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Jasmonic acid and abscisic acid play important roles in host–pathogen interaction between *Fusarium graminearum* and wheat during the early stages of fusarium head blight

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ABSTRACT

The effect of phytohormones on the defense response of wheat against *Fusarium graminearum* infection was investigated. Infection of heads with *F. graminearum* induced accumulation of salicylic acid (SA), jasmonic acid (JA), abscisic acid (ABA), and indole acetic acid (IAA). Exogenous phytohormone treatments showed crosstalk between them and a complex effect on expression of the genes *ATB2*, *ExpB6*, *LEA Td16*, *PR1*, *Pdf1.2*, *PR4*. JA treatment reduced *F. graminearum* growth and fusarium head blight (FHB) symptoms while an increase in FHB was observed with ABA. Transient down-regulation of allene oxide synthase (*AOS*) supports a complex role for JA in wheat head.

1. Introduction

Fusarium head blight (FHB), mainly caused by *Fusarium graminearum* Schwabe [teleomorph *Gibberella zeae* (Schwein) Petch], is a devastating disease of cereals worldwide [1]. FHB infection is largely dependent on air temperature and atmospheric humidity during flowering and early stages of kernel development [2]. *F. graminearum* is able to produce mycotoxins such as deoxynivalenol (DON) and its acetylated derivatives, 3- or 15-ADON. As a result of infection, grain yield is reduced and grains contaminated by mycotoxin become a hazard for human and animal health [3,4]. Many countries have imposed maximum allowable limits for DON levels in grain. Resistance to FHB is considered to be a complex and quantitative trait with many regions of the wheat (*Triticum aestivum*) genome contributing to it. In spite of large breeding efforts over the last three decades, few wheat cultivars highly resistant to FHB have been produced so far, insufficient genetic sources with resistance to FHB have been identified, and the molecular mechanisms of their resistance remain unknown.

Phytohormones play important roles in regulating developmental processes and signaling networks involved in plant responses to a wide range of biotic and abiotic stresses [5,6]. Three major signaling molecules, salicylic acid (SA), jasmonic acid (JA) and ethylene (ET), are recognized as major defense hormones against various pathogens [7–9]. SA is associated with resistance against biotrophic and hemibiotrophic pathogens, and with triggering systemic acquired resistance (SAR) in many species, including *Arabidopsis thaliana* and wheat [7,10,11]. Induction of SAR is accompanied by accumulation of SA and up-regulation of a set of genes encoding pathogenesis-related (PR) proteins in dicot plants,

Abbreviations: FHB, fusarium head blight; DON, deoxynivalenol; JA, Jasmonic acid; SA, salicylic acid; ET, ethylene; ABA, Abscisic acid; DPA, dihydrophaseic acid; ABAGE, abscisic acid glucose ester; PA, phaseic acid; 7′OH-ABA, 7′-hydroxy-abscisic acid; neoPA, neo-phaseic acid; IAA, indole-3-acetic acid; IAA-Ala, N-(indole-3-yl-acetyl)-alanine; IAA-Asp, N-(indole-3-yl-acetyl)-aspartic acid; IAA-Glu, N-(indole-3-yl-acetyl)-glutamic acid; RT-qPCR, reverse transcribed quantitative PCR; *AOS*, allene oxide synthase; BSMV, barley stripe mosaic virus.

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such as tobacco and *A. thaliana*, and provides protection against a broad range of pathogens [12–14]. In contrast, pathogens with a necrotrophic lifestyle are more sensitive to JA/ET-mediated defenses [9,10]. The interaction between SA- and JA/ET-dependent signaling is usually antagonistic in *A. thaliana* [6,15,16]. However, synergistic interactions between them have also been reported [6,9,17].

Recent research has also indicated that abscisic acid (ABA), auxins, gibberellins (GAs), cytokinins and brassinosteroids are associated with plant defense and interact with SA, JA and ET signaling pathways [6,18]. However, their roles in plant defense are less well understood than that of SA, JA and ET. Both positive and negative regulation of plant defense against various biotrophic and necrotrophic pathogens has been reported, yet the mechanisms allowing ABA, auxins, GAs, cytokinins and brassinosteroids to affect the balance of the hormone networks and fine tune defense responses specific to different pathogens remain unclear [5,6,19].

F. graminearum has a brief biotrophic-like relationship with its host before switching to the necrotrophic phase [20]. The necrotrophic stage is associated with an increase in vigor of colonization by the fungus and tissue death eventually leads to thorough colonization of the host substrate [1]. Evidences from *A. thaliana* and wheat suggest that SA signaling is activated in parallel with JA-mediated defense pathways following *F. graminearum* infection [16,21,22]. Overlapping of SA and JA pathways is also observed in maize defense response to *F. graminearum* [17]. SA application and SAR could enhance resistance to infection by *F. graminearum* in *A. thaliana*, while stimulation of JA signaling had a negative effect [16,23]. Yet, other observations in monocot and dicot plants indicate that JA is more important than SA in FHB resistance [24–26]. Previous research from our group showed that SA inhibits both spore germination and mycelial growth of *F. graminearum*, and suggested that the direct inhibiting effect of SA plays a role in FHB resistance in wheat [27]. Based on microarray analysis, Li and Yen [25] suggested that ET signaling also contributed to FHB resistance in wheat. However Chen et al. [28] showed that ET signaling facilitated the colonization of *F. graminearum* in both *A. thaliana* and wheat.

The work presented here is a more detailed characterization of the relationship between phytohormones, *F. graminearum* and FHB in a susceptible wheat cultivar, with a particular focus on JA and ABA.

2. Materials and methods

2.1. Plant material and growth conditions

The *T. aestivum* cultivar Roblin, which is very susceptible to *F. graminearum* infection, was used for the plant experiments. Plants were grown in climatically controlled chambers under 16: 8 h day–night cycles at 20: 16 °C, watered as needed and fertilized weekly with 20–20–20 (N–P–K).

2.2. Fungal strain and growth conditions

F. graminearum strain DAOM 180378 (Canadian Fungal Culture Collection, AAFC, Ottawa, ON, Canada) was used throughout. *F. graminearum* spores were induced on PDA (Potato Dextrose Agar, Difco, KS) plates as described previously [27].

The direct effects of JA and ABA (both from Sigma–Aldrich Canada, Oakville, Canada) on mycelial growth and spore germination were tested on modified SNA (Synthetischer Nährstoffarmer Agar [29]) plates (1 g KH_2PO_4, 1 g KNO_3, 0.5 g $MgSO_4$, 0.5 g KCl, 1 g glucose, 1 g sucrose and 20 g agar per liter). The modified SNA medium was supplemented with either 600 μM JA, or ABA at 285, 475 or 950 μM in methanol. For the effect on mycelium growth, each 60 mm Petri dish was inoculated with a 6 mm agar plug cut from the edge of 3–5 d-old cultures obtained from the same medium. Plates without treatment were also done as control. All plates inoculated with an *F. graminearum* plug were grown in a dark cabinet at 28 °C. Seven to 10 replicates were done for each treatment condition. The plates containing JA or ABA were sealed with parafilm while growing in the cabinet. The radial growth of mycelia was estimated at four positions/plate separated by approximately a 45° angle, by measuring the diameter of a unique colony for 2 consecutive d.

The direct effects of JA and ABA on spore germination were respectively tested in 10 ml (in 50 ml Falcon tubes) of a modified liquid SNA medium (1 g KH_2PO_4, 1 g KNO_3, 0.5 g $MgSO_4$, 0.5 g KCl, 5 g glucose and 5 g sucrose per liter) supplemented with ABA at 2.85 and 4.75 mM, or JA at 3.57 and 5.95 mM. The final concentration of spores in the media was 10^5 spores per ml. The cultures were grown at 180 rpm and 28 °C in the dark. One ml from each tube was then sampled and serially diluted in water to a concentration of 1000 spores/ml after 0, 2 and 4 h of treatment. Four hundred spores were uniformly distributed on the surface of modified SNA plates that were not supplemented with hormone. There were three biological replicates for each treatment, and three modified SNA plates were inoculated with spores for each biological replicate at each time point. The number of germinated spores was counted about 60 h after plating, following incubation at 28 °C in a dark cabinet. The plates with no visible growth were incubated at 28 °C for an extra week to confirm that the spores could not germinate.

2.3. Inoculations with F. graminearum

At mid-anthesis, two florets of a central spikelet from one head per plant were inoculated with a suspension containing hormone and 1000 spores of *F. graminearum*. The inoculated plants were subsequently placed in a misting greenhouse at 25–30 °C for 48 h, with 30 s of mist every 15 min, to facilitate infection, then transferred to a normal greenhouse at the same temperature. Two to 7 d after inoculation, disease symptoms were recorded by photography using a Nikon D70 digital camera. At 21 d post-inoculation, FHB disease levels were determined as the number of spikelets with visible symptoms of infection over the total number of spikelets per infected head. At least two independent inoculation experiments were performed for disease assessment, and 5 to 10 plants were used per treatment in each experiment.

To prepare samples for RNA extraction, two florets of each fully developed spikelet of a whole head at mid-anthesis were inoculated twice. First, 5 μl of a 10% (v/v) methanol: water solution supplemented or not (control) with hormone was inoculated followed immediately by 5 μl of a 10% methanol: water solution containing or not 1000 *F. graminearum* spores. After 48 h in the misting room, each floret was inoculated a second time with 10 μl of a 10% (v/v) methanol: water solution supplemented or not with hormone at half of the initial concentration. Initial hormone concentrations were 2 mM JA and 0.76 mM ABA. At 4 d after the initial treatment, the inoculated heads were harvested and ground in liquid nitrogen. Three biological replicates were performed using three heads from 3 plants per treatment. All of the solutions were inoculated into the florets in less than 1.5 h after preparation. JA and ABA solutions were stored on ice before use.

2.4. Hormone treatments on wheat heads

Two florets of each fully developed spikelet of a whole head were treated twice with 15 μl of a 10% methanol: water solution