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Indole-3-acetic acid in *Fusarium graminearum*: Identification of biosynthetic pathways and characterization of physiological effects

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ABSTRACT

Fusarium graminearum is a devastating pathogenic fungus causing fusarium head blight (FHB) of wheat. This fungus can produce indole-3-acetic acid (IAA) and a very large amount of IAA accumulates in wheat head tissues during the first few days of infection by *F. graminearum*. Using liquid culture conditions, we have determined that *F. graminearum* can use tryptamine (TAM) and indole-3-acetonitrile (IAN) as biosynthetic intermediates to produce IAA. It is the first time that *F. graminearum* is shown to use the L-tryptophan-dependent TAM and IAN pathways rather than the indole-3-acetamide or indole-3-pyruvic acid pathways to produce IAA. Our experiments also showed that exogenous IAA was metabolized by *F. graminearum*. Exogenous IAA, TAM, and IAN inhibited mycelial growth; IAA and IAN also affected the hyphae branching pattern and delayed macroconidium germination. IAA and TAM had a small positive effect on the production of the mycotoxin 15-ADON while IAN inhibited its production. Our results showed that IAA and biosynthetic intermediates had a significant effect on *F. graminearum* physiology and suggested a new area of exploration for fungicidal compounds.

Introduction

Fusarium graminearum Schwabe (*Gibberella zeae* (Schwein.) Petch) and closely related species are the main causal agents of fusarium head blight (FHB), one of the most important and destructive fungal disease in cereal crops around the world (Parry *et al.* 1995; Goswami & Corby 2004). In addition to causing devastating losses of yield in wheat, FHB causes

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Abbreviations; IAA, indole-3-acetic acid; L-TRP, L-tryptophan; TAM, tryptamine; IAN, indole-3-acetonitrile; TOL, tryptophol; IPA, indole-3-pyruvic acid; IAM, indole-3-acetamide; 15-ADON, 15-acetyldeoxynivalenol; NIV, Nivalenol

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significant contamination of grains by mycotoxins, including the trichothecenes deoxynivalenol (DON) and its acetylated derivatives 3-acetyldeoxynivalenol (3-ADON), 15-acetyldeoxynivalenol (15-ADON), and nivalenol (NIV), which are detrimental to human and livestock (Desjardins *et al.* 1993; Paulitz 1999; Bai & Shaner 2004). In Asia and Europe both DON and NIV are common contaminants of cereal grain, but in North America, DON and its derivatives are the primary mycotoxins in *Fusarium*-infected cereal grain (Ichinoe *et al.* 1983; Ward *et al.* 2008). To achieve better food and feed quality, we have to find effective measures to reduce the contents of DON during wheat production.

Indole-3-acetic acid (IAA), an auxin, is commonly known as a key plant hormone promoting multiple facets of development in various cell types (Davies 2004). Microorganisms, including bacteria and fungi, are also able to synthesis IAA (Tudzynski & Sharon 2002; Tsavkelova *et al.* 2006). Many microorganisms associated with plants as symbionts or parasites directly increase the IAA content in the infected plants and the IAA imbalance benefits the microorganisms (Gruen 1959; Gaudin *et al.* 1994; Tudzynski & Sharon 2002). We have recently shown that *F. graminearum* infection is associated with a very large accumulation of IAA in wheat head tissues during the first few days of infection, and that the IAA is likely from fungal origin (Qi *et al.* 2016).

In fungi, L-tryptophan (L-TRP)-dependent IAA biosynthesis has been documented using external feeding experiments with L-TRP and its metabolited intermediates (Tudzynski & Sharon 2002; Tsavkelova *et al.* 2006; Reineke *et al.* 2008). Some *Fusarium* species have been shown to produce IAA from L-TRP via indole-3-acetamide (IAM pathway); however *F. graminearum* has been shown to be missing the required genes for this pathway, tryptophan-2-monooxyngenase and indole-3-acetamide hydrolase, in its genome (Tsavkelova *et al.* 2012). Although it has been shown that *F. graminearum* can produce IAA in a L-TRP-dependent manner, it is not known if it uses one or more of the three other known pathways, the indole-3-pyruvic acid (IPA), the tryptamine (TAM) and the indole-3-acetonitrile (IAN) pathways (Zhao *et al.* 2002; Desroches. 2012). To date, no studies have examined the capability of *F. graminearum* to metabolize the phytohormone IAA. In the current study, we have focused on the identification of the biosynthetic pathway(s) used by *F. graminearum* to synthetize IAA, as well as on the characterization of the effects of exogenous IAA and its biosynthetic intermediates on *F. graminearum*.

Materials and methods

Fungal strains and culture conditions

Fusarium graminearum virulent strain DAOM 233423 (Canadian Fungal Culture Collection, AAFC, Ottawa, ON) was used for most experiments and should be assumed unless otherwise specified. Mycelia from that strain were kept on modified Synthetischer Nährstoffarmer Agar (SNA; 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) plates (Nirenberg 1976). To produce macroconidia, several plugs with mycelia from SNA plates were added to 100 mL carboxymethyl cellulose (CMC) liquid medium (Cappellini & Peterson 1965) and cultured with shaking at 28 °C in the dark for 72 h. The cultures were then filtered through one layer of cheesecloth (Fisher Healthcare, Houston, TX, USA); the collected macroconidia were washed twice by centrifugation at 4500 rpm for 10 min then resuspension of the pellets in sterile distilled water. Macroconidium suspensions were counted using a haemocytometer and their final concentration adjusted to 12.5×10^6 macroconidia/mL for experiments.

Feeding experiments

Fusarium graminearum liquid cultures were used to test the effects of IAA and possible biosynthetic intermediates. For each biological replicate, about 5×10^5 *F. graminearum* macroconidia were inoculated into 3.96 mL of first stage liquid medium (Taylor *et al.* 2008) in a six-well culture tray (BD Falcon, USA), the tray sealed with Parafilm (Bemis, USA) and shaken at 170 rpm, 28 °C in the dark for 24 h to produce mycelia. The mycelia grown in the first stage medium was harvested, washed with sterile distilled water, then transferred to 3.94 mL of second stage medium (Taylor *et al.* 2008) containing 40 μL of either methanol, or 100 mM stock solution of IAA or potential biosynthetic intermediate compounds. Controls with the same concentrations of IAA but without any fungal inoculum were also performed in second stage medium. Three biological replicates were grown for each treatment and time point of collection. At sampling time, the treated cultures were transferred to 15 mL Falcon tubes and centrifuged at 5000 rpm for 10 min. The supernatant was collected and filtered through 0.2 μm Nylon Syringe filters (Nalgene, Canada) and subjected to High Performance Liquid Chromatography (HPLC) analysis. For some experiments, the mycelia were harvested, dried and weighed. All compounds used for treatment or as HPLC reference compounds, including IAA, IPA, TAM, IAN, tryptophol (TOL) and 15-ADON, were purchased from Sigma–Aldrich Co. LLC, Canada.

To test potential biosynthetic intermediates for production of IAA and their effect on 15-ADON production, the following intermediates were added when the mycelia were transferred to the second stage medium: 1 mM IPA, 0.2 and 1 mM TAM, 0.2 and 1 mM IAN. A larger amount of IPA was used because of technical difficulties in detecting small amounts of IPA by HPLC, due to its instability. Time course experiments were performed and sampling was done by collecting the supernatants of fungal cultures in individual wells after incubation for up to 24 h. The IAN-treated cultures were collected in biological triplicates at 6 h, 12 h, and 24 h, while IPA- or TAM-treated cultures were collected in biological triplicates at 3 h, 6 h, 12 h, and 24 h.

To estimate the average amount of exogenous IAA utilized or biosynthesised by *F. graminearum*, fungal cultures and controls without fungus were treated at transfer in the second stage medium and for 24 h with either: 0.1, 0.2, 0.4, 0.8 or 1.0 mM IAA, or 1 mM IAN or TAM. The average IAA used was calculated as: ave IAA used = [ave IAA in controls − ave IAA with fungus] for a given concentration of IAA. The effect of IAA on 15-ADON production was calculated as follows: