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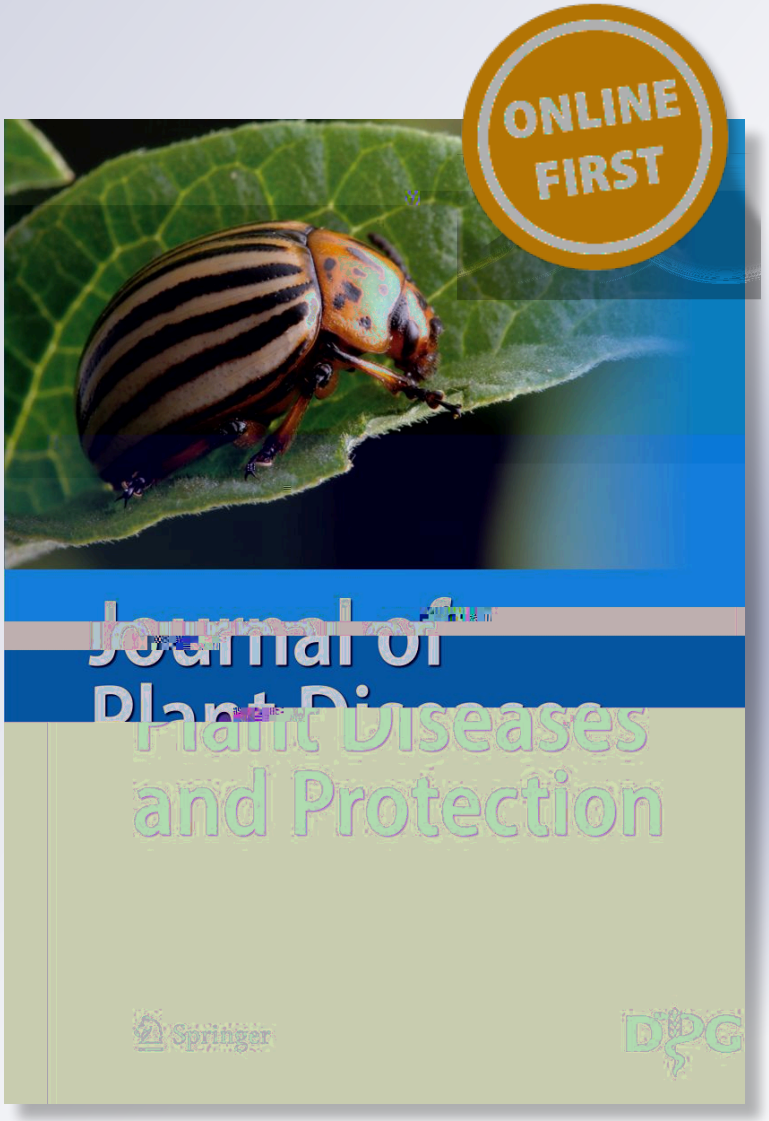

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ORIGINAL ARTICLE

Effect of *Sclerotinia sclerotior m* on lipid metabolism in *Arabidopsis thaliana*

Xiujuan Wang[1,2] · Junmin Li[2] · Peng Zhu[3]

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Abstract *Sclerotinia sclerotiorum* is a devastating fungal pathogen in economically important plants. We know not all but a lot about the molecular host defense to the fungus, but less direct for the pathosystem, which makes the fungal infection difficult to control. In this study, we analyzed the lipid changes in *S. sclerotiorum*-infected *Arabidopsis thaliana*, in the hope of shedding lights on plant defense response to the fungal infection. The full-scan mass spectrometry combined with principal component analysis showed that *A. thaliana* underwent significant lipid metabolic changes following *S. sclerotiorum* infection. The levels of oxylipin-containing PG (phosphatidylglycerol), oxylipin-containing DGDG (digalactosyldiacylglycerol), and lyso-MGDG (monogalactosyldiacylglycerol) were 3, 6, and 24 h after infection, implying that lipoxygenase acts directly on plastid-localized lipid species. *Sclerotinia sclerotiorum* infection also increased the levels of free fatty acids such as C18:3 all time point, which indicates that the high level of C18:3 is likely to be involved in the synthesis of jasmonic acid. In conclusion, *S. sclerotiorum*-induced lipid metabolism of *A. thaliana*, and the synthesized glycerolipids were oxidized to oxylipin-containing glycerolipids and free fatty acids.

Keywords Glycerolipids · Free fatty acids · Quadrupole time-of-flight mass spectrometry · *Sclerotinia sclerotiorum*

Introduction

Sclerotinia sclerotiorum is one of the most devastating fungal pathogens infecting more than 400 species of dicotyledonous plants (Boland and Hall 1994; Dickman 2007). It is a major pathogen of economically important plants including canola, soybean, sunflower, tomato, tobacco, and so on (Hegedus and Rimmer 2005). *Sclerotinia sclerotiorum* can induce the oxidative burst of host plant (Kim et al. 2008), and the reactive oxygen species (ROS) can attack proteins, lipids, and carbohydrates in the cell, causing lipid peroxidation and protein986Tm (cell,)-c98datiot (al.

(Buseman et al. 2006). In response to drought, total leaf lipid contents decreased progressively (Gigon et al. 2004). Recently, lipidomics has emerged as a powerful strategy to fully characterize lipid molecular species and their biological roles with respect to proteins and genes. Information on how lipids change and how the alterations are generated will help us understand the functions of lipids and membranes in plant in response to stress.

Arabidopsis thaliana is one of the most important research models in plant biology, which is also a model in the study of plant–pathogen interaction. Dickman and Mitra (1992) first used *A. thaliana* to study fungal pathogenesis. *Sclerotinia sclerotiorum* may activate jasmonic acid/ethylene-dependent basal defence of in Arabidopsis (Dai et al. 2006). Several signaling pathways including jasmonic acid, salicylic acid and ethylene signaling are involved in regulating defence against *S. sclerotiorum* in Arabidopsis (Guo and Stotz 2007). Jasmonic acid (JA) and its metabolites, commonly from C18 fatty acids, are important signals in plant responses to biotic and abiotic stress (Wasternack 2006). Thus, *A. thaliana* is good model to reveal the molecular mechanism regarding how the functional lipids change in plants after being infected by *S. sclerotiorum*, especially at the lipidomic level. In this work, we used ultra-performance liquid chromatography-electrospray ionization-quadrupole-time-of-flight mass spectrometry (UPLC-ESI-qTOF-MS) to determine the changes of lipids in *A. thaliana* in response to *S. sclerotiorum* infection, in the hope of providing insights into the relationship between the lipid metabolism and the defense response in Sclerotinia-infected plant.

Materials and methods

Chemicals and reagents

Acetonitrile, isopropanol, formic acid, and sodium formate were purchased from Sigma-Aldrich (liquid chromatography–mass spectrometry (LC–MS) grade, St. Louis, MO, USA). Distilled water was filtered through a Milli-Q system (Millipore, Bedford, MA, USA). Leucine-enkephalin was purchased from Sigma-Aldrich (St. Louis, MO, USA). Standards of phosphatidylcholine (PC), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), and phosphatidylinositol (PI) were obtained from the Avanti Polar Lipids, Inc. (Alabaster, AL, USA). Glycolipids standards including monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG) and sulfoquinovosyldiacylglycerol (SQDG) were purchased from Lipid Products (Red19.399999618(stl15.3

was 5 μL. An aliquot of each sample was injected into the column, and 25% of the effluent was split into the mass spectrometer.

Mass spectrometry analysis was performed as descr by Wang et al. (2014).

Analysis of free fatty acids (FFAs) by gas chromatography–mass spectrometry (GC–MS)

Free fatty acids were extracted from the *A. thaliana* tissues according to Kupper et al. (2006). The sample was dred under nitrogen gas. Fatty acids were methylated with boro tr3fluoride-methanol solution (14% in methanol) for 1 h at 60 °C. The fatty acids esters were analyzed on an Aglent Technolog3es 7890A gas chromatography system fitted with a SPB50 fused capillary silica column (30 m × 0.25 mm, 0.25 μm, Supelco, USA) coupled with an Agilent Technologies 5975C mass spectrometer (MS) (Aglent Technolog3es, USA). The injection temperature was 250 °C After injection, the column temperatur was kept at 100 °C for 3 min before being 3ncreased to 230 °C at a rate of 10 °C min^{-1} and kept for 10 min, increased to 240 °C at a rate of 1 °C min^{-1} and kept for 10 min, and increased to a final temperature of 280 °C at a rate of 5 °C min^{-1} and kept for 15 min. The injection volume was 1 μL with a split ratio of 5:1. Mass spectrometry was operated under the electron impact mode with 70 eV of electron energy. The ion soure temperature and interface temperature were set at 230 and 220 °C respectively. The scan range was from m/z 45–450. The analytes were identified on the basis of their retention times by comparing their mass spectra with those record in Nist 11 and DEMO. L Spectrometry Library and those related to the analysis of pure references are commercially available.

Data analysis

All data were presented as mean ± standard deviation (SD). The UPLC-MS data preprocessing was performed with MarkerLynx 4.1 software (Waters, USA). Pareto-scaled MarkerLynx matrices, including the peak number (based on the retention time and m/z), sample name, and the normalized peak intensity, were analyzed by projecting to latent structures with principal component analysis (PCA) using the SIMCA-P^+ software package (V.12.0, Umetrics AB, Umetric, Sweden). For each sampling time point, differences between the treatments and control were analyzed using one-way analyses of variance (ANOVA) followed by a post hoc multiple comparisons test (Dunnett's test). The content of lipid in *A. thaliana* was assessed by ANOVA. All statistical analysis was performed using SPSS 13.0 software. A value of $P < 0.05$ was considered statistically significant.

Results

Changes of lipid species after infection with *Sclerotinia sclerotior m*

Lipid samples extract from the control group (without *S. sclerotiorum* infection) and treatment group (with *S. sclerotiorum* infection) of *A. thaliana* were analyzed under the optimal UPLC-qTOF-MS conditions. Metabolic changes after infection with *S. sclerotiorum* were analyzed using PCA in both positive and negative ion scan modes, which distinguished differences among different treatment groups (Fig. 1a, b). The samples were classified into three clusters: the control group alone; *S. sclerotiorum* infection for 3 h alone; *S. sclerotiorum* infection for 6 and 24 h were classified into a cluster. The plants infected with *S. sclerotiorum* for 6 and 24 h were classified into the same cluster, indicating that the two groups have similar lipid profile. The lipid profile in this cluster was significantly different from that of the control (Fig. 1a, b).

Compared with the control, *S. sclerotiorum* infection caused significant changes in lipids in infected plants (Table S1; Fig. 2a, b). The levels of oxylipin-containing monogalactosyldiacylglycerol (MGDG) decrease significantly at 3 h and then increased significantly at 6 h after *S. sclerotiorum* infection, whereas the levels of sulfoquinovosyldiacylglycerol (SQDG) increased significantly at 3 h and then decrease significantly at 6 h. The levels of MGDG, and phosphatidylcholine (PC) decreased significantly at 3 h after *S. sclerotiorum* infection, whereas the levels of phosphatidylglycerol (PG) increased significantly at 3 h. The levels of phosphatidylethanolamine (PE), oxylipin-containing PG, oxylipin-containing DGDG, and lyso-monogalactosyldiacylglycerol (lyso-MGDG) increased at all the time points after *S. sclerotiorum* infection. (Table S1; Fig. 2a, b). These changes suggest *S. sclerotiorum* infection increases lipolytic activities.

Effects of *Sclerotinia sclerotior m* on free fatty acids (FFAs)

In order to assess the changes of free fatty acids in *A. thaliana* in response to *S. sclerotiorum* infection, GC–MS analyses were carried out to measure the levels of FFAs. A total of eight kinds of fatty acids were identified (Fig. 3). They were presented in both the treatment and control groups, consisting of C14:0, C16:0, C16:1, C16:3, C18:0, C18:1, C18:2, and C18:3 (Fig. 3). The C18:3 was the most abundant species in *A. thaliana*, followed by C16:3, C18:2. Compared with the control, all *S. sclerotiorum*-infected groups had increased the levels of free fatty acids (except C14:0 and C18:2) (Fig. 3).

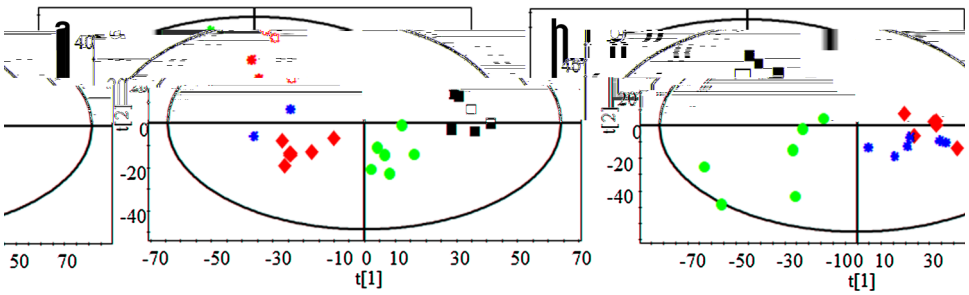

Fig. 1 PCA scores plot for the first two components of *Arabidopsis thaliana* in positive (**a**) and negative (**b**) ion scan mode. Samples were collected from control group(■), infection with *Sclerotinia sclerotiorum* for 1 h (●), 3 h (◆), and 6 h (*), respectively

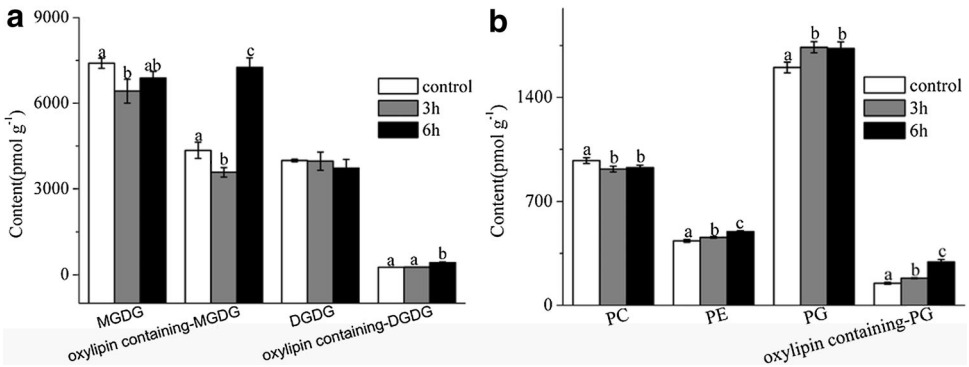

Fig. 2 Effect of *Sclerotinia sclerotiorum* on glycerolipid of *Arabidopsis thaliana* **a** galactolipids (MGDG (monogalactosyldiacylglycerol), DGDG (digalactosyldiacylglycerol), oxylipin-containing-MGDG, oxylipin-containing-DGDG); **b** Phospholipids (PC (phosphatidylcholine), PE (phosphatidylethanolamine), PG (phosphatidylglycerol), oxylipin-containing-PG). Different letters among treatments indicate significant differences (Dunnett's test, $P < 0.05$). *Arabidopsis thaliana* without treatment were used as the control (open columns) and compared to individuals treated with *S. sclerotiorum* for 3 and 6 h. The results are the mean of triplicate experiments and standard error

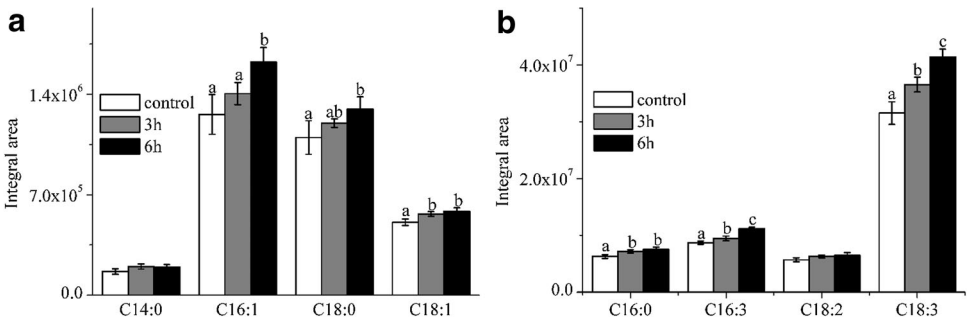

Fig. 3 Effects of *Sclerotinia sclerotiorum* on the level of free fatty acids (FFAs) in *Arabidopsis thaliana*. Different letters among treatments indicate significant differences (Dunnett's test, $P < 0.05$). *Arabidopsis thaliana* without treatment were used as the control (open columns) and compared to individuals treated with *S. sclerotiorum* for 3 and 6 h. The results are the mean of triplicate experiments and standard error

Discussion

We found that *S. sclerotiorum* infection increased the levels of oxylipin-containing PG, oxylipin-containing DGDG, and lyso-MGDG at 3 and 6 h following *S. sclerotiorum* infection. Oxylipins can be synthesized from free fatty acids, and they are also components of plastid-localized polar complex lipids in Arabidopsis (*A. thaliana*) (Buseman et al. 2006). For example, Buseman et al. (2006) reported the oxylipin-containing glycerolipids were accumulated in Arabidopsis leaves in response to wounding. Our previous study found that total level of oxylipin-containing glycolipids (MGDG and DGDG) decreased with increased time of treatment with oligochitosan (data not shown). Oligochitosan was a biological elicitor that can induce metabolic defense (Scheel and Parker 1990). In this study, we found that all the oxylipin-containing glycerolipids (oxylipin-containing MGDG, oxylipin-containing DGDG, oxylipin-containing PG) peaked at 6 h after infection with *S. sclerotiorum*, indicating that the lipid

peroxidation accrued in *A. thaliana* in response to *S. sclerotiorum* infection. MGDG species containing an 18:4-O and a 16:4-O chain and DGDG species containing two 18:4-O chains (as depicted in Table S1) are the major species formed in response to *S. sclerotiorum*, implying that lipoxygenase acts directly on plastid-localized lipid species (Table S1), rather than on free fatty acids released from these lipids. Tokumura et al. (2000) have reported that a lipoxygenase from soybean (*Glycine max*) can act directly on intact phospholipids. 18:3-16:3 MGDG and 18:3-18:3 DGDG are the most abundant galactolipid species in *A. thaliana* in this study. Thus, the high levels of 18:4-O-16:4-O MGDG production and 18:4-O-18:4-O DGDG production post *S. sclerotiorum* infection are consistent with the notion of direct conversion of esterified 18:3 and 16:3 to 18:4-O and 16:4-O, respectively.

The lipid can release free mono- and polyunsaturated fatty acids at *sn-1* or *sn-2* positions when plants are under stresses (Pohnert 2002; Kupper et al. 2006; Ritter et al. 2008). For example, Ritter et al. (2008) reported that copper could increase the release of free polyunsaturated fatty acids (C18:1, C18:2, C18:3, C20:4, C20:5) in *Laminaria digitata*. We found the free fatty acids (C14:0, C16:0, C16:1, C16:3, C18:0, C18:1, C18:2 and C18:3) were increased at all the time points following *S. sclerotiorum* infection. 18:3-16:3 MGDG and 18:3-18:3 DGDG are the most abundant galactolipid species in *A. thaliana*, and 18:3-18:3 MGDG and 18:3-16:3 DGDG are the next most abundant galactolipid species. Thus, by analyzing the structures of MGDG and DGDG at *sn-1* or *sn-2*, we speculated that the lipids may have released free fatty acids (C18:3). The high level of C18:3 is likely to be involved in the synthesis of jasmonic acid, an important signal molecule of plants against necrotrophic pathogens, such as *S. sclerotiorum* (Dai et al. 2006).

Taken together *A. thaliana* displays a strong lipid metabolism in response to *S. sclerotiorum* infection. We speculated that the lipid metabolic defense of *A. thaliana* against *S. sclerotiorum* was via oxidizing glycerolipids to oxylipin-containing glycerolipids and free fatty acids.

The knowledge of *A. thaliana* lipid metabolism in *S. sclerotiorum*-infected *A. thaliana* may help to better understand the interaction between plants and necrotrophic pathogens, which may facilitate the production of strategies for disease control in plants.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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