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HOST SPECIFICITY OF PUTATIVE ANTHRACNOSE TOXINS FROM *Colletotrichum gloeosporioides*

Angela T. Alleyne, Leonard O'Garro and Ashton J. Delauney

Department of Biology, The University of the West Indies, Cave Hill Campus, P.O. Box 64, Bridgetown, Barbados.

ABSTRACT

Anthracnose of crop plants caused by the fungus *Colletotrichum gloeosporioides* (PENZ) is a common disease in the Caribbean. The fungus has caused considerable decline in crop output, notably productivity. Putative toxins were extracted from culture filtrates of the fungus isolated from diseased yam (*Dioscorea alata* L. cv. White Lisbon), coffee, and anthurium plants and tested on a number of plant genotypes. Partially characterized, putative toxins from yam isolates of the fungus induced typical anthracnose symptoms on yam, but not on citrus (*Citrus* spp. L.), mango (*Mangifera indica* L.), avocado (*Persea americana* Mill.), soursop (*Anona muricata* L.) and anthurium plants. Similarly putative toxins induced foliar symptoms only on the respective host plant from which isolates were obtained. Furthermore, the viability of yam cell suspensions was also affected by the putative toxin.

INTRODUCTION

Anthracnose of yam (*Dioscorea alata*) caused by *Colletotrichum gloeosporioides* has been extensively documented since its first appearance in the 1960s, in the Caribbean (Gooding, 1960; Degras, 1993). Typical foliar symptoms appear as necrotic rust coloured or black irregular lesions, surrounded by a margin of chlorosis (Degras, 1983). The entire plant may become necrotic by the coalescence of spots. In the Caribbean, the yam cultivar White Lisbon is most severely affected by anthracnose (Purseglove, 1967), while cultivars such as Plembite, Kinayabayo and Welch have been reported as tolerant (Degras, 1983; Simmons, 1993).

Colletotrichum gloeosporioides is also responsible for several other anthracnoses on a range of tropical food crops (Sutton, 1992; Jefferies et al., 1992). This highly successful pathogen is found on such crops as avocado, mango, cacao, banana, sugar cane, coffee, pepper, tomato, and citrus. The variety of crops colonized by the fungus thus suggests a wide, non-specific host range.

Singh and Prasad (1967) proposed the term *C. gloeosporioides* F. *alatae* to describe isolates of the fungus affecting yam. This proposal however is not supported by other researchers (Sutton, 1992; Simmons, 1993). However, genetic studies including restriction fragment length polymorphisms (RFLP's), randomly amplified polymorhic DNA (RAPD's), and polymerase chain reaction (PCR) amplification and sequencing of highly conserved genomic sequences of ribosomal and mitochondrial DNA supports the proposal for host

specificity in *C. gloeosporioides* (Mills et al., 1992; Sreevinasprasad et al., 1993; Alakahoon et al., 1994; Sherriff et al., 1994). However, yam isolates of *C. gloeosporioides* were not included in these studies.

Culture filtrates of plant fungal pathogens have been used to isolate phytotoxic compounds of pathogenic origin (Strobel, 1982; Coleman, 1994). Several phytotoxic compounds including polysaccharides, glycoproteins (Goodman, 1960; Anderson, 1978; Frantzen, 1982; Ahoussou, 1989) and terpenoids (Gobhara et al., 1978) have been extracted from culture filtrates of *Colletotrichum* from different host plants. Glycoproteins produced by yam isolates of *C. gloeosporioides* show specific toxicity towards yam (Ahoussou, 1989).

Microbial toxins have been implicated in the induction of symptoms associated with plant diseases caused by phytopathogenic bacteria and fungi. Visible symptoms are normally characterized as necrosis, chlorosis and wilting. Generally, these toxins injure plants by inhibiting enzymes or altering permeability of cell membranes. The latter often results in electrolyte leakage. The present study reports on a phytotoxic nature of partially characterized extracts obtained from yam culture filtrates of *C. gloeosporioides* from yam, coffee and anthurium.

MATERIALS AND METHODS

Isolation and culture of the pathogen

Isolates of *C. gloeosporioides* were obtained from the yam cultivar White Lisbon in Barbados. *C. gloeosporioides* were isolated from White Lisbon by first excising leaf portions bearing typical symptoms. These were then surface-sterilized by washing in 95% ethanol for 15s, followed by sodium hypochlorite (1.25%) for 20 s and a final 5 s rinse in sterile distilled water (SDW). Leaf sections were then plated onto potato dextrose agar (PDA) and incubated for 3 d at 25 °C. *C. gloeosporioides* was then identified by typical conidial and mycelial morphology (Sutton, 1992). Fourteen other isolates from different host plants were provided by Dr L. O'Garro of the University of the West Indies, Cave Hill campus, Barbados. Single spore cultures of each isolate were obtained and stored on PDA slants at 4 °C until needed.

Precipitation of crude extract from culture filtrates

Richard's media (400 mL) (Ahoussou, 1989) were inoculated with 0.5 mL conidial suspension (1×10^6 conidia/mL) of *C. gloeosporioides* and incubated for 10–15 d on a shaker-incubator (GFL-Gesellschaft fur Labortechnik, Germany) set at 25 °C and 200 rpm. The fungal culture was then centrifuged at 1,200 *xg* and 4 °C for 15 min and the supernatant obtained pooled, lyophilized to 1/3 of its initial volume and then precipitated with 3 volumes of cold acetone for 48 h at 4 °C. The precipitate obtained was air dried, weighed and stored at -20 °C.

Purification of crude extract

Aqueous solutions (0.1mg/L), of the precipitated extract were dialyzed against SDW for 24 h and then fractionated using a 1-m column packed with Sephadex CL-6B. Samples (0.1 mg/L) of dialyzed extracts were loaded on the column, eluted with sodium acetate:sodium chloride (5 mM:50 mM) buffer and collected in 1-ml fractions. The fractions were analyzed colorimetrically (Dubois et al., 1956; Bradford, 1976) and those testing positive for carbohydrates and proteins were pooled and tested in this study.

Cell suspension culture

Cell suspensions of yam, pepper, tomato and tobacco were set up from callus cultures. Leaf explants, from 2 to 3-week-old seedlings of pepper and tomato were surface-sterilized by first immersing in 70% ethanol for 2 min, followed by 5% sodium hypochlorite for 4-7min and then rinsed five times in SDW. The explants were then placed on solid Murashighe and Skoog's basal medium with Gamborgh vitamins (MS), amended with phytohormones to induce callusing (Ammirato, 1983; Dixon, 1985). For pepper the hormones used were 2,4-dichlorophenoxyacetic acid (2,4-D) (3 mg/L) and kinetin (KI) (0.5 mg/L), (Arroyo, 1991) while the tomato medium contained KI (2 mg/L) and 2,4-D (0.5 mg/L) (Hamza, 1993). Yam leaf explants were obtained from tissue cultured plantlets of *D. alata* and cultured on MS medium amended with 4 mg/L 2,4-D (Tor, 1991)

Cell suspensions, were established by crushing 2-3 month-old friable calli in liquid MS medium, amended with phytohormones for each plan genotype as described above (Dixon, 1985). Cell suspension (100 mL) were maintained on an orbital rotary shaker at 25 °C by subculturing every 10 d with fresh culture medium.

Host treatment

a) Visible foliar response

Intercellular spaces of leaves were infiltrated with the original partially purified or boiled fungal extract (0.1 mg/L) using a sterile syringe (Swanson et al., 1988). Leaves serving as controls were similarly infiltrated with SDW or the growth medium (RM). Treated leaves were observed for visible foliar symptoms and the extent to which they were affected assessed as the portion of the leaf surface area covered by symptomatic tissue. Three leaves each from yam (White Lisbon), mango, citrus, avocado, anthurium and pepper and tomato were tested with extracts from two isolates of *C. gloeosporioides* from yam and one each from anthurium and coffee.

b) Electrolyte leakage from cell suspensions

Plant cell suspensions (5ml) were treated with 100 ug of the partially purified fungal extract and electrolyte leakage determined by conductivity measurements at 5 min intervals over a 2-h time period at 25 °C using a conductivity meter (Cole Palmer Co., Ill, USA). Cell

suspensions of yam, pepper and tomato were each tested with extracts produced by *C. gloeosporiodes* from yam, coffee and anthurium.

(c) Viability of host cell suspensions

Aliquots (0.5 mL) of ten-fold dilutions of fungal extract (1 mg/L), were tested on yam cell suspension (1 mL) in a repli-dish. The viability of the cell suspension was assessed microscopically on a daily basis following treatment with fluorescein diacetate (FDA). Only yam cell suspensions, tested with extracts from *C. gloeosporioides* from yam and coffee were used in this experiment. This experiment was repeated three times.

RESULTS

Purification of fungal extracts by gel filtration column chromatography

From the protein (Bradford, 1976) and carbohydrate (Dubois et al., 1956) assays, ten fractions (1 mL, each containing protein and or carbohydrate were obtained from the fractionation of the fungal extracts.

Host treatment

(a) Visible foliar response to fungal extracts

Necrosis was induced on yam within 36 h by partially purified extracts from yam isolates of *C. gloeosporioides* but not on other host plants. The yam cultivar, White Lisbon was considerably more severely affected than Plembite (Table 1). Furthermore, necrosis appeared much later on the latter cultivar. Fungal extracts from isolates from yam, anthurium and coffee induced necrosis only on their respective host plants from which the fungus was obtained. Generally, host plants were more seriously affected by crude extracts than the corresponding partially purified compound. Heat treated extracts failed to induce similar symptoms on all host plants investigated.

(b) Electrolyte leakage from cell suspensions

Fungal extracts induced electrolyte leakage from plant cells within 15 min (Table 2). However, the magnitude of electrolyte leakage from yam cell suspension was greater than that from pepper or tomato cell suspensions. Treated cell suspensions examined under the microscope had collapsed plasma membranes.

(c) Viability of yam cell suspensions

The viability of yam cells in suspension declined by 50% within 3–5 d after exposure to extracts from yam isolates of *C. gloeosporoides*. Viable cells gave a bright green fluorescence, while inviable cells appeared as dull yellow spots against a black background. Viable cells were undetectable after 5 d.

Table 1 Extent of necrosis* induced by partially characterized extracts from *C. gloeosporioides* on eight plant genotypes

Plant genotype	Origin of fungal extract			
	yam	yam	anthurium	coffee
Yam (White Lisbon)	86	69	0	0
Yam (Plembite)	31	22	0	0
Anthurium	0	0	35	0
Pepper	0	0	0	0
Mango	0	0	0	0
Citrus	0	0	0	0
Tomato	0	0	0	0
Avocado	0	0	0	0

*The extent of necrosis induced by fungal extracts was assessed as the proportion of leaf area covered by symptomatic tissue

Table 2 Extent of electrolyte leakage induced by partially characterized fungal extracts from *C. gloeosporioides*

Time (min)	Conductivity *u*mho/cm		
	Yam	Pepper	Tomato
0	2.0×10^3	1.2×10^3	1.6×10^3
5	4.1×10^3	1.5×10^3	1.6×10^3
10	4.4×10^3	1.5×10^3	1.8×10^3
15	4.9×10^3	1.7×10^3	2.0×10^3
60	5.0×10^3	2.0×10^3	1.9×10^3
120	5.0×10^3	2.0×10^3	2.0×10^3

DISCUSSION

The present study reports a phytotoxic property of partially purified extracts from cultures of *C. gloeosporioides* obtained from yam, coffee and anthurium. This property was manifested by the loss of electrolytes from cell suspensions, reduced viability of cell suspensions and the induction of necrosis on respective hosts. Furthermore, the extracts appear to be host-specific in activity. The host specific nature of toxins produced by *C. gloeosporioides* have been previously reported (Ahoussou, 1989). Initial studies on isolates from several hosts, including yam, using RAPD primers also suggested that *C. gloeosporioides* exhibits significant host-specificity.

Putative toxins produced by *C. gloeosporioides* are comprised of polysaccharides with one or more protein moieties. Heat treatment significantly abolished phytotoxicity, indicating that the protein components are essential for toxin activity.

Host-specific phytotoxins produced by *C. gloeosporioides* are expected to have utility as a selective agent for plant genotypes conferring resistance to anthracnose. The toxins can be used in this manner if toxin insensitive host cells are also resistant to infection by the fungus. We hope to use this approach to select transgenic yams encoding resistant genes to anthracnose.

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