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Progress and prospects of glucosinolate pathogen resistance in some brassica plants

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Abstract
Plants are constantly defending themselves against an array of assaults by pathogenic organisms. This has led to the evolution of precise and elaborate chemical defense systems involving glucosinolates (GSLs) in cruciferous plants. These GSLs and their hydrolysis products are biologically active and are implicated as enabling formidable plant defense processes in certain economically important members of Brassicaceae like broccoli, cabbage and mustard seed. This review provides a comprehensive report of how indole and aliphatic GSLs mitigate incidents of plant pathogenesis. By evaluating the roles of GSLs in plant-pathogen interaction of some brassica plants, this review highlights the associated mechanism that culminates in disease suppression. Moreover, seven economically important brassica pathogens were reviewed in terms of their ability to disrupt proper plant functioning as well as the mechanisms by which GSLs and their hydrolysis products in Brassica lower the susceptibility to them. Future perspectives of the application of GSLs in plant pathogen resistance using advanced molecular techniques are also discussed.

Keywords: Arabidopsis, Brassicaceae, Glucosinolates, Pathogens, Plant immunity, Secondary metabolites

INTRODUCTION

Plant biochemical defense mechanisms evolved overtime through phytochemical-mediated strategies to adapt and overcome antagonistic stress that may impair growth, development and reproduction (Dangl and Jones, 2001; Ausubel, 2005; Jones and Dangl, 2006, Chisholm *et al*., 2006). Some of the end products of this defense action include the production of reactive oxygen species (ROS), signal transduction, cell fortification, enzyme synthesis and production of diverse secondary metabolites. However, according to Dangl and Jones (2006), the lack of mobile defender cells and somatic adaptive immune systems ensures that plant often relies on the innate immunity of their individual cells as well as on systemic signals emanating from infection sites to initiate and coordinate defense response. Thus, the propagation of defense response culminates in effects beyond their site of initiation. Upon recognition of invading pathogens, opined that plant host cells respond by producing and accumulating ROS, which has been adversely studied not only for their role in plant development but also for eliciting immunity as a form of stress response (Lehmann *et al*., 2005; Torres *et al*., 2006; Vwioko *et al*., 2018). Although this sort of first response depends on the nature and severity of the pathogen and threat as well as the plant group, the multi-layered response system of plant, which in turn depends on the perceived signal and nature of the defense response, lead to microbe- or pathogen-associated molecular patterns or damage-associated molecular pat-

terns (Underwood, 2012). Nonetheless, the turnover of associated secondary metabolite such as glucosinolates (GSLs) is a suggestion of their roles in key interactions (Bednarek *et al*., 2009). For instance, the effector-triggered hypersensitive response (ET-HR) mechanism, which depends on indole and aliphatic glucosinolates, or their by-products have been implicated in delayed programmed cell death upon *Pseudomonas syringae* and *Hyaloperonospora arabidopsidis* inoculation in aliphatic glucosinolate-deficient *myb28* and *myb29* plants (Johansson *et al*., 2014; Andersson *et al*., 2015). These findings confirm that glucosinolates are associated with the ET-HR and ROS pathways.

Pathogens have significant effects on plant fitness and may regulate plant population and in turn lead to considerable economic damage (Rausher, 2001). After successful penetration, pathogens may directly benefit from the active metabolism of their host to complete their life cycle by either keeping the host cells alive during colonization (biotrophic strategy) or induce host cell disintegration after infection (necrotrophic strategy). Some pathogenic fungus utilizes hemibiotrophic strategy whereby they undergo a biotrophic phase and later switch to a necrotrophic phase (Horbach *et al*., 2011; Huckelhoven and Panstruga, 2011; Bolton *et al*., 2006). Williamson *et al*. (2007) posited that necrotrophic pathogens pose the significant economic challenge on Brassica crops because of their ability to cause lesions on nearly all harvestable parts of the plant. No completely resistant Brassica germplasm have been recorded for most of this necrotrophic fungus (Cao *et al*. 2016)

As a group of thioglucosides, including tryptophan -derived indole glucosinolates (IGSLs) and methionine, derived aliphatic glucosinolates (AGSLs), glucosinolates (GSLs) are important secondary metabolites in Brassica species. This plant health promoting, sulfur and nitrogen-containing group of phytochemicals can be found in several Brassica species including cauliflower, rapeseed, cabbage, broccoli, radish, rutabaga, baemuchae, kohlrabi, turnip, black mustard, Chinese cabbage, leaf mustard, and kale. In addition, Holst and Fenwick (2003) assert that these cruciferous plants containing GSLs have made invaluable contributions to human and animal diets as additives (mustard and wasabi), leafy vegetables (cabbage, swede), and livestock feedstuffs (rapeseed, kale, turnip). They are present in different concentrations in the different parts of the plant that may provide added insights into their site-dependent expression and functions (Moussaieff *et al*., 2013; Bhandari *et al*., 2015). GSLs also perform regulatory functions in inflammation, stress response, phase I metabolism, and antioxidant activities, as well as direct antimicrobial properties (Bischoff, 2016). In the same vein, some *Brassica* species containing GSLs have also been implicated in phytoremediation and biofumigation (Szczygłowska *et al*., 2011). Myrosinases coexist with GSLs but are stored separately in adjacent cells but mix upon sensing a pathogen attack (Redovnikov *et al*., 2008). The result is an hydrolysis of thioglucosidic GSLs bond to produce unstable aglucones, which decompose to various bioactive compounds, including isothiocyanates and thiocyanate with toxic effects on microorganisms, nematodes, insects and other pathogens (see Fig. 1; Lambrix *et al*., 2001; Burow and Wittstock, 2009; Bednarek *et al*., 2009; Clay *et al*., 2009; Wittstock and Burow, 2010; Stotz *et al*., 2011; Bednarek, 2012).

Some key defense mechanisms include a direct response to specific antimicrobial activities, metabolite biosynthesis, callose deposition, transcription of response genes, stomatal closure and programmed plant cell death signaling (Fig. 2). Previous workers have elucidated the roles of phytochemicals (chiefly secondary plant metabolites) in protecting plants against pathogens and pests (Cowan, 1999; Bennett and Wallsgrove, 1994). In the case of Brassica crops, they either produce phytochemicals as a component of their growth and development (i.e. inbuilt chemical barriers; structural barriers such as lignin, and pre-formed phytoanticipins such as GSLs) or de novo synthesis in response to pathogen attack or stress (phytoalexins) (Bennett and Wallsgrove, 1994).

Over the years, many studies have elucidated the GSL-triggered mechanisms by which plant immune systems respond upon attack by various pathogens (Table 1). These works suggest different operational mechanism, genes and vectors are involved in diverse GSL interactions. In addition, the indole glucosinolate biosynthesis pathway has been successfully bioengineered in *Nicotiana benthamiana*, a non-Brassica plant (Pfalz *et al*., 2011) and molecular techniques have also shown in detail how specific glucosinolates like glucoraphanin (4-methyl sulfinyl butyl GLS; 4-MSB) inhibit tumor cell growth in tobacco (Mikkelsen *et al*., 2010). However, limited information on the effects of specific pathogens on specific Brassica plants is available. This review presents a comprehensive investigation of some of the most important pathogens, which cause considerable damage to Brassica plants. This report will also highlight studies that successfully demonstrated the mechanisms by which GSLs mitigate pathogen effects on specific Brassica species. Some insights into future considerations for potential application in experiment and field studies will also be provided in this work.

***Xanthomonas campestris* pv. *campestris* (Pammel) Dowson:** *Xanthomonas campestris* pv. *campestris*, is the Gram-negative bacterium responsible for Black rot, one of the most devastating diseases of cruciferous crops

Table 1. Some studies showing the effects of specific GSLs on different pathogens and/or herbivores.

Vector/ herbivore	Pathogen/parasite	Specific GSL type/ metabolite	Results of study	Reference
Not mentioned	*Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc.	IGSLs	Microbe-associated molecular pattern (MAMP)-triggered immunity activates a genetically programmed cell death in the absence of the functional membrane-attack complex/perforin (MACPF) domain protein encoded by the Necrotic Spotted Lesion 1 NSL1) gene via Tryptophan (Trp)-derived secondary metabolite-mediated activation of the salicylic acid (SA) pathway.	Fukunaga *et al*. (2017)
	Botrytis cinerea Pers. *Dickeya dadantii* Samson and others	IGSLs	Enhanced accumulation of glucosinolates in response to exogenously applied nicotinamide adenine dinucleotide (NAD^+). In addition, an up-regulation of the glucosinolate-responsive genes *PEN2* (penetration-resistance gene 2) and 519 *CYP81F2* after direct NAD^+ treatment was observed, thus indicating NAD-specific regulation in Q -treated *nadC* of genes are involved in glucosinolate metabolism	Pétriacq *et al*. (2016)
Cabbage looper (*Trichoplusia ni* Hubner)	Not mentioned	Neoglucobrassicin and Glucobrassicin	Upon attack, the biosynthesis of both IGSs increased in a dose-dependent manner. The IGSs and their hydrolysis products showed significant inverse correlation with larval weight and survival after five days of treatment.	Ku *et al*. (2016)
Leaf-chewing insect (*Mamestra brassicae* L.)	Not mentioned	Aliphatic GSLs	Herbivore-induced ORA59-branch of the jasmonic acid (JA) signaling pathway and rhizobacterial colonization enhances the synthesis of aliphatic glucosinolates while synthesis of indole glucosinolates is suppressed.	Pangesti *et al*. (2016)
Brassica oleracea	(*Plectosphaerella cucumerina* Lindf. (W. Gams.))	IGSLs	MYB34, MYB51, and MYB122 transcription-mediated indole glucosinolate biosynthesis via PEN2 myrosinase	Frerigmann *et al*. (2016)
Pea aphids (*Acyrthosiphon pisum* Harris) Green peach aphids (*Myzus persicae*)	Parasitic dodder vines (*Cuscuta gronovii* Willd.)	Aliphatic GSLs, IGSLs	Elevated concentration of aliphatic and indole glucosinolates lowered parasitism by suppressing *cyp79B2 cyp79B3* factors	Smith *et al*. (2016)
	Escherichia coli (Migula), *Pseudomonas aeruginosa* (Schroeter) Migula, *Staphylococcus aureus* (Rosenbach) and *Listeria monocytogenes* (Murray) Pirie	Allylisothiocyanate (AITC) and 2- phenylethylisothiocyanate (PEITC)	Altered the membrane properties of the bacteria by decreasing their surface charge and compromising the integrity of the cytoplasmatic membrane with consequent potassium leakage and propidium iodide uptake	Borges *et al*. (2015)
	Xanthomonas campestris pv. Campestris (Dowson) Dye and others, *Pseudomonas syringae pv. Maculicola* (McCulloch) Dye and others, *Alternaria brassicae* and *Sclerotinia scletoriorum*	2-Propenyl (SIN) 3-Methylsulphinylpropyl (GIB) 4-Methylsulphinylbutyl (GRA) 2-Hydroxy-3-butenyl (PRO) 3-Butenyl (GNA) 4-Pentenyl (GBN) 4-Methylthiobutyl (GER) 4-Hydroxybenzyl (SNB) 2 -Phenylethyl (GST) Indole-3-ylmethyl (GBS)	A dose-dependent inhibition of all studied pathogens was demonstrated.	Sortelo *et al*. (2014)
	Botrytis cinerea (Pers.)	Allyl-isothiocyanate (AITC)	The AITC treatment reduced the decay caused by the pathogen by over 47.4% up to 91.5%, significantly different from the untreated fruit. Total phenolic content and antioxidant capacity estimated in treated and untreated strawberries showed no significant difference between control and AITC treated fruit.	Ugolini *et al*. (2014)
	Alternaria brassicae, *Hyaloperonospora brassicae* (Gaum.) Goker and others, and *Albugo candida* (Pers.) Roussel.	Aliphatic GSLs	Exposure of plants to elevated concentration of CO_2 (550 ppm) revealed lower incidence and severity of *Alternaria* blight while white rust infection increased. There was an increase in the concentration of total glucosinolates (GSs) under free-air CO_2 enrichment (FACE) in plants grown under elevated CO_2, but a decrease in their diversity.	Mathur *et al*. (2013)
	Broad spectrum	AITC	AITC-induced stomatal closure requires methyl jasmonate (MeJA) priming but not abscisic acid (ABA) priming, resulting in suppression of water loss and invasion of fungi through stomata	Khokon *et al*. (2011)
	Colletotrichum gloeosporioides and *Colletotrichum orbiculare* (Berk. and Mont.) Arx	IGSLs	The *Arabidopsis* indole glucosinolate pathway restricts entry of the non-adapted anthracnose fungi only when these pathogens employ hyphal tip-based entry (HTE). Arabidopsis mutants defective in indole glucosinolate biosynthesis or metabolism support the initiation of post -invasion growth of non-adapted *Colletotrichum gloeosporioides* and *Colletotrichum orbiculare*	Hiruma *et al*. (2010)
	Escherichia coli, Pseudomonas aeruginosa, *Listeria monocytogenes* and *Staphylococcus aureus*	Allylisothiocyanate, Benzylisothiocyanate and 2-phenylethylisothiocyanate	All isothiocyanates were more effective in inhibiting pathogen growth than phenolics. They inhibited the in vitro growth of the Gram-negative and Gram-positive pathogenic bacteria	Saavedra *et al*. (2010)
	Agrobacterium tumefaciens (Smith and Townsend), *Erwinia chrysanthem* (Burkholder and others), *Pseudomonas cichorii* (Swingle) Stapp., *Xanthomonas campestris* pv. *juglandis*	Aliphatic, aromatic and idolyl GSL hydrolysis products	The isothiocyanates were the most effective antibacterial components, showing a dose-dependent effect. 2-phenylethylisothiocyanate and sulphoraphane showed the highest inhibitory effects.	Aires *et al*. (2009)
Leaf-chewing insect (*Mamestra brassicae* L.)	Not mentioned	Aliphatic GSLs	Aliphatic GSLs biosynthesis solely regulated by *myb28/myb29* transcription factors. *Myb28/Myb29* double mutant was devoid of any aliphatic GSLs.	Beekwilder *et al*. (2008)
Polyphagous aphid (*Myzus persicae* Sulzer)	Not mentioned	IGSLs	IGSLs biosynthesis pathway was mediated by *CYP79B2* and *CYP79B3* genes	Kus´nierczyk *et al*. (2007)

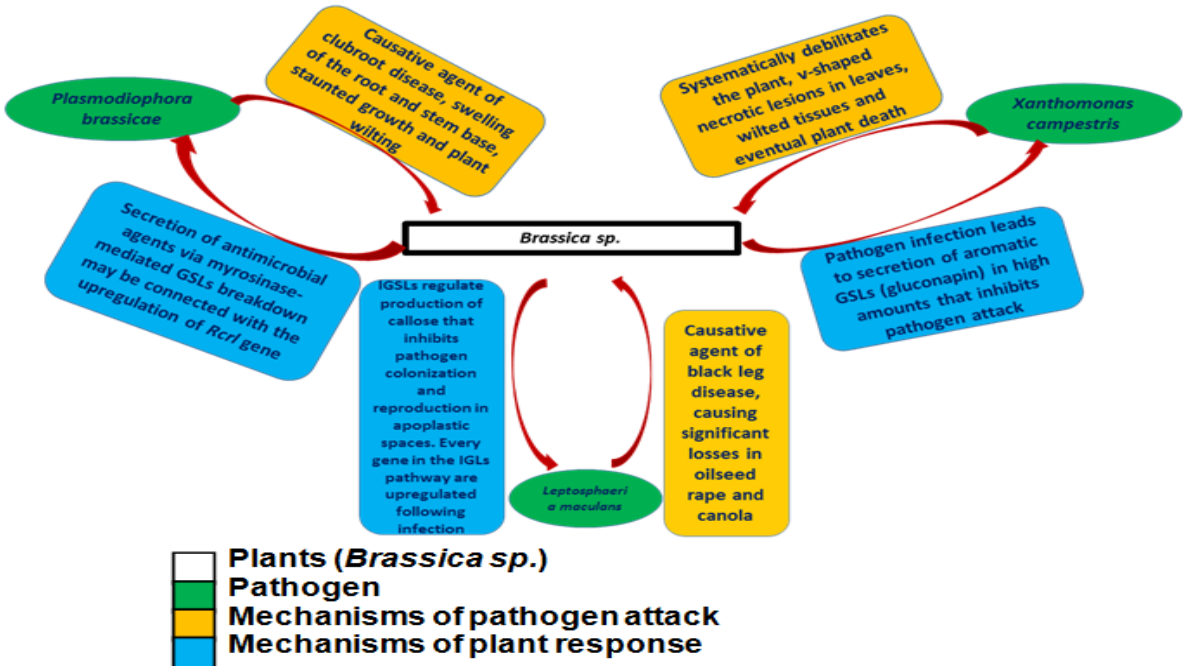

Fig. 1. *GSLs and hydrolysis products' response mechanisms to attacks by X. campestris, P. brassicae and L. maculena.*

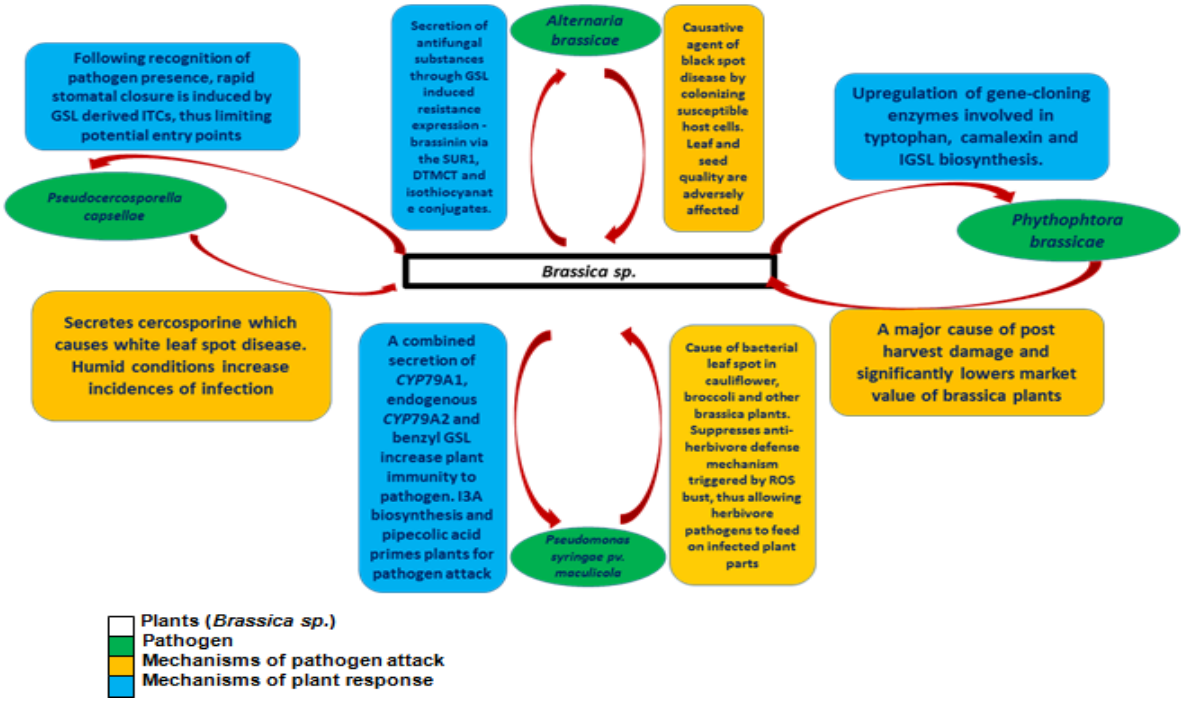

Fig. 2. *GSLs and hydrolysis products' response mechanisms to attacks by P. maculicola, A. brassicae, P. capsellae and L. maculans.*

worldwide especially *Brassica oleracea* var. *capitata* and *B. rapa* ssp. *rapa* L. in warm and humid climate (Dias *et al*., 2010; Velasco *et al*., 2013; Vicente and Holub, 2013). The bacterium is a seed-borne pathogen, as well as transmitted through infection or natural openings (Goss *et al*., 2017). The systemic vascular disease debilitates the plant, thus favoring the attack of other pathogens but even in mild attack, can cause several V-shaped necrotic lesions on leaves, which decrease the quality of the product for fresh market (Velasco *et al*., 2013). Symptoms include yellow lesions, wilted tissue, necrosis, defoliation and plant death (Fig. 3).

The roles of GSLs and their respective hydrolysis products (benzyl isothiocyanate, 2-phenyl ethyl

Fig. 3. *Black rot caused by Xanthomonas campestris pv. Campestris on turnip (Brassica rapa ssp. rapa L.). Source: Mullen (2012)*

Fig. 4. *Clubbing of Brassica oleraceae var. capitata root caused by Plasmodiophora brassicae Source: Holmes (2003).*

Fig. 5. *A Brassica plant infected by blackleg disease caused by Leptosphaeria maculans Source: Pscheidt and Ocamb (2019).*

Fig. 6. *Foliar lessons on turnip (Brassica rapa ssp. rapa) caused by Pseudocercosporella capsellae Source: Holmes (2010).*

Fig. 7. *Black spot of cabbage caused by Alternaria brassicae Source: Wells (2018).*

Fig. 8. *Leaf spot of Cauliflower caused by Pseudomonas syringae pv. maculicola Source: Lazarev (2009).*

isothiocyanate and sulforaphane), against *X. campestris* infection on Brassica plants have been highlighted by the report of Aires *et al*. (2011). They concluded that GSLs play complex roles disease resistance, particularly in the early growth stages when the young plants are in metabolic flux. Nonetheless, they successfully showed the susceptibility of Brassica to *X. campestris* is generally higher in Brassica species with lower contents of aromatic GSLs and 4-methyl sulfinyl butyl (glucoraphanin). These compounds are effective inhibitors of *X. campestris in vitro*. More so, experiment results of Velasco *et al*. (2013) showed that butl-3-enylglucosinolate and isothiocyanate have direct antimicrobial activities on *X. campestris.* Their results demonstrated that gluconapin and its ITC possess antibacterial effect on the development of *X. campestris* and this effect strongly correlate with the concentration of the above compounds.

***Plasmodiophora brassicae* Woronin:** *Plasmodiophora brassicae* is the causative agent of club rot disease that affect the root and lower stem of Brassica crops such as the Canadian canola (*Brassica napus* L.) and cabbage *Brassica oleraceae* var. *capitata* L. (Fig. 4). It is a soilborne, obligate, and biotrophic pathogen that is capable of causing significant yield losses (Dixon, 2009). The pathogen causes the root and stem base to swell and form characteristic clubs, which inhibit xylem and phloem roles, stunt the growth of the plant and wilting. After weeks of infection, the clubbed root, weakening the support of the plant. (Voorrips, 1995).

P. brassicae influence glucosinolate levels in both root and aerial tissues during primary, secondary and mature gall formation stage disease development (Islam and Guest, 2010). In the opinion of Voorrips (1995), the evidence of a correlation between IGSL content and club root susceptibility is conflicting because no relation between IGSL content and clubroot resistance has been found. He opined that although the auxin production from IGSL is somewhat important in clubroot development, the processes occurring during pathogenesis, the mechanisms responsible for resistance were unclear. However, Islam and Guest (2010) found that GSLs levels remain unchanged in aerial tissues but significantly increased (1.5 times) in root tissues during symptom development. The concentration difference might implicate a role for GSL in *P. brassicae* pathogenesis.

More so, Song *et al*. (2016) reported a myrosinase -mediated breakdown of GSLs to be one of the identified biological processes in resistant samples where they were up-regulated for host-defense responses. Their results implicate several phytoalexins as putatively deriving from the GSL metabolism in *B. rapa* roots carrying *Rcr1 (the club root resistant gene)* upon *P. brassicae* infection, which suggest the possibility for antimicrobial agents via the GSL-myrosinase metabolism pathway. Recently, Zhao *et al*. (2017) found that in the response of *A. thaliana* to *P. brassicae* infection, the expression of GSL genes and terpenoid biosynthesis significantly increased in the metabolism pathway. Further study may be required to elucidate the resultant pathway in Brassica crops.

***Leptosphaeria maculans* (Sowerby) Karst:** This hemibiotrophic fungal pathogen is the causal agent of blackleg disease in *B. napus* L. (canola, oilseed rape), which causes a significant global yield loss (Becker *et al*., 2017). *B. napus* is the second-highest produced oilseed crop worldwide and is under constant threat of the blackleg disease (Fitt *et al*., 2006). This underscores the economic effects of *L. maculans,* which affects the stems and leaves of Brassica plants (Fig. 5).

According to Hiruma *et al*. (2013), IGSLs are required for resistance against hemibiotrophic fungi. However, their role in the *B. napus - L. maculans*

pathosystem was unclear at the time. Considering that IGSLs promote the production of callose that likely prevents *L. maculans* colonization and reproduction in apoplastic spaces within cotyledons thereby confering resistance on *B. napus* (Becker *et al*. 2017). This agrees with Aist and Bushnell (1991), who proposed that callose deposition is a conserved defense response in plants that is controlled by GSL metabolism through acting as a physical barrier in the cell wall. Data from Becker *et al*. (2017) also showed activation of the complete IGSL biosynthetic pathway in resistant *B. napus* cotyledons inoculated with *L. maculans*. This confirms the previous study by Clay *et al*. (2009) who stated that in resistant hosts, every gene of the IGSL biosynthetic pathway was upregulated following *L. maculans* infection, whereas in the susceptible genotype, several genes required for IGSL production were downregulated during infection. Future investigation may be directed at exposing specific interactions within the callose of major Brassica that act under stress conditions from *L. maculans* to promote defense (Fig. 4).

***Pseudocercosporella capsellae* (Ellis and Everh.) Deighton:** *Pseudocercosporella capsellae* causes white leaf spot across a wide range of Brassica plants including oilseed, vegetable condiment and forage Brassicas worldwide (Fig. 5; Crossan, 1954; Deighton, 1973; Koike *et al*., 2007). *P. capsellae* produces a purple-pink coloured toxin called cercosporin, which has been implicated in white leaf spot disease initiation in brassica crops (Gunasinghe *et al*., 2016). *P. capsellae may be carried across seasons within thick-walled mycelium on crop debris and produces conidia when the weather is favourable. Conidia infect plants causing white or pale beige lesions on leaves. Infections are favoured by wet weather conditions (Petrie et al., 1985; Barbetti and Khangura, 2000).*

Several GSL-derived ITCs induce stomatal closure in *A. thaliana in a dose-dependent manner (Hossain et al., 2013; Khokon et al., 2011). More so,* rapid stomatal closure occurs in resistant *B. carinata* following recognition of pathogen presence, a characteristic considered a winning pre-invasive defence barrier developed by plants (Ton *et al*., 2009). By limitation potential entry ports by resistant *B. carinata,* this appears to be a major mechanism of resistance against *P. capsellae.* Hence, efforts geared at elucidating the phytochemicals associated with this structural-resistant response may characterize future research (Fig. 6).

***Alternaria brassicae* (Schwein) Wiltshire:** Black spot disease of some Brassica crops like broccoli, oil seed rape and cabbage is caused by the fungus *Alternaria brassicae* (Fig. 7). This facultative parasite colonizes susceptible hosts as well as dead plant material secreting host-specific toxins. This disease results in a considerable reduction of both yield and seed quality (Sotelo *et al*., 2014). *A. brassicae* is the prime causative agent of *Alternaria* blight disease, which affects most Brassica crops globally, causing economic losses with no proven source of transferrable resistance in any of its hosts. In tropical regions, this pathogen is most destructive in the wet season (Meena *et al*., 2010).

The report of Giri *et al*. (2013) suggested that the production of antifungal substance(s) in resistant *B. juncea* leaves was responsible for reduced infection by *Alternaria brassicae.* This includes GSLs that affect the differential expression of resistance across different plant species, lines as well as cultivars of the same species or within different tissues of a plant (Osbourn, 1996). The antifungal byproducts are not formidable in resistance of these pathogens (Meena *et al*., 2010, Zhou *et al*., 2002), however, they remain a key defense system in many Brassica plants. GSLs and their hydrolysis products have also been shown previously to have antimicrobial properties (Tierens *et al*., 2001). Recently, more specific pathways of this antifungal mechanism were reported by Klein and Sattely (2017). These researchers identified some key enzymes required for the synthesis of the parent phytoalexin of Brassica plants called Brassinin from well-studied GSLs. Some of the brassinin-type phytoalexins may be more tightly linked to the biosynthetic pathway of IGSLs. The carbon-sulfur lyase SUR1 processes cysteine–isothiocyanate conjugates, as well as the S-methyltransferase DTCMT that methylates the resulting dithiocarbamate, together completing a pathway to brassinin. Also, the β-glucosidase BABG that is present in *Brassica rapa* but absent in Arabidopsis was shown by these researchers to act as a myrosinase and may be a determinant of plants that synthesize phytoalexins from IGSLs.

***Phytophthora brassicae* De Cock and Man in't Veld:** *Phytophthora brassicae* is an economic and notorious oomycete pathogen. It has a narrow host range restricted to Brassica plants such as Chinese cabbage (*B. rapa* subsp. *pekinensis*), Brussels sprouts (*B. oleracea* var. *gemmifera*) and rutabaga (*B. napus* var. *napobrassica*) (Semb, 1971, Fagertun and Semb, 1986). *P. brassicae* is responsible for post-harvest damage that lowers the marketability of cabbage to around 90 % losses (Fagertun, 1987, Mauch *et al*, 2009). *P. brassicae* is one of the few *Phythophthora* species that infect *Arabidopsis* plant both naturally and under laboratory conditions (Koch and Slusarenko, 1990).

The susceptibility of the double mutant *pen2-1 pad3-1*, demonstrates that both camalexin and product of IGSL hydrolysis are important for

P. brassicae disease resistance in Brassica (Schlaeppi *et al*., 2010). Transcript analysis from Schlaeppi *et al*. (2008) showed that genes encoding enzymes involved in tryptophan, camalexin and IGSL biosynthesis coordinate induced response to *P. brassicae*. On the other hand, the double mutant *cyp79B2 cyp79B3* is highly susceptible to *P. brassicae as it is unable* to convert tryptophan into indole-3-acetaldoxime (IAOx), the precursor of camalexin and IGSLs (Zhao *et al*. 2002; Wang *et al*. 2013a,b). These authors also opined that the susceptibility of the double mutant *cyp79b2cyp79b3* to *Phytophthora capsici* could be attributed to the deficiency of IGSLs and camalexin, thus IGSLs confer resistance against *P. brassicae.* *P. brassicae* disease resistance may be established by the sequential activity of phytoanticipin IGSL and phytoalexin camalexin (Fig – No figure yet.).

***Pseudomonas syringae* pv. *Maculicola* (McCullock) Young, Dye and Wilke:** *Pseudomonas syringae* pv. *maculicola* (PSM) causes bacterial leaf spot in cauliflower, broccoli, brussels sprouts and other Brassicas (Fig. 8; Young, 2010; Sotelo *et al*., 2014). GSLs interactions trigger plant immune response against PSM. Brader *et al*. (2006) showed that *Arabidopsis,* which expresses the sorghum gene *CYP79A1,* endogenous *CYP79A2* gene or benzyl GSL respectively, showed increased resistance towards PSM. Using a series of physiological and genetic tools, Groen *et al*. (2015) showed that PSM enhances the feeding of infected plant parts by the herbivore, *Scaptomyza flava* partly by suppressing anti-herbivore defense mechanisms triggered by ROS burst.

Stahl *et al*. (2016) showed that indol-3-ylmethylamine (I3A) was one of the three major accumulating compounds and is also produced via IGSL breakdown by pathways dependent and independent of the myrosinase *PEN2*. Their report also showed that salicylic acid defense hormone produce I3A at the expense of its precursor indol-3-ylmethylglucosinolate (I3M), and the SAR regulator pipecolic acid primes plants for enhanced PSM-induced activation of distinct branches of indolic metabolism. The report of Jiang *et al*. (2016) suggest the biosynthesis of GSL from tryptophan and aliphatic GSL biosynthesis side chain may be triggered following PSM infection. More so, differential co-expression is a common phenomenon during plant attack (Hsu *et al*., 2015 Gaiteri *et al*., 2014). These findings put together suggest the existence of an effective pathway by which GSLs and their metabolites may be manipulated for formidable defense response to bacterial pathogens such as PSM (Fig. 8).

Some future perspectives: The practical application of GSLs induced pathogen resistance response in Brassica will culminate in enhanced crop yield and preserve biodiversity. In plant breeding, the above techniques may be applied to propagate resistant varieties by exploiting individual and plant part based GSL concentration. Although, the signaling pathways involved in regulating GSL biosynthesis are unknown in some Brassica crops, which merit further investigations to advance our understanding in this regard. According to Xu *et al*. (2016), more omics studies will elucidate how antimicrobial activities of GSL biosynthesis can be linked with the apoptotic stimulation of programmed cell death in major fungal pathogens. No doubt, this would provide insights on the development of a new range of potent fungicides and fungal-based drugs (Shlezinger *et al*., 2011). The GSL biosynthesis product, 4-methylsulfinylbutyl isothiocyanate (sulforaphane) does not only activate defense in naïve tissues but provide protection against virulent isolates (Andersson *et al*., 2015). This suggests that GSL byproducts products are involved in cell-to-cell signaling and are prime bacteriostatic molecules albeit their applications warrant more in-depth studies. Furthermore, the findings of Zhang *et al*. (2016) suggests that directly searching for resistance loci may not be the best approach at improving resistance in Brassica to necrotrophic pathogen, rather it may be necessary to have a broader perspective of the effects of resistance loci.

Conclusion

In future, the measuring of plant response to pathogen using transcriptional approach is likely to be more available, which will permit the analysis of large scale sizable expression data with a view to achieving more robust results. In the meantime, the flourish of transcriptional data allows us to answer specific biological questions in the context of differential co-expression. For instance, the comparative analysis of differential co-expression during plant immune responses to different pathogens is an important topic. Differential co-expression analysis can boost the study of plant immune response-related transcriptomics and provide new insights into deciphering the molecular mechanisms of plant-pathogen interactions (Jiang *et al*., 2016). More qualitative studies has the potential to give further insights into the synergistic effects of ROS and GSL metabolites in view of improving plant immunity (Groen *et al*., 2015; Groen *et al*., 2013; Gloss *et al*., 2014).

In conclusion, studies reported in this review suggest diverse complex perspectives of how aliphatic and IGSLs interact to confer immunity to plants using the model plant, *Arabidopsis thaliana* as well as in some Brassica crops. The trend thus far clearly shows that our view of GSLs have tremendously improved over the years. Despite advances in recent years, much is yet to be known and

understood as to how GSLs and their hydrolysis products interact with other non-toxic plant components and plant parts. It is anticipated that more molecular (especially "-omics") studies will pave way for more effective strategies aimed at developing more resistant, tolerant and high yielding plants. Further applications of these studies in enhancing food security are also needed as the global population is projected to soar in the next twenty years and global issues such as climate change are now receiving a more synergistic and strategic response from several governments. It is anticipated that these considerations will give GSL research a more holistic application in the biotechnology, food, pharmaceutical and biomedical industries.

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