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Original Article

Chitinases as markers for red rot resistance in sugarcane

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Abstract

Red rot disease caused by *Colletotrichum falcatum* Went is one of the major constraints to sugarcane cultivation in India. Detailed studies were conducted on the involvement of important biochemical indices viz., pathogenesis related proteins *i.e.* the chitinases and phytoalexins in red rot resistance with a set of sugarcane cultivars varying in resistance. In the present investigation, quantitative as well as qualitative analyses showed that chitinases were specifically induced in disease resistant cultivars upon pathogen inoculation. Chitinases of 20, 23, 24 and 26 kDa were identified as PR-proteins specifically involved in sugarcane red rot resistance.

Introduction

Chitinases are the major PR-proteins produced in plants in response to pathogen infection. These are the group of enzymes that hydrolyze chitin, the major component of fungal wall. Chitinases along with β-1,3-glucanases are shown to inhibit *in vitro* growth of many fungi (Mauch *et al*., 1988). In sugarcane also, involvement of PR-proteins in systemic induced resistance mediated by *Pseudomonas* spp. against *C. falcatum* causing red rot disease has been reported (Viswanathan and Samiyappan, 2001). Detailed studies were carried out on the specific induction of chitinases involved in sugarcane for red rot resistance.

Materials and Methods

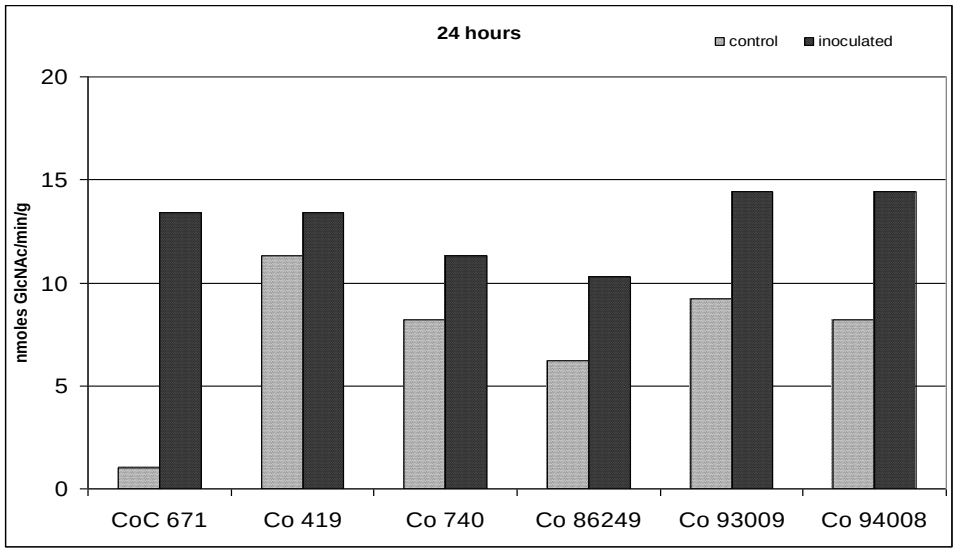
The pathogen was inoculated on cut leaves of susceptible (CoC 671, CoC 9oo63, CoC 92061, Co 419 and Co 740) and resistant (Baragua, Bo-91, Co 86249, Co 93009 and Co 94008) sugarcane cultivars by pinprick method and incubated in moist chambers at 25°C. Later the samples were drawn at 24 and 48h after inoculation for protein extraction. Induction of chitinase was studied quantitatively using colloidal chitin as a substrate by spectrophotometric analysis at 585 nm (Boller *et al*., 1983) and the enzyme activity was expressed in nmoles of N-Acetyl glucosamine released/min/mg of tissue. For qualitative analysis protein samples were run on homogenous 12% SDS-PAGE and the separated proteins were transblotted from acrylamide gels to polyvinylidene fluoride (PVDF) membrane using semi-dry transfer apparatus. Then the membrane was

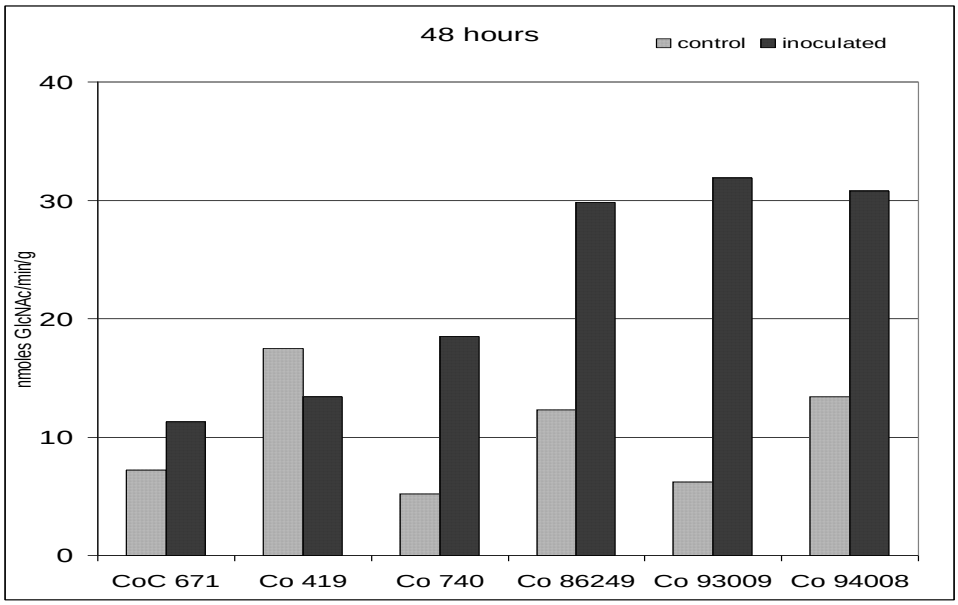
immunoblotted with barley chitinase antiserum and chitinase expression was recorded.

Results and Discussion

The spectrophotometric analysis revealed that chitinase activity was induced upon pathogen inoculation and its level in sugarcane leaves varied with degree of resistance. The enzyme activity increased rapidly in disease resistant cultivars after pathogen inoculation and by 48 h 100% increase in enzyme activity was found (Fig. 1).

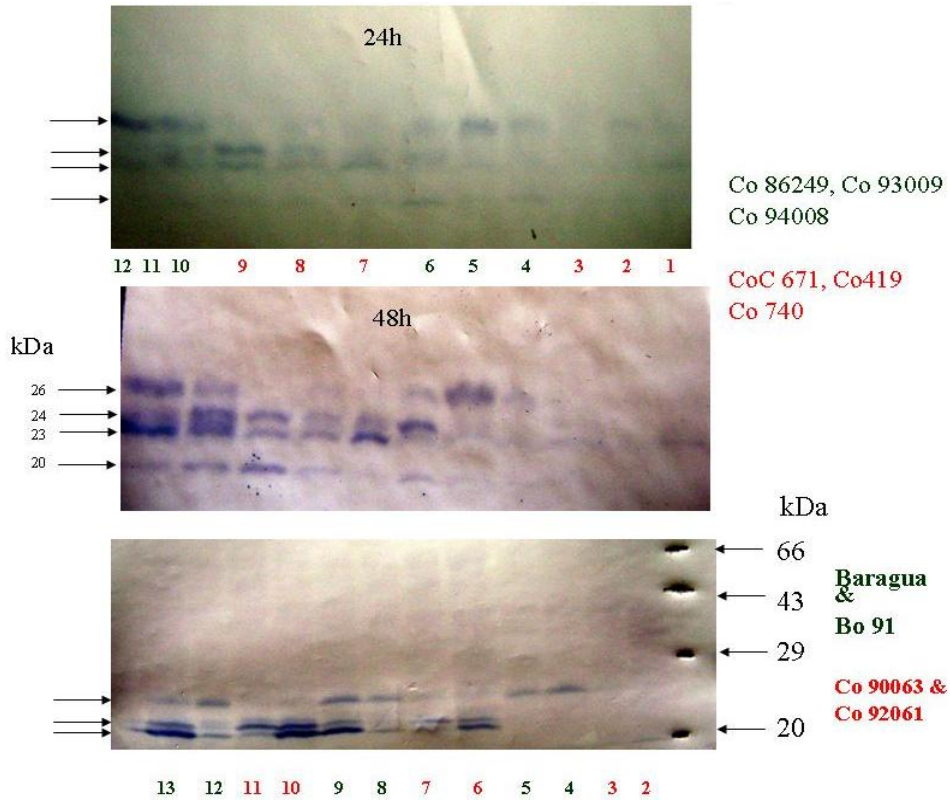
Fig.1. Induction of chitinases in sugarcane varieties varying in red rot resistance after *C. falcatum* infection





Results of the Western blot analysis showed that in resistant varieties chitinases induction increased with time from 24 to 48h after inoculation and 3 to 4 chitinase proteins with molecular masses of 20 to 29 kDa were found to be associated with red rot resistance. It was interesting to note that in uninoculated sugarcane tissues no chitinase was found in susceptible cultivars, while in resistant cultivars 23, 24 and 26 kDa chitinases were present at very low intensities and they were induced to higher levels after inoculation. Irrespective of cultivars, a 20 kDa chitinase was induced at lower level whereas 23 and 24 kDa chitinases were induced at higher levels in resistant cultivars than in susceptible ones (Fig. 2). These results revealed that a 26 kDa chitinase in resistant cultivars is induced at higher level along with 23 and 24 kDa chitinases. Further Western blot assays with 12 resistant and susceptible cultivars each revealed a similar pattern of chitinase induction after pathogen interaction.

Fig.2. Differential induction of chitinases in sugarcane in response to *C. falcatum* infection



Above findings clearly demonstrated the differential induction of chitinases in sugarcane cultivars varying in red rot resistance in response to pathogen infection. The study clearly pointed out that constitutive expression of these proteins is observed only in resistant cultivars and their induction requires specific signals in

susceptible cultivars. Also a rapid induction of chitinases quantitatively and qualitatively was noticed in cultivars with high degree of resistance. Many workers demonstrated early induction of PR-proteins in different host-pathogen interactions (Van Loon and Van Strien, 1999). This is the first detailed report of the specific involvement of chitinases in red rot resistance. Earlier differential induction of chitinases and thaumatin like proteins in sugarcane in response to red rot infection has been demonstrated in cane tissue (Viswanathan et al., 2005). Our studies also showed a negative correlation between symptom development on inoculated leaves and induction of chitinases with a set of resistant and susceptible cultivars. The present studies establish suppression of symptom development in resistant cultivars due to chitinases and other defense related proteins.

Conclusion

Results of the present investigation clearly proved the presence of chitinases constitutively in resistant cultivars and their specific induction after pathogen inoculation. Further studies have been done on purification and characterization of these chitinases, which would ultimately lead to identification of disease resistance markers and genetic engineering of sugarcane for red rot resistance.

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