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CHARACTERIZATION OF 14-3-3 LIKE PROTEIN GENE FROM *COLLETOTRICHUM FALCATUM* CHALLENGED SUGARCANE USING RACE-PCR

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Red rot is one of the most devastating diseases of sugarcane caused by the fungal pathogen *Colletotrichum falcatum*. It has wiped out a large number of elite commercial sugarcane varieties in the past. Although the inheritance of red rot resistance in sugarcane has not been understood very clearly, the possible factors that might contribute for red rot resistance in disease resistant varieties have been established through differential induction of pathogenesis related proteins such as chitinase and thaumatin like proteins (Viswanathan *et al.,* 2005) and 3-deoxyanthocyanidin phytoalexins such as luteolinidin, apigeninidin and caffeic acid ester of 5-0-apigeninidin (Viswanathan *et al.,* 1996) in disease resistant and susceptible varieties. Recently, differential display RT-PCR (DD-RT-PCR) approach was used to study the *interaction* between sugarcane and *C. falcatum* in cane tissues as well as between sugarcane cell suspension and *Cf*-elicitor (isolated from fungal mycelium) at the institute. This study for the first time, established that *in vitro* interaction between sugarcane cell suspension and *Cf*-elicitor mimics the molecular interaction in sugarcane and *C. falcatum* in cane tissuesThe present study reports the full length sequencing of 14-3-3 like protein transcript from DD-RT-PCR. The gene was characterized using the RNA ligase mediated rapid amplification of cDNA ends (RLM-RACE-PCR) technique using GeneRacer kit (Invitrogen, USA).

A 1094 bp, 14-3-3 like protein transcript (Genbank Acc. no. HO222097) was identified from the RACE-PCR. The sequence revealed the presence of conserved 14-3-3 superfamily domain in the CDD search. The ORF finder tool from the NCBI, revealed the presence of a 771 bp ORF, flanked by 86 bp 5'-UTR, and 237 bp 3'-UTR in the full length sequence. The gene product was 256 amino acids in length and the calculated molecular weight was found to be 28.8 kDa. In plants, the earliest studies revealed the role of 14-3-3 like proteins in binding phosphorylated nitrate reductase (NR), in a physiological setting (i.e. the response to inhibition of photosynthesis in leaves). They were further linked to the regulation of the plasma membrane H^+-ATPase, and have been found to bind to a number of proteins (including VP1 and EmBP1) which mediate abscisic acid-induced gene expression. The studies involving host-pathogen interaction, wherein the 14-3-3 plays significant role is by regulating target proteins with functions of either signalling or transcription activation or defense. Earlier studies implicated the regulation of 14-3-3s in the non-host hypersensitive response (HR) between barley and *Blumeria graminis f.sp. tritici*. It was found that 14-3-3s bind to and activate H^+-ATPase, creating a binding site for the phytotoxin FC, and FC-binding activity of an epidermal microsomal fraction increases upon the pathogen attack, suggesting that 14-3-3s are involved in an epidermis-specific response to the fungus, probably through activating the proton pump (H^+-ATPase) to stimulate the HR. The phenylpropanoid pathway components namely caffeic acid/5-hydroxyferulic acid o-methyltransferase (OMT1) and ascorbate peroxidase that are implicated in plant defense or oxidative stress were identified to interact with 14-3-3s (Zhang *et al.,* 1997). This is particularly significant in the

present context, as it has been well established that in sugarcane the phenylpropanoid pathway is associated with the red rot defense (Viswanathan *et al.,* 1996). The role of the 14-3-3s can thus be presumed to be in some way involved in the induction of resistance response in sugarcane. It has been identified as being induced in sugarcane cell suspension, as well as cane tissues in the differential display studies. Thus the role of 14-3-3s in sugarcane *C. falcatum* interaction is very much in line with their roles in several other crops in defense responses.

In order to further understand the position of the sugarcane 14-3-3s to those of other crops, the already known sequences were used for comparison. Among plants the dicotyledonous model plant *Arabidopsis* has the most complete and largest 14-3-3 family consisting of 15 members classified into two evolutionary branches, the -group and the non- group. Rice 14-3-3s (GF14b,GF14c,GF14e & GF14f) have been known to be involved in stress responses, while some are known to be differentially regulated during interaction between with rice blast fungus *Magnaporthe grisea* and bacterial blight *Xanthomonas oryzae* pv. *oryzae* (Xoo), defense signalling compounds and diverse abiotic stress stimuli (Chen *et al.,* 2006). Thus in a monocotyledonous system, the rice 14-3-3s having been studied extensively and is an ideal candidate for comparison with sugarcane 14-3-3s. The phylogenetic analysis was hence carried out using the sequences from rice 14-3-3 proteins, GF14a-h (total-8 nos.). The study utilized 14-3-3 sequence from our study along with three EST clusters (SCCCLR1022D05.g, SCCCRZ1001D02.g and SCBFLR 1026E02.g) similar to 14-3-3 proteins found in the sugarcane EST genome project database. The multiple sequence alignment and their dendrogram revealed that the isolated 14-3-3 was more similar to rice GF14C followed by sugarcane cluster SCBFLR1026E02.g from SUCEST database (Fig. 1).

The GF14c was found to be induced in early incompatible and compatible rice-*Magnaporthe grisea* interactions along with GF14b, GF14e and GF14f, and this induction was stronger in the incompatible interactions. Also the GF14c was induced during rice-Xoo interaction as well (Chen et al., 2006). Thus the identification of 14-3-3 during the incompatible interaction between sugarcane - *C. falcatum* is consistent with the expression pattern of defence-related genes during the host-pathogen interactions. The GF14c was also found to be induced significantly in response to post-ethephon treatment (ETH, a precursor of ethylene) after 24 h, and slightly induced by BTH and MeJA. Also a low H_2O_2 (0.01 mM) concentration was found to induce a higher GF14c response in rice. During salt and drought stress conditions the GF14c was found to be induced very rapidly (2-8 h) as in the case with ABA treatment.

The study by Papini-Terzi *et al.* (2009) identified four 14-3-3 proteins of the GF14 type (SCCCLR1022D05.g, SCCCRZ1001D02.g, SCEQRT 1031D02.g and SCEQRT1025D06.g) that were expressed at lower levels in mature internodes of sugarcane. An earlier report had suggested that 14-3-3 proteins, together with a SnRK1 (SNF-like kinases), phosphorylate and inhibit the enzyme sucrose phosphate synthase (SPS) *in vitro* in sugarcane. Thus the reduced expression of 14-3-3 in mature internodes may suggest an increased accumulation of sucrose content. Thus the interplay between different components of protein regulatory mechanism suggests a complex modulatory role for different isoforms of 14-3-3s thus playing an important role in response to a specific stimulus.

The present study on 14-3-3 like protein indicated a specific induction upon interaction with *C. falcatum*,which suggest an implication for 14-3-3 to play an important role in sugarcane disease

resistance mechanism. The information on the different isoforms of the 14-3-3 like protein in sugarcane is unavailable and future studies towards greater understanding of its role and their interplay needs to be established as in other crops.

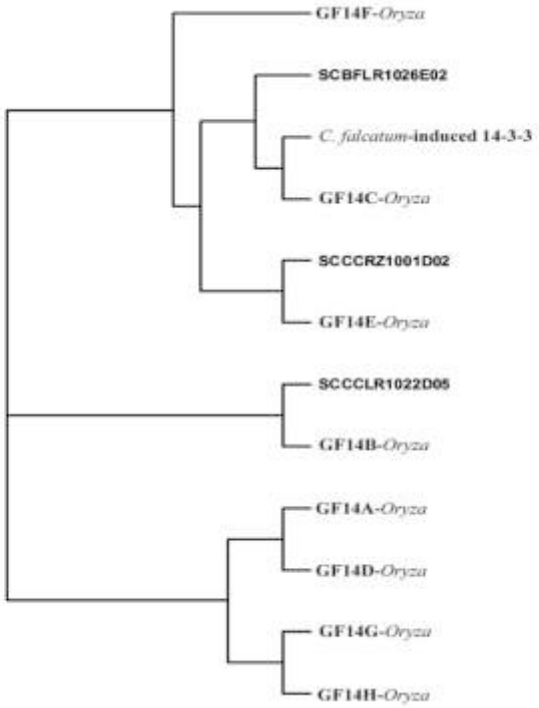

Fig 1. Phylogenetic tree analysis of *C. falcatum* induced 14-3-3 like protein with rice 14-3-3 like proteins by Neighbor-joining method

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