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DISEASE RESISTANCE IN SUGARCANE – AN OVERVIEW

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ABSTRACT - Sugarcane is one of the important commercial crops cultivated world-wide both under tropical and sub-tropical conditions. The crop gains economic importance by virtue of its industrial potential in terms of products like crystal white sugar, bagasse, pressmud, power etc. Among the various production constraints of the crop, diseases are seen as a major threat for sustaining the productivity of sugarcane. Conventional Breeding is a lengthy process and it involves almost more than 10 years for the release of a commercial variety. Many varieties with superior agronomical traits have succumbed to diseases like red rot and smut during the course of cultivation, which hitherto at the time of release were rated to be resistant. The breakdown of disease resistance is attributed to the possible emergence of new virulent pathotypes. This situation has warranted a pertinent need to have a thorough understanding on inheritance pattern and mechanism of disease resistance in sugarcane, which would aid for quick screening of disease resistant clones and successful management of the diseases, respectively. Overall, there is a paradigm shift in the understanding of plant disease resistance, thanks to the advent of robust molecular tools. An integration of the tools of "Omics" namely genomics, proteomics, metabolomics etc. has further strengthened in deciphering plant-pathogen interactions at the molecular level. With the accomplishments in elucidating sugarcane ESTs, which was ably supported by employing the next generation sequencing platforms to unlock the secrets of pathogenomics in sugarcane, it is now made possible to further improve our understanding on disease resistance in sugarcane. Giving the scenario, the future looks evenmore promising, wherein convincing results are in the offing to thoroughly unravel the enigmatic relationship between sugarcane and its important pathogens.

Key words: sugarcane, disease resistance, red rot, smut.

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INTRODUCTION

Sugarcane is one of the important commercial crops cultivated throughout the world mostly in countries with a tropical/sub-tropical climate. Sugarcane is the primary source for manufacturing crystal sugar, which is a predominant commodity in the global food industry. Besides, the production of crystal sugar as a main product, ethanol, bagasse, pressmud and co-generation of power are the other useful bye-products. Presently, sugarcane is also looked upon as a feed stock for biofuels and would be one of the major sources of energy for the future. Diseases are a major constraint affecting the sugarcane productivity world-wide, which can be broadly classified as fungal, bacterial, viral and of phytoplasmal origin (Table 1).

Disease resistance in crop plants is an enigma to be unravelled, in spite of advances made in plant biology. Understanding plant-pathogen interaction precisely is still a fascinating area, which forms a basis to develop disease resistance varieties in Agriculture. The science of plant disease resistance has undergone a paradigm shift in understanding starting from the gene for gene concept to the present age of guard decoy model to decipher disease resistance genes in crop plants.

Brief history of plant disease resistance

During the course of co-evolution, both plants and fungi have developed their molecular combat system in a see-saw manner, which ultimately dictates the winner of this arms race. Plants developed their surveillance system by means of R genes (receptors) to recognize the enemy's signatures at the cell surface and intracellular level, whereas the fungus produced a repertoire of effectors that modulate the functions of warriors engaged in host defense and thus colonizing the tissues.

When we look into the history, the understanding of disease resistance has emerged with the pioneering observations and demonstrations on disease resistance since the beginning of 19th century (Table 2). However, the understanding on the concepts and mechanism of disease resistance has gained momentum only with the hypothesis "Gene for gene" proposed by H.H. Flor in 1946. The hypothesis states that for each resistance gene in a host, there is a corresponding gene for avirulence in the pathogen conferring resistance and vice versa. With the basis of Flor's concept various models (Table 3) have been proposed during different time periods to explain the mechanism of activation/induction of defense or disease resistance. However, no single model could explain all the phenomena of defense activation.

TABLE 1. List of important diseases of sugarcane.

Sl. No.	Name of the disease	Causal agent	Causal organism
1.	Red rot	Fungus	*Colletotrichum falcatum*
2.	Smut	Fungus	*Sporisorium scitamineum*
3.	Wilt / Top rot / Pokkah Boeng	Fungus	*Fusarium sacchari*
4.	Sett rot	Fungus	*Ceratocystis paradoxa*
5.	Rust	Fungus	*Puccinia melanocephala* *Puccinia kuehnii*
6.	Leaf spot (Eye leaf spot)	Fungus	*Helminthosporium sacchari*
7.	Leaf scald disease	Bacteria	*Xanthomonas albilineans*
8.	Ratoon stunting disease	Bacteria	*Leifsonia xyli* sub sp. *xyli*
9.	Sugarcane Mosaic Virus disease	Virus	Sugarcane Mosaic Virus (SCMV)
10.	Yellow leaf disease	Virus	Sugarcane Yellow leaf (SCYLV)
11.	Grassy shoot disease	Phytoplasma	Sugarcane Phytoplasma

Deciphering disease resistance in sugarcane is a subject of interest for the all-time pathologists. The genesis of "Sugarcane Breeding Institute" in India, which was established in 1912 by Dr. C.A. Barber has encompassed in it an important objective, is that to breed for superior sugarcane clones for the Tropical India with red rot

resistance. Many of the superior interspecific hybrids released as commercial varieties succumbed to diseases like red rot and smut over a period of time. These varieties were evaluated as resistant at the time of release, however subsequently fell prey to these diseases. This breakdown of disease resistance in sugarcane could be attributed to the emergence of new and virulent pathotypes. Knowledge on disease resistance is very important, as it is a pre-requisite to evolve breeding strategies to develop disease resistance varieties in sugarcane. The present scenario has made it very clear that continuous research efforts are required to understand the evolution of pathogenicity of sugarcane pathogens and the mechanism of disease resistance in sugarcane, so as to sustain the productivity of the released varieties for commercial cultivation. This review will be covering the available information more precisely on red rot and smut disease resistance mechanisms, besides touching upon the literature available in other important diseases of sugarcane. A thematic figure depicting the strategies for enhancing disease resistance and their presumptive molecular mechanism in sugarcane is given hereunder as presented in Figure 1 for better interpretation.

Table 2. Era of observations and demonstrations on the concept of disease resistance (1901-1940).

Year	Researcher	Observation/demonstration
1901	Beauverie J	Testing of immunization of plants against fungal diseases
1901	Ray J	Fungal diseases of plants (observation of inducible defenses)
1902	Ward HM	Observed necrotic active defense in Poaceae, a form of induced local resistance (ILR) (later termed as HR)
1907	Biffen	Inheritance of resistance in Mendelian fashion
1915	Stakman	Termed "Hypersensitive Response" for ILR induced by *Puccinia graminis* in resistant plants
1930	Newton	Inheritance of pathogenicity in Mendelian fashion
1933	Chester KS	Reviewed the observations of inducible defenses as "acquired physiological immunity"
1940	Muller K & Borger H	Established the phenomenon of ILR in potatoes to late blight (*Phytophthora infestans*)

TABLE 3. Conceptual disease resistance models based on 'gene for gene' hypothesis.

Mode of Perception	Model	Proposed researcher/References
Direct perception	Elicitor-Receptor model	Anderson-Prouty and Albersheim (1975) NT Keen (1982)
	Suppressor-Receptor model	Bushnell and Rowell (1981) Heath (1982)
	Dimer model	Ellingboe (1982)
	Ion-Channel model	Gabriel et al. (1988)
Indirect perception	Guard model	Van der Biezen and Jones (1998) Dangl and Jones (2001)
	Decoy model	Van Der Hoom and Kamoun (2008)

Red rot resistance in sugarcane:

Red rot of sugarcane caused by *Colletotrichum falcatum* Went is one of the devastating diseases of sugarcane causing significant loss to sugarcane production in India and other Asian countries. The disease gains importance in terms of its potential damage to yield and reducing quality of sugar recovery in sugarcane. Natarajan et al. (2001) reported that the wild species *S. spontaneum* significantly contributes to red rot resistance and its incorporation as parents in the hybridization program will result in development of commercial cultivars with red rot resistance. However, complex polyploidy and lack of information on inheritance to red rot in sugarcane make breeding for red rot resistance more difficult. Besides that many important commercial cultivars released as red rot resistant hitherto were found to succumb to the disease.

This breakdown of disease resistance is attributed to the emergence of new virulent races of *C. falcatum*, which further complicates the management of the disease. Mohanraj and Kaverinathan (2011) developed a suitable methodology to screen sugarcane genotypes with field tolerance to red rot disease. The method involves inoculation of planted setts with the pathogen inoculum multiplied on sorghum grain under field conditions. The study indicated the potential to identify such field tolerant genotypes using certain standardized inoculation and evaluation procedures. Further the possibility of identifying superior commercial clones or genetic stocks with field tolerance to red rot from the early seedling stage in the selection process has been indicated. Hence, a thorough understanding of host resistance and pathogen biology is imperative to evolve successful strategies for red rot management in sugarcane.

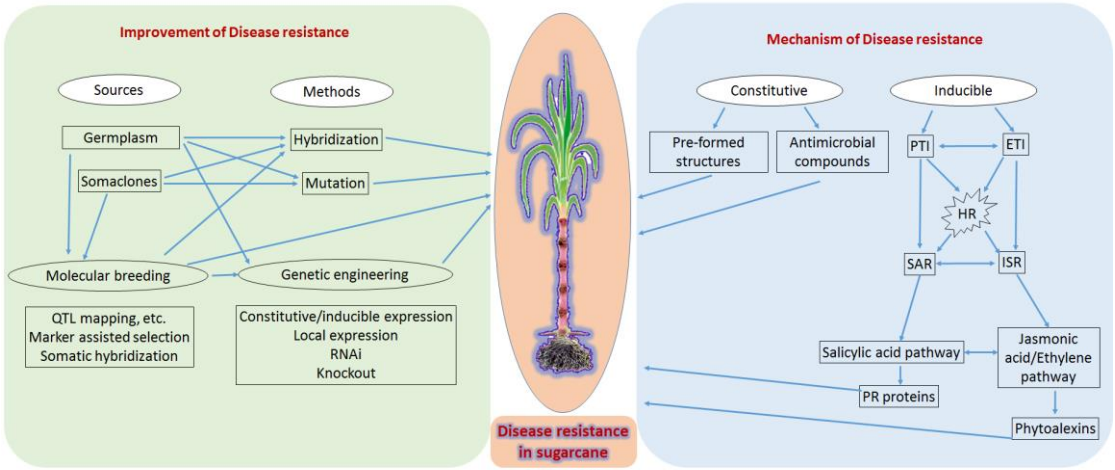

FIGURE 1 – Pictorial depiction of a hypothetical disease resistance mechanism in sugarcane.

Induced resistance:

Plants have developed a very sophisticated mechanism to resist the attack of pathogens through constitutive and inducible defenses. Constitutive defenses are pre-formed barriers are otherwise called as passive defense, whereas inducible defenses / induced resistance (IR) is an active form of defense, that induces defense at two different modes. Generally, the outcome of constitutive defenses are characterized by high degree of localized expression of defense such as Pathogenesis-related (PR) proteins, phytoalexins, hypersensitive response (HR), etc. during an incompatible interaction, while IR is characterized by the activation of systemic defense with the expression of PR proteins, phytoalexins, etc. during a compatible interaction. The two established forms of IR are Systemic acquired resistance (SAR) and Induced systemic resistance (ISR). Both the strategies were well demonstrated in sugarcane against red rot, a major devastating fungal disease in many tropical countries.

Breeding for red rot resistance has been complicated by the frequent emergence of new pathogenic variants, which overpowered the elite erstwhile ruling varieties viz., Co 419, Co 997, Co 1148, Co 6304, Co 7805, CoC 671, CoC 92061, CoJ 64, etc., and resulted in withdrawal from commercial cultivation (VISWANATHAN et al., 1997). Many of the attempted fungicides were not practically successful in controlling red rot at grand growth and maturity phases, even though some of which could significantly afford protection against the soil/sett-borne inoculum at germination phase (RAMESH SUNDAR et al., 2012a). Hence, the constant demand for a promising eco-friendly alternative has driven to explore "IR" for the efficient management of red rot. SAR and ISR has been an established phenomenon in sugarcane under green house and field conditions, ably supported by the results of biochemical and molecular analyses undertaken by the pathology group at SBI, Coimbatore, India.

Systemic acquired resistance (SAR):

SAR is a unique trait of a plant for enhancing the inherent defense potential or innate immunity with response to an external stimuli. It is systemic, durable, and broad spectrum, which can be induced by 'priming' with both synthetic and biotic elicitors/inducers/activators (DURRANT AND DONG, 2004; VLOT et al., 2008; GOZZO AND FAORO, 2013). SAR activation is mediated with the signal molecule salicylic acid (SA) and associated with an accumulation of PR proteins both locally and systemically. SAR in sugarcane is one of the promising strategies in managing red rot in elite commercial cultivars under field conditions by employing both biotic and synthetic inducers. Understanding SAR metabolism components is an important concern regarding plant breeding, as it would help in identification of SAR-responsive clones of sugarcane.

Synthetic elicitors like Benzothiadiazole (BTH), SA, Isonicotinic acid (INA) and Succinic acid induced SAR response and substantially restricted C. falcatum

colonization on cane stalk tissues with an induction of defense-related compounds (RAMESH SUNDAR et al., 2001, 2006). Cf elicitor, a glycoprotein extracted and purified from the cell wall of *C. falcatum* induced many defense-related compounds and PR proteins, similar to BTH priming and effectively restricted pathogen colonization (RAMESH SUNDAR; VIDHYASEKERAN, 2002a, 2003; RAMESH SUNDAR et al., 2002b, 2008, 2009). Further, transcriptional profiling analyses to elucidate the molecular basis of SAR priming indicated that BTH, SA and Cf elicitor upregulate the expression of several defense-related transcription factors (TFs) (MUTHIAH et al., 2013), phenylpropanoid pathway genes and resistant gene analogues (RGAs) at the earlier stage of infection in the red rot susceptible cultivar, CoC 671 (SELVARAJ et al., 2014). Ramesh Sundar et al (2014) presented a comprehensive update on the status of Induced resistance in sugarcane for disease management during the 7th Brazilian Meeting on Induction of Plant Resistance to Pathogens. Analysis of differential expression of proteins with response to BTH and Cf elicitor priming by 2DGE identified proteins that are involved in redox reactions, signal transduction, stress signaling, oxidative folding of defense proteins and programmed cell death (PCD), which together provided the glimpse of activation of SAR at molecular level. Currently, the investigatory group has identified two putative PAMPs from the culture filtrate of C. falcatum that can elicit host defense upon priming (Personal communication).

Induced systemic resistance (ISR):

ISR can be defined as the defense potentiated by certain strains of plant growth promoting rhizobacteria (PGPR) and some saprophytic fungi. ISR relies on the signals of jasmonic acid (JA) and ethylene (ET) and increases phytoalexin levels during pathogen challenge. In sugarcane, the induction of ISR by Pseudomonas strains against red rot pathogen was investigated in detail. Viswanathan and Samiyappan (2002) established the involvement of the enzymes of phenylpropanoid pathway and oxidative pathway in ISR. Studies of Viswanathan et al. (2001) have also shown strong anti-fungal activities of sugarcane chitinases purified from systemically protected stalk tissues against *C. falcatum*. Further, involvement of different PR-proteins such as β-1.3-glucanases, chitinases and thaumatin-like proteins (TLPs) were also found to be associated with *Pseudomonas*-mediated ISR (VISWANATHAN et al., 2003a). The results clearly demonstrated that bacterium treated disease susceptible sugarcane was able to restrict disease development to a level equivalent to moderately resistant varieties and many PR-proteins are involved in that ISR response. Characterization of *Pseudomonas* strains revealed that production of different metabolites/antibiotics such as SA, auxins, siderophores, pyocyanine, pyoluteorin and 2.4-diacetyl phloroglucinol and hydrolytic enzyme chitinase contribute to suppression of *C. falcatum*, IR and growth promotion in sugarcane (VISWANATHAN; SAMIYAPPAN, 2004).

Biochemical basis of red rot resistance

Preliminary studies based on biochemical tools identified possible involvement of certain parameters in red rot resistance. The studies revealed the role of enzymes of the phenyl-propanoid pathway viz. Peroxidase (POX), Polyphenol oxidase (PPO), Phenyl-alanine ammonia lyase (PAL) etc. PR proteins viz. Chitinases, β-1.3 glucanases, Thaumatin-like proteins etc. and 3-deoxyanthocyanidin phytoalexins especially luteolinidin and apigeninidin in red rot resistance.

Higher accumulation of 3-deoxyanthocyanidins, luteolinidin, apigeninidin and the caffeic acid ester of 5-0-apigeninidin was reported in disease resistant genotypes, than in susceptible genotypes and it was hypothesized that these phytoalexin compounds might contribute to red rot resistance in sugarcane (VISWANATHAN et al., 1996). Anthocyanin extracts from sugarcane cultivars had an inhibitory effect on the conidial germination of *C. falcatum* and the results suggested a possible involvement of these metabolites in resistance of sugarcane against red rot (VISWANATHAN et al., 2000). The differential expression pattern of these different 3-deoxyanthocyanidin fractions even among the resistant cultivars, indicated the multiple modes of phytoalexin-mediated resistance mechanism operating in sugarcane. The study clearly established that luteolinidin may be the major phytoalexin, which determines host resistance to *C. falcatum* which needs further investigation (GANESH KUMAR et al., 2015). Further, the study indicated that induction of either luteolinidin or apigeninidin compounds alone or in combination at higher concentrations in the resistant cultivars may enable the effective arrest of pathogen invasion and further development inside the stalk tissues.

Thirupathraja et al. (2004) analysed time course accumulation of POX and reported differential response between resistant and susceptible varieties. The study further indicated that higher and early response of red rot resistant varieties to POX levels could be one of the determinants of disease resistance. Viswanathan et al. (2005) observed differential induction of chitinases and thaumatin-related proteins between resistant and susceptible varieties and could be possible be used as a biochemical marker in sugarcane to identify red rot resistance. Select strains of *Trichoderma viride* isolated from sugarcane rhizosphere caused lysis of red rot pathogen mycelium and the inhibitory effect was much more pronounced using chitin or cell wall of *C. falcatum* as a carbon source (VISWANATHAN et al., 2003b). An endochitinase gene isolated from T. harzianum might be responsible for the antagonistic activity of the bio-control agent against *C. falcatum* (VISWANTHAN et al., 2006). Incorporation of chitin in *Pseudomonas*-based talc formulation substantially reduced *C. falcatum* colonization in sugarcane stalks. Viswanathan and Samiyappan (2007) identified select strains of *P. fluorescens* with strong antagonism against *C. falcatum*.

Molecular basis of red rot resistance

Studies using semi-quantitative RT-PCR after pathogen inoculation from sugarcane cultivars varying in

red rot resistance, revealed differential accumulation of transcripts of the flavanoid biosynthetic pathway like coumarate-4-hydroxylase, chalcone synthase, chalcone reductase, flavanoid 3′-5′ hydroxylase and flavanoid glycosyl transferase and this transcript analysis, further confirmed the role of sugarcane phytoalexins in red rot resistance. Similarly the role of PR- proteins like chitinase and β-1.3-glucanase was established at the transcript level. Viswanathan et al., (2009) detected transcripts of Resistant gene analogues (RGAs), Transcription factors (TFs), defense-related genes and few signalling-related genes up-regulated specifically during sugarcane - *C. falcatum* interaction. Detailed molecular studies using differential display (DD-RT-PCR) identified expression of more number of differentially expressed transcripts during the host-pathogen interaction. Full length sequences of many potential transcripts were identified and are being characterized (VISWANATHAN et al. 2012). Similarly, Prathima et al., (2013) identified 300 differentially expressed transcripts, in which the defense/stress/signaling group was the largest group, with clones homologous to genes known to be actively involved in various pathogenesis-related functions in plant species. The information provided a set of candidate genes for detailed molecular dissection of signaling and defense responses in tropical sugarcane during the onset of red rot resistance. The expression of chitinases in sugarcane cultivars varying in red rot resistance, their characterization and theoretical 3D structure prediction through transcriptomic and bioinformatics tools were reported by Rahul et al (2013). Sathyabhama et al. (2015) identified a network of early defence responses and associated signals for the first time in a red rot resistant sugarcane cultivar in response to C. falcatum infection through Suppression subtractive hybridization (SSH). The study has identified a total of 139 EST's which were functionally categorized as belonging to recognition and signal transduction, oxidative stress, redox maintenance, membrane trafficking and transport, defence and programmed cell death, energy and photosynthesis, metabolism, secondary metabolite biosynthesis, cell/nuclear structure and unknown categories. To identify specific proteins involved in host resistance using two dimensional electrophoresis, Amalraj et al. (2010) established a reference stalk proteome in sugarcane, which served as the first time report to further demonstrate defense proteomics-related work in sugarcane-pathogen interactions. Further, present proteomics studies on red rot resistance has been summed up on the comprehensive review on sugarcane proteomics by Barnabas et al. (2015).

Smut resistance in sugarcane

Smut of sugarcane caused by *Sporisorium scitamineum* (formerly known as *Ustilago scitaminea*) is one of the most important diseases of sugarcane worldwide. Albeit various management methods, cultivating resistant varieties is considered as the most economic and effective strategy. However, sugarcane smut resistance still remains as one of the major research areas that are yet to be thoroughly understood. A number of studies have been carried out in the past few decades, which predominantly focused on correlating levels and activities of various defense-related biochemical compounds and enzymes to sugarcane smut resistance. Ramesh Sundar et al. (2012b) comprehensively reviewed the various biochemical and molecular studies carried out on sugarcane smut from 1964 to 2009 as a means of providing an update in smut resistance during the period. Sugarcane smut resistance is demonstrated to be heritable. Two types of resistance behaviour were reported in sugarcane against the smut pathogen. One is an external resistance, mediated by a chemical or physical barrier in the sugarcane bud and an internal resistance, which is speculated to be governed during host-pathogen interaction. It was observed that conventional dip inoculation method is useful for studying external resistance mechanism - probably a chemical or physical barrier in the sugarcane bud. However, Injection method is appropriate to determine internal resistance mechanism, which is governed by interaction of plant and fungus within the plant tissue. It is postulated that presence of bud phenylpropanoids and glycosyl-flavonoids might play role in imparting physiological resistance. However, smut resistance is governed by multifactorial process and a comprehensive knowledge on factors governing smut resistance is to be understood thoroughly.

Lloyd and Pillay (1980) reported inhibition of smut teliospores by flavonoid group of compounds. Alexander and Rao (1981) identified sources for smut resistance and reported that more resistance could be observed in exotic clones such as Puerto Rico (PR), Queensland (Q) and Canal Point (CP) varieties. Wild species of *Saccharum* namely *S. spontaneum* was found to possess more of smut resistance than *S. officinarum*, *S. sinense*, *S. robustum* and *S. barberi*. Also, they have established a linear relationship between smut resistance and the content of glycosidic substances in the bud scales (LLOYD; NAIDOO, 1983). Jalaja et al. (1987) indicated that somaclonal variation could be an alternate system for improving smut resistance in high yielding commercial cultivars. Padmanaban and Alexander (1988) correlated increased levels of total phenolics, reducing sugars and total free amino acids with smut susceptibility in sugarcane. Padmanaban and Mohanraj (1989) correlated certain bud characters with smut reaction of sugarcane genotypes viz. morphological and biochemical. Susceptible clones exhibited loose-bud scales, apical germination and prominent bud grooves, however resistant types showed tight bud scales, sub-apical germination and less pronounced grooves. Bud scale diffusates containing non-phenolic glycoside from the resistant varieties inhibited teliospore germination. Alexander and Padmanaban (1992) made efforts to study the pathogenicity mechanism of smut infection, so as to understand the host resistance. Temporal and spatial analysis of the sugarcane-smut pathogen interaction established that the infection was systemic and the hyphae progressed in the upper internodes, which culminated in the formation of sori and teliospores.

Biochemical basis of smut resistance:

Legaz et al. (1998) established a relationship between phenolics-conjugated polyamines viz. SH-spermidine and SH-cadaverine and sensitivity of sugarcane to smut. Syringic acid is the main phenol associated with the smut infection; however, ferulic acid seems to be the main hydroxyl cinnamic acid derivative in the whip. Sugarcane in response to *S. scitamineum* infection resulted in a remarkable increase of both free and conjugated polyamines. The resistant buds mainly produce free putrescine, however the sensitive cultivars produced acid soluble-conjugated as well as acid insoluble-conjugated sperrnidine and spermine in response to smut infection. Variation of cadaverine, also produced by sugarcane buds, did not show any clear correlation with smut development (PINON et al., 1999). Santiago et al. (2008) separated elicitor active fractions from the smut pathogen by capillary electrophoresis, which enhanced the accumulation of free phenolics, mainly hydroxycinamic acids in leaves of sugarcane cultivars namely cv. Mayarí 5514 (susceptible), and cv. Barbados 42231 (resistant). An important difference observed was the enhancement of peroxidase in the resistant cultivar, an enzyme that uses free phenolics as substrates for the activation of important mechanisms of resistance of sugarcane leaves to the fungal pathogen.

Further, the accumulation of smut pathogen-responsive soluble and cell wall-bound phenolics in the same set of sugarcane cultivars was studied by Santiago et al. (2008). Cell wall-bound phenolics, such as ferulic, caffeic, and syringic acids increased in the resistant and not so in the case of susceptible variety. This could result in a better capacity to cv. Barbados 42231 for an increase in the frequency of bridges between lignin fragments through ester-ether linkages for reinforcing the cell wall and major resistance to smut disease. This reinforcement of the cell wall could provide an effective barrier to pathogen entry and spread. It was further hypothesised that the pathway of hydroxybenzoic acids is activated, once the level of p-coumaric acid justifies the accumulation of hydroxycinnamic acids required for reinforcing the cell wall after inoculation.

Santiago et al. (2010) investigated the role of Caffeic acid (CA) as a possible phytotoxin affecting sugarcane and the smut fungal growth and physiology. The effect of CA upon *S. scitamineum* growth cycle was showed to be time and concentration dependent. Inhibition was more evident after 24 h or 28 h incubation of teliospores in CA solution, which at a concentration of 20 mg ml_1 reduced both germination of teliospores and production of haploid sporidia, but did not have any significant effect on dikaryotic mycelium appearance after 24-h incubation. Santiago et al. (2012) recorded increased production of lignin to about 29% in the smut resistant sugarcane cultivar and only 13% in the susceptible cultivar after inoculation compared to uninoculated plants. The results demonstrated that the resistance of My 5514 to smut is likely derived, at least in part, to a marked increase of lignin concentration by the activation of coniferyl alcohol dehydrogenase (CAD) and sinapyl alcohol dehydrogenase (SAD).

β-1.3- glucanase and chitinase, as well as secreted glycoproteins from sugarcane caused spore agglutination and inhibition of teliospore germination of the smut fungus. These glycoproteins impede teliospore motility towards the infection sites. Motility inhibition seems to be related to the inhibition of contractile ATPases similar, or identical, to myosin II that interacts with the F-actin cytoskeleton promoting contraction-relaxation episodes, which might contribute to teliospore displacement (LEGAZ et al., 2005). Fontaniella et al. (2002) proposed a hypothesis about the possible role of high molecular mass glycoproteins (HMMG) and medium molecular mass glycoproteins (MMMG) as defence metabolites, which significantly inhibited the teliospore germination of *S. scitaminuem*. The ability of these glycoproteins to produce cytoagglutination is attributed for the reduced teliospore activity. Binding of fluorescein-labelled glycoproteins was studied by fluorescence microscopy, which showed that staining of cells was not uniform, but mainly in the contact zone between two individual teliospores when aggregated. Smut pathogen inoculation increased the production of sugarcane glycoproteins of HMMG and decreased the amount of those of mid-molecular mass MMMG recovered from stalks cell-free extracts. Glycoproteins that accumulate in the parenchymatous cells of sugarcane stalks regulate cell polarity of S. scitaminea teliospores. These glycoproteins increases after inoculation of sugarcane plants with smut teliospores, induce homotypic adhesion and inhibit teliospore germination. Results of the study indicated that smut teliospores seem to be able to change the pattern of glycoprotein production by sugarcane, thereby promoting the synthesis of different glycoproteins that activate polarization after binding to their cell wall ligand (MILLANES et al., 2005). Further results indicated that peptide fraction of HMMG and MMMG bind to this amino sugar in the polysaccharide moiety of smut pathogen ligands (MILLANES et al., 2008).

Inheritance and screening of smut disease resistance:

Breeding varieties for smut resistance still stands tall as the most viable option for successfully managing the smut disease. Raboin et al. (2001) initiated a study genetic determinism underlying sugarcane smut resistance, wherein a genetic mapping strategy was followed involving a cross between cultivar R 570 (resistant) and cultivar MQ 76/53 (highly susceptible), which showed segregation for smut resistance. AFLP markers linked to smut resistance through QTL mapping have been identified (BUTTERFIELD et al., 2004). Smut resistance is hypothesized to be controlled by many genes with smaller effects, and for detection of markers using association mapping, larger populations are to be used (ZHU et al., 2008). Quantitative trait loci (QTL) a classical approach was used to identify regions of sugarcane genome that contains genes controlling smut resistance, which involves generation of a linkage map. Aitken et al., (2013) established that smut resistance in sugarcane is

governed by a major gene. The results are expected to identify candidate genes determining smut resistance, which could be further used as a markers assisting breeding for smut resistance. It was suggested that integrating datasets from phenotyping experiments and marker assisted selection (MAS) would be more effective in developing smut disease resistance in sugarcane. Identification of a major QTL is the first step, followed by validation in different genetic backgrounds for further carrying forward in breeding programs.

The unexpected incursion of sugarcane smut in Queensland, Australia in 2006 caused a huge economic impact in the country's sugar industry. This prompted a strategic formulation of a focussed breeding programme named as "Smut Buster". This was to enable accelerated development of smut-resistant clones with high agronomic value, which includes a substantial research component addressing a wide range of screening methods (COX et al, 2011). Taking into cognizance of the need for a methodology to support smut resistance breeding programs, Purcell et al. (2010) devised a rapid, non-destructive, on-site screening technique based on NIR spectroscopy for rating the sugarcane clones for smut resistance. Varietal resistance to smut was predicted using NIR spectra collected from sugarcane bud scale tissue and were subsequently extrapolated with chemometric data treatment methods. NIR-predicted smut ratings could be correlated with traditionally derived ratings obtained from field trials. With improvement in robustness and throughput, this technology would find major applications in early screening of varieties for smut resistance in the future. Zhou (2013) suggested that Conventional Breeding in South Africa is promising and holds the key for successfully managing the smut disease in sugarcane. A five stage concerted breeding program is implemented in the varietal development process. More than 60 varieties have been released from the breeding programs, which includes strategies such as introgression, family evaluation, selection models and use of molecular markers. Bhuiyan et al. (2013) demonstrated that parental clones selected for the study possessed both internal and external mechanisms of smut resistance. Different types of resistance mechanism between varieties have been postulated. These findings will benefit breeders in selecting parent materials in their crossing programs to develop smut-resistant cultivars. By understanding the disease resistance mechanism of parent clones, sugarcane breeders will be able to formulate a breeding strategy to develop smut-resistant varieties. Resistance screening for sugarcane smut by artificial inoculation method or natural infection should be carried out in different geographical location to identify the new biotypes or races into different geographical regions. Effort should be made to use a mixture of spores collected from various geographical regions for artificial inoculation in future screening trials. Nevertheless, the Australian sugar industry needs to be prepared for possible loss of resistance of cultivars to sugarcane smut (BHUYIAN et al., 2015).

Molecular basis of smut resistance

Several studies employing various molecular techniques including cDNA-AFLP (THOKOANE; RUTHERFORD, 2001; LAO et al., 2008), differential display techniques (HIDALGO et al., 2005), etc. have also accumulated information on differentially expressed transcripts of sugarcane in response to S. scitamineum challenge. Differentially regulated transcripts in smut infected buds were identified by Zhu et al. (2008) from the RNAseq data. Such qPCR validated genes need to be mapped to the segregating population to determine the extent of variation as explained by the candidate genes. Lao et al. (2008) reported differential expression of transcript-derived fragments (TDFs) on the *Saccharum* spp. – *S. scitamineum* pathogenic interaction. A majority (67.2%) of the differential TDFs up-regulated was recorded in the resistant M31/45 cultivar, representing major genes involved in oxidative burst, defensive response, ethylene and auxins pathways during the first 72 h post-inoculation. Results obtained suggested a key role for genes involved in the oxidative burst and the lignin pathways in the initial sugarcane defense against the S. scitamineum infection. cDNA - AFLP technique was employed by Que et al. (2011a) to identify transcripts that were differentially expressed in a resistant variety in response to *S. scitamineum* challenge. Around 136 TDFs were found to be differentially expressed in response to pathogen challenge. Among the 40 TDFs that were consistent, 34 TDFs were newly induced and 6 TDFs were significantly upregulated after inoculation and its expression levels were further confirmed by semi quantitative PCR.

In sugarcane - smut pathogen interaction, few literatures are available on some key defense genes and TFs, which are discussed as hereunder: Xiong et al. (2008) cloned six NBS-LRR type resistance gene analogs (RGAs) from sugarcane. Homology analysis was also conducted to evaluate the relationship between sugarcane RGAs and known plant R genes. Finally, the full-length cDNA of cRGA1 (Accession number: EF155648), termed SNLR gene, has been cloned and its expression profile under the treatment of Ustilago scitaminea, SA and H_2O_2 was investigated by real-time RT PCR (Accession number: EF155654). The results showed that SNLR gene could be influenced to some extent by Ustilago scitaminea and SA, but not by H_2O_2.

Considering the established roles of NPR1 gene (non-expressor of pathogenesis related genes 1) in salicylic acid (SA)-mediated plant defense, Chen et al. (2012) identified and characterized a sugarcane NPR1 (ScNPR1) gene. The full length coding sequence of 2184 bp shared considerable homology with maize ZmNPR1 gene and its expression was increased significantly in response to SA and *S. scitamineum* challenge and was downregulated upon treatment with methyl jasmonate and ethylene.

Two β-1.3-glucanase genes (ScGluA1 and ScGluD1) from sugarcane located in apoplast exhibited different expression patterns in smut infection stress. The gene expression patterns were similar to response to abiotic stresses and against smut infection. It was

postulated by Su et al. (2013) that these two β-1.3 glucanases may function in sugarcane defense mechanism for *S. scitamineum*. The positive responses of ScGluA1 and the negative responses of ScGluD1 to biotic and abiotic stresses indicated that they play different roles in interaction between sugarcane and biotic or abiotic stresses. Su et al. (2014) observed high levels of correlation between catalase activity and smut resistance. The catalase gene (ScCAT1) encoding the protein localized in the plasma membrane and cytoplasm was found to be induced in response to many stress situations. Expression profiling indicated relatively high level of expression of Sc CAT1 in the buds as compared to the stem epidermis and stem pith, which indicated the possible role of ScCAT1 in imparting smut resistance in sugarcane.

Esh et al. (2014) observed variation in the levels of six PR proteins (polyphenol oxides, phenylalanine ammonia lyase, peroxidase, esterase, chitinase and β-1.3 glucanase) in sugarcane clones that are resistant and susceptible to smut. Su et al. (2015) determined the structural properties and profiled the expression patterns of ten differentially expressed chitinase genes (belonging to class I~VII) obtained from RNA-seq analysis of incompatible and compatible interactions between sugarcane and S. scitamineum. Among the ten, expression of seven chitinases (ScChiI1, ScChiI2, ScChiI3, ScChiIII1, ScChiIII2, ScChiIV1 and ScChiVI1) in resistant cultivar was higher compared to the susceptible variety. Liu et al. (2012) isolated a 1003 bp gene encoding WRKY protein from sugarcane. This Sc WRKY gene was strongly induced by S. scitamineum, salicylic acid (SA), NaCl and PEG, which suggests that this gene might play an important role in smut-resistant, drought-tolerant and salt-tolerant mechanism.

Employing NGS platforms, transcriptome profiling for smut resistance resulted in identification of highly expressed genes that could be mapped back to the segregating population. Wu et al. (2013) was the first to apply NGS- based platform to study sugarcane – S. scitamineum interaction, wherein which high-throughput tag-sequencing (tag-seq) analysis by Solexa technology was performed. Among the 2015 differentially expressed transcripts, 1125 were up regulated and 890 were down-regulated in response to pathogen challenge with *S. scitamineum*. Functional categorization of the differentially expressed transcripts indicated that majority of transcripts were related to several cellular processes representing various metabolic pathways. Following this, Que et al. (2014) analyzed the transcriptome of smut resistant and susceptible sugarcane cultivars challenged with S. scitamineum using an Illumina-based platform HiSeqTM 2000. Transcriptome profiling at 24, 48 and 120 hours post inoculation and functional categorization of differentially expressed transcripts indicated that up-regulation of defense related genes occurred earlier in the resistant variety. Pathway enrichment analysis indicated that majority of differentially expressed genes were related to plant hormone signal transduction, flavonoid biosynthesis, cell wall fortification and other defense-associated metabolic pathways. The genome of *S. scitamineum* sequenced recently by Que et al. [2014] and Taniguti et al. [2015] has provided insights on genome organization and its synteny with other closely related smut fungi - *Sporisorium reilianum* and *Ustilago maydis*. Transcriptome profiling during distinct stages of infection (5 and 200 dpi) has resulted in identification of several effectors and other genes with putative roles in pathogenicity and virulence (TANIGUTI et al., 2015).

A proteomic analysis on the interaction between sugarcane and *S. scitamineum* employing 2-DE coupled with MALDI-TOF/TOF-MS by Que et al. (2011b) resulted in identification of 23 proteins, that were differentially expressed in a resistant and susceptible sugarcane in response to pathogen challenge. Functional annotation of these proteins established them to have been associated with functions such as photosynthesis, signal transduction, and disease resistance. This study represents the first time report and provides reference information on sugarcane response to S. scitamineum stress at the protein level.

In parallel with the ongoing research on sugarcane smut, our group at ICAR-Sugarcane Breeding Institute, India has been probing this interaction using proteomics tools to address the proteomic level changes that occur in sugarcane and the smut fungus during its interaction. In addition, results of another study focused on examining the alterations in the *in vitro* secretome of *S. scitamineum* using 2-DE-MALDI-TOF/TOF has also provided information on the secretome level alterations of this smut fungus in response to sugarcane meristem tissue and has resulted in identification of secretory proteins with putative roles in pathogenicity and virulence.

Other diseases

Besides red rot and smut, other diseases though not considered as much important under Indian context are quite significant in other parts of the world. However, globally other diseases like Pokkah Boeng, Rust, Leaf scald, Mosaic, Yellow leaf disease, etc. are of economic importance and hence the available literature on these diseases are briefly reviewed over here:

Strobel (1973) reported that clones of sugarcane susceptible to the toxin produced by *Helminthosporium sacchari* (organism causing eyespot disease) possessed a membrane protein that binds the toxin. However, the protein from the resistant clone - H50-7209, which varied with few amino acid residues, did not bind the toxin, thus indicating that disease resistance is directly associated with the structurally altered membrane-binding protein. Heritability estimates for pokkah boeng disease reaction in sugarcane were very high, indicating that genetic differences among populations were responsible for most of the observed differences in the disease reaction. A study indicated that the frequency of pokkah boeng susceptibility within F1 populations can be accurately predicted, if the degree of susceptibility of the parental clones is known (LYRENE et al., 1977).

Mc Ghie et al. (1997) monitored the cellular response in sugarcane, when primed with a heat-derived elicitor preparation from *Pachymetra chaunorhiza*, which causes a root rot in sugarcane. Pre-treatment with the *P.*

chaunorhiza elicitor induced marked changes in the biochemistry of both resistant (Q114) and susceptible (Q90) sugarcane cell lines in terms of PAL, POX activities and the production of additional phenolic compounds. Induced enzyme activities also differed between the cell lines with Q90 (susceptible) showing a large and transitory increase in PAL activity, that was far greater than that observed for Q114 (resistant). POX activity increased more in Q114 than in Q90, although the differences between the resistant and susceptible cell lines were not as great as for PAL.

Amplified fragment length polymorphism (AFLP) display of complementary DNA (cDNA) was used to identify genes from sugarcane somaclones expressed during the interaction with the rust pathogen -Puccinia melanocephala. The isolated TDFs correspond to genes involved in the resistance process. Genes related with recognition, signalling and general response were identified through BLAST search (CARMONA et al., 2004). Oloriz et al. (2012) reported a HR-mediated resistance to the brown rust pathogen – *P. melanocephala* in a sugarcane mutant obtained by chemical mutagenesis. Differentially expressed genes in response to *P. melanocephala* challenge inoculation was identified using SSH. Genes coding for a putative no apical meristem protein, S-adenosylmethionine decarboxylase, non-specific lipid transfer protein, and GDP-L-galactose phosphorylase involved in ascorbic acid biosynthesis were up-regulated in the incompatible interaction at the onset of haustorium formation, and may contribute to the HR-mediated defense response in the rust resistant mutant. Medeiros et al. (2014) studied changes in the transcription profile obtained by cDNA-AFLP analysis involving two sugarcane varieties contrasting to SCMV resistance, when challenged with a severe virus strain. A total of 392 TDFs were verified in the resistant variety against 380 in the susceptible one. Ten out of 23 sequenced TDFs (unique from the resistance variety), showed identity with known plant sequences, mostly related to plant defense mechanisms against pathogens. Casu et al. (2005) reviewed the status of sugarcane Expressed sequence Tags (ESTs) and gene expression associated with maturation and sucrose accumulation. Arencibia et al. (2006) assigned a new role for the plant growth-promoting nitrogen-fixing endophytic bacteria *Gluconacetobacter diazotrophicus* during its involvement in the sugarcane - *Xanthomonas albilineans* pathogenic interactions. It was observed that *G. diazotrophicus* produce elicitor molecules, which activated the sugarcane defense response resulting in the plant resistance to *X. albilineans*. A set of differentially expressed TDFs were identified by cDNA-AFLP, which shared significant homologies to genes of the ethylene signaling pathway (26%), proteins regulates by auxins (9%), β-1.3 glucanase proteins (6%) and ubiquitin genes (4%), all major signaling mechanisms. Results confirmed *G. diazotrophicus* mediated ISR in sugarcane against the leaf scald bacterium. Legaz et al. (2011) recorded that sugarcane glycoproteins may act as signals for the production of xanthan in the plant-associated bacterium *X. albilineans*, causing leaf scald disease. It was postulated that these glycoproteins might inhibit bacterial proteases after pathogen infection. The results indicated the existence of a positive feedback loop, in which plant-produced glycoproteins act as a cell-to-bacteria signal that promotes xanthan production, by protecting some enzymes of xanthan biosynthesis against from bacterial proteolytic degradation.

CONCLUSIONS

Understanding disease resistance in sugarcane has witnessed a paradigm shift from the conventional approaches like histopathological and biochemical to the application of robust NGS technology. The basic step in managing any plant disease is to have a thorough knowledge on host resistance and the pathogen dynamics. The knowledge thus gained would help in unlocking the secrets underlying any host-pathogen interaction. This is expected to ultimately lead to development of appropriate strategies for successful management of plant diseases. The twentieth century has been productive for the science of plant pathology and the field of host-parasite interactions—both in understanding how pathogens and plant defense work and in developing more effective means of disease control. Plant pathology rapidly adopted molecular cloning and its spin-off technologies, and these have fuelled major advances in our basic understanding of host-pathogen interactomics. This growing knowledge and the development of efficient technologies based on the tools of "Omics" viz. Genomics, Proteomics, Metabolomics etc. quite convincingly indicate that plant-pathogen interaction will be better elucidated in the future. The gained knowledge and the development of efficient technologies for producing transgenic plants convey optimism that plant diseases will be more efficiently controlled in the twenty-first century.

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