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Induction of pathogenesis-related proteins in sugarcane leaves and cell-cultures by a glycoprotein elicitor isolated from *Colletotrichum falcatum*

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Abstract

The induction of pathogenesis-related (PR) proteins in sugarcane (*Saccharum officinarum* L.) leaves and suspension-cultured cells in response to treatment with a glycoprotein elicitor isolated from *Colletotrichum falcatum* (the red rot pathogen) was investigated. Treatment of leaves and cells with the elicitor resulted in a much marked increase in the activities of chitinase and β-1,3-glucanase in red rot resistant (BO 91) than susceptible (CoC 671) sugarcane cultivar. SDS-PAGE analysis revealed that *C. falcatum* elicitor induced the accumulation of several proteins in suspension-cultured cells of resistant cultivar (BO 91); among them the 35 kDa protein was predominant. Whereas, a 27 kDa protein was induced predominantly in the cells of susceptible cultivar upon treatment with the elicitor. When sugarcane leaves were treated with *C. falcatum* elicitor, two proteins with apparent molecular masses of 25 and 27 kDa were induced both in the resistant and susceptible cultivars. However, the induction was stronger in the resistant than the susceptible cultivar. Immunoblot analysis for chitinase indicated that a protein with an apparent molecular mass of 37 kDa cross-reacting with barley chitinase antiserum was strongly induced in the suspension cultured cells of both the cultivars. The induction of 37 kDa chitinase was more in the cells of resistant cultivar than in the susceptible cultivar. Western blot analysis revealed that a 25 kDa thaumatin-like protein (TLP) cross-reacting with bean TLP antiserum was strongly induced in leaves and cultured cells of both resistant and susceptible cultivars due to elicitor treatment.

Additional key words: chitinase, β-1,3-glucanase, red rot pathogen, *Saccharum officinarum*, thaumatin-like protein.

Introduction

Plants respond to pathogen infection by producing a number of proteins believed to be important in protecting them from the deleterious effects of the pathogen. These proteins described as pathogenesis-related (PR) proteins are known to be selectively extractable in a buffer of low pH, and are highly resistant to proteolytic degradation (Van Loon and Gerritsen 1989). The PR proteins have been classified into 14 families based on the amino acid sequences, serological relationship and/or enzymatic or biological activity (Van Loon and Van Strien 1999). Many PR proteins purified from plants exhibit direct antifungal activity against a wide range of fungal pathogens (Datta *et al.* 1999). Furthermore, it has been demonstrated that genetically engineered over-expression of PR proteins can increase resistance in plants (Broglie *et al*. 1991, Datta *et al*. 2001, Velazhahan and Muthukrishnan 2004). The activation of defense mechanisms in plants is considered to be consequent upon an initial recognition event in which the host plant detects molecular components of the pathogen, known as elicitors (Van't Slot and Knogge 2002). Several biotic elicitors including proteins, glycoproteins, peptides and lipids have been detected in germination fluid, culture fluids and cell walls of many phytopathogenic fungi (Ebel and Cosio 1994, Darwill and Albersheim 1984, Kiba *et al*. 1999). These elicitor molecules bind to a receptor(s) on the plasma membrane of plant cells and activate the signaling events required for the onset of the defense responses (Umemoto *et al*. 1997). Previously we reported purification of a high molecular mass glycoprotein elicitor from the mycelial walls of *Colletotrichum falcatum*, the red rot pathogen of sugarcane which induced phenylpropanoid metabolites and active oxygen species in suspension-cultured

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Abbreviations: PR proteins - pathogenesis related proteins, TLP - thaumatin like protein.
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sugarcane cells (Ramesh Sundar *et al*. 2002). In the present study, the induction of PR proteins in sugarcane leaves and suspension-cultured cells of red rot resistant (BO 91) and susceptible (CoC 671) sugarcane cultivars in response to treatment with *C. falcatum* elicitor was demonstrated for the first time. Rapid induction of PR proteins in cells and leaves of resistant cultivar than the susceptible cultivars was observed. The possible role of PR proteins in the defense of sugarcane against *C. falcatum* is discussed.

Materials and methods

Isolation of cell wall elicitor: The fungus *Colletotrichum falcatum* was grown on oatmeal broth under room temperature (28 ± 2 °C). Mycelia were harvested from 8-d-old liquid cultures and homogenised for 60 s using 5 cm^3 of water g^{-1}(fresh mass of mycelia). The homogenate was filtered through a coarse sintered glass funnel and the residue obtained on the filter was saved. The residue was homogenized three more times in water, once in a mixture of chloroform and methanol (1:1) and finally in acetone. The residue was air-dried and elicitor was extracted and purified as described earlier (Ramesh Sundar *et al*. 2002).

Plants: Sugarcane (*Saccharum officinarum* L.) cvs. CoC 671 (highly susceptible to red rot disease) and BO 91 (a standard resistant cultivar) were raised in cement pots containing 5 kg of potting mixture containing sand: red earth: farmyard manure, 1:1:1, under standard greenhouse conditions (25 ± 2 °C, 250 μmol m^{-2} s^{-1}), using two budded sets as the planting material. Ideal growing conditions were ensured by providing nutritional supplements and proper irrigation in order to obtain healthy cane material to be used for the study (Sundara 1998).

Suspension-cultured sugarcane cells/leaf samples and elicitor treatment: Embryogenic calli were initiated from inner two whorls of young leaves of sugarcane cvs. BO 91 and CoC 671 on Murashige and Skoog (1962; MS), basal medium supplemented with 1.0 mg dm^{-3} of 2,4-di-chlorophenoxyacetic acid. Rapidly growing embryogenic cell suspensions were established from one month old embryogenic calli as described by Ho and Vasil (1983). After transfer to fresh medium, sugarcane cell cultures were incubated for 6 d prior to treatment with 1 cm^3 of filter sterilized fungal cell wall elicitor (60 μg in terms of glucose equivalents per cm^3 cells) (Ramesh Sundar and Vidhyasekaran 2003). Controls were treated with 1 cm^3 of sterile distilled water. Immediately following the addition of elicitor, the cultures were returned to standard growth conditions. Samples were collected at different time intervals for analysis of chitinase and β-1,3 glucanase.

Similarly, leaf samples were collected from 3-month-old sugarcane plant of both the cultivars. Leaves were dipped in 30 cm^3 of sterile distilled water containing 1 cm^3 of elicitor solution (60 μg glucose equivalents) in a conical flask. Analogous treatments with water alone served as control. Treated and control leaves were kept in a *Percival* (Perry, IA, USA) dew chamber at 20 °C under continuous irradiance of 500 μmol m^{-2} s^{-1}. Samples were collected at periodical intervals for analyses of different parameters (Ramesh Sundar *et al*. 2002).

Samples from each treatment were analyzed thrice, and the experiment was performed twice. The treatment values from two independent experiments were averaged and plotted by time. For the analysis of PR-proteins by SDS-PAGE and Western blotting was done at 24 h after elicitor treatment.

Assay of chitinase: Suspension-cultured cells/leaves (1 g) were homogenized in 5 cm^3 of 0.1 M sodium citrate buffer (pH 5.0). The homogenate was centrifuged for 10 min at 10 000 *g* at 4 °C and the supernatant was used in the enzyme assay. Colloidal chitin was prepared according to Berger and Reynolds (1958) from crab shell chitin (*Sigma,* St. Louis, MO, USA). The commercial lyophilized snail gut enzyme (helicase, obtained from *Sepracor*, Louvres, France) was desalted as described by Boller and Mauch (1988). For the colorimetric assay of chitinase, 0.01 cm^3 of 1 M sodium acetate buffer (pH 4.0), 0.4 cm^3 of enzyme extract and 0.1 cm^3 of colloidal chitin (1 mg) were pipetted into a 1.5 cm^3 Eppendorf tube. After 2 h at 37 °C, the reaction was stopped by centrifugation at 10 000 *g* for 3 min. An aliquot of the supernatant (0.3 cm^3) was pipetted into a glass reagent tube containing 0.03 cm^3 of 1 M potassium phosphate buffer (pH 7.1) and incubated with 0.02 cm^3 of desalted snail gut enzyme for 1 h. The resulting monomeric N-acetyl-glucosamine (GlcNAc) was determined according to Reissig *et al*. (1955) using internal standards of GlcNAc in the assay mixtures for calculations. Enzyme activity was expressed as nmol(GlcNAc equivalents) mg^{-1}(f.m.) min^{-1}.

Assay of β-1,3-glucanase activity: β-1,3-glucanase activity was colorimetrically assayed by the laminarin dinitrosalicylic acid method (Pan *et al.* 1991). Leaves/ suspension-cultured cells (1 g) were extracted with 5 cm^3 of 0.05 M sodium acetate buffer (pH 5.0) by grinding at 4 °C using a pestle and mortar. The extract was then centrifuged at 10 000 *g* for 15 min at 4 °C and the supernatant was used in the enzyme assay. The reaction mixture consisted of 0.0625 cm^3 of 4 % laminarin and 0.0625 cm^3 of enzyme extract. The reaction was carried out at 40 °C for 10 min. The reaction was then stopped by adding 0.375 cm^3 of dinitrosalicylic reagent and heating for 5 min on a boiling water bath. The resulting colored

solution was diluted with 4.5 cm^3 of distilled water, vortexed and its absorbance at 500 nm was determined. Enzyme activity was expressed as nmol mg^{-1}(f.m.) min^{-1}.

SDS-PAGE and Western blot analysis: Sugarcane leaves/suspension-cultured cell tissues were collected 24 h after elicitor treatment. Proteins extracts were prepared by grinding 1 g of cells or tissues in 5 cm^3 of 0.1 M potassium phosphate buffer (pH 6.5) containing 0.5 mM phenylmethylsulphonyl fluoride (PMSF) (Velazhahan *et al*. 1998). Proteins (100 µg) in aliquots of extracts were separated on 12 % SDS-PAGE and stained using Coomassie brilliant blue (Laemmli 1970) using *BioRad* (Hercules, CA, USA) mini gel apparatus. The separated proteins were electroblotted onto polyvinylene difluoride (PVDF) membrane (pore size 0.45 µm) using *Bio-Rad* semidry transblot unit in accordance with the manufacturer's instructions. The membrane was then blocked for 3 h at room temperature in Tris buffered saline-*Tween 20* (TBST) containing 5 % (m/v) casein. The membrane was soaked in the diluted primary antibody at 1:1000 dilution overnight in TBST. Antiserum raised against a barley chitinase and an antiserum raised against bean thaumatin-like protein (TLP; a gift of Dr. S. Muthukrishnan, Kansas State University, USA) were used as the primary antibodies. After incubation, the membranes were washed thrice with TBST for 10 - 15 times each to remove the unbound antibody. The membranes were then incubated in secondary antibody for 2 h. Affinity purified goat anti-rabbit immunoglobulin (IgG) alkaline phosphatase conjugate (*Sigma*) was used as a secondary antibody at a dilution of 1:4000. After washing with TBST, immunological reaction was visualized by soaking the membranes in alkaline phosphatase colour development reagents. Immediately after colour development, the membranes were washed with distilled water and the reaction was stopped with 20 mM EDTA and the membrane dried. Apparent molecular masses of proteins were determined by comparison with molecular mass standards (*Rainbow markers*, *Amersham Pharmacia*, Piscataway, NJ, USA). Protein concentrations were determined by Bradford assay (Bradford 1976).

Results

Significant increase in chitinase activity was detected in leaves of both resistant and susceptible cultivars of sugarcane 24 h after elicitor treatment. The enzyme activity gradually increased throughout the experimental period of 72 h. However, the activation of chitinase was more rapid and to a greater extent in leaves of BO 91 than in CoC 671. A 3 and 2.5 fold increase in chitinase activity was recorded after 72 h in elicitor treated leaves of BO 91 and CoC 671 respectively when compared to control (Fig. 1*A*). A similar induction of chitinase activity was observed in the suspension-cultured cells of sugarcane in response to elicitor treatment. The maximum enzyme activity was recorded 48 h after the treatment and thereafter a gradual decline in enzyme activity was noticed. The levels of chitinase activity were higher in cells of resistant cultivar than in the susceptible cultivar. A 2.5 and 1.7 fold increase in chitinase activity was observed in cells of resistant and susceptible cultivars respectively at 48h after elicitor treatment (Fig. 1*B*). Immunoblot analysis for chitinase indicated that a chitinase with an apparent molecular mass of 37 kDa cross-reacting with barley chitinase antiserum was constitutively expressed in the control cells, but strongly induced after elicitor treatment in the suspension cultured cells of both the cultivars and the induction was more in the cells of resistant cultivar than in the susceptible cultivar (Fig. 2).

β-1,3-glucanase activity increased rapidly by 24-h elicitor treatment in leaves of BO 91, reaching a level two-fold higher than the corresponding control. In CoC 671, β-1,3-glucanase activity increased significantly by

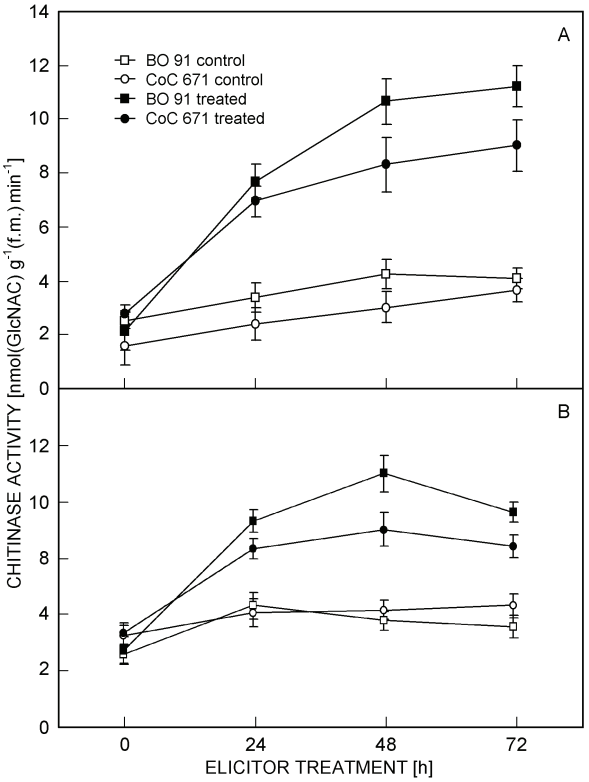

Fig. 1. Changes in chitinase activity in sugarcane leaves (*A*) and suspension cultures (*B*) in response to treatment with *Colletotrichum falcatum* elicitor. Chitinase activity was determined colorimetrically. Data are means from three independent samples. *Bars* indicate standard deviation.

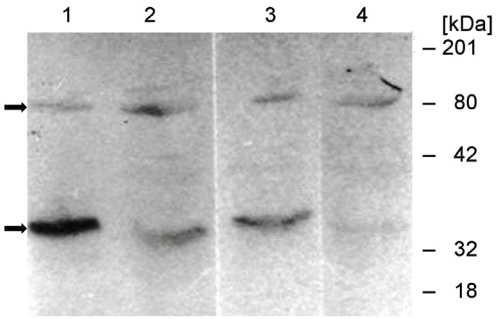

Fig. 2. Western blot showing induction of chitinase in sugarcane suspension-cultured cells in response to treatment with *C. falcatum* elicitor. Total proteins (100 µg) extracted from elicitor-treated cultured cells of resistant (R) and susceptible (S) sugarcane cultivars 24 h after treatment were separated by 12 % SDS-PAGE. After electrophoresis, the proteins were electrotransferred to a polyvinylene difluoride membrane and the chitinase was detected using barley chitinase antibody. *Lane 1* - elicitor treated BO 91 (R) cells, *lane 2* - control BO 91 (R) cells, *lane 3* - elicitor treated CoC 671 (S) cells, *lane 4* - control CoC 671 (S) cells. *Arrows* indicates the position of induced chitinase.

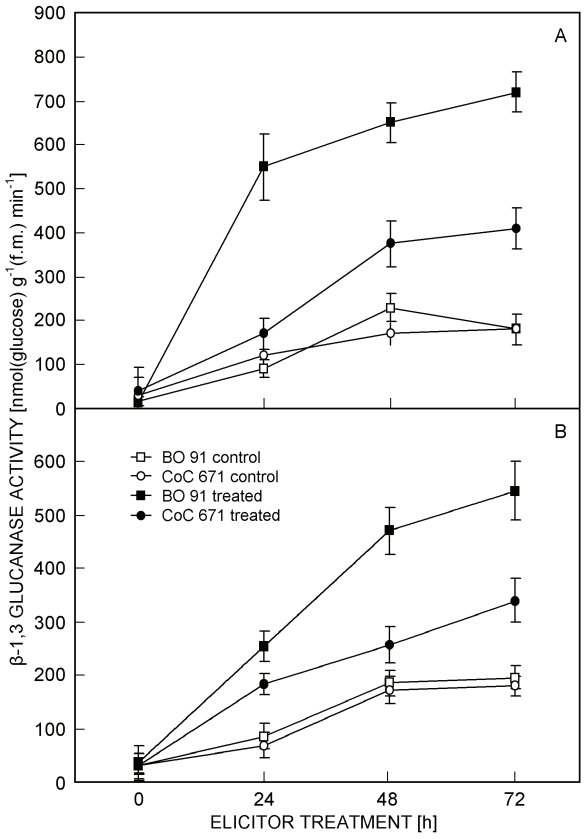

Fig. 3. Changes in β-1,3-glucanase activity in sugarcane leaves (*A*) and in suspension-cultured cells (*B*) in response to treatment with *C. falcatum* elicitor. β-1,3-glucanase activity was determined colorimetrically. Data are means from three independent samples. *Bars* indicate standard deviation.

48-h treatment reaching a level of 2.2 folds higher than the corresponding control (Fig. 3*A*). A similar response was observed in the case of suspension cultured cells treated with the fungal elicitor, though the rate of increase is different from that observed in leaf tissue. β-1,3-glucanase activity significantly increased in cells of both cultivars 24 h after elicitor treatment and the enzyme activity continuously increased throughout the experimental period of 72 h (Fig. 3*B*). However, the enzyme activity was higher in the cells of BO 91 when compared to CoC 671. A 3-fold increase in β-1,3 glucanase activity was recorded 72 h after elicitor treatment in cells of BO 91 compared to control.

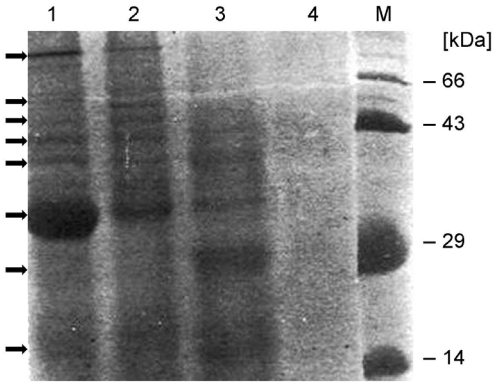

Fig. 4. SDS-PAGE analysis of protein extracts from elicitor-treated suspension-cultured cells of sugarcane. *Lane 1* - elicitor treated BO 91 (R) cells, *lane 2* - control BO 91 (R) cells, *lane 3* - elicitor treated CoC 671 (S) cells, *lane 4* - control CoC 671 (S) cells, *lane M* - molecular mass marker. *Arrows* indicate the induced proteins.

Changes in the protein profile of sugarcane leaves and cultured cells after elicitor treatment were analyzed by SDS-PAGE. Differential accumulation of PR proteins between resistant and susceptible cultivars was observed in elicitor treated cells. *C. falcatum* elicitor induced the accumulation of several proteins in suspension-cultured cells of BO 91, among them the 35 kDa was predominant (Fig. 4). The 35-kDa protein was constitutively present at a lower amount in control cells but strongly induced after elicitor treatment. Several new proteins appeared in the cultured cells of CoC 671 after elicitor treatment. Polypeptides with molecular masses of 17, 22, 27, 31, 35, 37, 40 45 and 70 kDa could be detected in the elicitor treated CoC 671 cells, which were absent from the control cells. Among them the 27 kDa protein was predominant. The 27 kDa protein was not induced in the cells of B0 91 upon treatment with the elicitor (Fig. 4). When sugarcane leaves were treated with *C. falcatum* elicitor, two proteins with apparent molecular masses of 25 and 27 kDa were induced in both cultivars. However, the induction was stronger in the resistant than the susceptible cultivar (Fig. 5).

Western blot analysis revealed that a 25 kDa thaumatin like protein cross reacting with bean TLP

antiserum was strongly induced in leaves (Fig. 6) and cultured cells (Fig. 7) of both resistant and susceptible cultivars due to elicitor treatment. This 25 kDa TLP could not be detected in the control leaves and cells. Similarly enhanced induction of chitinase isoforms were observed in elicitor treated cell suspensions of both cultivars. However, BO 91 harbored more of constitutive TLP as compared to CoC 671.

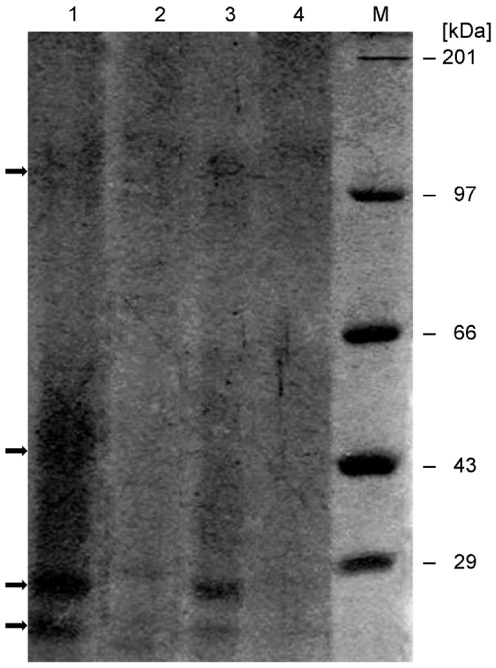

Fig. 5. SDS-PAGE analysis of protein extracts from elicitor treated leaves of sugarcane. Total proteins (100 µg) extracted from elicitor-treated leaves of resistant (R) and susceptible (S) sugarcane cultivars 24 h after treatment were separated by 12 % SDS-PAGE and then stained with Coomassie Brilliant Blue. *Lane 1* - Elicitor treated BO 91 (R) leaves, *lane 2* - control BO 91 (R) leaves, *lane 3* - elicitor treated CoC 671 (S) leaves, *lane 4* - control CoC 671 (S) leaves, *lane M* - molecular mass marker. *Arrows* indicate the induced proteins.

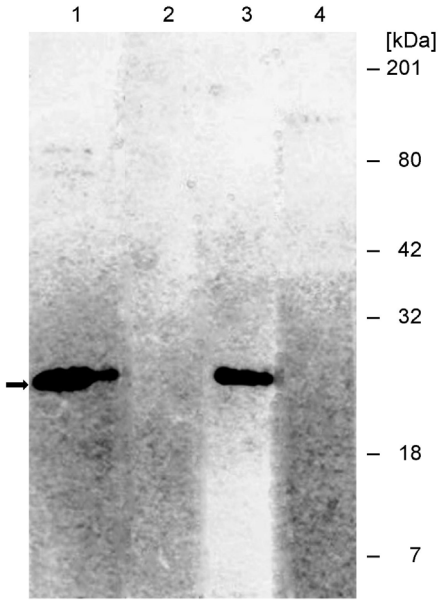

Fig. 6. Induction of thaumatin like proteins (TLP) in sugarcane leaves in response to treatment with *C. falcatum* elicitor. Total proteins (100 µg) extracted from elicitor-treated leaves of resistant (R) and susceptible (S) sugarcane cultivars 24 h after treatment were separated by 12 % SDS-PAGE. After electrophoresis, the proteins were electrotransferred to a polyvinylene difluoride membrane and the TLP was detected using bean TLP antibody. *Lane 1* - Elicitor treated BO 91 (R) leaves, *lane 2* - control BO 91 (R) leaves, *lane 3* - elicitor treated CoC 671 (S) leaves, *lane 4* - control CoC 671 (S) leaves. *Arrow* indicates the position of TLP.

Discussion

Although few sugarcane cultivars exhibit resistance to *C. falcatum*, the molecular mechanisms underlying the resistance are poorly understood. For studying the defence gene activation in sugarcane, the intact plant-pathogen system may not be ideal because the time course of pathogen infection cannot be monitored precisely. Cell cultures instead of whole plants and elicitors instead of live pathogens are found to be suitable models to study the defence gene activation in bean (Edington *et al.* 1991), rice (Velazhahan and Vidyasekaran 2000), grapevine (Repka 2006) and in sugarcane (McGhie *et al*. 1997, Ramesh Sundar and Vidhyasekaran 2003). Further, each cell in the culture is uniformly exposed to the elicitor and hence the response is relatively uniform. In the present study, differential induction of PR proteins between resistant and susceptible cultivars of sugarcane was observed in elicitor treated cells and leaves. SDS-PAGE analysis revealed that *C. falcatum* elicitor induced the accumulation of several proteins in suspension-cultured cells of resistant cultivar (BO 91), among them the 35 kDa protein was predominant. In contrast, a 27 kDa protein was induced predominantly in the cells of susceptible cultivar. When sugarcane leaves were treated with *C. falcatum* elicitor, two proteins with apparent molecular masses of 25 and 27 kDa were induced both in the resistant and susceptible cultivars. However, the induction was stronger in the resistant than the susceptible cultivar. The chitinase activity increased significantly from 24 h after elicitor treatment and reached the maximum 72 h after treatment in leaves of both cultivars of sugarcane. However, the activation of chitinase was more rapid and to a greater extent in leaves of resistant than in susceptible cultivar. A similar induction of chitinase activity was observed in the suspension-cultured cells in response to elicitor treatment. Immunoblot analysis for chitinase indicated that a

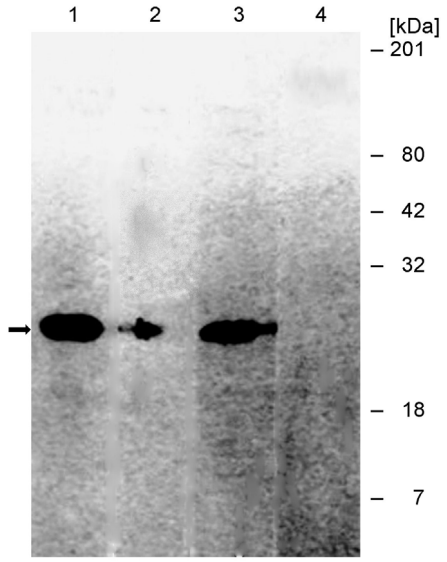

Fig. 7. Induction of thaumatin like proteins (TLP) in sugarcane suspension-cultured cells in response to treatment with *C. falcatum* elicitor. Total proteins (100 µg) extracted from elicitor-treated cultured cells of resistant (R) and susceptible (S) sugarcane cultivars 24 h after treatment were separated by 12 % SDS-PAGE. After electrophoresis, the proteins were electrotransferred to a polyvinylene difluoride membrane and the TLP was detected using bean TLP antibody. *Lane 1* - elicitor treated BO 91 (R) cells, *lane 2* - control BO 91 (R) cells, *lane 3* - elicitor treated CoC 671 (S) cells, *lane 4* - control CoC 671 (S) cells. *Arrow* indicates the position of TLP

chitinase with an apparent molecular mass of 37 kDa was induced in the suspension cultured cells of both the cultivars. However, the induction of 37 kDa chitinase was stronger in the cells of BO 91 than in CoC 671. Chitinases, belonging to PR-3 group of PR proteins catalyze the hydrolysis of β-1,4 linkages of the N-acetyl-D-glucosamine polymer, called chitin, which is a major component of the cell walls of many fungi (Collinge *et al.* 1993). Many purified plant chitinases have been demonstrated to have antifungal activity *in vitro* (Velazhahan *et al.* 2000b). It has been demonstrated that constitutive overexpression of chitinase in transgenic plants can enhance disease resistance (Broglie *et al*. 1991, Lin *et al*. 1995, Punja *et al*. 1996, Datta *et al*. 2001). In addition, the chitinases can release elicitors from the fungal cell walls by their enzymatic action and these elicitors induce various defence responses in plants (Ren and West 1992). Elicitor induced chitinase activity in suspension-cultures has been reported in bean cells treated with *Colletotrichum lindemuthianum* elicitor (Daugrois *et al*. 1990), in mungbean cells treated *Macrophomina phaseolina* elicitor (Ramanathan and Vidhyasekaran 1997), and in rice cells treated with *Rhizoctonia solani* elicitor (Velazhahan *et al.* 2000 b). The consistent and rapid increase in chitinase activity in the resistant cultivar of sugarcane after elicitor treatment suggests a possible role for chitinases in the defence mechanism of sugarcane against *C. falcatum*.

Elicitor treatment also increased the activity of β-1,3-glucanase in leaves and suspension-cultured cells of both the cultivars. β-1,3-glucanase activity increased rapidly by 24-h elicitor treatment in leaves of BO 91, whereas in CoC 671, β-1,3-glucanase activity increased significantly by 48-h treatment. A direct role for β-1,3-glucanases in the defence of plants against pathogens has been proposed, because the substrate for the enzyme, β-1,3-glucan is a major component of the cell walls of many fungi (Wessels and Sietsma 1981). Furthermore, β-1,3-glucanases are known to release oligosaccharides from the walls of fungi, which in turn, act as signals in the elicitation of host defence responses (Sharp *et al*. 1984, Yoshikawa *et al*. 1990, Takeuchi *et al*. 1990, Ham *et al*. 1991). A correlation between constitutive β-1,3-glucanase level and resistance has been detected in leaves of tomato lines infected with *Alternaria solani* (Lawrence *et al*. 1996). In *Fusarium*-infected musk-melon, there was evidence of higher β-1,3-glucanase activity in resistant plants than in susceptible plants (Ward *et al*. 1991). It has been demonstrated that β-1,3-glucanase is involved in the defence response controlled by the *Lr35* gene in wheat to leaf rust caused by *Puccinia recondita* f. sp. *tritici* (Anguelova-Merhar *et al*. 2001). Similar reports on fungal elicitor induced glucanase activity are available in bean (Edington *et al*. 1991), alfalfa (Dalkin *et al.* 1990), rice (Velazhahan 1995) and in mungbean (Ramanathan and Vidhyasekaran 1997). It has been demonstrated that both chitinases and β-1,3-glucanases from pea tissues acted synergistically in partial degradation of isolated fungal cell walls (Mauch *et al*. 1988). Further, it has been demonstrated that transgenic plants with elevated levels of chitinase and glucanase expression are more resistant to fungal pathogens (Lin *et al*. 1995, Datta *et al*. 2001). In sugarcane, synthetic elicitors namely acibenzolar S-methyl and salicylic acid have been found to increase considerably activities of these two hydrolytic enzymes after inoculation with the red rot pathogen (Ramesh Sundar *et al.* 2001). Similarly the role of chitinases in *Pseudomonad*-induced resistance to *C. falcatum* in sugarcane has been established (Viswanathan and Samiyappan 2001). Molecular elucidation of the primed cells in the present study indicate the possibility of β-1,3-glucanase in combination with chitinase play a pivotal role in imparting defence against sugarcane red rot pathogen.

Western blot analysis revealed that a 25 kDa thaumatin-like protein (TLP) was induced in elicitor treated suspension-cultured cells and leaves upon treatment with the fungal elicitor. TLPs have been demonstrated to have antifungal activity by disrupting fungal plasma membrane in *in vitro* assays (Roberts and Selitrennikoff 1990, Vigers *et al*. 1991, Jayaraj *et al*. 2004). Furthermore, it has been demonstrated that transgenic plants constitutively expressing high levels of TLPs showed an enhanced disease resistance (Liu *et al*. 1994, Datta *et al.* 1999, Velazhahan and Muthukrishnan 2004). The induced TLP in sugarcane treated with elicitor

might have involved in resistance against *C. falcatum*.

The results, therefore, suggest a possible role of PR-proteins in defence mechanism of sugarcane against the red rot pathogen. The PR proteins are activated in leaves and cultured cells of both resistant and susceptible sugarcane cultivars by *C. falcatum* elicitor. Cultured cells respond to fungal elicitor in a manner similar to intact plant tissue-elicitor system. Hence the detached leaf-elicitor system can be used for studying molecular basis of disease resistance in sugarcane.

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