Molecular characterisation of defence of *Brassica napus* (Oilseed rape) to *Rhizoctonia solani* AG2-1 confirmed by functional analysis in *Arabidopsis thaliana*

Isabelle Sims[1], Dasuni Jayaweera[1], Kamal Swarup[1] and Rumiana V. Ray[1*]

[1]Division of Plant and Crop Sciences, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, Leicestershire, LE12 5RD

*Corresponding author: Rumiana.Ray@nottingham.ac.uk

Funding: The PhD of Isabelle L. Sims was funded by the BBSRC Doctoral Training Programme at the University of Nottingham

Abstract

Rhizoctonia solani is a necrotrophic, soil-borne fungal pathogen associated with significant establishment losses in *Brassica napus* (Oilseed Rape; OSR). The Anastomosis Group (AG) 2-1 of *R. solani* is most virulent to OSR, causing damping-off, root and hypocotyl rot, and seedling death. Resistance to *R. solani* AG2-1 in OSR has not been identified, and the regulation of OSR defence to its adapted pathogen, AG2-1, has not been investigated. In this work, we used confocal microscopy to visualise the progress of infection by sclerotia of AG2-1 on *B. napus* varieties with contrasting disease phenotypes. We defined their defence response using gene expression studies and functional analysis with *Arabidopsis thaliana* mutants. Our results showed existing variation in susceptibility to AG2-1 and plant growth between OSR varieties, and differential expression of genes of hormonal and defence pathways related to auxin, ethylene, jasmonic acid, abscisic acid, salicylic acid, and reactive oxygen species regulation. Auxin, abscisic acid signalling, and the MYC2 branch of jasmonate signalling contributed to susceptibility to AG2-1, whilst induced systemic resistance was enhanced by NAPDH RBOHD, ethylene signalling and the ERF/PDF branch of jasmonate signalling. These results pave the way for future research, which will lead to the development of *Brassica* crops that are more resistant to AG2-1 of *R. solani* and reduce dependence on chemical control options.

Keywords: *Brassica napus*, *Rhizoctonia solani*, resistance, necrotroph, auxin, jasmonates, ethylene

Introduction

Brassica napus L., known as oilseed rape (OSR), is a valuable crop species, primarily grown for use as rapeseed oil, animal feed or biofuel. *Rhizoctonia solani* J.G. Kühn is a soil-borne, fungal species complex divided into 13 reproductively isolated Anastomosis Groups (AGs) (Carling et al. 2002), of which, AG2-1 is most virulent to *B. napus*. *R. solani* survives in the soil as sclerotia (resting bodies of compacted mycelia), which in the presence of a susceptible host, rapidly germinate to produce infectious hyphae colonising host tissues, and forming infection cushions with hyphal pegs underneath to penetrate the host (Kataria and Verma 1992). On pre-germinated seedlings, symptoms of the developing damping off disease appear as hypocotyl/root rot and necrotic lesions, although the pathogen is also known to inhibit seed germination pre-emergence. Artificial inoculation of OSR with *R. solani* AG2-1 has shown a reduction in establishment by 61% and a yield reduction of 41% (Jayaweera and Ray 2022). Control is usually attempted through chemical and cultural methods, although there are currently no approved chemical seed treatments, and genetic resistance has not yet been identified (Brown et al. 2021; Jayaweera and Ray 2022).

Most of the information on the modulation of defence against *R. solani*, causing damping off, has been provided by functional studies with the model plant, *Arabidopsis thaliana* (L.) Heynh., challenged with AG8, or hypovirulent isolates of *R. solani* (Foley et al. 2013; Sharon et al. 2011; Kumar et al. 2020; Kidd et al. 2021). Defence against *R. solani* AG8 has been shown to involve jasmonic acid (JA) and ethylene (ET) pathways since mutations in JA (*coi1*), ET (*ein2*, *ers1* or *ers2*) and *pen2* reduced plant survival under AG8 inoculation (Kidd et al. 2021). The NADPH oxidases (NOXs) double mutant *rbohd rbohf* was also highly susceptible to *R. solani* AG8 (Foley et al. 2013; Kumar et al. 2020). In contrast to JA and ET responses to AG8, auxin (Bartz et al. 2012) and ABA (Cordovez et al. 2017) mediated signalling patwhays have been identified as potentially increasing host susceptibility to various other AGs of *R.*

solani. Transcriptomics experiments showed that exposure to volatile organic compounds released by *R. solani* AG2-2 IIIB induced upregulation of ABA and auxin signalling genes in *A. thaliana*, while ET and JA signalling pathways were down-regulated (Cordovez et al. 2017). Furthermore, isolates of AG1 IA, AG3 and AG4 have been shown to produce the auxin, phenyl acetic acid (PAA) and its derivatives (Bartz et al. 2012; Iacobellis and DeVay 1987; Mandava et al. 1980; Lakshman et al. 2006). PAA is a natural auxin with an overlapping regulatory role with indole-3-acetic acid (IAA) (Sugawara et al. 2015) and in the host interaction with *R. solani*, PAA production has been associated with increased disease severity on susceptible hosts (Bartz et al. 2012). It is currently unknown if *R. solani* AG2-1 isolates produce PAA, however the pathogen has been shown to produce IAA (Furukawa and Syono, 1998) suggesting that these auxins play a role in the disease biology of OSR. Studies using hypovirulent binucleate *Rhizoctonia* (Ru18-1, Ru89-1 [AG-B(o)], Rh521, and Ru56-8 (AG-A)) have provided information on the early defence response to AG4 (HG-1), as increased expression of genes PR5, PDF1.2, LOX2, LOX1, CORI3 involved in induced systemic resistance and PAD3 of the phytoalexin production pathway was observed (Sharon et al. 2011). Whilst previous studies with *A. thaliana* challenged by AG8 or hypovirulent *R. solani* have contributed to our understanding of non-host defence regulation, further molecular studies are needed to define the host-specific interactions in defence of OSR to AG2-1.

Here we provide new insights on OSR infection and the defence response to AG2-1 using inoculation experiments with three contrasting OSR varieties and further functional studies with *A. thaliana* mutants for key genes involved in hormonal regulation. This work aimed to first quantify and characterise variation in the tolerance of small range of commercial varieties of *B. napus* to *R. solani* AG2-1. We investigated this by quantifying disease symptoms and root growth, as well as imaging the initial stages of the infection process with AG2-1 sclerotia. Varieties with contrasting resistance responses were used for molecular characterisation to

identify differences in their defence pathways. We hypothesised that host susceptibility is associated with increased expression of genes of the SA and auxin response, whilst enhanced defence to AG2-1, like to AG8, with increased expression of genes of the JA and ET pathways. We used RT-qPCR to investigate changes in gene expression and confirmed gene functionality using *A. thaliana* mutant lines under inoculation with AG2-1.

Materials and methods

Fungal inoculum preparation

R. solani AG2-1 (isolate 1934 from the University of Nottingham isolate collection) was used for inoculum production. AG2-1 was cultured on potato dextrose agar plates (PDA; Sigma-Aldrich, UK) at room temperature (18°C). Inoculation was carried out using 6 mm diameter AG2-1 cultured agar plugs from plates that were grown for 6-10 days before the production of sclerotia. For microscopy experiments using sclerotia, plates were prepared in the same manner and kept at room temperature for 4 weeks.

AG2-1 inoculation in *Brassica napus*

Light expanded clay aggregate (LECA) particles were used for AG2-1 inoculation in *B. napus* as roots of young seedlings were kept clean and intact for further analysis. This experiment was carried out using a randomized block design, with 2 factors resulting in 10 treatment combinations in 4 replications. The factors were: commercially available OSR variety (Anastasia (LG seeds), Campus (KSW), SY Saveo (Syngenta), SY Sensia (Syngenta) or Skye (Elsom seeds)) and pathogen inoculation (non-inoculated or AG2-1 inoculated). 40 pots (9 cm in diameter) were filled one third with LECA particles (size 4-10mm; Saint-Gobain Weber Limited, UK) and five either AG2-1 colonised (inoculated) or clean (non-inoculated) 6 mm diameter PDA plugs before filling with the remaining two thirds of LECA particles. Seeds were pre-germinated on filter paper in petri dishes with 5 ml sterile distilled water for three days in

the dark at room temperature (18°C). Three pre-germinated seedlings were added to each LECA pot. The pots were supplemented with 25% Hoagland's (Sigma-Aldrich, UK) in 0.5 L of purified water in equal amounts once only at the start of the experiment. Plants were kept in a controlled environment chamber at 20°C with 12h photoperiod and a relative humidity of 60% under photosynthetically active radiation of 300 μmol m^{-2} s^{-1}.

Disease assessment

The symptoms of *Rhizoctonia* infection include damping-off, root rot and stem rot. The hypocotyl and roots show necrotic lesions which become water soaked, soft and incapable of supporting the plant. Disease assessment was conducted 7 days post inoculation using a scoring scale of 0-4 for both hypocotyl and root; with 0 = symptomless, 1 = 25% symptoms, 2 = 50% symptoms, 3 = 75% of symptoms, and 4 = plant death, modified from Drizou et al. (2017). Root length was measured using photographs and the SmartRoot plugin for ImageJ (Schneider et al. 2012; Lobet et al. 2011).

Gene expression analysis

Whole plant samples of *B. napus* were collected at 8, 24 and 48 hours post inoculation (hpi) for RNA extraction, using RNeasy Plant kit (Qiagen) with TRIzol reagent (Invitrogen) as described in Ajigboye et al. (2021). First strand cDNA was synthesized using iScript cDNA Synthesis Kit (Bio-Rad). Quantitative reverse transcription PCR (RT-qPCR) with Sybr Green (Bio-Rad) was conducted using CFX96 Touch Real-Time PCR Detection System (BioRad) consisting of 95°C for 1 minute, followed by 40 cycles with 15 seconds at both 95°C and 60°C. The primers are listed in supplementary Table S1. Relative quantification, with actin used as the reference gene, for inoculated and non-inoculated plants (control) was calculated using the $2^{-\Delta\Delta C_T}$ method (Livak and Schmittgen 2001). The disease-free, non-inoculated, plant samples (control) were used as the calibrator, so that target gene expression can be interpreted relative

to the internal control in the inoculated plants compared with the non-inoculated (control) plants (Schmittgen and Livak, 2008) allowing the calculation of fold change in gene expression due to inoculation. Arithmetic means and standard errors were calculated with three biological (each with three technical) replicates per sample.

Wheat Germ Agglutinin (WGA)-Alexa Fluor/ Propidium Iodide staining and microscopy

B. napus seedlings were surface sterilised for 8 minutes using a sterilisation solution containing 70% sodium hypochlorite (Parazone, Jeyes Limited, UK) and 0.2% Tween-20 and then washed three times with sterile distilled water and plated on 10% (w/v) water agar. They were cold stratified for 3 days then moved to a controlled environment room with 16h light at 21°C, 8h dark at 15°C for 25 days. *R. solani* sclerotium were added next to (as close as touching) to the roots of the plants to allow fast infection. Roots were sampled at 8, 24 and 48 hpi and stored in 100% ethanol to undergo bleaching upon collection. A minimum of 9 samples were examined for each variety at each time point (average: 17). Ethanol was then replaced with 10% potassium hydroxide and incubated at 85°C for 1.5 hours. Samples were washed five times in phosphate buffer saline (PBS) pH7.4. Staining solution, made with 20µg/ml propidium iodide, 10µg/ml WGA-Alexa Fluor 488 conjugate (Thermo Fisher) and 0.1% (v/v) Tween-20 in PBS pH7.4. Propidium Iodide was used to stain plant cell walls of root tissues and Alexa Fluor was used to stain fungal hyphae. Vacuum infiltration at atmospheric pressure was completed 3 times for 5 minutes each with 5-minute intervals between them. Samples were washed twice with PBS before visualisation with the Leica SP5 Confocal microscope (Leica Microsystems, Germany) (Redkar *et al*. 2018).

AG2-1 inoculation in *Arabidopsis thaliana*

R. solani AG2-1 is virulent to *A. thaliana*. Seeds of *A. thaliana* were obtained from NASC, UK, Dr Ranjan Swarup and Dr Jorge Vicente Conde, University of Nottingham, and were

surface sterilised as above. The seeds were transferred to 50% MS pH 5.8 (Murashige and Skoog Basal Medium, Sigma-Aldrich, UK), 1% agar plates and cold stratified at 4°C for 3 days. The plates were then moved to a controlled environment room with 16h light at 21°C, 8h dark at 15°C and grown vertically for 11days. Seedlings were transplanted into 3x4 well trays containing a mix of M3 compost (Levington, Everris Limited, UK), vermiculite and perlite in a 4:2:1 ratio. Three days later, the plants were transferred into experimental trays with 10 *R. solani* AG2-1 colonised or non-inoculated 6 mm diameter PDA plugs per well added 3cm from the top of the well. Trays were kept in a controlled environment chamber at 22°C with 16 h photoperiod. Photographs were taken 11 days post inoculation, from above at constant distance, using a digital camera and the green area for each plant was measured using ImageJ (Schneider et al. 2012). A ruler was included in all photographs to set the scale for measurements.

Infection and imaging of Jas9:VENUS plants

A. thaliana Jas9:VENUS seeds were obtained from NASC (Stock code: N2105629) and were surface sterilised, cold stratified, and grown on 50% MS pH 5.8 1% agar plates as described above. After 8 days growth, *R. solani* mycelium from a PDA plate was added close to the plant roots, and the plants were imaged 20 h after inoculation. Images were taken using a Leica SP5 Confocal microscope (Leica Microsystems, Germany).

Infection and imaging of $IAA2_{pro}$:GUS plants

A. thaliana Ws $IAA2_{pro}$:GUS lines were obtained from Dr Ranjan Swarup, University of Nottingham and were grown on 50% MS pH 5.8 1% agar plates as described above, for 7 days before AG2-1 inoculation. The plants were spaced at least 1 cm apart and 3 cm from the top of the plate. Plates were inoculated using 6mm PDA plugs colonised with *R. solani* AG2-1. Three plugs were used per plate, spaced equally, 2 cm from the bottom of the plate. The fungal growth was close to, but not touching the roots, by 3 dpi. Samples were attempted at later time points

but after the fungus reached the plants, the roots were not able to be removed from the plates and stained effectively without breaking.

GUS buffer was prepared with 100mM pH7.0 sodium phosphate buffer, 0.5M EDTA, 1mM potassium ferricyanide, 1mM potassium ferrocyanide, 0.5% (w/v) 5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid (x-gluc; thermo scientific) dissolved in 1ml dimethylformamide (DMX) and 0.1% (v/v) Triton x-100. Whole plants were harvested and immediately placed in the prepared GUS buffer on ice until all samples were collected. Samples were incubated with GUS buffer at 37°C for 30 minutes wrapped in foil. Samples were transferred to fresh tubes with 25% ethanol overnight, before increasing the ethanol percentage over subsequent days (50%, 70%, 90%, 100%), then the samples were stored in 50% glycerol until microscopy. Samples were viewed using the Leica CTR5000 microscope (Leica Microsystems, Germany).

Statistical Analysis

Statistical analysis for all experiments were carried out using Genstat® Version 19 for windows (VSN International Ltd, UK). Analysis of Variance (ANOVA) with t-test was conducted on the data for the *B. napus* root and hypocotyl disease scores and on the proportional decrease of root length due to inoculation. Student t-test was used to compare fold changes in gene expression due to inoculation with AG2-1 for each OSR genotype at each timepoint. The decrease in plant leaf areas of the *A. thaliana* mutants relative to their non-inoculated controls were evaluated using student t-tests with differences considered significantly different at $P < 0.05$.

Results

Phenotypic comparison of *Brassica napus* varieties

Differences in the symptom severity between *B. napus* varieties inoculated with *R. solani* AG2-1 were determined at 7 days post-inoculation (dpi) (Figure 1A, B). All varieties showed root and hypocotyl symptoms and a reduction in root length under inoculation, but plants of cv. Anastasia were most susceptible, showing extensive necrosis on both the root and hypocotyl (root: 4/4, hypocotyl: 3.5/4) and total root length reduction by 95% under inoculation (Figure 1B, C). Campus showed the fewest symptoms on both the root and the hypocotyl and was significantly more resistant to hypocotyl damping off than Saveo or Anastasia (Figure 1B). Furthermore, Campus exhibited significantly lower reduction of root length due to inoculation, compared to Anastasia (Figure 1C). Skye showed more severe symptoms than Campus but grew the longest roots under inoculation, despite an 85% reduction in length (Supplementary Figure 1 and Figure 1C). Symptom severity and root length reduction in Saveo and Sensia were comparable to Anastasia and Skye, respectively (Figure 1B, C).

Anastasia, Skye, and Campus were chosen for further investigation using confocal microscopy as these conventional genotypes represented contrasting disease severity phenotypes as shown on Figure 1. Anastasia was identified as highly susceptible, Campus as most resistant and Skye was intermediate due to some tolerance to disease since root growth was least inhibited despite developing severe symptoms. Anastasia, Skye, and Campus roots were infected using *R. solani* AG2-1 sclerotia and stained using Propidium Iodide and Alexa Fluor Wheat Germ Agglutinin 488 to visualise the infection (**Error! Reference source not found.**). Propidium iodide stains plant cell walls, and the Alexa Fluor stains fungal hyphae. Hyphal networks forming from germinated sclerotia and infection cushions were observed at 8, 24 and 48 hours post-inoculation (hpi) on Anastasia (Figure 2). Sclerotia germination was observed on Skye at 8hpi with smaller infection cushions, than observed on Anastasia, developing by 48hpi. Germination from sclerotia was rarely seen at 8hpi on Campus, but some surface hyphal growth was

observed at 24 and 48hpi. There were no infection cushions observed in Campus at any time point of the microscopic investigation carried out up to 48hpi.

Characterisation of defence and hormonal responses in *Brassica napus* and *Arabidopsis thaliana*

RT-qPCRs were conducted using cDNA from whole plant RNA extractions with Log2 fold changes of gene expression due to infection of Anastasia, Campus, and Skye shown at 8, 24 and 48hpi (Figures 3, 5, 7-10). AG2-1 reduces plant vigour, growth, and development of *A. thaliana* (Supplementary Figure 2). To functionally confirm hormonal defence responses, *A. thaliana* mutants were soil-inoculated with *R. solani* AG2-1 and assessed for disease effects on plant growth above ground (Supplementary Figure 3), expressed as proportional reduction due to inoculation before comparison to the wild type *A. thaliana* (WT, Col-0) (Figures 3, 5, 7-10). Differential gene expression of OSR varieties for hormonal pathways and differences in growth, due to inoculation, between WT and Arabidopsis mutants are described in relation to the proposed defence diagram shown in Figure 11.

We first assessed the expression of AUX1 involved in auxin transport in plants, and AXR1 and TIR1 as two major genes of auxin signalling. AUX1 was significantly upregulated in Anastasia compared to Skye at 8 and 24hpi and declined in expression at 48hpi remaining significantly less expressed in Skye compared to Campus (Figure 3A). AXR1 showed similar expression pattern, although differences between varieties were not found to be statistically significant at any time point (Figure 3B). TIR1 and the auxin-responsive gene IAA7 also had similar expression patterns, with Anastasia upregulating both genes significantly more than Campus at 24hpi (Figure 3C, D). The least auxin gene responsive variety at 24hpi was Campus, followed at 48hpi by Skye with both showing repressed gene expression compared to Anastasia (Figure 3C, D). These results showed that inoculation significantly increased the expression of

genes of auxin transport, signalling and response in the susceptible Anastasia compared to the other two more tolerant genotypes. When gene functionality was tested in *A. thaliana* (Figure 3E), results showed that differences in above ground growth reduction of the mutants compared to WT plants were small and not significant at $P<0.05$. However, auxin signalling in the susceptible WT *A. thaliana* roots increased by pathogen infection as *A. thaliana* $IAA2_{pro}$:GUS lines showed more intense staining over the course of infection (Figure 4).

The basic-helix-loop-helix (bHLH) transcription factor, MYC2, differentially regulates jasmonic acid (JA)-responsive defence to pests and pathogens (Kazan and Manners 2013). JA is synthesised from α-linolenic acid and is converted to its bioactive form JA-Ile by JAR1 (Wang et al. 2021). JA-Ile promotes the ubiquitination and degradation of JAZ proteins via the E3-ligase complex SCF-COI1. This causes the de-repression of MYC2 and ERF1, which form two distinct, antagonistic signalling pathways (Kazan and Manners 2013). Inoculation significantly upregulated JAR1 in Skye and Campus in contrast to Anastasia where gene expression was repressed at 8hpi (Figure 5A). At 24hpi only Campus showed positive increase in gene expression whilst a significant decrease was observed in Skye and Anastasia. At 48hpi, Anastasia upregulated JAR1 significantly compared to Skye. MYC2 was highly expressed in Anastasia at 24 and 48hpi compared to Campus and Skye (Figure 5B), suggesting that the susceptible phenotype follows this branch of the JA signalling pathway. The *A. thaliana* mutant *coi1-4* showed a significantly greater reduction in growth under inoculation compared to the WT (Figure 5C), supporting the role of JA-regulated defence response to *R. solani*. To visualise JA response in the susceptible interaction, *A. thaliana* Jas9:VENUS lines were imaged under inoculation with AG2-1 (**Error! Reference source not found.**) (Larrieu et al. 2015). In the tissues near the root tip where *R. solani* had colonised, no fluorescence was observed, indicating the complete degradation of JAZ9 proteins. However, in the susceptible WT root

tissues further away from the infection, JAZ9 appeared to be stabilised, indicating a reduction in JA activity.

MYC2 is positively regulated by abscisic acid (ABA) (Kazan and Manners 2013). ABA is synthesised from zeaxanthin, with the final two steps involving the conversion of xanthoxin by a short chain alcohol dehydrogenase (ABA2) into abscisic aldehyde, and oxidation into ABA by abscisic aldehyde oxidase (AAO3) (Finkelstein 2013). ABA can then form a complex with ABA receptors (PYR/PYL/RCAR), which interact with PP2Cs, causing the de-repression of SnRK2s and leading to the transcription of ABA-induced genes (Finkelstein 2013). AAO3 was significantly downregulated in Anastasia compared to Skye at 8hpi, however by 48hpi Anastasia significantly upregulated AAO3 compared to Campus or Skye (Figure 7A). ABI4 is an ABA-regulated transcription factor, which has increased expression in the presence of ABA, but is repressed by auxin (Saini et al. 2013). All varieties exhibited negative log2 fold change of ABI4 however Anastasia showed >15-fold repression at 8 and 24hpi compared to the other two cultivars (Figure 7B). The *A. thaliana* mutants *aba2-1* and *abi4-1* showed similar proportional reduction in growth due to inoculation and were as susceptible as the Col-0 to AG2-1 (Figure 7C).

The transcription factor, ERF1, is negatively regulated by MYC2, but positively regulated by ethylene (ET) synthesised from methionine (Wang et al. 2002). In high ET, EIN2 activates ET signalling (Wang et al. 2002) with EIN3/EIL proteins promoting ERF1, which leads to downstream ET-responsive transcription (Wang et al. 2002). ERF1 is a downstream component of both ET and JA pathways, and can be activated by both independently, or synergistically (Lorenzo et al. 2003). All varieties upregulated EIN2, however Anastasia showed greater log2 fold change of gene expression compared to Skye at 48hpi (Figure 8A). There were no significant differences between varieties for ERF1 expression at 8 or 24hpi, however at 48hpi, in response of infection, Campus and Skye increased ERF1 expression

significantly compared to Anastasia where expression was repressed 5-fold due to infection (Figure 8B). PDF1.2, downstream of ERF1, showed significantly increased log2 fold change in expression in Skye at 8hpi, Skye and Campus at 24hpi, and just for Campus at 48hpi compared to Anastasia or Skye (Figure 8C). These results indicated that the ERF1 pathway contributes to defence against *R. Solani* AG2-1. The ET signalling mutant *Atein3eil1* showed a significant reduction in growth compared to WT *A. thaliana* (Figure 8D), suggesting that plants with reduced ET- responsive transcription were highly susceptible to *R. solani* AG2-1 infection.

Mutually antagonistic crosstalk is known to exist between JA and salicylic acid (SA) and the balance of action between these two hormones is particularly important in the host defence responses to biotrophic and necrotrophic pathogens (Robert-Seilaniantz et al. 2011). SA is synthesised via the ICS and PAL pathways (Lefevere et al. 2020). ICS1 (also known as SID2) catalyses the first reaction of chorismate to isochorismate in the ICS pathway. PAL4 catalyses the reaction of phenylalanine to trans-cinnamic acid (tCA) that can also lead to lignin biosynthesis (Zheng et al. 2019; Lefevere et al. 2020). In response to inoculation at 8hpi and in contrast to Campus, Anastasia downregulated both ICS1 and PAL4 (Figure 9A, B). ICS1 and PAL4 expression increased by 20- and 10-fold, respectively at 24hpi in Anastasia compared to the other two genotypes with higher expression maintained by Anastasia at 48hpi (Figure 9A, B). High cytosolic SA leads to a redox change, causing cytosolic NPR1 oligomers to monomerise and translocate to the nucleus. There, NPR1 enables the transcription of SA-responsive genes enabling systemic acquired resistance (SAR) responses, and is then targeted for degradation (Ding and Ding 2020). NPR1 is also required for induced systemic resistance (ISR) independent of SA accumulation but requiring responsive JA or ET defence pathways (Withers and Dong 2016). Anastasia and Campus upregulated NPR1 in response to infection at 24 and 48hpi compared to Skye, in which gene expression was repressed (Figure 9C). The

final step of the biosynthesis of the phytoalexin, camalexin, derived from tryptophan, is catalysed by PAD3 (Zhou et al. 1999; Schuhegger et al. 2006). In our studies, PAD3 expression increased over time in Anastasia showing significantly higher log2 fold change compared to the other two varieties at 48hpi (Figure 9D). The *A. thaliana* mutants *sid2* and*npr1* showed similar reduction in growth due to inoculation as the WT *A. thaliana* plants (Figure 9E). However, the *A. thaliana pad3* mutant showed a significant reduction in growth under inoculation compared to Col-0 suggesting that a reduction in camalexin biosynthesis increased susceptibility to AG2-1.

NOXs known as respiratory burst oxidase homologs (RBOHs), mediate signal transduction pathways via production of reactive oxygen species (ROS) and participate in the regulation of plant development and growth processes, in addition to defence to biotic stress (Hu et al. 2020). RBOHC, known as RHD2, is a key regulator of ROS accumulation in the roots involved in root hair formation, and primary root elongation and development by regulating cell expansion (Chapman et al. 2019; Hu et al. 2020). RBOHD is a membrane protein, which undergoes conformational changes and phosphorylation during the influx of Ca^{2+} after pathogen perception resulting in the production ROS (Lee et al. 2020). RBOHD can be directly phosphorylated by DORN1 (Hu et al. 2020), which has been previously shown to enhance the resistance of *A. thaliana* to *R. solani* AG8 (Kumar et al. 2020). RBOHC expression was significantly higher in Anastasia compared to the other two genotypes at any time and decreased over time (Figure 10A). Inoculation failed to affect the expression of RBOHC in Skye or Campus. In contrast, RBOHD was significantly repressed in Anastasia at all time points compared to the other two cultivars with Skye and Campus upregulating RBOHD at 48hpi (Figure 10B), supporting the hypothesis that RBOHD activity is involved in enhanced resistance responses to AG2-1.

Discussion

To our knowledge, this is the first study to molecularly characterise the defence response of OSR to its pathogen, *R. solani* AG2-1. The OSR varieties used here showed contrasting disease phenotypes and defence pathways of their response to AG2-1. Using *A. thaliana* mutants, we evaluated the functionality of key hormonal defence genes against *R. solani* AG2-1 to support our gene expression studies. Our results showed that in contrast to auxin, JA and ET signalling enhanced the resistance response to *R. solani* AG2-1.

Currently there are no *R. solani* AG2-1 resistant OSR varieties, however, we have shown that some cultivar variation in susceptibility exists, with Anastasia being the most susceptible OSR variety used in our studies, whilst Campus was the most resistant. These results were confirmed using microscopy visualising the infection caused by sclerotia of *R. solani* on roots of *B. napus*. We showed that infection cushions developed quickly and more abundantly on the more susceptible host agreeing with previous research (Verma 1996) demonstrating that *R. solani* hyphae penetrated rapidly in compatible interactions. Indeed, Anastasia exhibited severe root rot and death quickly, whereas necrotic lesions formed slowly on the more resistant Campus and Skye, with the latter two genotypes continuing to grow despite the infection suggesting that seedling vigour and growth can be useful tolerance traits against the damping off effects of AG2-1.

Molecular studies using RT-qPCRs showed different defence responses amongst the varieties, which we supported with functional studies with Arabidopsis mutants. Our results showed a strong link between increased auxin signalling and susceptibility to *R. solani* AG2-1. IAA is synthesised from chorismate, via the tryptophan (Trp)-dependent or Trp-independent pathways (Mano and Nemoto 2012) and functions through interactions with the E3-ligase complex SCF-TIR1, which promotes the ubiquitination of AUX/IAA proteins and their subsequent

degradation (Sugawara et al. 2015). This leads to the de-repression of ARFs and the transcription of auxin-responsive genes. LAX transporters, such as AUX1, are IAA influx carriers, and are vital for IAA concentration gradients (Sugawara et al. 2015). The increased auxin signalling in the susceptible hosts was demonstrated here with increased expression of key auxin responsive genes and *A. thaliana* $IAA2_{pro}$:GUS lines supporting the hypothesis that an auxin produced by the fungus is likely to be involved in the modification of host development and defence. Indeed, previous work has identified a link between the production of the auxin, phenyl acetic acid (PAA), by *R. solani* and its pathogenicity (Bartz et al. 2012). The role of PAA in plants is less studied than IAA, but it is known to be synthesised from phenylalanine, and is found at higher endogenous levels than IAA in various *A. thaliana* plant tissues (Sugawara et al. 2015). In general, auxin-responsive genes can be regulated by both IAA and PAA (Sugawara et al. 2015). It will thus be vital to uncover if *R. solani* AG2-1 is producing PAA, and how this auxin and IAA produced by the fungus may be contributing to the virulence of the pathogen, and to host susceptibility.

When plants are induced by exogenous auxin and the auxin–TIR–AUX/IAA–ARF signalling is activated, JA synthesis is induced (Yang et al. 2019). JAR1 converts JA to JA-Ile with the latter promoting the ubiquitination and degradation of JAZ proteins via the E3-ligase complex SCF-COI1(Wang et al. 2021) causing the release of MYC2 and ERF1, forming two distinct, antagonistic signalling pathways (Kazan and Manners 2013). MYC2 plays a substantial role in the crosstalk between growth and hormonal defence regulating pathways. For example, MYC2 is known to repress PLETHORA1 (PLT1) and PLT2 transcription factors, facilitating the interaction between JA and auxin to inhibit root growth (Chen et al. 2011). This may have contributed to the almost complete inhibition of root growth in inoculated Anastasia plants. Furthermore, when using Jas9:VENUS lines under inoculation with AG2-1, we observed the depletion of JA indicated by an increased Jas9:VENUS signal away from infection. Based on

407 the expression of the key genes of the JA signalling pathway and the functional analysis in
408 Arabidopsis, the MYC2 branch of the pathway was shown to contribute to increased
409 susceptibility to AG2-1.

410 The JAZs-MYC2 components play an important role in the crosstalk between JA and ABA
411 signalling pathways, affecting plant growth and defence (Chen et al. 2011). ABA enhances the
412 interaction between PYRABACTIN RESISTANCE1-Like protein (PYL6) and JAZ, activating
413 transcription of MYC2, which in turn activates the expression of the JA responsive gene VSP2
414 against herbivore damage, thus linking ABA and JA defence responses (Aleman et al. 2016).
415 However, this activity negatively regulates the ERF1/ORA59-PDF1.2 branch of the JA
416 pathway required for pathogen defence (Kazan and Manners 2013). The ABA biosynthesis
417 mutants (*aao3* and *aba2*), as well as the ABA insensitive mutant (*abi4*) showed increased
418 susceptibility to the soil-borne oomycete *Pythium irregulare*, however, these mutants were
419 more resistant to the necrotroph *Botrytis cinerea* (Adie et al. 2007). We observed that *abi4-1*
420 and *aba2-1* showed similar susceptibility to the WT Arabidopsis however ABI4 expression
421 was downregulated >15-fold in the susceptible Anastasia under infection, compared to Campus
422 or Skye, suggesting that ABA signalling likely repressed by auxin (Saini et al. 2013) aided
423 susceptibility to *R. solani* AG2-1.

424 JAZs-MYC2 and EIN3/EIL1 of the JA and ET pathways, respectively, co-ordinate the plant
425 defence response against necrotrophic pathogens by activating the expression of the defence
426 protein PDF1.2 through ERF1 and ORA59 (Yang et al. 2019). ERF1 has previously been
427 shown to regulate resistance to many necrotrophic fungi including *B. cinerea*, *Plectosphaerella*
428 *cucumerina*, *Fusarium oxysporum* f. sp. *conglutinans* and *F. oxysporum* f. sp. *lycopersici*
429 (Berrocal-Lobo and Molina 2004). Similarly in our studies, the increased expression of ERF1
430 and PDF1.2 in Campus and Skye, in contrast to Anastasia, together with functional analysis
431 using *Atein3eil1* supported our conclusion that the ET signalling pathway is a key regulator in

the host defence response to *R. solani* AG2-1.Molecular genetics approaches have shown evidence that ET and NADPH oxidases act to co-ordinate plant responses to both abiotic and biotic stress (Xia et al. 2015). In relation to biotic stress regulation, EIN2-mediated signalling has been shown to be required for flagellin-induced RBOHD-dependent ROS accumulation against bacterial pathogens (Xia et al. 2015). The *A. thaliana* double mutant *rbohf rbohd* also exhibits almost complete loss of resistance to *R. solani* AG8 (Foley et al. 2013). Taken together with our results showing upregulation of RBOHD only in the more resistant Campus and Skye, in contrast to repression in the susceptible Anastasia we suggest that ROS produced via RBOHD is part of an effective defence response to *R. solani*. In contrast, RBOHC was upregulated in Anastasia at all time points. The activity of RBOHC has been mostly associated with regulation of root growth and development rather than the response to pathogenic attack and its role in resistance or susceptibility to soil-borne pathogens is unknown. We can speculate based on the expression of our contrasting varieties that RBOHC is not likely to be as effective as RBOHD in the defence to AG2-1.

SA, synthesised via the ICS and PAL pathways, also plays a key role in ROS production through the regulation of RBOH transcription, and can create a feedback loop where ROS also regulate SA signalling (Lukan and Coll 2022). In our studies ICS1 and PAL4 were upregulated significantly more in Anastasia than in Campus or Skye and although the *A. thaliana* mutant *sid2* showed similar susceptibility as the WT plants to the disease these results together suggest that SA biosynthesis is likely to contribute to susceptibility. Previously OsPAL4 was identified as contributing to resistance to *R. solani* AG2 (Tonnessen et al. 2015) and BnPAL4 activity was increased in resistant OSR during *Verticillium longisporum* infection (Zheng et al. 2019). The role of PAL4 in secondary metabolism in the interaction with AG2-1 requires further investigation as we observed increased expression in the most resistant variety, Campus, compared to the tolerant Skye. The importance of NPR1 for the response to *R. solani* or other

soil-borne pathogens including *P. irregulare* (Adie et al. 2007) has been demonstrated in various reports. For example, tissue-specific expression of AtNPR1 in rice has been shown in previous research to confer resistance to *R. solani* AG1-1A (Molla et al. 2016). Similarly expression of BjNPR1 in mungbean also increased resistance to *R. solani* (AG not specified) (Vijayan and Kirti 2012). However, here differences in NPR1 expression of OSR varieties and the growth phenotype of the *A. thaliana npr1* mutant under inoculation suggested that NPR1 may play a dual role in the disease response depending on the simultaneous activity of other hormones. Thus, the increased expression of NPR1 in the susceptible Anastasia was associated with NPR1 enabled transcription of SA-responsive genes for SAR (Ding and Ding 2020) in contrast to Campus, where NPR1 functionality was required for ISR that is independent of SA accumulation, but dependant on responsive JA/ET defence pathways (Withers and Dong 2016). PAD3 encodes the cytochrome P450 enzyme CYP71B15 that catalyses the last step of camalexin biosynthesis, and camalexin plays an important role in both SAR and ISR (Nguyen et al. 2022). SA, but not JA or ET, has been shown to be required for ISR associated with camalexin accumulation. Whilst *Atpad3* mutants were susceptible to *R. solani* suggesting a link between camalexin production and defence, greater PAD3 activity was observed in the susceptible Anastasia. Whilst contradicting, these results are in part explained by the ability of *R. solani* AG2-1 to effectively detoxify camalexin (Pedras and Liu 2004), suggesting an advantageous pathogen adaptation strategy where SAR or ISR involving camalexin are rendered ineffective against AG2-1.

In conclusion, our investigations have shown that susceptibility responses to *R. solani* AG2-1 vary among commercially available varieties of *B. napus*. Cv. Anastasia was most susceptible, Campus showed fewest symptoms, while Skye showed a degree of tolerance under inoculation. Investigating the relative expression of known defence pathway genes in the *B. napus* varieties, mutants and transgenic lines in *A. thaliana* has suggested that auxin signalling plays a role in

susceptibility to AG2-1 of *R. solani*. ABA signalling, likely modified by auxin in compatible interactions, and the MYC2 component of JA signalling were also associated with susceptibility (Figure 11). In contrast, increased defence response was driven by JA/ET signalling and RBOHD (Figure 11). Further studies examining the genetic differences between the OSR varieties used here can identify genes or markers that will inform breeding programs, and lead to the development of more resilient OSR varieties. The broad host range of *R. solani* AG2-1 makes this research findings important for other crops within the same family such as vegetable Brassicas. With limited chemical control options available, it is vital that *R. solani* resistant crops are developed to maintain future yields in fields with high inoculum.

Acknowledgements

Isabelle L. Sims was funded by BBSRC via the University of Nottingham Biotechnology and Biological Sciences Doctoral Training Programme.

References

Adie, B.A.T., Pérez-Pérez, J., Pérez-Pérez, M.M., Godoy, M., Sánchez-Serrano, J.J., Schmelz, E.A. and Solano, R., 2007. ABA is an essential signal for plant resistance to pathogens affecting JA biosynthesis and the activation of defenses in Arabidopsis. *Plant Cell* [Online], 19(5), pp.1665–1681. Available from: https://doi.org/10.1105/tpc.106.048041.

Ajigboye, O.O., Jayaweera, D.P., Angelopoulou, D., Ruban, A. V., Murchie, E.H., Pastor, V., Summers, R. and Ray, R. V., 2021. The role of photoprotection in defence of two wheat genotypes against Zymoseptoria tritici. *Plant Pathology* [Online], 70(6), pp.1421–1435. Available from: https://doi.org/10.1111/ppa.13392.

Aleman, F., Yazaki, J., Lee, M., Takahashi, Y., Kim, A.Y., Li, Z., Kinoshita, T., Ecker, J.R. and Schroeder, J.I., 2016. An ABA-increased interaction of the PYL6 ABA receptor with MYC2 Transcription Factor: A putative link of ABA and JA signaling. *Scientific Reports* [Online], 6(June), pp.1–7. Available from: https://doi.org/10.1038/srep28941.

Bartz, F.E., Glassbrook, N.J., Danehower, D.A. and Cubeta, M.A., 2012. Elucidating the role of the phenylacetic acid metabolic complex in the pathogenic activity of Rhizoctonia solani anastomosis group 3. *Mycologia* [Online], 104(4), pp.793–803. Available from: https://doi.org/10.3852/11-084.

Berrocal-Lobo, M. and Molina, A., 2004. Ethylene Response Factor 1 Mediates Arabidopsis Resistance to the Soilborne Fungus Fusarium oxysporum. *Molecular Plant-Microbe*

Interactions [Online], 17(7), pp.763–770. Available from: https://doi.org/10.1094/mpmi.2004.17.7.763.

Brown, M., Jayaweera, D.P., Hunt, A., Woodhall, J.W., and Ray, R.V. 2021. Yield Losses and Control by Sedaxane and Fludioxonil of Soilborne Rhizoctonia, Microdochium, and Fusarium Species in Winter Wheat. *Plant disease* [Online] 105, pp.2521-2530. Available from: https://doi.org/10.1094/PDIS-11-20-2401-RE

Carling, D.E., Kuninaga, S. and Brainard, K.A., 2002. Hyphal anastomosis reactions, rDNA-internal transcribed spacer sequences, and virulence levels among subsets of Rhizoctonia solani anastomosis group-2 (AG-2) and AG-BI. *Phytopathology* [Online], 92(1), pp.43–50. Available from: https://doi.org/10.1094/PHYTO.2002.92.1.43.

Chapman, J.M., Muhlemann, J.K., Gayomba, S.R. and Muday, G.K., 2019. RBOH-Dependent ROS Synthesis and ROS Scavenging by Plant Specialized Metabolites to Modulate Plant Development and Stress Responses. *Chemical Research in Toxicology* [Online], 32(3), pp.370–396. Available from: https://doi.org/10.1021/acs.chemrestox.9b00028.

Chen, Q., Sun, J., Zhai, Q., Zhou, W., Qi, L., Xu, L., Wang, B., Chen, R., Jiang, H., Qi, X., Palme, K. and Li, C., 2011. The Basic Helix-Loop-Helix Transcription Factor MYC2 Directly Represses PLETHORA Expression during Jasmonate-Mediated Modulation of the Root Stem Cell Niche in Arabidopsis. *The Plant Cell*, 23, pp.3335–3352.

Cordovez, V., Mommer, L., Moisan, K., Lucas-Barbosa, D., Pierik, R., Mumm, R., Carrion, V.J. and Raaijmakers, J.M., 2017. Plant phenotypic and transcriptional changes induced by volatiles from the fungal root pathogen Rhizoctonia solani. *Frontiers in Plant Science* [Online], 8(July), pp.1–14. Available from: https://doi.org/10.3389/fpls.2017.01262.

Ding, P. and Ding, Y., 2020. Stories of Salicylic Acid: A Plant Defense Hormone. *Trends in Plant Science* [Online], 25(6), pp.549–565. Available from: https://doi.org/10.1016/j.tplants.2020.01.004.

Drizou, F., Graham, N.S., Bruce, T.J.A. and Ray, R. V., 2017. Development of high-throughput methods to screen disease caused by Rhizoctonia solani AG 2-1 in oilseed rape. *Plant Methods* [Online], 13(1), pp.1–14. Available from: https://doi.org/10.1186/s13007-017-0195-1.

Finkelstein, R., 2013. Abscisic Acid Synthesis and Response. *The Arabidopsis Book* [Online], 11, p.e0166. Available from: https://doi.org/10.1199/tab.0166.

Foley, R.C., Gleason, C.A., Anderson, J.P., Hamann, T. and Singh, K.B., 2013. Genetic and Genomic Analysis of Rhizoctonia solani Interactions with Arabidopsis; Evidence of Resistance Mediated through NADPH Oxidases. *PLoS ONE* [Online], 8(2). Available from: https://doi.org/10.1371/journal.pone.0056814.

Furukawa, T., Syono, K. 1998. Increased Production of IAA by Rhizoctonia solani is Induced by Culture Filtrate from Rice Suspension Cultures, *Plant Cell Physiol* [Online], 39 (1), pp.43–48, Available from: https://doi.org/10.1093/oxfordjournals.pcp.a029287

Hu, C.H., Wang, P.Q., Zhang, P.P., Nie, X.M., Li, B. Bin, Tai, L., Liu, W.T., Li, W.Q. and Chen, K.M., 2020. NADPH Oxidases: The Vital Performers and Center Hubs during Plant Growth and Signaling. *Cells* [Online], 9(2), pp.1–41. Available from: https://doi.org/10.3390/cells9020437.

Iacobellis, N.S. and DeVay, J.E., 1987. Studies on pathogenesis of Rhizoctonia solani in

beans: an evaluation of the possible roles of phenylacetic acid and its hydroxy derivatives as phytotoxins. *Physiological and Molecular Plant Pathology* [Online], 30(3), pp.421–432. Available from: https://doi.org/10.1016/0885-5765(87)90021-X.

Jayaweera, D., and Ray, R.V. 2022. Yield loss and integrated disease control of Rhizoctonia solani AG2-1 using seed treatment and sowing rate of oilseed rape. *Plant disease* [Online] First look, Available from: https://doi.org/10.1094/PDIS-08-22-1817-RE

Kataria, H.R. and Verma, P.R., 1992. Rhizoctonia solani damping-off and root rot in oilseed rape and canola. *Crop Protection* [Online], 11(1), pp.8–13. Available from: https://doi.org/10.1016/0261-2194(92)90072-D.

Kazan, K. and Manners, J.M., 2013. MYC2: The master in action. *Molecular Plant* [Online], 6(3), pp.686–703. Available from: https://doi.org/10.1093/mp/sss128.

Kidd, B.N., Foley, R., Singh, K.B. and Anderson, J.P., 2021. Foliar resistance to Rhizoctonia solani in Arabidopsis is compromised by simultaneous loss of ethylene, jasmonate and PEN2 mediated defense pathways. *Scientific Reports* [Online], 11(1), pp.1–14. Available from: https://doi.org/10.1038/s41598-021-81858-5.

Kumar, S., Tripathi, D., Okubara, P.A. and Tanaka, K., 2020. Purinoceptor P2K1/DORN1 Enhances Plant Resistance Against a Soilborne Fungal Pathogen, Rhizoctonia solani. *Frontiers in Plant Science* [Online], 11(September), pp.1–10. Available from: https://doi.org/10.3389/fpls.2020.572920.

Lakshman, D.K., Liu, C., Mishra, P.K. and Tavantzis, S., 2006. Characterization of the arom gene in Rhizoctonia solani, and transcription patterns under stable and induced hypovirulence conditions. *Current Genetics* [Online], 49(3), pp.166–177. Available from: https://doi.org/10.1007/s00294-005-0005-6.

Larrieu, A., Champion, A., Legrand, J., Lavenus, J., Mast, D., Brunoud, G., Oh, J., Guyomarc'H, S., Pizot, M., Farmer, E.E., Turnbull, C., Vernoux, T., Bennett, M.J. and Laplaze, L., 2015. A fluorescent hormone biosensor reveals the dynamics of jasmonate signalling in plants. *Nature Communications* [Online], 6(May 2014), pp.1–9. Available from: https://doi.org/10.1038/ncomms7043.

Lee, D.H., Lal, N.K., Lin, Z.J.D., Ma, S., Liu, J., Castro, B., Toruño, T., Dinesh-Kumar, S.P. and Coaker, G., 2020. Regulation of reactive oxygen species during plant immunity through phosphorylation and ubiquitination of RBOHD. *Nature communications* [Online], 11(1), p.1838. Available from: https://doi.org/10.1038/s41467-020-15601-5.

Lefevere, H., Bauters, L. and Gheysen, G., 2020. Salicylic Acid Biosynthesis in Plants. *Frontiers in Plant Science* [Online], 11(April), pp.1–7. Available from: https://doi.org/10.3389/fpls.2020.00338.

Livak, K.J. and Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. *Methods* [Online], 25(4), pp.402–408. Available from: https://doi.org/10.1006/meth.2001.1262.

Lobet, G., Pagès, L. and Draye, X., 2011. A novel image-analysis toolbox enabling quantitative analysis of root system architecture. *Plant Physiology* [Online], 157(1), pp.29–39. Available from: https://doi.org/10.1104/pp.111.179895.

Lorenzo, O., Piqueras, R., Sánchez-serrano, J.J. and Solano, R., 2003. ETHYLENE RESPONSE FACTOR1 integrates signals from Ethylene and Jasmonate pathways in plant defense. [Online], 15, pp.165–178. Available from: https://doi.org/10.1105/tpc.007468.signaling.

Lukan, T. and Coll, A., 2022. Intertwined Roles of Reactive Oxygen Species and Salicylic Acid Signaling Are Crucial for the Plant Response to Biotic Stress. *International Journal of Molecular Sciences* [Online], 23(10). Available from: https://doi.org/10.3390/ijms23105568.

Mandava, N.B., Orellana, R.G., David Warthen, J., Worley, J.F., Dutky, S.R., Finegold, H. and Weathington, B.C., 1980. Phytotoxins in Rhizoctonia Solani: Isolation and Biological Activity of m-Hydroxy-and m-Methoxyphenylacetic Acids. *Journal of Agricultural and Food Chemistry* [Online], 28(1), pp.71–75. Available from: https://doi.org/10.1021/jf60227a009.

Mano, Y. and Nemoto, K., 2012. The pathway of auxin biosynthesis in plants. *Journal of Experimental Botany* [Online], 63(8), pp.2853–2872. Available from: https://doi.org/10.1093/jxb/ers091.

Molla, K.A., Karmakar, S., Chanda, P.K., Sarkar, S.N., Datta, S.K. and Datta, K., 2016. Tissue-specific expression of Arabidopsis NPR1 gene in rice for sheath blight resistance without compromising phenotypic cost. *Plant Science* [Online], 250, pp.105–114. Available from: https://doi.org/10.1016/j.plantsci.2016.06.005.

Nguyen, N.H., Trotel-Aziz, P., Villaume, S., Rabenoelina, F., Clément, C., Baillieul, F. and Aziz, A., 2022. Priming of camalexin accumulation in induced systemic resistance by beneficial bacteria against Botrytis cinerea and Pseudomonas syringae pv. tomato DC3000 . *Journal of Experimental Botany* [Online], 73(11), pp.3743–3757. Available from: https://doi.org/10.1093/jxb/erac070.

Pedras, M.S.C. and Liu, J., 2004. Designer phytoalexins : probing camalexin detoxi fi cation pathways in the phytopathogen Rhizoctonia solani †. pp.1–7.

Redkar, A., Jaeger, E. and Doehlemann, G., 2018. Visualization of Growth and Morphology of Fungal Hyphae in planta Using WGA-AF488 and Propidium Iodide Co-staining. *Bio-Protocol* [Online], 8(14), pp.1–7. Available from: https://doi.org/10.21769/bioprotoc.2942.

Robert-Seilaniantz, A., Grant, M. and Jones, J.D.G., 2011. Hormone crosstalk in plant disease and defense: More than just JASMONATE-SALICYLATE antagonism. *Annual Review of Phytopathology* [Online], 49, pp.317–343. Available from: https://doi.org/10.1146/annurev-phyto-073009-114447.

Saini, S., Sharma, I., Kaur, N. and Pati, P.K., 2013. Auxin: A master regulator in plant root development. *Plant Cell Reports* [Online], 32(6), pp.741–757. Available from: https://doi.org/10.1007/s00299-013-1430-5.

Schmittgen, T., Livak, K. 2008. Analyzing real-time PCR data by the comparative CT method. *Nat Protoc* [Online], 3, pp.1101–1108. Available from: https://doi.org/10.1038/nprot.2008.73

Schneider, C.A., Rasband, W.S. and Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of Image Analysis. *Nat Methods* [Online], 9(7), pp.671–675. Available from: https://doi.org/10.1007/978-1-84882-087-6_9.

Schuhegger, R., Nafisi, M., Mansourova, M., Petersen, B.L., Olsen, C.E., Svatoš, A., Halkier, B.A. and Glawischnig, E., 2006. CYP71B15 (PAD3) catalyzes the final step in camalexin biosynthesis. *Plant Physiology* [Online], 141(4), pp.1248–1254. Available from: https://doi.org/10.1104/pp.106.082024.

Sharon, M., Freeman, S. and Sneh, B., 2011. Assessment of resistance pathways induced in

649 Arabidopsis thaliana by hypovirulent Rhizoctonia spp. isolates. *Phytopathology*
650 [Online], 101(7), pp.828–838. Available from: https://doi.org/10.1094/phyto-09-10-
651 0247.

652 Sugawara, S., Mashiguchi, K., Tanaka, K., Hishiyama, S., Sakai, T., Hanada, K., Kinoshita-
653 Tsujimura, K., Yu, H., Dai, X., Takebayashi, Y., Takeda-Kamiya, N., Kakimoto, T.,
654 Kawaide, H., Natsume, M., Estelle, M., Zhao, Y., Hayashi, K.I., Kamiya, Y. and
655 Kasahara, H., 2015. Distinct characteristics of indole-3-acetic acid and phenylacetic
656 acid, two common auxins in plants. *Plant and Cell Physiology* [Online], 56(8), pp.1641–
657 1654. Available from: https://doi.org/10.1093/pcp/pcv088.

658 Tonnessen, B.W., Manosalva, P., Lang, J.M., Baraoidan, M., Bordeos, A., Mauleon, R.,
659 Oard, J., Hulbert, S., Leung, H. and Leach, J.E., 2015. Rice phenylalanine ammonia-
660 lyase gene OsPAL4 is associated with broad spectrum disease resistance. *Plant*
661 *Molecular Biology* [Online], 87(3), pp.273–286. Available from:
662 https://doi.org/10.1007/s11103-014-0275-9.

663 Verma, P.R., 1996. Biology and control of Rhizoctonia solani on rapeseed : A review.
664 *Phytoprotection* [Online], 77(3), pp.99–111. Available from:
665 https://doi.org/10.7202/706106ar.

666 Vijayan, S. and Kirti, P.B., 2012. Mungbean plants expressing BjNPR1 exhibit enhanced
667 resistance against the seedling rot pathogen, Rhizoctonia solani. *Transgenic Research*
668 [Online], 21(1), pp.193–200. Available from: https://doi.org/10.1007/s11248-011-9521-
669 y.

670 VSN International, 2019. GenStat for Windows 20th Edition [Online]. Hemel Hempstead,
671 UK. Available from: Genstat.co.uk.

672 Wang, K.L.C., Li, H. and Ecker, J.R., 2002. Ethylene biosynthesis and signaling networks.
673 *Plant Cell* [Online], 14(SUPPL.), pp.131–152. Available from:
674 https://doi.org/10.1105/tpc.001768.

675 Wang, Y., Mostafa, S., Zeng, W. and Jin, B., 2021. Function and Mechanism of Jasmonic
676 Acid in Plant Responses to Abiotic and Biotic Stresses. *International Journal of*
677 *Molecular Sciences* [Online], 22(8568). Available from: https://doi.org/https://doi.org/
678 10.3390/ijms22168568.

679 Withers, J. and Dong, X., 2016. Posttranslational Modifications of NPR1: A Single Protein
680 Playing Multiple Roles in Plant Immunity and Physiology. *PLoS Pathogens* [Online],
681 12(8), pp.1–9. Available from: https://doi.org/10.1371/journal.ppat.1005707.

682 Xia, X.J., Zhou, Y.H., Shi, K., Zhou, J., Foyer, C.H. and Yu, J.Q., 2015. Interplay between
683 reactive oxygen species and hormones in the control of plant development and stress
684 tolerance. *Journal of Experimental Botany* [Online], 66(10), pp.2839–2856. Available
685 from: https://doi.org/10.1093/jxb/erv089.

686 Yang, J., Duan, G., Li, C., Liu, L., Han, G., Zhang, Y. and Wang, C., 2019. The Crosstalks
687 Between Jasmonic Acid and Other Plant Hormone Signaling Highlight the Involvement
688 of Jasmonic Acid as a Core Component in Plant Response to Biotic and Abiotic
689 Stresses. *Frontiers in Plant Science* [Online], 10. Available from:
690 https://doi.org/10.3389/fpls.2019.01349.

691 Zheng, X., Koopmann, B. and von Tiedemann, A., 2019. Role of salicylic acid and
692 components of the phenylpropanoid pathway in basal and cultivar-related resistance of
693 oilseed rape (Brassica napus) to Verticillium longisporum. *Plants* [Online], 8(11).

Available from: https://doi.org/10.3390/plants8110491.

Zhou, N., Tootle, T.L. and Glazebrook, J., 1999. Arabidopsis PAD3, a gene required for camalexin biosynthesis, encodes a putative cytochrome P450 monooxygenase. *Plant Cell* [Online], 11(12), pp.2419–2428. Available from: https://doi.org/10.1105/tpc.11.12.2419.

Figure Legends

Figure 1. *Brassica napus* disease symptom scores and damping off effects on root length at 7 days post inoculation with *Rhizoctonia solani* AG2-1. A) Plants were scored from 0-4; 0: symptomless, 1: 25% symptoms, 2: 50% symptoms, 3: 75% symptoms, 4: death. B) Average disease symptom scores for five commercially available *B. napus* varieties. Dark grey bars show average hypocotyl symptom scores and light grey bars show average root symptom scores. Non-inoculated data not shown as no individuals showed symptoms. C) Proportional reduction in root length (relative to non-inoculated plant roots) due to AG2-1 measured using ImageJ and SmartRoot plugin. The total length includes lateral roots. Error bars indicate standard error (SE). Different letters above the bars indicated significant differences using t-test, $p < 0.05$.

Figure 2. Confocal microscopy images showing *Rhizoctonia solani* AG2-1 sclerotia infection on different *Brassica napus* varieties up to 2 days post-inoculation. Alexa Fluor Wheat Germ Agglutinin 488 and Propidium Iodide staining of fungal tissues and *B. napus* roots, respectively, showing the development of infection structures in Anastasia, Skye, and Campus. Yellow arrowheads indicate infection cushions. Red shows staining with Alexa Fluor Wheat Germ Agglutinin 488. Blue shows staining with Propidium Iodide. The images shown were chosen as representatives from a minimum of nine samples of infected roots for each variety. Scale bar = 250μm.

Figure 3. Effect of *Rhizoctonia solani* AG2-1 inoculation on auxin transport and signalling genes (A) AUX1, (B) AXR1, (C) TIR1 and (D) IAA7 in *Brassica napus* varieties Anastasia, Skye and Campus at 8, 24 and 48 hours post inoculation, and (E) proportional reduction in plant leaf area (relative to non-inoculated plants) of *Arabidopsis thaliana* mutant plants at 11 days post inoculation with *Rhizoctonia solani* AG2-1. The data represent log2 fold change of differential gene expression using non-inoculated plants as internal calibrant. Error bars indicate standard error (SE). Asterisk indicates a significant difference according to Student t test, $p < 0.05$.

Figure 4. Response of *Arabidopsis thaliana* $IAA2_{pro}$:GUS lines to *Rhizoctonia solani* AG2-1 at 1 and 3 days post-inoculation. Light microscopy images showing GUS staining in Ws $IAA2_{pro}$:GUS plants. At the time of sampling, *R. solani* growth had not reached the root of plants. Scale bar = 100µm.

Figure 5. Effect of *Rhizoctonia solani* AG2-1 inoculation on Jasmonic acid signalling genes (A) JAR1 and (B) MYC2 in *Brassica napus* varieties Anastasia, Skye and Campus at 8, 24 and 48 hours post inoculation, and (C) proportional reduction in plant leaf area (relative to non-inoculated plants) of *Arabidopsis thaliana* mutant plants at 11 days post inoculation with *Rhizoctonia solani* AG2-1. The data represent log2 fold change of differential gene expression using non-inoculated plants as internal calibrant. Error bars indicate standard error (SE). Asterisk indicates a significant difference according to Student t test, $p < 0.05$.

Figure 6. Confocal microscopy images of *Arabidopsis thaliana* Jas9:VENUS seedlings showing Jasmonic acid response to *R. solani* AG2-1 inoculation. Images are ordered left to right reflecting their location within the taproot relative to the root tip, i.e. Farther right is the root tip, and left is furthest from the root tip. Fluorescence from the Jas9:VENUS biosensor is shown in green. Scale bar = 100µm.

Figure 7. Effect of *Rhizoctonia solani* AG2-1 inoculation on Abscisic acid biosynthesis genes (A) AAO3 and (B) ABI4 in *Brassica napus* varieties Anastasia, Skye and Campus at 8, 24 and 48 hours post inoculation, and (C) proportional reduction in plant leaf area (relative to non-inoculated plants) of *Arabidopsis thaliana* mutant plants at 11 days post inoculation with *R. solani* AG2-1. The data represent log2 fold change of differential gene expression using non-inoculated plants as internal calibrant. Error bars indicate standard error (SE). Asterisk indicates a significant difference according to Student t test, $p < 0.05$.

Figure 8. Effect of *Rhizoctonia solani* AG2-1 inoculation on Ethylene signalling genes (A) EIN2, (B) ERF1 and (C) PDF1.2 in *Brassica napus* varieties Anastasia, Skye and Campus at 8, 24 and 48 hours post inoculation, and (D) proportional reduction in plant leaf area (relative to non-inoculated plants) of *Arabidopsis thaliana* mutant plants at 11 days post inoculation with *R. solani* AG2-1. The data represent log2 fold change of differential gene expression using non-inoculated plants as internal calibrant. Error bars indicate standard error (SE). Asterisk indicates a significant difference according to Student's t test, $p < 0.05$.

Figure 9. Effect of *Rhizoctonia solani* AG2-1 inoculation on Salicylic acid signalling genes (A) ICS1, (B) PAL4, (C) NPR1 and (D) PAD3 in *Brassica napus* varieties Anastasia, Skye and Campus at 8, 24 and 48 hours post inoculation, and (E) proportional reduction in plant leaf area (relative to non-inoculated plants) of *Arabidopsis thaliana* mutant plants at 11 days post inoculation with *R. solani* AG2-1. The data represent log2 fold change of differential gene expression using non-inoculated plants as internal calibrant. Error bars indicate standard error (SE). Asterisk indicates a significant difference according to Student's t test, $p < 0.05$.

Figure 10. Effect of *Rhizoctonia solani* AG2-1 inoculation on respiratory burst oxidase homologs (RBOHs) genes mediating signal transduction via reactive oxygen species production (A) RBOHC and (B) RBOHD in *Brassica napus* varieties Anastasia, Skye and

767 Campus at 8, 24 and 48 hours post inoculation. The data represent log2 fold change of
768 differential gene expression using non-inoculated plants as internal calibrant. Error bars
769 indicate standard error (SE). Asterisk indicates a significant difference according to Student t
770 test, $p < 0.05$.

771 Figure 11. Proposed diagram of hormonal defence pathways involved in the Brassica-
772 *Rhizoctonia solani* AG2-1 interaction. Underlined genes have been tested in this work. Auxin
773 signalling and abscisic acid (ABA) signalling, with the latter likely modified by auxin in
774 compatible interactions, and the MYC2 component of jasmonic acid (JA) signalling together
775 with RBOHC were associated with susceptibility of Brassica hosts to AG2-1 of *R. solani*. In
776 contrast, JA/ethylene (ET) signalling and RBOHD enhanced the defence response to the
777 pathogen. NPR1 functionality dependant on responsive JA/ET defence pathways contributed
778 to induced systemic resistance (ISR) and enhanced defence to AG2-1. In contrast, SAR or ISR
779 by salicylic acid and PAD3 activity were associated with susceptible responses.

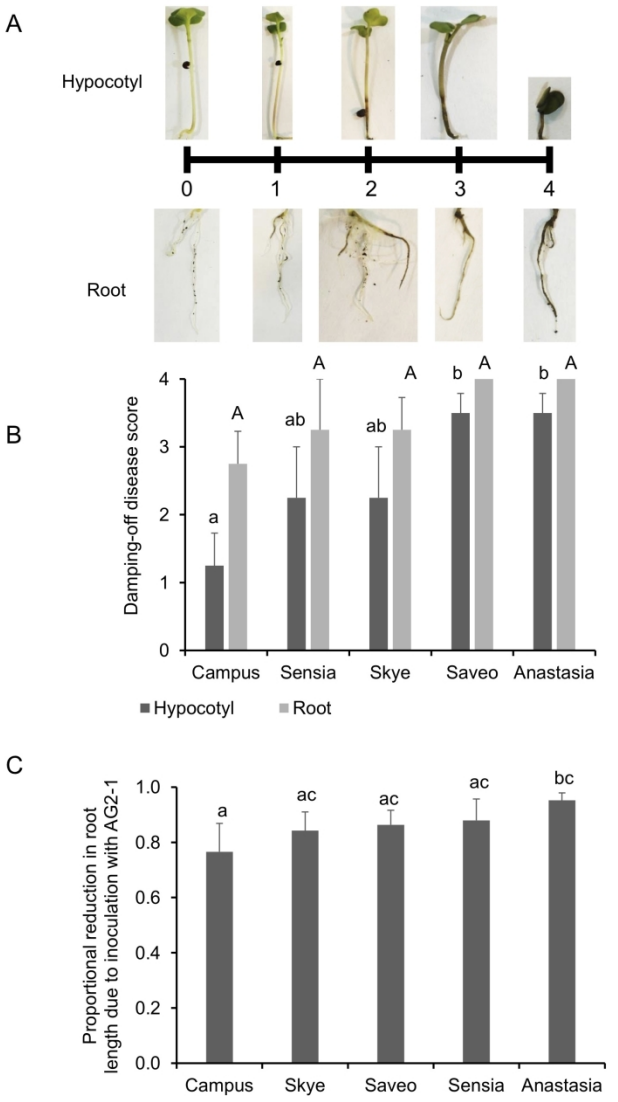
780 Supplementary Figure 1. Effect of *Rhizoctonia solani* AG2-1 inoculation on root length (cm)
781 of cvs. Campus, Skye, Saveo, Sensia and Anastasia of *Brassica napus* (oilseed rape). Error
782 bars indicate standard error (SE). Asterisk indicates a significant difference according to
783 Student's t test, $p < 0.05$.

784 Supplementary Figure 2. Effect of *Rhizoctonia solani* AG2-1 inoculation on (A) plant leaf area
785 of *Arabidopsis thaliana* (Col-0). Images (B) of inoculated and healthy plants shown at 11 days
786 post inoculation. Error bars indicate standard error (SE). Asterisk indicates a significant
787 difference according to Student's t test, $p < 0.05$. Scale bar = 1 cm.

788 Supplementary Figure 3. Effect of *Rhizoctonia solani* AG2-1 inoculation on plant leaf area of
789 Columbia-0 (Col-0) and mutants of *Arabidopsis thaliana* at 11 days post inoculation. Error

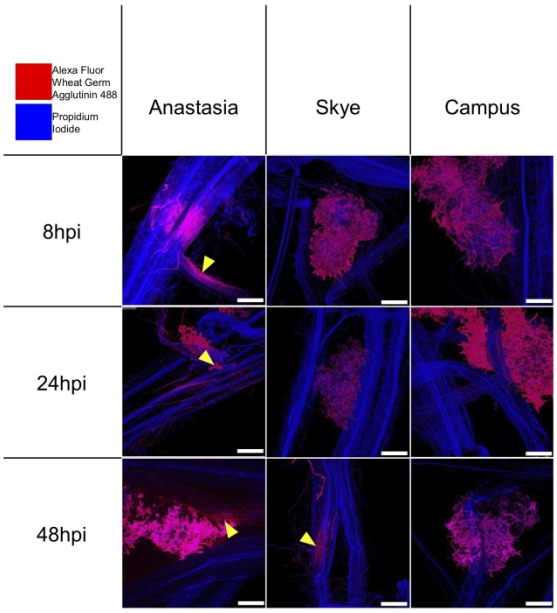
790 bars indicate standard error (SE). Asterisk indicates a significant difference according to

791 Student's t test, $p < 0.05$.



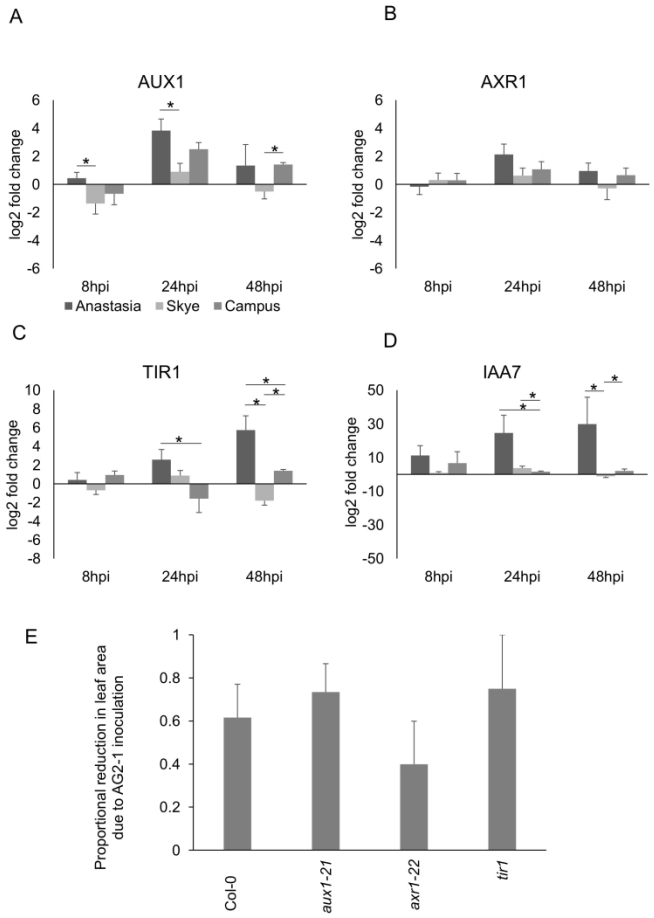
Brassica napus disease symptom scores and damping off effects on root length at 7 days post inoculation with Rhizoctonia solani AG2-1. A) Plants were scored from 0-4; 0: symptomless, 1: 25% symptoms, 2: 50% symptoms, 3: 75% symptoms, 4: death. B) Average disease symptom scores for five commercially available B. napus varieties. Dark grey bars show average hypocotyl symptom scores and light grey bars show average root symptom scores. Non-inoculated data not shown as no individuals showed symptoms. C) Proportional reduction in root length (relative to non-inoculated plant roots) due to AG2-1 measured using ImageJ and SmartRoot plugin. The total length includes lateral roots. Error bars indicate standard error (SE). Different letters above the bars indicated significant differences using t-test, $p < 0.05$.

190x275mm (300 x 300 DPI)



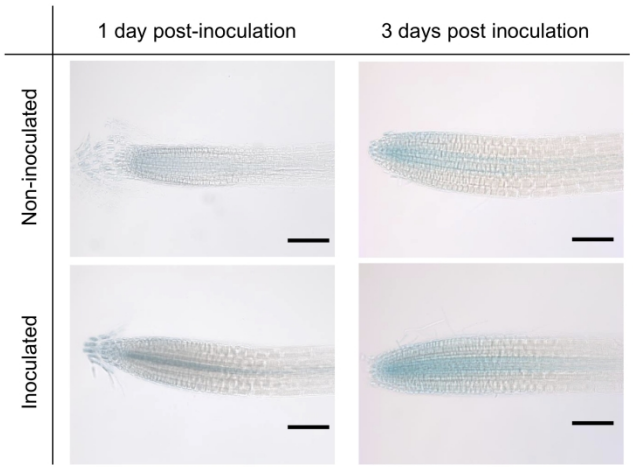
Confocal microscopy images showing Rhizoctonia solani AG2-1 sclerotia infection on different Brassica napus varieties up to 2 days post-inoculation. Alexa Fluor Wheat Germ Agglutinin 488 and Propidium Iodide staining of fungal tissues and B. napus roots, respectively, showing the development of infection structures in Anastasia, Skye, and Campus. Yellow arrowheads indicate infection cushions. Red shows staining with Alexa Fluor Wheat Germ Agglutinin 488. Blue shows staining with Propidium Iodide. The images shown were chosen as representatives from a minimum of nine samples of infected roots for each variety. Scale bar = 250µm.

275x190mm (300 x 300 DPI)



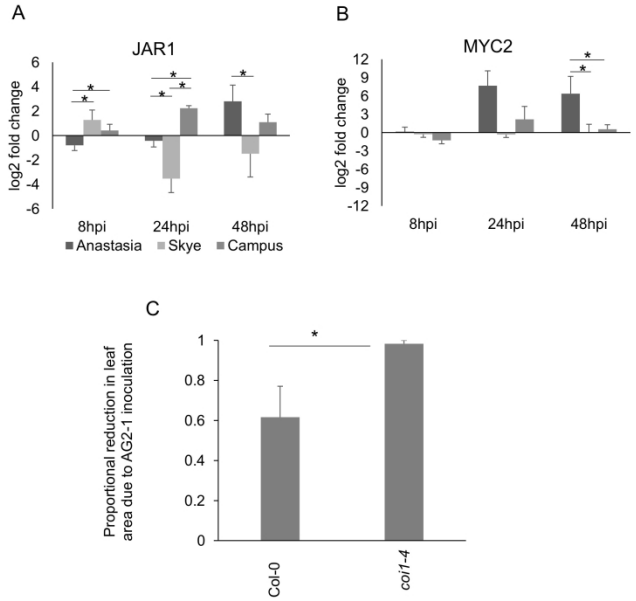
Effect of Rhizoctonia solani AG2-1 inoculation on auxin transport and signalling genes (A) AUX1, (B) AXR1, (C) TIR1 and (D) IAA7 in Brassica napus varieties Anastasia, Skye and Campus at 8, 24 and 48 hours post inoculation, and (E) proportional reduction in plant leaf area (relative to non-inoculated plants) of Arabidopsis thaliana mutant plants at 11 days post inoculation with Rhizoctonia solani AG2-1. The data represent log2 fold change of differential gene expression using non-inoculated plants as internal calibrant. Error bars indicate standard error (SE). Asterisk indicates a significant difference according to Student t test, $p < 0.05$.

190x338mm (300 x 300 DPI)



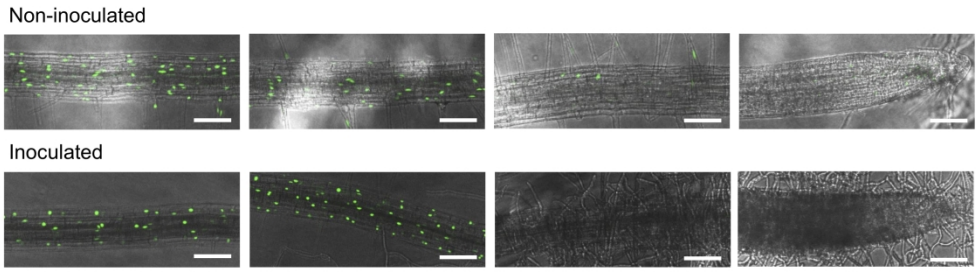
Response of Arabidopsis thaliana IAA2pro:GUS lines to Rhizoctonia solani AG2-1 at 1 and 3 days post-inoculation. Light microscopy images showing GUS staining in Ws IAA2pro:GUS plants. At the time of sampling, R. solani growth had not reached the root of plants. Scale bar = 100μm.

275x190mm (300 x 300 DPI)



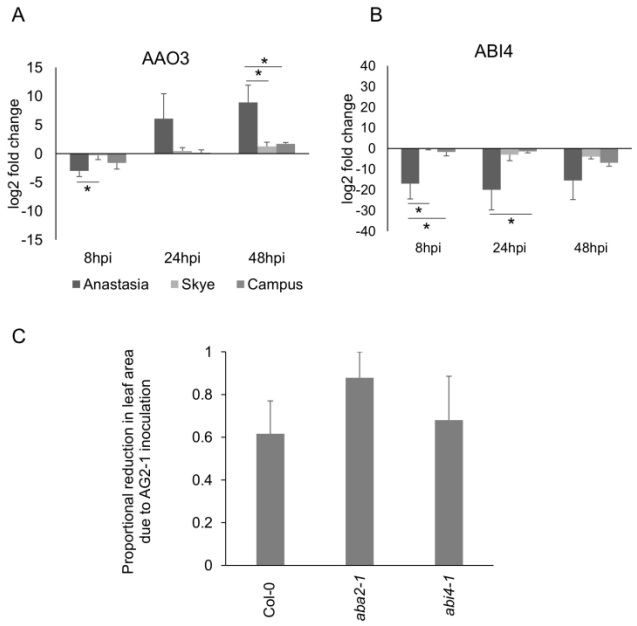
Effect of Rhizoctonia solani AG2-1 inoculation on Jasmonic acid signalling genes (A) JAR1 and (B) MYC2 in Brassica napus varieties Anastasia, Skye and Campus at 8, 24 and 48 hours post inoculation, and (C) proportional reduction in plant leaf area (relative to non-inoculated plants) of Arabidopsis thaliana mutant plants at 11 days post inoculation with Rhizoctonia solani AG2-1. The data represent log2 fold change of differential gene expression using non-inoculated plants as internal calibrant. Error bars indicate standard error (SE). Asterisk indicates a significant difference according to Student t test, $p < 0.05$.

190x338mm (300 x 300 DPI)



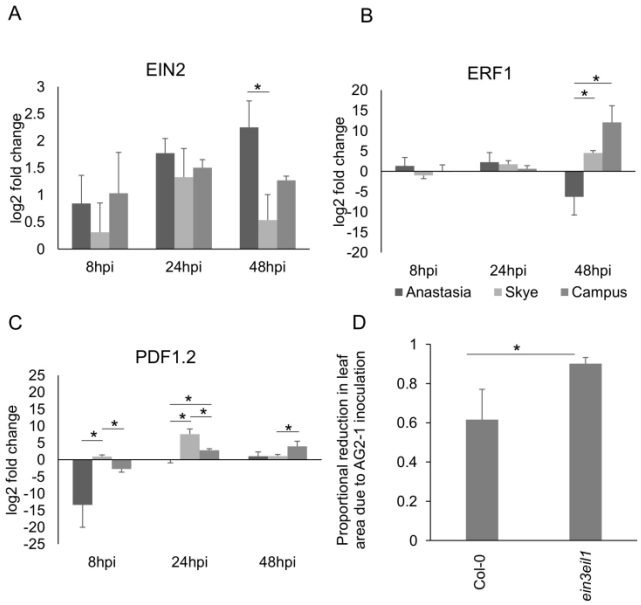
Confocal microscopy images of Arabidopsis thaliana Jas9:VENUS seedlings showing Jasmonic acid response to R. solani AG2-1 inoculation. Images are ordered left to right reflecting their location within the taproot relative to the root tip, i.e. Farther right is the root tip, and left is furthest from the root tip. Fluorescence from the Jas9:VENUS biosensor is shown in green. Scale bar = 100μm.

275x190mm (300 x 300 DPI)



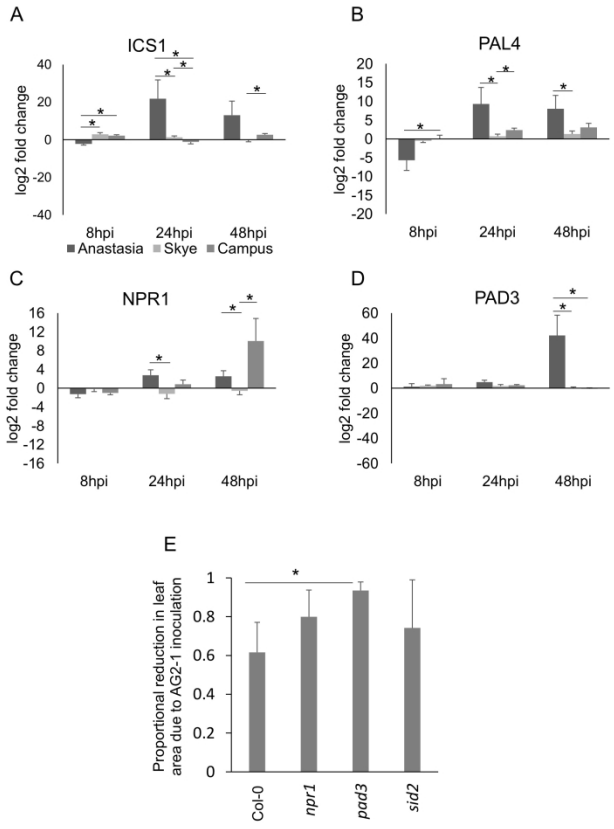
Effect of Rhizoctonia solani AG2-1 inoculation on Abscisic acid biosynthesis genes (A) AAO3 and (B) ABI4 in Brassica napus varieties Anastasia, Skye and Campus at 8, 24 and 48 hours post inoculation, and (C) proportional reduction in plant leaf area (relative to non-inoculated plants) of Arabidopsis thaliana mutant plants at 11 days post inoculation with R. solani AG2-1. The data represent log2 fold change of differential gene expression using non-inoculated plants as internal calibrant. Error bars indicate standard error (SE). Asterisk indicates a significant difference according to Student t test, $p < 0.05$.

190x338mm (300 x 300 DPI)



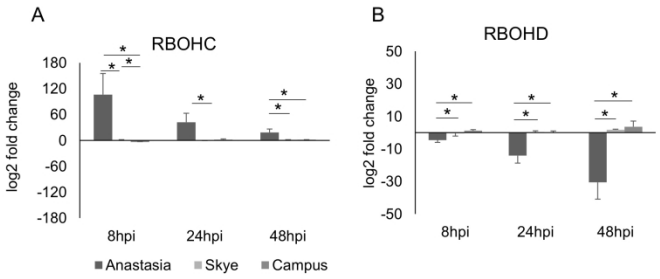
Effect of Rhizoctonia solani AG2-1 inoculation on Ethylene signalling genes (A) EIN2, (B) ERF1 and (C) PDF1.2 in Brassica napus varieties Anastasia, Skye and Campus at 8, 24 and 48 hours post inoculation, and (D) proportional reduction in plant leaf area (relative to non-inoculated plants) of Arabidopsis thaliana mutant plants at 11 days post inoculation with R. solani AG2-1. The data represent log2 fold change of differential gene expression using non-inoculated plants as internal calibrant. Error bars indicate standard error (SE). Asterisk indicates a significant difference according to Student's t test, $p < 0.05$.

190x338mm (300 x 300 DPI)



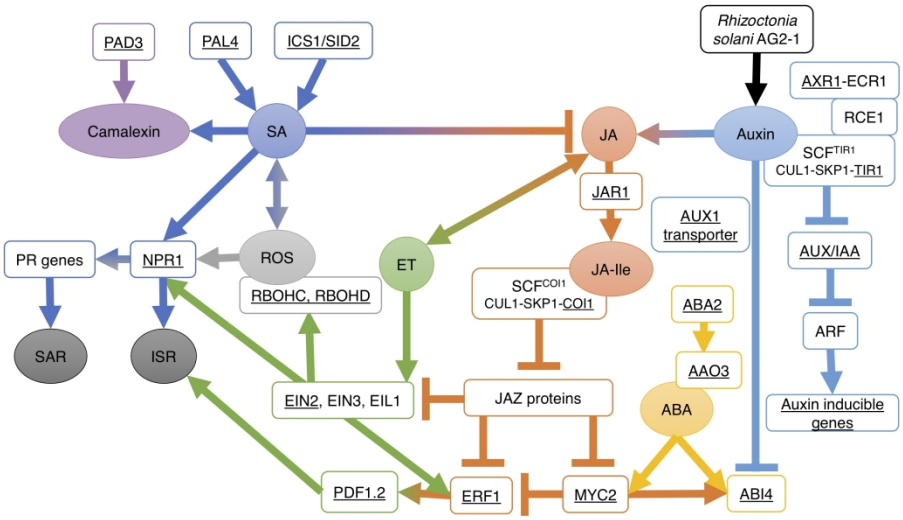
Effect of Rhizoctonia solani AG2-1 inoculation on Salicylic acid signalling genes (A) ICS1, (B) PAL4, (C) NPR1 and (D) PAD3 in Brassica napus varieties Anastasia, Skye and Campus at 8, 24 and 48 hours post inoculation, and (E) proportional reduction in plant leaf area (relative to non-inoculated plants) of Arabidopsis thaliana mutant plants at 11 days post inoculation with R. solani AG2-1. The data represent log2 fold change of differential gene expression using non-inoculated plants as internal calibrant. Error bars indicate standard error (SE). Asterisk indicates a significant difference according to Student's t test, $p < 0.05$.

190x338mm (300 x 300 DPI)



Effect of Rhizoctonia solani AG2-1 inoculation on respiratory burst oxidase homologs (RBOHs) genes mediating signal transduction via reactive oxygen species production (A) RBOHC and (B) RBOHD in Brassica napus varieties Anastasia, Skye and Campus at 8, 24 and 48 hours post inoculation. The data represent log2 fold change of differential gene expression using non-inoculated plants as internal calibrant. Error bars indicate standard error (SE). Asterisk indicates a significant difference according to Student t test, $p < 0.05$.

190x338mm (300 x 300 DPI)

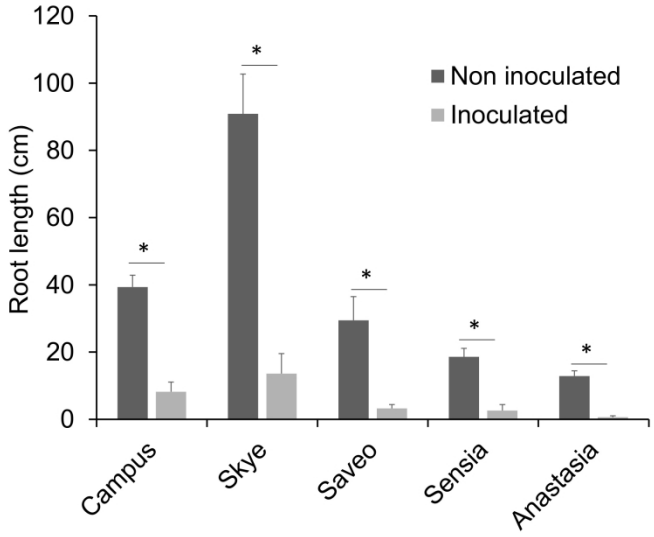


Proposed diagram of hormonal defence pathways involved in the Brassica-Rhizoctonia solani AG2-1 interaction. Underlined genes have been tested in this work. Auxin signalling and abscisic acid (ABA) signalling, with the latter likely modified by auxin in compatible interactions, and the MYC2 component of jasmonic acid (JA) signalling together with RBOHC were associated with susceptibility of Brassica hosts to AG2-1 of R. solani. In contrast, JA/ethylene (ET) signalling and RBOHD enhanced the defence response to the pathogen. NPR1 functionality dependant on responsive JA/ET defence pathways contributed to induced systemic resistance (ISR) and enhanced defence to AG2-1. In contrast, SAR or ISR by salicylic acid and PAD3 activity were associated with susceptible responses.

275x190mm (300 x 300 DPI)

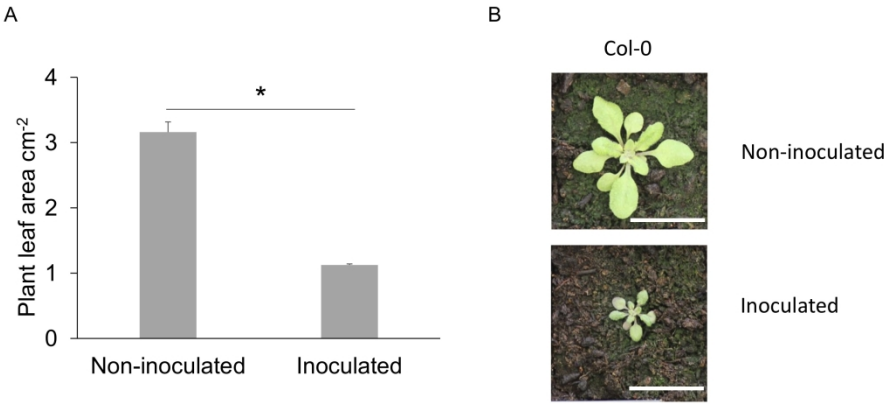
Supplementary Table 1: Primers used for RT-qPCR analysis. All primers were designed using NCBI Primer-BLAST, except the PAL4 primers which were taken from (Zheng *et al.*, 2019).

Gene	Gene name	Forward primer	Reverse primer
AAO3	Abscisic Aldehyde Oxidase 3	TTCCAGCGTGGACTGATGAC	CACTCACATACGGCAATGCG
ABI4	ABA Insensitive 4	GGCCGTTGTTGATCCGGTTA	TTGACCGACCTTTAGGGTTCC
AUX1	Auxin Resistant 1	GCTGCCATCTTCTGGGTTCA	GGGTCCTTTAGTTCTCACTTGC
AXR1	Auxin Resistant 1	TGGCTTGAAGCACAGAGAAGA	CGGCTGAATCGTCCTGAACA
EIN2	Ethylene Insensitive 2	CCAATGGGTTGAAGAAGGACC	GAGGTTTCGACTCTTCGGCT
ERF1	Ethylene Responsive Factor 1	TGTTCAGTCACCGTTCTCCG	CGGAACGTTTTGCTGTGTGG
IAA7	Indole-3-Acetic Acid 7	TGTTCAACCATATGACGGGTTCT	TCCACACCTCACTGGTAACAT
ICS1	Isochorismate Synthase 1	AGCAACCCAACCTCAGAGTG	ACACACTGATTCTCTATTACCCCA
JAR1	Jasmonate Resistant 1	GGGGAAACAGAGGAGAGACC	CAACGTCACCAAGCCGGTAT
MYC2	MYC2, Jasmonate Insensitive 1	GATTGGAGTACCCGAGCAGG	CCGGATTCGGGTTTTCGATG
NPR1	Nonexpresser of PR genes 1	CCCGTGATGGTGTTACAGAGTT	GTGCATGAACGTTGCCAAAC
PAD3	Phytoalexin Deficient 3	TTGGGGATTGCCTGAGAAGG	ACAGCTACCTAAGAATAATACACCC
PAL4	Phenylalanine Ammonia-Lyase 4	GGCACGGACAGTTATGGAGT	GCCGACTTAGGTAGCGTGAG
PDF1.2	Plant Defensin 1.2	CATCACCCTTCTCTTCGCTGC	ATGTCCCACTTGACCTCTCGC
RBOHC	Respiratory Burst Oxidase Homolog C	ACTCCGACGCCGAAAGCAG	TTCCGACCCGGGGGATTTG
RBOHD	Respiratory Burst Oxidase Homolog D	GACGAGGGAATTCAGGAACC	TTCGTTGTCGGAGTTGGTGT
TIR1	Transport Inhibitor Response 1	TCAACCATGAGGGTTTGCCA	GGGCGATGATGAACAGGATTG



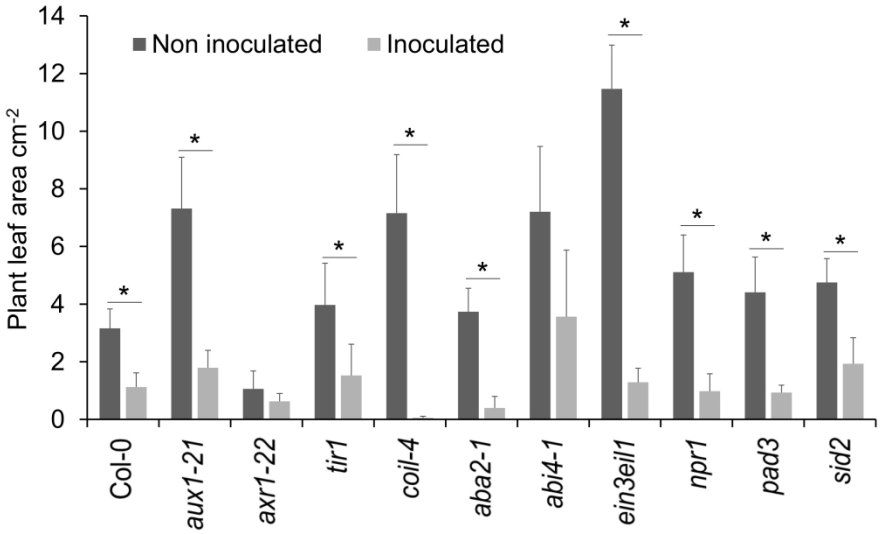
Effect of Rhizoctonia solani AG2-1 inoculation on root length (cm) of cvs. Campus, Skye, Saveo, Sensia and Anastasia of Brassica napus (oilseed rape). Error bars indicate standard error (SE). Asterisk indicates a significant difference according to Student's t test, $p < 0.05$.

275x190mm (300 x 300 DPI)



Effect of Rhizoctonia solani AG2-1 inoculation on (A) plant leaf area of Arabidopsis thaliana (Col-0). Images (B) of inoculated and healthy plants shown at 11 days post inoculation. Error bars indicate standard error (SE). Asterisk indicates a significant difference according to Student's t test, $p < 0.05$. Scale bar = 1 cm.

275x190mm (300 x 300 DPI)



Effect of Rhizoctonia solani AG2-1 inoculation on plant leaf area of Columbia-0 (Col-0) and mutants of Arabidopsis thaliana at 11 days post inoculation. Error bars indicate standard error (SE). Asterisk indicates a significant difference according to Student's t test, $p < 0.05$.

275x190mm (300 x 300 DPI)