Chen, R., Wang, T., Liu, J., Yang, W., Ye, Z., 2012: S-allylcysteine induces cell cycle arrest and apoptosis in androgen-independent human prostate cancer cells. Mol. Med. Report 5, 439-443. DOI: 10.3892/mmr.2011.658

Ludwig-Mueller, J., Bennett, R., Kiddle, G., Ihmig, S., Ruppel, M., Hilgenberg, W., 1999: The host range of Plasmodiophora brassicae and its relationship to endogenous glucosinolate content. New Phytol. 141, 443-458. DOI: 10.1046/j.1469-8137.1999.00368.x

Ludwig-Müller, 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. DOI: 10.1016/S0031-9422(96)00498-0

Maathuis, F.J.M., 2009: Physiological functions of mineral macronutrients. Curr. Opin. Plant Biol. 12, 250-258. DOI: 10.1016/j.pbi.2009.04.003

Malhi, S.S., Gan, Y., Raney, J.P., 2007: Yield, seed quality, and sulfur uptake of Brassica oilseed crops in response to sulfur fertilization. Agron. J. 99, 570-577. DOI: 10.2134/agronj2006.0269

Ménard, R., Laure, J.P., Silué, D., Thouvenot, D., 1999: Glucosinolates in cauliflower as biochemical markers for resistance against downy mildew. Phytochemistry 52, 29-35. DOI: 10.1016/S0031-9422(99)00165

Merah, O., 2015: Genetic variability in glucosinolates in seed of *Brassica juncea*: Interest in mustard condiment. J. Chem. Volume 2015, Article ID 606142, 6 pages. DOI: 10.1155/2015/606142

Metwally, D.M., Al-Olayan, E.M., Alanazi, M., Alzahrany, S.B., Semlali, A., 2018: Antischistosomal and anti-inflammatory activity of garlic and allicin compared with that of praziquantel in vivo. BMC Complement. Altern. Med. 18, 135. DOI: 10.1186/s12906-018-2191-z

Mikhaiel, A.A., 2011: Potential of some volatile oils in protecting packages of irradiated wheat flour against *Ephestia kuehniella* and *Tribolium castaneum*. J. Stored Prod. Res. 47, 357-364. DOI: 10.1016/j.jspr.2011.06.002

Monde, K., Sasaki K., Shirata, A., Takasugi, M., 1991: Methoxybrassenins A and B, sulphur-containing stress metabolites from *Brassica oleracea* var. capitata. Phytochemistry 30, 3921-3922. DOI: 10.1016/0031-9422(91)83435-N

Monde, K., Sasaki, K., Shirata, A., Takasugi, M., 1990: 4-Methoxybrassinin, a sulphur-containing phytoalexin from Brassicaoleracea. Phytochemistry 29, 1499-1500. DOI: 10.1016/0031-9422(90)80108-S

Monde, K., Takasugi, M., Shirata, A., 1995: Three sulphur-containing stress metabolites from Japanese radish. Phytochemistry 39, 581-586. DOI: 10.1016/0031-9422(95)00011-U

Monde, K., Taniguchi, T., Miura, N., Kutschy, P., Curillova, Z., Pilatova, M., Mojzis, J., 2005: Chiral cruciferous phytoalexins: preparation, absolute configuration, and biological activity. Bioorganic Med. Chem. 13, 5206-5212. DOI: 10.1016/j.bmc.2005.06.001

Moreira-Rodríguez, M., Nair, V., Benavides, J., Cisneros-Zevallos, L., Jacobo-Velázquez, D.A., 2017: UVA, UVB Light, and methyl jasmonate, alone or combined, redirect the biosynthesis of glucosinolates, phenolics, carotenoids, and chlorophylls in broccoli sprouts. Int. J. Mol. Sci. 18, 11 pii: E2330. DOI: 10.3390/ijms18112330

Mortensen, J., Eriksen, J., Nielsen, J.D., 1992: Sulfur defiency and ainino acid composition in seeds and grass. Phyton (Horn, Austria) 32, 85-90.

Mugford, S.G., Lee, B.R., Koprivova, A., Matthewman, C., Kopriva, S., 2011: Control of sulfur partitioning between primary and secondary metabolism. Plant J. 65, 96-105. DOI: 10.1111/j.1365-313X.2010.04410.x. Epub 2010 Nov 22

Nowsad, A., Mondal, R., Islam, M., 2009: Effectiveness of neem, garlic and red chili against adult dermestid beetle in sun dried fish. Progress. Agric. 20, 133-142.

Park, I.K., Choi, K.S., Kim, D.H., Choi, I.H., Kim, L.S., Bak, W.C., Choi, J.W., Shin, S.C., 2006: Fumigant activity of plant essential oils and components from horseradish (*Armoracia rusticana*), anise (*Pimpinella anisum*) and garlic (*Allium sativum*) oils against *Lycoriella ingenua* (Diptera: Sciaridae). Pest Manag. Sci. 62, 723-728. DOI: 10.1002/ps.1228

Pedras, M.S., Yaya, E.E., Glawischnig, E., 2011: The phytoalexins from cultivated and wild crucifers: chemistry and biology. Nat. Prod. Rep. 28, 1381-1405. DOI: 10.1039/c1np00020a

Pedras, M.S.C., Sarwar, M.G., Suchy, M, Adio, A.M., 2006a: The phytoalexins from cauliflower, caulilexins A, B and C: isolation, structure determination, syntheses and antifungal activity. Phytochemistry 67, 1503-1509. DOI: 10.1016/j.phytochem.2006.05.020

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. DOI: 10.1016/S0031-9422(97)00362-2

Pedras, M.S.C., Sorensen, J.L., Okanga, F.I., Zaharia, I.L., 1999: Wasalexins A and B, new phytoalexins from wasabi: isolation, synthesis, and antifungal activity. Bioorganic Med. Chem. Lett. 9, 3015-3020. DOI: 10.1016/S0960-894X(99)00523-5

Pedras, M.S.C., Suchy, M., Ahiahonu, P.W., 2006: Unprecedented chemical structure and biomimetic synthesis of erucalexin, a phytoalexin from the wild crucifer *Erucastrum gallicum*. Org. Biomol. Chem. 4, 691-701. DOI: 10.1039/b515331j

Pedras, M.S.C., Yaya, E.E., 2010: Phytoalexins from Brassicaceae: News from the front. Phytochemistry, Supporting Data 71, 1191-1197. DOI: 10.1016/j.phytochem.2010.03.020

Pedras, M.S.C., Zheng, Q.A., Gadagi, R.S., 2007b: The first naturally occurring aromatic isothiocyanates, rapalexins A and B, are cruciferous phytoalexins. Chem. Comm., 368-370. DOI: 10.1039/b615424g

Pedras, M.S.C., Zheng, Q.A., Sarwar, M.G., 2007a: Efficient synthesis of brussalexin A, a remarkable phytoalexin from Brussels sprouts. Org. Biomol. Chem. 5, 1167-1169. DOI: 10.1039/B702156A

Pedras, M.S.C., Zheng, Q.A., Schatte, G., Adio, A.M., 2009: Photochemical dimerization of wasalexins in UV-irradiated Thellungiella halophila and in vitro generates unique cruciferous phytoalexins. Phytochemistry 70, 2010-2016. DOI: 10.1016/j.phytochem.2009.09.008

Pedras, M.S.C., Zheng, Q.A., Strelkov, S., 2008: Metabolic changes in roots of the oilseed canola infected with the biotroph *Plasmodiophora brassicae*: Phytoalexins and phytoanticipins. J. Agric. Food Chem. 56, 9949-9961. DOI: 10.1021/jf802192f

Pilatova, M., Sarissky, M., Kutschy, P., Mirossay, A., Mezencev, R., Curillova, Z., Suchy, M., Monde, K., Mirossay, L., Mojzis, J., 2005: Cruciferous phytoalexins: antiproliferative effects in T-Jurkat leukemic cells. Leuk. Res. 29, 415-421. DOI: 10.1016/j.leukres.2004.09.003

Pitann, B., Heyer, C., Mühling, K.H., 2017: The effect of sulfur nutrition on glucosinolate patterns and their breakdown products in vegetable crops. In: Kok, L., Schnug, E., Hawkesford, M. (eds.), Sulfur metabolism in higher plants – fundamental, environmental and agricultural aspects, 61-73. Springer Verlag, Berlin.

Radojcic Redovnikovic, I., Glivetic, T., Delonga, K., Vorkapic-Furac, J., 2008: Glucosinolates and their potential role in plant. Periodicum Biologorum 110, 297-309.

Rausch, T., Wachter, A., 2005: Sulfur metabolism: A versatile platform for launching defence operations. Trends Plant Sci. 10, 503-509. DOI: 10.1016/j.tplants.2005.08.006

Rennenberg, H., Herschbach, C., 2012: Sulphur compounds in multiple compensation reactions of abiotic stress responses. Sulphur Metabolism in Plants. Proc Int Plant Sulphur Workshop 1, 203-215.

Rostás, M., Bennett, R., Hilker, M., 2002: Comparative physiological responses in Chinese cabbage induced by herbivory and fungal infection. J. Chem. Ecol. 28, 2449-2463. DOI: 10.1023/A:102142791

Sabol, M., Kutschy, P., Siegfried, L., Mirossay, A., Suchy, M., Hrbkova, H., Dzurilla, M., Maruskova, R., Starkova, J., Paulikova, E., 2000: Cytotoxic effect of cruciferous phytoalexins against murine L1210 leukemia and B16 melanoma. Biologia 55, 701-707.

Saito, K. 2004: Sulfur assimilatory metabolism. The long and smelling road. Plant Physiol. 136, 2443-2450. DOI: 10.1104/pp.104.046755

Saladino, F., Bordin, K., Luciano, F.B., Franzón, M.F., Mañes, J., Meca, G., 2016: Antimicrobial activity of the glucosinolates. In: Mérillon, J.M., Ramawat, K. (eds.), Glucosinolates. Reference Series in Phytochemistry. Springer, Cambridge.

Samadi, S., Keusgen, M., 2013: Effectiveness of bulb extracts of Allium species on some selected plant pathogenic fungi. Planta Med. 79–PN97. DOI: 10.1055/s-0033-1352439

Schnug, E., Haneklaus, S., 2005: Sulphur deficiency symptoms in oilseed rape (*Brassica napus* L.) – the aesthetics of starvation. Phyton. 45, 79-95.

Schonhof, I., Blankenburg, D., Müller, S., Krumbein, A., 2007: Sulfur and nitrogen supply influence growth, product appearance, and glucosinolate concentration of broccoli. J. Plant Nutr. Soil Sci. 170, 65-72. DOI: 10.1002/jpln.200620639

Strehlow, B., Bakowsky, U., Pinnapireddy, S.R., Kusterer, J., Mielke, G., Keusgen, M., 2016: J. Pharm. Drug Deliv. Res. 5, 1. DOI: 10.4172/2325-9604.1000143

Tada, Y., Spoel, S.H., Pajerowska-Mukhtar, K., Mou, Z., Song, J., Wang, C., Zuo, J., Dong, X., 2008: Plant immunity requires conforma-

tional changes [corrected] of NPR1 via S-nitrosylation and thioredoxins. Science 2008, 321, 952-956. DOI: 10.1126/science.1156970

TAKASUGI, M., KATSUI, N., SHIRATA, A., 1986: Isolation of three novel sulphur-containing phytoalexins from the Chinese cabbage *Brassica campestris* L. ssp. Pekinensis (Cruciferae). J. Chem. Soc. Chem. Commun. 1077-1078. DOI: 10.1039/C39860001077

TAKASUGI, M., MONDE, K., KATSUI, N., SHIRATA, A., 1988: Novel sulfur-containing phytoalexins from the Chinese cabbage *Brassica campestris* L. ssp. pekinensis (Cruciferae). Bull. Chem. Soc. Jpn. 61, 285-289. DOI: 10.1246/bcsj.61.285

VENDITTI, A., BIANCO, A., 2018: Sulfur-containing secondary metabolites as neuroprotective agents. Curr. Med. Chem. DOI: 10.2174/0929867325666180912105036

WANG, K., LIU, N., ZHANG, P., GUO, Y., ZHANG, Y., ZHAO, Z., LUAN, Y., LI, S., CAI, J., CAO, J., 2016: Synthetic methods of disulfide bonds applied in drug delivery systems. Curr. Org. Chem. 20, 1477-1489. DOI: 10.2174/1385272820666151207194002

YU, C.S., HUANG, A.C., LAI, K.C., HUANG, Y.P., LIN, M.W., YANG, J.S., CHUNG, J.G., 2012: Diallyl trisulfide induces apoptosis in human primary colorectal cancer cells. Oncol. Rep. 28, 949-954. DOI: 10.3892/or.2012.1882

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