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Secondary metabolite profile and phytotoxic activity of genetically distinct forms of *Colletotrichum gloeosporioides* from yam (*Dioscorea* spp.)

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ABSTRACT

Highly virulent, slow-growing grey (SGG); moderately virulent, fast-growing salmon (FGS); and avirulent/weakly virulent, fast-growing grey (FGG) forms of *Colletotrichum gloeosporioides* have been described from yam (*Dioscorea* spp.), but little is known about their chemodiversity or the role of toxins in their pathogenesis. Secondary metabolite profiles in high performance tlc (hptlc) showed that the pathogenic SGG and FGS forms have a chemotype (A or B) that is distinct from the non-pathogenic FGG form (chemotype C). Crude extracts of 35-d-old Czapek–Dox yeast broth cultures of FGS and SGG isolates caused tissue necrosis on treated yam leaves but not those of FGG isolates. Extracts from uninoculated broth cultures showed no phytotoxic activity. Toxicity of the culture filtrate was not host specific and toxic substances were thermostable. *Dioscorea* genotypes with varying levels of resistance to anthracnose differed in their sensitivity to crude toxin extract of FGS (Cg33) and SGG (Cg25) isolates, indicating that these extracts may be useful in evaluating host resistance to anthracnose *in vitro*. Analysis of two toxin fractions unique to the pathogenic FGS and SGG forms using hlpc, mass spectrometry and nuclear magnetic resonance suggested the presence of a low molecular weight amide peptide. However, possibly due to low yield and the presence of impurities, the chemical structure of the compound(s) could not be fully elucidated.

Introduction

Anthracnose is among the most widespread and economically important diseases of water yam (*Dioscorea alata*) worldwide. The disease is caused by *Colletotrichum gloeosporioides* (teleomorph, *Glomerella cingulata*) (Abang *et al.* 2002). It attacks all above-ground plant parts; die-back of shoots and eventual death of affected plants may result in over 80 % yield loss

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when susceptible varieties are grown under high disease pressure and conducive environmental conditions (Nwankiti *et al.* 1984; Green 1998; McDonald *et al.* 1998; Ano *et al.* 2002).

The presence of a distinct chlorotic zone surrounding dark brown anthracnose lesions on yam suggests the likely effect of a pathogen-induced toxin (Molina & Krausz 1989; Abang *et al.* 2003). The possibility that toxic metabolites play a role in the development of anthracnose on yam has been suggested (Amusa *et al.* 1993, 2000; Alleyne 2001; Moura-Costa *et al.* 1993), and efforts have been made to extract and test the phytotoxic principle on yams with varying levels of resistance to anthracnose. The advantages of using *in vitro* plantlets rather than whole plants for assaying phytotoxins have been reviewed (Amusa 2006; Daub 1986; Soledade & Pedras 2004; švábová & Lebeda 2005). Experiments with pathogen culture filtrates have shown that tissue response *in vitro* may correlate with disease reaction of the host species/variety and, where this occurs, the use of culture fitrates may allow selection of important traits in disease resistance *in vitro* (Amusa 2006; Daub 1986; Moura-Costa *et al.* 1993).

Amusa *et al.* (1993) and Ahoussou (1989, cited in Moura-Costa *et al.* 1993) extracted phytotoxic substances from *C. gloeosporioides*-infected yam leaves, which induced necrotic lesions similar to that produced by the pathogen on yam leaves. The toxin extracted by Amusa *et al.* (1993) gave a fluorescent band similar to that produced by toxic metabolites of the pathogen in culture, indicating that it is a vivotoxin. In preliminary studies on the chemical characterization of culture filtrates, Ahoussou (1989, cited in Moura-Costa *et al.* 1993) showed that phytotoxicity of toxic fractions of a West African isolate of *C. gloeosporioides* could be attributed to an, as yet, unidentified glycoprotein and an associated polysaccharide. Similar results were obtained by Alleyne (2001) who partially purified and characterized phytotoxic metabolites exuded by *C. gloeosporioides* from yam in Barbados. Water-soluble, glycoprotein-type compounds were observed, and the protein fraction of the toxin complex showed host selectivity when tested on a wide range of yam and non-yam hosts. The chemical structure of the phytotoxin(s) remains elusive.

Three forms of *C. gloeosporioides* have been described from yam (Abang *et al.* 2002). Sequence analysis of the rDNA ITS region confirmed that the moderately virulent, fast-growing salmon (FGS) and the highly virulent, slow-growing grey (SGG) strains, which cause typical anthracnose symptoms, belong to *C. gloeosporioides* (Abang *et al.* 2002). The avirulent/weakly virulent, fast-growing grey (FGG) strain appeared unrelated to *C. gloeosporioides* and it remains unclear whether it should be retained within the *C. gloeosporioides* species complex (Abang *et al.* 2002; Johnston & Jones 1997). Secondary metabolites could be used as an additional criterion in chemotaxonomic studies of pathogenic and non-pathogenic forms of *C. gloeosporioides* from yam (Sutton 1992; Frisad *et al.* 2008). Although the 'blueprint' of each form is represented by the genome, its behaviour is expressed as its phenotype, i.e. growth characteristics, cell differentiation, response to the environment, the production of secondary metabolites and enzymes. Therefore, the profile of (secondary) metabolites — fungal chemodiversity — is important for understanding functional genomics in this pathosystem and the elucidation of host–pathogen interactions.

In this paper, genetically distinct forms of *C. gloeosporioides* from yam were further characterized based on their secondary metabolite profile. Also, bioassays were used to determine the phytotoxicity of toxin fractions both *in vitro* and *in vivo*, and the response of diverse *Dioscorea* genotypes to anthracnose was evaluated using the toxins. Attempts were made to purify two toxin fractions from a virulent FGS strain, and elucidate their chemical structure using mass spectrometry (MS) and nuclear magnetic resonance (NMR).

Materials and methods

Fungal cultures, growth media, and metabolite extraction procedure

Twenty-seven isolates, representing the FGS, SGG, and FGG forms of *Colletotrichum gloeosporioides* were used in this study (Table 1). Morphological, virulence, and molecular-genetic characteristics of these morphotypes were described previously (Abang *et al.* 2002, 2003, 2004, 2005, 2006). These strains

Table 1 – Designation, morphotype, rDNA ITS group, and chemotype of slow growing grey (SGG), fast growing salmon (FGS), and fast growing grey (FGG) forms of *Colletotrichum gloeosporioides* based on their secondary metabolite profile in high performance thin layer chromatography (hptlc)[a]

Isolate	Morpho type	rDNA ITS group	Hptlc		Chemo type
			UV (360 nm)	Anis-aldehyde staining	
Cg1	FGS	1	–	+	B
Cg2	FGS	1	+	+	A
CgS2	FGS	1	+	+	A
CgS3	FGS	1	+	+	A
CgS5	FGS	1	+	+	A
CgS6	FGS	1	–	+	B
Cg9	FGS	1	+	+	A
Cg10	FGS	1	+	+	A
Cg11	FGS	1	+	+	A
Cg12	FGS	1	+	+	A
Cg13	FGS	1	–	+	B
Cg14	SGG	1	–	+	B
Cg15	FGG	2	+	–	C
Cg16	FGG	2	+	–	C
Cg17	FGS	1	+	+	A
Cg18	FGG	2	+	–	C
Cg19	FGG	2	+	–	C
Cg20	FGS	1	+	+	A
Cg21	FGS	1	+	+	A
Cg22	FGS	1	+	+	A
Cg25	SGG	1	–	+	B
Cg26	SGG	1	–	+	B
Cg29	FGS	1	+	+	A
Cg33	FGS	1	+	+	A
Cg35	FGS	1	+	+	A
Cg66	SGG	1	–	+	B
Cg219	SGG	1	–	+	B

a Based on previous morphotype and rDNA ITS analysis (Abang *et al.* 2002).