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Lead paper 2

Sugarcane pathology in India: application of molecular techniques to understand pathogen variability, disease diagnosis and management

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Introduction

About 100 diseases are reported in the sugarcane and the crop suffers due to fungal, bacterial, phytoplasmal and viral diseases in the country. In spite of all the efforts of breeding for disease resistant varieties, this crop is becoming more and more prone to many diseases. About 55 diseases of sugarcane caused by fungi, bacteria, viruses, phytoplasmas and nematodes have been reported from India (Rao et al 2002; Rott et al 2000). The crop suffers from different diseases in almost all the sugarcane growing states (Anon. 2013) (Table 1). Among them red rot, smut, wilt and pineapple disease (sett rot) are the important fungal diseases. Bacterial diseases like leaf scald disease (LSD) and ratoon stunting disease (RSD) are found to cause considerable yield loss in India. Among the viral diseases, mosaic and yellow leaf disease (YLD) are prevalent in almost all parts of the country. Besides these, grassy shoot (GSD) caused by phytoplasmas is also a potential disease, which can cause considerable damage to sugarcane production. Foliar diseases such as yellow spot, brown spot, brown stripe, eye spot, ring spot, rust etc may cause loss to sugarcane depending on the prevailing environmental conditions (Viswanathan 2012a; Viswanathan and Rao 2011). Many promising varieties were removed from cultivation in the past since they succumbed to new virulent pathogenic variants of red rot pathogen. Also slow buildup of many non-fungal diseases in sugarcane causes decline in varietal performance and results in varietal degeneration (Viswanathan, 2012a). Since the crop is grown throughout the year and vagaries of environment affect disease intensity, spread and development of disease epidemics.Conventional disease management relies more on disease resistant varieties and agrochemicals. Recently, molecular approaches are being used to characterize the pathogen variation, understand host resistance and to develop sensitive diagnostics in different crops and this review summarises application of molecular techniques in sugarcane pathology.

Complexities in pathogen identity

Mosaic disease

Sugarcane mosaic is a very common disease in India because of the virus perpetuation through vegetative cuttings and was first reported from Pusa in 1921, since then virus isolates causing mosaic disease on sugarcane in India continue to be a potential threat to sugarcane industry as the disease causes interveinal chlorotic specks, streaks or stripes especially on young leaves of sugarcane. In India, incidence of mosaic disease is almost 100%. It results in significant yield losses considering the vast area under sugarcane cultivation (Agnihotri 1996; Bhargava 1975; Viswanathan and Balamuralikrishnan 2005). In the past, there were assumptions that mosaic does not cause significant yield loss in sugarcane in the country, however the author has observed severe expression of the disease in the popular varieties like Co 740, Co 7219, CoA 92081, CoC 671, CoC 92061, CoJ 64, CoS 767 etc in different regions. Earlier Agnihotri (1996) reported that SCMV causes an appreciable damage in susceptible varieties and even 10-15 per cent yield

loss due to this disease is highly significant because of extensive cultivation of susceptible vari... of th... crop.

In India up to 1990's sugarcane mosaic disease was supposed to be caused by different ...ains ... *Sugarcane mosaic virus* (SCMV) (Rao et al 2002). Hemaet al (1999) reported *Sugarcane strea... mosaic virus* (SCSMV) as the new casual virus of the mosaic disease in tropical India. Since there was a ...nfusio... on causative virus associated with mosaic, detailed molecular studies were conducted on the as...ociate... viruses and established that both SCMV and SCSMV cause mosaic in India either separately or ...gethe... (Viswanathan et al 2007). Subsequently SCSMV was characterized as a new genus "Susmovirus" in th... family *Potyviridae* based on its distinct coat protein genome (Viswanathan et al 2008a). Later ICTV, h... renamed the genus as *Poacevirus* based on host range of the species in the genus. Recently co...plet... nucleotide sequence of an SCSMV isolate from India, SCSMV-IND was determined. It is a linear sing... stranded positive sense RNA genome of 9786 nucleotides in length (excluding the Poly A tail) and ... comprises a large open-reading frame encoding polyprotein of 3131 amino acid residues (Parameswa...et ... 2013). Among these two viruses causing sugarcane mosaic, SCSMV is found to occur in more frequ...ncie... than SCMV (Rao et al 2006; Viswanathan and Karuppaiah 2010). Earlier Viswanathan et al (2009a) speculated that low distribution of SCMV as compared to SCSMV in sugarcane across the Indian subcontinent, perhaps due to the comparatively less amino acid variations in the hyper variable region of th... genome than the same region of SCSMV.

Yellow leaf disease (YLD)

The disease earlier described as yellow leaf syndrome (YLS) is characterized by a yellowing of the midrib and lamina occurred in most of the sugarcane growing regions of the country. Viswanathan (2002) reported spread of YLD in sugarcane in different regions and recorded disease intensity up to 100 per cent in certain susceptible varieties. *Sugarcane yellow leaf virus* (SCYLV) a *Polerovirus* and *Sugarcane yellow phytoplasma* (ScYP) are the associated pathogens. In India, SCYLV has been found to be the major causal agent in all the states. However recent studies revealed association of ScYP with the disease both in the tropical and subtropical regions (G.P. Rao, Personal communication).

Detailed molecular analyses of partial genome of SCYLV isolates revealed the existence of five genotypes of SCYLV based on the geographical location where it was first detected. Studies of Viswanathan et al (2008b) reported the fifth genotype of SCYLV viz. IND from India along with three other genotypes *viz*., CUB, IND and BRA based on partial sequences encoding for ORFs1 and 2. Of them, probably the genotype IND might be found only in India besides CUB and BRA-PER (Viswanathan et al 2008b; Singh et al 2011). Recent studies on complete genome of the virus isolates from India revealed that IND genotype shares close similarity with CHN1 genotype reported from China. In addition, comparison of phylogenetic relation of reported complete genomic sequences worldwide established that IND and CHN1 originated from Asia grouped together in a cluster and other genotypes reported from America and Africa separated in another cluster (Chinnaraja et al 2013).

Leaf fleck

During 1990s suspected occurrence of *Sugarcane bacilliform virus* (SCBV) was reported in *Saccharum officinarum* and other *Saccharum* spp clones of World Sugarcane Germplasm Collection maintained at SBI Research Centre, Kannur, Kerala. Though foliar symptoms indicated the suspected virus it was not clear until confirmation by ISEM studies (Viswanathan et al 1996). These studies gave authentic confirmation on SCBV infection in sugarcane from India. The virus exhibited enormous variation

symptoms on different genotypes of *Saccharum* spp,*Pennisetum* sp and cultivated varieties. ELISA assays helped to detect the virus suspected clones; however, genomic variation in the causative virus could not be brought out during that time. Using the assays the virus infection in sugarcane germplasm was established (Viswanathan and Premachandran 1998). Recently, molecular studies conducted at SBI clearly demarcated five different SCBV species infecting sugarcane and SCBV genome ranged from 7553 to 7884 nucleotides in size. The Indian SCBV isolates share identities of 69-85% for the complete genomic sequence, indicating wide genetic diversity among them, and share 70-82% identity with *Sugarcane bacilliform IrengMaleng virus* (SCBIMV) and *Sugarcane bacilliform Morocco Virus* (SCBMV), as well as 43-46% identity with *Banana streak virus* (BSV) and BSV-related SCBV species and this variation indicates the distinctness of Indian SCBV population. It is concluded that the symptoms associated with badnaviruses in sugarcane in India are caused by at least three new species, SCBBBV, SCBBOV and SCBBRUV, besides SCBIMV and SCBMV (Karuppaiah et al 2013; Karuppaiah and Viswanathan 2012).

Grassy shoot (GSD)

The disease has been recorded in most sugarcane growing areas of India (Rishi and Chen 1989; Viswanathan 2000). This disease is characterized by the production of a large number of thin, slender, adventitious tillers from the base of the affected stools and this seriously affect millble cane production in the field. In addition to the virus and bacterial pathogens, grassy shoot phytoplasma infection can cause 35% reduction in stalk height, 15% reduction in stalk girth, 50-60% reduction in length of the internodes. Above all, 50-75% plant crop infection resulted in 100% failure in millable cane production in the ratoon crop in different varieties in the field (Viswanathan and Rao 2011). Nucleotide sequence analysis of 16S rRNA genes revealed that Sugarcane grassy shoot (SCGS) -phytoplasma affecting sugarcane crop in India is very closely related to Sugarcane white leaf (SCWL) agent and is, thus, a member of the RYD phytoplasma group. SCGS and SCWL phytoplasmas shared a 16S rDNA sequence similarity which varied from of 97.5 to 98.8%. The Bermuda grass white leaf (BGWL) -phytoplasmas and Brachiaria grass white leaf (BraWL) agents share 97.3 and 97.1% 16S rDNA sequence similarity, respectively with SCGS phytoplasmas (Rao et al 2008) and are grouped in the same phylogenetic groups. Although there were significant variations in phenotypic expression of grassy shoot in sugarcane, no genotypic variations could be established (Nasare et al 2007; Rao et al 2008; Viswanathan et al 2011a).

Sugarcane wilt

The disease was reported for the first time in India by Butler (1906) from Bihar. Later the disease was reported to cause damages in the states of Uttar Pradesh, Punjab and Haryana in subtropical India. Butler and Khan (1913) studied wilt in detail and described *Cephalosporium sacchari* as the associated pathogen. Subsequently, several workers reported *Fusarium moniliforme* var *subglutinans* as the causative pathogen. Gams (1971) coined a new species *Fusarium sacchari* (Butler) W. Gams to which both *Cephalosporium sacchari* and *Fusarium moniliforme* var *subglutinans* were made synonyms. Besides *F. sacchari*, Singh and Singh (1974) reported isolation of *Acremoniumimplicatum* and *A. furcatum* from wilt infected samples in subtropical India. However, studies of Viswanathan *et al.* (2006) failed to recover *Acremonium* from nodal tissues from various cane samples from tropical and subtropical regions in India and only *Fusarium* could be recovered from both nodal and internodal tissues. Although wilt of sugarcane is known in India for a long time, very less research work has been done. The causal organism was found to vary with time and investigator and could not reproduce the disease under artificial conditions in the field. Detailed studies of Viswanathan et al (2011b) established the identity, the pathogen diversity of fungi

causing wilt in sugarcane and compared the pathogenic vs nonpathogenic *Fusarium* recovered from sugarcane stalk tissues. Results of pathogenic and molecular variation separated pathogenic *F. sacchari* isolates from non-pathogenic *F. proliferatum, F. napiforme* and *F. subglutinans* isolates and their studies proved *F. sacchari* as the pathogen associated with sugarcane wilt.Phenotypical characterization of the pathogen was carried out at SBI, Coimbatore based on growth rate, pigmentation, texture, nature of phialides and types of conidia produced using 117 isolates collected from 13 sugarcane growing states. It was concluded that the extensive variation in cultural and morphological characters of the isolates is probably due to their origin from varied climatic conditions and hosts in the country (Poongothai et al., 2014a,b). Since conventional mycological techniques were inadequate to clearly discriminate the Fusariums from sugarcane stalk, four molecular tools were employed to distinguish them. In this morphologically distinct isolates formed separate clusters and isolates of *F. sacchari* grouped together in a cluster. Within this cluster, due to intraspecific variation *F. sacchari* isolates were further grouped into many sub-clusters. Species other than *F. sacchari* viz., *F. proliferatum, F. subglutinans* and *F. napiforme* clustered away from *F. sacchari* (Viswanathan et al2012). During the recent years sudden outbreak of *pokkahboeng* across the country was noticed on several varieties. It was found that *Fusarium* sp associated with *pokkahboeng* also causes stalk infections and produces wilt in certain varieties (Viswanathan, 2013a,b). It is likely that application of molecular tools will resolve Fusaria associated with *pokkahbeong* and wilt and epidemiology of wilt in sugarcane, especially on survival of *F. sacchari* and its possible manifestation as foliar as well as stalk disease.

Applications of genomics and proteomics research in sugarcane pathology

Pathogen variation in Colletotrichum falcatum

The frequent break down of resistance in sugarcane varieties under commercial cultivation has been suggesting the possible continuous development of new variants of the pathogens. The causative fungus red rot exhibits a wide array of variation in cultural characters and virulence. Based on the variations in cultural characters and that of fruiting structures, different isolates have been characterized. The development of physiological races has been attributed to hybridization, mutation, conidial and hyphal fushion and heterokaryosis. Earlier variation in the pathogen was broadly grouped as light and dark races. Among them the light race produced abundant conidia and proved more virulent than the dark race. Detailed studies conducted at SBI, Coimbatore revealed a clear variation in serological and vegetative compatibility grouping among *C. falcatum* pathotypes (Viswanathan et al 2003). Currently, the *C. falcatum* isolates are grouped based on their pathogenic reaction on a set of 14 host differentials at 12 sugarcane research centres in the country. Studies conducted so far revealed existence of 11 pathotypes (CF01 to CF11), seven from subtropical region and four from tropical region (Viswanathan 2010).

Molecular variation

As genetic diversity in *C. falcatum* is very rapid and thus confusing, molecular biological tools were used to resolve the variation among the pathotypes. Existence of variation at molecular level in different pathotypes of *C. falcatum* was established by RAPD-PCR techniques (Mohanraj et al 2002; Suman et al 2006). Molecular analyses of a set of nine *C. falcatum* pathotypes representing tropical and subtropical regions showed limited variation in 5.8s-ITS region in nucleotide sequences and they varied in one base pair difference (Malathi et al 2010). However, phylogenetic analysis clearly showed two genetically diverged groups, which almost correspond to the tropical (clade I), and sub-tropical (Clade II) conditions except for one isolate Cf7717 which belonged to tropical pathotypes clade. Malathi et al (2011) and Malathi and

Viswanathan (2012) identified the genetic divergence on the basis of phylogenetic analysis of ITS sequences under three distinct molecular groups as Group I, II and III. Multi-locus analysis with three gene sequences of housekeeping genes like actin, calmodulin and glyceraldehydes-3 phosphate dehydrogenase with their introns for conserved proteins among 25 isolates implicated existence of Group III and least genetic support for the movement of gene sequences between Groups I and II.

Mechanism of resistance in sugarcane to C. falcatum

Although inheritance of red rot resistance has not been explained in detail due to genome complexities of sugarcane, considerable progress has been made to understand resistance mechanism to red rot in sugarcane. Studies conducted during the previous decades indicated possible role of oxidative enzymes, pathogenesis related (PR) proteins and 3-deoxyanthocyanidin phytoalexins in governing red rot resistance. Application of genomic and proteomic tools has led to better understanding of host-pathogen interaction (Viswanathan et al., 2009b). Recent studies involving semiquantitativeRT-PCR assays from sugarcane cultivars varying in red rot resistance post pathogen challenge, revealed differential accumulation of transcripts of the flavanoid biosynthetic pathway like coumarate-4-hydroxylase, chalcone synthase, chalcone reductase, flavanoid 3`-5` hydroxylase and flavanoidglycosyltransferase and this transcript analysis, further confirmed the role of sugarcane phytoalexins in red rot resistance. Similarly, the role of PR-proteins like chitinase and β-1,3-glucanase was established at the transcript level. Detailed molecular analysis applying differential display (DD)-RT-PCR identified expression of more number of differentially expressed transcripts during the host pathogen interaction. Further northern and reverse northern blot analyses using radioactive α [^{32}P]dCTP confirmed differential expression of potential DD transcripts. Full length sequences of many potential transcripts were identified and are being functionally annotated. Recently subtractive libraries were developed to identify specific transcripts involved in red rot resistance in sugarcane (Viswanathan 2010, 2012b; Viswanathan et al, 2014a).

Defence related transcription factors involved in host-pathogen interaction are also identified (Muthumeena et al., 2013). Protein extraction protocols for 2-dimentional electrophoresis (DE) from sugarcane stalk tissues were standardized to identify specific proteins involved in host resistance in sugarcane (Ramesh Sundar et al., 2010). Recently ~125 up/down regulated proteins during host-pathogen interaction were characterized by peptide mass finger printing, some of the identified important proteins were putative callose synthase, R2R3-MYB transcription factor MYB6, p-coumarate 3-hydroxylase, PrLTP1 and PISTILLATA-like protein (Viswanathan 2012b). These studies have established that both genomic and proteomic analyses have the potential to provide significant insights into the molecular events that occur during sugarcane–*C. falcatum* interactions. Further validation of the differential expression of identified proteins/transcripts by qPCR and RNA blots are in progress.

Disease diagnosis in sugarcane

Vegetative propagation in sugarcane favours transmission of various diseases through planting materials. Incipient infections of *Colletotrichum falcatum* and *Sporisorium scitamineum*, causing red rot and smut, respectively are very common in the planting materials (setts). These infections go unnoticed during planting and this favours introduction of the diseases in the field. However, non-fungal diseases caused by bacteria, virus and phytoplasmas accumulate systemically in the stalks and depending on the pathogen titre varying symptoms occur in the field. Hence planting disease-free materials is recommended to manage the disease in sugarcane. In recent years there is an increased production and use of tissue culture

derived seedlings to multiply new sugarcane varieties. In tissue culture also, if mother plant is infected progrs will be uniform disease introduction in the field through the seedlings. This will have a catastrophic effect on crop hygiene and performance. At SBI for the past two decades, detailed studies were conducted to develop sensitive diagnostic tools to detect sugarcane pathogens. Initially serological techniques such as ISEM, ELISA, FAT, dot-blot and tissue-blot were standardized for the diagnosis. Recently different molecular techniques were standardized for the sensitive diagnosis of sugarcane pathogens.

Viral pathogens

Earlier, serology based diagnostic techniques were followed to detect sugarcane pathogens at SBI, Coimbatore (Viswanathan, 1997). However, when compared to molecular diagnostic approach like RT-PCR, ELISA technique was found to be less sensitive (Balamuralikrishnan et al 2004). Hence in the recent years molecular techniques are preferred in sugarcane quarantine. After detailed molecular characterization of the viruses diagnostic techniques for SCMV, SCSMV, SCYLV and SCBV were standardized (Balamuralikrishnan and Viswanathan 2005; Karuppaiah and Viswanathan 2012; Viswanathan et al 2007, 2008a, 2009c; Viswanathan andKaruppaiah, 2010). Further studies were conducted to detect both SCMV and SCSMV associated with mosaic viruses in a single reaction. For this a new set of primers were designed from the coat protein region of the viruses to suit duplex-RT-PCR and the conditions were standardized to amplify the target viruses in this assay (Viswanathan et al 2008c). Similar assay was developed for the simultaneous detection of SCSMV and SCYLV in sugarcane.

The efficiency of the SCYLV diagnostic primers in RT-PCR assay was validated with a set of sugarcane samples collected before and after yellow leaf symptom expression and established that 97.73 % of the samples were found to be infected with SCYLV and the diagnostic primers efficiently detected all the SCYLV population even in asymptomatic plants (Viswanathan et al 2009c). A multiplex-RT-PCR was developed for the detection of SCMV, SCSMV and SCYLV, three of the major RNA viruses widely prevailing in the sugarcane growing regions across the globe. The primers designed from the respective viruses were able to specifically amplified fragments of ~860 bp (SCMV), ~690 bp (SCSMV) and 615 bp (SCYLV) of the target viruses in sugarcane and sugarcane aphid, *Melanaphis sacchari* in multiplex-RT-PCR (Viswanathan et al 2010).

Recently SCSMV coat protein (CP) was expressed in an expression vector along with maltose binding protein (MBP) as a fusion protein (Viswanathan et al., 2011c). Later polyclonal antisera were produced against recombinant SCSMVcp and the serum detected the recombinant MBP-SCSMV-CP fusion protein upto 1:40,000 dilutions in direct antigen coating (DAC)-ELISA and sensitivity of the antiserum was found to be as low as 2.5 ng in ELISA. Further, in the assays the serum detected the virus antigen from infected leaf extracts diluted to 1:10,000. Subsequently the efficiency of the new antiserum was validated with 349 leaf samples collected from 332 sugarcane cultivars showing asymptomatic to varying degrees of mosaic/streak mosaic symptoms by DAC-ELISA. This assay established that the SCSMVcp antiserum is highly efficient to detect SCSMV infection in naturally infected / asymptomatic sugarcane field samples (Viswanathan et al., 2013a).Subsequently a duplex immunocapture reverse transcription (IC-RT) PCR assay sensitive to detect both SCMV and SCSMV was standardized (Viswanathan et al., 2013b). Although DAC-ELISA is simple and cost effective to diagnose these viruses, in samples with very low virus titre, IC-RT-PCR would be more sensitive. Recently quantitative PCR (q-PCR) assays were developed to detect exact copy number of the viruses in the plant tissues. In sugarcane reverse transcriptase (RT)-qPCR assay was

standardized to assess the virus population in asymptomatic plants and meristem culture derived seedlings (Chinnaraja et al., 2014). This assay very clearly established that meristem culture either completely eliminate the virus population in sugarcane seedlings or near to complete elimination of the virus.

Phytoplasma/bacteria

The phytoplasma associated with GSD was detected in sugarcane by ELISA and immunofluorecence. Later, universal phytoplasma-specific primer pairs were used for phytoplasma detection in sugarcane and its reported leafhopper vector *Deltocephalus vulgaris* (Srivastava et al 2006; Viswanathan et al. 2005, 2011a). DAC-ELISA techniques were found reliable for the detection of RSD in infected sugarcane samples in India (Viswanathan, 2001). Dot-blot as well as tissue blot immunoassays were developed to detect directly the bacterial infection from the field samples (Viswanathan 2004, 2012a). Ratoon stunting (RSD) bacterium *Leifsoniaxylli*subsp*xylli* is also detected using a set of nested primers. However, for our routine diagnosis of RSD, dot-blot or tissue blot techniques are followed due to their simplicity and specificity. An ELISA based assay was standardized for the detection of *Xanthomonas albilineans* causing leaf scald (LSD) in sugarcane (Viswanathan et al 1998a). Later polyclonal antiserum was produced against the bacterium and it was found to be highly sensitive to detect the low bacterial titre in Indirect ELISA (Viswanathan and Ramesh Sundar 2004).

Fungal pathogens

Since the polyclonal antisera were not completely specific to *C. falcatum*, specific antisera to 101-kDa polypeptide of *C. falcatum* were developed. In ELISA, the tissues such as buds, root eyes and leaf scar were found to be more frequently colonized by red rot pathogen in high titre in an infected stalk (Viswanathan et al 1998b). Recently Malathi and Viswanathan (2012) identified *C. falcatum* specifically as compared to other species of *Colletotrichum*. Results on PCR amplification with three specific primers and they were able to detect the *C. falcatum* specifically in mixed state of infection in sugarcane. The infection of smut fungus can be detected well before symptom expression of the disease through trypan blue staining and this technique is useful to identify the symptomless plants (Nallathambi et al 1998). Recently PCR assays were developed to detect smut infections in sugarcane at SBI. The assays targeted an amplicon of 459 bp of *bE* mating type gene. When compared to histopathological examination of apparently healthy tissue samples by employing microscopy, this PCR-based method was found to be more rapid and early in detecting the latent infection (Ramesh Sundar et al., 2012)

Disease Management

After YLD attained epidemic status, serious efforts were made to manage the disease under field conditions. The studies revealed that meristem tip-culture combined with molecular diagnosis was found to be more appropriate to eliminate the virus from mother plant and tissue culture seedlings derived through this process performed well under field conditions due to freedom of the virus (Viswanathan and Rao, 2011). Detailed studies conducted by the author have established varietal degeneration in sugarcane in India due to non-fungal diseases and this is amenable for management (Viswanathan, 2012c). The pathogens causing RSD and GSD can be eliminated through heat treatment from the infected canes. Alternatively, use of tissue culture derived seedlings also eliminates these pathogens from the infected seed canes. Careful management of seed canes is essential to manage the diseases under Indian conditions. The varietal degeneration is also addressed by replacing the sick varieties.However, replacing well established

commercial varieties will be a huge task and the success depends upon availability of suitable repl ement varieties.

Conclusion and future strategies

Since growing resistant varieties remain the mainstay in disease management and there is a ced to scientific introduction of varieties in the field. Recently a large number of red rot and smut resistant rieties are identified and they need to be adopted in endemic regions in the country to manage the d caused Management strategies were already developed to reduce severity of *pokkahboeng* and rust. Possil lity o same fungal pathogen causing *pokkahboeng* and wilt has emerged in the recent studies conducted at NI (Viswanathan et al., 2014b). However, further studies are required through molecular markers to cor olotel characterize the Fusariums associated with stalk and foliage tissue damages in sugarcane and their elatio to disease(s) epidemiology

New effective fungicides were identified against red rot. The major concern of poor fungicid uptak inside the setts is addressed by developing mechanized treatment device and it needs to be demo strate under field conditions. Varietal degeneration in sugarcane affects longevity of proven varieties and manage this, meristem culture combined with virus diagnosis is established. Multiplication of dise e-fr seedlings through tissue culture needs new thrust. Hence, industry associations should come for ard create common tissue culture laboratories to produce healthy seedlings. The sugar industry and tissue cultu production units may utilize virus-indexing service from SBI to produce disease-free planting m erial Establishing disease-free nursery chains of sugarcane varieties across the country would facilitate ealth sugarcane in the field and varietal vigour will also be maintained in the long run. This process wo d ul ensure development of healthy planting materials supply-chain and sustain sugarcane productivit in th country.

Future research should most likely be focused on management of the disease, through ide tifyin disease resistance in germplasm and developing resistant varieties through conventional breeding t ul using biotechnological methods. Also more focus to be given on the impact of climate change i ues build-up of disease epidemics and increase in severity of minor diseases in sugarcane.

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Table 1 Current sugarcane disease situation in different sugarcane growing states in the country

State	Major fungal diseases	Minor fungal diseases	Non-fungal diseases
Uttar Pradesh	Red rot- Trace to moderate (1-20%) in CoLk 8102, CoSe 92423, CoSe 95422, CoS 91269, CoSe 95436; smut - trace to moderate (1-10%) in Co 0238, Co 0239, CoS 88230, CoSe 92423, CoS 97264, CoSe 01235, CoSe 01424, CoSe 96436, CoSe 96275, CoSe 98231, CoS 767, CoS 95255, UP 9530, BO 91 and 35-40% in CoSe 98231; wilt - trace (1-2%) in CoSe 92423, UP 9530	Moderate *pokkahboeng*in CoS 92423, Co 0238	Leaf scald in CoLk 8102, Co 0238; GSD in CoS 767, Co 0238, CoSe 92423, CoLk 94184, CoSe 98231, CoS 95255, CoS 8436, UP 9530, UP 0097, CoS 97261; RSD in CoS 767; Bacterial soft rot in CoSe 92423
Punjab	Red rot - trace to 8% in CoS 8436, CoJ 64, CoJ 85; traces of smut and wilt in Co 89003;	Traces to 3% *Pokkahboeng* in Co 0238, and traces to 2% red stripe/top rot in CoJ 85	Moderate GSD in Co 0238
Bihar	Red rot along with wilt in CoS 91269, CoS 8436, BO 145 and wilt alone in Co 0118, BO137, BO 147, Co 0235; smut - traces BO 137, BO 147, CoS 8432, CoSe 98231 and Co 0238;	*Pokkahboeng* in BO 137, BO 147, CoLk 9418, Co 0238, Co 0235, BO 141; Sett rot in BO 141	Spike disease in CoJ 64
Haryana	Red rot - Traces to 40% in CoS 8436; smut - traces (1%) in Co 89003, Co 0238, CoH 119, CoH 150, CoH 133, CoH 116, Co 0116; wilt - in Co 7717, Co 767, Co 1148, CoJ 64, CoH 119;	Top rot was observed in CoH 152, CoS 8436, Co 0238, CoJ 85 and traces to 12% of *Pokkahboeng* in CoH 151, CoH 119, CoH 152, CoS 8436	GSD in CoS 8436, CoH 152, CoS 767, Co 89003; YLD in Co 84212, CoS 8436, Co 89003, CoH 119, CoJ 64
Uttaranchal	Red rot - traces in CoPant 97222, CoS 8436, CoS 8432, CoPant 99214, CoS 767, CoPant 99259, Co 1148; smut mild	Low to mild *Pokkahboeng*andbanded sclerotial disease	Mild incidences of RSD, GSD, YLD
Madhya Pradesh	Red rot - 1-9% in CoJ 64;	Highest (5-7%) *pokkahboeng* in Co 99004	GSD in Co 62175 and YLD in traces
Gujarat	Red rot - traces in Co 86002, Co 86032, Co 92020, CoM 9011 and severe in CoC 671;	*Pokkahboeng* observed in Co 99004	GSD in Co 86032 and YLD in Co 86032, Co 99004

	smut - severe (11%) in CoSi 95071, Co 86002 and also observed in Co 97009, Co 99004, CoN 07071; wilt - moderate (6%) in CoC 671, Co 86032, Co 86002, CoSi 95071. Maximum red rot and wilt occurred in CoC 671		
Tamil Nadu	Red rot - severe (10-25%) in Co 92012, CoSi 6, Co 87012, Co 97009, CoV 09356; smut - moderate to severe (10-15%) incidences of smut in Co 97009, CoC 22, CoA 92081; wilt - moderate (10-15%) in Co 86032	-	Severe YLD in Co 86032, C[illegible] 92102, CoV 09356
Andhra Pradesh	Red rot - 10-40% in Co 62175, 81 A 99, 93 V 297, 81 V 48; smut - severe (10-60%) in CoA 92081, CoV 05356, 91 V 83, 97 R 83; wilt - severe (10-40%) in Co 8368, 87 A 380, Co 7219, 91 V 83, CoA 92081, Co 62175, 81 A 99	Top rot, ring spot and rust	Severe YLD and GSD
Karnataka	smut in Co 8011, Co 740, Co 86032	Severe rust in CoM 0265, CoC 671, Co 8011, Co 86032, Co 2001-15, Co 94012, Co 740	Severe YLD in 86032 and low i[illegible] Co[illegible] 671, Co 92005; GSD in Co 940[illegible]2
Maharashtra	smut - 5% in Co 7527 and 10-12% in Co 86032, Co 7219 &CoC 671	Severe rust throughout in CoM 0265, CoVSI 9805, Co 86032, Co 92005, CoC 671, Co 94012; Co 7527, CoVSI 434; *Pokkahboeng*in CoVSI 9805, CoC 671, Co 7527, Co 94012, CoM 0265, CoVSI 434, Co 86032; Eye spot in CoM 0265, CoC 671, Co 7527, Co 94012, Co 8014, Co 740, Co 7219, Co 86032; Brown spot in CoM 0265, Co 86032; Ring spot also observed	YLD in Co 86032; Moderate GS[illegible] throughout in CoC 671, Co 8603[illegible], C[illegible] 8014, CoM 0265, Co 94012, VSI 434 severemosaic in Co 740, Co 7219 Co 94012, CoC 671, Co 86032, VSI 434; banded chlorosis in CoC 671, Co 86032, CoM 0265
Kerala	-	Ring spot and *Pokkahboeng*	Mild YLD and mosaic