Necrotrophic fungal infection affects indolic glucosinolate metabolism in Brassica rapa

Aghajanzadeh, Tahereh A.; Watanabe, Mutsumi; Tohge, Takayuki; Hawkesford, Malcolm J.; Fernie, Alisdair R.; Hoefgen, Rainer; Elzenga, J. Theo M.; de Kok, Luit J.

Published in:
Acta Physiologiae Plantarum

DOI:
10.1007/s11738-023-03546-3

Publication date:
2023

Acta Physiologiae Plantarum (2023) 45:64
https://doi.org/10.1007/s11738-023-03546-3

ORIGINAL ARTICLE

Necrotrophic fungal infection affects indolic glucosinolate metabolism in *Brassica rapa*

Tahereh A. Aghajanzadeh[1] · Mutsumi Watanabe[2,4] · Takayuki Tohge[2,4] · Malcolm J. Hawkesford[3] · Alisdair R. Fernie[4] · Rainer Hoefgen[4] · J. Theo M. Elzenga[5] · Luit J. De Kok[5]

Received: 25 August 2020 / Revised: 8 February 2023 / Accepted: 16 March 2023 / Published online: 22 March 2023

Abstract

Brassica species contains sulfur-containing secondary compounds including glucosinolates which might protect plants from pathogens. In the present investigation, the first leaves of *Brassica rapa* were grown in different situations such as sulfate-sufficient and deprived conditions, and infected with two types of fungi namely, *Alternaria brassicicola* and *Botrytis cinerea* as the specialist Brassica pathogen and generalist pathogen, respectively. The glucosinolates level was locally increased mainly due to indolic glucosinolates when the plant was infected with both fungi. This increase was in line with the increase in the expression of the genes including *CYP79B2*, *CYP79B3*, and *CYP83B1* which are responsible for the biosynthesis of indolic glucosinolates and their regulation (*MYB34* and *MYB51*). However, the locally induced indolic glucosinolates in plants infected with *A. brassicicola* were substantially higher than those of the plants infected with *B. cinerea.* The expression of the genes responsible for the biosynthesis of indolic glucosinolates was increased by infection of plant with *A. brassicicola*. The increase in the content of indolic glucosinolate occurred in the second leaf and roots, demonstrating a systemic response to fungal infection. Upon infection of plants with fungi, the content of both glucosinolates was reduced, while the expression of the most genes responsible for the biosynthesis of indolic glucosinolates was enhanced in plants infected with *A. brassicicola.* This may indicate that indolic glucosinolates are important in response to necrotrophic fungi in Brassica.

Keywords *Alternaria brassicicola* · *Botrytis cinerea* · *Brassica rapa* · Glucosinolate metabolism · Necrotrophic fungi · Sulfur metabolism

Introduction

Fungi cause disease in many crops leading to significant economic losses (Jimenez-reyes et al. 2019). Based on the different lifestyles, plant pathogenic fungi can be divided into biotrophs and necrotrophs (Brader et al. 2001; Tierens et al. 2001; Glazebrook 2005; Pandaranayaka et al. 2019). Necrotrophic pathogenic fungi grow in the dead tissues of the host and they can be classified into host-specific and broad-host-range species (Horbach et al. 2011; Faris and Friesen 2020). *Botrytis cinerea*, a broad-host-range fungus, infects a lot of crop plants and leads to huge losses worldwide (Wang et al. 2014). *Alternaria brassicicola*, a Brassica-specific necrotrophic fungus, causes a foliar disease in many crucifer species (Meur et al. 2015).

Replacing fungicides with compounds that have low environmental risks has been of great interest in recent years. Sulfur-containing compounds such as cysteine, sulfur-rich proteins (defensin and thionins), glutathione,

Communicated by E. Kuzniak-Gebarowska.

✉ Tahereh A. Aghajanzadeh
t.aghajanzadeh@umz.ac.ir

1 Department of Plant Sciences, Faculty of Science, University of Mazandaran, Babolsar, Iran

2 Graduate School of Biological Science, Nara Institute of Science and Technology (NAIST), Ikoma, Japan

3 Plant Sciences Department, Rothamsted Research, Harpenden, UK

4 Department of Molecular Plant Physiology, Max Planck Institute of Molecular Plant Physiology, Potsdam, Germany

5 Laboratory of Plant Physiology, Groningen Institute for Evolutionary Life Sciences, University of Groningen, Groningen, The Netherlands

phytoalexins, and glucosinolates are involved in the increase in resistance of plants against fungal pathogens (Bloem et al. 2005; Künstler et al. 2020; Nakai and Maruyama-Nakashita 2020; Zechmann 2020). Glucosinolates classify into three classes including aliphatic group, derived from alanine, leucine, isoleucine, methionine or valine, and the aromatic group, derived from tyrosine or phenylalanine, and the indolic group, derived from tryptophan (Seo and Kim 2017; Sikorska-Zimny and Beneduce 2021). Furthermore, the most abundant glucosinolates in *Brassica* are methionine-derived aliphatic glucosinolates (Mithen et al. 2003; Kumar et al. 2019; Gohain et al. 2021).

The glucosinolates breakdown products e.g. isothiocyanate, thiocyanate, nitrile, epithionitrile, and oxazolidinethione can occur only if tissue is damaged in the hydrolysis reaction with myrosinase (Bones and Rossiter 1996; Chhajed et al. 2020). It seems that the breakdown products of plant glucosinolates partially control the infection of plants by necrotrophic fungi (Brader et al. 2001; Tierens et al. 2001; Kliebenstein 2004). In addition, the purified glucosinolate breakdown products showed an inhibitory impact on the growth of necrotrophic fungi species (Troncoso et al. 2005; Troncoso-Rojas et al. 2005; Báez-Flores et al. 2011; Chen et al. 2020). In addition to the level of the plant tissues' glucosinolate, the composition of glucosinolate could be important in plant resistance to fungal pathogens (Stotz et al. 2011).

An increase in indolic glucosinolates has been shown in plants upon biotic and abiotic stresses. The level of indolic glucosinolates is affected by different abiotic stresses such as temperature, salinity, heavy metals, and also fertilizer applications (Park et al. 2018; Aghajanzadeh et al. 2018, 2020a, b; Ljubej et al. 2021). In addition, an increase in indolic glucosinolates and their breakdown products have been observed in plants exposed to biotic stress like fungal diseases such as Agerbirk et al. (2009) *B. cinerea, Plectosphaerella cucumerina* and *Phytophthora brassicae* (Sanchez-Vallet et al. 2010; Schlaeppi et al. 2010; Buxdorf et al. 2013). It has been also indicated that specialists' pathogens for Brassicaceae may have adapted to the presence of glucosinolates or evolved mechanisms to limit the toxicity of glucosinolate breakdown products (Bednarek et al. 2009; Buxdorf et al. 2013; Nambiar et al. 2021).

In the present study, the significance of glucosinolate in the *Brassica rapa* against necrotrophic fungal infection at different sulfate conditions (sulfate-sufficient and sulfate-deprived) was studied. In addition, the impact of fungal infection on the expression of the genes which are responsible for the biosynthesis of glucosinolates and their corresponding MYB transcription factors were investigated.

Materials and methods

Fungal growth

Two fungi entitled *A. brassicicola* strain CBS 125,088 and *B. cinerea* strains CBS 124.58 were prepared from the Fungal Biodiversity Centre (CBS) in the Netherlands. Fungal suspensions (200 µl) were spilled to a specific medium in Petri dishes. *A. brassicicola* was grown on oatmeal agar. Oatmeal flour (30 g) and agar (20 g) were mixed with distilled water (1 l). Then it was autoclaved at 121 °C for 20 min. Then, the culture medium was distributed into sterile Petri dishes (Bello and Epstein 2013; Su'udi et al. 2013). Hay medium agar was used to grow *B. cinerea*. Finely hashed hay was added to distilled water (50 g l^{-1}) and boiled for 10 min and subsequently filtered through Whatman paper. Then agar was added (20 g l^{-1}) and autoclaved for 20 min. Finally, the culture medium was distributed into sterile Petri dishes. Both strains were incubated for two weeks. The temperature during the day (14 h) and night (10 h) was 21 and 18 °C (± 1 °C), respectively (Kiran et al. 2018). Before infection, the spores were prepared in sterile water and counted in a hemocytometer.

Plant growth condition

Seeds of *B. rapa* cv. Komatsuna were prepared from Van der Wal, Hoogeveen, The Netherlands. The seeds germinated in the same situation as the fungi growth condition, see above). The seedlings (ten-day-old) were grown for 5 days in a nutrient solution with 0.5 mM sulfate. Then the seedlings were transferred to a fresh nutrient solution without sulfate (−S) or 0.5 mM sulfate (+ S) in 30 l containers (30 plants in each container). One day after the plant got used to the hydroponic environment (at this stage the plants had two leaves), the first leaf was treated with two droplets of the conidial suspension and that of the control plant with distilled water. Two drops of conidial suspension were placed on the upper surface of the first leaf on the left and right side of the middle leaf vein to create a uniform and effective distribution of contamination in the leaf. Each 5 µl droplet contained 10^8 spore ml^{-1} (Macioszek et al. 2018). Three days after infection, the plants were harvested and the first and second leaves and the roots were separated, weighed, and kept in a freezer at − 80 °C. Leaf area and lesion area size were measured using an Epson Desktop Scanner and the WinRhizo software (Regents Instruments, Quebec, Canada).

Glucosinolate content

The glucosinolate content and composition were measured by the liquid chromatography-electrospray ionization-mass spectrometry (LC/ESI–MS) method (Giavalisco et al. 2011; Krueger et al. 2011; Tohge et al. 2016). The frozen grounds samples (100 mg) were extracted by methanol (300 µl) and chloroform (200 µl) solutions and centrifuged at 14,000 rpm for 10 min. The pellet was solved in methanol (10 µl of 80%) containing isovitexin (5 µg ml^{-1}) on ice subsequently sonicated and followed by centrifugation for 15 min at 14,000 rpm at 4 °C (Tohge and Fernie 2010). 3 µl of the supernatant was injected for LC/ESI–MS analysis. Furthermore, the following UPLC gradient profile was used: after 1 min of isocratic run at 99% A, a linear 12 min gradient was applied to 65% A. Immediately followed a 1.5 min gradient to 30% A, before a 1 min gradient to 1% A. Then, a 1.5 min isocratic period at 1% A followed, before switching back to 99% A to re-equilibrate the column for 2.5 min, before the next sample could be injected. LC/ESI–MS was comprised of an ultra-performance liquid chromatography (UPLC) system and a mass spectrometer system equipped with an electrospray ionization interface. Liquid chromatographic separation was carried out using HSS T3 C18 reversed-phase column (100 mm × 2.1 mm × 1.8 µm particles), buffer A (a mix of water and formic acid at a final concentration of 0.1% formic acid v/v) and buffer B (a mix of acetonitrile and formic acid at a final concentration of 0.1% formic acid v/v) with flow rate 400 µl min^{-1}. The subsequent mass spectrometric analysis was performed in positive or negative ionization mode. The capillary temperature was 250 °C; the spray voltage was 3 kV with a mass range of 100–1500 *m*/*z*. The contents of the different glucosinolates were calculated with sinigrin as a standard (Aghajanzadeh 2015).

RNA extraction

The total RNA of freeze-dried samples (leaves and root) was extracted using a hot (80 °C) phenol/ Tris–HCl extraction buffer. The extraction was continued using chloroform and isoamyl alcohol. Then, the total RNA of the samples was precipitated by Lithium Chloride (Verwoerd et al. 1989). To remove the genomic DNA contamination, DNAse treatment (Promega, Cat # M6101, USA) was applied. The RNA quality was determined by Thermo NanoDrop 2000 and its integrity was detected by electrophoresis gel.

Real-time quantitative PCR

The synthesis of DNA was done using 1 µg of RNA via a cDNA synthesis kit (Promega, USA, GoScript™ Reverse Transcriptase). The primers of the genes responsible for the biosynthesis of glucosinolates and their regulation (MYB transcriptional factors), were designed using CDS of *A. thaliana* genes to query homologous *B. rapa* sequences. The gene of *B. rapa* actin 2 was used as a reference gene. The genome sequence of *B. rapa* is available in the portal http://www.brassica-rapa.org (Aghajanzadeh 2015). The full-length sequences of these genes are shown by the accession number listed in Table 1 along with gene-specific primer sets (Table 1).

SYBR Green master mix kit (Thermo Scientific) was applied to perform RT-PCR on Applied BioSystems' 7300 real-time PCR system. The transcript level of the actin and target gene was measured using the comparative Ct method. Analysis of qPCR data was performed using three biological replicates from separate plant tissue with two technical replicates (Aghajanzadeh 2015).

Statistical analysis

Data on lesion area, glucosinolate content, and relative expression represent the mean of four biological replicates (3 plants in each replicate), four biological replicates (18 plants in each replicate) and three biological replicates with two technical replicates for each (± SD), respectively. To perform the statistical analyses, SPSS software 20 for Windows 7 was used. Based on the results of the heterogeneity test, a one-way analysis of variance (ANOVA or Welch) was used. Tukey's HSD all-pairwise comparisons at the level of $p \leq 0.01$ as a post-hoc test was performed to compare the treatment means. In addition, GraphPad Prism Software (San Diego, CA, USA) was used for making graphs.

Results

lesion area size of sulfate-sufficient and sulfate-deprived plants infected with *A. brassicicola* and *B. cinerea*

The leaves of *B. rapa* developed visible lesion formation at the site of fungi spore application at the same time after inoculation of the plant with both fungi. However, the rate of lesion formation was faster by *A. brassicicola* than *B. cinerea*. The lesion formation appeared to develop after 48 h and spread more on the leaves of *B. rapa* within 72 h by both fungi. The infection of the first leaf by *A. brassicicola* resulted in a threefold greater lesion area of the leaves of *B. rapa* than that of *B. cinerea* (Fig. 1). In sulfate- deprived condition there was a higher lesion area size on the first leaf (locally induced) with both *A. brassicicola* (1.3-fold increase) and *B. cinerea* (1.9-fold increase) infections compare to relevant sulfur sufficient plants (Fig. 1).

Table 1 The primers and accession number of full-length sequences of the genes responsible for the biosynthesis of glucosinolates and their regulation (MYB transcription factors)

Gene	Primer sequences (5′–3′)	Accession number of full-length sequences
MAM1/MAM3	F- TCAAAGCMAACACTCCTG* R-CCACTTCTTTCRCCTATTCC*	Bra029355, Bra018524, Bra029356, Bra021947, Bra013009, and Bra013011
CYP79F1/ CYP79F1	F-CTCCTGGACCACCAGGAT** R-CTCGAGCGATCTCGTCAG**	AT1G16410 and Bra026058
CYP83A1	F-GGATGGGCCAAAAAATACGG R-TGCGTCTTGAGAAGCTCTTT	AT4G13770 and Bra032734
MYB28	F-GCATCTAGTTCCGACAARCR R-RGKGTTGAAACCGGAGG	AT5G61420 and Bra029311
MYB29	F-GACTCAAACCCGAGTAACC R-GGAGTTAAAGGAACCATAGTTTCT	AT5G07690 and Bra005949
CYP79B2	F-GGCTCCACAGCATCATGAA R-GAGCGTCTTGTTGCTTGAGT	AT4G39950 and Bra011821
CYP79B3	F-ATGCTTACGGGATTGGATCTAAAC R-GGTTTGATTTCATCAGCGGTAA	AT2G22330 and Bra030246
CYP83B1	F-CGAACCAGTCATCCCAATTCTT R-CGGCTGTGTCACGAGAAA	AT4G31500 and Bra034941
MYB34	F-GATCCAACCACTCACAAACC R-GCGATGWTTGTGGAGTTTC	AT5G60890, Bra035954, Bra029350, Bra029349, and Bra013000
MYB51	F-CAAGTGTCACCGTTGACTC R-GAG RCGACGTAGCGTTA	AT1G18570, Bra016553 and Bra031035
Actin	F-AGCAGCATGAAGATCAAGGT R-GCTGAGGGATGCAAGGATAG	ATNM112764.3 and JN120480.1

F forward, *R* reverse

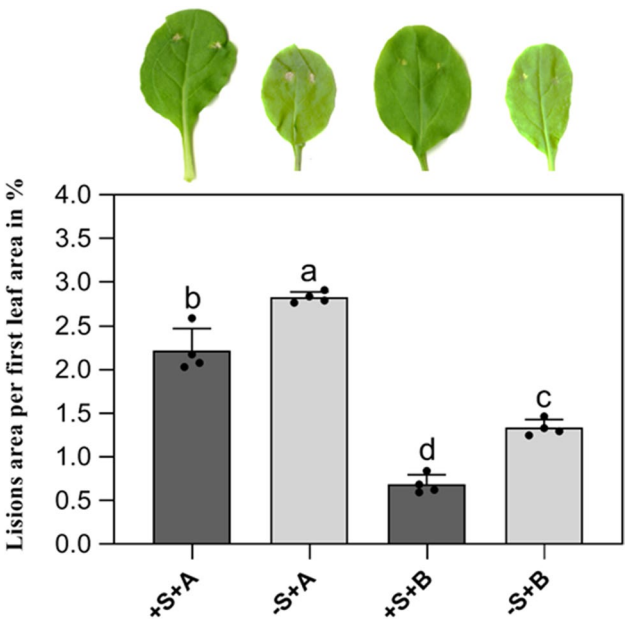

Fig. 1 Lesion area size of the first leaf of *B. rapa* infected with *A. brassicicola* (+A) and *B. cinerea* (+B) at sulfate-sufficient (+S) and sulfate-deprived (−S) conditions. The sulfate-sufficient and sulfate-deprived conditions are presented in dark and light gray bars, respectively. Different letters indicate significant differences between treatments ($P \leq 0.01$).

Content and composition of glucosinolates in plants grown in sulfate-sufficient and deprived conditions

The content of glucosinolate in the roots is higher than that of the first and second leaves (Fig. 2). The glucosinolate content of the first leaf was 50% lower than that of the second leaf (Fig. 2). In the first and the second leaves, gluconapin (3-butenyl glucosinolate; for all chemical names of the glucosinolates see Table 2) and glucobrassicanapin were the major aliphatic glucosinolates and glucobrassicin was the major indolic glucosinolate (Fig. 2). The total aliphatic glucosinolates pool in both the first and second leaves almost entirely made up of short-chain aliphatic glucosinolates (glucoerucin, gluconapin, and glucobrassicanapin). In the roots, the level of short-chain aliphatic glucosinolates was almost twofold higher than that of the long-chain glucosinolates (glucoberteroin and glucolesquerellin; Fig. 2). Furthermore, in roots glucoerucin and glucoberteroin were the most important aliphatic glucosinolates and 4-methoxyglucobrassicin was the major indolic glucosinolate.

Sulfate deprivation strongly decreased the glucosinolate content of the roots and the first and the second leaves, with the second leaf being more affected than the first (Fig. 2). The levels of aliphatic glucosinolates were reduced more than those of indolic glucosinolates. A 4-day sulfate deprivation resulted in decreases of aliphatic glucosinolates by 75,

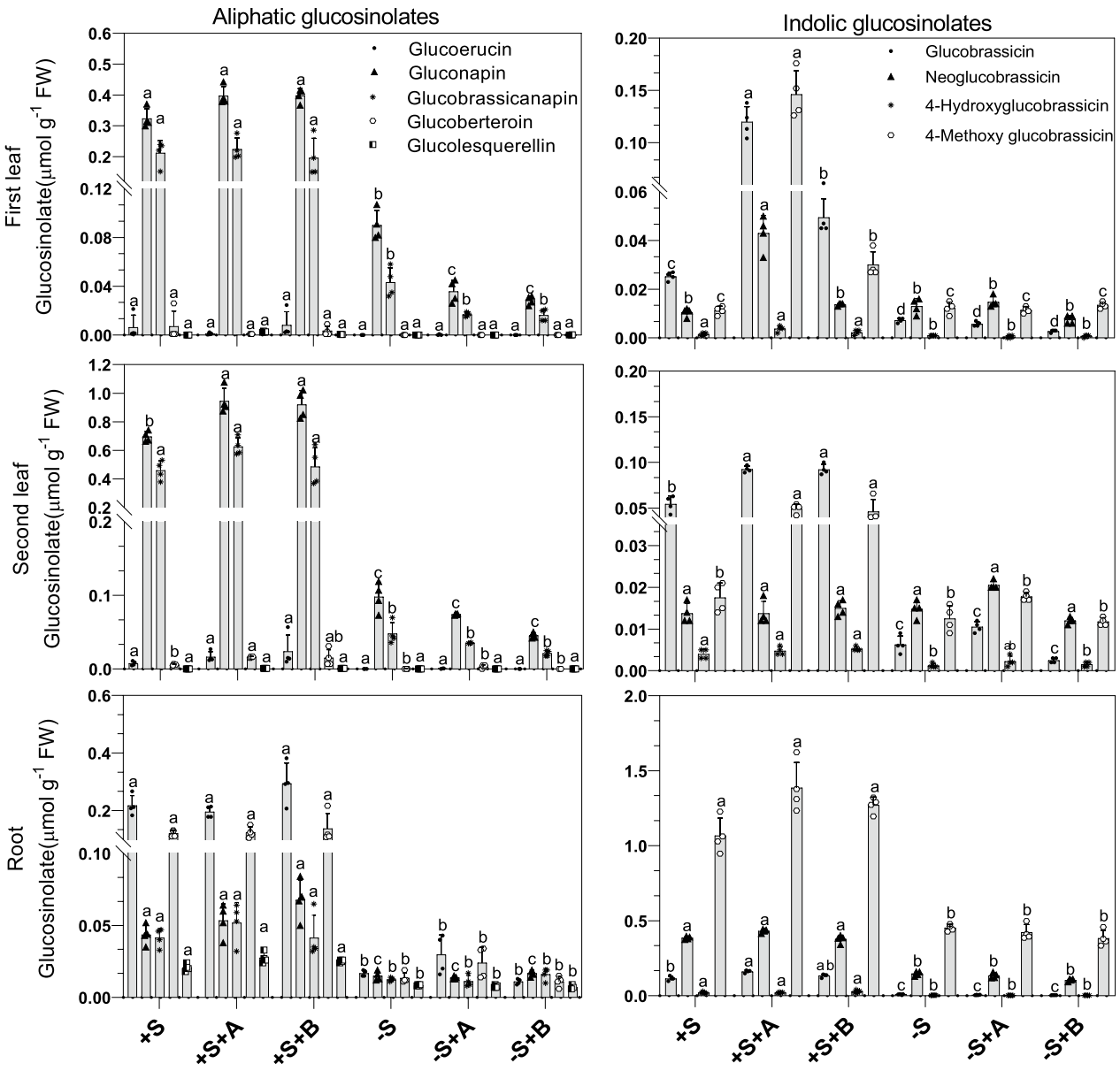

Fig. 2 The content and composition of aliphatic and indolic glucosinolates in the different organs of sulfate-sufficient (+S) and sulfate-deprived (−S) *B. rapa* infected with *A. brassicicola* (+A) and *B. cinerea* (+B). Different letters indicate significant differences between treatments ($P \leq 0.01$).

88, and 84%, in the first leaf, the second leaf, and the roots, respectively, while the indolic glucosinolate contents were reduced by 32, 64, and 61%, respectively (Fig. 2).

Content and composition of glucosinolates in fungal-infected sulfate-sufficient plants

The infection of sulfate-sufficient *B. rapa* with both fungi hardly affected the content of aliphatic glucosinolates in the first leaf (Fig. 2). In the second leaf, infection of plants with *A. brassicicola* and *B. cinerea* resulted in 33 and 17% increases in total aliphatic glucosinolate, respectively (Fig. 2). The biomasses of the first leaf, second leaf, and roots were not affected by the infection (Table 3), which showed that the increase in the content of aliphatic glucosinolate could not be attributed to growth dilution. An increase in the content of gluconapin, a short-chain aliphatic glucosinolate, was responsible for the change in aliphatic glucosinolate content of the second leaves (Fig. 2). In the first leaf and roots, however, the content of aliphatic glucosinolate was hardly changed after infection of the plants with either fungus (Fig. 2).

However, the level of indolic glucosinolates was significantly increased in plant tissues upon infection of

Table 2 Nomenclature of the individual glucosinolates (GSL) identified in *B. rapa*

GSL type	Trivial name	Chemical name
Aliphatic		
	Glucoerucin	4-4-Methylthiobutyl GSL
	Gluconapin	3-Butenyl GSL
	Glucobrassicanapin	4-Pentenyl GSL
	Glucoberteroin	5-Methylthiopentyl GSL
	Glucolesquerellin	6-Methylthiohexyl GSL
Indolic		
	Glucobrassicin	Indol-3-ylmethyl GSL
	Neoglucobrassicin	1-Methoxy-indol-3-ylmethyl GSL
	4-Hydroxy glucobrassicin	4-Hydroxy-indol-3-ylmethyl GSL
	4-Methoxyglucobrassicin	4-Methoxy-indol-3-ylmethyl GSL

sulfate-sufficient *B. rapa* with either fungus (Fig. 2). The infection of plants with *A. brassicicola* and *B. cinerea* resulted in 6.5 and 2-fold increases in the total indolic glucosinolate content of the first leaf, respectively (Fig. 2). Likewise, infection of *Brassica rapa* with either *A. brassicicola* or *B. cinerea* led to a 1.7-fold increase of total indolic glucosinolate content of the second leaf (Fig. 2). However, infection of plants with either fungus only resulted in a slight increase in the indolic glucosinolate content of the roots (Fig. 2). The increase in the content of indolic glucosinolate was, for the greater part, due to increased levels of 4-methoxy glucobrassicin and somewhat less to glucobrassicin in both leaves of the infected plants and to a lesser extent in the roots, which indicates a systemic response of the plant to fungal infection (Fig. 2).

Content and composition of glucosinolates in fungal-infected sulfate-deprived plants

When sulfate-deprived *B. rapa* was infected with *A. brassicicola* and *B. cinereal*, the content of aliphatic glucosinolate of the first leaves was reduced by 60 and 66%, respectively (Fig. 2). The composition of glucosinolates showed that the reduction of aliphatic glucosinolate content in the first was mostly due to a decrease in the content of gluconapin and glucobrassicanapin (Fig. 2). In the second leaf and roots, however, the aliphatic glucosinolate content was hardly reduced after infection of the sulfate-deprived plants with both fungi (Fig. 2).

Infection of sulfate-deprived plants with both fungi hardly affected the indolic glucosinolate content of the first leaf, second leaf, and roots (Fig. 2).

Expression of the genes involved in the biosynthesis of glucosinolate

The expression of the genes responsible for the biosynthesis of the core structure (*CYP79F* and *CYP83A1*) and side chain (*MAM*) of the aliphatic glucosinolates (Wittstock and Halkier 2002), either in leaves or roots was significantly reduced in plants exposed to sulfate deprived condition (Fig. 3). The expression levels of these genes were hardly affected upon infection of plants with either *A. brassicicola* or *B. cinerea in* sulfate sufficient and/or deprived conditions. However, the transcript level of *MAM* was enhanced in the second leaves of the sulfate-sufficient plants infected with *A. brassicicola* (Fig. 3).

The genes expression of the enzymes involves in the biosynthesis of glucosinolates (indolic and aromatic) including *CYP79B2, CYP79B3,* and *CYP83B1* (Wittstock and Halkier 2002), in sulfate-deprived plants was also significant in the leaves and slightly in the roots reduced (Fig. 4). The expression of these genes in the first leaf of sulfate-sufficient plants was increased in plants infected with *A. brassicicola* and with a lower level with *B. cinerea* (Fig. 4). The expression of genes including *CYP79B2, CYP79B3,* and *CYP83B1* in the first leaf of sulfate-sufficient plants infected with *A. brassicicola* was increased 11.5-, 6.5- and 5.5-fold, respectively. While the expression of these genes was enhanced twofold in sulfate-sufficient plants infected with *B. cinerea.* In the second leaf, the transcript level of *CYP83B1* was significantly increased in sulfate-sufficient plants upon fungal infection while that of the genes such as *CYP79B2* and *CYP79B3* was hardly changed. (Fig. 4). The expression of these genes was hardly changed in the roots of sulfate-sufficient plants upon infection with *B. cinerea* (Fig. 4).

The transcript level of *CYP79B2, CYP79B3* and *CYP83B1* in the sulfate-deprived plants, was remarkably increased in the first leaf of *A. brassicicola* infected plants

Table 3 Impact of *A. brassicicola* (+A) and *B. cinerea* (+B) infection on the biomass (g FW) of different plant organs of sulfate-sufficient (+S) and sulfate-deprived *B. rapa* (−S)

Biomass (g FW)	+S	+S+A	+S+B	−S	−S+A	−S+B
First leaf	0.35 ± 0.01a	0.33 ± 0.02a	0.34 ± 0.03a	0.21 ± 0.01b	0.19 ± 0.02b	0.21 ± 0.01b
Second leaf	0.45 ± 0.02a	0.45 ± 0.012a	0.44 ± 0.02a	0.23 ± 0.02b	0.22 ± 0.03b	0.26 ± 0.02b
Root	0.27 ± 0.01a	0.26 ± 0.023a	0.26 ± 0.02a	0.22 ± 0.03a	0.24 ± 0.04a	0.25 ± 0.02a

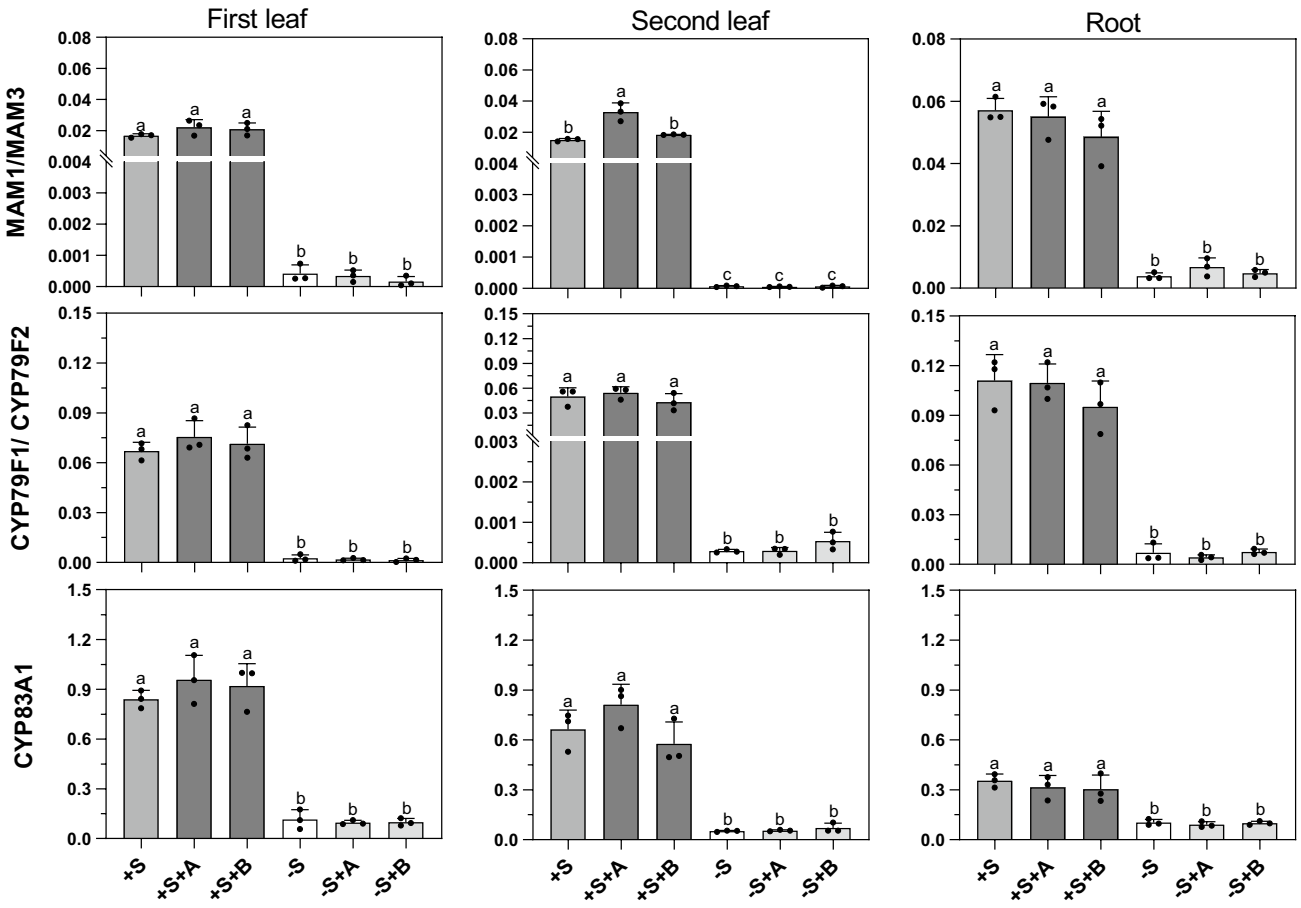

Fig. 3 Transcript levels of *MAM*, *CYP79F* and *CYP83A1* in the different organs of sulfate-sufficient (+S) and sulfate-deprived (−S) *B. rapa* infected with *A. brassicicola* (+A) and *B. cinerea* (+B). Different letters indicate significant differences between treatments ($P \leq 0.01$).

by 34-, 25- and 18-fold respectively but not in the second leaf and in roots. While transcript levels of these genes were hardly changed in the leaves and roots of infected plants with *B. cinerea* under sulfate-deprived conditions (Fig. 4).

Expression of MYB transcription factors involved in the regulation of the biosynthesis of glucosinolate

The expression of the genes including *MYB28* and *MYB29* involved in the regulation of aliphatic glucosinolates biosynthesis (Wittstock and Halkier 2002), were decreased by 50% and 80% in the first leaves, and 50% and 95% in the second leaves of sulfate deprived plants, respectively (Fig. 5). While in the roots, the gene expression of *MYB28* was enhanced fourfold but that of the *MYB29* was hardly changed in the sulfate-deprived plants (Fig. 5). The gene expression of *MYB28* and *MYB29* was hardly changed in the plant tissues upon infection with both fungi either in sulfate-sufficient or deprived plants (Fig. 5).

The gene expression of *MYB34* and *MYB51* involved in the regulation of indolic glucosinolates biosynthesis (Wittstock and Halkier 2002), were hardly affected in all tissues of plants under the sulfate-deprived condition (Fig. 6). There were substantial increases in transcript levels of *MYB34* and *MYB51* by 3- and 14-fold in the first leaves of plant infected with *A. brassicicola* under sulfate sufficient condition, respectively (Fig. 6). The transcript level of *MYB34* was increased threefold in the first leaf of sulfate-sufficient plants upon infection with *B. cinerea* while that of the *MYB51* remained unaffected (Fig. 6). The transcript level of *MYB34* was increased threefold in the second leaves of sulfate-sufficient plants upon infection with *A. brassicicola* or *B. cinerea*, whereas that of the *MYB51* remained unaffected (Fig. 6). The gene expression of *MYB34* and *MYB51* was remained unaffected in the roots of plant infected with *A. brassicicola* or *B. cinerea* under sulfate-sufficient condition (Fig. 6). The transcript levels of *MYB34* and *MYB51* were hardly affected upon infection of sulfate-deprived plants with *A. brassicicola* or *B. cinereal*. However, the gene expression of *MYB51* was enhanced 25-fold in the first

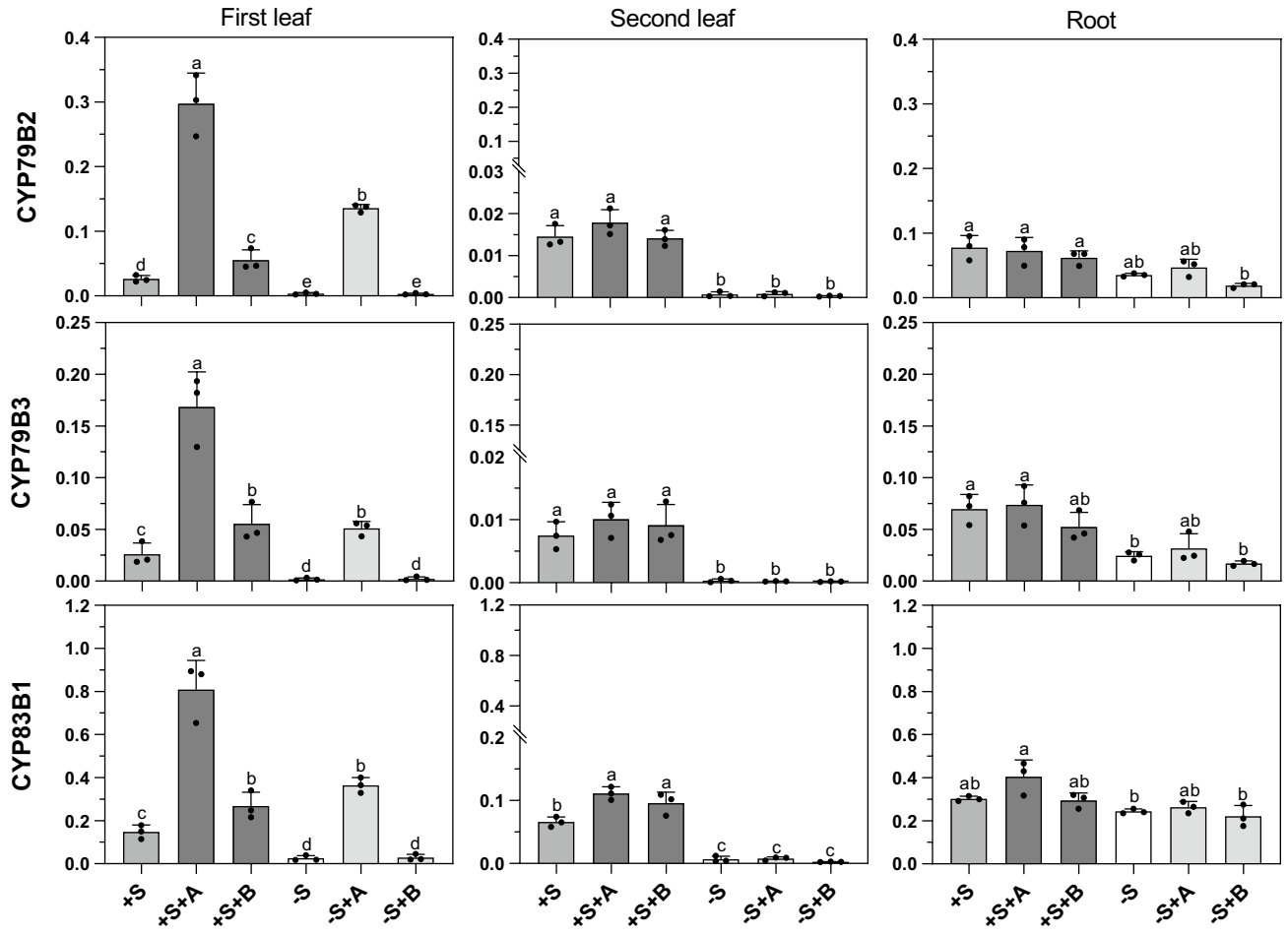

Fig. 4 Transcript levels of *CYP79B2*, *CYP79B3* and *CYP83B1* in the different organs of sulfate-sufficient (+S) and sulfate-deprived (−S) *B. rapa* infected with *A. brassicicola* (+A) and *B. cinerea* (+B). Different letters indicate significant differences between treatments ($P \leq 0.01$).

leaves of plants infected with *A. brassicicola* under sulfate deprived condition (Fig. 6).

Discussion

In the current study, the significance of glucosinolates in response to fungal infection of *B. rapa* with the necrotrophic fungi *B. cinerea*, a broad-spectrum pathogen, and *A. brassicicola*, a Brassica specialist pathogen, was studied. It is assumed that specialist pathogens are able to develop mechanisms to overcome plant defense responses and one might expect that *A. brassicicola* would already have been adapted to the presence of high levels of glucosinolates (Aghajanzadeh 2015). Pathogens may have mechanisms for detoxification of glucosinolates, involving conversion to less toxic derivatives (VanEtten et al. 1989; Pedras et al. 2002, 2009; Pedras and Yaya 2010; Pedras and Hossain 2011; Chen et al. 2020; Sun et al. 2020). The current study displayed strong responsiveness of the indolic glucosinolates to infection with both specialist and generalist necrotrophic pathogens. This may suggest that indolic glucosinolates, in general, contribute to a greater extent to plant resistance to fungi than aliphatic glucosinolates. This is corroborated by previous studies that suggested that indolic glucosinolates fulfill a defensive function in *B. napus* cultivars in response to *Alternaria brassicae* (Doughty et al. 1991) and infection of different cultivars of *B. napus* with *S. sclerotiorum* resulted in increased contents of both indolic and aromatic glucosinolates (Li et al. 1999). Furthermore, the Brassica specialist *A. brassicicola* was more effective in the infection of *B. rapa* than the non-specialist *B. cinerea*, indicating that infection by *A. brassicicola* was hardly hindered by the potentially toxic effects of glucosinolates breakdown products by either detoxifying or even metabolizing them (Buxdorf et al. 2013; Calmes et al. 2015; N'Guyen et al. 2021).

Furthermore, the observed induction of indolic glucosinolates in the second leaf of *B. rapa* upon both necrotrophic fungi infections could be additional supportive evidence for the antifungal properties of glucosinolates and systemic

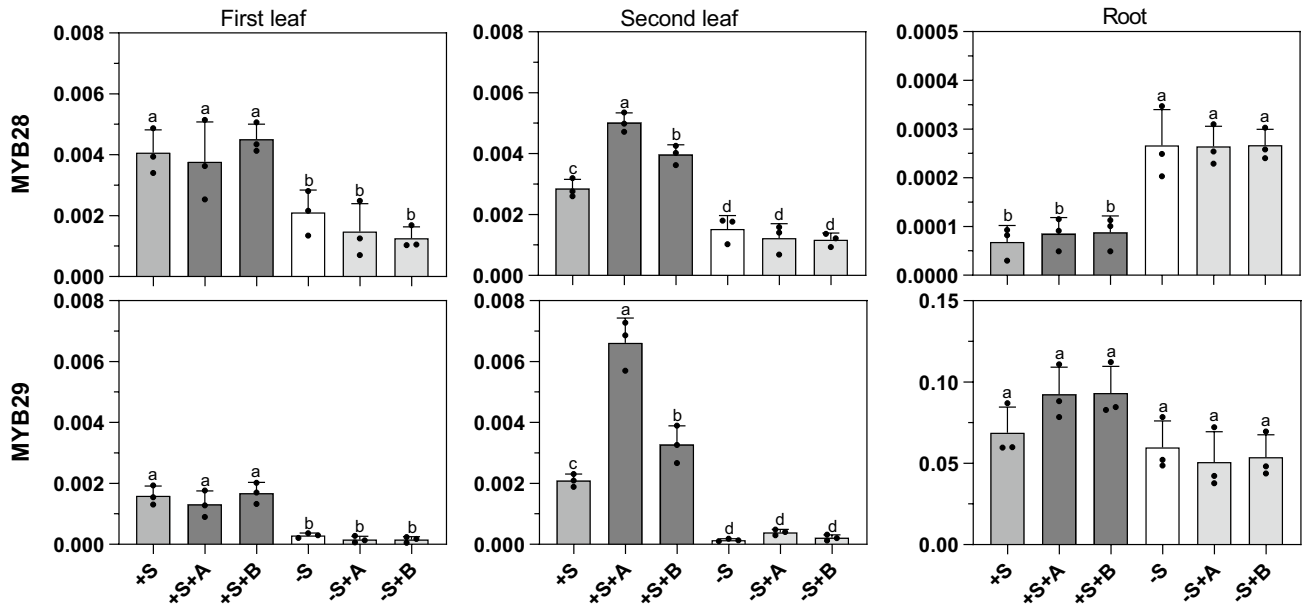

Fig. 5 Transcript levels of *MYB28* and *MYB29* in the different organs of sulfate-sufficient (+S) and sulfate-deprived (−S) *B. rapa* infected with *A. brassicicola* (+A) and *B. cinerea* (+B). Different letters indicate significant differences between treatments ($P \leq 0.01$)

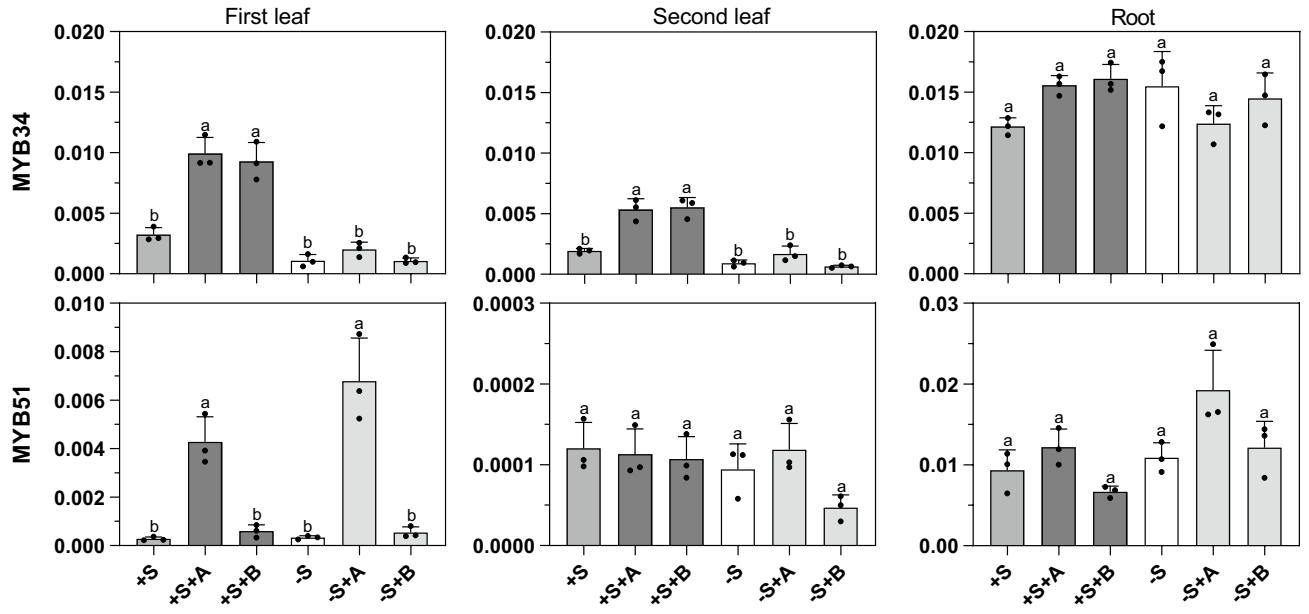

Fig. 6 Transcript levels of *MYB28* and *MYB29* in the different organs of sulfate-sufficient (+S) and sulfate-deprived (−S) *B. rapa* infected with *A. brassicicola* (+A) and *B. cinerea* (+B). Different letters indicate significant differences between treatments ($P \leq 0.01$)

resistance as well. Infection of *B. rapa* with *Leptosphaeria maculans* as well as *S. sclerotiorum* also resulted in a systemic accumulation of indolic glucosinolates (Li et al. 1999; Abdel-Farid et al. 2010). In addition, 4-methoxy-indol-3-ylmethyl was induced at a much higher level than the other indolic glucosinolates upon exposure to both necrotrophic fungi which might indicate especially high antifungal effects of breakdown products of 4-methoxy-indol-3-ylmethyl (Bednarek et al. 2009; Clay et al. 2009). It has been observed in *A. thaliana*, that 4-Methoxyglucobrassicin is being degraded upon fungal attack to an intermediate compound (4-methoxylated indole dithiocarbamate), which is formed from the isothiocyanate and glutathione. This intermediate compound is presumed to be toxic to fungal

pathogens (Bednarek et al. 2009). The current study showed that the roots of *B. rapa* have a relatively high proportion of glucosinolates synthesis or accumulation and especially indolic glucosinolates. However, a very low increase of indolic glucosinolates in the roots of sulfur-sufficient *B. rapa* upon exposure to *A. brassicicola* may display lower importance of the roots in systemic resistance.

The indolic glucosinolates biosynthesis upon infection of *B. rapa* with both necrotrophic fungi was promoted by up-regulation of the transcription factors *MYB34* and *MYB51* regulating the pathway of their target genes involved in the biosynthesis of the indolic glucosinolates, of which we showed induction of *CYP79B2, CYP79B3,* and *CYP83B1*. Transcript levels of the genes including *MAM*, *CYP79F*, and *CYP83A1* and their regulatory transcription factors including *MYB28* and *MYB29* were hardly changed with both *A. brassicicola* and *B. cinerea* in the first leaf and roots which coincides with the fact, that also the level of aliphatic glucosinolates was not altered. However, transcript levels of *MAM, MYB28* and *MYB29* in the second leaf, were increased upon exposure to both *A. brassicicola* and *B. cinerea* which was in concordance with the increase in the content of gluconapin. The latter observation might suggest a systemic response to the infections. The involvement of *MYB28* and *MYB29* has been observed in the systemic defense against the necrotrophic fungus *Alternaria brassicae* (Nongbri 2013).

Sulfur deficiency led to a decrease in the transcript level of the genes responsible for the biosynthesis of glucosinolates and their regulation which subsequently resulted in a decrease in the biosynthesis of both aliphatic and indolic glucosinolates in *B. rapa* leaves. However, the transcript level of *MYB28* in the roots of sulfate-deprived *B. rapa* was significantly increased. The response of different MYB transcription factors at sulfur deficiency can be due to the activation of different signaling components which act upstream of MYBs (Frerigmann and Gigolashvili 2014). It was also indicated in Arabidopsis plants that the low levels of glucosinolates may act as a signal to promote their synthesis (Smolen and Bender 2002; Mugford et al. 2009). It was proposed MYB transcription factors regulate each other and an increase in the transcription level of MYB28 may result in a decrease in the transcript level of MYB29 and MYB76 (Sønderby et al. 2010; Li et al. 2013).

Sulfate-deprived *B. rapa* was more sensitive to infection with either *A. brassicicola or B. cinerea* compared to the sulfate-sufficient plant. The low content of glucosinolate in sulfate-deprived *B. rapa* is in line with the reduction of the transcript level of the genes involved in the biosynthesis of glucosinolate renders the plant more susceptible to infection which has been shown also in mutant plant approaches where glucosinolates contents were reduced (Buxdorf et al. 2013; Piślewska-Bednarek et al. 2018; Chen et al. 2020). Glucosinolate reduction under sulfate deprivation might be a means to reroute sulfur to primary metabolism (Aarabi et al. 2016). Despite this, an overall substantial up-regulation was observed in genes including CYP79B2, CYP79B3, and CYP83B1 (the gene responsible for the biosynthesis of indolic glucosinolates), as well as the MYB transcription factor, MYB51 in the first leaves of plants infected with *A. brassicicola*. This may support the opinion that *B. rapa* hardly adapted to specialist fungal pathogens and indolic glucosinolates are possibly involved in plant defense against *A. brassicicola.* The up-regulation of indolic glucosinolate biosynthetic genes and relative transcriptional factors have been observed during pathogen attacks (Iven et al. 2012; van de Mortel et al. 2012; Wei et al. 2016). It has been suggested that different MYB transcription factors have specific responses due to the activation of the upstream of the MYB transcription factor via various specific signaling compounds (Frerigmann and Gigolashvili 2014). The glucosinolate is involved in plant defense that is accompanied by different signaling hormones such as jasmonic acid, salicylic acid and ethylene which are important in eliciting plant defense response upon pathogen attack (Brader et al. 2001; Dombrecht et al. 2007; Guo et al. 2013; Augustine and Bisht 2015; Yi et al. 2016). For example, MYB51 is the key major regulator of indolic glucosinolate biosynthesis upon salicylic acid and ethylene signaling (Frerigmann and Gigolashvili 2014). Furthermore, indole-3-acetaldoxime as an intermediate exists in a branching point for the biosynthesis of indolic glucosinolate, as well as the pathways that lead to the biosynthesis of camalexin (Frerigmann et al. 2016). The up-regulation of *MYB51* and genes involved in the biosynthesis of indolic glucosinolates may indicate the contribution of camalexin in defense against fungi pathogens.

Sulfur metabolism is of great significance in plant resistance to fungal pathogens and several sulfur metabolites are presumably involved in the protection of plants against a fungal attack including glutathione, phytoalexins, defensins and thionins, phytoalexins, elemental sulfur, cysteine and glucosinolates including all types of aliphatic, indole and aromatic glucosinolates (Bloem et al. 2005). In the current study, it was obvious that indolic glucosinolates were involved in the local response and both aliphatic and indolic glucosinolates in the systemic response of *Brassica rapa* upon infection to the fungi *A. brassicicola* and *B. cinerea.*

Author contribution statement TAA and LJK designed the research. TAA carried out the experiments and analyzed the data and wrote the manuscript. MW, TT, ARF and RH measured the glucosinolates and commented on the manuscript. LJK, MJH and JTME commented on the manuscript.

Acknowledgements The authors would like to thank Sue Steele, Rothamsted Research for the critical reading of the manuscript and Änne Eckhardt, MPI-MP for the LC-MS measurements. Work at Rothamsted Research is supported via the 20:20 Wheat® Programme (BBS/E/C/00005202) funded by the UK Biotechnology and Biological Science Research Council. Work at the MPI-MP is supported by institutional funds of the Max Planck Society. The paper is part of my Ph.D. thesis which can be accessed via the University of Groningen in the respective repository under weblink (https://pure.rug.nl/ws/portalfiles/portal/25086155/Complete_thesis.pdf).

Data availability Data have been clearly represented in the manuscript in graphs and tables. However, raw data which support this study will be available from the corresponding author upon reasonable request.

References

Aarabi F, Kusajima M, Tohge T, Konishi T, Gigolashvili T, Takamune M, Sasazaki Y, Watanabe M, Nakashita H, Fernie AR, Saito K, Takahashi H, Hubberten HM, Hoefgen R, Maruyama-Nakashita A (2016) Sulfur deficiency-induced repressor proteins optimize glucosinolate biosynthesis in plants. Sci Adv 7:2(10):e1601087. https://doi.org/10.1126/sciadv.1601087

Abdel-Farid IB, Jahangir M, Mustafa NR, Van Dam NM, Van den Hondel CA, Kim HK, Choi YH (2010) Verpoorte R.: glucosinolate profiling of *Brassica rapa* cultivars after infection by *Leptosphaeria maculans* and *Fusarium oxysporum*. Biochem Sys Ecol 38:612–620. https://doi.org/10.1016/j.bse.2010.07.008

Agerbirk N, De Vos M, Kim JH, Jander G (2009) Indole glucosinolate breakdown and its biological effects. Phytochem Rev 8:101–120. https://doi.org/10.1007/s11101-008-9098-0

Aghajanzadeh TA (2015) Sulfur metabolism, glucosinolates and fungal resistance in Brassica. Dissertation, University of Groningen

Aghajanzadeh TA, Reich M, Kopriva S, De Kok LJ (2018) Impact of chloride (NaCl, KCl) and sulphate (Na_2SO_4, K_2SO_4) salinity on glucosinolate metabolism in *Brassica rapa*. J Agron Crop Sci 204:137–146. https://doi.org/10.1111/jac.12243

Aghajanzadeh TA, Dharmendra HP, Burow M (2020a) Copper toxicity affects indolic glucosinolates and gene expression of key enzymes for their biosynthesis in Chinese cabbage. Arch Agron Soil Sci 66:1288–1301. https://doi.org/10.1080/03650340.2019.1666208

Aghajanzadeh TA, Dharmendra HP, Burow M (2020b) Differential partitioning of thiols and glucosinolates between shoot and root in Chinese cabbage upon excess zinc exposure. J Plant Physiol 244:153088. https://doi.org/10.1016/j.jplph.2019.153088

Augustine R, Bisht NC (2015) Biotic elicitors and mechanical damage modulate glucosinolate accumulation by co-ordinated interplay of glucosinolate biosynthesis regulators in polyploid *Brassica juncea*. Phytochemistry 117:43–50. https://doi.org/10.1016/j.phytochem.2015.05.015

Báez-Flores ME, Troncoso-Rojas R, Osuna MAI, Domínguez MR, Pryor B, Tiznado-Hernández ME (2011) Differentially expressed cDNAs in *Alternaria alternata* treated with 2-propenyl isothiocyanate. Microbiol Res 166:566–577. https://doi.org/10.1016/j.micres

Bednarek P, Pi'slewska-Bednarek M, Svatovs A, Schneider B, Doubsk`y J, Mansurova M, Humphry M, Consonni C, Panstruga R, Sanchez-Vallet A, Molina A, Schulze-Lefert P (2009) A glucosinolate metabolism pathway in living plant cells mediates broad-spectrum antifungal defense. Science 323:101–106. https://doi.org/10.1126/science.1163732

Bello M, Epstein L (2013) Clades of γ-glutamyltransferases (GGTs) in the Ascomycota and Heterologous expression of *Colletotrichum graminicola* CgGGT1, a member of the pezizomycotina-only GGT clade. J Microbiol 51:88–99

Bloem E, Haneklaus S, Schnug E (2005) Significance of sulfur compounds in the protection of plants against pests and diseases. J Plant Nutr 28:763–784. https://doi.org/10.1081/PLN-200055532

Bones AM, Rossiter JT (1996) The myrosinase-glucosinolate system, its organisation and biochemistry. Physiol Plant 97:194–208. https://doi.org/10.1111/j.1399-3054

Brader G, Tas E, Palva ET (2001) Jasmonate-dependent induction of indole glucosinolates in Arabidopsis by culture filtrates of the nonspecific pathogen *Erwinia carotovora*. Plant Physiol 126:849–860

Buxdorf K, Yaffe H, Barda O, Levy M (2013) The effects of glucosinolates and their breakdown products on necrotrophic fungi. PLoS ONE 8:e70771. https://doi.org/10.1371/journal.pone.0070771

Calmes B, N'Guyen G, Dumur J, Brisach CA, Campion C, Iacomi B et al (2015) Glucosinolate-derived isothiocyanates impact mitochondrial function in fungal cells and elicit an oxidative stress response necessary for growth recovery. Front Plant Sci 6:414. https://doi.org/10.3389/fpls.2015.00414

Chen J, Ullah C, Reichelt M, Beran F, Yang ZL, Gershenzon J, Hammerbacher A, Vassão DG (2020) The phytopathogenic fungus *Sclerotinia sclerotiorum* detoxifies plant glucosinolate hydrolysis products via an isothiocyanate hydrolase. Nat Commun 11:3090. https://doi.org/10.1038/s41467-020-16921-2

Chhajed S, Mostafa I, He Y, Abou-Hashem M, El-Domiaty M, Chen S (2020) Glucosinolate biosynthesis and the glucosinolate–myrosinase system in plant defense. Agronomy 10(11):1786. https://doi.org/10.3390/agronomy10111786

Clay NK, Adio AM, Denoux C, Jander G, Ausubel FM (2009) Glucosinolate metabolites required for an *Arabidopsis* innate immune response. Science 323:95–101

Dombrecht B, Xue GP, Sprague SJ, Kirkegaard JA, Ross JJ, Reid JB, Fitt GP, Sewelam N, Schenk PM, Manners JM (2007) MYC2 differentially modulates diverse jasmonate-dependent functions in Arabidopsis. Plant Cell 19:2225–2245

Doughty KJ, Porter AJR, Morton AM, Kiddle G, Bock CH, 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. https://doi.org/10.1111/j.1744-7348.1991.tb05648.x

Ko EJ, Shin YH, Hyun HN, Song HS, Hong JK, Jeun YC (2019) Bio-Sulfur pre-treatment suppresses Anthracnose on cucumber leaves inoculated with *Colletotrichum orbiculare*. Mycobiology 47:308–318. https://doi.org/10.1080/12298093

Frerigmann H, Gigolashvili T (2014) Update on the role of R2R3-MYBs in the regulation of glucosinolates upon sulfur deficiency. Front Plant Sci 5:626. https://doi.org/10.3389/fpls.2014.00626

Frerigmann H, Pi´slewska-Bednarek M, Sánchez-Vallet A, Molina A, Glawischnig E, Gigolashvili T, Bednarek P (2016) Regulation of pathogen-triggered tryptophan metabolism in arabidopsis thaliana by Myb transcription factors and indole glucosinolate conversion products. Mol Plant 9:682–695

Faris JD, Friesen TL (2020) Plant genes hijacked by necrotrophic fungal pathogens. Curr Opin Plant Biol 56:74–80. https://doi.org/10.1016/j.pbi.2020.04.003

Giavalisco P, Li Y, Matthes A, Eckhardt A, Hubberten HM, Hesse H, Segu S, Hummel J, Köhl K, Willmitzer L (2011) Elemental formula annotation of polar and lipophilic metabolites using ^{13}C, ^{15}N and ^{34}S isotope labelling, in combination with high-resolution mass spectrometry. Plant J 68:364–376. https://doi.org/10.1111/j.1365-313X.2011.04682.x

Glazebrook J (2005) Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annu Rev Phytopathol 43:205–227. https://doi.org/10.1146/annurev.phyto.43.040204.135923

Gohain B, Kumar P, Malhotra B, Augustine R, Pradhan AK, Bisht NC (2021) A comprehensive Vis-NIRS equation for rapid quantification of seed glucosinolate content and composition across diverse Brassica oilseed chemotypes. Food Chem 354:129527. https://doi.org/10.1016/j.foodchem.2021.129527

Guo R, Shen W, Qian H, Zhang M, Liu L, Wang Q (2013) Jasmonic acid and glucose synergistically modulate the accumulation of glucosinolates in *Arabidopsis thaliana*. J Exp Bot 64(18):5707–5719

Horbach R, Navarro-Quesada AR, Knogge W, Deising HB (2011) When and how to kill a plant cell: Infection strategies of plant pathogenic fungi. J Plant Physiol 168:51–62. https://doi.org/10.1016/j.jplph.2010.06.014

Iven T, König S, Singh S, Braus-Stromeyer SA, Bischoff M, Tietze LF, Braus GH, Lipka V, Feussner I, Dröge-Laser W (2012) Transcriptional activation and production of tryptophan-derived secondary metabolites in Arabidopsis roots contributes to the defense against the fungal vascular pathogen *Verticillium longisporum*. Mol Plant 5(6):1389–1402

Jimenez-reyes MF, Carrascob H, Oleab AF, Silva-Morenoa E (2019) Natural compounds: a sustainable alternative to the phytopathogens control. J Chil Chem Soc 64:4459–4465. https://doi.org/10.4067/S0717-97072019000204459

Kiran GVNSM, Thara SS, Pavan S (2018) Physiological characterisation of *Alternaria brassicicola* causing *Alternaria* leaf spot in cabbage. Int J Curr Microbiol Appl Sci 7(10):2727–2734. https://doi.org/10.20546/ijcmas.2018.710.317

Kliebenstein D (2004) Secondary metabolites and plant/environment interactions: a view through *Arabidopsis thaliana* tinged glasses. Plant Cell Environ 27:675–684. https://doi.org/10.1111/j.1365-3040.2004.01180.x

Krueger S, Giavalisco P, Krall L, Steinhauser MC, Büssis D, Usadel B, Flügge UI, Fernie AR, Willmitzer L, Steinhauser DA (2011) Topological map of the compartmentalized *Arabidopsis thaliana* leaf metabolome. PLoS ONE 6(3):e17806. https://doi.org/10.1371/journal.pone.0017806

Kumar R, Lee SG, Augustine R, Reichelt M, Vassão DG, Palavalli MH, Allen A, Gershenzon J, Jez JM, Bisht NC (2019) Molecular basis of the evolution of methylthioalkylmalate synthase and the diversity of methionine-derived glucosinolates. Plant Cell 31(7):1633–1647. https://doi.org/10.1105/tpc.19.00046

Künstler A, Gullner G, Ádám AL, Kolozsváriné Nagy J, Király L (2020) The versatile roles of sulfur-containing biomolecules in plant defense—a road to disease resistance. Plants 9(12):1705. https://doi.org/10.3390/plants9121705

Li Y, Kiddle G, Bennett R, Wallsgrove R (1999) Local and systemic changes in glucosinolates in Chinese and European cultivars of oilseed rape (*Brassica napus* L.) after infection with *Sclerotinia sclerotiorum* (stem rot). Ann Appl Biol 134:45–58. https://doi.org/10.1111/j.1744-7348.1999.tb05234.x

Li Y, Sawada Y, Hirai A, Sato M, Kuwahara A, Yan X, Hirai MY (2013) Novel insights into the function of Arabidopsis R2R3-MYB transcription factors regulating aliphatic glucosinolate biosynthesis. Plant Cell Physiol 54:1335–1344. https://doi.org/10.1093/pcp/pct085

Ljubej V, Radojčić Redovniković I, Salopek-Sondi B, Smolko A, Roje S, Šamec D (2021) Chilling and freezing temperature stress differently influence glucosinolates content in *Brassica oleracea* var. acephala. Plants 10(7):1305. https://doi.org/10.3390/plants10071305

Macioszeka VK, Lawrenceb CB, Kononowicz AK (2018) Infection cycle of *Alternaria brassicicola* on *Brassica oleracea* leaves under growth room conditions. Plant Pathol 67:1088–1096

Meur G, Shukla P, Dutta-Gupta A, Kirti PB (2015) Characterization of *Brassica juncea–Alternaria brassicicola* interaction and jasmonic acid carboxyl methyl transferase expression. Plant Gene 3:1–10. https://doi.org/10.1016/j.plgene.2015.06.001

Mithen R, Faulkner K, Magrath R, Rose P, Williamson G, Marquez J (2003) Development of isothiocyanate-enriched broccoli and its enhanced ability to induce phase 2 detoxification enzymes in mammalian cells. Theor Appl Gen 106:727–734

Mugford SG, Yoshimoto N, Reichelt M, Wirtz M, Hill L, Mugford ST, Nakazato Y, Noji M, Takahashi H, Kramell R, Gigolashvili T, Flügge UI, Wasternack C, Gershenzon J, Hell R, Saito K, Kopriva S (2009) Disruption of adenosine-5-phosphosulfate kinase in Arabidopsis reduces levels of sulfated secondary metabolites. Plant Cell 21:910–927

Nakai Y, Maruyama-Nakashita A (2020) Biosynthesis of sulfur-containing small biomolecules in plants. Int J Mol Sci 21:3470. https://doi.org/10.3390/ijms21103470

Nambiar DM, Kumari J, Augustine R, Kumar P, Bajpai PK, Bisht NC (2021) GTR1 and GTR2 transporters differentially regulate tissue-specific glucosinolate contents and defence responses in the oilseed crop *Brassica juncea*. Plant Cell Environ 44(8):2729–2743. https://doi.org/10.1111/pce.14072

N'Guyen GQ, Raulo R, Porquier A, Iacomi B, Pelletier S, Renou J-P, Bataillé-Simoneau N, Campion C, Hamon B, Kwasiborski A, Colou J, Benamar A, Hudhomme P, Macherel D, Simoneau P, Guillemette T (2021) Responses of the necrotrophic fungus *Alternaria brassisicola* to the indolic phytoalexin brassinin. Front Plant Sci 11:611643. https://doi.org/10.3389/fpls.2020.611643

Nongbri PL (2013) The role of glucosinolates in the Arabidopsis/Piriformospora indica interaction. Dissertation, University of Jena

Pandaranayaka EP, Frenkel O, Elad Y, Prusky D, Harel A (2019) Network analysis exposes core functions in major lifestyles of fungal and oomycete plant pathogens. BMC Genom 20:1020. https://doi.org/10.1186/s12864-019-6409-3

Park YJ, Lee HM, Shin M, Arasu MV, Chung DY, Al-Dhabi NA, Kim SJ (2018) Effect of different proportion of sulphur treatments on the contents of glucosinolate in kale (*Brassica oleracea* var. acephala) commonly consumed in Republic of Korea. Saudi J Biol Sci 25(2):349–353. https://doi.org/10.1016/j.sjbs.2017.04.012

Pedras MSC, Yaya EE (2010) Phytoalexins from Brassicaceae: news from the front. Phytochemistry 71:1191–1197. https://doi.org/10.1016/j.phytochem.2010.03.020

Pedras MSC, Hossain S (2011) Interaction of cruciferous phytoanticipins with plant fungal pathogens: indole glucosinolates are not metabolized but the corresponding desulfo-derivatives and nitriles are. Phytochemistry 72:2308–2316. https://doi.org/10.1016/j.phytochem.2011.08.018

Pedras MSC, Nycholat CM, Montaut S, Xu Y, Khan AQ (2002) Chemical defenses of crucifers: elicitation and metabolism of phytoalexins and indole-3-acetonitrile in brown mustard and turnip. Phytochemistry 59:611–625. https://doi.org/10.1016/s0031-9422(02)00026-2

Pedras MSC, Minic Z, Sarma-Mamillapalle VK (2009) Substrate specificity and inhibition of brassinin hydrolases, detoxifying enzymes from the plant pathogens *Leptosphaeria maculans* and *Alternaria brassicicola*. FEBS 276:7412–7428. https://doi.org/10.1111/j.1742-4658.2009.07457.x

Piślewska-Bednarek M, Nakano RT, Hiruma K, Pastorczyk M, Sanchez-Vallet A, Singkaravanit-Ogawa S, Ciesiołka D, Takano Y, Molina A, Schulze-Lefert P, Bednarek P (2018) Glutathione transferase U13 functions in pathogen-triggered glucosinolate metabolism. Plant Physiol 176:538–551. https://doi.org/10.1104/pp.17.01455

Sanchez-Vallet A, Ramos B, Bednarek P, López G, Pi'slewska-Bednarek M, Schulze-Lefert P, Molina A (2010) Tryptophan-derived secondary metabolites in *Arabidopsis thaliana* confers non-host resistance to necrotrophic *Plectosphaerella cucumerina* fungi. Plant J 63:115–127. https://doi.org/10.1111/j.1365-313X.2010.04224.x

Schlaeppi K, Abou-Mansour E, Buchala A, Mauch F (2010) Disease resistance of Arabidopsis to *Phytophthora brassicae* is established by the sequential action of indole glucosinolates and camalexin. Plant J 62:840–851. https://doi.org/10.1111/j.1365-313X.2010.04197.x

Seo M-S, Kim JS (2017) Understanding of MYB transcription factors involved in glucosinolate biosynthesis in Brassicaceae. Molecules 22(9):1549. https://doi.org/10.3390/molecules22091549

Sikorska-Zimny K, Beneduce L (2021) The glucosinolates and their bioactive derivatives in *Brassica*: a review on classification, biosynthesis and content in plant tissues, fate during and after processing, effect on the human organism and interaction with the gut microbiota. Crit Rev Food Sci Nutr 61(15):2544–2571. https://doi.org/10.1080/10408398.2020.1780193

Smolen G, Bender J (2002) Arabidopsis cytochrome P450 cyp83B1 mutations activate the tryptophan biosynthetic pathway. Genetics 160:323–332

Sønderby IE, Burow M, Rowe HC, Kliebenstein DJ, Halkier BA (2010) A complex interplay of three R2R3 MYB transcription factors determines the profile of aliphatic glucosinolates in Arabidopsis. Plant Physiol 153:348–363. https://doi.org/10.1104/pp.109.149286

Stotz HU, Sawada Y, Shimada Y, Hirai MY, Sasaki E, Krischke M, Brown PD, Saito K, Kamiya Y (2011) Role of camalexin, indole glucosinolates, and side chain modification of glucosinolate-derived isothiocyanates in defense of Arabidopsis against *Sclerotinia sclerotiorum*. Plant J 67:81–93. https://doi.org/10.1111/j.1365-313X.2011.04578.x

Sun R, Gols R, Harvey JA, Reichelt M, Gershenzon J, Pandit SS, Vassão DG (2020) Detoxification of plant defensive glucosinolates by an herbivorous caterpillar is beneficial to its endoparasitic wasp. Mol Ecol 29:4014–4031. https://doi.org/10.1111/mec.15613

Su′ udi M, Park JM, Park SR, Hwang DJ, Bae SC, Kim S, Ahn IP (2013) Quantification of *Alternaria brassicicola* infection in the *Arabidopsis thaliana* and *Brassica rapa* subsp.pekinensis. Microbiol 159:1946–1955. https://doi.org/10.1099/mic.0.068205-0

Textor S, Gershenzon J (2008) Herbivore induction of the glucosinolate-myrosinase defense system: major trends, biochemical bases and ecological significance. Phytochem Rev 8(1):149e170

Tierens KFJ, Thomma BP, Brouwer M, Schmidt J, Kistner K, Porzel A, Mauch-Mani B, Cammue BP, Broekaert WF (2001) Study of the role of antimicrobial glucosinolate-derived isothiocyanates in resistance of Arabidopsis to microbial pathogens. Plant Physiol 125:1688–1699

Tohge T, Fernie AR (2010) Combining genetic diversity, informatics and metabolomics to facilitate annotation of plant gene function. Nat Protoc 5:1210–1227. https://doi.org/10.1038/nprot.2010.82

Tohge T, Wendenburg R, Ishihara H, Nakabayashi R, Watanabe M, Sulpice R, Hoefgen R, Takayama H, Saito K, Stitt M, Fernie AR (2016) Characterization of a recently evolved flavonol-phenylacyl transferase gene provides signatures of natural light selection in Brassicaceae. Nat Commun 7:12399. https://doi.org/10.1038/ncomms12399

Troncoso R, Espinoza C, Sánchez-Estrada A, Tiznado M, Garćia HS (2005) Analysis of the isothiocyanates present in cabbage leaves extract and their potential application to control Alternaria rot in bell peppers. Food Res Int 38:701–770. https://doi.org/10.1016/j.foodres

Troncoso-Rojas R, Sánchez-Estrada A, Ruelas C, Garc'ia HS, Tiznado-Hernández M (2005) Effect of benzyl isothiocyanate on tomato fruit infection development by *Alternaria alternata*. J Sci Food Agric 85:1427–1434. https://doi.org/10.1002/jsfa

van de Mortel JE, de Vos RC, Dekkers E, Pineda A, Guillod L, Bouwmeester K, van Loon JJ, Dicke M, Raaijmakers JM (2012) Metabolic and transcriptomic changes induced in Arabidopsis by the rhizobacterium *Pseudomonas fluorescens* SS101. Plant Physiol 160(4):2173–2188

VanEtten H, Matthews D, Matthews PS (1989) Phytoalexin detoxification: importance for pathogenicity and practical implications. Annu Rev Phytopathol 2:143–164

Verwoerd TC, Dekker BM, Hoekema A (1989) A small-scale procedure for the rapid isolation of plant RNAs. Nucl Acids Res 17:23–62

Wang X, Jiang N, Liu J, Liu W, Wang G (2014) The role of effectors and host immunity in plant–necrotrophic fungal interactions. Virulence 7:722–732. https://doi.org/10.4161/viru.29798

Wei L, Jian H, Lu K, Filardo F, Yin N, Liu L, Qu C, Li W, Du H, Li J (2016) Genome-wide association analysis and differential expression analysis of resistance to *Sclerotinia stem* rot in *Brassica napus*. Plant Biotechnol J 14(6):1368–1380. https://doi.org/10.1111/pbi.12501

Wittstock U, Halkier BA (2002) Glucosinolate research in the Arabidopsis era. Trends Plant Sci 7:263–270. https://doi.org/10.4161/viru.29798

Yi GE, Robin AHK, Yang K, Park JI, Hwang BH, Nou IS (2016) Exogenous methyl jasmonate and salicylic acid induce subspecies-specific patterns of glucosinolate accumulation and gene expression in *Brassica oleracea* L. Molecules 21:1417. https://doi.org/10.3390/molecules21101417

Zechmann B (2020) Subcellular roles of glutathione in mediating plant defense during biotic stress. Plants 9:1067. https://doi.org/10.3390/plants9091067