

Pathogen toxin-induced electrolyte leakage and phytoalexin accumulation as indices of red-rot (*Colletotrichum falcatum* Went) resistance in sugarcane

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Summary. A phytotoxin produced by the sugarcane red-rot fungus *Colletotrichum falcatum* Went was partially purified. The phytotoxin caused increased electrolyte leakage in susceptible sugarcane varieties and higher levels of phytoalexins (3-deoxyanthocyanidins) in resistant sugarcane varieties. This relationship between phytotoxin induced changes and disease reaction could possibly be used as an additional index to rapidly identify red-rot resistant varieties.

Key words: *Saccharum* sp., phytotoxin, phytoalexin induction, resistance.

Introduction

Red-rot (*Colletotrichum falcatum* Went; perfect stage, *Physalospora tucumanensis* Speg. Arx and Muller) is one of the most serious diseases of sugarcane, causing substantial losses in crop yield and quality. Management of the disease is mainly by the use of disease resistant/tolerant varieties. However, screening for disease resistance by means of natural infection or artificial inoculation often gives inconsistent results due to interaction between host, pathogen and environment (Mohanraj *et al.*, 1997). The existence of different populations of the pathogen further complicates the problem (Mohanraj *et al.*, 2002a). Hence, physiological indices are

considered to be an important means to identify resistant sugarcane *Saccharum* sp., in addition to artificial inoculation with selected strains of the pathogen. The red-rot pathogen produces a phytotoxin that reproduces some of the symptoms of the disease (Olufolaji and Bomgboye, 1986; Mohanraj *et al.*, 2002b). This phytotoxin also induces an accumulation of phytoalexins (anthocyanidin pigments) in treated canes similar to that caused by the pathogen (Viswanathan *et al.*, 1996b). In this paper, phytoalexin accumulation and electrolytic leakage in sugarcane treated with phytotoxin were examined to detect a relation between the toxin, red-rot symptoms and screening for red-rot resistance.

Materials and methods

Toxin purification

Colletotrichum falcatum was isolated from sus-

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ceptible sugarcane cv. CoC 671 and grown on Czapek's liquid medium with sugarcane extract instead of sucrose as carbon source (30 ml l⁻¹). Phytotoxin from actively growing liquid culture of the fungus was purified by a modified form of the solvent extraction procedure of Olufolaji and Bomgboye (1986) and Mohanraj *et al.* (1995). The modification involved the concentration of original culture filtrates to 1:10 volume (instead of 1:20) under reduced pressure. It was further fractionated in a Sephadex G-75 column and the toxin fraction with the greatest biological activity (based on symptoms produced in a susceptible sugarcane variety) was separated and pooled. This fraction was condensed and dried under reduced pressure to obtain the partially purified toxin as a dry powder.

Phytoalexin accumulation and electrolytic leakage

Phytoalexin accumulation was studied on nodal tissue and electrolytic leakage on leaf tissue. The choice of tissue was dictated by the following consideration: phytoalexin normally enters the leaf by the nodal region, so that its level here would give the best indication of the degree of resistance of the

varieties. In sugarcane affected with red-rot, on the other hand, it is the leaves that show the typical yellowing and drying symptoms even though the pathogen does not actually colonize the leaves; this suggests that translocated pathogen metabolites cause these symptoms. Since the toxin induces the same leaf symptoms, its effect in relation to electrolyte leakage was studied on the leaf tissues of susceptible and resistant sugarcane varieties.

Toxin treatment for phytoalexin induction

Sugarcane plants (6–7 months old) from 15 resistant and 15 susceptible sugarcane varieties (Table 1), were studied. Plants were maintained in a humidity chamber with a relative humidity of >90% at 32°C. The topmost nodes of selected stalks from which the leaf sheath could be removed without injury to the nodal region were exposed. A 0.2% toxin solution in distilled water (Mohanraj and Padmanaban, 2001) was prepared. Thin absorbent cotton strips 2 cm wide and 10 cm long dipped in the toxin solution were wound around the exposed nodes of the stalks in a way similar to the standard nodal inoculation

Table 1. Electrolyte leakage from the leaf tissues of various sugarcane varieties in response to treatment with red-rot toxin.

Resistant variety	Electrolyte leakage $\mu\text{S cm}^{-1}$ (26°C) ^{a, b}	Susceptible variety	Electrolyte leakage $\mu\text{S cm}^{-1}$ (26°C) ^{a, b}
BO 91	110 m	CoC 671	356 fgh
Co 7201	245 ijk	CoC 92061	392 a–e
CoV 92102	193 m	Co 6806	397 a–d
Co 86249	121 m	Co 8011	412 ab
CoG 95076	244 ijk	Co 8338	336 efg
CoG 93076	262 ij	Co 86007	406 abc
Co 85019	267 hij	Co 87003	423 ab
Co 86011	317 gh	Co 86010	340 d–g
Co 87013	278 hi	Co 87044	432 a
Co 93009	244 ijk	Co 87013	351 c–g
Co 87045	216 jk	Co 85007	367 b–g
Co 8021	118 kl	Co 8371	386 a–e
Co 94008	234 ijk	Co 97009	391 a–e
Co 94003	227 ijk	Co 86032	371 b–g
Co 90014	253 lm	CoC 90063	381 a–f

^a Values followed by the same letter in a column are not significantly different from each other according to Duncan's multiple range test.

^b Measures were taken at 26°C.

method with the pathogen (Mohanraj *et al.*, 1994). The treated plants were incubated in the humidity chamber.

Phytoalexin assay

After 72 h of incubation, small tissue samples (2 mm², 1 mm thick) were collected from the surface of the toxin-treated nodes. Five hundred mg of each group of tissue samples was quickly immersed in 2.5 ml of HPLC grade methanol. The pigments in the samples were allowed to leach into the solvent for 12 h overnight under constant agitation at 4°C, after which the methanol was decanted, centrifuged at 10,000 rpm (8,000 g) for 15 min and its absorbance read at 525 nm with a spectrophotometer (Shimadzu-UV 1601, Kyoto, Japan) (Viswanathan *et al.*, 1994).

Electrolyte leakage

The effect of the toxin on electrolyte leakage in the sugarcane varieties was studied in the leaf tissues following the method of Balasubramanian (1994). The third fully opened leaf from the top was selected and 100 mg of tissue from the

middle of each leaf blade was collected and enclosed in thin muslin cloth. After rinsing in running distilled water and air drying the samples were immersed in 25 ml toxin solution at a concentration of 500 mg ml⁻¹ (Mohanraj and Padmanaban, 2001), and vacuum infiltrated under negative pressure for 10 min. The electrolytic conductivity of the solution was then measured with a high sensitivity conductivity meter (Denver Instrument 1000, Model 30, Denver, CO, USA) and recorded as μScm^{-1} at 26°C. Earlier studies (unpublished) in this laboratory had indicated that healthy tissues or tissues not treated with pathogen toxin from different resistant and susceptible sugarcane varieties did not show differences among each other in electrolytic leakage.

Analysis of data

For each test there were three replications, each with tissue from different stalks/leaves. Differences in phytoalexin accumulation and in electrolytic leakage between resistant and susceptible varieties were compared using analysis of variance and Duncan's multiple range test (DMRT).

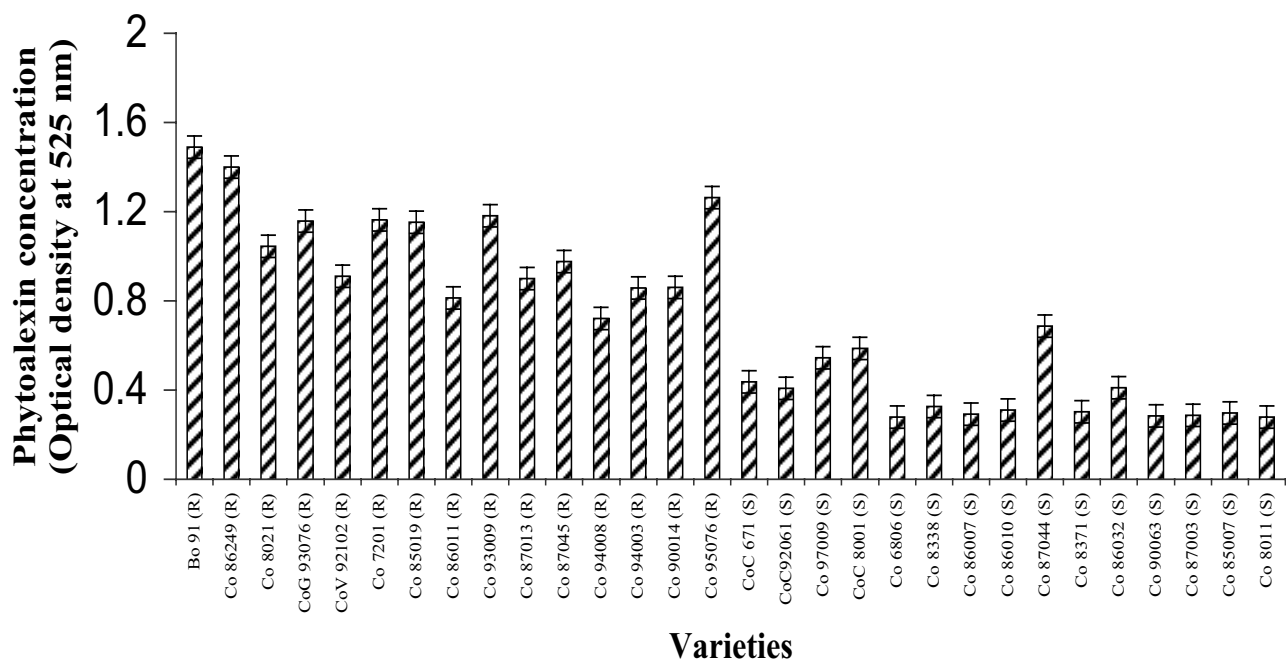


Fig 1. Phytoalexin (3-deoxyanthocyanidins) accumulation in the nodal tissues of sugarcane varieties in response to *Colletotrichum falcatum* toxin application. R, resistant variety; S, susceptible variety.

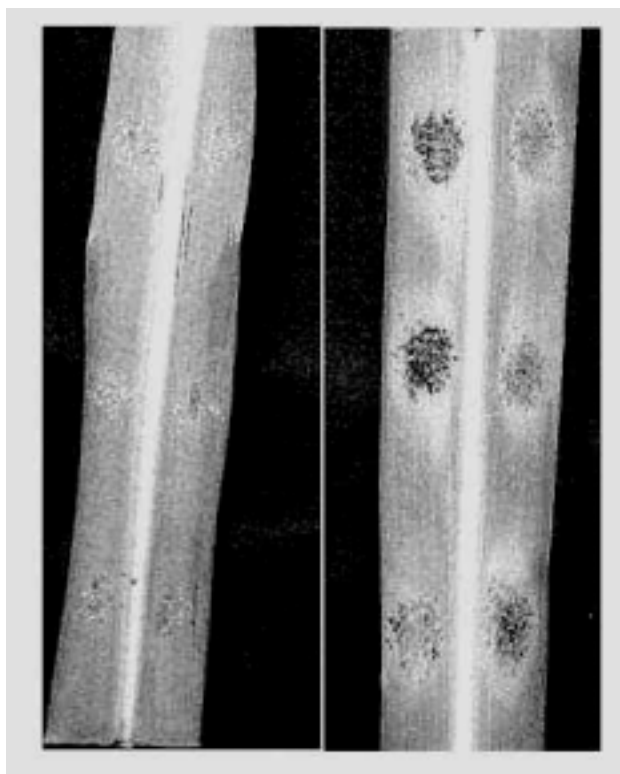


Fig 2. Phytotoxin-induced lesions on leaves of a susceptible sugarcane variety. Left, control; right, toxin treatment at 500 mg ml⁻¹

Results and discussion

Phytoalexin accumulation

The total amount of anthocyanidins eluted per unit quantity of young sugarcane nodal tissue treated with the pathogen toxin was consistently greater ($P=0.05\%$) in red-rot resistant varieties than in susceptible ones. Induction of anthocyanidin pigments was smallest in the susceptible Co 8011, greatest in the resistant BO 91 (Fig. 1).

Electrolyte leakage

The effect of red-rot toxin on electrolyte leakage in each variety was inversely related to the red-rot resistance of that variety, with susceptible varieties showing much greater electrolyte leakage ($P=0.05\%$) than resistant varieties (Table 1). The greatest electrolyte leakage in response to toxin treatment was in the susceptible Co 87044, the smallest in the resistant BO 91.

The nature of necrotic foliar lesions produced by toxin treatment suggesting the role of electrolytic leakage in a susceptible sugarcane variety is shown in Fig. 2.

Analysis of variance on the DMRT data showed that resistant and susceptible varieties differed significantly with regard to phytoalexin accumulation and electrolyte leakage in response to toxin treatment.

Species of *Colletotrichum* causing a range of plant diseases are known to produce phytotoxins (Gohbara *et al.*, 1978; Godderd *et al.*, 1979). However, little is known about how disease symptoms or host responses are induced. Godshall and Lonergan (1987) reported that 3-deoxyanthocyanidin pigments such as apigeninidin and luteolinidin are synthesized in sugarcane in response to infection by the red-rot pathogen. Sugarcane varieties constitutively contain colourless leucoanthocyanidins or proanthocyanidins as precursors of coloured anthocyanidin pigments, which are synthesized in response to stress (Godshall *et al.*, 1996). Production of red anthocyanidin pigments increased more markedly in resistant than in susceptible sugarcane genotypes (Nallathambi *et al.*, 1999). Viswanathan *et al.*, (1996a) found that levels of 3-deoxyanthocyanidin pigments in response to *C. falcatum* infection were much higher in resistant sugarcane varieties and that this factor could be used to identify resistant cultivars. The present study indicates that the *C. falcatum* phytotoxin examined elicits a similar response. In a comparable host-pathogen system, anthracnose stalk rot of maize and sorghum caused by *C. graminicola*, the level of phytoalexins also indicates the level of resistance in the plant (Yamaoka *et al.*, 1990). Pathogen-toxin induced phytoalexin synthesis has also been reported in some other plant diseases (Ransom *et al.*, 1992).

The higher levels of electrolytic leakage suggest that in susceptible varieties this leakage plays a role in red-rot development, which is characterized by symptoms such as necrosis, disintegration and drying of tissues. One way in which the phytotoxins of some plant-pathogenic fungi act is by disrupting the membrane function of the host, resulting in enhanced electrolyte leakage from affected tissues and contributing to symptom development (Scheffer and Liv-

ingston, 1980). In sugarcane itself increased electrolyte leakage is reported to be caused by the phytotoxin of the leaf-spot pathogen *Helminthosporium sacchari* (Steiner and Byther, 1971).

The findings of the study suggest that phytoalexin accumulation and electrolytic leakage in response to red-rot phytotoxin treatment may be used as indices to identify red-rot resistance in sugarcane varieties. These indices can serve as additional means to precisely and rapidly recognize red-rot resistance, along with the host reaction, in response to pathogen inoculation. These indices would be particularly valuable when disease reactions following pathogen inoculation in the field are inconsistent due to lack of ideal environmental conditions or pathogen variability.

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