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# Resistance of *Fusarium poae* in *Arabidopsis* leaves requires mainly functional JA and ET signaling pathways

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ABSTRACT


*Fusarium poae* has been considered as a minor species among those that cause the FHB disease but in recent years several researchers have documented a high frequency of occurrence in several crops. We evaluated the ability of *F. poae* to produce symptoms in *A. thaliana* leaves. Moreover, we analyzed the defense of *A. thaliana* against *F. poae* using SA, JA, and ET mutants and we monitored the expression level of genes involved in the main signaling pathways related to plant defense. Symptoms were observed in the inoculated leaves demonstrating the ability of *F. poae* to infect *A. thaliana* leaves. Moreover, the *npr1-1* mutants presented low symptoms compared to Col-0, *etr2-1*, and *coi1-1* and that the *coi1-1* mutant was the most susceptible genotypes followed by *etr2-1* genotypes. The RT-PCR revealed that *PDF1.2*, *CHI/PR3*, and *ERF1*, three important JA-ET responsive genes and *NPR1* and *PR1*, which are regulated by SA signaling, were expressed upon *F. poae* inoculation. Our results suggest that JA and ET could play a key role in *Arabidopsis* leaves defense against *F. poae* representing the first evaluation of the response of the main *A. thaliana* phytohormones involved in plant defense in the presence of *F. poae*.


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## Introduction

In interaction with the environment, plants are often exposed to different types of stress including abiotic stress caused by temperature or water availability, and biotic stress such as diseases caused mainly by viruses, bacteria or fungi. Disease represents a major cause of the negative impacts of biotic stress on crop yields. One of the most important fungal diseases of small grain cereals is Fusarium Head Blight (FHB) by reducing barley, wheat and oat production and seed quality because of the ability of *Fusarium* species to produce mycotoxins harmful to both human and animal health (McMullen


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et al. 1997; Desjardins 2006). Among *Fusarium* species, *Fusarium graminearum*, *Fusarium poae*, *Fusarium avenaceum*, and *Fusarium culmorum* have been frequently isolated from plant tissue exhibiting FHB symptoms whose occurrence depend on the environment conditions in the moment that disease develops (Nicholson et al. 2003). Commonly, *F. poae* is considered as a minor species due to be less pathogenic and aggressive than other FHB pathogens as *F. graminearum*, but in the last years several researchers have documented a high frequency of occurrence worldwide (Audenaert et al. 2009; Stenglein et al. 2012; Lindblad et al. 2013). Infantino et al. (2012) demonstrated that *F. poae* was the most dominant species isolated from Italian wheat. Recently, Nielsen et al. (2014) showed that *F. poae* is the prevalent species in barley grain in the United Kingdom affecting the quality and safety of malt and beer.

*Fusarium poae* is a necrotrophic pathogen able to produce several mycotoxins not only type A such as HT-2, T-2 and diacetoxyscirpenol (DAS) and B trichothecenes such as nivalenol (NIV) with harmful effects on human and animal health but also other minor mycotoxins such as enniatins, beauvericin, neosolaniol with minor effects on consumers but equally important (Gutleb et al. 2002; Thrane et al. 2004; Meca et al. 2010). Moreover, *F. poae* is considered the most important NIV producer on barley, wheat, maize, and oats (Vogelgsang et al. 2008).

Plants produce several hormones essential for the regulation of plant growth, development, reproduction, and survival. Phytohormones include auxins (AUX), gibberellins (GA), abscisic acid (ABA), cytokinins (CK), salicylic acid (SA), ethylene (ET), jasmonates (JA), brassinosteroids (BR), and peptide hormones, which change their levels during pathogen infection (Adie et al. 2007; Bari & Jones 2009). The induced defense responses are regulated by a network of interconnecting signal transduction pathways in which SA, JA, and ET play key roles. Therefore, the plant resistance to biotrophic pathogens is thought to be mediated through SA signaling, while resistance to necrotrophic pathogens is mediated by JA/ET (Glazebrook 2005).

Several plants have been used as model system to study the plant–pathogen interaction. The most recognized system is the crucifer *Arabidopsis thaliana* L. which has several characteristics that facilitate *Arabidopsis* genome manipulation providing different signaling pathway mutants and transgenic lines useful for plant–pathogen interaction studies (Dangl 1993). Several authors have evaluated the interaction between *Fusarium* species and *Arabidopsis*. Chen et al. (2006) and Makandar et al. (2010) evaluated the behavior of *F. graminearum* on different ecotypes and several mutants of *Arabidopsis* in the main signaling pathways associated with plant defense, respectively. Moreover, Pantelides et al. (2013) used this model to study the *Fusarium oxysporum* pathogenicity. To our knowledge, no previous studies have evaluated the interaction between *F. poae* and *Arabidopsis*. Such studies would provide valuable information regarding the signaling pathways involved in plant defense against *F. poae*. Therefore, the objectives of this study were 1) to test the ability of *F. poae* to infect and to produce symptoms in *A. thaliana* leaves by using two different methods, 2) to evaluate the role of SA, JA, and ET role in the plant defense by testing pathogen virulence on mutant plants deficient in signaling pathways and evaluating expression levels of several genes involved in plant defense.

## Materials and methods

### *Plant material and growth conditions*

Seeds of *Arabidopsis thaliana* ecotype Columbia (Col-0) as wild type (WT) and *npr1-1* [CS3796], *coi1-1* [CS68796], and *etr2-1* [CS67924] mutants were used. All the mutants used were in Col-0 background. Seeds were surface sterilized with 50 % ethanol for 3 min, then in 2 % sodium hypochlorite for 3 min and finally rinsed three times in sterile distilled water. All seeds were vernalized at 4 °C and were sown into 8-cm-diameter pots, each containing approximately 200 $cm^3$ of sterilized soil: perlite: vermiculite mixture (4:1:1) at 20 °C–24 °C with 16 h of light (150 $\mu E\ m^{-2}\ s^{-1}$) in a controlled environmental growth chamber. The plants were watered as needed.

### *Fungal isolates and inoculum preparation*

A total of four *Fusarium poae* isolates were selected based on high level of nivalenol production according to the *in vitro* nivalenol production evaluated by Dinolfo et al. (2012) and were conserved on Spezieller Nährstoffarmer Agar (SNA) slants according to Leslie & Summerell (2006). Before being used for inoculation, fungal isolates were cultured in Petri dishes containing potato dextrose agar (PDA) at 25 °C under 12 h light/dark conditions for 5–7 days. Conidial harvest was taken by flooding the plates with 5 ml of distilled water and dislodging the conidia with a bent glass rod. The resulting suspension of the mixture of the four isolates selected was filtered through cheesecloth and the conidial suspension was adjusted to $1 \times 10^5$ conidia per ml according to Brennan et al. (2007) using a haemacytometer (Neubauer) and a binocular microscope. Tween® 20 (0.05 %) (Biopack) was added as surfactants.

### Fusarium poae – Arabidopsis thaliana *assays*

Four week old plants were used for inoculation. Two methods were used to assess virulence. First, the adaxial surfaces of the leaves were wounded and the inoculum was deposited on the wound site as described by Chen et al. (2006). Second, the conidial suspension was infiltrated into the *A. thaliana* abaxial leaf surfaces with a syringe according to Makandar et al. (2010). In both cases, control plants were inoculated with sterile distilled water plus Tween® 20 (0.05 %) (Biopack) and all the plants were covered with polythene bags to maintain high relative humidity. A total of three leaves per plant were inoculated with *F. poae*. The experiment was repeated three times with 20 replicates per experiment. Disease severity (DS) was evaluated by a disease score according to Chen et al. (2006) and was daily recorded for 30 days after inoculation to generate the disease progress curve. The DS index was scored visually and the rating used was: 0, no symptoms; 1, chlorotic lesion restricted to the inoculation site; 2, chlorotic lesion covering 25 of the leaf area; 3, chlorotic lesion covering 25–50 % of the leaf area; 4, chlorotic lesion covering 50–75 % of the leaf area; 5, chlorotic lesion covering the entire leaf (Chen et al. 2006). The area under the disease curves (AUDPC) was calculated by the trapezoidal integration method (Campbell &

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