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ISSCT XII PATHOLOGY WORKSHOP

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Programme schedule

Code no.	Date	Time	Title of the paper	Page No.
	03-09-18	**8:30-10:00**	**Inaugural Session**	
BP1	03-09-18	10:20-11:00	Current research interests in sugarcane pathology *P. Rott*	
	03-09-18	11:00-11:30	Break	
BP2	03-09-18	11:30-12:00	Genomic approaches for red rot management in sugarcane *R.K. Singh*	
BP3	03-09-18	12:00-12:10	About ISSCT *Asha Dookun Saumtally*	
Host resistance				
BP4	03-09-18	12:10-12:30	Disease management through host resistance in sugarcane: Screening methodologies developed for different diseases in India *R. Viswanathan*	
BP5	03-09-18	12:30-12:50	Breeding strategies for incorporating red rot resistance in commercial varieties *G. Hemaprabha et al.*	
	03-09-18	12:50-14:00	Lunch	
BP6	03-09-18	14:00-14:20	Identification and utilization of source of red rot resistance available in national breeding gene pool for sugarcane improvement in India *Anna Durai et al.*	
BP7	03-09-18	14:20-14:40	Screening of Sugarcane genotypes for Red rot resistance and incorporation in the hybridization program *Rajeswari et al.*	
BP8	03-09-18	14:40-15:00	Genome wide association studies in sugarcane for yellow leaf disease resistance *Parameswari et al.*	
BP9	03-09-18	15:00-15:20	Mining novel genic SSR markers for WRKY transcription factors and disease resistance proteins for use in sugarcane breeding programmes *Shanthi et al.*	
BP10	03-09-18	15:20-15:40	Genomic selection approaches for red rot resistance in sugarcane *Manimekalai et al.*	
BP11	03-09-18	15:40-16:00	Broadening the genetic base of sugarcane:	

			introgression of red rot resistance from the wild relative *Erianthus procerus* *Mohanraj et al.*	
	03-09-18	16:00-16:30	Break	
BP12	03-09-18	16:30-16:50	Red rot resistance in interspecific hybrids of sugarcane derived from diverse cytotypes of *Saccharum spontaneum* L. *Suganya et al.*	
BP13	03-09-18	16:50-17:10	The sugarcane pathology program at CTC, Brazil *Aline Zavaglia et al.*	
BP14	03-09-18	16:50-17:10	Identification of sources of resistance to red rot and smut in *Saccharum* germplasm *Alarmelu et al.*	
BP15	03-09-18	17:10-17:30	Characterization of sugarcane micro-transcriptome in response to *Colletotrichum falcatum* inoculation *Sangeeta Srivastava et al.*	
	03-09-18	18:00-19:30	CULTURAL PROGRAMME	
	03-09-18	19:30-21:00	DINNER	
Pathogen variation				
BP16	04-09-18	08:20-08:40	Presence of diverse sugarcane bacilliform viruses infecting sugarcane in China revealed by pairwise sequence comparisons and phylogenetic analysis *Kashif Ahmad et al.*	
BP17	04-09-18	08:40-09:00	Genomic characterization of *sugarcane mild mosaic virus* *Denis Filloux et al.*	
BP18	04-09-18	09:00-09:20	Identification of two sugarcane yellow leaf virus genomic recombinants in the germplasm collection of Guadeloupe *Emmanuel Fernandez et al.*	
BP19	04-09-18	09:20-09:40	Dynamics of pathogenic variation in *Colletotrichum falcatum*, red rot pathogen of sugarcane *Viswanathan et al.*	
BP20	04-09-18	09:40-10:00	Molecular identification and characterization of *Fusarium sacchari* associated with wilt and pokkah boeng disease. *Balaji et al.*	
BP21	04-09-18	10:00-10:20	Complete genome characterisation of six isolates of Sugarcane yellow leaf virus from Mauritius *Nawshad Joomun et al.*	
BP22	04-09-18	10:20-10:40	Genetic variability of phytoplasma associated with weeds grown in proximity of sugarcane	

			fields infected with grassy shoot disease *Tiwari and Rao*	
Diagnosis & new reports				
BP23	04-09-18	10:40-11:00	The use of shotgun RNA metagenomics as a tool for viral detection in sugarcanes grown in quarantine *Malapi-Wight et al.*	
	04-09-18	11:00-11:30	Break	
BP24	04-09-18	11:30-11.50	Development of a fast and reliable molecular-based detection method for sugarcane white leaf disease (WLD) phytoplasma *Dayasena et al.*	
BP25	04-09-18	11:50-12:10	Status of pokkah boeng disease of sugarcane in sub-tropical India *Chhabra et al.*	
Biosecurity & current status				
BP26	04-09-18	12:10-12:30	Testing of sugarcane clones in quarantine in Mauritius: Improvement during the last decade *Nawshad Joomun et al.*	
BP27	04-09-18	12:30-12:50	Leaf fleck, an emerging viral disease in sugarcane: surveillance under various geographical locations in India *Sanju Balan et al.*	
	04-09-18	12:50-14:00	Lunch	
BP28	04-09-18	14:00-14:20	Current situation of the sugarcane yellow leaf disease in Colombia *Garcés-Obando et al.*	
BP29	04-09-18	14:20-14:40	A summary of biosecurity research in Papua New Guinea: 2009-2017 *Thompson et al.*	
BP30	04-09-18	14:40-15:00	A decade of orange rust in Florida (USA): where we were and where we are going. *Raid et al.*	
BP31	04-09-18	15:00-15:20	Investigations into sugarcane diseases in Florida *P. Rott*	
BP32	04-09-18	15:20-15:40	Sugarcane smut – current status of research in India and emerging prospects *Ramesh Sundar et al.*	
BP33	04-09-18	15:40-16:00	How spread is the leaf scald in the Brazilian sugarcane industry? *Andreato, C.; Silva, M. F.; Coraini, N. F., Urashima, A.S.*	
	04-09-18	16:00-16:30	Break	
BP34	04-09-18	16:30-16:50	Unravelling sugarcane pokkah boeng disease caused by *Fusarium* species complex in China	

			Muqing Zhang and Wei Yao	
BP35	04-09-18	16:50-17:10	New strategy for production virus free tissue culture plant through direct regeneration technique in sugarcane *D. Neelamathi et al.*	
Biocontrol and Disease management				
BP36	04-09-18	17:10-17:30	*Trichoderma*: an effective biocontrol agent for management of red rot of sugarcane *Deeksha Joshi et al.*	
BP37	04-09-18	17:30-17.50	Efficient management of fungal diseases in sugarcane by enhanced fungicide delivery in the planting materials *Malathi et al.*	
Short Presentation (Host resistance, Biocontrol & new reports, Epidemiology)				
BP47	04-09-18	17:50-18:00	Identification of novel sources of resistance to red rot *Adhini et al.*	
BP58	04-09-18	18:00-18:10	Status of sugarcane yellow leaf resistance in *Saccharum* hybrid populations and parental clones in India *Nithya et al.*	
BP49	04-09-18	18:10-18:20	Biotherapy –a tool for the management of red rot in sugarcane *K. Krishnamma*	
Disease management				
BP38	07-09-18	08:40-09:00	Controlling of sugarcane white leaf disease with hot tetracycline treatment in Thailand *Udomsak Lertsuchatavanich et al.*	
BP39	07-09-18	09:00-09:20	Recent Approaches in Diagnosis and Management of Sugarcane Phytoplasma Diseases *Rao GP*	
BP40	07-09-18	09:20-09:40	Healthy sugarcane to achieve targeted yield - A farmer's perspective *Karanjeet Singh Dhaliwal*	
BP41	07-09-18	09:40-10:00	Management of sugarcane red rot with liquid formulated endophytic *Bacillus subtilis* *Raguchander and Prema Ranjitham*	
BP42	07-09-18	10:00-10:20	Elimination of yellow leaf disease by combined method of heat treatment, meristem culture and nursery systems in Rajshree sugars command area *Vijayakumar et al.*	
Host-pathogen interaction				

BP43	07-09-18	10:20-10:40	Identification of effectors as molecular markers to genotype isolates of *puccinia kuehnii*, the causal agent of sugarcane orange rust *Rodrigues Porto et al.*	
BP44	07-09-18	10:40-11:00	*Chitinase* gene expressions in response to red rot pathogen *Colletotrichum falcatum* infection by RT-qPCR *Singh et al.*	
	07-09-18	11:00-11:30	Break	
BP45	07-09-18	11:30-11:50	Rhizospheric microbial diversity in different sugar profile varieties of sugarcane *Dinesh Singh et al.*	
BP46	07-09-18	11:50-12:10	Transcriptome characterization and expression profiles of the pathogenicity related genes in *Colletotrichum falcatum*, causing red rot in sugarcane *Naveen Prasanth et al.*	
	07-09-18	12:10-14:00	Lunch	
Short presentation 10 minutes (Disease management, Biocontrol, Host-pathogen interaction, Pathogen variation, Yield losses and Diagnosis)				
BP50	07-09-18	14:00-14:10	Disease monitoring and epidemiology of fungal diseases in *Saccharum officinarum* clones *Gopi et al.*	
BP51	07-09-18	14:10-14:20	Efficacy of different thermotherapy conditions to control ratoon stunt with different titers of *Leifsonia xyli* subsp. *xyli* *Uzan et al.*	
BP52	07-09-18	14:20-14:30	Biological control approach – serves as an interface to identify antifungal/ pathogenicity related proteins during tritrophic interactions *Elamathi et al.*	
BP53	07-09-18	14:30-14:40	Temporal variation of the orange rust pathogen, *Puccinia kuehnii*, and its mycoparasite *Sphaerellopsis filum* at a sugarcane breeding center in Brazil *Leite F.A.C. Urashima A.S.*	
BP54	07-09-18	14:40-14:50	Pathogenicity correlation between red rot pathotypes in resistance evaluation of sugarcane genotypes *Singh et al.*	
BP55	07-09-18	14:50-15:00	Identification and characterization of genes/proteins related to *Colletotrichum falcatum* pathogenesis in sugarcane *Kaverinathan et al.*	
BP56	07-09-18	15:00-15:10	Occurrence of red rot of sugarcane and variation	

			in pathogenicity of *Colletotrichum falcatum* in coastal Tamil Nadu *Ravichandran et al.*	
BP57	07-09-18	15:10-15:20	Deterioration in economical traits of sugarcane due to pokkah boeng disease *Vishwakarma et al.*	
BP58	07-09-18	15:20-15:30	Ultrasensitive nanogold-labelled immunoassay for the detection of sugarcane streak mosaic virus *Raja et al.*	
	07-08-18	15:30-16:00	Break	
Poster session				
BP59	07-09-18		Taking RSD diagnosis closer to the field edge *Sa. Mcfarlane et al.*	
BP60	07-09-18		Confirmation of a new rust, *Macruropyxis fulva* (*pucciniales*) infecting sugarcane in southern Africa *R S Rutherford et al.*	
BP61	07-09-18		Mapping and marker identification for red rot resistance in biparental segregating populations of sugarcane using microsatellite markers. *Selvi et al.*	
BP62	07-09-18		Induction and antifungal activities of 3-deoxyanthocyanidins phytoalexin compounds as host response against invading Colletotrichum falcatum in sugarcane *Nandakumar et al.*	
BP63	07-09-18		Comparative secretome analysis of *Colletotrichum falcatum* identifies potential proteins that induces systemic resistance in sugarcane *Ashwin et al*	
BP64	07-09-18		Assessing resistance of sugarcane clones to brown rust in the field *Selvakumar et al.*	

BP01 KEY NOTE SPEAKER ABSTRACT

CURRENT RESEACH INTERESTS IN SUGARCANE PATHOLOGY

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Key Words: Host-pathogen interactions, metagenomics, molecular markers, new diseases, resistance.

Growing resistant cultivars is considered the best approach for sustainable control of sugarcane diseases. Efficient development of disease resistant sugarcane relies on a good knowledge of plant-pathogen interactions. The advent of the -Omics (Genomics, metagenomics, proteomics, metabolomics, etc.) techniques has tremendously improved our understanding of sugarcane diseases and development of diagnostic methods. Entire genomes of several sugarcane pathogens have been sequenced, including viruses, bacteria, and fungi. This information is now used for functional genomics and investigations into evolution of these pathogens. Metagenomics approaches were successfully employed to study the prevalence of known viruses such as *Sugarcane yellow leaf virus* and to discover new viruses such as Sugarcane white streak virus and Sugarcane striate virus. Use of metagenomics also resulted in the identification of a *Cercozoa* as the causal agent of an old disease called chlorotic streak. *In situ* localization of pathogens using tagged bacteria revealed unexpected features of the causal agents of leaf scald and ratoon stunting. Sequencing the genome of sugarcane and other *Poaceae* contributed to identification of genes involved in host response to pathogen colonization, including resistance genes (smut, red rot). Molecular markers for resistance to brown rust, orange rust, and yellow leaf have been or are currently being developed. Besides molecular biology-based research, investigating conditions that favor disease appearance, spread, and progress in the field remains essential for good management of sugarcane diseases.

BP02 KEY NOTE SPEAKER ABSTRACT

GENOMIC APPROACHES FOR RED ROT MANAGEMENT IN SUGARCANE

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Sugarcane, a major source of sugar, is an important crop for tropical and subtropical regions of the world. Sugarcane is a cost-effective renewable resource with its multifaceted use as food, feed, fiber, and energy. The present-day cultivated sugarcane is a result of interspecific hybridization involving *Saccharum officinarum*, *S. spontaneum*, and other related genera. Being a long duration crop sugarcane has to cope with different abiotic and biotic stresses. Among the biotic stress, the most common threat to sugarcane is red rot disease. Red rot is a devastating disease of sugarcane caused by the fungus *Colletotrichum falcatum* Went, [Perfect stage *Glomerella tucumanensis* (Speg.)] that has a colossal damage potential. The disease, under epiphytotic conditions, may lead to total crop failure. The impact of the disease is severe in the Indian sub-continent where red rot is considered as a major threat to the profitable cultivation of sugarcane. The fungus keeps on producing new pathogenic strains leading to the breakdown of resistance in newly released varieties and hence the deployment of linked markers for marker-assisted selection for resistance to this disease can fine tune the breeding programme. Due to this multispecies origin, sugarcane is considered to possess one of the most complex plant genomes. The genetic manipulation of sugarcane for red rot resistance using traditional breeding has been cumbersome due to its heterozygous complex polyploid genome which does not permit an easy and predictable gene introgression. Under these circumstances, modern genomic tools could very well aid conventional breeding in overcoming genetic bottlenecks and thereby enhancing breeding efficiency for red rot resistance.

A fundamental pre-requisite of a genomics-based breeding is the identification of trait-associated molecular markers. Genetic mapping utilizing lineage and association mapping has made remarkable attention to recognize genes responsible for different traits with agricultural and evolutionary significance in many crops including sugarcane. In order to decipher the marker-trait associations (MTAs) for resistance to red rot, we used a panel of 119 diverse sugarcane genotypes consisted of cultivated varieties, advanced breeding lines, and genetic stocks. All the 119 genotypes were screened for resistance against three (*Cf01*, *Cf08*, and *Cf09*) races of *C. falcatum*. Genotyping was performed based on a set of 115 polymorphic SSR primers which generated a total of 944 dominant loci. Marker-trait associations for resistance to three races of red rot were studied employing general linear model (GLM) and mixed linear model (MLM) approaches containing population structure and kinship as co-factor and detected four MTAs that were able to explain 10-16% of the trait variation, individually. Utilizing the advantage of synteny between sorghum and sugarcane genome we identified the candidate genes potentially involved in plant-pathogen/stress interactions in the vicinity of the sorghum homologs of sugarcane markers. Among the four MTAs, EST sequences diagnostic of three could be BLAST searched to the sorghum genome with significant sequence homology. Several genes encoding important plant defense-related proteins, viz., cytochrome P450, Glycerol-3-phosphate transporter-1, MAP Kinase-4, Serine/threonine-protein kinase, Ring finger domain protein and others were localized to the vicinity of these MTAs. These positional candidate genes are worthy of further investigation and possibly these could contribute directly to red rot resistance, and may find a potential application in marker-assisted sugarcane breeding.

BP03

BP04

DISEASE MANAGEMENT THROUGH HOST RESISTANCE IN SUGARCANE: SCREENING METHODOLOGIES DEVELOPED FOR DIFFERENT DISEASES IN INDIA

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Key Words: Screening for red rot resistance, field method, controlled condition screening

Fungal diseases such as red rot, smut, wilt, pokkah boeng and rust and non-fungal diseases like grassy shoot, yellow leaf disease (YLD) and mosaic cause substantial losses to productivity in sugarcane in India. To successfully manage these diseases, host resistance plays a vital role hence screening techniques have been developed to select resistant clones in the germplasm and in the progenies. Red rot caused by *Colletotrichum falcatum* is a destructive pathogen and currently varieties with red rot resistance alone are recommended for commercial cultivation. Sugarcane progenies are screened for red rot resistance during different stages of selection process both under field conditions as well as under controlled conditions. Evaluation of red rot resistance by screening method provides basis for recommending elite sugarcane varieties for commercial release in the country. To assess red rot resistance at very early stages of varietal improvement programme, the clones are tested under controlled conditions from seedling stage onwards. This rapid screening methodology ensures screening of large number of progenies every year at the Institute. Resistance to smut in the progenies is assessed under field conditions by following standard sett dip inoculation method of the pathogen. Screening of sugarcane varieties for wilt resistance is being done at few centres in the country where wilt is a serious disease. Recently screening the clones for YLD resistance has been adopted in all the sugarcane research stations using 0-5 rating scale developed by the institute. Since diseases such as brown rust and pokkah boeng have become major constraints in few states, screening methodologies were standardized to screen the clones for resistance to these diseases. Overall, development of

disease resistant varieties has been fully supported by screening programmes in different sugarcane research stations in the country and ensures release of commercial varieties with disease resistance to major diseases.

BP05

BREEDING STRATEGIES FOR INCORPORATING RED ROT RESISTANCE IN COMMERCIAL VARIETIES

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Key Words: Red Rot, resistance, pre-breeding, phenotyping, multi trait genetic stock.

Major diseases of importance in Indian context are red rot and smut. The concerted efforts of breeders and pathologists have helped to contain these diseases through developing resistant cultivars. The process of breeding for red rot resistance is continuous, considering the pathogen specialization that breaks down the resistance of the host. All the parental lines used for sugarcane improvement are systematically screened for red rot resistance with the most virulent pathotype for identification resistant parents and this resulted in developing resistance varieties for tropical India. A glance on the performance of Coimbatore canes (Co canes) bred since 2006 revealed a high proportion (>90%) as resistant/ moderately resistant to red rot. This has been possible by adopting crossing programs guided by the resistance reaction of the parents along with the yield and quality of the parental clones. Standardization of controlled condition testing technique has been a boon to sugarcane breeders. In the second clonal stage with a population size of 1000 clones downsized through selection in two early stages were screened for red rot and disease reaction formed a major selection parameter. During the process several parents /cross combinations could be identified as donor parents/ proven crosses in breeding. Genetic enhancement with new and hitherto unutilized genetic resources of *Saccharum* species and allied genus *Erianthus* has been in focus since 1980. Several interspecific hybrids have been identified with red rot resistance. These have been incorporated in base broadening that has resulted in several hybrids of diverse genetic background for use in breeding programs. In the process 11 genetic stocks for multitraits have been identified with proven breeding value. Red rot and smut are given due importance in pre-breeding for climate resilience. Eight founder parents, which are genetically diverse and carry many abiotic and biotic stress resistance, and agronomic traits relevant to sugarcane improvement were intercrossed and two-way populations were developed. The selected two-

way cross progenies were phenotyped for drought tolerance and red rot resistance and 25.0% clones were relatively drought tolerant and 45.7% were red rot resistant. A high proportion of progeny from the cross Co 95005 (*S. robustum* base) x CYMA 09-1369 (having *Erianthus* cytoplasm) were resistant to red rot and seven clones viz., TWC 50, TWC 88, TWC 38, TWC 51, TWC 69, TWC 75, TWC 84 combined both drought and red rot resistance and most of them were from the cross CYM 08-922 x ISH 176. These form a valuable resource for developing climate-resilient cultivars with a broad genetic base.

BP06

IDENTIFICATION AND UTILIZATION OF SOURCE OF RED ROT RESISTANCE AVAILABLE IN NATIONAL BREEDING GENE POOL FOR SUGARCANE IMPROVEMENT IN INDIA

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Key Words: Red rot, resistance, hybridisation, *Colletotrichum falcatum.*

The studies on red rot resistance breeding search for more resistant parents in hybridization to increase the proportion of resistant progenies. The national breeding gene pool maintained in ICAR-SBI, Coimbatore serves as a source for generating genetic variability for sugarcane varietal development in India. Identification of resistant parents to the prevailing pathotypes is required since they are used directly in the breeding programme without any pre-breeding effort. The resistance sources from various locations which had region specific choice of parents, selection and evaluation of the progenies may be of immense value to increase red rot resistance in sugarcane. About 417 genotypes including those from five different zones of sugarcane cultivation in India, exotic hybrids and interspecific hybrids were initially tested for their resistance against the tropical (mixed inoculum of CF06 [*Cf*671] and CF12 [*Cf*94012]) pathotypes of *Colletotrichum falcatum,* the red rot pathogen followed by the subtropical pathotype CF08 (*Cf*64). We found the highest number (44 of 158) of resistant (R) or moderately resistant (MR) were parents from North West Zone (27.85%) followed by North Central and North East Zone (17 of 36), Peninsular Zone (16 of 134) and East Coast Zone (2 of 34). Only one exotic parent (CP 61-23) among the 17 was found to be MR to both pathotypes of *C. falcatum*. Overall, the sub-tropical parents had higher proportion of R types while the tropical parents had more of susceptible types. A case study on breeding potential of one of the resistant parent Co 12014 identified in the study, resistant to the major *C. falcatum* pathotypes in the tropical India was done by obtaining the progenies of Co 12014 through open pollination. The randomly chosen 174 progenies were screened for red resistance at the clonal stage which revealed that among the 174 progenies 23 were R while 86 MR, 39 were moderately susceptible, 18 were susceptible and eight were highly susceptible. From this study, it is

inferred that Co 12014 has a greater potential to be used as resistant parent in the sugarcane improvement. Further, an appraisal on breeding potential of these parents in developing new genotypes indicated importance of these parents in imparting red rot resistance to sugarcane.

BP07

SCREENING OF SUGARCANE GENOTYPES FOR RED ROT RESISTANCE AND INCORPORATION IN THE HYBRIDIZATION PROGRAM

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Key Words: Red rot, resistant cultivars, yield losses, germplasm, screening program

Red rot caused by *Colletotrichum falcatum* is one of the widest spread sugarcane disease in the country, which has been in existence affecting the productivity for 100 years in India. This pathogen affects the valuable stalk tissues of sugar cane leading drastic changes in the juice quality which causes huge economic loss. Many wonder varieties have gone out of cultivation due to red rot disease. Breeding and identification of red rot resistant variety is inevitable to suitably manage the disease and avoid epiphytotics of red rot. At E.I.D. Parry, sustained efforts were made to develop new sugarcane varieties with high yield and high sugar along with disease resistance in an ongoing breeding program. Red rot screening program was carried out in an isolated Pathology farm, located at Thyagavalli, Cuddalore. In the past 3 years, 155 sugarcane genotypes were screened by inoculating mixtures of red rot pathogen, *C. falcatum* containing three pathotypes viz., *Cf*671,*Cf*419 and *Cf*997. Plug and nodal methods of inoculations were followed. Among 155 genotypes tested for red rot resistance, identified three genotypes under resistant and 17 under moderately resistant categories. 73 genotypes were rated as MS (moderately susceptible) and the remaining 62 genotypes were considered as susceptible and highly susceptible using plug method of inoculation. In Nodal method of testing 6 genotypes have shown resistant and 17 in moderately resistant category. Also observed 62 under MS (moderately susceptible) and 70 under susceptible and highly susceptible categories. The resistant and moderately resistant genotypes were included as one of the parents in hybridization

program and effected 55 crosses. 5580 seedlings were produced from these crosses and are under evaluation for developing red rot resistant cultivars.

BP08

GENOME WIDE ASSOCIATION STUDIES IN SUGARCANE FOR YELLOW LEAF DISEASE RESISTANCE

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Key Words: Yellow leaf, genome wide association studies GWAS, *Sugarcane yellow leaf virus*

Yellow leaf disease (YLD) caused by *Sugarcane yellow leaf virus* (SCYLV) is the major viral disease affecting sugarcane in India and causes 43 to 39% reductions in plant growth and 34 to 30% losses in juice yield in susceptible cultivars. Currently healthy seed nursery programme is suggested to manage the disease; however iidentifying host resistance is the sustainable strategy to manage this disease. Earlier, few attempts on SCYLV resistance was attempted based on QTL approach and the first major quantitative trait allele named Ryl1 was tagged through a biparental cross. Compared to QTL approach, Genome wide association study (GWAS) based on linked disequilibrium in diverse genetic samples is a relatively new approach which offers higher resolution mapping that under optimal conditions can pinpoint causal genes underlying quantitative trait variation. Based on the GWAS, two resistant markers were identified from sugarcane germplasm of CIRAD, France for resistance to SCYLV. Recently, GWAS has been taken up for identifying genomic regions influencing resistance to YLD from sugarcane germplasm used for current breeding programme of ICAR-SBI, Coimbatore. Detailed surveys for YLD incidence and severity on 4066 genotypes/varieties maintained by the institute at Coimbatore and its three research centres were recorded using the newly developed YL disease rating scale 0 to 5 and we have identified 463 resistant sources in the hybrid clones and 773 in *Saccharum* spp. Among the parental clones BO 91, Co 678, Co 976, CoPant 97222, CoJ 89, CoP 9302 and ISH 76 were found to be resistant to YLD. In case of *Saccharum* spp, 86% of *S. robustum* were resistant to YLD followed by *S. sinense* (80%), *S. officinarum* (78%) and *S. barberi*

(76%). Further work is in the progress to identify YLD resistant markers linked to genomic regions of these germplasm through genome wide association mapping.

BP09

MINING NOVEL GENIC SSR MARKERS FOR WRKY TRANSCRIPTION FACTORS AND DISEASE RESISTANCE PROTEINS FOR USE IN SUGARCANE BREEDING PROGRAMMES

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Key Words: SSR markers, resistance, molecular markers, WRKY.

Genic SSR markers for economically important traits have immense potential in a polypoid crop like sugarcane as they specifically target the transcribed region of the genome which are more likely to be conserved between related species. The plant WRKY transcription factors comprising a large family of regulatory proteins play an important role in response to various stresses. A study was carried out to characterise transcript repeat motifs for WRKY transcription factors and disease resistance proteins in the coding region, and assess their molecular diversity in a germplasm panel of twenty-four sugarcane clones. Unigene datasets such as the gene indices from the Institute of Genomic research (TIGR) were used for mining novel microsatellites. 18275 SSRs were analysed for gene ontology alignment and for functional gene identification. Higher numbers of repeat motifs were located in the ORFs than those found in 5' and 3' UTRs. Twenty-one primer sets were designed from eleven WRKY family transcription factors and 10 disease resistance proteins. AMOVA based on SSR allelic frequencies showed that the two groups (wild and cultivated species) as well as the taxa within the group were significantly different. Gene diversity among the wild relatives was high for the loci WRKY 1b, WRKY 3, WRKY 7, DRP 2, DRP 3, DRP 4, DRP 7, DRP 8, DRP 11a compared to the other group (domesticated group). Erosion of alleles WRKY 1a, WRKY 4, WRKY 9a, WRKY 10, DRP 6, and DRP among the cultivated group is an important finding that indicates the changes in population structure under selection pressure. Neutrality test on allelic frequencies characterised the selection sweeps happening in the transcribed region and resolved the WRKY alleles under positive and diversifying selection. WRKY 1b, WRKY 8, WRKY 9b, WRKY 11 DRP 7 and DRP 10 exhibited substantial heterozygote deficiency that is indicative of positive selection. Evidence for linkage disequilibrium was observed between WRKY 4 and WRKY 1a (r^2 =1.00 and P = 0.004), DRP3 and WRKY 1a (r^2 =1.00 and P = 0.005), DRP 3 and WRKY 4 (r^2 =1.00 and P = 0.006). Structure analysis based on SSR allelic datasets

revealed the presence of five different populations in the selected core set. Of the 24 genotypes, 14 (58.33%) have more than 0.70% membership in any given five clusters. Out study identified microsatellite markers for WRKY transcription factors and disease resistance proteins having potential applications in sugarcane breeding and selection programmes.

BP10

GENOMIC SELECTION APPROACHES FOR RED ROT RESISTANCE IN SUGARCANE

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Key words: Red rot resistance, genomic selection, SNPs

Sugarcane is an important commercial crop for sugar and of late it is being recognized for a biomass crop for bio fuel production. The red rot resistance mechanism is still unclear in sugarcane because of its polyploidy. Genomic Selection (GS) aimed at selecting preferred individuals based on genomic estimated breeding values predicted by GBLUP, BayesA and BayesB based on the relationship between phenotypes and genotypes. The present study utilizes the GS approaches for red rot resistance breeding. A bi parental cross population involving red rot resistant and susceptible parents (BO 91 X Co 775) was developed. The seedlings were planted in replicated block design (RBD) with two replications. Controlled Condition Testing (CCT) method was employed for scoring the red rot resistance against *Colletotrichum falcatum* pathotype CF06 (*Cf*671). Seven month old healthy stalks from each clone were used and the conidial suspension was applied on the nodal region. The inoculated canes were incubated in the humidity controlled moist chamber with ~90% relative humidity and the temperature was maintained at 32°C. The rating for the disease expression was done 10 days after inoculation as highly susceptible, susceptible, moderately susceptible, moderately resistant and resistant. The average of three scoring was taken for analysis. Chi square test for goodness of fit was worked out for monogenic inheritance involving parents of R x S types. The results indicated that the progenies segregated in the expected ratio of 1:1 with significance. The progenies were phenotyped for other traits such as drought tolerance index, brix %, sucrose %, CCS %, CCS yield and cane yield. We have estimated the BLUPs for the brix and sucrose for generating genomic selection models with SNP markers. We expect that GS could increase genetic gain per unit time for sugarcane breeding for imparting red rot resistance.

BP11

BROADENING THE GENETIC BASE OF SUGARCANE: INTROGRESSION OF REDROT RESISTANCE FROM THE WILDRELATIVE *ERIANTHUS PROCERUS*

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Key Words: Red rot, disease resistance, *Erianthus procerus*, genetic base

Red rot of sugarcane caused by *Colletotrichum falcatum* is one of the important stalk diseases causing substantial yield losses in India. Host plant resistance plays a vital role in managing the disease and considerable importance has been given in different stages of varietal development. Exploitation of new and diverse sources of variation is needed to enhance the disease resistance and wild relatives with resistance/tolerance to multiple stresses provide important sources of genetic diversity. Traditionally *S. spontaneum* had been used as a source for imparting high productivity and tolerance to biotic and abiotic stresses in sugarcane varieties. Of late, considerable attention has been given towards the incorporation of *Erianthus* spp. which had been identified as valuable source for many traits such as ratoonability, tolerance to environmental stresses, vigour, and disease resistance including red rot resistance. An intergeneric hybrid between *E. procerus* and *Saccharum* (GU 04(28) EO-2) has been identified as potential source for diversifying the genetic base of the sugarcane varieties in view of its yield potential, red rot resistance and drought tolerance. The hybrid was further backcrossed with two commercial clones Co 06027 and Co 775, and 32 BC1 hybrids were developed during 2012. The F1 along with 32 BC1 hybrids were evaluated for their red rot resistance under controlled condition testing using the isolate cf 671 during 2014 and using more virulent mixed inoculum of cf671+ cf94012 during 2015. The results showed that the F1 was resistant (R) against both cf 671 and the mixed isolate, whilst 70% of the BC1 hybrids showed resistance against cf671 and 62.5% were resistant against more virulent mixed inoculum. Only 6.82% of the progenies were highly susceptible for mixed inoculum. The BC1 hybrids GU 12-20, GU 12-21 and GU 12-33 had enhanced red rot resistance and could be a potential source for developing sugarcane varieties with Erianthus base of red rot resistance.

BP12

RED ROT RESISTANCE IN INTERSPECIFIC HYBRIDS OF SUGARCANE DERIVED FROM DIVERSE CYTOTYPES OF *SACCHARUM SPONTANEUM* L.

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Key Words: Red rot, resistance, hybrids, cytotype, *S. spontaneum*.

Red rot is the oldest disease of sugarcane and caused major losses to the crop for more than hundred years ago. It is caused by the fungal pathogen *Colletotrichum falcatum* Went.. In sugarcane breeding, development of resistant varieties through inter-varietal hybridization is a major challenge, due to narrow genetic base in the varieties. Exploitation of wild relatives is an ideal approach to incorporate resistance. *Saccharum spontaneum*, the wild species of sugarcane has been utilized in sugarcane breeding since 1912 owing to their wider adaptability to biotic and abiotic stresses and cytomorphological diversity with 41 cytotypes from 2n = 40-128. If cytologically diverse clones with different ploidies are incorporated, hybrids with sustained resistance for red rot can be obtained. In the present study, 588 hybrids derived from 37 interspecific crosses involving eight commercial varieties and 16 clones of *S. spontaneum* with eight cytotypes (2n=40, 56, 60, 64, 72, 80, 88, 112) were evaluated for red rot resistance at 6th month under controlled condition using the pathotype CF06 (*Cf*671). In each cross 10 - 29 hybrids were tested. Since the cytotype 64 is the predominant type, 248 hybrids from 7 clones with 2n=64 were included. The parents exhibited a differential reaction to pathogen. Among the 588 hybrids tested, 44.4 % of progenies were moderately resistant (MR) and resistant (R). Among the 170 MR hybrids, 32.0-39.5 % of progenies derived from the cytotypes 2n=40, 72, 88 and 112, while 29.0 % involved the cytotype 2n=64. In resistance, the cytotype 64, 80, 88 and 112 generated 19.7, 14.0, 18.3 and 24.0 % of resistant progenies. The contribution of lower cytotypes (2n=40, 56 and 60) for imparting resistance is limited with 5.2 - 7.2% resistant progenies. In the susceptible female parent (Co 1148, Co 8371 and BO 102) involved crosses, resistance noticed in the progenies with the cytotype 2n=64, 80, 88 and 112. The cytotype 80 incorporated resistance at higher

frequency and this indicates that enhanced ploidies with genome size increases resistance. The best resistant hybrids were backcrossed with sugarcane. Several promising backcross hybrids with sustained resistance could be observed and are further utilized.

BP13

THE SUGARCANE PATHOLOGY PROGRAM AT CTC, BRAZIL

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CTC runs 6 breeding and selection programs, aimed at three major agro-climatic regions, and 2 planting/harvest cycles in each region (early season harvest and late season harvest). In addition, CTC has a genetic transformation pipeline to produce transgenic sugarcane varieties. The pathology program provides crucial support to these core business areas, in addition to providing *ad hoc* advice to sugar mills when requested, and a strategic monitoring of plant health issues in Brazil. Some of the specific activities conducted include:

- Routine testing for RSD and Leaf Scald in stalk material entering transformation or multiplication.
- Inoculation of all seedlings entering the breeding program (~500,000 per year) with brown and orange rust, and discard of seedlings before entering the first field testing phase of the breeding program.
- Artificial inoculation screening trials for Smut and Leaf Scald for promising clones.
- Field screening using spreader rows for Mosaic virus and Rusts for promising clones.
- Research in using qPCR to develop a laboratory screening test for resistance to Leaf Scald.
- Research in alternative screening methods for Mosaic virus and Smut resistance.
- We have also recently started to screen varieties for Red Rot using the methodology developed at SBI, Coimbatore.

These will be presented and discussed in more detail.

BP14

IDENTIFICATION OF SOURCES OF RESISTANCE TO RED ROT AND SMUT IN *SACCHARUM* GERMPLASM

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Key Words: Red rot, resistance, *Saccharum*, smut

Red rot, wilt and smut are major diseases that seriously affect sugarcane production in India. The increase in climate variability and drastic change in climate patterns have great impact on occurrence of diseases and in sugarcane, resistance breeding is the only solution to tackle this problem. In an attempt to increase diversity of wild germplasm through introgression in base broadening programmes, variability within *Saccharum* species was assessed to identify different sources of resistance to red rot and smut for use in breeding programmes to identify resistant cultivars. The 136 BC_1 hybrids from two crosses of improved *S.officinarum* x improved *S. robustum* and 100 BC_2 clones involving BC_1× Co 86011, Co 09014 were evaluated for red rot (cf 671 by CCT) and assessed for natural incidence of smut during 2016 and 2017 seasons. Out of 100 BC_1 hybrids with improved *S.officinarum* as recurrent parent, 62 clones (45.58 %) had no natural incidence of smut and 32 clones (23.52 %) showed MR or R reaction to red rot. Of the 80 tested BC_2 lines involving commercials as a backcross parent, 18 (22.5 %) were resistant to moderately resistant to red rot and 31 clones (38.75 %) were free from smut incidence. BC_1 progenies had more resistant types than BC_2. Eleven clones from this group *viz.*,12-15,12-44,13-36,13-44,13-69,13-251,14-48,14-14,14-144,14-125,14-102 combined resistance to red rot and with no natural smut incidence in trials. Along with resistance these clones combined yield and sucrose % at 360 days. About 11.2 % of the BC_1 progenies of (Co 8371 x *S.barberi* (Pathri) x Co 0209 were moderately resistant to red rot and clones 13-176 and 14-60 combined sucrose, red rot resistance and yield.The F_1 progenies (281) involving improved *S. spontaneum* had 27.78 % MR 4.62 % R and 3.20% MS clones to red rot. Large number of resistant progenies were found in the cross involving both red rot susceptible parents and three crosses produced predominantly

resistant progenies involving 96-38,96-195,97-12, 96-319, 96-137, 96-77 as donors. The study suggests that resistant hybrids involving improved *S. robustum* and S. *barberi* germplasm could also be used as source of smut and red rot resistance in sugarcane breeding programs apart from *S. spontaneum*. The new genetic stocks identified with multiple disease resistance and other desirable traits are to be exploited by the breeders to combat the future climate challenges to identify clones with stable resistance.

BP15

CHARACTERIZATION OF THE SUGARCANE MICRO-TRANSCRIPTOME IN RESPONSE TO *COLLETOTRICHUM FALCATUM* INOCULATION

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Key Words: Red rot, Saccharum, High-throughput sequencing, miRNA, small RNA

The plant microRNAs (miRNAs) are implicated in response to many biotic and abiotic stresses and play a critical role in plant adaptation. Several miRNAs either are up- or down-regulated by stresses, suggesting that they may be involved in regulation of stress-responsive gene expression and stress adaptation. Stress-induced small RNAs in general down-regulate their target genes, which may encode negative regulators of stress responses. Conversely, down-regulation of small RNAs in response to stress leads to the accumulation of their target mRNAs, which may contribute positively to the adaptation to stress. Red rot disease caused by the fungus *Colletotrichum falcatum* is one of the most important diseases of sugarcane in India especially in the subtropical belt, and yield losses due to red rot can reach 100 per cent. Since only a little is known about the miRNA population in sugarcane, their identification from red rot challenged sugarcane would be a critical step towards understanding small RNA-guided gene regulation. The present study, therefore was done on two sugarcane genotypes, one resistant and one susceptible to red rot, to identify differentially expressed small RNAs in sugarcane challenged with*C. falcatum* using deep sequencing technology. Total RNA was isolated at different time intervals from the cane stalks inoculated with *C. falcatum* along with control samples. Four small RNA libraries were constructed and sequenced individually. The conserved, novel and differentially expressed miRNAs were identified by bioinformatics and target genes were predicted. Details would be discussed in paper.

BP16

PRESENCE OF DIVERSE SUGARCANE BACILLIFORM VIRUSES INFECTING SUGARCANE IN CHINA REVEALED BY PAIRWISE SEQUENCE COMPARISONS AND PHYLOGENETIC ANALYSIS

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Key Words: Badnaviruses; sugarcane bacilliform virus; genetic diversity; sequence analysis; reverse transcriptase/ribonuclease H

Sugarcane bacilliform viruses (SCBV), which belong to the genus *Badnavirus*, family *Caulimoviridae*, are an important DNA virus complex that infects sugarcane. To explore the genetic diversity of the sugarcane-infectingbadnavirus complex in China, we tested 392 sugarcane leaf samples collected from Fujian, Yunnan, and Hainan provinces for the occurrence of SCBV by polymerase chain reaction (PCR) assays using published primers SCBV-F and SCBV-R thattarget the reverse transcriptase/ribonuclease H (RT/RNase H) regions of the viral genome. A total of 111 PCR-amplified fragments (726bp)from 63 SCBV-positive samples were cloned and sequenced. A neighbor-joiningphylogenetic tree was constructed based on the SCBV sequences from this study and 34 published sequences representing 18 different phylogroups or genotypes (SCBV-A to -R). All SCBV-tested isolates could be classified into 20 SCBV phylogenetic groups from SCBV-A to -T. Out of nine SCBV phylogroups reported in this study two novel phylogroups are proposed, SCBV-S and SCBV-T that share 90.0-93.2% sequence identity and show 0.07-0.17 genetic distance with each other in the RT/RNase H region. SCBV-S had 57.6-92.2% sequence identity and 0.09-0.66 genetic distance, while SCBV-T had 59.0-93.2% sequence identity and 0.11-0.63 genetic distance compared with the published SCBV phylogroups. Additionally, two other *Badnavirus* species, *Sugarcane bacilliform MO virus* (SCBMOV) and *Sugarcane bacilliform IM virus* (SCBIMV), which originally clustered in phylogenetic groups SCBV-E and SCBV-F, respectively, are reported by first time in China. These findings will help to understand the level of genetic heterogeneity present in the complex of *Badnavirus* species that infect sugarcane.

BP17

GENOMIC CHARACTERIZATION OF *SUGARCANE MILD MOSAIC VIRUS*

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Key Words: SCMMV, metagenomics, germplasm, quarantine, Ampelovirus.

Sugarcane mild mosaic virus (SCMMV) that was first discovered by Lockhart et al. (1992) was provisionally assigned to the genus *Ampelovirus*, family *Closteroviridae* (Martelli et al 2002). Since the initial characterization of SCMMV using enzyme immunosorbent assay and immunosorbent electron microscopy, no new information about SCMMV has been obtained. Recently, without a priori metagenomics-based approaches (including virion-associated nucleic acid and siRNAs sequencing) were used for viral screening of sugarcane varieties from sugarcane quarantine and germplasm collections. The combination of both metagenomics approaches yielded 918 contigs sharing homologies with large parts of the genome of representative members of the *Ampelovirus* genus and potentially covering 80.6% of typical full-length ampelovirus genomes. In addition, RNASeq or whole transcriptome shotgun sequencing approach based on total RNA extracted from an ampelovirus infected sugarcane variety yielded a 12,408 nt long scaffold of SCMMV. Resequencing PCR, including a gene walking approach and RACE PCRs yielded the full-length genome sequence of SCMMV with a size of 13,144 nt. The most closely related ampelovirus to the novel ampelovirus is *Plum bark necrosis stem pitting-associated virus* with only 43.4% identity, suggesting, as expected, that the agent identified is a novel ampelovirus. High-throughput sequencing data were used to design specific detection primers located within the HSP70 gene for diagnosis. Using these primers, 16 % of sugarcane varieties from the CIRAD sugarcane

quarantine program tested positive for SCMMV, providing a first vision of the geographic distribution (Argentina, Barbados, Ecuador, Guadeloupe, Philippines, Réunion, Senegal, and USA), prevalence, and diversity of this virus. Phylogenetic analysis of the HSP70 gene sequence grouped these isolates into four genetic groups. Immuno-capture PCR using SCMMV antibodies developed by B.E.L. Lockhart detected the three genetic groups for which single strain infection was available, confirming that SCMMV and the newly identified ampelovirus are the same entity.

Lockhart, B. E. L., Autrey, L. J.-C., and Comstock, J. C. 1992. Phytopathology 82:691-695

Martelli G. P., Agranovsky A. A., Bar-Joseph M., Boscia D., Candresse T., et al. 2002.Arch. Virol. 147:2039–44.

BP18

IDENTIFICATION OF TWO SUGARCANE YELLOW LEAF VIRUS GENOMIC RECOMBINANTS IN THE GERMPLASM COLLECTION OF GUADELOUPE

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Key Words: SCYLV, genotypes, metagenomics, microevolution.

Three hundred varieties from the CIRAD sugarcane collection from Guadeloupe Island were processed using the Virion-associated nucleic acids (VANA) metagenomics-based approach. Sugarcane yellow leaf virus (SCYLV) related reads were identified in 134 sugarcane varieties. Among the 47 varieties having >200 SCYLV-related reads, 8 plants had sequences belonging to different genotypes, based on analysis of ORF1 and ORF3 partial sequences, suggesting occurrence of SCYLV genotypes coinfection in these varieties. Further analysis of all available aligned sequences for these 8 plants co-infected by at least two SCYLV genotypes revealed that at least two varieties putatively contained genotype recombinant SCYLV isolates. For these two isolates, Sanger sequences were generated encompassing the putative recombinant zones and confirmed the recombinant events. In addition, recombinant events were also confirmed using the RDP4 software. While one recombinant isolate (infecting the ROC7 variety) presumably arose from a SCYLV CUB isolate (major parent) and a SCYLV REU isolate (minor parent)[(1)], the second recombinant isolate (infecting the PR1059 variety) presumably arose from a SCYLV BRA isolate (major parent) and a SCYLV REU isolate (minor parent). *P* values of both recombinant events (ROC7 and PR1059) under RPD process were $9.374\ 10^{-68}$ and $2.470\ 10^{-17}$, respectively. This result indicates possible adaptive microevolution of SCYLV by genotype recombination on Guadeloupe Island.

[(1)] Roumagnac P., Mollov D., Daugrois JH., Filloux D. 2018. Viral metagenomics and sugarcane pathogens. In: Achieving sustainable cultivation of sugarcane Volume 2: Breeding, pests and diseases, Rott Philippe (ed.). Cambridge: Burleigh Dodds Science Publishing, 1-19. (Burleigh Dodds Series in Agricultural Science) ISBN 9781786761484 http://dx.doi.org/10.19103/AS.2017.0035.20

BP19

DYNAMICS OF PATHOGENIC VARIATION IN *COLLETOTRICHUM FALCATUM*, RED ROT PATHOGEN OF SUGARCANE

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Key words: *Colletotrichum falcatum*, pathotypes, virulence, host adaptation

Red rot caused by *Colletotrichum falcatum* was reported more than 120 years ago in India and still it continues to be a major threat to sugarcane cultivation in the country. Continuous evolution of new variants and their adaptation to the new selected varieties contributed to the appearance of new pathogenic variants of *C. falcatum*. Earlier the variants were categorized into 'light' and 'dark' isolates and the former was considered as highly sporulating and virulent and the latter as less sporulating and less virulent. Later differential host varieties were identified to assess pathogenic variation in the vast *C. falcatum* population and more than 10 pathotypes were found in the country. The newly identified pathotypes were found to be virulent and represent the prevailing pathogenic population in the ecosystem like the recently identified pathotype CF12 which was found to be highly virulent over the erstwhile predominant pathotype CF06 in the tropical region. Further, the pathogenic population exhibited a very clear cut with a tropical and subtropical grouping of their pathogenic behaviour. Characterizing a large population of *C. falcatum* isolates from tropical and subtropical regions by documenting their pathogenic behaviour at tropical and subtropical locations revealed that the isolates exhibit comparatively low virulence in the subtropical conditions. Recent studies on the behaviour of 12 isolates of tropical region on 20 popular varieties revealed that the isolates more than 30 years old were lesser in their virulence than the isolates originating after 2000. Our studies clearly evidenced a huge diversity for virulence and year to year variation indicating an instability in the pathogenic behaviour of *C.*

falcatum. Probable stable and unstable behavior in the pathogenic virulence of the isolates could be attributed to their host variety and prevailing environment during host-pathogenic interaction. Further, continuous evaluation of the isolates for many seasons revealed their stability in their behaviour and gain/ loss of virulence and their attempt to suppress host resistance, possibly leading to emergence of new variants.

BP20

MOLECULAR IDENTIFICATION AND CHARACTERIZATION OF *FUSARIUM SACCHARI* ASSOCIATED WITH WILT AND POKKAH BOENG DISEASE

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Key Words: Wilt, Pokkah boeng, *Fusarium sacchari*

Wilt is an important disease of sugarcane reported nearly 100 years ago in India that was responsible for the elimination of many elite varieties in both tropical and subtropical regions. Cultural, morphological and molecular tools established that *Fusarium sacchari* is the causal agent. Severe Pokkah boeng (PB) caused by different species of *Fusarium* such as F. *sacchari*, *F. verticillioides*, *F.andiyazi*, *F. subglutinans and F. semitectum*drastically reduced inter nodal elongation of canes. Though both wilt and PB diseases occur independently in the field, now it is recorded that they occur together in the same sugarcane plant. Characterization of *Fusarium* isolates of wilt and PB affecting sugarcane varieties was done by sequencing TEF1-a gene which has been widely used for species identification. Most of the earlier studies results revealed that *F. sacchari* is the major causal organism of wilt disease and *F. proliferatum* and other *Fusarium* species are associated with pokkah boeng disease. Gene sequencing and phylogenetic analysis of 48 isolates revealed that 44 isolates of wilt and PB were *F. sacchari* and the remaining four isolates of PB were *F. proliferatum*. Wilt and PB isolates of Co 0238 and MS 901 cultivars were only *F. sacchari* and the several other varieties also exhibited progressive disease severity through different phases of PB and that resulted in wilt development. Thus, *F.sacchari* is the major causative agent of two distinct diseases present in sugarcane plant in India viz., wilt and PB.

BP21

COMPLETE GENOME CHARACTERISATION OF SIX ISOLATES OF *SUGARCANE YELLOW LEAF VIRUS* FROM MAURITIUS

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Key Words: Genotype, recombinant, sequence similarity

Sugarcane yellow leaf virus (SCYLV- genus Polerovirus; family Luteoviridae) is present in most sugarcane growing regions worldwide and is responsible for yellow leaf disease. Eight genotypes have been described based on geographical origins. In Mauritius previous studies based on amplification of genotype specific fragments have shown predominance of REU genotype in commercial plantations. Here, we report the assembly of the complete 5.8 kb sequence of six SCYLV isolates from three varieties namely M 2024/88 (1), R579 (2) and R570 (3). The six isolates were phylogenetically close to each other with 97.1- 99.8 % sequence similarity. Two isolates, each from M 2024/88 and R570, were identified as REU genotype (98.6 and 98.8% sequence similarity with the reference REU-YL1). The two other isolates from R570 could not be characterised using genotype-specific primers. These two isolates shared 97.2 and 97.4 % similarity with REU-YL1 and were identified as recombinants, using Recombination Detection Programme RDP4, with REU-YL2 as major parent and CHN-GD-ZJ4 (Bra) as minor parental sequences. They were phylogenetically distinct and are described as a novel genotype (MU). Two isolates assembled from R579 clustered within MU, with between 98.0-98.3% sequence similarities with R570-MU. Increased ScYLV diversity is expected to be observed with further characterisation of local genomes. This diversity may have implications in diagnostic tests used for genotype characterisation.

BP22

GENETIC VARIABILITY OF PHYTOPLASMA ASSOCIATED WITH WEEDS GROWN IN PROXIMITY OF SUGARCANE FIELDS INFECTED WITH GRASSY SHOOT DISEASE

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Key words: Sugarcane grassy shoot disease, 16Sr XI phytoplasmas, weed hosts

Sugarcane grassy shoot disease (SCGS) disease is one of the most harmful disease threats to sugarcane cultivation and production in India. So far, the 16Sr XI phytoplasmas have been reported as the major phytoplasma group associated with SCGS disease followed by the 16Sr I group as the minor one. No alternative weed host has been reported as a natural reservoir of SCGS in India. Hence the present study was undertaken to screen several weed species for phytoplasma association in proximity to sugarcane fields infected with SCGS disease. Keeping in view their possible role as a natural reservoir of sugarcane phytoplasmas, the study was planned to analyze the major weed species showing suspected phytoplasma symptoms for phytoplasma association with PCR and nested assays using phytoplasma specific primer pairs in central and eastern Uttar Pradesh. In the present investigation, seven weeds species (*Cynodon dactylon, Oplimenus burmani, Croton bonplandianum*, *Cannabis sativa*, *Arundo donax*, *Ocimum cannum, Aclypha indica)* showing suspected phytoplasma symptomsof leaf chlorosis, leaf yellowing and witches' broom,grown near sugarcane grassy shoot infected field were collected. A total of 14 leaf samples (two of each weed) of different symptomatic weeds were collected along with two SCGS samples and processed by PCR and nested PCR assays with phytoplasma universal primer pairs (P1/P6 & R16F2n/R16R2). All the symptomatic weed samples (14) along with SCGS samples tested were found positive. Positive amplicons of nested PCR (1.2Kb) were directly sequenced and a phylogeny was constructed. High genetic variability was observed in 16Sr DNA sequences of the analyzed weed samples. Three different groups (16Sr I, VI, & XIV) were identified and characterized from the 14 weeds samples.

However, 16Sr XI group was identified associated with the SCGS samples of variety UP 05125 from Pilibhit on the basis of comparison of 16Sr DNA sequences. Further, *in silco* RFLP analysis of 16Sr DNA sequences of weed phytoplasmas confirmed association of 16Sr I-B with *A. donax*, *A indica, O. cannum, O. burmani*, 16Sr VI-D with *C. bonplandianum*. Also, the 16Sr XIV-A sub group was found associated with *Cannabis* and *Cynodon*. The aforesaid analysis confirmed that none of the weed species analyzed in the present study were infected by the 16Sr XI group which is the major group reported associated with SCGS and SCWL worldwide. Our results suggest the tested symptomatic weeds had no direct role in the transmission of SCGS phytoplasma in study area. However, the weeds reported as host of the 16S I-B subgroup in the present study may play role in transmission of a sugarcane phytoplasma recently reported in Uttar Pradesh to be of the 16Sr I-B subgroup. . The 16Sr XIV group of phytoplasma identified in *Cannabis* and *Cynodon* are phylogenetically close to 16Sr XI group and may be responsible for natural transmission of these phytoplasmas s from sugarcane to weeds and *vice versa*, which needs an immediate attention. Further, more weed species in and around sugarcane fields need to be indexed against phytoplasma to identify their role as reservoirs of SCGS related phytoplasmas.

BP23

THE USE OF SHOTGUN RNA METAGENOMICS AS A TOOL FOR VIRAL DETECTION IN SUGARCANES GROWN IN QUARANTINE

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Key Words: Sugarcane, metagenomics, quarantine, viruses

The USDA-APHIS Plant Germplasm Quarantine Program (PGQP) is the only Federal Quarantine Program in the United States authorized to import sugarcane clones. Sugarcanes imported by PGQP are tested for multiple pathogens using a combination of 21 molecular methods (*e.g.* PCR and qPCR), serological tests, and bioassays. However, these tests require previous knowledge of the pathogens infecting each crop since the diagnosis relies on pathogen-specific tests. Metagenomics, the genomic analyses of a population of microorganisms, has provided a powerful alternative for the detection and identification of microorganisms without prior knowledge of their presence in a sample. Our goal at PGQP is to establish shotgun RNA metagenomics as a routine diagnostic tool to detect known and novel pathogens in each imported plant accession. In this study, we used Illumina sequencing-by-synthesis technology to in-house sequence >100 quarantined sugarcanes and grasses. In order to assess how environmental factors affect viral identification, samples derived from plants maintained at PGQP as positive controls were sequenced at different times of the year. Ribosomal-depleted RNA libraries were generated using the TruSeq Stranded Total RNA kit and sequenced on an Illumina NextSeq 500 platform.Sequence reads were processed using CLC Genomics Workbench and analyzed by mapping them to a custom plant viral database of 83,409 sequences. RNA metagenomics not only detected all DNA and RNA viruses identified by laboratory diagnostic tests used routinely by PGQP, but also detected novel pathogens that are not targeted by the lab-based tests, such as two *Caulimoviridae*, a new *Mastrevirus*, and a novel *Luteovirus*, among others. Further, the turnover time for metagenomics testing for 12 samples was five days compared to several months of the complete conventional diagnostics

testing. This presentation will describe the advantages and challenges of using metagenomics as a tool for the diagnosis of plant viruses in Quarantine Programs.

BP24

DEVELOPMENT OF A FAST AND RELIABLE MOLECULAR-BASED DETECTION METHOD FOR SUGARCANE WHITE LEAF DISEASE (WLD) PHYTOPLASMA

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Key Words: PCR, Phytoplasma, Sri Lanka, Sequencing, White Leaf Disease

White leaf disease (WLD) caused by phytoplasma is one of the devastating diseases in sugarcane plantations in Sri Lanka. A fast and reliable method for detecting the phytoplasma is essential to prevent the spread of the disease through infected seed material. Currently, the Sugarcane Research Institute, Sri Lanka, has been using the phytoplasma-specific universal primers with nested PCR reactions for detecting the WLD phytoplasma. This paper presents a protocol developed for detecting the WLD phytoplasma in sugarcane by running a single PCR programme only, with high level of reliability. The PCR reaction was performed using the DNA extracted by CTAB method and SPP1 forward and SPP2 reverse primers. The used primer annealing temperature of 53°C, was determined with the gradient PCR programme. The PCR products were analysed in 2% Agarose gel electrophoresis and then stained in ethidium bromide before sequencing. The similarity of the query sequence with subject sequences was examined by performing NCBI web nucleotide BLAST analysis. One hundred sugarcane plants that were visually-positive or negative for WLD, obtained from infected planting material, were subjected to the above-mentioned procedure to identify its reliability in detecting the WLD phytoplasma. According to the PCR results, the amplified bands were clearly visible in 2% agarose gel and the sequencing results confirmed that the size of the PCR product is 287 bp. The nucleotide BLAST results confirmed that the query sequence was 94% identical with part of the sequence of the16S rRNA gene of a sugarcane white leaf disease phytoplasma strain (Genbank accession number KT270948.1) with the lowest E-value. Out of the 100 plants tested, 96 were proven positive for WLD by this method and 32 plants were detected positive by visual detection method. Thus, this method can be accepted as a fast and reliable molecular method for detecting WLD phytoplasma.

BP25

STATUS OF POKKAH BOENG DISEASE OF SUGARCANE IN SUB-TROPICAL INDIA

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Key Words: Pokkah Boeng, disease incidence, subtropical India

Pokkah boeng (PB) caused by *Fusarium* spp.has beenreported in almost all the countries where sugarcane is grown. This disease was reported for the first time in sugarcane by Wakker and Went (1896) in Java and during 1983 in India from Maharashtra. In recent years, its widespread incidence has been noticed in almost all sugarcane varieties and areas of the country. To know current scenario of the disease in sub-tropical India, fields of 33 sugar mills were surveyed during 2011-2018 crop seasons. Data generated over the years revealed that PB has increased many folds in cultivated varieties and now attained the status of a major disease. In Haryana maximum disease incidence (10.0–40.0%) was recorded in cvs CoS 8436 and CoH 160, whereas in cvs Co 0238, Co 0118, Co 0239, Co 89003, CoH 116, CoH 119, CoH 160, CoH 152, CoH 156, CoJ 85, CoJ 88 and CoS 8432 incidence ranged between traces to 12.0%. Similarly, top rot phase was recorded by 2.0-40% on the cvs CoJ 85, CoH 150, CoS 8436, CoJ 85, CoH 119 and CoH 152 in the state. In Punjab PB and top rot incidence was recorded to 0.1-16% and traces to 5.0% on cultivated varieties, respectively. By and large, disease also prevailed in most of the varieties grown in Uttar Pradesh and incidence of PB and top rot ranged between traces to 35% and upto 10.0%, respectively. In few fields combined infection of PB along with top borer was noticed in the cvs Co 0238, CoS 8436 and CoH 119, but no association was found between PB and top borer incidences.

BP26

TESTING OF SUGARCANE CLONES IN QUARANTINE IN MAURITIUS: IMPROVEMENT DURING THE LAST DECADE

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Key Words: Sugarcane disease, latent infection, molecular diagnostic.

Quarantine facilities for introducing sugarcane germplasm exist in Mauritius since 1928, and the introduction of graminaceous crops that share common pathogens with sugarcane are restricted. These measures, together with rigorous disease testing in quarantine, have allowed for a fairly healthy sanitary status of the crop in the island and important diseases, present elsewhere namely; mosaic, streak mosaic, Fiji gall, white leaf, grassy shoot and downy mildew have not been observed. Over the years, new molecular diagnostics tools have been introduced and improved to provide an efficient way for intercepting sugarcane pathogens in quarantine. In the early 2000, the application of reverse transcription Polymerase chain reaction (RT-PCR) coupled with virus elimination using tissue culture techniques helped in the release of *Sugarcane yellow leaf virus* (SCYLV)-free germplasm. Testing for SCYLV over the last decade was further improved with application of real-time RT-PCR and genotype-specific tests. Subsequently, the number of pathogens being tested using different molecular tools (RT-PCR, Real Time PCR) has increased and include mosaic viruses, *Sugarcane streak mosaic virus*, *Xanthomonasalbilineans*, *Leifsonia xyli* subsp. *xyli*, and phytoplasmas. These new tools have allowed interception of SCMV, SrMV and SCYLV genotypes. The constraints and challenges as well as the advantages of application of molecular tools for sugarcane quarantine will be discussed from the Mauritius perspective. These implemented tools could be useful for other countries.

BP27

LEAF FLECK, AN EMERGING VIRAL DISEASE IN SUGARCANE: SURVEILLANCE UNDER VARIOUS GEOGRAPHICAL LOCATIONS IN INDIA

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Key words: Leaf fleck, disease symptomatology, SCBV, molecular diagnosis

Sugarcane bacilliform virus (SCBV) causing leaf fleck was reported three decades ago in sugarcane and for many years its symptoms have not been clearly described. Initially, the symptoms of fleck, mottling and mild mosaic were suspected to be caused by virus infections in *Saccharum* spp clones and no clear information was available on virus infections and disease symptoms in hybrid varieties. As reported earlier, PCR assays targeting a 221bp amplicon is not reliable for virus diagnosis from the SCBV-suspected clones of sugarcane. Hence a comprehensive study was made on leaf fleck symptomatology in *Saccharum* spp clones in germplasm and *Saccharum* spp hybrid varieties under cultivation and new PCR assays for precise diagnosis of the virus were developed. Surveys to determine the incidence, diversity and distribution of SCBV were conducted in different geographical locations at Kerala, Tamil Nadu and Andhra Pradesh. It was observed that the leaf fleck symptoms varied among the *Saccharum* spp clones and hybrid varieties. When compared to *Saccharum* spp clones, hybrid varieties exhibited a severe expression of the disease. Symptoms initiated as sparse or intense flecks in the lamina progressed to chlorotic stripes or distinct chlorotic streaks, intense yellow streaks/ blotches, reddening of the streaks and pre-mature drying of leaves. The average disease incidence was observed between 12 and 42 % in Kerala and higher in other states. The newly designed virus specific primers were found to be highly specific and detected the virus in symptomatic and asymptomatic plants. The studies revealed extensive spread of the virus across *Saccharum* spp genotypes and hybrid varieties.

BP28

CURRENT SITUATION OF SUGARCANE YELLOW LEAF DISEASE IN COLOMBIA

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Key Words: Yellow leaf, SCYLV, transmission, resistance, diagnosis, seed cane.

The use of healthy seed cane is the basis to establish commercial fields of sugar cane (*Saccharum officinarum*) with higher productivity and profitability. CENICAÑA carries out the production and delivery of vegetative seed cane of CENICAÑA-Colombia (CC) cultivars through the conventional method, *in vitro* culture (Conventional and Immersion system) and the increase of plants through single buds system. CENICAÑA seed cane increase service has delivered more than six million plants between 2004 and 2017. CENICAÑA offers growers diseases diagnostic service where the levels of incidence of the main systemic diseases, including *Sugarcane yellow leaf virus* (SCYLV), are determined through the use of serological (Tissue Blot Enzyme Immunoassay-TBIA) and molecular techniques (RT-PCR). With the results delivered, the growers can select in a reliable and timely manner healthy seed cane and commercial fields for commercial planting. In seed cane fields the incidence of SCYLV from 2015 to 2016 increased from 6 to 11%; however, the SCYLV incidence decreased during 2016 to 2017 from 11 to 8.7%.Even though a reduction of incidence has been observed, if a highly productive but SCYLV-susceptible variety is widely grown then more SCYLV-resistant varieties will be required. To test SCYLV resistance in different cultivars, a mass vector rearing was established and vector preference, SCYLV transmission, and virus quantification was assessed by molecular (RT-PCR and RT-qPCR) and immunological (TBIA) techniques. . The number of aphids per plant and preference did not correlate with susceptibility to the disease. When comparing serological and molecular techniques, the virus was detected in 36%, 48% and 72% of the varieties by TBIA, RT-PCR and RT-qPCR

respectively. Resistant varieties related to low levels of incidence and systemic infection with the disease, were identified. The varieties CC 01-746, CC 01-678, CC 01-1228, CC 99-2282, CC 01-1940 and CC 93-7711 were found to be resistant to the virus-vector transmission. These results will give more control strategies for SCYLV in Colombia.

BP29

A SUMMARY OF BIOSECURITY RESEARCH IN PAPUA NEW GUINEA: 2009-2017

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Key Words: Papua New Guinea, downy mildew, ramu stunt, moth borers

Papua New Guinea (PNG) is the centre of diversity for several species in the *Saccharum* genus, including *S. officinarum*, which constituted the first commercial sugarcane in Australia. PNG is also the home to major disease and pest threats that are a risk to sugarcane production both domestically in PNG and in other neighbouring countries. Over the past 8 years Australian pathology and entomology researchers have been involved in a series of research projects in PNG to study some of the main biosecurity threats: Downy mildew (caused by oomycete *Peronosclerospora* sp.), Ramu stunt (caused by a newly described Tenuivirus) and moth borers (*Sesamia grisescens*, *Chilo terrenellus*, *Scirpophaga excerptalis*). This paper will summarise the work done in these main areas, drawing on previously published work from the Australian Society of Sugarcane Technologists (ASSCT), ISSCT and journal articles, as well as as-yet-unpublished findings. We conclude that significant outputs from the research include a much better understanding of the causal agents, a range of specific diagnostic tests, an understanding of pest and diseases distributions, and improved methods for varietal resistance screening.

BP30

A DECADE OF ORANGE RUST IN FLORIDA (USA): WHERE WE WERE AND WHERE WE ARE GOING

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Key Words: Brown rust, orange rust, host-plant resistance, *Puccinia melanocephala, Puccinia kuehnii*

Orange rust caused by *Puccinia kuehnii*, was first observed in Florida during 2007 on a very important cultivar, CP80-1743. Resistant to brown rust incited by *P. melanocephala*, this cultivar occupied nearly one third of the FL hectarage and was favored for its high early sucrose. Its phase out began immediately, but its replacements, namely CP88-1762 and CP89-2143 soon succumbed to the disease, presumably due to the presence of rust variants. By 2013, nearly 70% of the Florida hectarage was planted to orange rust susceptible cultivars. Thus, during the early years of orange rust, growers were forced to rely on newly registered fungicides to prevent significant losses to this disease. In more recent years, however, growers have chosen to plant a cultivar resistant to orange rust but susceptible to brown rust, CP96-1252, primarily for its high yield and good ratooning ability. With cane possessing a narrower window of susceptibility for brown rust, the choice to chemically control brown rust rather than orange rust is an economic one, requiring fewer applications. Hence, the sugarcane hectarage susceptible to brown rust has actually increased rather than decreased since reaching a low of 14% in 2007, the year of orange rust's introduction. However, as we move forward with more precise decision tools regarding fungicide applications, the Florida sugarcane breeding program shows signs of making progress regarding more durable host-plant resistance, reducing the orange rust susceptible hectarage from 70% in 2013 to a low of 24% in 2018.

BP31

INVESTIGATIONS INTO SUGARCANE DISEASES IN FLORIDA

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Key Words: Epidemiology, brown rust, orange rust, *Saccharum* spp., yellow leaf, yield losses.

Sugarcane is an important crop in Florida, and Florida's climate conditions are very conducive to disease epidemics. Understanding how sugarcane diseases emerge and progress is essential for developing efficient and sustainable methods of control. The goal of our research and extension program is to understand the biology and genetics of plant pathogen interactions, and to impart this knowledge to the growers and other stakeholders in order to improve the management of sugarcane diseases in Florida and to limit their impact on sugarcane production. This work includes basic and applied research on diseases currently limiting sugarcane production or that are potential threats to the industry such as brown rust (caused by *Puccinia melanocephala*), orange rust (caused by *P. kuehnii*), and yellow leaf (caused by sugarcane yellow leaf virus or SCYLV). At least two races of *P. kuehnii* occur in Florida, and both brown and orange rust need to be controlled using fungicides because several cultivars currently grown by the industry are susceptible to one of these diseases. Without fungicide management, it was shown that yield losses can be higher than 40%. Weather conditions favorable to disease progress have been identified which will help to improve management of rust diseases. At least two strains of SCYLV are present in Florida. This virus is widespread in Florida and can cause yield losses greater than 20%. Besides sugarcane, Columbus grass (*Sorghum almum*) and grain sorghum (*S. bicolor*) are two natural hosts of SCYLV. However, the importance of these plants in disease spread is not yet known. In recent field studies, re-infection levels of healthy sugarcane plants by SCYLV did not exceed 33% after three crops (plant cane and two ratoons). This suggests that planting clean seed cane is currently the best approach to limit incidence and yield losses due to yellow leaf in Florida.

BP32

SUGARCANE SMUT – CURRENT STATUS OF RESEARCH IN INDIA AND EMERGING PROSPECTS

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Key words: *Sporisorium scitamineum*, lifestyle transitions, host resistance, proteome

The sugarcane-smut pathogen interaction has been investigated in detail at ICAR-SBI, Coimbatore during the past decade by employing the tools of "*Omics*" viz. transcriptomics and proteomics. Probing this intriguing interaction has generated interesting leads on various aspects of the subject. Lifestyle transitions of the smut pathogen both at *in vitro* and *in planta* stages has been clearly elucidated. Genetic and pathogenic variability analysis established a clear discrimination of a representative set of *Sporisorium scitamineum* isolates, based on the differential responses of a set of sugarcane genotypes. SSR molecular markers could delineate isolates based on geographical location to a certain extent. Putative orthologs of *Ustilago maydis* effectors were screened from the genome of the sugarcane smut fungus and the study identified candidate *S. scitamineum* orthologs with more sequence identity with *Sporisorium reilianum* than *U. maydis* sequences. Alterations in the *in vitro* secretome of *S. scitamineum* in response to synthetic and sugarcane meristem-tissue amended growth media were analysed and it revealed that ten exclusively secreted and six abundant proteins were secreted differentially in response to signals in the host extract medium. Proteome-level alterations occurring in the meristem of a *S. scitamineum* infected susceptible sugarcane cultivar (Co 96007) at the whip emergence stage indicated that most proteins represented metabolic and biosynthetic pathways, and also lignin biosynthesis, phenylpropanoid, and auxin metabolism pathways. This is the first detailed report on proteome level alterations in the meristem of a susceptible sugarcane variety in response to *S. scitamineum* at the whip emergence stage. Identification of putative effector orthologs and host-responsive *in vitro* secreted proteins of

S. scitamineum, serves as a valuable resource for further studies to understand molecular aspects of pathogenesis. In conclusion, our results have provided novel insights, considerably improving our understanding of sugarcane smut and allow us to envisage new directions for future research.

BP33

HOW SPREAD IS THE LEAF SCALD IN THE BRAZILIAN SUGARCANE INDUSTRY?

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Key Words: *Saccharum* spp.; *Xanthomonas albilineans*; dissemination; distribution

Leaf Scald (LS) (*Xanthomonas albilineans*) is one major disease of sugarcane in many countries due to yield loss and difficulty of accurately diagnose latent infected plants, which is only possible by laboratory techniques. Contaminated propagating materials are its main primary inoculum source, as it is the case of the Ratoon Stunt (RSD) (*Leifsonia xyli* subsp. *xyli*). However, a routine diagnostic test for LS is not established in Brazil, differently from RSD. Therefore, the present study aimed to examine the prevalence of LS in the Brazilian sugarcane industry. Sugarcane sap sent by growers was used for RSD and LS serological diagnostic tests and allowed comparison of disease incidence between the two diseases. LS was diagnosed by serology or isolation on culture medium, or PCR. Samples from 13 fields representing three sugarcane regions, distant from 520 to 2,700 km from each other: São Paulo state (SP), Cerrado, and Northeast Brazil. Our data showed that: 1) LS is present in all these regions; 2) The three varieties analyzed were highly susceptible, since they exhibited high *X. Albilineans* population in stalk*;* 3) The within field incidence (amount of diseased stalk in a field) of LS was higher in the Northeast and Cerrado; 4) There was no correlation between LS and RSD and individual diagnostic test is necessary for each disease; 5) Higher LS severity (bacteria's concentration in a stalk) was found in Cerrado and Northeast regions than SP, while higher RSD severity occurred in SP.

BP34

UNRAVELLING SUGARCANE POKKAH BOENG DISEASE CAUSED BY *FUSARIUM* SPECIES COMPLEX IN CHINA

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Pokkah boeng disease (PBD) is one of the most serious and devastating diseases of sugarcane in China. *Fusarium* species complex (FSC) is the major causal agent of this disease around the world. A total of over 200 isolates were recovered from the pokahh boeng samples collected from five major sugarcane production areas in China throughout 2012 and 2017. Two *Fusarium* species, i.e. *F. verticillioidesis* (Fv), and *F. proliferatum* (Fp), have been identified by morphological observation, pathogenicity test and phylogenetic analysis, of which the whole genomes were sequenced and assembled at the chromosome level. The assembled genome of *F. verticillioides* CNO-1 was 44.59 Mb with 19 contigs that 12 chromosomes ranged from 1.02 Mb to 6.44 Mb (N50 of 4.3 Mb; GC-content is 47.81%; 283-fold coverage), but some of these key genome features are differences from the reported for *F. verticillioides* 7600 (Ma et al, 2010). The assembled genome of *F. proliferatum* YN41 was 44.05 Mb with 26 contigs that 12 chromosomes ranged from 0.68 Mb to 6.34 Mb. A total of 14,670 and 14,796 protein coding gene models were predicted using a combination of gene prediction tools. The coordination of these related gene clusters and accumulation of gibberellin metabolites and production of mycotoxins were deciphered to be associated with nitrogen application and low temperature based on transcriptome, proteome and metabolome. This integrated analysis allowed us to uncover additional information for a more comprehensive understanding of biological events relevant to fungal secondary metabolic regulation in response to variations in nitrogen availability and temperature response. Many studied on disease investigations, breeding of disease resistant varieties and strategy of disease control have also been carried out in China.

BP35

NEW STRATEGY FOR PRODUCTION VIRUS FREE TISSUE CULTURE PLANT THROUGH DIRECT REGENERATION TECHNIQUE IN SUGARCANE

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Key Words: Tissue culture, virus free, direct regeneration.

In sugarcane apical meristem tip culture is the only time-consuming method currently available for the production of virus free plantlets. But, direct regeneration where young leaf disc or segments are used as explant technique, is faster and cost effective than meristem tip culture. An efficient high frequency of shoot regeneration method was developed from young leaf segment of sugarcane. Multiple shoots were induced in young leaf segment of variety Co 86032 on the modified half Murashige and Skoog media supplemented with NAA (5 mg/l) ,GA 3 (0.5 mg /l) and 3% sucrose.Shoots were produced directly from the leaf segment without intermediate callus formation. By this method was possible to develop 12- 15 shoots from a single shoot within 45-60 days<, where as a single shoot only was develop from the meristem tip. However, explants other than meristem tip are likely to contain virus particles in the vascular tissue. Then, antiviral effect of ribavarin in elimination of sugarcane viruses through direct regeneration were assessed, leaf whorl of variety Co 86032 were inoculated in MS medium containing different concentration of ribavarin viz., 10, 11 and 12 mg/l. The regenerated shoots from above treatments were multiplied and the *in vitro* plantlets were found to be free from SCMV and SCSMV. Results indicated that increasing ribavarin concentration to 12 mg/l reduced shoot formation from leaf whorls. Also, plantlets of the variety Co 86032 comprising one meristem tip cultured plantlet, one direct regenerated plantlet from leaf whorls without ribavirin treatment and three direct regenerated plantlets with ribavirin treatment (10mg, 11mg and 12mg) were tested for somaclonal variation or genetic stability. Ten sets of primer from the core set of sugarcane microsatellite primers were used to characterize the plantlets. The profiles obtained were uniform for all the primers tested. Direct

regenerated plants developed from young leaf whorls with ribavarin treatment viz., 10,11 and 12 mg/l were planted in the field.There was no significant difference in terms of NMC,cane height ,girth of cane, internode number, length of internode, single cane weight and Brix % in 10 month old crop. However, numerically increases of cane height (207.3 cm), length of internode (13.85 cm) and single cane weight (1.35 kg) were noticed in the 12 mg/l ribavarin treatment crop compared to 10 mg, 11 mg ribavarin and control. Use of antiviral chemicals ribavarin in culture medium during regeneration of shoots from young leaf segment was found to be an alternate and quick method for *in vitro* culture establishment to produce virus-free tissue culture plants.

BP36

TRICHODERMA: AN EFFECTIVE BIOCONTROL AGENT FOR MANAGEMENT OF RED ROT OF SUGARCANE

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Key Words: Red rot, *Trichoderma*, Induced systemic resistance

Red rot is a major disease of sugarcane in India. Multiple control approaches have been explored for the management of this disease. The use of disease-resistant varieties has been successful in the field, but due to the frequent development of new variants of the fungus, the newly released varieties succumb to the pathogen over time. In recent years there has been a growing emphasis on exploring alternative disease management options like biological control using *Trichoderma* spp. Studies were carried out at ICAR-Indian Institute of Sugarcane Research, Lucknow, to explore the potential of indigenous *Trichoderma* isolates for management of red rot. Sixty six *Trichoderma* isolates, previously established from rhizosphere soil samples of sugarcane, were screened extensively under laboratory (potential to produce diffusible inhibitory metabolites, cellulase and chitinase) and field conditions for their antagonistic activity against *C. falcatum* and promising isolates were identified. Selected isolates were further characterized using a multiplex PCR assay. The results of PCR assay indicated *T. harzianum* to be the predominant species in the sugarcane rhizosphere followed by *T. longibrachiatum*. Field studies were carried out to evaluate the two most promising *Trichoderma* isolates (STr-83 & STr-108) for management of red rot in the field (var. Co 1148; pathotype CF01) over two crop seasons (2016-2018). Talc formulations of these isolates were prepared and evaluated under different delivery methods. All treatments resulted in considerable reduction in red rot (~30% to 52%) over control in both years. Overall, application of *Trichoderma* isolates as a combination of sett and soil treatment was most effective in suppressing red rot (> 50%) along with significant increases in yield in both crop seasons. Biochemical changes (total phenol content and

defence enzymes) associated with *Trichoderma* mediated red rot suppression were also assessed. Considerable increases in total phenol contents as well as all defence enzymes was recorded in all *Trichoderma* treatments as compared to control. Based on these studies we have identified promising *Trichoderma* isolates for red rot management and established the role of induced systemic resistance in *Trichoderma* mediated red rot suppression. Further studies on growth promoting potential of *Trichoderma* isolates are in progress.

BP37

EFFICIENT MANAGEMENT OF FUNGAL DISEASES IN SUGARCANE BY ENHANCED FUNGICIDE DELIVERY IN PLANTING MATERIALS

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Key Words: Mechanized sett treatment, fungicide delivery, disease management

Vegetative propagation in sugarcane favours pathogen infection in the stalks and their transmission through seed cane leading to disease epiphytotics. Although the major fungal diseases causing red rot, smut and wilt are managed largely by deploying resistant varieties, in many situations there is a need to manage these diseases under field conditions due to break down of resistance or other factors. To effectively manage these diseases, primary sources of pathogen inoculum carried through seed canes should be targeted. Conventional sett treatment with fungicides is ineffective due to poor uptake of the fungicides inside the setts. Hence an efficient fungicide delivery method was developed to diffuse fungicides inside the setts using a mechanized-vacuum infiltration approach. The treatment method has resulted in more effective diffusion of the fungicides into sugarcane setts and it facilitated killing of sett borne infections of red rot and smut pathogens. Further, disease development from soil borne inoculum of *Colletotrichum falcatum* was also arrested in the field. A set of field trials conducted under field conditions at Coimbatore and disease-endemic regions validated disease management by improved fungicide delivery through sugarcane setts. In addition, the same method of sett treatment has been found efficiently to deliver different agrochemicals and macro and micro nutrients.This study established that the mechanized sett treatment has several additional advantages such as portability, operational simplicity, simultaneous delivery of compatible inputs before planting, etc. This new fungicide delivery system has opened up new opportunities for managing red rot in sugarcane under field conditions.

BP38

CONTROLLING OF SUGARCANE WHITE LEAF DISEASE WITH HOT TETRACYCLINE TREATMENT IN THAILAND

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Key Words: Sugarcane white leaf disease, phytoplasma, hot antibiotic treatment.

Sugarcane white leaf phytoplasma (SCWL) disease is one of the most destructive diseases of sugarcane in Thailand. Symptoms include total leaf chlorosis, plant stunting, and eventual death of the entire plant. SCWL was first reported in Thailand in 1962 and is still present today. Disease spread through planting of infected cane is the most important source of primary inoculum in Thailand. In 1978, hot tetracycline treatment was reported to control SCWL with some success, but this technique was not promoted to the farmer. In this study, we tested the efficacy of tetracycline HCl (500 ppm at 54 ^{o}C for 30 min) for control of SCWL in infected cane stalks. A popular sugarcane cultivar "Khon Kaen3" was used as plant material for this study. Mildly infected SCWL stalks were chosen and treated as per the protocol, and treatment efficacy was tested using PCR detection and symptom development. The technique was also trialed by planting in a farmer's field. The results showed this treatment can control SCWL in cane stalks, and reduced SCWL in the field experiment. This technique is suitable for Thai's small scale farmer due to it being a simple method that can be done with limited expertise, low cost and a short time when compared with elimination using tissue culture technique. If combined with other strategies, such as insect vector control, this could be a method for control of SCWL in Thailand.

BP39

RECENT APPROACHES IN DIAGNOSIS AND MANAGEMENT OF SUGARCANE PHYTOPLASMA DISEASES

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Key Words: grassy shoot disease, white leaf disease, nested PCR, multilocus specific genes

Phytoplasmas have been reported to be associated with two major sugarcane diseases viz., grassy shoot disease (GSD) and white leaf disease (WLD). Sugarcane yellow leaf syndrome is a new syndrome of sugarcane caused by a luteovirus and a phytoplasma. Sugarcane green grassy shoot (SCGGS) and ramu stunt are also associated with sugarcane from Thailand and Papua New Guinea, respectively. GSD and WLD are causing significant economic losses to sugarcane yield and sugar recovery in Asian countries. Both these phytoplasmas have been spreading very rapidly to newer locations with the help of infected seed material and leafhopper vectors. Hence, it would be important to diagnose and manage these phytoplasmas at an early stage of sugarcane growth to avoid further spread and significant losses caused by them. Because of unreliable and unspecific symptoms, the identification and characterization of the associated phytoplasma at an early stage of plant growth is problematic and unreliable. The introduction of molecular genetic methods into plant mycoplasmology about 20 years ago greatly improved the diagnosis of phytoplasma infections in plant and insect hosts. PCR offers several advantages over other methods including versatility, relative simplicity, specificity and high sensitivity, which can be increased by a two-step PCR (nested PCR). It has also become possible to differentiate, characterize and classify the phytoplasmas on a phylogenetic basis, using mainly sequence analysis of ribosomal DNA (rDNA). Further, utilization of multilocus specific genes like *secA, secY, lesS, rp, gyr A, leuS, gyB, dna B* genes have made amplification of sugarcane phytoplasma more specific from the phytoplasma infected plant and insect samples. LAMP assays has also been developed to detect phytoplasma at field level without PCR assay involvement, hence, are being used at a large scale to characterize these pathogens at an early stage of growth and for screening planting seed

materials for possible elimination and management of theses phytoplasma at an early stage of their growth and propagation. We summarize research being conducted on characterization and management of these phytoplasma by novel biotechnological and molecular approaches.

BP40

HEALTHY SUGARCANE TO ACHIEVE TARGETED YIELD - A FARMER'S PERSPECTIVE

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Key Words: Seed, soil health, germination.

Sugarcane agriculture is challenged by different biotic and abiotic stress factors in India. Furthermore, sugarcane productivity in the subtropical region of the country is low due to various factors including use of poor quality seed and ignoring soil health. To sustain sugarcane productivity and continue sugarcane farming, the farmers have to adopt scientific cultivation practices. In order to maximize sugarcane productivity in the Tarai region of Uttar Pradesh in subtropical India, detailed experiments were conducted with various improved agronomic practices such as: use of healthy seed of popular sugarcane variety Co 0238; applying organic manures; wide row planting; balanced fertilizer application; use of biocontrol agents, etc. By planting healthy seed of the cv Co 0238 the crop was maintained as disease-free in the plant and ratoon crops. In the trials, adoption of scientific cultivation practices gave a yield of ~200 tonnes of cane per hectare, which is a record rarely achieved in this part of the country showing yield improvement close to 100%. It was realized that selection of seed is very important as good quality disease free seed results in good sett germination and better establishment of the crop. Other important practices need to be followed are treating the setts in the solution of fungicide before planting, and selection of seed at right age of the crop i.e. by avoiding over mature seed canes. With the developments in information technology the success stories are being disseminated to the farming community with the aim to maximize sugarcane productivity and doubling the farmer's income in the near future.

BP41

MANAGEMENT OF SUGARCANE RED ROT WITH LIQUID FORMULATED ENDOPHYTIC *BACILLUS SUBTILIS*

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Key Words: Sugarcane, *Colletotrichum falcatum*, endophytic bacteria

Sugarcane cultivation in Tamil Nadu is hindered by many biotic diseases, of which red rot caused by *Colletotrichum falcatum i*s the most important destructive disease. Among the different management practices, planting of resistant varieties is the best way to overcome the problem. However, due to fungal variation, the released resistant varieties becomes susceptible after some year of cultivation. Biological control is an option for managing the red rot disease. Twenty endophytic bacterial strains isolated from the leaves and stalks of sugarcane and five strains collected from Department of Plant Pathology, TNAU, Coimbatore were tested for its efficacy in managing the red rot disease of sugarcane. The molecular characterization through amplifying 16S rDNA confirmed that all strains were *Bacillus.* The PCR analysis for the presence of antibiotic gene in the *Bacillus* strains revealed that eighteen strains of *Bacillus* had the gene for iturin A. Fourteen strains had the gene for surfactin, twenty one strains had the gene for bacillomycin D and only two strains had the gene for the zwittermicin A. Among the 25 strains of *B. subtilis*, two strains SUB4 and EPC5, showed higher per cent inhibition under in vitro conditions by dual plate assays. The secondary metabolites from these two strains were analyzed through GC/MS and the compounds were identified as benzoic acid, 1-Lecyl-d-leucine, Actinomycin, 5a-cholestane-3a,7a,12a, 25-Tetrol TMS ether, DL-proline, 1,8 Diazacyclotetradecane-2, Piperazinedione, Thiophene, Cyclohexadiene, Heptenoic acid, Pyrrolizidine-3-one, 1,2-benzenedicarboxylic acid, 2,2-DI-N-butylcyclohexanone and butryic acid. The SUB4 strain had higher inhibition of *C. falcatum* presumably because of the higher amount of antibiotic, siderophore, IAA activity and volatile compound production. A liquid formulation of *B. subtilis* SUB4 was developed with 2 per cent glycerol as preservative and tested for its efficiency in managing the disease both in glass house and field condition. The biocontrol strains were delivered

through sett treatment with liquid formulation of *B. subtilis* SUB4 @ 5ml/l of water + soil application @ 5ml/kg of farm yard manure with apparently healthy setts. The disease management trials were conducted under disease endemic area in Cuddalore district, Tamil Nadu and the studies found moderate reduction in the incidence of red rot under field conditions in a susceptible variety.

BP42

ELIMINATION OF YELLOW LEAF DISEASE BY COMBINED METHOD OF HEAT TREATMENT, MERISTEM CULTURE AND NURSERY SYSTEMS IN RAJSHREE SUGARS COMMAND AREA

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Key Words: YLD, meristem culture, nursery system

Sugarcane (*Saccharaum officinarum)* is an important cash crop which plays a pivotal role in India's agriculture and industrial economy. Poor quality seed material, environmental factors, genetic deterioration of the varieties are responsible for transmission of diseases in sugarcane. The viral disease viz., Yellow leaf disease (YLD) is serious problem in most of the sugarcane varieties in the recent times which leads to poor yield and juice quality. Rajshree Sugars and Chemicals Limited is producing YLD free seed cane through the breeder nursery program by combined adoption of thermotherapy of shoot tops and apical meristem culture for production of disease free tissue culture seedlings. The cultures produced by this method are indexed for yellow leaf virus by RT-PCR at Sugarcane Breeding Institute and the negative cultures are multiplied in to further stages. Three tier nursery programs viz., breeder nursery, primary nursery and commercial nursery systems are followed for multiplication of the quality healthy seed material for bulk cane planting to ensure higher cane and sugar yield. Through continuous measures for the past ten years, the percentage of achieving YLD free seedlings have gradually improved from 3.5% in 2009-10 to 100% in 2018-19. To assess the seed cane quality, various stages of nursery and bulk crops were selected and observed for disease incidence. The data revealed that 100% YLD free in the breeder nurseries and in the bulk crop, the YLD incidence got reduced from 24.83 % in 2013-14 to 5.40 % in 2017-18 in the Rajshree cane command area. Hence combining heat treatment of shoot tops, apical meristem culture with sound nursery system rejuvenates the varieties and maintains the sugarcane crop from disease incidence.

BP43

IDENTIFICATION OF EFFECTORS AS MOLECULAR MARKERS TO GENOTYPE ISOLATES OF *PUCCINIA KUEHNII*, THE CAUSAL AGENT OF SUGARCANE ORANGE RUST

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Key Words: Bioinformatics, effector genes, primer design, *Saccharum* spp.

Puccinia kuehnii, the causal agent of orange rust, is a pathogen that affects photosynthesis, thus causing significant losses to the sugarcane crop. In Brazil, orange rust is mainly controlled using resistant sugarcane cultivars and the success of this control method relies on knowledge of pathogen variation. *P. kuehnii*uses a specialized infection structure called a haustorium which facilitates nutrient acquisition. During this process, the pathogen can also secrete disease-related effector proteins. The main objective of this study was to identify molecular markers that could be deployed to characterize the genetic variation of *P. kuehnii*. The publicly available genome sequence and effector annotations from the wheat rust fungus *P. striiformis* were used to identify conserved effector genes in the genomes of *P. kuehnii* 1040 and 2143 from Florida. These latter genomes were recently sequenced (Cano et al., unpublished data) and contained sequences homologous to gene *Shr2* (PSTG_01062) from *P. striiformis*. Gene *Shr2* encodes a secreted protein known to suppress the hypersensitive response in plant tissues. A partial fragment (501 bp) of gene *Shr2* was amplified by polymerase chain reaction from six single-pustule isolates of *P. kuehnii*: four isolates from susceptible sugarcane cultivar SP89-1115 in Brazil (State of Sao Paulo) and two from susceptible cultivar CL85-1040 in Florida. Amplicons obtained for all six isolates were sequenced and resulting sequences were aligned to identify nucleotide changes. No sequence polymorphism was found among the six isolates of *P. kuehnii*. Six additional

putative effector genes were identified in the genomes of *P. kuehnii* isolates 1040 and 2143. This information will be used to further investigate genetic variation among rust isolates from Brazil and the USA (FAPESP grant 2017/25455-1).

BP44

CHITINASE GENE EXPRESSIONS IN RESPONSE TO RED ROT PATHOGEN *COLLETOTRICHUM FALCATUM* INFECTION

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Key Words: Red Rot, *Colletotrichum falcatum*, RT-qPCR, gene expression.

Sugarcane plant responds to pathogen infection by inducing expression of PR genes comprising *Chitinase* (*Chi*). *Chi*gene induced by pathogenesis is directly or indirectly involved in plant defense, leading to pathogen death or inducing immunity. Study was conducted by using two chemicals to induce the expression of *Chi* gene in sugarcane varieties CoS 08272 (Resistant) and CoJ 64 (Susceptible). Two chemicals viz; salicylic acid (SA) @5 mM and iso-nicotinic acid (INA)@140 ppm were used for the induction of *Chi* gene using drenching and spraying mode of application before inoculation of *Colletotrichum falcatum*. The pathogen was inoculated in stalk tissues, samples of stalk tissue and leaf were harvested at 0, 24, 48, 72, 120 h after inoculation. Expression of *Chi* gene was assessed by reverse transcription quantitative real time PCR (RT-qPCR) using SYBR Green chemistry. RT-qPCR results revealed that expression of *Chi* gene was greater at 48 h based on Ct (Cycle threshold) value followed by 72 h and 120 h in CoS 08272. *Chi* gene was also expressed in CoJ 64 at 48 h followed by 120 h in treated stalk while not expressed in untreated plant. Induction of *Chi* gene increased rapidly in resistant variety by both chemicals (SA and INA) at 48 h whereas comparative low induction was noticed in susceptible plant. RT-qPCR results showed that *Chi* gene was highly expressed in adjacent area of inoculated stalk tissues. Results revealed that *Chi* gene expression in CoS 08272 found consistently higher than CoJ 64, indicating that a positive correlation may exist between *Chi* and red rot resistance. Also plant immunity is enhanced in sugarcane by the application of SA and INA. Hence, expression of *Chi* gene could be ultimately utilized as disease resistance marker for red rot resistance and both chemicals could be also recognized as growth inhibitor of *C. falcatum* in sugarcane plant.

BP45

RHIZOSPHERIC MICROBIAL DIVERSITY IN DIFFERENT SUGAR PROFILE VARIETIES OF SUGARCANE

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Key Words: Sugarcane varieties, Rhizosphere, fungal mycoflora association

Rhizospheric engineering was the objective to work out the novel and environmentally sound strategies for diseases management and growth promotion of sugarcane under changing scenario of cropping system. For the same, nine sugarcane varieties having different sugar content namely CoJ 64, CoLk 94184, CoS 8436, Co 1148, CoLk 8102, CoSe 92423, SES 594, Baragua and Khakai were used for rhizospheric sampling. Out of these nine varieties, SES 594, Baragua and Khakai were the standard check. Baragua belongs to *Saccharum officinarum*; SES 594 belongs to *Saccharum spontaneum* and Khakai belongs to *Saccharum sinense*. Six different rhizospheric soil sampling was conducted for isolation of microbes for growth promotion, nutrient and disease management in sugarcane. The sampling schedules followed were 50 days after planting; 100 days after planting; 150 days after planting; 200 days after planting; 250 days after planting and 300 days after planting. Conclusively, a high sugar early maturing variety (CoLk 94184) was found associated with maximum number of bacteria in their rhizospheric region followed by CoJ 64 which is also a high sugar variety. Whereas regarding fungal mycoflora association of rhizospheric region, CoJ 64 (high sugar early maturing) variety yielded the maximum number of mycoflora followed by Co 1148 (medium sugar medium maturing) variety. The association of mycoflora in rhizospheric region keeps on building with the age of crops up to 250 days. Grand growth phase of the crop resulted highest numbers of rhizospheric microbial association with all the experimental varieties including three different checks. The rhizospheric zone of 50 days age crop was found associated with 13 fungal genera namely *Fusarium*, *Alternaria*, *Rhizoctonia*, *Trichoderma*, *Penicillium*, *Cladosporium*, *Acremonium*, *Chaetomium*, *Rhinocladiella*, *Candida*, *Aspergillus*, *Rhizopus* and *Mucor*. At the stage of advance tillering phase (100 days of crop age), fifteen mycoflora genera were found associated in rhizospheric region. Out of these fifteen genera, 13 were found

associated with root zone at the stage of fifty days crops whereas *Verticillium* and *Nigrospora* were the two new associations increased during the tillering Phase. One more association namely *Curvularia* got increased during the grand growth phase of the crop (150 days), resulting 16 in numbers of mycoflora genera. Later on the mycoflora genera, *Candida* was not found associated during ripening and maturation phase of the crop from rhizospheric microbial association (250 days crop age) where simple sugars (monosaccharide *viz.,* fructose and glucose) are converted into cane sugar (sucrose, a disaccharide). *Curvularia*, *Pythium*, *Verticillium*, *Nigrospora*, *Paecilomyces* and *Epicoccum* were the genera which were not associated during germination and establishment phase (at 50 days) to starting of tillering Phase. These genera of mycoflora got build up in rhizospheric microbial association during the grand growth phase and keep on continuing up to ripening and maturation phase of the crop especially with the high sugar early maturing varieties as compare to low sugar varieties.

BP46

TRANSCRIPTOME CHARACTERIZATION AND EXPRESSION PROFILES OF THE PATHOGENICITY RELATED GENES IN *COLLETOTRICHUM FALCATUM*, CAUSING RED ROT IN SUGARCANE

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Key Words: Transcriptome, *Colletotrichum falcatum*, pathogenicity

Red rot of sugarcane, caused by *Colletotrichum falcatum*, is a major disease of sugarcane (*Saccharum* spp.). Systemic infections of red rot in sugarcane are often considered as destructive and the mechanism of disease resistance is poorly understood. Genetic complexities of sugarcane and lack of transcriptomic and genomic information hinder our understanding of the molecular mechanisms underlying the sugarcane and *C. falcatum* interaction. Here, we studied the host response of the sugarcane during interactions with *C. falcatum* through transcriptomic approach andcomparative transcriptome analysis helped us to understand the pathogenicity genes expressed during this interaction. We have used massively parallel high-throughput sequencing of cDNA (RNA-Seq) to generate a high-resolution map of the *C. falcatum* transcriptome under different time intervals (3, 5, 7 dpi) and identified several transcriptionally active regions (TARs) such as plant cell wall degrading enzymes (PCWDE), Candidate secretory effector proteins (CSEPs), Carbohydrate active enzymes (CAZy), membrane transporters and secondary metabolites. Interestingly, the expression of many of these TAR is regulated in a condition specific manner and putatively identified as biotrophy-necrotrophy switch (BNS) genes. The four major class of genes which contribute pathogenicity in sugarcane are CSEPs, Secondary metabolites, CAZy and Membrane transporter were found to govern virulence by inducing proteases, peptidases and Cytochrome p450. This study also helped us to identify the regulation of melanin biosynthetic genes during appressorium formation of *C. falcatum*. This comprehensive transcriptome analysis significantly enhances the current genome annotations of *C. falcatum*, a necessary framework for a complete understanding of the molecular mechanisms of pathogenesis for this important sugarcane stalk pathogen.

BP47

IDENTIFICATION OF NOVEL SOURCES OF RESISTANCE TO RED ROT

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Key Words: red rot, germplasm, *Saccharum spontaneum*

Sugarcane is an important commercial crop of India occupying 5 million ha cultivated in several states under varied agro climatic conditions and factors that affect productivity. Among the biotic factors affecting sugarcane red rot caused by *Colletotrichumfalcatum*Went. is a serious threat. Hence, red rot resistance is an essential selection criterion in developing varieties in addition to high sucrose content and cane yield. Moreover, development of resistant varieties is the only solution to manage this disease and a number of elite varieties combining high sucrose content, high cane yield and resistance to red rot have been developed. Evolution of new races of pathogen is the major factor for the breakdown of resistance in shorter time and reducing the varietal longevity. (Viswanathan and Samiyappan, 2000). Then, identification of novel and diverse sources of resistance is needed to incorporate durable resistance in the new varieties. Sugarcane Breeding Institute is the custodian of the world collection of *Saccharum* and related grasses. At Coimbatore, a total of 2054 wild germplasm of *Saccharum* complex is maintained and the gene pool is enriched every year with the collections from hitherto unexplored areas of *Saccharum* diversity. Targeted explorations have been carried out in the states of Maharashtra and Punjab & Haryana during 2015 and 2016, respectively. In an attempt to search for novel sources of resistance in the *S. spontaneum* germplasm collected from these states were evaluated for their reaction to red rot against the CF06 (*Cf*671) pathotype. A total of 43 accessions viz., 14 from Maharashtra, 14 from Haryana and 15 from Punjab were evaluated for their reaction to red rot through artificial inoculation under controlled condition testing and graded

as various levels of susceptibility and resistance using a standard disease index. Out of the 14 *S. spontaneum* accessions from Maharashtra tested for CF06, five were resistant (R), eight were moderately resistant (MR) one was susceptible (S). From the Haryana collections, the accessions segregated into two R, 10 MR and one each MS and S. Out of the 16 accessions from Punjab, two were R, 11 were MR, one each MS and S. In total, out of 43, nine were R(20.9%), twenty nine MR(67.44%), three MS (6.97%) and two S(4.65%). These identified new sources of resistance could be utilized in future pre-breeding programme to develop resistant varieties to red rot for enhancing the productivity and sustainability.

BP48

STATUS OF SUGARCANE YELLOW LEAF RESISTANCE IN *SACCHARUM* HYBRID POPULATIONS AND PARENTAL CLONES IN INDIA

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Key Words: YLD, germplasm screening, resistance

Sugarcane yellow leaf disease caused by *Sugarcane yellow leaf virus* (SCYLV) has spread throughout the sugarcane growing regions of India. The disease severity has reached epidemic levels in some of elite varieties under cultivation. It is primarily spread through planting material and secondary spread through aphid vector, *Melanaphis sacchari*. Since its first report in India, various aspects of the disease like epidemiology, diagnosis, variation in SCYLV genome, vector transmission and the disease management strategies were studied in detail. Developing yellow leaf resistant varieties could also be a sustainable approach to manage the disease effectively. In this regard, YL resistance in sugarcane germplasm maintained by the institute at Kannur, Agali, Coimbatore and Karnal were assessed earlier. Subsequently, SCYLV resistance in different *Saccharum* hybrid populations, parents and advanced varietal populations were screened during the last three seasons using a 0-5 YL rating scale. Clones were categorised as R (0.0-1), MR (1.1-2), MS (2.1-3), and S (3.1-5). In advanced varietal populations, ~46.35% were found to be YL resistant and the remaining 53.65% were moderately resistant to susceptible. Assessing YL severity in ~600 parental clones revealed that YL resistance ranged from 88.88 to 92.09% during the last three years. Among the 3351 *Saccharum* hybrid populations maintained at Agali centre, 2642 were identified as YL resistant. Although YL resistance has been identified in all these *Saccharum* populations, further studies are in progress to identify true resistance sources through real time q-PCR assays. For sustainable management of the disease, host resistance needs to be given importance; hence information generated from this work on host resistance suggests that the YL resistant parents can be effectively utilised in the future breeding programme to develop resistant varieties.

BP49

BIOTHERAPY –A TOOL FOR THE MANAGEMENT OF RED ROT IN SUGARCANE

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Key Words: Red rot, biocontrol, *Trichoderma viride*, *Pseudomonas aeruginosa*, sugarcane.

Eleven fungal species and ten bacteria from rhizosphere, phylloplane and internal stalk tissue of canes were isolated. The efficacy of native fungal and bacterial isolates were studied *in vitro*. Among the fungal isolates *Trichoderma viride (Tv)* and bacterial isolates *Pseudomonas aeruginosa (Pa)*were found significantly superior in mycelial growth inhibition of *Colletorichum falcatum ie., (Cf)* 84.1 and 81.7 %, respectively. It was also observed that *Tv*was overgrown on the mycelial growth of *Cf*(Mycoparasitism) where in pathogenic hyphae coiled with the hyphae of *Tv* was observed under scanning electron microscope. Whereas in case of *Pa*, clear lysis of hyphae of *Cf* due to interaction with *Pa* was observed. Hence these two native potential antagonists were selected for *in vivo*. Field experiments were conducted using these native antagonistic organisms to determine the most effective method of application in reducing the intensity of red rot disease. These two biocontrol agents were applied as sett – treatment, soil application alone and soil application in combination with other treatments separately. Among the treatments, soil application of *Tv* or *Pa* alone and soil application in combination with other treatments significantly reduced the disease intensity resulting in improvement in quantitative and qualitative parameters, and registered higher values in effective treatments. Survival studies of applied bioagents in rhizosphere at 60 days after planting (DAP), 150 DAP and at harvest revealed the population density in the individual treatments was proportionate to the quantity of the bioagent added to the soil. The results revealed that these native potential bioagents while interacting with roots could induce systemic resistance in sugarcane against red rot disease, might be early establishment of increased population of bioagents in rhizosphere.

Viswanathan, R. and R. Samiyappan (2000). Red rot disease in sugarcane: Challenges and prospects. Madras Agricultural Journal, 87: 549-559.

BP50

DISEASE MONITORING AND EPIDEMIOLOGY OF FUNGAL DISEASES IN *SACCHARUM OFFICINARUM* CLONES

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Key Words: Epidemiology, foliar diseases, germplasm.

About 55 diseases have been reported in sugarcane and several of these are of concern for crop production and productivity in India. ICAR-Sugarcane Breeding Institute, Kannur, Kerala, maintains the largest collection of sugarcane germplasm with wide diversity for morphological and quality traits. Of more than 3300 maintained clones of sugarcane germplasm and related genera, 757 are *Saccharum officinarum*. Considering the importance of germplasm in future breeding programmes for the development of new cultivars, diseases occurring in different clones of *S. officinarum* were monitored regularly and disease incidence and severity were assessed. Among the various diseases, ring spot caused by *Leptosphaeria sacchari* was predominant, followed by stalk rot (*Phaeocytostroma sacchari*) and pokkah boeng (*Fusarium sacchari*). Maximum ring spot incidence was recorded in 28 NG 260, 28 NG 18, 28 NG 20, Kham, Awela Green sport and Azul de Cazaandtwo clones IJ 76-322 and IK 76-70 recorded pokkah boeng. Stalk rot was observed in more than 35 clones and only at the end of the cropping season this disease was detected. It was also found that the disease was aggravated by insect infestation, other diseases, weak and damaged clones. The incidence of ring spot and pokkah boeng started with the onset of rainfall during June and increased gradually. Top rot symptoms were noticed in IK 76-70 and both *S. officinarum* clones showed complete wilting symptoms during January. *S. officinarum* clones IJ 76-322 and IK 76-70 recorded pokkah boeng incidence of 25% with severities of 16% and 25%, respectively. Ring spot and pokkah boeng incidences were positively correlated with minimum temperature and rainfall and negatively correlated with maximum temperature and relative humidity. These studies revealed that though the prevailing climate was conducive for occurrence of these foliar diseases, the diverse germplasm of *S. officinarum* exhibited variation for disease resistance and we could readily identify clones with resistance to these fungal diseases.

BP51

EFFICACY OF DIFFERENT THERMOTHERAPY CONDITIONS TO CONTROL RATOON STUNT WITH DIFFERENT TITERS OF *LEIFSONIA XYLI* SUBSP. *XYLI*

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Key Words: *Saccharum* spp., serological test, heat treatment, germination.

Ratoon stunt, caused by the fastidious bacterium *Leifsonia xyli* subsp. *xyli* (*Lxx*), is one of the most important diseases of sugarcane worldwide due to yield loss and control difficulty. Heat-treatment of propagating materials is one major strategy to its control. The long hot water treatment (LHWT) at 50 °C for 2 h is employed in most countries whereas in Brazil is used the short hot water treatment (SHWT) at 52 °C for 30 min. Reasons for this choice include fear over bud germination of the first combination and a greater number of seed canes that can be treated. On the other hand, the 52°C and 30 min., combination does not eliminate *Lxx* completely. To clarify the control of initial *Lxx* titer in propagating materials was study the effect of two thermotherapy combinations with different initial *Lxx* titer on cultivar RB86-7515. Diseased buds of RB867515, with 10^6 and 10^7cfu.mL^{-1}, were submitted to three conditions: LHWT at 50ºC/2h and SHWT at 52ºC/30min and non-treated control. Subsequently, they were planted in 600-liter boxes in a completely randomized design, with four replications and allowed to grow in natural conditions. Parameters evaluated were: germination (from day 6 to 20), plant height (at day 30), yield and *Lxx* control (9th month). Our data showed that the efficacy of the SHWT is dependent of the initial *Lxx* titer in seed canes; only the LHWT at 50ºC/2h combination provided RSD control, regardless of *Lxx* titer; the xylem sap from canes of nine months old and low titer of *Lxx* (10^7 cfu.mL^{-1}) was not detected by serological diagnostic test; even though thermotherapy affected germination, the SHWT at 52ºC/30min significantly cause less effect in germination and resulted in intial higher plant height (Up to the 20th day); however, the thermotherapy treatment did not provide yield increase in plant cane of cultivar RB86-7515.

BP52

BIOLOGICAL CONTROL APPROACH – SERVES AS AN INTERFACE TO IDENTIFY ANTIFUNGAL/PATHOGENICITY RELATED PROTEINS DURING TRITROPHIC INTERACTIONS

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Key Words: Biocontrol, tritrophic interactions, antifungal genes

In vivo tritrophic interaction was studied in standing canes to confirm the biological suppression of the red rot pathogen *Colletotrichum falcatum* in sugarcane by the fungal antagonist *Trichoderma harzianum*. Inoculation of pathogen and antagonist in canes and sampling were standardized and subjected for proteomic analysis. During tritrophic interaction, the growth and proliferation of *T. harzianum* over *C. falcatum* was confirmed by suppression of symptom production, duplex PCR, tissue bioassay, and microscopic observations. Results on proteomic analysis revealed that, most of the differentially expressed proteins involved during three way interaction were found to be sugarcane origin and were categorized into defense and stress responsive proteins (s-adenosyl methionine synthetase, translation initiation factor FA, ascorbate peroxidase, caffeic acid 3-o-methyltransferase), metabolism protein (glutamine synthetase) signaling protein (mucleoside diphosphate kinase-1-like protein) etc. Besides suppression of pathogenicity related proteins viz., cytochrome p450 and Hsp20 were observed. Since antifungal proteins could not be traced in standing canes, proteomic analyses on two-way interaction of *C. falcatum* and *T. harzianum* on sugarcane tissue was performed. Results from this study, showed expression of 17 unique protein spots which were found to be defence and stress responsive proteins *viz*., disulfide isomerase, pyruvate decarboxylase, peroxidase, Hex1, Cu/zn superoxide dismutase, hypothetical protein etc., of *T. harzianum*. Like antagonistic isolates, *C. falcatum* also expressed superoxide dismutase proteins and Hypothetical proteins during two way interaction. Expression analysis on transcripts of identified proteins revealed that, defence genes of sugarcane and candidate genes of *T. harzianum* were upregulated by the induction of *T. harzianum* in sugarcane either alone or along with pathogen. Interestingly, the pathogenicity / virulence related proteins *viz*., cytochrome p450 and Hsp20 *C. falcatum* were down regulated during interaction with *T. harzianum* in sugarcane.

BP53

TEMPORAL VARIATION OF THE ORANGE RUST PATHOGEN, *PUCCINIA KUEHNII*, AND ITS MYCOPARASITE *SPHAERELLOPSIS FILUM* AT A SUGARCANE BREEDING CENTER IN BRAZIL

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Key Words: biological control; *Saccharum* spp; mycoparasitism, weather

Orange rust (*Puccinia kuehnii*) is a serious threat to sugarcane industry in Brazil infecting various commercially grown cultivars. Chemical protection is recommended in highly conducive conditions for susceptible cultivars. At the same time, field observations showed *Sphaerellopsis filum* in great number on orange rust pustules, with sporulation greatly affected. The current concern for environment protection leads us to examine the possibility to control orange rust of sugarcane by biological means. To initiate this project the present work aimed to survey the temporal variation of orange rust severity, its relationship with viability of urediniospores, and the level of parasitism by *S. filum*. This work began December 2017 by weekly collecting 60 leaves +2 from the susceptible cultivar SP89-1115. Laboratory assay evaluated the orange rust severity by image analysis with Assess in 140 $cm^2$of the most diseased area, and the degree of mycoparasitism by percentage of pustules parasitized by *S. filum* in 2 cm^2 of a new infectious area. Viability of *P. kuehnii* urediniospores was carried out biweekly on water agar media with 100µl of spore suspension at concentration of 10^5spores/ml and incubation at 18ºC under continuous illumination for 24h (five replications), when percentage of 100 spores germination was evaluated. Our data showed that orange rust increased steadily during summer months until reaching a peak of 42.9% in the end of March, and then, severity decreased continuously down to 3% in late May, coinciding with long period of no rainfall. In most cases, viability of urediniospores varied from 35 to 45%, even when disease severity decreased. mycoparasitism by *S. filum* showed a very similar tendency of orange rust, with incidence of parasitism varying from 30 to 50%, declining to zero in late May. This work still continues.

BP54

PATHOGENICITY CORRELATION BETWEEN RED ROT PATHOTYPES IN RESISTANCE EVALUATION OF SUGARCANE GENOTYPES

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Key Words: Sugarcane, red rot, *Colletotrichum falcatum*, pathotypes, resistance.

Red rot pathogen (*Colletotrichum falcatum* Went.) displays huge disparity in pathogenicity on host crop. Resistant varieties are most effective to manage red rot and the emergence of new races of pathogen is the main hurdle for long term resistance in promising high sugar yielding cultivars. The development of varieties having stable resistance is prerequisite and needs vigorous testing against red rot pathotypes prevailing in the area. This study was carried out at Sugarcane Research Institute, Shahjahanpur during 2016-2017. Thirty-five newly developed varieties were tested against red rot pathotypes common in Northern West zone of India (CF07, CF08, CF09). Plants were inoculated with each pathotype and their composite inoculum by plug method. Results revealed that, all the pathotypes and their mixture produced almost similar disease index. Over all varieties, disease indexing means were computed as 4.61, 4.24, 4.29, and 4.33 for CF07, CF08, CF09 and their composite inoculum, respectively. Means of disease index for medium resistant varieties were 3.34, 3.01, 3.33, and 3.07 for CF07, CF08, CF09 and their mixture, respectively. Based on correlation coefficient study, highly significant positive correlation (0.889) was found between CF08 and CF09. Disease indexing indicates that all the pathotypes displayed almost the similar significant positive correlation among them. Hence, selection of genotypes with single existing pathotype could well discriminate resistance for effective and economic screening process of red rot resistant varieties at least for the northern zone of India.

BP55

IDENTIFICATION AND CHARACTERIZATION OF GENES/PROTEINS RELATED TO *COLLETOTRICHUM FALCATUM* PATHOGENESIS IN SUGARCANE

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Red rot caused by *Colletotrichum falcatum* Went is one of the major constraints in the profitable cultivation of sugarcane in many states of India. Although no variety is being released without red rot resistance, frequent breakdown of released resistant cultivars occurs due to emerging new variants of the pathogen. The isolates were distinguished at morphological, cultural, serological, genetic, pathogenicity and molecular level. However, no correlation could be made with pathogen virulence and the Indian isolates exhibited high phenotypic with little genotypic variation. Hence proteomic and genomic approaches were employed to identify pathogenicity related genes/proteins differentially expressed between the phylogenetically differentiated virulent isolate, *Cf*671 and the least virulent isolate, *Cf*92020. In the proteomic approach, mycelial proteins, secreted proteins and proteins from host-pathogen interaction of both the isolates were analyzed by 2-D gel electrophoresis and unique proteins from the virulent isolate were selected and analyzed by MALDI-TOF. Similarly, under the genomic approach, suppression subtractive hybridization (SSH) was employed to identify differentially expressed genes from the virulent isolate. Results indicated that, among the characterized unique proteins in response to virulent isolate, there were 27 proteins from mycelium, eight proteins from secretome and 12 proteins from host-pathogen interaction were found to be matched with the unique transcripts identified under SSH approach. Besides, ten identified proteins were found to be common to mycelium and host-pathogen interaction and four proteins *viz.*, Bys1 family protein, two methyltransferases and 3-isopropylmalate dehydrogenase were present in all the mycelial, secretome and host-pathogen interaction analyses. Furthermore, the candidate genes were able to differentiate the pathogen virulence in *in*

vitro and during host pathogen interaction in response to host resistance. Conclusively, the unique genes/proteins obtained from our study might play a significant role during *C. falcatum* pathogenesis in sugarcane.

BP56

OCCURRENCE OF RED ROT OF SUGARCANE AND VARIATION IN PATHOGENICITY OF *COLLETOTRICHUM FALCATUM* IN COASTAL TAMIL NADU

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Key Words: Sugarcane, red rot, pathogenic variability

Red rot is the most common disease of sugarcane in Tamil Nadu, caused by the fungus, *Colletotrichum falcatum* Went. Surveys were conducted in the coastal districts of Tamil Nadu *viz*., Cuddalore, Kanchipuram, Villupuram, Nagapattinum and Puducherry to record incidence of this disease from 2015-2018. Red rot disease was noticed in varieties *viz.*, CoC 24, TNAU Si7, TNAU Si 8, and CoV 09356 (2 to 62%) in 2015-16; varieties *viz.*, CoC 24, CoC 23 and Co 91017 (2 to 54 %) in 2016-17 and in varieties *viz.*, CoC 24 and Co 91017 (2 to 22 %) in 2017-18. Red rot pathogen *C. falcatum* isolated from varieties (CoC 23, CoC 24, Co 91017, CoSi 6, TNAU Si 8) and the designated pathotype CF 06 were inoculated in nineteen differential varieties to identify the pathogenic variability in *C. falcatum*. Isolate of *C. falcatum* from variety CoC 24 had showed limited variation in pathogenicity from the designated pathotype CF 06. In the differential variety BO 91, isolate CoC 24 showed an intermediate reaction, while CF 06 had a resistant reaction. In the Co 1148 differential variety, the isolate CoC 24 showed susceptible reaction while CF 06 was resistant. In the differential CoJ 64, the isolate from CoC 24 showed susceptible reaction while CF 06 was intermediate. In the differential variety CoS 767, the isolate from CoC 24 showed intermediate in reaction while CF 06 was resistant. All the other isolates behaved similar in reaction to pathotype CF 06

BP57

DETERIORATION IN ECONOMICAL TRAITS OF SUGARCANE DUE TO POKKAH BOENG DISEASE

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Key Words: Sugarcane, Pokkah Boeng, qualitative, quantitative traits.

During recent years Pokkah boeng is an emerging disease of sugarcane which has been recently found to cause losses in sugar and cane yield related traits. An experiment was conducted for three consecutive years from 2012-13 to 2014-15 at Sugarcane Research Institute, Shahjahanpur, UP. The impact of pokkah boeng disease was evaluated by comparison of healthy and infected canes of same field in natural condition. The results of three years mean data for twelve promising sugarcane varieties revealed that the reduction in yield and quality contributing parameters are due to incidence of pokkah boeng disease. The most important traits which play a prime role in yield enhancement such as cane height and stalk diameter were reduced due to this disease. Cane height decreased numerically ranging from 4.85 to 17.40% and stalk diameter reduced from 7.14 to 19.35%. Other yield related parameters i.e. number of green leaves, internode length and number of internodes also decreased ranging from 40 to 66%, 5.0 to 35.0% and 12.5 to 54.5%, respectively. Reduction in quality parameters were also observed in all promising sugarcane varieties. Pol percent in cane and sucrose percent in juice reduced from 3.65 to 10.48% and 0.60 to 5.80 %, respectively. Reduction in associated quality traits such as purity coefficient and extraction percent were also computed from 0.20 to 2.45% and 2.18 to 11.87%. Thus, pokkah boeng is responsible for economic losses for Indian farmers as well as millers due to reductions in yields and quality of sugarcane.

BP58

ULTRASENSITIVE NANOGOLD-LABELLED IMMUNOASSAY FOR THE DETECTION OF *SUGARCANE STREAK MOSAIC VIRUS*

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Key Words: virus diagnosis, gold nanoparticles, lateral flow immunoassay

Molecular assays, especially PCR based diagnostic assays are regularly employed in India to diagnose different viruses and phytoplasmas infecting sugarcane. Although these assays are sensitive, there is a need to develop field diagnostic kits to detect the pathogens. Lateral flow assay (LFA) using gold nanoparticles (GNPs) has been found to be a valuable assay for field diagnosis of viruses in different crop plants. In this study we have standardized an LFA for diagnosing viruses causing mosaic in sugarcane. In this assay, *Sugarcane streak mosaic virus*(SCSMV)specific antibodies were blotted and immobilized on nitrocellulose membrane in a spot, followed by incubation with suspected virus samples. After the incubation, the target viral antigen was allowed to hybridize with gold nanoparticles (GNPs) (~27nm) labeled SCSMV-specific antibody probes. Here, GNPs were synthesized by a standard citrate method and the gold labelled antibody probes were prepared by covalent conjugation using EDC/NHS linkers. Conjugation was confirmed by both UV-Vis spectral and gel retardation assays. Hybridization of the target with the nanogold labeled probes resulted in a change in spot colour. The detection limit was found up to one nanomolar concentration of SCSMV antigens in the samples. However, the detection limit can be further increased up to femtomolar concentration by the silver enhancement method. The new assay has been found to be fast and requires less consumables. Above all, it is portable and cost-effective. This new development in virus diagnostics would further strengthen production of healthy planting materials in sugarcane.

TAKING RSD DIAGNOSIS CLOSER TO THE FIELD EDGE

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Key Word: Diagnosis, ratoon stunt, *Leifsonia xyli* subsp. *xyli*, LAMP

Ratoon stunt (formerly ratoon stunting disease - RSD - *Leifsonia xyli* subsp. *xyli*) is one of the most important sugarcane diseases in South Africa. The identification of the bacterium *L. xyli* subsp. *xyli* as the causal agent of RSD in 1973 made diagnosis based on the microscopic examination of xylem sap possible. Phase contrast microscopy was introduced to routinely diagnose RSD in South Africa in 1977 and played a critical role in the development and implementation of management strategies for the disease. An evaporative binding-enzyme immunoassay (EB-EIA) replaced PCM for routine diagnosis in 1998. This method proved to be more sensitive, more efficient and resulted in reduced transport costs since xylem sap could be extracted and stored at remote locations before delivery to a central laboratory at Mount Edgecombe for analysis. While the EB-EIA currently remains the standard method of diagnosis in South Africa, assay sensitivity is relatively low compared to the molecular diagnostic methods that are now available, expensive equipment is required and sap samples are prone to degradation during transportation. As a result, alternative methods that are suitable for in- or near-to-field diagnosis are currently being investigated. A modified loop-mediated isothermal (65°C) amplification (LAMP) assay was developed to detect *L. xyli* subsp. *xyli* in 30 min. To improve reliability and suitability for near-to-field applications, carryover contamination that resulted in false positives was successfully eliminated by incorporating uracil nucleoside glycosylase in the mastermix and incubating for 37°C for 10 minutes. Diagnosis was based on the detection of LAMP products using lateral flow devices rather than a subjective colorimetric change. Samples of

exudate from leaf sheath discs were tested as an alternate template for the LAMP assay but results were found to be less reliable than xylem sap. A pre-prepared master mix, which would improve accuracy and efficiencies when preparing the assays, could be stored for up to 4 months at -20°C without any reduction in performance. While these changes made the assay more suitable for the near-to-field detectionof *L. xyli* subsp. *xyli* in centres with basic facilities, further modifications to reduce the requirement for a qualified technician to perform the assays are required.

BP60

CONFIRMATION OF A NEW RUST, *MACRUROPYXIS FULVA* (*PUCCINIALES*) INFECTING SUGARCANE IN SOUTHERN AFRICA

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Key Words: *Macruropyxis fulva*, tawny rust, *Miscanthidium capense*, host-range expansion, touchdown PCR.

The presence of a unique rust pathogen, *Macruropyxis fulva* (L.A. Martin, S.A. McFarl. and L.A. Castl.), causal agent of tawny rust in sugarcane, was confirmed. Phylogenetic analysis revealed a super-clade containing all three sugarcane rusts, namely *M. fulva*, *P. melanocephala* (brown rust) and *P. kuehnii* (orange rust). This super-clade contains three main groupings; the clades labelled *Macruropyxis*, *Puccinia* I and *Puccinia* II. Although *P. melanocephala* and *P. kuehnii* fall into the *Puccinia* II clade, this clade breaks into two sub-clades very near the split between *Puccinia* I and II, indicating that *P. melanocephala* and *P. kuehnii* are relatively divergent from one another. *Miscanthidium capense* is thought to represent the native original host of *M. fulva* after the pathogen was confirmed to be the causal agent of similar symptoms on this grass species in a mountainous region of KwaZulu-Natal, South Africa. A study on the Andropogoneae within the family Poaceae indicated that *Miscanthidium* was about 2 million years divergent from sugarcane. In this study, the three African species, *M. capense*, *Miscanthidium junceum* and *Narenga porphyrocoma* were shown to be the closest relatives to sugarcane. *Miscanthidium* is not chromosomally compatible with *Saccharum* (n=15 for *Miscanthidium*) but five of these chromosomes are ancestral to the core Saccharinae, whilst ten chromosomes

share a direct common ancestor with *Saccharum*. While *Miscanthidium* is unlikely to hybridize with *Saccharum* in the wild, it lies within the 3.4 million year window where wild hybridization is possible and human-mediated hybridization is likely. In KwaZulu-Natal however, it is by far the most closely related species to sugarcane, increasing the potential for adaptation and host range expansion of native *M. fulva* to sugarcane where distributions overlap. A touchdown PCR assay specific for *M. fulva* was developed to distinguish *M. fulva* from *P. melanocephala* and *P. kuehnii*. This assay will allow the rapid and accurate diagnosis of tawny rust in the event of incursions in other countries.

BP61

MAPPING AND MARKER IDENTIFICATION FOR RED ROT RESISTANCE IN BIPARENTAL SEGREGATING POPULATIONS OF SUGARCANE USING MICROSATELLITE MARKERS

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Key Words: Red rot, *Colletotrichum falcatum*, molecular markers, microsatellite, resistance.

Sugarcane production and productivity in the country depends on various biotic and abiotic factors which differ from one region to another. Among the biotic factors red rot followed by smut are the major diseases that cause considerable yield loss in sugarcane. Breeding for resistant varieties requires precise screening methodologies and selection for resistant plants, which are often laborious and require extensive knowledge on plant-pathogen interactions. Molecular markers tightly linked to the genes conferring resistance will be of advantage in such situations. This study was attempted to identify markers that are associated with the component traits of red rot resistance in a biparental mapping population. The mapping population of ninety individuals were generated from the cross CoC 671 X BO 91 and were screened for their resistance and susceptibility. The progenies were screened for three components viz., Lesion width, Nodal transgression and white spots. The results showed a clear distinction between the resistant and the susceptible clones with a segregation ratio of 1:1 for lesion width and nodal transgression whereas the segregation for the white spots was slightly skewed towards resistance. The mapping population was screened with 60 microsatellite primer pairs that showed polymorphisms in the parents. The primer pairs were selected in a way that they permit recovery of maximum polymorphisms in the progenies. Accordingly the 60 primer pairs amplified a total of 850 markers in the parents and progenies. Chi squared analysis of the marker segregation in the progenies revealed that majority of the marker segregation followed the 1:1 segregation and was considered as markers segregating in single dose. A total of 322 single dose markers were used for linkage analysis, of which 167 were male parent specific and 155 were female parent specific. Markers were identified for lesion width and for nodal segregation whereas markers for the white spots remained unlinked.

BP62

INDUCTION AND ANTIFUNGAL ACTIVITIES OF 3-DEOXYANTHOCYANIDINS PHYTOALEXIN COMPOUNDS AS HOST RESPONSE AGAINST INVADING *COLLETOTRICHUM FALCATUM* IN SUGARCANE

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Key Words: Sugarcane, red rot, resistance, 3-deoxyanthocyanidin phytoalexin

The fungal disease red rot, caused by *Colletotrichum falcatum*, causes severe yield loss and poor juice quality of sugarcane, thereby leading to economic loss and elimination of many elite sugarcane varieties especially in the Asian continent. The resistance mechanism of sugarcane is complex to understand due to its polyploidy nature. Previous studies on this host-pathogen interaction revealed the expression of various transcripts involved in the flavonoid biosynthesis pathway and the differential accumulation of 3-deoxyanthocyanidin phytoalexin compounds as a result of the defense response. To further establish the role of phytoalexin accumulation in host defense, HPLC studies were carried out. The results clearly showed the differential accumulation of 3-deoxyanthocyanidin compounds including apigeninidin, luteolinidin, cyanidin and some uncharacterized compounds, in varying concentrations corresponding to the clones resistance to the pathogen. Accumulation of phytoalexin compounds had a direct role in restricting the pathogen progression in the host tissue. Conidial germination assays carried out with the HPLC fractions showed that luteolinidin fraction inhibits germ tube elongation and appressorium formation. The results indicate that induction and accumulation of phytoalexins compounds during host-pathogen interaction leads to restriction of pathogen progression in resistant varieties. The role of these compounds will be studied in detail using mass spectrometric technique and their specific function characterized in future studies.

BP63

COMPARATIVE SECRETOME ANALYSIS OF *COLLETOTRICHUM FALCATUM* IDENTIFIES POTENTIAL PROTEINS THAT INDUCES SYSTEMIC RESISTANCE IN SUGARCANE

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Colletotrichum falcatum, a hemibiotrophic fungal pathogen causes one of the major devastating diseases of sugarcane - red rot. Repeated *in vitro* sub-culturing of *C. falcatum* under dark condition alters morphology and reduces virulence of the culture. *Hitherto*, no information is available on this phenomenon at molecular level. In this study, the *in vitro* secretome of *C. falcatum* that were cultured under light and dark conditions were analyzed using 2DE coupled with MALDI TOF/TOF MS. Comparative secretome analysis identified nine differentially expressed proteins and revealed a major portion of alterations (9 out of 10 proteins) occurred in the range of low molecular weight (LMW) proteins (<30KDa). In dark cultured sample, most of the LMW proteins (7 proteins) were less abundant or absent, except a ceratoplatanin protein called CfEPL1 (eliciting plant response like protein 1), which was the only and very high abundant LMW protein. Whereas, in light cultured *C. falcatum*, a novel protein called CfPDIP1 (plant defense inducing protein 1) found to be highly abundant. Subsequent functional characterization of distinct domains of CfEPL1 and CfPDIP1 by *in vitro* expression and purification indicated that CfEPL1ΔN1-92 and CfPDIP1ΔN1-21 induces HR in tobacco and systemic resistance against red rot in sugarcane. Priming assay and co-infiltration assay of *in vitro* expressed and purified CfPDIP1 protein indicated that this putative effector has a dichotomous functional role. It induces host defense when primed by means

of foliar spray, while suppresses host defense during co-infiltration with *C. falcatum* spores in sugarcane leaves. Comprehensively, the study has identified proteins that putatively contribute to virulence of *C. falcatum* and for the first time, demonstrated the potential role of PAMPs/Effectors of *C. falcatum* inducing PAMP-triggered immunity (PTI)/effector-triggered immunity (ETI) in sugarcane.

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ASSESSING RESISTANCE OF SUGARCANE CLONES TO BROWN RUST IN THE FIELD

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Recently, brown rust of sugarcane (*Puccinia melanocephala* Syd.&P.Syd) has become a serious disease under field conditions of different parts of Peninsular India. Studies were initiated to assess adult plant resistance in sugarcane clones by categorizing response to brown rust infection in the field as Screening of 275 sugarcane clones for rust resistance revealed that ~60% of clones remained free from rust and 20% exhibited MR reactions. Of the 20% susceptible group, ~13% were MS and 7% susceptible. Further studies on influence of various weather parameters on rust development revealed that temperature, rainfall and relative humidity greatly affect the disease development. The age of crop and position of leaves also influenced the rust severity in the field. Area under disease progress curve (AUDPC) was developed by recording brown rust severity of each leaf for two susceptible cvs (Co 0218 and Co 0403) at 10-day intervals and plotting disease severity over time. The Average AUDPC value of Co 0218 was 1613 which was five times greater than AUDPC value (305) of Co 0403. Therefore, of these varieties, cv Co 0218 was found to be more susceptible than cv Co 0403. In both varieties, the lower leaves showed more rust than the upper leaves of the same plant. The AUDPC of lower leaves in Co 0218 was higher (3380) whereas the top leaves showed less than 50. In the case of Co 0403, even the lower leaves showed only 1312 and many of the new leaves were free from any rust at the time of maturity. The AUDPC can be utilized to identify varieties that have slower rust progression, enabling better selection of varieties for release.