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# Effect of salicylic acid on *Fusarium graminearum*, the major causal agent of fusarium head blight in wheat

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ABSTRACT

Salicylic acid (SA) is one of the key signal molecules in regulating plant resistance to diverse pathogens. In *Arabidopsis thaliana*, it is predominantly associated with resistance against biotrophic and hemibiotrophic pathogens, and triggering systemic acquired resistance. In contrast, the effect of SA on the defence efficiency of wheat against fusarium head blight (FHB) and its causal agent, *Fusarium graminearum*, is still poorly understood. Here we show that the *F. graminearum* mycelial growth and conidia germination were significantly inhibited, and eventually halted in the presence of increasing concentration of SA in both liquid and solid media. Addition of SA also significantly reduced the production of the mycotoxin deoxynivalenol (DON). However the inhibitory effect of SA required acidic growth conditions to be observed while basic conditions allowed *F. graminearum* to use SA as a carbon source. High performance liquid chromatography (HPLC) analysis confirmed the capacity of *F. graminearum* to metabolize SA. To better understand the effect of SA on *F. graminearum* mycelial growth, we have compared the expression profiles of SA-treated and untreated *F. graminearum* liquid cultures after 8 and 24 h of treatment, using an *F. graminearum* custom-commercial microarray. The microarray analysis suggested that *F. graminearum* can metabolize SA through either the catechol or gentisate pathways that are present in some fungal species. Inoculation of *F. graminearum* conidia in a SA-containing solution has led to reduced FHB symptoms in the very susceptible *Triticum aestivum* cv. Roblin. In contrast, no inhibition was observed when SA and conidia were inoculated sequentially. The expression patterns for the wheat *PR1*, *NPR1*, *Pdf1.2*, and *PR4* genes, a group of indicator genes for the defence response, suggested that SA-induced resistance contributed little to the reduction of symptoms in our assay conditions. Our results demonstrate that, although *F. graminearum* has the capacity to metabolize SA, SA has a significant and direct impact on *F. graminearum* through a reduction in efficiency of germination and growth at higher concentrations.



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*Abbreviations; DON, deoxynivalenol; 3- and 15-ADON, 3- and 15-acetyl-deoxynivalenol; FHB, fusarium head blight; SA, salicylic acid;* PR1 *and* PR4, *pathogenesis-related proteins 1 and 4;* NPR1, *nonexpress of PR genes 1; PDA and PDB, potato dextrose agar and broth media; qRT-PCR, quantitative RT-PCR*

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## Introduction

*Fusarium graminearum* is the principal causal agent of fusarium head blight (FHB), a major disease of cereal crops worldwide (Goswami & Kistler 2004). FHB infection is favoured by warm and humid conditions during flowering and early stages of kernel development (Gilbert & Tekauz 2000). Besides the negative effect on grain yield, the deposition of mycotoxins, such as deoxynivalenol (DON) and its acetylated derivatives (3- and 15-acetyldeoxynivalenol (3- and 15-ADON)), results in the contamination of harvested grain (Xu & Berrie 2005). Many countries have adopted maximum allowable limits for the mycotoxin DON in grain. DON is a potent inhibitor of protein synthesis, and thereby presents hazards for both human and animal health (Desjardins *et al.* 1993; Paulitz 1999). In the last three decades, wheat breeding has focused in part on the identification and characterization of genetic sources of resistance to FHB; however only a small number of FHB-resistant genotypes have been identified and the molecular mechanisms for their resistance are still unknown.

Plant hormones play a critical role in defence against pathogens, and salicylic acid (SA), jasmonic acid, and ethylene are recognised as key players (De Vos *et al.* 2005; Koornneef & Pieterse 2008). SA is predominantly associated with resistance against biotrophic and hemibiotrophic pathogens, and with triggering systemic acquired resistance (SAR) in many species, including *Arabidopsis thaliana* and wheat (Görlach *et al.* 1996; De Vos *et al.* 2005; Glazebrook 2005). SAR provides protection against a broad range of pathogens. Induction of SAR by SA is accompanied by the expression of a set of genes encoding pathogenesis-related (PR) proteins in dicot plants, such as tobacco and *A. thaliana* (Ward *et al.* 1991; Uknes *et al.* 1992). However the role of SA in the defence response of monocot species, including wheat, is not as clearly defined.

Standing out against the plant's ability to produce SA for defence, five genera of fungi, i.e. *Aspergillus*, *Trichosporon*, *Trichoderma*, *Glomerella*, and *Rhodotorula*, have been shown to metabolize SA (reviewed in Wright 1993). Directly or through intermediates, those species convert SA into catechol and/or gentisate. These compounds are then catabolised *via* parallel *ortho* ring-cleavage pathways to 3-oxoadipate, which can be metabolized through the TCA cycle. The presence of enzymes for the catechol and protocatechuate degradation pathways has been demonstrated in *Fusarium* species (Cain *et al.* 1968; Dodge & Wackett 2005).

The contribution of SA to the wheat/*F. graminearum* interaction is still not well understood. Constitutive overexpression of *A. thaliana* Nonexpresser of PR Genes 1 (*NPR1*), leads to upregulation of genes regulated by SA and enhanced FHB resistance in transgenic wheat (Makandar *et al.* 2006). However, exogenous application of SA or its functional analogue benzothiadiazole have produced contradictory results on FHB resistance in wheat (Yu & Muehlbauer 2001; Makandar *et al.* 2006, 2008). Li & Yen (2008) have shown that jasmonic acid and ethylene, rather than SA, could increase resistance to FHB. Preliminary work comparing the SA and jasmonic acid levels accumulating in *F. graminearum*-infected wheat tissues of three cultivars ranging from very susceptible to very resistant to FHB showed that the more resistant cultivar (Wuhan 1) had the least increase in SA and jasmonic acid levels following *F. graminearum* infection (Rocheleau *et al.* 2009). The development of novel strategies to control FHB in cereals will benefit from studies to clarify the relationship between SA and FHB.

In the present work, we have investigated the direct effect of SA on *F. graminearum* conidia germination, mycelial growth, and production of DON, as well as the capacity of *F. graminearum* to metabolize SA. Finally, we have carried out inoculation experiments in wheat to compare the effects on FHB disease development of the direct interaction between SA and *F. graminearum*, *versus* the contribution of SA through *in planta* stimulation of plant defence mechanisms.

## Materials and methods

### *Plant materials and growth conditions*

Plants of three *Triticum aestivum* cv. Roblin, NuyBay, and Wuhan 1 were grown in climatically controlled chambers under 16:8 h day—night cycles at 20:16 °C. Plants were watered as needed and fertilized weekly with 20-20-20 (N-P-K). Roblin, NuyBay, and Wuhan 1 are highly susceptible, moderately resistant, and very resistant wheat cultivars, respectively.

### *Fusarium* graminearum *strains and growth conditions*

*Fusarium graminearum* virulent strain DAOM180378 (Canadian Fungal Culture Collection, AAFC, Ottawa, ON) was used throughout, except for the experiment measuring the effect of SA on DON production for which the strain DAOM233423 was used. The two strains have similar virulence and DON production level on wheat; however the in plate-DON production assay used was optimised for DAOM233423. Production of conidia was carried out on Potato Dextrose Agar (PDA, Difco) plates incubated at 25 °C under mixed ultraviolet and fluorescent light (TLD 36 − 08; TL 40 W-33RS; Philips) for 7 d. Conidia were harvested from the plates with the aid of a sterile glass rod and sterile water, and then filtered through autoclaved cheesecloth. For the DON experiment, conidia were produced in CarboxyMethyl Cellulose (CMC) media at 28 °C, 180 rpm for 5 d. The concentration of conidia was determined with a haemocytometer by microscopy.

The direct effect of SA on the growth of mycelia and germination of conidia was tested on modified Synthetischer Nährstoffarmer Agar (SNA; Nirenberg 1976) 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 litre). Efficiency of conidia germination and mycelium growth was tested on PDA plates as well. These media were amended with SA (Sigma) at 0, 0.1, 0.2, 0.4, 0.6, 0.8, 1, 2, 2.5, 3, 4, 5, 10, and 20 mM from a 1 M stock solution in methanol. Plates without SA were used as controls. Each Petri dish (90 mm) was inoculated with a 6 mm agar plug cut from the edge of 3–5 d old cultures obtained from the same medium. The radial growth of mycelia was estimated at four positions/plate separated by approximately a 45° angle, by measuring the diameter of the unique colony for five consecutive days. To study the effect on conidia germination, 400