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Original Article

Fungal pathogenicity gene expression as tool to measure defense gene expression during Sugarcane X *Colletotrichum falcatum* interaction

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Abstract

Genomic and proteomic approaches are being attempted to understand the mechanism of host resistance against sugarcane red rot, the most serious disease caused by *Colletotrichum falcatum*. Study on differential expression of functional genes of both host and pathogen would help us to identify target genes of pathogenecity and host defense reactions. The functional genes responsible for host defense (Chitinase) and fungal pathogenicity (Chitin Synthase) characterized in our earlier studies were selected for studying their expression in response to host resistance. Differential induction of these two genes was monitored by PCR during *C. falcatum* pathogenesis in stalk tissue with increase in length in response to host resistance. Expression study in the adjoining tissue of inoculation point at different intervals indicated that there was differential expression of chitinase in response to host resistance after pathogen inoculation. Regarding fungal functional gene, the expression was delayed and then stopped at 6th day in resistant cultivar, while it was earlier and prolonged after 6th day in susceptible cultivar. Based on results, it is inferred that the Chitin Synthase gene expression could be used as one of the tool to measure pathogenecity and correspondingly it is easy to monitor expression level of defense related genes.

Introduction

The red rot disease of sugarcane, caused by *Colletotrichum falcatum* is the most serious disease of both tropical and sub-tropical India. Recently attempts are being made at genomic and proteomic level to understand the mechanism of host resistance during host pathogen interaction. Study on differential expression of functional genes of both host and pathogen in relation to defense mechanism and pathogenicity correspondingly will help us to identify target genes of pathogenicity and host defense reactions involved in particular host pathogen interaction. At the institute, expression of various defense related genes have been identified as major defense response against *C. falcatum* infection in sugarcane (Viswanathan *et al*., 2009). Of all the genes, expression of chitinases was dominated and hence it has been cloned and characterized for its full length. Similarly the chitin synthase gene has been cloned from *C. falcatum* and well characterized and functionally analysed

for its role in pathogenicity (Malathi *et al*., 2012). In the present investigation these two functional genes chitinase and chitin synthase were analysed for their simultaneous expression in relation to host defense and pathogenicity reactions.

Sugarcane Chitinase

Chitin is an important component of the cell wall of many fungal pathogens, and chitinase has been shown to inhibit hyphal growth of several fungi *in vitro*. Thus one of the postulated functions attributed to chitinases in plants is as a defense against fungal infection. In sugarcane differential induction of chitinases was demonstrated during host pathogen interaction with *C. falcatum*. Higher accumulation and early induction were found to be associated with resistance reaction (Viswanathan *et al*., 2005). Further sugarcane chitinase has been characterized for differential expression in sugarcane stalk tissues during pathogenesis of *C. falcatum* causing red rot in sugarcane were established by reverse transcription (RT)-PCR studies and through differential display (Viswanathan *et al*., 2009).

Colletotrichum falcatum - Chitin Synthase

The chitin synthase has been well characterized in various *Colltotrichum* spp. and other pathogens as functional gene required for chitin synthesis and thereby plays important role in cell wall morphogenesis, hyphal growth, spore germination and pathogenicity. Such functional gene has been characterized as chitin synthase 1A in *C. falcatum*, which is one among the different classes of chitin synthase (Malathi *et al*., 2012).

Materials and Methods

Resistant and susceptible cultivars were inoculated with *C. falcatum* spore suspension by plug method of inoculation. RNA was extracted surrounding the inoculated portion of standing canes of both the cultivars at 24h intervals by TRIZOL method; the quality was checked and quantified using spectrophotometer. Then translated into cDNA using reverse transcriptase and normalized based on GAPDH expression from plant and pathogen and subjected to RT-PCR with specific primers of both the genes.

Nucleotide sequence of chitinase and chitin synthase used for amplification in RT-PCR studies varied in their size. Size of nucleotide sequence amplified for expression studies was 830bp for chitinase which includes full length, while 181bp for chitin synthase which is designed from the 5'region of the full length having 2800bp. Sequence size of chitin synthase transcript (181bp) differs from the DNA amplification (239bp) is due to the exclusion of 58bp of intron size.

Results and Discussion

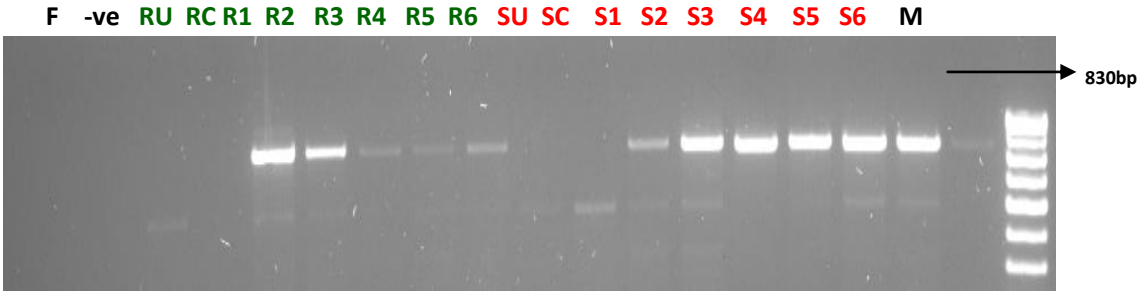
Expression of functional genes during host pathogen interaction

Results on defense gene expression showed that there was no constitutive expression of chitinase in both susceptible and resistant cultivars, while there was induction in injured canes of susceptible cultivar and not in resistant cultivar. Under inoculated conditions, there was differential expression in both the cultivars. In resistant canes the expression was found to be high at 24 and 48h intervals and thereafter it was reduced and finally stopped at 144h. In susceptible cultivar, there was equal expression upto 120h and then it was reduced. Regarding fungal functional gene, the expression was delayed (48h) and then stopped at 144h in resistant cultivar, while it was found to be earlier (24h) and prolonged

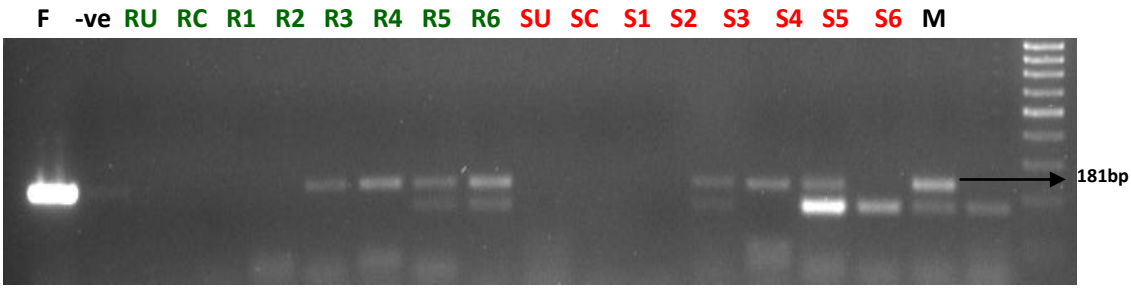
with increased level upto 144h in susceptible cultivar (Fig.).

Fig. RT-PCR studies on expression of functional genes during host pathogen interaction

a. Chitinase from the host



b. Chitin synthase from pathogen



R-Resistant; S- Susceptible; 1-24h, 2-48h, 3-72h, 4-96h, 5-120h, 6-144h;

Synchronized expression of functional genes in response to red rot resistance

The expression of chitinase is limited in resistant cultivar which might be due to that the sufficient level of earlier induction against the pathogenesis and correspondingly reduction in the expression of pathogenicity gene. In contrast, there was concurrent expression of both the defense gene chitinase and pathogen functional gene chitin synthase with additional transcript in susceptible cultivar. Based on the results, it is inferred that the chitin synthase gene expression could be used as one of the tool to measure pathogenicity for host resistance and it can be very well utilized to determine the expression of defense genes. Benito *et al*. (1998) utilized fungal actin and β-tubulin genes as probes to measure the fungal biomass in different portions of tomato leaf infected with *Botrytis cinerea* and proved its synchronous expression. They also studied the kinetics of PR-proteins (chitinase, β -1,3-glucanase and PR-1) expression from tomato plant in response to *B. cinerea* simultaneously. Jin *et al*. (1999) also studied actin gene expression during compatible interaction of round-leaved mallow, *Malva pusilla* and *Colletotrichum gloeosporioides* f. sp. *malvae* from both host and pathogen.

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