Selenium Improved Phenylacetic Acid Content in Oilseed Rape and Thus Enhanced the Prevention of Sclerotinia sclerotiorum by Dimethachlon

Huan Zhang 1,2,†, Qin Cheng 1,†, Xu Wang 2, Wei Jia 1, Jiatao Xie 1, Guocheng Fan 3, Chuang Han 1 and Xiaohu Zhao 1,*

1 State Key Laboratory of Agricultural Microbiology, College of Resources and Environment, Huazhong Agricultural University, Wuhu 430070, China
2 Institute of Quality Standard and Monitoring Technology for Agro-Products of Guangdong Academy of Agricultural Sciences, Guangzhou 510640, China
3 Fujian Key Laboratory for Monitoring and Integrated Management of Crop Pests, Fuzhou 350003, China
* Correspondence: xhzhao@mail.hzau.edu.cn
† These authors contributed equally to this work.

Abstract: Sclerotinia sclerotiorum is a broad-spectrum necrotrophic phytopathogen that can infect many plant species worldwide. The application of fungicides is a common measure for controlling Sclerotinia sclerotiorum. Due to the risk of developing resistance to fungicides, it is imperative to find ways to be environmentally friendly and even effective. Using bioactive compounds in plants to reduce the amounts of fungicides has become a clean and sustainable strategy of controlling Sclerotinia sclerotiorum. Our study found that selenium in soil mediated the phenylacetic acid-related metabolic pathway in oilseed rape and reduced the incidence rate of Sclerotinia sclerotiorum. The growth-inhibition rates of Sclerotinia sclerotiorum were observed at 25.82%, 19.67%, and 52.61% for treatments of 0.8 mg L⁻¹ dimethachlon, 0.1 mg mL⁻¹ phenylacetic acid, and dimethachlon (0.8 mg L⁻¹) + phenylacetic acid (0.1 mg mL⁻¹), respectively. Phenylacetic acid reduced the application amount of dimethachlon and enhanced the inhibition effect for Sclerotinia sclerotiorum. Results also suggested that phenylacetic acid severely damaged the morphological structure, changed the electrical conductivity, and reduced the capacity of acid production and oxalic acid secretion of Sclerotinia sclerotiorum mycelium. Further studies revealed that phenylacetic acid increased the gene-expression level of Ssodc1, Ssodc2, CWDE2 and CWDE10 in mycelium while decreasing the expression level of SsGgt1, and phenylacetic acid + dimethachlon reduced the relative expression level of SsBil. These findings verified that phenylacetic acid could partially replace the amount of dimethachlon, as well as enhance the prevention of Sclerotinia sclerotiorum by dimethachlon, which provides evidence for developing an environment-friendly method for Sclerotinia sclerotiorum control.

Keywords: Sclerotinia sclerotiorum; oilseed rape; inhibition; dimethachlon; phenylacetic acid; gene expression

1. Introduction

Sclerotinia sclerotiorum (S. sclerotiorum) is an aggressive pathogenic fungus with a broad host range and a worldwide distribution that can infect over 400 plant species, including many economically important crops and vegetables, such as rapeseed, sunflower, common bean, soybean, and canola [1,2]. Sclerotinia stem rot (SSR), caused by Sclerotinia, is the primary fungal disease of rapeseed, which severely affects the yield and quality of rapeseed and endangers the production safety of rapeseed in many areas of Canada, America and China [3].

Recently, the methods for controlling plant diseases caused by S. sclerotiorum have mainly focused on agronomic regulation and bio-chemical control [4,5]. S. sclerotiorum...
has a wide host range and no host specificity, therefore, many agricultural practices, such as crop rotation, timely sowing, drainage of drainage ditches and rational fertilization are effective methods against *S. sclerotiorum* [6]. However, agricultural control cannot permanently control sclerotinia disease because sclerotia is resistant to stress and crop rotation. In addition, biological control is a common strategy but is unstable in field-crop sclerotinia-disease prevention and control. Currently, chemical agents are used primarily for the prevention and control of sclerotinia [7]. Unfortunately, with the frequent use of fungicides, sclerotia developed resistance to some commonly used chemical fungicides, leading to a decline in the effect of chemical control, for example, sclerotia strains resistant to dimethachlon (DIM) [8]. Furthermore, environmental pollution and pesticide residues caused by unscientific application of chemical pesticides are becoming increasingly serious. Therefore, reducing the use of pesticides and using plant-derived substances, which is a clean and sustainable strategy, as well as low-cost plant disease control, have increasingly attracted attention.

Recently, compound preparation has played an important role in improving the effectiveness of *S. sclerotiorum* control, expanding the germicidal spectrum, reducing the dosage, delaying the drug resistance of pathogenic bacteria, and prolonging the service life and health of fungicides [9]. The use of fungicides for biologically active compounds of microbial or plant origin together could become a promising approach in crop protection [10–12]. The resulting synergistic or additive effect of both components provided a significant reduction in the efficient concentrations of chemical fungicides. For instance, a high fungicidal activity of the mycelial of *P. chrysogenum* F-24-28 (DMP) was demonstrated [13]. In addition, a combined application of DMP (0.3 g·L\(^{-1}\)) and azoxystrobin at low dosage (2.5 mg·L\(^{-1}\)) showed a high suppressing activity towards *S. sclerotiorum* (even 100% growth inhibition), including inhibition of a sclerotia formation [14].

Many studies have shown that Se can alleviate the damage to plants caused by stress conditions such as heavy metal toxicity, salt damage, drought, and diseases [15,16]. The application of Se in plant protection can improve the yield and quality of crops while ensuring agricultural safety [17]. Under in vitro culture conditions, Se has been shown to inhibit the growth of a variety of plant pathogens [18,19]. A previous study conducted by our group indicated that up-regulation of metabolite (PA) content derived from rape straw pretreated with selenium (Se) in soil improved the inhibition of *S. sclerotiorum* growth [20]. Another study found that PA could be a good alternative chemical reagent to replace the use of DIM [21]. However, the key metabolic pathways affected by Se-mediated up-regulation of PA in dissolved organic matter (DOM) from rape straw were not clear, and whether these metabolic pathways were directly related to the incidence rate of *S. sclerotiorum* needs to be further revealed. Moreover, PA, as a key metabolite in various metabolic pathways of rape, is also a compound of plant origin. How much DIM can be replaced by PA, as well as the action mechanism, needs to be revealed further.

Therefore, the objectives of this study were: (1) to clarify the effect of the PA-related metabolic pathway mediated by Se on the reduction incidence rate of *S. sclerotiorum*; (2) to examine the effect of PA enhancing the prevention of *S. sclerotiorum* by DIM; (3) to illuminate the structure change and biochemical responses of mycelia to PA and DIM, and (4) to investigate the change of pathogenicity gene-expression level in mycelia when treated with PA and DIM. All of these studies could help us to reveal the effect of PA on the growth of *S. sclerotiorum*. The results could provide a novel method and evidence for reducing the use of fungicides and improving the effects of *S. sclerotiorum* control.

2. Materials and Methods

2.1. Pathogens and Reagent Preparation

*S. sclerotiorum* (JZJL-13) was obtained from the Key Laboratory of Crop Disease Monitoring and Safety Control, College of Plant Science and Technology, Huazhong Agricultural University. To obtain a new mycelium of *S. sclerotiorum*, the blade was sterilized and used to cut the sclerotia, then the sclerotia was placed into the medium. After growing
the sclerotia, taking the distance of mycelium at the same radius as the medium, we then inoculated it onto a new PDA and incubated at 23 °C for 48 h.

PA was an analytical reagent purchased from Guangzhou Card Finn Biological Technology Co., Ltd. (Guangzhou, China). DIM, as wettable powder with an effective content of 40%, was purchased from Zhejiang Shengtong Bio Chemical Limited Co., Ltd. (Zhejiang, China). PA and DIM were selected in this study based on the results.

2.2. Metabolite Note and KEGG Pathway Analysis

A field trial with Se treatments (0, 1.12 kg·ha⁻¹) was conducted, rape straw was collected at the mature stage, DOM from rape straw (RSDOM) was obtained, and the RSDOM metabolites of Se₀ and Se₁.₁₂ treatment were investigated by gas chromatography-time-of-flight mass spectrometry [20]. On this basis, to illustrate the relationship between the metabolic pathway of rape regulated by Se and disease resistance further, the metabolites were noted and the KEGG pathway of RSDOM was analyzed with and without Se treatment.

2.3. EC₅₀ of DIM and PA

The effective medium concentrations (EC₅₀) of DIM and PA were determined by the mycelial growth rate method first. EC₅₀ represents a concentration of 50% of maximal effect. DIM and PA solutions were added to PDA medium, the final series concentrations of DIM in PDA were 0, 0.5, 1.0, 2 mg·L⁻¹, and the final series concentrations of PA were 0, 0.05, 0.1, 0.2, 0.4 mg·mL⁻¹. After the medium was cooled and solidified, the mycelium obtained from pathogen preparation was inoculated in the center of the medium. After that, the PDA plate was put upside down in a 23 °C incubator for 48 h. Then, the diameter of each colony was measured, and the inhibition rate and EC₅₀ value were calculated.

2.4. Mycelial Growth Assay and Sclerotial Formation

To examine the effect of PA and DIM on the growth of S. sclerotiorum, 0.8 mg·L⁻¹ DIM and 0.1 mg·mL⁻¹ PA were chosen according to the results of EC₅₀ and previous study [16]. The effects of DIM and PA on mycelial growth of S. sclerotiorum were assayed in PDA medium with different treatments (CK, 0.8 mg·L⁻¹ DIM, 0.1 mg·mL⁻¹ PA, 0.8 mg·L⁻¹ DIM + 0.1 mg·mL⁻¹ PA). Growth of S. sclerotiorum was measured after incubation at 23 °C in darkness for 36 h. The inhibitory ratio (% = (dₜreated − dₜreated)/dₜried). “dₜried” was the treatment with DIM or PA. Each treatment was set for three repetitions, and two identical groups were set to analyze sclerotial formation. To record the number of sclerotia on each PDA plate, each plate was incubated at 23 °C in the dark for 10 d. Then, all the sclerotia on each plate were moved into empty plates, and the fresh weight was recorded.

2.5. Measurement of Pathogenicity on Detached Leaves of Rape

The pathogenicity of S. sclerotiorum was studied on detached leaves of oilseed rape. The leaves were picked from the field experiment that was performed at the eco-agriculture base (30°28′26″ N, 114°2′15″ E), Huazhong Agricultural University, Wuhan, China. Leaves were inoculated with PDA mycelial plugs that were produced by a different treatment (the treatments were the same as the above). In order to help facilitate the infection of mycelia into leaves, all leaves were the same size and wounded with sterile needle tips before inoculation. Then, the leaves were incubated in a growth chamber (23 °C, relative humidity 80%). After 36 h, lesion diameters on each leaf were recorded.

2.6. Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) Analysis

The membrane structure changes of mycelia response to PA and DIM were determined by SEM and TEM. S. sclerotiorum mycelium was cultured with and without treatment on PDA medium that was covered with cellophane in an incubator of 23 °C for 48 h. Then, glass paper was cut into small pieces and placed in tubes containing glutaraldehyde solution.
After 24 h, gradient alcohol series (30%, 50%, 70%, 80%, 90%, 100%) were used only for dehydration [22]. Next, mycelium samples were dried with a critical point dryer in Wuhan (Hitachi HCP-2 from Japan) for 0.5 h. The dried samples were sputter-coated with gold for 100 s at 20 mA using a low-vacuum coater in Wuhan (Leica EM ACE 200 from Germany), generating an approximately 10 nm coating thickness. Finally, mycelia were observed with SEM (Hitachi SU-8010) under an accelerating voltage of 3.0 KV. The TEM (TEM, H-7650, Hitachi, Japan) observation was taken according to a modification method [23]. Sclerotia was collected from four treatments grown at 23 °C for 15 d on PDA medium. Samples were fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH = 7.2) for 4 h at 4 °C and shook several times, followed by a thorough rinse with phosphate buffer for 4 h. Then, the samples were placed into 1% osmium tetroxide at 4 °C for 2 h. After that, the samples were dehydrated in graded acetone series for 4 h and were allowed to immerse in a mixture solution with grade acetone and resin for 4 d. Samples were cut using a Leica Ultracut UCT ultramicrotome and collected into 200-mesh copper grids. After that, samples were dyed with uranyl-acetate and lead citrate for 30 min, and the grids were examined using TEM under an accelerating voltage of 80 kV.

2.7. Electrical Conductivity Assay

The electrical conductivity of the mycelia was measured [24]. Cultivated in PDA medium for 3 days, mycelial plus (5 mm in diameter) from fresh edges of 3-day-old colonies were transferred into 50 mL PDB medium and incubated at 23 °C for 7 days. Mycelia were supposed to be centrifuged at 4000 rpm for 10 min and washed three times with sterilized water. Then, 3 g of mycelia was placed into tubes containing DIM or PA solutions. The electrical conductivity of the solution was monitored by an electrical conductivity meter (DDS-307A, Shanghai Leici Instrument Inc., Shanghai, China) at 0, 10, 20, 30, 60, 120, 180 min, respectively. Three replicates were performed for each treatment.

2.8. Acid Production Determination

The effect of DIM or PA on acid production was determined by PDB medium study, following the method with slight modification [24]. Mycelial plugs (6 mm diameter) were cut from fresh edges of 2-day-old colonies in PDA medium. Then, the samples were transferred into a 50 mL flask with PDB medium with different treatments (CK, DIM, PA, DIM + PA) and incubated at 23 °C in the dark for 48 h. Each 50 mL flask contained five mycelial plugs. Subsequently, the PDB solution was centrifuged (5000 × g, 5 min) and the acid of the liquid was determined by the Seven2Go pH meter S2-Std-Kit (Mettler Toledo instruments Co., Ltd., Shanghai, China). The pH in PDB medium was measured to investigate the change of acid production in mycelium due to different treatments.

2.9. Oxalic Acid (OA) Content Measurement

OA content measurements were carried out with S. sclerotiorum mycelium that was cultured in potato dextrose broth (PDB) medium containing different treatments (CK, DIM, PA, DIM + PA). The supernatant was used to identify the OA content [20]. The OA content was identified using a colorimetric method. Briefly, 0.4 mL supernatant was moved to a tube with 0.1 mL 0.5 mg·mL⁻¹ Fe³⁺ standard solutions, 1 mL KCl-HCl solution (3.7 g·L⁻¹ KCl and 5.4 g·L⁻¹ HCl, pH 2.0) and 0.06 mL 0.5% sulfosalicylic acid (w/v). After 20 min, the absorbance at 510 nm was read from a UV-5200 ultraviolet spectrophotometer, and sodium oxalate served as the standard. Each treatment contained three replicates.

2.10. RNA Isolation and Quantitative Real-Time PCR (qRT-PCR) Analysis

The relative gene-expression levels of six pathogenic genes of S. sclerotiorum (S sodc1, S sodc2, CWDE2, CWDE10, SsBil1, SsGgt1) were assessed by qRT-PCR. Target primer sequences are given in Table 1. S. sclerotiorum mycelium was obtained with different treatments (CK, 0.8 mg·L⁻¹ DIM, 0.1 mg·mL⁻¹ PA, 0.8 mg·L⁻¹ DIM + 0.1 mg·mL⁻¹ PA), and each treatment contained five replicates. Total RNA was extracted using NI-Sclerotinia...
sclerotiorum RNA Reagent (Newbio Industry, Tianjin, China), then RNA was reverse transcribed with reagent from Trans Gen Biotech, Beijing (EasyScript One-Step gDNA Removal and cDNA Synthesis SuperMix). qRT-PCR was performed using an ABI Q6 Flex system (Applied Biosystems, USA). Each sample was repeated three times. The relative expression level of target genes was calculated by $2^{-\Delta\Delta CT}$ method.

Table 1. Primers used for quantitative real-time PCR of target genes [20].

| Gene Name | Forward Primer (5′-…-3′) | Reverse Primer (5′-…-3′) |
|-----------|--------------------------|--------------------------|
| Ssodc1    | GATGAGGGCACCACCTACGGT    | CGGGAGTGCTACGCTTACGGG    |
| Ssodc2    | ATTTCTTGCCAACGCCCAAC     | TGGGCCACGTAAGTTTGGAA     |
| CWDE2     | ATGGCTCTTTCACCACAACC     | ACACCCCCCATTCGCATAATA    |
| CWDE10    | CCAATGGAGATCAGCAAGGT      | TTGTCACATTGCGGAAAAACCCAA |
| SnBi1     | TCAATTCCAGCAACCACATAA    | GAAACATGACACCAACCCACAGAG |
| SsGgt1    | AGATCGCCCAACTTCACTCG     | TTCCTCTCTGAAAGTCGCC      |
| β-tublin  | TTGGATTTGCTCCTTTGACCAG   | AGCGGCCATCATGTTCTTGG     |

2.11. Statistical Analysis

GC-TOF-MS analysis was conducted [25]. Chroma TOF 4.3X Software (Leco), (Carl Schultz, America) equipped with the Leco-Fiehn Rtx5 database was used to identify metabolites. All data analysis was performed with SPSS software version 22.0 and the results were reported as the mean ± standard error (S.E.) of three replicates. One-way analysis of variance (ANOVA) was carried out to analyze the results, which included mycelial growth assay. Differences between different treatments were subjected to the LSD test by Duncan’s multiple comparison ($p < 0.05$). Commercial databases including KEGG [20] and MetaboAnalyst [20] were utilized to search for the pathways of metabolites.

3. Results

3.1. Metabolic Pathway Analysis

Differential metabolites were shown in our study, PA content derived from rape straw was up-regulated with Se added into soil [20]. KEGG pathway enrichment analysis was conducted according to the consequences of differential metabolites (Table 2). Differential metabolites were enriched in 35 pathways, and Se mainly regulated phenylalanine metabolism, alanine, aspartate and glutamate metabolism, lysine biosynthesis, arginine and proline metabolism and the purine metabolism pathway (Figure 1A). PA was mainly involved in tyrosine metabolism and phenylalanine metabolism pathways. A schematic diagram was structured showing that Se mediated the PA-related metabolic pathway and reduced the incidence rate of S. sclerotiorum (Figure 1B).

---

Figure 1. Cont.
Figure 1. (A) KEGG metabolic pathway analysis for DOM from rape straw between group Se0 and Se1.2. The darker the color, the smaller the P value and the more significant the enrichment degree. (B) A schematic diagram showing that Se regulated the metabolic pathway that is relevant to disease resistance and reduced the incidence rate of S. sclerotiorum. Se regulated multiple metabolism pathways and specific metabolites, which were relevant to the effects of systemic resistance.

Table 2. KEGG pathway of different Metabolite.

| Pathway Description | # Compounds (dem) | Compounds (dem) | # Compounds (All) | Compounds (All) |
|---------------------|-------------------|-----------------|-------------------|-----------------|
| Metabolic pathways-Brassica rapa (field mustard) | brp01100 | C00049; C00407; C00042; C00257; C00034; C00036; C00042; C00257; C00034; C00036; C00042 | 36 | C00049; C00407; C00042; C00257; C00034; C00036; C00042; C00257; C00034; C00036; C00042 |
| Biosynthesis of secondary metabolites-Brassica rapa (field mustard) | brp01110 | C00049; C00407; C00042; C00257; C00122; C00047; C00253; C00198; C00188; C00187; C0077; C001762; C00187; C00949 | 13 | C00049; C00407; C00042; C00257; C00122; C00047; C00253; C00198; C00188; C00187; C0077; C001762; C00187; C00949 |
| ABC transporters-Brassica rapa (field mustard) | brp02010 | C00049; C00407; C00121; C00333; C00540; C00047; C00188; C00077; C00064 | 9 | C00049; C00407; C00121; C00333; C00540; C00047; C00188; C00077; C00064 |
| Biosynthesis of amino acids-Brassica rapa (field mustard) | brp01230 | C00049; C00407; C00047; C00188; C00077; C00449; C00064 | 7 | C00049; C00407; C00047; C00188; C00077; C00449; C00064 |
| Alanine, aspartate and glutamate metabolism-Brassica rapa (field mustard) | brp00250 | C00049; C0034; C00042; C00122; C00334; C00438; C00064 | 6 | C00049; C0034; C00042; C00122; C00334; C00438; C00064 |
### Table 2. Cont.

| Pathway | Description | # Compounds (dem) | Compounds (dem) | # Compounds (All) | Compounds (All) |
|---------|-------------|------------------|-----------------|------------------|-----------------|
| brp00760 | Nicotinate and nicotinamide metabolism-Brassica rapa (field mustard) | 6 | C00049; C001042; C00122; C00334; C00394; C00402; C00422; C01022; C00334; C01384; C00253; C002532; C032722 | 8 | C00049; C003042; C00122; C00334; C01384; C00253; C002532; C032722 |
| brp01200 | Carbon metabolism-Brassica rapa (field mustard) | 6 | C00049; C001042; C00257; C001122; C001198; C00184 | 20 | C001037; C003049; C004042; C002057; C00098; C001122; C001126; C001419; C002058; C001258; C01198; C001199; C002032; C00160; C01013; C01172; C00026; C000979; C00184; C00036; C003037; C00294; C0030212; C003087; C000359; C00242; C00559; C00479; C01762; C00385; C00064 |
| brp00230 | Purine metabolism-Brassica rapa (field mustard) | 5 | C000294; C00212; C00242; C01762; C00064 | 12 | C00183; C003037; C000409; C001428; C002047; C00407; C000388; C001052; C00064 |
| brp00970 | Biochemistry-Brassica rapa (field mustard) | 5 | C00049; C001047; C00047; C00188; C00064 | 9 | C00183; C003049; C001428; C002047; C000388; C001052; C00064 |
| brp01210 | 2-Oxocarboxylic acid metabolism-Brassica rapa (field mustard) | 5 | C00049; C001047; C00047; C00226; C00077 | 12 | C00183; C003049; C001428; C002047; C000388; C001052; C00064 |
| brp00053 | Ascorbate and aldarate metabolism-Brassica rapa (field mustard) | 4 | C000979; C00858; C00333; C00942 | 8 | C00137; C008879; C00018; C00333; C00199; C00026; C001040; C005422 |
| brp00220 | Arginine biosynthesis-Brassica rapa (field mustard) | 4 | C00049; C001122; C00077; C00064 | 7 | C00049; C001122; C003027; C00026; C00064; C00077; C00064 |
| brp00240 | Pyrimidine metabolism-Brassica rapa (field mustard) | 4 | C001016; C00299; C00438; C00064 | 10 | C00106; C00299; C00178; C00055; C00099; C00113; C000296; C000326; C000438; C00064 |
| brp00350 | Tyrosine metabolism-Brassica rapa (field mustard) | 4 | C00402; C001122; C01384; C000547 | 8 | C00402; C001122; C01384; C00232; C000811; C000547; C00642; C00536 |
| brp00380 | Phenylalanine metabolism-Brassica rapa (field mustard) | 4 | C00954; C02669; C02172; C005831 | 10 | C00954; C02043; C02470; C02693; C01717; C01164; C02172; C07322; C05635; C05831 |
| brp00410 | Beta-Alanine metabolism-Brassica rapa (field mustard) | 4 | C00049; C001106; C00334; C005670 | 8 | C00049; C001106; C00334; C00099; C00113; C001073; C005670; C03722 |
| brp00650 | Butanone metabolism-Brassica rapa (field mustard) | 4 | C00042; C001122; C00334; C00064 | 8 | C00042; C00189; C00223; C00334; C001884; C00232; C000206; C01089 |
| brp00030 | Pentose phosphate pathway-Brassica rapa (field mustard) | 3 | C00257; C00121; C00198 | 7 | C00257; C00121; C00126; C00258; C00198; C00199; C003752 |
| brp00290 | Biosynthesis-Brassica rapa (field mustard) | 3 | C00407; C00188; C020226 | 6 | C00183; C00407; C002033; C00188; C00411; C02226 |
| brp00300 | Biosynthesis-Brassica rapa (field mustard) | 3 | C00049; C00047; C00464 | 5 | C00049; C00047; C00263; C00026; C000449 |
| brp00330 | Arginine and proline metabolism-Brassica rapa (field mustard) | 3 | C000334; C011877; C00077 | 9 | C00148; C00334; C0111877; C013942; C0131177; C00077; C004031; C00581; C035147 |
| brp00360 | Phenylalanine metabolism-Brassica rapa (field mustard) | 3 | C00042; C00122; C070866 | 8 | C00180; C00042; C001122; C070866; C00811; C00642; C000601; C02137 |
| brp00460 | Beta-Alanine metabolism-Brassica rapa (field mustard) | 3 | C00049; C00407; C005670 | 8 | C00183; C00037; C000409; C00407; C005670; C00152; C01401; C02512 |
| brp00960 | Citrate cycle (TCA cycle)-Brassica rapa (field mustard) | 3 | C00407; C00047; C00253 | 4 | C00407; C00047; C00253; C01479 |
| brp00020 | Oxidative phosphorylation-Brassica rapa (field mustard) | 2 | C00042; C00122 | 6 | C00042; C00122; C001499; C00158; C00026; C00036 |
| brp00190 | Glycine, serine and threonine metabolism-Brassica rapa (field mustard) | 2 | C00042; C00122 | 2 | C00042; C00122 |
| brp00260 | Monobactam biosynthesis-Brassica rapa (field mustard) | 2 | C00049; C00188 | 7 | C00037; C00049; C05519; C00258; C00188; C00263; C00581 |
| brp00261 | Lysine degradation-Brassica rapa (field mustard) | 2 | C00049; C00188 | 3 | C00049; C00059; C00188 |
| brp00310 | Lysine degradation-Brassica rapa (field mustard) | 2 | C00049; C00188 | 7 | C00037; C00049; C00489; C00449; C002727; C00431 |
### Table 2. Cont.

| Pathway | Description | # Compounds (dem) | Compounds (dem) | # Compounds (All) | Compounds (All) |
|---------|-------------|------------------|----------------|------------------|----------------|
| brp00480 | Glutathione metabolism-Brassica rapa (field mustard) Pyruvate | 2 | C05422; C00077 | 4 | C00037; C05422; C00077; C00051 |
| brp00620 | metabolism-Brassica rapa (field mustard) Glyoxylate and dicarboxylate | 2 | C00042; C00122 | 4 | C00042; C00122; C00149; C00036 |
| brp00630 | metabolism-Brassica rapa (field mustard) C5-Branched dibasic acid | 2 | C00042; C00064 | 12 | C00037; C00042; C00149; C00258; C00158; C00209; C00160; C01732; C00026; C00036; C00098; C00064 |
| brp00660 | metabolism-Brassica rapa (field mustard) Pantothenate and CoA biosynthesis-Brassica rapa (field mustard) Nitrogen | 2 | C02341; C02226 | 5 | C04090; C02341; C01732; C00026; C02226 |
| brp00770 | metabolism-Brassica rapa (field mustard) | 2 | C00049; C00106 | 4 | C00183; C00049; C00106; C00099 |
| brp00910 | metabolism-Brassica rapa (field mustard) | 2 | C00192; C00064 | 2 | C00192; C00064 |

#### 3.2. Inhibitory EC\(_{50}\) of PA and DIM on S. sclerotiorum

The S. sclerotiorum inhibitory EC\(_{50}\) of DIM and PA were determined. The inhibitory effects of DIM and PA on S. sclerotiorum growth are presented in Figure 2A,B. The DIM and PA EC\(_{50}\) of S. sclerotiorum were 1.12 mg·L\(^{-1}\) and 0.2091 mg·mL\(^{-1}\), respectively. In the previous study, the inhibitory ratio of S. sclerotiorum was 35.5% and 68.73% with 1 mg·L\(^{-1}\) DIM and 1 mg·L\(^{-1}\) DIM + 0.1 mg·mL\(^{-1}\) PA treatment, respectively [16].

**Figure 2. Inhibitory effects and inhibitory concentration of DIM and PA for S. sclerotiorum.** (A) Baseline of DIM sensitivity to S. sclerotiorum. (B) Baseline of PA sensitivity to S. sclerotiorum.

Combining with the results of EC\(_{50}\), the amount of DIM was reduced; 0.8 mg·L\(^{-1}\) DIM and 0.1 mg·mL\(^{-1}\) PA were chosen to investigate the inhibition of S. sclerotiorum growth, morphological structure changes of S. sclerotiorum, biochemical responses of mycelia and pathogenic gene-expression levels further.

#### 3.3. Effects of PA on S. sclerotiorum Growth and Sclerotial Formation

Compared with the control, all the treatments significantly inhibited the growth of S. sclerotiorum (Figure 3A), while DIM + PA and DIM showed higher growth-inhibition rates than PA. The growth-inhibition rates were 25.82% and 19.67% with treatment of 0.8 mg·L\(^{-1}\) DIM and 0.1 mg·mL\(^{-1}\) PA, respectively (Figure 3B). Compared with the treatment of 1 mg·L\(^{-1}\) DIM, the growth-inhibition rate increased from 35.5% to 52.61% with 0.8 mg·L\(^{-1}\) DIM + 0.1 mg·mL\(^{-1}\) PA. In other words, 0.1 g of PA in 1L solution could replace 20% of DIM and increase the 17.11% growth-inhibition rate of S. sclerotiorum (Table 3).
Increased PA content (g) 

Table 3. Combined inhibition of different concentration of DIM and PA on S. sclerotiorum growth.

| Name     | Treatments | Mycelium Growth (cm) | Inhibitory Ratio (%) |
|----------|------------|----------------------|----------------------|
| CK1      | None       | 6.09 ± 0.00a         | /                    |
| T1       | 1.0 mg/L DIM | 3.96 ± 0.14b         | 35.50%               |
| T2       | None       | 1.92 ± 0.09c         | 68.73%               |
| CK2      | None       | 5.28 ± 0.03a         | /                    |
| T3       | 0.8 mg/L DIM | 3.91 ± 0.07d         | 25.82%               |
| T4       | 0.8 mg/L DIM | 2.50 ± 0.00e         | 52.61%               |
| T4/T1    | Reduced DIM content (mg) (1L solution) | Δ Mycelium growth (cm) | Increased Inhibitory ratio (%) |
|          | None       | 2.13/2.78            | 17.11%               |

Notes: Data were analyzed by one-way ANOVA and shown as means ± SD. Different letters indicate statistically significant differences within the same concentration of DIM (p < 0.05). T4/T1 means that compared with T1, there is a variation in DIM content, PA content, mycelium growth and inhibitory ratio in T4.

In addition, the weight of sclerotia with different treatment after 10 d was determined. Interestingly, compared with the control, the weights of sclerotia in the treatments of DIM and DIM + PA were increased by 37.72% and 73.52%, respectively (Figure 3C).

3.4. Effect of PA on Lesion Development on Detached Leaves

As shown in Figure 3D, the lesion diameter on leaves treated with PA was significantly decreased by 7.4%, while the lesion diameter on leaves showed no significant difference with DIM. Using DIM and PA together inhibited the virulence of mycelia significantly (p < 0.05), and the disease lesion diameter was decreased by 18.9% when compared with the control.
3.5. Morphological Structure Changes of *S. sclerotiorum*

The surface of mycelia in the control treatment was smooth and the connection between mycelia was normal from the scanning electron microscope (Figure 4(Aa,Ba)). After DIM treatment, mycelia were twisted and fractured (Figure 4(Ab,Bb)), while after PA treatment, mycelia were twisted (Figure 4(Ac,Bc)). When treated with DIM and PA, mycelia were shrunk and torn (Figure 4(Ad,Bd)). The mycelia’s inner structure was observed using TEM. The cell wall around the inner cells of untreated mycelia was round and regular in shape, and the outer side of the cell wall was separated from other cells by the intercellular layer (Figure 5(Aa,Ba)). When treated with DIM or PA, the cytoplasm contracted and aggregated, and the cell wall was deformed (Figure 5b,c). After DIM and PA treated together, the cytoplasm was degraded and the cell wall was cracked (Figure 5d).

**Figure 4.** Effect of DIM or PA treatments on structural appearance changes of sclerotia. Scanning electron microscope (SEM) images of mycelia in *S. sclerotiorum* receiving DIM or PA. (A): bars = 50 μm, ×450; (B): bars = 10 μm, ×1000). The treatments were: (a): CK, (b): DIM (0.8 mg·L−1), (c): PA (0.1 mg·mL−1), and (d): DIM (0.8 mg·L−1) + PA (0.1 mg·mL−1), respectively.
Figure 5. Effect of DIM or PA treatments on ultrastructural changes of sclerotia. Representative transmission electron microscopy (TEM) images of sclerotia cells selected from 2 specimens (A,B) in each treatment. Bar = 1 μm. The treatments were: (a): CK, (b): DIM (0.8 mg·L⁻¹), (c): PA (0.1 mg·mL⁻¹), and (d): DIM (0.8 mg·L⁻¹) + PA (0.1 mg·mL⁻¹), respectively.

3.6. Electrical Conductivity, Acid Production and Oxalic Acid Content of Mycelia Analysis

Compared with the control, both DIM and PA significantly inhibited the hydrogen production capacity of mycelia, which was decreased by 80.8%, 19% and 89.6%, respectively. The inhibition effect was stronger when DIM and PA were used together (Figure 6A). Compared with the control, both DIM and PA inhibited the oxalic acid secretion of mycelia; similarly, PA obviously enhanced the inhibition effect when it was used together with DIM. The oxalic acid (OA) secretions were decreased by 3.1%, 4.4% and 7.3%, respectively (Figure 6B). As shown in Figure 6C, the electrical conductivity was increased in 120 min and remained constant after 120 min. Additionally, compared with the control, the electrical conductivity was increased in 120 min and remained constant after 120 min. Additionally, compared with the control, the electrical conductivity was increased in 120 min and remained constant after 120 min. Additionally, compared with the control, the electrical conductivity was increased in 120 min and remained constant after 120 min. Additionally, compared with the control, the electrical conductivity was increased in 120 min and remained constant after 120 min. Additionally, compared with the control, the electrical conductivity was increased in 120 min and remained constant after 120 min. Additionally, compared with the control, the electrical conductivity was increased in 120 min and remained constant after 120 min.
conductivity of *S. sclerotiorum* mycelium under PA and DIM + PA treatments increased by 12.6% and 27.8% at 120 min, respectively. However, it decreased with DIM treatment. It is speculated that DIM at low concentration thickens the mycelium cell wall by membrane exostosis and making substances accumulate, thus reducing the permeability of the cell membrane. The correlation analysis between the disease lesion diameter and OA secretion showed that the Pearson product-moment correlation coefficient was 0.95064, and the R square was 0.9037 (Figure 6D).

**Figure 6.** Effect of Se on the physiological property of *S. sclerotiorum* mycelia. The treatments were: CK, DIM (0.8 mg·L⁻¹), PA (0.1 mg·mL⁻¹), and DIM (0.8 mg·L⁻¹) + PA (0.1 mg·mL⁻¹), respectively. (A) The electrical conductivity of *S. sclerotiorum* mycelia. (B) Acid production of *S. sclerotiorum* mycelia. (C) OA secretion of *S. sclerotiorum* mycelia. (D) The correlation between OA secretion and the disease lesion diameter. Vertical bars indicate standard deviations (SD). Different letters (a, b, c, d) indicate statistically significant differences among the different treatments (p < 0.05).

### 3.7. Changes of Pathogenic Gene-Expression Levels

The relative gene-expression levels of six pathogenic genes of *S. sclerotiorum* were assessed by qRT-PCR. It showed that DIM or PA treatment significantly improved relative expression levels of *Ssodc2, CWDE2, CWDE10*, while the *SsBil1* and *SsGgt1* gene expressions were down-regulated. Furthermore, compared with the control, the expression levels of *Ssodc2, CWDE2, and CWDE10* with DIM treatment were up-regulated by 950%, 660% and 250%, respectively (Figure 7B–D). Obviously, gene-expression levels of *SsGgt1* were decreased by 36.1% in DIM treatment (Figure 7F). Similarly, compared with control, PA and DIM + PA did evoke obvious increases in *Ssodc1, Ssodc2, CWDE2*, and *CWDE10* gene-expression levels, and PA up-regulated the expressions of these genes by 341% and 560%, 80.3% and 76%, respectively. DIM + PA up-regulated the expressions of these genes by 209% and 250%, 48.6% and 147%, respectively (Figure 7A–D). Differently, the relative
gene-expression levels of SsGgt1 with DIM and PA were decreased by 36.1% and 26.7%, while the expression level of SsGgt1 with DIM + PA was increased by 33.4% (Figure 7F).

Figure 7. Relative expression level of six target genes of S. sclerotiorum mycelia incubated for 48 h in PDA medium containing different treatments. Different bars with different letters (a, b, c) are significantly different ($p < 0.05$). The treatments were: CK, DIM (0.8 mg·L$^{-1}$), PA (0.1 mg·mL$^{-1}$), and DIM (0.8 mg·L$^{-1}$) + PA (0.1 mg·mL$^{-1}$), respectively. (A–F) indicated that the relative expression level of Ssode1, Ssode2, CWDE2, CWDE10, SsBil1 and SsGgt1.

4. Discussion

4.1. PA Related-Metabolic Pathway of Rape Regulated by Se Was Relevant to Disease Resistance

In recent years, fungicide application has still been the main method for controlling S. sclerotiorum [26,27]. Many fungicides with specific modes of action, including benzimidazole fungicides carbendazim, dicarboximide fungicides (DCFS) dimethachlon, and other groups, lack durability due to the development of resistance, as well as induced environmental pollution and food safety [28–30]. There is an urgent need to find new alternative fungicides with different modes of action for consistent control of S. sclerotiorum. Our previous research has proved that Se can reduce S. sclerotiorum incidence of rape by increasing plant Se concentration, shifting soil microbial community and functional profiles [31]. Another research study showed that in a field experiment, when sclerotia was retreated with Se (VI), the pathogenicity to oilseed rape leaves was reduced [32]. Interestingly, dissolved organic matter derived from rape straw pretreated with selenium in soil improves the inhibition of S. sclerotiorum growth, and metabolites up-regulated in DOM showed significant
inhibition on *S. sclerotiorum* growth [20]. Therefore, plant-derived metabolites can be used as an environmentally friendly fungicide. In this study, Se regulated multiple metabolism pathways of rape straw (Figure 1A), and the phenylacetic acid in phenylalanine metabolism was one of the up-regulated metabolites as it could affect the content of phenylpropanoids in phenylalanine metabolism (Figure 1B). Phenylpropanoids are ubiquitous plant phenolics that occur in defense root exudates, which are often detected as biomarkers of fungal infections; research has found that resistance to Fusarium graminearum attack in barley is based on the rapid accumulation and secretion of phenylpropanoids (cinnamic acid derivatives) after fungal infection [33–35]. Additionally, the contents of fumarate, an intermediate in the TCA cycle, and succinate, an intermediate in the metabolism of alanine, aspartate and glutamate metabolism, were significantly affected by phenylacetic acid. A study on Brassica rape has shown high levels of fumarate and phenylpropanoids in fungus-infected plants, which is related to the effects of systemic resistance [20,36]. Therefore, the regulation of the PA and PA-related metabolic pathway by Se was beneficial in reducing the incidence rate of *S. sclerotiorum* (Figure 1B).

4.2. PA Reduced Application Amount of DIM and Enhanced Inhibition Effect for *S. sclerotiorum*

PA is an intermediate in organic synthesis of medicine and pesticides [37], and itself is also a pesticide [38] and plant growth hormone [39,40]. It should be noted that PA is harmful when inhaled, ingested or absorbed through the skin. Therefore, the appropriate dosage should be considered in the actual process of use, and the toxicity test should be carried out in advance to avoid its toxicological harm. To compare the inhibitory effect of DIM and PA alone as well as using DIM and PA together on *S. sclerotiorum*, the baseline sensitivity of *S. sclerotiorum* to DIM and PA was established at first. The EC<sub>50</sub> value of the distribution of populations was a key reference for evaluating resistance risk and monitoring resistance levels in the pathogen populations. In this research, *S. sclerotiorum* was tested for sensitivity to different concentrations of DIM and PA through testing the inhibition of mycelial growth. The EC<sub>50</sub> value of DIM was greater than that of PA, which indicated that DIM has stronger resistance than PA (Figure 2A, B). Numerous researchers have reported that a variety of plant pathogens are resistant to DCFs [30].

The mycelial growth, weight of sclerotia in medium and the disease lesion diameter of rape leaves in vitro were measured using 0.8 mg·L<sup>−1</sup> DIM and 0.1 mg·mL<sup>−1</sup> PA, and the results showed that DIM or PA inhibited mycelial growth (Figure 3B). Compared with the treatment of 1 mg·L<sup>−1</sup> DIM, the growth-inhibition rate was increased with 0.8 mg·L<sup>−1</sup> DIM +0.1 mg·mL<sup>−1</sup> PA, which indicated that PA can enhance the prevention of *S. sclerotiorum* by dimethachlon. Similar inhibition effects of fungicides such as flusilazole and demethylation inhibitors (DMIs) observed on hyphae growth and sclerotia development have been reported [41]. Phenylacetic acid is an organic chemical material. Studies have shown that the inhibition rate of filament fungi, *D. bryoniae*, was above 25% when treated with 0.1 mg·mL<sup>−1</sup> phenylacetic acid [42]. Interestingly, the application of 0.8 mg·L<sup>−1</sup> DIM and 0.1 mg·mL<sup>−1</sup> PA obviously improved the *S. sclerotiorum*-inhibition rate and weight of sclerotia after 10 days compared to DIM and PA alone, which indicated that the use of DIM and PA was an effective method for inhibiting *S. sclerotiorum* and that PA could reduce the application amount of DIM. The formation of sclerotia requires appropriate external conditions, such as nutrition, light, pH, humidity and temperature, among which pH is the most critical factor affecting sclerotia formation and the pathogenicity of sclerotia [43]. In this study, compared with the control, the weight of sclerotia increased after 10 days of treatment. This may be due to the number of sclerotia increased in DIM and PA treatment (Figure 3C,D).

4.3. The Inhibitory Effect Mechanism of PA and DIM on *S. sclerotiorum*

When investigating the inhibition effects of DIM and PA on *S. sclerotiorum*, it might involve the following mechanistic processes (Figure 8):
4.3. The Inhibitory Effect Mechanism of PA and DIM on S. sclerotiorum

Previous studies have shown that fungicides (flusilazole and fludilamide) can change the surface morphology and internal structure of the mycelia \cite{28,44}. In this study, morphological changes of mycelial cells were observed. Mycelia were found to be twisted and fractured after DIM treatment and twisted after PA treatment using scanning electron microscopy, and mycelia shrank and tore when DIM and PA were used together. Transmission electron microscopy showed that the cytoplasm contracted and aggregated and the cell wall deformed after DIM and PA treatment. When DIM and PA were used together, the cytoplasm was degraded and the cell wall was cracked. The inhibition of the fungicide on nuclear discs showed that fungicide induced more branches at the tip of the mycelia, which slows the spread of the fungus \cite{41}. The main function of the cell membrane is to increase cellular permeability and participate in intracellular energy as a protein matrix, targeting signal transduction, solute transport and DNA activities such as replication \cite{45}. Results indicated that DIM and PA play a role by destroying cell walls and reaching the cell membrane, thus promoting a series of changes in cells. Ion homeostasis not only plays an essential role in maintaining intracellular energy stability, but also plays an important role in solute transport, metabolic regulation, cell turgor and dynamic equilibrium \cite{46}. When the integrity of the cell membrane is destroyed, leakage occurs in the cells \cite{47}.

1. PA and DIM damaged the cell structure and inhibited the growth of S. sclerotiorum.

Previous studies have shown that fungicides (flusilazole and fludilamide) can change the surface morphology and internal structure of the mycelia \cite{28,44}. In this study, morphological changes of mycelial cells were observed. Mycelia were found to be twisted and fractured after DIM treatment and twisted after PA treatment using scanning electron microscopy, and mycelia shrank and tore when DIM and PA were used together. Transmission electron microscopy showed that the cytoplasm contracted and aggregated and the cell wall deformed after DIM and PA treatment. When DIM and PA were used together, the cytoplasm was degraded and the cell wall was cracked. The inhibition of the fungicide on nuclear discs showed that fungicide induced more branches at the tip of the mycelia, which slows the spread of the fungus \cite{41}. The main function of the cell membrane is to increase cellular permeability and participate in intracellular energy as a protein matrix, targeting signal transduction, solute transport and DNA activities such as replication \cite{45}. Results indicated that DIM and PA play a role by destroying cell walls and reaching the cell membrane, thus promoting a series of changes in cells. Ion homeostasis not only plays an essential role in maintaining intracellular energy stability, but also plays an important role in solute transport, metabolic regulation, cell turgor and dynamic equilibrium \cite{46}. When the integrity of the cell membrane is destroyed, leakage occurs in the cells \cite{47}.

2. PA and DIM reduced pathogenicity of S. sclerotiorum by changing pathogenic factors and growth environment.

After DIM treatment, the conductivity increased with the time of mycelium immersion in deionized water, and the conductivity was significantly lower than that of the control after DIM treatment at any time ($p < 0.05$), which might be due to the resistance of sclerotinia to DIM. However, compared with control, only using PA or using DIM and PA together increased the conductivity, indicating that using PA alone and the use of DIM and PA together may promote electrolyte exosmosis in mycelium cells; this result is consistent with that of Zhou et al. (2014). Similarly, it was found that in the study of the inhibiting mechanism of sclerotinia, a chemical pesticide was used to prevent S. sclerotiorum and the leakage of electrolytes in the cell membrane of S. sclerotiorum \cite{29}. pH is the most critical factor affecting sclerotia formation and the pathogenicity of sclerotinia \cite{43}. In this
study, acid production was decreased after treatment, which induced the improvement of pH and inhibited the pathogenicity of sclerotinia. Oxalic acid is a major pathogenic factor for sclerotinia [48]. OA secretion was reduced obviously after treatment in our study, which also explained that acid production had decreased. The study showed that the pH of the surrounding environment around the infection site is required to determine whether pathogens can successfully infect host plants [24,49]. Importantly, we found that disease lesion diameter was remarkably correlated linearly with OA secretion (R² = 0.951) (Figure 6), which also illustrated that OA secretion is a direct pathogenic factor.

(3) PA and DIM regulated the expression level of the pathogenicity gene and affected the pathogenicity of *S. sclerotiorum*.

Recently, the roles of OA and cell wall-degrading enzymes (CWDEs) have been focused on the molecular aspects of the pathogenicity of *S. sclerotiorum* [50]. CWDEs can help with the penetration and degradation of host cell walls [51]. Odc1 and Odc2 are two putative oxalate decarboxylase genes involved in oxalic acid degradation [52]. Compared with the control, the relative expression levels of *Ssodc1* and *Ssodc2* were increased in our study, which further explained the decrease in OA secretion. The cell wall takes the lead in limiting the infiltration of pathogens and the spread barrier of infection [53]. CWDEs are an essential extracellular enzyme secreted by pathogens, and they can degrade plant cell walls and penetrate plant tissue for infection [54], however, *CWDE2* and *CWDE10* were increased after treatment. Up-regulated CWDE gene expression was associated with low virulence [55]. Similarly, Xu et al. (2015) found that the increase of the CWDE gene did not promote the spread of *sclerotinia* on pea leaves [24]. This may be due to CWDE being influenced by the environment, such as high-pH conditions [56]. In spite of OA and CWDES, *Ggt1* and *Bi1* were related to pathogenic factors. Yu et al. (2015) has proved that the *Bi1* code was a hypothetical BAX inhibitory protein. The protein can induce the full toxicity of sclerotinia [57]. The expression level of *SsBil1* was decreased obviously after using DIM alone as well as using DIM and PA together, which reduced the pathogenicity of *S. sclerotiorum*. The expression level of *SsBil1* showed no significant difference, which might be because PA changed the pH of the environment. In addition, *SsGgt1* (Gamma glutamyl transpeptidase) is the regulation of the antioxidation system of sclerotinia [58]. The expression level of *SsGgt1* was decreased after DIM and PA treatment, while there was no significant difference after using DIM and PA together. Recent research has reported that some secretory proteins related to their virulence do exist in sclerotinia, which may affect the gene-expression levels of *SsGgt1* [59].

5. Conclusions

The Se-mediated PA-related metabolic pathway improved the PA content in oilseed rape and reduced the incidence rate of *S. sclerotiorum*. PA significantly inhibited mycelial growth and pathogenicity as well as enhanced the growth inhibition of *S. sclerotiorum* by DIM. The mycelia morphological structure damage response of DIM was increased by PA. DIM changed the pathogenic factors of *S. sclerotiorum*, and PA enhanced the changes induced by DIM. Further studies revealed that PA and DIM applications regulated the expression level of pathogenic genes. The findings will be helpful to provide evidence that PA has promising potential as an alternative to novel fungicides for sclerotinia in oilseed rape.

**Author Contributions:** H.Z.: Methodology, Data curation, Formal analysis and Writing—original draft. Q.C.: Investigation, Methodology, Data curation. X.W., W.J., J.X., G.F., C.H.: Writing—review and editing. X.Z.: Conceptualization, Funding acquisition, Supervision, Project administration. All authors have read and agreed to the published version of the manuscript.
References

1. Hou, Y.-P.; Mao, X.-W.; Qu, X.-P.; Wang, J.-X.; Chen, C.-J.; Zhou, M.-G. Molecular and biological characterization of Sclerotinia sclerotiorum resistant to the anilinopyrimidine fungicide cyprodinil. Pestic. Biochem. Physiol. 2018, 146, 80–89. [CrossRef] [PubMed]

2. Wei, W.; Pierre-Pierre, N.; Peng, H.; Ellur, V.; Vandemark, G.; Chen, W. The D-galacturonic acid catabolic pathway genes differentially regulate virulence and salinity response in Sclerotinia sclerotiorum. Fungal Genet. Biol. 2020, 145, 103482. [CrossRef] [PubMed]

3. Dong, X.; Ji, R.; Guo, X.; Foster, S.J.; Chen, H.; Dong, C.; Liu, Y.; Hu, Q.; Liu, S. Expressing a gene encoding wheat oxalate oxidase enhances resistance to Sclerotinia sclerotiorum in oilseed rape (Brassica napus). Planta 2008, 228, 331–340. [CrossRef] [PubMed]

4. Zhao, J.; Peltier, A.J.; Meng, J.; Osborn, T.C.; Grau, C.R. Evaluation of Sclerotinia stem rot resistance in oilseed Brassica napus using a petiole inoculation technique under greenhouse conditions. Plant Dis. 2004, 88, 1033–1039. [CrossRef]

5. Li, C.X.; Li, H.U.A.; Sivasithamparam, K.; Fu, T.D.; Li, Y.C.; Liu, S.Y.; Barbetti, M. Expression of field resistance under Western Australian conditions to Sclerotinia sclerotiorum in Chinese and Australian Brassica napus and Brassica juncea germplasm and its relation with stem diameter. Aust. J. Agric. Res. 2006, 57, 1131–1135. [CrossRef]

6. Peltier, A.J.; Bradley, C.A.; Chilvers, M.I.; Malvick, D.K.; Mueller, D.S.; Wise, K.A.; Esker, P.D. Biology, Yield loss and Control of Sclerotinia Stem Rot of Soybean. J. Integr. Pest Manag. 2012, 3, 1–7. [CrossRef]

7. Wang, Z.; Fang, H.; Chen, Y.; Chen, K.; Li, G.; Gu, S.; Tan, X. Overexpression of BnWRKY33 in oilseed rape (Brassica napus) enhances resistance to Sclerotinia sclerotiorum: Enhanced resistance to Sclerotinia sclerotiorum. Mol. Plant Pathol. 2014, 15, 677–689. [CrossRef]

8. Zhou, F.; Zhang, X.-L.; Li, J.-L.; Zhu, F.-X. Dimethachlon Resistance in Sclerotinia sclerotiorum in China. Plant Dis. 2014, 98, 1221–1226. [CrossRef]

9. Xu, T.; Yao, F.; Liang, W.-S.; Li, Y.-H.; Li, D.-R.; Wang, H.; Wang, Z.-Y. Involvement of alternative oxidase in the regulation of growth, development, and resistance to oxidative stress of Sclerotinia sclerotiorum. J. Microbiol. 2012, 50, 594–602. [CrossRef]

10. Dzhavakhiya, V.; Scherbakovka, L.; Semina, Y.; Zhemchuzhina, N.; Campbell, B. Chemosensitization of Plant Pathogenic Fungi to Agricultural Fungicides. Front. Microbiol. 2012, 3, 87. [CrossRef]

11. Kim, K.; Lee, Y.; Ha, A.; Kim, J.-I.; Park, A.R.; Yu, N.H.; Son, H.; Choi, G.J.; Park, H.W.; Lee, C.W.; et al. Chemosensitization of Fusarium graminearum to Chemical Fungicides Using Cyclic Lipopeptides Produced by Bacillus amyloliquefaciens Strain JCK-12. Front. Plant Sci. 2017, 8, 2010. [CrossRef]

12. Shcherbakova, L.; Mikityuk, O.; Arslanova, L.; Stakhcheev, A.; Erokhin, D.; Zavriev, S.; Dzhavakhiya, V. Studying the Activity of Thymol to Improve Fungicidal Effects of Tebuconazole and Difenoconazole Against Some Plant Pathogenic Fungi in Seed or Foliar Treatments. Front. Microbiol. 2021, 12, 629429. [CrossRef] [PubMed]

13. Karpova, N.V.; Yaderets, V.V.; Glagoleva, E.V.; Petrova, K.S.; Ovchinnikov, A.I.; Dzhavakhiya, V.V. Antifungal Activity of the Dry Biomass of Penicillium chrysogenum F-24-28 and Its Application in Combination with Azoxystrobin for Efficient Crop Protection. Agriculture 2021, 11, 935. [CrossRef]

14. Yaderets, V.V.; Karpova, N.V.; Glagoleva, E.V.; Ovchinnikov, A.I.; Petrova, K.S.; Dzhavakhiya, V.V. Inhibition of the Growth and Development of Sclerotinia sclerotiorum (Lib.) De Bary by Combining Azoxystrobin, Penicillium chrysogenum VKM F-4876d, and Bacillus Strains. Agronomy 2021, 11, 2520. [CrossRef]

15. Cui, M.; Hu, C.; Wang, X.; Zhao, Y.; Jia, W.; Sun, X.; Elyamine, A.M.; Zhao, X. Selenium induces changes of rhizosphere bacterial characteristics and enzyme activities affecting chromium/selenium uptake by pak choi (Brassica campestris L. ssp. Chinensis Makino) in chromium contaminated soil. Environ. Pollut. 2019, 249, 716–727. [CrossRef]

16. Tripp, R.C., 3rd; Pilon-Smits, E.A.H. Selenium transport and metabolism in plants: PHYtoRediation and biofortification. J. Hazard. Mater. 2021, 404, 124178. [CrossRef]

17. Sarwar, N.; Akhtar, M.; Kamran, M.A.; Imran, M.; Riaz, M.A.; Kamran, K.; Hussain, S. Selenium biofortification in food crops: Key mechanisms and future perspectives. J. Food Compos. Anal. 2020, 93, 103615. [CrossRef]
18. Shakibaie, M.; Forootanfar, H.; Golkar, Y.; Mohammadi-Khorsand, T.; Shakibaie, M.R. Anti-biofilm activity of biogenic selenium nanoparticles and selenium dioxide against clinical isolates of Staphylococcus aureus, Pseudomonas aeruginosa, and Proteus mirabilis. J. Trace Elem. Med. Biol. 2015, 29, 235–241. [CrossRef]

19. WH, L.; CH, C.; CW, H.; CC, W.; VH, L. Selenite enhances immune response against Pseudomonas aeruginosa PA14 via SKN-1 in Caenorhabditis elegans. PLoS ONE 2014, 9, e105810. [CrossRef]

20. Jia, W.; Hu, C.; Xu, J.; Ming, J.; Zhao, Y.; Cai, M.; Sun, X.; Liu, X.; Zhao, X. Dissolved organic matter derived from rape straw pretreated with selenium in soil improves the inhibition of Sclerotinia sclerotiorum growth. J. Hazard. Mater. 2019, 369, 601–610. [CrossRef]

21. Cheng, Q.; Jia, W.; Hu, C.; Shi, G.; Yang, D.; Cai, M.; Zhan, T.; Tang, Y.; Zhou, Y.; Sun, X.; et al. Enhancement and improvement of selenium in soil to the resistance of rape stem against Sclerotinia sclerotiorum and the inhibition of dissolved organic matter derived from rape straw on mycelium. Environ. Pollut. 2020, 265, 114827. [CrossRef] [PubMed]

22. Jia, W.; Hu, C.; Ming, J.; Zhao, Y.; Xin, J.; Sun, X.; Zhao, X. Action of selenium against Sclerotinia sclerotiorum: Damaging membrane system and interfering with metabolism. Pestic. Biochem. Physiol. 2018, 150, 10–16. [CrossRef] [PubMed]

23. Yu, Y.; Jiang, D.; Xie, J.; Cheng, J.; Li, G.; Yi, X.; Fu, Y. Ss-SL2, a novel cell wall protein with PAN modules, is essential for sclerotial development and cellular integrity of Sclerotinia sclerotiorum. PLoS ONE 2012, 7, e34962. [CrossRef] [PubMed]

24. Firoz, M.J.; Xiao, X.; Zhu, X., Fu, Y.P.; Jiang, D.H.; Schnabel, G.; Luo, C.X. Exploring mechanisms of resistance to dimethachlone in Sclerotinia sclerotiorum. Pest Manag. Sci. 2016, 72, 770–779. [CrossRef]

25. Xu, L.; Xiang, M.; White, D.; Chen, W. pH dependency of sclerotal development and pathogenicity revealed by using genetically defined oxalate-minus mutants of Sclerotinia sclerotiorum. Environ. Microbiol. 2015, 17, 2896–2909. [CrossRef]

26. Kind, T.; Wohlgemuth, G.; Lee, D.Y.; Lu, Y.; Palazoglu, M.; Shahbaz, S.; Fiehn, O. FiehnLib: Mass Spectral and Retention Index Libraries for Metabolomics Based on Quadrupole and Time-of-Flight Gas Chromatography/Mass Spectrometry. Anal. Chem. 2009, 81, 10038–10048. [CrossRef]

27. Li, J.; Kang, T.; Talab, K.M.A.; Zhu, F.; Li, J. Molecular and biochemical characterization of dimethachlone resistant isolates of Sclerotinia sclerotiorum. Pestic. Biochem. Physiol. 2017, 138, 15–21. [CrossRef]

28. Liu, X.; Yin, Y.; Yan, L.; Michailides, T.J.; Ma, Z. Sensitivity to iprodione and boscalid of Sclerotinia sclerotiorum isolates collected from rapeseed in China. Pestic. Biochem. Physiol. 2009, 85, 106–112. [CrossRef]

29. Wang, Y.; Duan, Y.-B.; Zhou, M.-G. Baseline sensitivity and efficacy of fluazinam in controlling Sclerotinia stem rot of rapeseed. Eur. J. Plant Pathol. 2015, 144, 337–343. [CrossRef]

30. Wang, Y.; Hou, Y.-P.; Chen, C.-J.; Zhou, M.-G. Detection of resistance in Sclerotinia sclerotiorum to carbendazim and dimethachlor in Jiangsu Province of China. Australas. Plant Pathol. 2014, 43, 307–312. [CrossRef]

31. Liu, K.; Cai, M.; Hu, C.; Sun, X.; Cheng, Q.; Jia, W.; Yang, T.; Nie, M.; Zhao, X. Selenium (Se) reduces Sclerotinia stem rot disease incidence of oilseed rape by increasing plant Se concentration and shifting soil microbial community and functional profiles. Environ. Pollut. 2019, 254, 113051. [CrossRef] [PubMed]

32. Cheng, Q.; Hu, C.; Jia, W.; Cai, M.; Zhao, Y.; Tang, Y.; Yang, D.; Zhou, Y.; Sun, X.; Zhao, X. Selenium reduces the pathogenicity of Sclerotinia sclerotiorum by inhibiting sclerotial formation and germination. Ecotoxicol. Environ. Saf. 2019, 183, 109503. [CrossRef] [PubMed]

33. Tan, J.; Bednarek, P.; Liu, J.; Schneider, B.; Svatova, A.; Hahlbrock, K. Universally occurring phenylpropanoid and species-specific indolic metabolites in infected and uninfected Arabidopsis thaliana roots and leaves. Phytochemistry 2004, 65, 691–699. [CrossRef] [PubMed]

34. Abdel-Farid, I.B.; Jahangir, M.; van den Hondel, C.A.M.J.J.; Kim, H.K.; Choi, Y.H.; Verpoorte, R. Fungal infection-induced metabolites in Brassica rapa. Plant Sci. 2009, 176, 608–615. [CrossRef]

35. John, K.M.M.; Jung, E.S.; Lee, S.; Kim, J.-S.; Lee, C.H. Primary and secondary metabolites variation of soybean contaminated with Aspergillus sojae. Food Res. Int. 2013, 54, 487–494. [CrossRef]

36. de Oliveira, C.S.; Lião, L.M.; Alcantara, G.B. Metabolic response of soybean plants to Sclerotinia sclerotiorum infection. Phytochemistry 2019, 167, 112099. [CrossRef] [PubMed]

37. Hwang, B.K.; Lim, S.W.; Kim, B.S.; Lee, J.Y.; Moon, S.S. Isolation and in vivo and in vitro antifungal activity of phenylacetic acid and sodium phenylacetate from Streptomyces humissis. Appl. Environ. Microbiol. 2001, 67, 3739–3745. [CrossRef] [PubMed]

38. Jiao, M.; He, W.; Ouyang, Z.; Shi, Q.; Wen, Y. Progress in structural and functional study of the bacterial phenylacetic acid catabolic pathway, its role in pathogenicity and antibiotic resistance. Front. Microbiol. 2022, 13, 964019. [CrossRef]

39. Iwase, A.; Takebayashi, A.; Aoi, Y.; Favero, D.S.; Watanabe, S.; Seo, M.; Kasahara, H.; Sugimoto, K. 4-Fluorobutyric acid promotes plant regeneration as an auxin by being converted to phenylacetic acid via an IBR3-independent pathway. Plant Biotechnol. 2022, 39, 51–58. [CrossRef]

40. Maki, Y.; Soejima, H.; Sugiyama, T.; Watahiki, M.K.; Sato, T.; Yamaguchi, J. 3-Phenyllactic acid is converted to phenylacetic acid and induces auxin-responsive root growth in Arabidopsis plants. Plant Biotechnol. 2022, 39, 111–117. [CrossRef]

41. Hou, Y.-P.; Mao, X.-W.; Wu, L.-Y.; Wang, J.-X.; Mi, B.; Zhou, M.-G. Impact of fluazinam on morphological and physiological characteristics of Sclerotinia sclerotiorum. Pestic. Biochem. Physiol. 2019, 155, 81–89. [CrossRef] [PubMed]

42. Mao, S.; Lee, S.-J.; Hwangbo, H.; Kim, Y.-W.; Park, K.-H.; Cha, G.-S.; Park, R.-D.; Kim, K.-Y. Isolation and characterization of antifungal substances from Burkholderia sp. culture broth. Curr. Microbiol. 2006, 53, 358–364. [CrossRef] [PubMed]
43. Wu, J.; Zhao, Q.; Yang, Q.; Liu, H.; Li, Q.; Yi, X.; Cheng, Y.; Guo, L.; Fan, C.; Zhou, Y. Comparative transcriptomic analysis uncovers the complex genetic network for resistance to Sclerotinia sclerotiorum in Brassica napus. Sci. Rep. 2016, 6, 19007. [CrossRef] [PubMed]

44. Liang, H.-J.; Di, Y.-L.; Li, J.-L.; Zhu, F.-X. Baseline sensitivity and control efficacy of fluazinam against Sclerotinia sclerotiorum. Sci. Rep. 2016, 6, 19007. [CrossRef] [PubMed]

45. Theis, T.; Stahl, U. Antifungal proteins: Targets, mechanisms and prospective applications. Cell. Mol. Life Sci. 2004, 61, 437–455. [CrossRef] [PubMed]

46. Tao, N.; Jia, L.; Zhou, H. Anti-fungal activity of Citrus reticulata Blanco essential oil against Penicillium italicum and Penicillium digitatum. Food Chem. 2014, 153, 265–271. [CrossRef]

47. Lionetti, V.; Fabri, E.; De Caroli, M.; Hansen, A.R.; Willats, W.G.T.; Piro, G.; Bellincampi, D. Three Pectin Methylesterase Inhibitors Protect Cell Wall Integrity for Arabidopsis Immunity to Botrytis. Plant Physiol. 2017, 173, 1844–1863. [CrossRef] [PubMed]

48. Kim, Y.T.; Prusky, D.O.V.; Rollins, J.A. An activating mutation of the Sclerotinia sclerotiorum pac1 gene increases oxalic acid production at low pH but decreases virulence. Mol. Plant Pathol. 2007, 8, 611–622. [CrossRef]

50. Rahmanpour, S.; Backhouse, D.; Nonhebel, H.M. Reaction of glucosinolate-myrosinase defence system in Brassica plants to pathogenicity factor of Sclerotinia sclerotiorum. Eur. J. Plant Pathol. 2010, 128, 429–433. [CrossRef]