transduction and the expression of different functional stress-responsive genes (Urano et al., 2010). Therefore, the use of genes encoding regulatory proteins is one of the most promising strategies to obtain transgenic plants tolerant to drought.

Genetic engineering applied to increased drought tolerance has been done in many plants species. Starting with *Arabidopsis* and tobacco that are model plants used to study the effects and function of the genes. However, economically important crops, such as wheat, rice and maize have also been used for concept-proof to drought tolerance.

In the case of sugarcane, stable transformation is not a routinely achieved procedure. Low transformation efficiency, transgene inactivation, somaclonal variation in tissue culture are some of the major bottlenecks. The first published report on sugarcane transformation for drought tolerance used the overexpression of the disaccharide trehalose. Physiological characterization of plants submitted to drought stress, such as bound water/free water rate, plasma membrane permeability, malondialdehyde content, chlorophyll *a* and *b* contents, and activity of superoxide dismutase and peroxidase in excised leaves, suggested that overexpression of trehalose is involved in enhanced drought tolerance. Another example is the increase of drought tolerance in transgenic sugarcane overexpressing proline in response to stress. Transformed plants were protected against the oxidative stress caused by water deficit. The higher tolerance of those transgenic plants was assessed by higher biomass yields after 12 days of withholding water.

The *DREB* genes are well studied transcription factors linked to stress related genes expression. The DREB proteins interacts with *cis*-acting dehydration-responsive element/C-repeat (DRE/CRT) present in the promoter region of many functional genes related to drought, activating there expression. Our research group has developed transgenic sugarcane plants with *AtDREB2A* gene driven by stress-inducible (*ZmRAB17*) and constitutive (*ZmUbi-1*) promoters. Preliminary results showed that events *AtDREB2A* were tolerant to drought stress when submitted to water withholding treatment. Currently, physiological and agronomical evaluations of the selected events are being conducted under greenhouse conditions.

S-IX-O1

PROTEOMIC ANALYSIS OF SUGARCANE - *COLLETOTRICHUM FALCATUM* INTERACTION

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Red rot of sugarcane caused by the fungus *Colletotrichum falcatum* is one of the oldest and severe diseases of sugarcane. Studies have been carried out for the past several decades to understand sugarcane-red rot interaction. These include biochemical studies on the accumulation of different types of secondary metabolites including phytoalexins and its fractions (Viswanathan *et al.,* 1996). The induction of different phenylpropanoid pathway enzymes was also established in sugarcane red rot resistance reaction. Differential induction of pathogenesis related proteins like chitinase, â-1, 3-

Table 1. MS identified Sugarcane stalk proteins, differentially expressed during *C. falcatum* inoculation

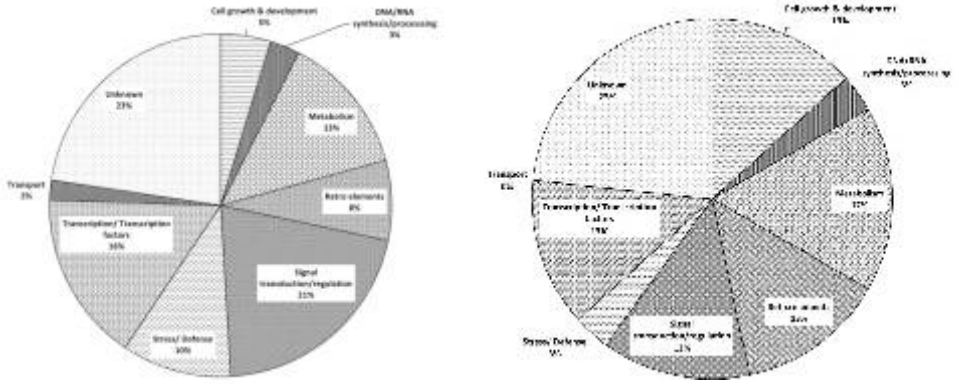
S.No	Protein Description	Organism	Protein ID	Regulation					
				R	RC	RI	S	SC	SI
	Cell growth & development								
1	PAN1	*Z. mays*	gi|209915972		+	+	-	+	+
2	Auxin-responsive family protein	A. thaliana	gi|15236187		-	-	-	+	+
3	Green ripe-like 2	S. lycopersicum	gi|87244451		-	-	+	-	-
	Metabolism								
4	Polyphenoloxidase	*C. nitidissima*	gi|222093457		+	+	+	-	-
5	Putative Decarboxylating dehydrogenases	*R. communis*	gi|255599835		+	-	+	-	+
	Signal transduction / regulation								
6	LOC100281197 (C gated ion channel protein)	*Z. mays*	gi|226531770	**ne**	+	+	**ne**	**ne**	**ne**
7	Protein containing Serine/Threonine kinase domain	*Z. mays*	gi|224030305		+	+	-	-	+
8	Os12g0246700 (containing NB-ARC domain)	*O. sativa*	gi|115488014		+	+	-	+	+
9	Flagellar associated protein	*Micromonas*	gi|255078576		+	+	-	-	-
10	Putative calcium dependent protein kinase	*N. tabacum*	gi|28866015		+	+	-	-	-
11	Protein containing Serine/Threonine kinase domain	*Z. mays*	gi|224030305		+	+	**ne**	**ne**	**Ne**
12	NB-ARC domain containing protein	*O. sativa*	gi|77556296		+	+	-	+	-
13	Putative disease resistance protein	*A. thaliana*	gi|6721164		+	+	-	-	-
14	Putative ACC synthase	*R. communis*	gi|255539669		-	-	+	+	+
15	Flagellar associated protein	*Micromonas*	gi|255078576	**ne**	**ne**	**ne**	+	-	**ne**
	Stress / Defense								
16	Os05g0458300 (Similar to Laccase)	*O. sativa*	gi|115464289	**ne**	+	+	**ne**	**ne**	**ne**
17	PrLTP1	*P. radiata*	gi|2507619		+	+	-	+	+
18	CYTOKININ OXIDASE / cytokinin dehydrogenase	*A. thaliana*	gi|15241997		+	+	+	-	-
19	Putative DNAJ heat shock protein	*A. thaliana*	gi|15231987	**ne**	+	+	+	+	+
20	Putative Callose synthase	*G. hirsutum*	gi|4588012		-	-	-	+	+
	Transcription / Transcription factors								
21	R2R3-MYB transcription factor MYB6	*P. glauca*	gi|147744712		nc	nc	-	-	-
22	Hypothetical protein (containing WRKY DNA binding domain)	*Sorghum*	gi|242069753		-	+	-	-	-
23	Maturase-like protein	*A. volckmannii*	gi|5817720	**ne**	+	+	**ne**	**ne**	**ne**
24	Putative Pentatricopeptide repeat-containing protein	*R. communis*	gi|255580386		+	-	+	-	+

glucanase and thaumatin like proteins was also reported in many studies involving red rot resistant and susceptible varieties (Viswanathan, 2010).

Genomic studies on sugarcane-red rot interaction are very limited except, for the work done at SBI, Coimbatore. Ramesh Sundar *et al.,* (2012) reported the induction of defense related transcripts like POX, PPO, PAL, TAL, SOD and LOX in semi quantitative RT-PCR. DD-RT-PCR studies conducted in the lab identified differentially regulated transcripts, during red rot interaction with resistant sugarcane cultivar Co 93009 (Viswanathan, 2010). The study was able to throw light on the interaction of sugarcane *C. falcatum* at the transcript level for the first time. Studies by Gupta *et al.,* (2009) identified several defense related transcripts that were differentially regulated in the red rot resistant sugarcane cultivar BO 91. The large genome size of cultivated sugarcane (~3000 Mbp) makes its genomic studies difficult, further aggravated by the polyploidy and aneuploidy nature of *Saccharum* genomes. Even when genomics studies are carried out, they can only reveal what could theoretically happen, whereas the proteome-level investigations (proteomics) provide insights into the actual players involved in mediating specific cellular processes. In addition, the study of proteins introduces another level of complexity at the level of the post-translational modifications (PTM) and the biological relevance of such modifications. These changes in PTM during the growth and development or in response to stress (including disease) cannot be deduced from studies investigating genome sequences and/or transcript abundance. Such changes can only be deciphered through proteomics and it is a powerful tool in understanding which proteins are abundant/present in particular tissue under given conditions. Proteomic studies in sugarcane are very limited. The only proteome study reported, was on the sugarcane suspension cells treated under saline conditions and for drought tolerance in sugarcane leaves. The work by Ramesh sundar *et al.,* 2010 involving two dimensional gel electrophoresis (2-DE) resulting in the identification of important proteins playing critical role in the systemic acquired resistance mechanism (SAR) of sugarcane against red rot pathogen gave the necessary impetus to detailed proteomic study in sugarcane.

The objective of the present study is to understand the changes in the proteome profile of resistant and susceptible sugarcane cultivar in response to pathogen challenge. The samples were collected 24 h after pathogen inoculation from the stalk tissues of two sugarcane cultivars viz., Co 93009 (resistant to red rot) and CoC 671 (susceptible to red rot). The protein samples were subjected to standardized 2-DE protocol. The analysis of the proteome profile revealed the up-regulation/induction of about 106 proteins and down-regulation of about 30 proteins, in response to pathogen inoculation. The differentially regulated protein spots were then characterized by peptide mass finger printing using eLD-IT-TOF-MS/MS analysis. The MS analysis revealed the differential regulation of important proteins involved in PAMPs triggered innate immunity, signal transduction and few resistance gene analogs. (Table1). Based on the putatively assigned functions, the identified spots were categorized into 8 groups and represented in the pie chart (Fig.1). The major portion of the up-regulated proteins belongs to the signal transduction (21%) and transcription/transcription factors. The proteins related to metabolism (13%) and defense/stress was also found to be the other major categories induced by pathogen challenge. Among the down regulated proteins, major portions belonged to metabolism (17%) and cell growth & development (13%) categories. The present study on the proteome-level changes that occur during *C. falcatum*- sugarcane interaction using 2-DE is the first attempt to standardize proteome analysis and to identify specific proteins involved in red rot resistance in sugarcane. Among the differentially regulated proteins, quantitative difference in expression was

noticed between resistant and susceptible cultivars. In conclusion, tight regulation typical of resistance response against biotropic pathogens was observed in the resistant variety. Whereas, in the susceptible variety the response was more general and placid, a favorable situation for necrotropic phase of the pathogen. This is the first such study, concerning host-pathogen interaction in sugarcane involving 2-DE. The results clearly demonstrated the suitability of proteomics based analysis in deciphering components involved in host pathogen interaction.



Note: Regulation relative to the respective healthy sample were indicated with + and symbols for up and down regulation respectively. Regulation indicated for S is relative to corresponding R sample. R- resistant healthy, RC resistant mock inoculated control, RI- resistant pathogen inoculated, S- susceptible healthy, SC susceptible mock inoculated control, SI- susceptible pathogen inoculated.

Fig 1. Pie chart showing functional classification of down regulated and up regulated proteins after pathogen inoculation based on putative functions

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