Functional Plant Science and Biotechnology ©2012 Global Science Books

Sugarcane Wilt: New Insights into Pathogen Identity, Variability and Pathogenicity

Rasappa Viswanathan* • **Muthusamy Poongothai** • **Palaniyandi Malathi** • **Amalraj R. Sundar**

Sugarcane Breeding Institute, Indian Council of Agricultural Research, Coimbatore 641007, India

Corresponding author: * **rasaviswanathan@yahoo.co.in**

ABSTRACT

Wilt of sugarcane, a fungal disease is known to cause significant damage to sugarcane production and productivity in India and other countries for the past one century. Although sugarcane wilt is known in India for a long time, research work on this important disease is totally lacking. The causal organism was found to vary with time and investigator and could not reproduce the disease under artificial conditions in the field. We have made detailed disease surveys in 13 major sugarcane growing states in the country and 263 *Fusarium* isolates were isolated. We have established the variation in *Fusarium* isolates associated with sugarcane wilt, based on cultural, morphological, pathogenic and molecular characterization of 117 isolates. Critical observations in the conventional techniques combined with molecular biological approaches clearly established that *Fusarium sacchari* as the causal agent of the disease. Other *Fusarium* sp isolated from wilt infected sugarcane stalks were found to be either secondary invaders or non-pathogenic in nature. We have developed an artificial simulation technique to induce wilt in sugarcane under field conditions and efficient management strategy through biocontrol was also developed.

Keywords: *Fusarium sacchari*, IGS-RFLP, ITS, ISSR, RAPD
Abbreviations: IGS-RFLP, inter-generic sequences-restricted fragment length polymorphism; **ISSR**, inter simple sequence repeats; **ITS**, internally transcribed region; **RAPD**, randomly amplified polymorphic DNA

CONTENTS

INTRODUCTION 30
DISEASE SITUATION IN INDIA 31
SYMPTOMATOLOGY 31
IMPACT OF WILT ON SUGARCANE 31
ASSOCIATED PATHOGENS 33
Pathogen variability 33
EPIDEMIOLOGICAL STUDIES 34
Abiotic factors 34
Biotic factors 34
SCREENING FOR WILT RESISTANCE 36
MANAGEMENT 37
Cultural practices 37
Disease surveillance 37
Chemical control 37
Use of resistant varieties 37
Biological control 37
CONCLUSIONS 38
REFERENCES 38

INTRODUCTION

Butler and Khan (1913) for the first time described a stem rot disease in India in sugarcane under the term 'wilt' and noted *Cephalosporium sacchari* as the causal agent. However, Bourne (1922) recorded a stem rot disease of the basal portions of unwounded sugarcane stems having a species of *Fusarium* associated with the disease in Barbados. During the same period, Fawcett (1922) also recorded a *Fusarium* sp. associated with a dry rot and ratoon rot of this crop in Argentina. Earlier Nowell (1920) reported the widespread occurrence of the disease in Barbados without causing much loss to crop whereas in the island of Nevis, the damage was more severe and the effects of the disease were similar to those of red rot. Bourne (1933) also noted a stem disease in Florida which was causing a 'wilt' disease of seedling varieties similar to that originally described by Butler and Khan (1913) in India. Abbott (1932) noted a purple species of *Fusarium* constantly associated with diseased cuttings in Louisiana and also reported the presence of a purple and white strain of *Fusarium* in seed cuttings. The purple form of *Fusarium* was also common in growing stalks following borer injury. Rands and Abbott (1938) reported a purplish coloured rotting of both standing crop and seed cane in Louisiana due to *Fusarium.* Banked cane, badly bored seed cane and cuttings planted in low, wet areas were found to be more susceptible. Ayson (1938) recorded the *Fusarium* stem rot in standing canes, seed canes and stubbles and

Received: 12 April, 2011. Accepted: 12 December, 2011.

Invited Review

suggested that it was the same disease Butler and Khan had described in India. Bourne (1953) recorded the *Fusarium* stem rot as prevalent during the period 1949-52 in the Everglades region of Florida, and considered it was just as important to breed canes resistance to it as to red rot itself. Wilt epidemics during early decades of the last century resulted in elimination of many commercial varieties like 'Co 245', 'Co 321', 'Co 527', 'Co 951', 'Co 1107' and 'Co 1223', from cultivation (Kirtikar *et al*. 1972). In parts of Tamil Nadu in tropical India, the disease caused serious damage during 1955-56 to varieties: 'Co 419', 'Co 449', 'Co 453', 'Co 527' and 'Co 1122'. In many instances, complete loss to cane production had occurred, as in 'CoC 671' in Gujarat state, in 'Co 89003' in Punjab and in other varieties in Bihar state (Agnihotri and Rao 2002). Country-wide disease assessment in India revealed that wilt of 60% on 'Co 7717', 5-10% in 'CoJ 64', 'CoJ 79' and 'CoS 767' in Uttar Pradesh, severe wilt incidence in combination with red rot noticed on major varieties in Bihar, severe wilt incidence on 'Co 89003' and moderate wilt on 'Co 7717', 'CoS 8436' and 'CoS 88230' in Punjab, varying levels of wilt in most of the varieties in cultivation viz., 'Co 85002' 'Co 85004', 'Co 85019' 'Co 86002', 'Co 86010' 'Co 86032', 'Co 97009' 'CoC 671', 'CoC 91061' 'CoJn 86141', 'CoSi 95071' and 'CoSi 98071' in South Gujarat, mild wilt on 'Co 740', 'Co 7257', 'Co 8011' 'CoC 671',and 'Co 86032' in Maharashtra, 5-19% wilt incidence recorded in 'Co 1148', 'CoLk 8001', 'CoC 671', 'Co 1305' and 'Co 1307' in Madhya Pradesh. Several commercial varieties were withdrawn from cultivation in North and southern parts of India due to wilt (Agnihotri and Rao 2002; Viswanathan and Rao 2011).

DISEASE SITUATION IN INDIA

The disease had prevailed to varying intensities for the past 100 years in India. Although there were reports on the disease incidences in different periods, only recently Viswanathan *et al*. (2006) made detailed surveys in all the sugarcane growing areas and they reported that the disease severity is highest in Gujarat state, east coastal regions in Tamil Nadu, Andhra Pradesh and Orissa in tropical India. In subtropical regions, Bihar, Uttar Pradesh, Haryana and Punjab states showed severe incidence of wilt (**Fig. 1**). They recorded wilt in most of the sugarcane varieties under cultivation in endemic locations. The most affected varieties were 'Co 7805' and 'Co 86032' in Andhra Pradesh and Orissa, 'Co 86032', 'Co 85036', 'CoSi 95071' and 'CoC 671' in Gujarat, 'Co 89003' in Punjab and Haryana, 'CoJ 64' and 'CoS 767' in Uttar Pradesh. Trace incidence of wilt was observed in different varieties in Tamil Nadu and Maharashtra. In Bihar most of the varieties exhibited wilt disease. They also reported that the disease incidence ranged from 5 to 30% in cultivated varieties under subtropical conditions. Sugarcane cv. 'Co 89003', a major variety in Punjab, Haryana, Uttar Pradesh and Uttarakhand exhibited wilt incidence in all the surveyed fields and had a maximum of incidence of 30%. The other major varieties, 'CoJ 64' and 'CoS 767', in these states recorded a maximum incidences up to 10%. However, at many locations newer varieties under multiplication or recent introductions *viz*., 'Co 0121', 'Co 86032' and 'CoV 94102', recorded very severe disease intensities up to 75%. In subtropical regions disease intensity of more than 5% was noticed in Punjab, Haryana, Uttar Pradesh and Bihar except Uttarakhand and Madhya Pradesh where the disease intensity was low. In tropical regions, higher disease intensities of 5 to 60% on 'Co 7805' and 'Co 86032' were detected in different locations of Andhra Pradesh and Orissa. In general, the disease intensity was low in other parts of peninsular India. Here only in very few locations very severe incidence was noticed which was always influenced by different biotic and abiotic factors.

SYMPTOMATOLOGY

As other crops showing wilt symptoms, leaves of affected stalks gradually turn yellow and dry. Symptoms first appear on 4-5-months-old sugarcane. Eventually plants become completely dry and die leaving hollow stalks leading to loss of millable canes. Externally the affected canes show shriveling and breaking of stalk at any point depending on the severity. Disease found to be severe in fields having stress conditions which may be biotic or abiotic. Typical internal symptoms can be observed after splitting the diseased stalks longitudinally. The internal pith tissue, particularly of the lower internodes, is light to dark purplish-brown. Varying shades of pinkish red or brownish red tissue discoloration can be seen in the internodes (**Figs. 2, 3**). The intense discoularation of tissues at the nodes might be due to the profuse ramification of fungal hyphae in the tissues immediately above the growth ring. The vascular tissues appear as dark reddish-brown streaks that pass through the internodal tissues from one internode to another. The wilt affected canes do not emit a foul odour and there are no white spots in internodal tissues that are typical of red rot infection caused by *Colletotrichum falcatum*. However, shrinkage of internodal tissues takes place due to the loss of moisture resulting in the formation of longitudinal, spindle-shaped cavities in the middle of the internodes (**Fig. 4**). Tissue discolouration and boat-shaped cavity formation are relatively more prevalent in the affected canes and these are characteristic diagnostic symptoms of the disease. Initially, the roots of affected plants do not display distinct symptoms but subsequently the affected roots die and vascular bundles of the shoot become brownish as the stalks die. Wilted canes may also show root borer tunnels with borer larva inside, mostly at lower internodes. In association with red rot, we cannot distinguish the symptoms as the infected stalk found to show symptoms of both the diseases.

IMPACT OF WILT ON SUGARCANE

Wilt is a serious constraint to sugarcane production in India and is next to red rot caused by *C. falcatum* in causing economic losses. During 1965-1967 wilt caused severe damage to sugarcane crop in the Deccan plateau. Edgerton and Moreland (1920) working with the white strain of *Fusarium* in Louisiana got consistent reductions in germination of cuttings from both top and bottom halves of 'D74' and purple varieties which had been inoculated. In one inoculation test with 'POJ 213' in Louisiana, Abbott (1935) showed that the purple strain of *Fusarium* reduced the germination 41% below that of the control. However, since red rot was also present, the organism was regarded as only weakly pathogenic.

Losses due to wilt are usually computed on the basis of quantum of canes, dried or dead, found in the field, after harvest and they may vary from 2 to 10 tonnes/ha (Parthasarathy 1972). Diseased plants produce less number of tillers as compared to healthy ones (Agnihotri and Singh 1989). In association with stalk borer, the disease has been reported to bring a loss of about 8.75 tonnes per hectare (Kulshreshtha and Avasthy 1959). Sarma (1976) reported that loss in the yield might go as high as 65% and the incidence of the disease is more in ratoon as compared to the plant crop.

Wilt fungus in association with some insect pests of sugarcane particularly stalk borer and scale insects causes significant damage to the crop. Singh (1973) reported that the decline in weight was 24.9%, when the mean incidence of stalk borer-wilt complex was 51.4%. Waraitch (1981) reported high incidence of wilt (90%) in association with stalk borer in cultivar 'Co 1148' and the crop was almost unfit for milling. Conservative estimates of loss of 3-6 tonnes/ha, the disease may cause a loss of 12.7-25.4 million tonnes annually in different years. Hence the loss to sugarcane production would be between Rs. 12500-25000 million rupees per annum in India (Viswanathan *et al*. 2006). The loss in production is accounted to the farmers and the

Fig. 1 Severely wilt infected sugarcane crop showing complete drying in subtropical India. **Fig. 2** Internal symptoms: Wilt infected sugarcane show pinkish discolouration at the nodal region. **Fig. 3** Internal symptoms: Wilt infected sugarcane show brownish discolouration at the internodal region. **Fig. 4** Internal symptoms: Typical wilt symptoms of brownish/pinkish tissue discolouration with boat shaped cavities in the internodes. **Fig. 5** Variation in cultural characters of *Fusarium sacchari* isolates on potato dextrose agar. Left: mycelial growth pattern among 9 isolates from Andhra Pradesh. Right: pigmentation pattern on the reverse side of the plates. **Fig. 6** Sugarcane stalks showing combined infections of red rot and wilt. **Fig. 7** Root borer infested canes showing typical symptoms of wilt. **Fig. 8** Artificial simulation of wilt under field conditions by plug method of pathogen inoculation. Reproduction of typical wilt symptoms in cv 'Co 95020' in Gujarat. **Fig. 9** Artificial simulation of wilt under field conditions by plug method of pathogen inoculation. Reproduction of partial wilt symptoms in cv 'Co 975' in Karnal. **Fig. 10** Bioassay of *Trichoderma* isolate against *F. sacchari* in Petri plates. *Trichoderma* isolates T1-T10 corresponding to 1-10 were tested against *F. sacchari* isolate Fs 032 TN4L2 in potato dextrose agar (PDA) medium. PDA plate with only *F. sacchari* without antagonistic fungi served as control (C). Growth rate of both the fungal cultures and inhibition were recorded 10 days later. The plates were incubated at 28°C.

sugar industry. The impact of wilt infected canes on sugar loss in the mill is not assessed properly and sugar mills experience these unaccounted losses every year.

The deterioration in juice quality is due to the decrease in sucrose content (Khanna and Chacravarti 1949) and an increase in reducing sugars, gums, titrable acidity, flavonoids and soluble salts (Singh and Waraitch 1981), which adversely affect processing of white sugar in the mills. Besides reduction in cane yield, the wilt causes 14.6 to 25.8% reduction in juice extraction and 3 to 20% in sugar recovery (Gupta and Gupta 1976). Subba Raja and Natarajan (1972) recorded 9.97% reduction in recovery, when the disease incidence was only 6% in commonly cultivated canes – 'Co 658' and 'Co 449'. Mill tests at Motihari showed sugar recoveries to lower by 9.97% when the crop crushed had 6% incidence of the disease whereas at Motipur (Bihar) it was lower by 10.61% with 13% wilt affected canes.

Although wilt pathogen causes enormous damage to sugarcane, research work on wilt of sugarcane had received limited attraction among the sugarcane pathologists in India. Mostly scientists prefer to work on red rot or smut owing to the relative advantage in reproducing these diseases and the pathogens are well characterized. Wilt either alone or in combination with red rot causes significant damage to cane yield and quality. Synergistic activity of red rot and wilt pathogens on many varieties is well established and also wilt aggravates red rot susceptibility in some of the varieties. Moreover the loss caused by the pathogen is witnessed mostly during harvest and wilt infected canes are counted as dead canes; hence impact of the disease infection in the main field is ignored. So far no detailed study has been conducted to assess the actual loss caused by the wilt pathogen in sugarcane in India (Viswanathan, unpublished).

Losses caused by wilt go unnoticed in the field since the disease infection takes place after maturity stage of the crop. The loss could be noticed only at the time of harvest hence under normal situations the crop is harvested as healthy one but still it suffers from the disease. However in epidemics, disease incidence is noticed during grand growth phase i.e., 5 to 6 month canes. The studies of Viswanathan *et al*. (2006) revealed that disease prevails in sugarcane in all the sugarcane growing regions in the country to varying intensities. The fungi associated with wilt were isolated and were characterized based on cultural, morphological, pathogenic and molecular characters.

ASSOCIATED PATHOGENS

Wilt in sugarcane is caused by the fungal pathogen, *Fusarium sacchari* (Butler) W. Gams. 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. Later Nirenberg (1976) distinguished two varieties of *Fusarium sacchari* namely, *F. sacchari* var *sacchari* and *F. sacchari* var *subglutinans*, the former having mostly aseptate conidia in the aerial mycelium, no sporodochia, while the latter with 1-3 septate conidia, macroconidia more commonly formed often in sporodochia. Besides *F. sacchari*, Singh and Singh (1974) reported isolation of *Acremonium implicatum* and *Acremonium furcatum* from wilt infected samples in subtropical India. However, no further reports are available on the association of *Acremonium* sp. with sugarcane wilt. Studies by Viswanathan *et al*. (2006) at SBI, Coimbatore on pathogen recovery revealed that out of 95 different wilt-infected samples subjected for isolation, only 53 samples yielded wilt pathogens and all are found to be species of *Fusarium* and *Acremonium* was not isolated in any of the samples. Of the 53 varietal infected samples, 18 yielded wilt fungi in both nodal and internodal tissue samples. However, in the remaining cases the fungus could be isolated either from nodal or internodal samples. In 24 of 53 varieties, wilt fungi could be recovered only from nodal samples whereas only in 11 varieties recovery could be made from internodal samples. Their studies clearly indicated that the pathogen colonized nodal tissues at a higher level since 42 of 53 samples are colonized at the nodes.

Pathogen variability

Detailed studies have been carried out on phenotypic and genotypic variability at this Institute (Viswanathan *et al*. 2009). Phenotypical characterization of the pathogen was done based on growth rate, pigmentation, texture, nature of phialides and conidia produced. Cultural characteristics of 117 isolates have been studied and they were grouped in to three groups based on the growth rate. Based on the mycelial colour, the isolates were categorized into 7 groups viz., white, orange, orange-pink, pink, dark pink, pinkish violet and reddish brown (**Fig. 5**). More than 75% of the isolates showed typical pinkish pigmentation and other cultures exhibited varying shades of pinkish pigmentation. However, when reverse pigmentation was compared, the isolates were further categorized into 21 groups. Morphological characterization of 50 isolates was recorded. Both micro and macroconidia were observed at higher frequencies in some cultures and at low frequencies in other cultures. Individually also the frequency varied either high or low. Studies based on morphological examinations of phialides, conidial arrangement and spore germination indicated that the isolates may be mono- or polyphialidic or in chains (Viswanathan *et al*. 2009). The extensive variation in cultural and morphological characters of the isolates may probably due to their origin from varied climatic conditions and hosts in the country. However, Nelson (1994a) reported that mere cultural and morphological data based on growth and reproductive structures produced are insufficient to characterize the isolates.

As the phenotype of the pathogen depends on varying environmental conditions, reliability and repeatability of the identity as seen earlier is doubtful. Molecular tools based on DNA analyses are being currently used as an alternative to cultural and morphological characters to characterize the variants of fungal species. Additionally the use of molecular tools for characterization would save time and are more reliable compared to phenotypic characters which vary with time and conditions. Hence further molecular studies were conducted with 50 isolates of the 117 isolates, characterized initially. In all the four different molecular tools viz., sequencing of internally transcribed region (ITS), randomly amplified polymorphic DNA (RAPD), inter-generic sequences-restricted fragment length polymorphism (IGS-RFLP) and inter simple sequence repeats (ISSR) used for characterization, 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 subclusters. In 3 different molecular tools used viz., RAPD, IGS-RFLP and ISSR, the chain-forming species separated in a cluster. In RAPD, except FsSi 071 TN1, 5 other chain-forming isolates were grouped in a cluster. In IGS-RFLP the 6 chain forming isolates separated from *F. sacchari* in small groups of 3 clusters viz., cluster I, III and IV. In ISSR, all the 6 chain-forming isolates were grouped together in a cluster. rDNA ITS sequencing did not separate the *F. moniliforme* isolates rather it grouped them together with other isolates. However, rDNA ITS sequencing helped in confident prediction of species other than *F. sacchari* in a separate group. Species other than *F. moniliforme* viz., *F. proliferatum*, *F. subglutinans* and *F. napiforme* clustered away from *F. sacchari.* The isolate FsNG 159 K4 which matched with *F. subglutinans* in its ITS sequence separated in RAPD and ISSR but not in IGS. The isolates Fs 805 AP1L1 and FsV 048 AP1 that were similar to *F. proliferatum* separated in ISSR but RAPD and IGS failed to separate FsV 048 AP1. FsV 048 AP3 that formed chlamydospore separated in ISSR but this isolate was grouped together with *F. sacchari* in RAPD and IGS RFLP. FsBln 175 B2 that produced papillate macroconidia matched with *F. napiforme* in ITS sequence and this isolate separated in all the 4 molecular tools used. However, the basis of intraspecific variation within *F. sacchari* isolates could not be brought out (Poongothai 2010). As many reported works on molecular characterization using different molecular tools correlate with secondary metabolite production like gibberellins or toxin production like Fumonisin, trichothecene, zearlenone, etc. (Nelson *et al.* 1994a, 1994b; Mitter *et al*. 2002), the basis for intraspecific grouping in our *F. sacchari* isolates might also be chemotypic which further has to be studied in detail. In our studies cultural and morphological characters of the

pathogen also correlated with the dendrograms constructed, however pigment production had no influence on the dendrogram. Grouping of isolates in our dendrogram was mainly based on morphology and pathogenicity. However source of sample viz., internodal or nodal origin, soil and source variety had no influence on grouping of the isolates. To some extent, geographical origin of the pathogen also correlated with the grouping. The degree of polymorphism (98%) was greater for ISSR, compared to the other two methods which was also reflected in ISSR dendrogram with the least similarity value of 0.51. RAPD and IGS-RFLP had least similarity of 0.57 and 0.58 respectively and the percent of polymorphism was 97 and 92%, respectively. The grouping of isolates in the dendrogram and the percent of polymorphism observed implies that ISSR is a more reliable technique compared to RAPD and IGS RFLP. Correlation of the dendrogram generated by cultural and morphological characters with the molecular dendrograms revealed that the dendrogram generated by phenotypical data separated the different species to a limited extent however not all the isolates that belong to different species were grouped out by cultural and morphological data. Our grouping based on RAPD, IGS-RFLP, ISSR and ITS sequencing which confidently proved that the isolates that belong to species other than *F. sacchari* formed a cluster. The reason for higher polymorphic pattern in the tools used is probably due to collection of the isolates across the country and wide variety of sugarcane hosts used for isolation of the pathogen. This is the first time that a report is being made using different molecular tools with such a high degree of polymorphism among the isolates collected and a clear cut demarcation of *F. sacchari* from the isolates that belonged to other species and this very clearly proves that *F. sacchari* is the causal agent of sugarcane wilt in India.

EPIDEMIOLOGICAL STUDIES

The pathogen(s) is primarily transmitted through infected seed canes in the field. Also the fungus surviving in sugarcane debris in the soil serves as a source of inoculum to infect sugarcane from soil. The wilt fungus can survive in soil for 2.5 to 3 years. Secondary spread from field to field occurs through rain and irrigation water. Wilt is very common in certain locations where conducive environment and susceptible hosts are available. However, the disease expression and its severity are being influenced by various biotic and abiotic factors in the field. Probably no other sugarcane disease of sugarcane has such unique influence of abiotic and biotic factors which influence wilt development.

Abiotic factors

The wilt pathogen survives in the soil and injury to the underground portion of stalk facilitates the entry of wilt pathogen. Experimental evidences are available on the influence of soil conditions such as moisture, pH and organic matter content on pathogen perpetuation and pathogenesis. Depending on the C: N ratio acidic or alkaline soil conditions influence the wilt occurrence. Moisture stress during summer months coupled with high day temperature and low humidity may favour increase in wilt incidence. Since drought and water logging have impact on wilt incidence their influence on wilt incidence was studied under the role of varietal resistance with the following varieties 'Co 419', 'Co 6304', 'Co 86249', 'Co 86032', 'CoC 671', 'CoC 90063' and 'CoC 92061'. From the results we found that 'Co 419' and 'CoC 92061' were most susceptible followed by 'Co 6304' irrespective of abiotic conditions imposed. Drought at pre- and post inoculation periods influences the wilt incidence significantly and varietal behaviour differs depending on the stress conditions even in uninoculated conditions. Variation in symptom development was found to be high under normal conditions followed by drought (Viswanathan *et al*. 2008). Experience of sugarcane pathologists on wilt occurrence in India is that it is strongly influenced by extreme drought during summer with high temperature and water logging during subsequent monsoon season. However, severity of the disease is always dictated by varietal resistance. For example, sugarcane cv. 'Co 7805' succumbs to wilt in Coastal Andhra Pradesh when other varieties perform very well under the same environment (Viswanathan, unpublished). Similarly, Viswanathan (unpublished) observed severe wilt outbreak in 'Co 86032' planted under pit method in Coimbatore District. Under normal conditions the upland situation in Coimbatore District, Tamil Nadu do not favour wilt development, however, continuous water logging for 2 months in the field favoured disease development.

Biotic factors

Although abiotic factors play a decisive role on disease severity, biotic agents facilitates entry of the pathogen inside the root or stalk. Hence role of biotic factors are of paramount important in wilt initiation, development and severity. Wilt is more dangerous and causes enormous damage to crop in association with red rot, pineapple disease, borers or scale insects. These disease complexes have become a threat to sugarcane growers in both tropical and sub-tropical regions.

1. Red rot

Different workers have recorded occurrence of both the diseases in sugarcane. More damage to sugarcane by the combined infection is also recorded in the field. Butler and Khan (1913) who first described sugarcane wilt mention that *Cephalosporium sacchari* was several times found associated with *Colletotrichum falcatum*. Abbott (1935) noted the presence of white and purple strains of *Fusarium* in Louisiana associated with the red rot, but considered them to be at the most weakly parasitic and not of much importance on the varieties studied. However, in the 1940s in South Africa constant association of *c. falcatum* with *C. sacchari*, later reported as *F. moniliforme*, was found. There, *F. moniliforme* was considered to play an important role rather than as being merely a secondary invader in causing deterioration of sugarcane. In Australia, also *fusarium* has been found associated with red rot. It thus becomes evident that in all those countries where red rot has been studied thoroughly in the last century *Fusarium* or *Cephalosporium* has been found associated with the disease and in many instances almost constantly found together with *C. falcatum* (**Fig. 6**).

Bourne (1953) has established that *C. falcatum* and *F. moniliforme* very frequently occur in diseased stalks in Florida. He found that when *Fusarium* is predominant in red rot affected stalks the tissues are dark reddish purple and contrast with the lighter reddening found in case of *C. falcatum* alone. He also confirmed that *F. moniliforme* isolated from cane stalks and that causing typical 'pokkah boeng' are identical morphologically and culturally. Srinivasan and Vijayalakahmi (1961) found natural infection *C. falcatum* with *F. moniliforme* in sugarcane. In artificial inoculation, they found synergistic effect of combined inoculation in disease susceptible clones. *Fusarium* produced a mild infection, which did not extend beyond the inoculated internode even after three months. However, in combination with *C. falcatum* it led to severe infection on all the varieties and the advance of the lesion is greater in the combined infection than infection by *C. falcatum* alone. They suggested inter dependence of these pathogens for their growth and establishment inside the stalks. During the recent years sugarcane varieties suffered severely due to *pokkah boeng* in both tropical and subtropical regions in India. Detailed studies on the pathogen identify using molecular techniques revealed that both *F. verticilloides* (*Gibberella moniliformis*) and *F. sacchari* are associated with the disease (Viswanathan, unpublished). Further studies are in

progress to relate the pathogenicity of these isolates on foliar and stalk tissues of sugarcane to establish the dual role of the pathogen in causing *pokkah boeng* and wilt in the same crop.

Under field conditions, Viswanathan (2010) observed more damage to sugarcane caused by the combined infection in different varieties. Even in severe red rot epiphytotics, more damage to sugarcane varieties occurred when both of them infect together. In such a situation, *C. falcatum* infects the cane first either through primary or secondary infections. *Fusarium* follows it immediately and here deterioration of the infected canes is faster. Wherever red rot epidemics noticed like in Gujarat, Punjab, Haryana, Uttar Pradesh, Uttarakhand, Orissa and Andhra Pradesh by Viswanathan (unpublished) in the past 15 years he found that *Fusarium* affects the cane significantly. Although *Fusarium* alone cannot cause any damage in certain cane varieties red rot infection, aggravate the cane for wilt infection. However, he observed that during severe red rot epiphyotics in Tamil Nadu and Pondicherry in 'CoC 671', 'CoC 85061', 'CoC 86062', 'CoC 92061', etc. in the 1990s *C. falcatum* as the chief invader and destroyer except in stray instances *Fusarium* was also noticed. Interestingly it was found that deterioration of 'CoC 671' in Gujarat has been mainly due to the combined infection of both the pathogens. Also in adjacent fields of 'CoC 671' having combined infections, 'Co 86032' exhibiting wilt alone and 'Co 92020' with red rot alone were not uncommon in that region (Viswanathan *et al*. 2006). This type of situation exists in different States and this situation indicates the existence of variation in varietal resistance to both the pathogens. There are also possibilities of complementing factors between the two may be there. It is possible that *C. falcatum* may make available some growth-promoting factor needed by *Fusarium*. In Thailand, *C. falcatum* and *F. moniliforme* are reported to be associated with the red rot-Fusarium stem rot. The fungi reduced sett germination and caused shoot mortality. In highly susceptible varieties, the pathogens are able to infect all stages of crop growth while susceptible and moderately susceptible varieties are affected up to 5 months after planting. The latent period of the disease has been estimated to be between one and two months (Ouvanich *et al*. 1995).

2. Insect pests

Wherever root borer, *Emmalocera depressella,* infestation is severe, more wilt infection is noticed. Varieties like 'Co 85036' and 'CoC 671' in Gujarat and 'Co 89003' in subtropical region showed such root borer-wilt complex (**Fig. 7**). However such borer-wilt complex was rarely noticed in the tropical region except, Gujarat state. The borer-wilt complex was confined to certain varieties. Two varieties namely 'Co 86032' and 'CoV 94102' under large-scale multiplication in Meerut district of Uttar Pradesh showed a clear positive association of borer-wilt complex. In this case nearly 80-90% borer infestation resulted in about 60% wilt infection. These observations made by Viswanathan (unpublished) clearly indicate that restriction of tropical varieties in sub-tropical regions in the country may probably due to their susceptibility to root borer or wilt or both. Earlier, Sardana *et al*. (2000) found a positive association between root borer and wilt in the varieties 'Co 7717', 'Co 8347', 'Co 89003', 'Co 94018' and 'CoC 671'. They also observed that these varieties had tendency to pick up wilt infection as there was less of root borer infestation and more of wilt infection alone indicating an association between root borer and wilt. The varieties like 'Co 85036', 'Co 7314' and 'CoJ 64' showed less of wilt infection in spite of more root borer infestation alone and had no tendency to pick up wilt infection, thus a non-significant association between root borer and wilt. They felt that the association of wilt with other pathogens or insect pests of sugarcane makes the study of wilt syndrome very complex and cumbersome but challenging at the same time. In addition to root borer association of scale insect (Avasthy 1978) and stalk borer (Kulshreshtha and Avasthy 1959) has been reported to influence wilt infections. Although root borer infestation was very rare in Tamil Nadu, Viswanathan (unpublished) recently found a serious outbreak in cv 'Co 86032' in isolated pockets in Perambalur District. This infestation facilitated wilt development in such fields in upland conditions. This is the first such observation in Tamil Nadu on the root borer and wilt complex. Similarly Viswanathan (unpublished) has observed such root borer-wilt complex in 'Co 94012' during 2005 and in cvs 'Co 86032' during 2007 in Satara and Ahmednagar Districts, respectively in Maharashtra. During our surveys we noticed that severe outbreak of internode borer *Chilo sacchariphagous indicus* in cv 'Co 86032' leads to build up severe wilt incidence in Krishna District of Andhra Pradesh. In the trial on influence of abiotic factors, borer infestation was found to be high under complete drought or drought followed by waterlogging and it was high in cvs. 'Co 86032' and 'Co 86249'. Internodal borer infestation was found to cause reddening in the wilt infected canes and there is no evidence on pathogen progress in less susceptible cultivars (Viswanathan *et al*. 2008).

3. Nematodes

No definite role of nematodes in stimulating the wilt syndrome in sugarcane is established. However Rashid and Singh (2002) reported a possible association between nematode and wilt fungi. They recorded highest (65%) wilt incidence and reduction in fresh plant weight when sugarcane plants were inoculated with nematodes and wilt fungi (*Fusarium sacchari* and *Acremonium implicatum*) together. The next higher wilt incidence was observed in cane plants grown in wilt sick soil and also inoculated with nematodes (1000/pot). The findings suggest that aggravated wilt incidence in presence of good population of plant parasitic nematodes around the roots of sugarcane plants. Further studies on the role of nematodes in natural conditions are required as in the case of borer pests.

4. Other agents

In addition to red rot and root borer, other biotic agents such as ratoon stunting, leaf scald disease, red stripe and internode borer were also associated with wilt in certain varieties. Frequent association of ratoon stunt caused by *Leifsonia xyli* susp *xyli* in 'CoS 767', red stripe caused by *Pseudomonas rubrilineans* (Lee *et al.* 1925) Stapp in 'CoJ 85' and leaf scald caused by *Xanthomonas albilineans* (Ashby) Dowson in 'CoS 96268' under subtropical conditions were noted (Agnihotri and Rao 2002). However, when the crop is affected by wilt and one of these bacterial pathogens, the combined damage was not drastic as we noticed in the case of red rot.

Overall our surveys and studies clearly indicated that the disease severity was associated with factors such as susceptibility of varieties, favourable environmental conditions and infestation of root-borer. In case of first one it was evident from 'Co 7805' in Andhra Pradesh and Orissa and 'Co 89003' in Haryana and Punjab. Although other varieties did show significant wilt in these regions, only these two varieties showed very high susceptibility to the disease leading to high level of disease build up. The varietal susceptibility coupled with typical deltaic conditions like flooding during monsoon season and deep alluvial soils may aggravate the disease build up. In Andhra Pradesh, 'Co 7805' showed severe wilt incidence of up to 60% in East Godavari District, where the crop cultivation is in low land. However, the same variety showed trace to 10% disease incidence in West Godavari district, where the cultivation is under upland condition. 'Co 86032', the predominant cultivar in tropical India, it was found that the disease is present from trace levels to 5-6% in most of the upland regions of Tamil Nadu and Maharashtra whereas in parts of Andhra Pradesh, Gujarat and Orissa disease incidence of up to 10-20% was recorded in deltaic regions. The root borer infestation is a factor,

which increases the disease susceptibility in many areas. In this situation varietal susceptibility to root borer and wilt complex increases the chances of disease to epidemic levels. In case of 'Co 89003', root borer infestation predisposes to wilt infestation. If root borer is managed by chemicals or combination of chemicals and neem cake, damage by both root borer and wilt get reduced (Viswanathan *et al*. 2006).

SCREENING FOR WILT RESISTANCE

The sett or stalk borne and soil-borne nature of the pathogen favours the pathogenicity testing by treating all the primary sources with artificial inoculum of wilt pathogen. It includes sett treatment/ stalk inoculation with spore suspension and soil inoculation with soil based medium containing the pathogen. Naturally it can be tested in endemic areas having favourable conditions and also by artificially stimulating the abiotic stress viz., drought and water logging. Depending on the method the disease severity has been noted on sett germination, plant growth, per cent stalks infected and length of diseased stalk by different workers. Since this disease causes both yield and quality losses under certain conditions, earlier workers have used different methods for evaluating pathogenicity of wilt pathogen. Initially the intensity of wilt incidence in different varieties in varying situations was expressed as per cent diseased stalks over the total number of stalks counted (Srinivasan 1956; Sathyanarayana 1975) and the grading based on this was used to evaluate the level of resistance in sugarcane genotypes. Alexander and Lal (1967) evaluated the disease severity after artificial inoculations as per cent length of the cane stalk colonized by the fungi, resulting in visible internal lesions. It was also reported by Singh *et al*. (1975). Agnihorti (1983) developed four different methods of pathogenicity evaluation using setts, cut canes, standing canes and soil by artificial inoculation of various isolates. By sett treatment method, the setts are treated with spore suspension and the effect was recorded on germination, tillering, growth and yield besides disease intensity. For soil application of pathogen inoculum also these effects could be studied. But for cut canes and standing canes, the disease development was monitored only based on the disease development internally after inoculating with the pathogen by plug method. However, it is usually observed that in the wilt affected fields, apparently healthy canes also yield the associated fungi, when cultured on suitable media. Similarly, when the fungi are artificially inoculated on the stalk, there is internal spread of the disease from symptomless tissue to severe necrotic wilt. Hence quantitative numerical index for rating wilt severity based on all the effects of disease incidence on stalks was developed by Mohanraj and Alexander (1984). They have used 100 clumps of 10-month-old sugarcane cv 'CoJ 64' from wilt infected field, split open the stalks along the roots and examined for the development of internal and external symptoms. Based on the pathological effects of disease on different degrees, they developed the 0 to 4 scale. The grouping of different symptoms clearly indicated the possibility of assigning specific grade values to the varying levels of wilt severity. The grading system rendered it possible to distinguish physiological wilt induced by water stress or injury from pathological wilt by a comprehensive evaluation of the symptoms and relating them to each other. However, this system didn't include the effect of diseased setts/soil on sett germination or plant growth. The effect of source of inoculum cannot be judged. Also in ratoons and situations with lower disease index, this system is not applicable. Screening for wilt under artificial inoculation is not practiced in different stations. In centers like Navasari, Lucknow and Ludhiana screening under sick-plot is being followed. Different workers opined that artificial screening technique developed by Mohanraj and Alexander (1984) at Coimbatore is cumbersome and impractical although it is valid for various conditions.

More recent studies at SBI, Coimbatore by Viswanathan *et al*. (2006, 2007, 2008) indicated the effects of various testing methods of pathogenicity under *in vitro* and *in vivo* conditions under endemic situations and also in disease free areas with abiotic stress. Detailed studies to induce wilt pathogenesis under field conditions were conducted at Coimbatore, Karnal and Surat region in Gujarat during 2006 to 2009 (Viswanathan *et al*. 2009). In all the three locations, pathogen was inoculated by the standard plug method during 6-8 months of crop age. Evaluation of canes 3-5 months after inoculation revealed a clear reproduction of the disease under Gujarat conditions (**Fig. 8**). The cvs 'Co 86002', 'Co 97008', 'Co 95020', 'Co 98010' and 'Co 0323' exhibited susceptible reaction at Gujarat whereas the cv 'Co 86032 and genotype 94764 showed moderately susceptible reaction. Inoculation of wilt pathogens in the fields of Karnal exhibited only mild symptoms (**Fig. 9**). This proved that pathogen entry was favoured by climatic conditions and other environmental factors also govern the disease build up. However, by imposing drought and water logging they were able to reproduce the disease under Coimbatore conditions. In the field trial, pathogen inoculation combined with abiotic stress factors especially drought predisposed wilt in the cvs 'Co 6304', 'Co 86032' and 'Co 86249' at Coimbatore. The cvs 'CoC 90063' and 'CoC 92061' showed partial symptoms. Weakening of the host tissue by abiotic factors induces pathogen entry in some cultivars and in certain cultivars further weakening by pests was found more effective in inducing the disease. This situation reflects the observation made during disease surveys where borer pest infestations increase the wilt manifestation. A clear varietal response to disease resistance or susceptibility was found. Probably this is the first information on the reproduction of this disease under field conditions. Further validation needs to be done to use this method for routine field testing. It was also reported that the cut canes were able to produce partial symptoms under lab conditions.

Our studies clearly established that the plug method of inoculation with spore suspension is ideal in inducing wilt compared to the soil inoculum. In plug method, pathogen gained easy access to the vascular system; however, soil inoculum has to cross several physical barriers. *Fusarium* remained dormant and only under favourable conditions exhibited pathogenic symptoms when applied as soil inoculum. The pathogen inoculum was also applied in the setts by four methods viz., vacuum infiltration of spore suspension, swabbing of spore-talc mixture on the setts, dipping of setts in the spore suspension and transpirational uptake of spores by settlings. Although infiltration of pathogenic spores into the planting setts caused germination failure and caused death of buds, the disease symptoms were not obvious as in the canes. The death of buds may be due to direct pathogenic effect of the pathogen or its metabolites on the tissues. Similar to infiltration of spores, swabbing of spore-talc mixture on the setts and dipping of setts in spore suspension were effective in causing death to buds and germination failure. Similarly treatment of germinated buds by transpirational uptake of spores also proved to induce the disease and was confirmed by reisolation of the pathogen from infected tissues like roots. In this method, of the 3 pathogen isolates viz., Fs 032 G1, Fs 805 AP1L1 and FsC 671 G1, only Fs 032 G1 proved pathogenic.

Koch's postulate was proved by isolation of the pathogen from infected canes and reproduction of the disease by artificial inoculation of the isolated pathogen. Reisolation of the pathogenic and nonpathogenic isolates from the artificially inoculated canes and molecular characterization using RAPD, ISSR and IGS-RFLP markers confirmed that variation in molecular profile of the isolates correlated with pathogenecity and taxonomy. Clustering of *Fusarium* isolates in RAPD, IGS-RFLP and ISSR revealed that isolates of *F. moniliforme*, *F. proliferatum*, *F. napiforme* or *F. subglutinans* are non pathogenic or less virulent (Viswanathan *et al*. 2011). However, except for a few, most of the isolates of *F. sacchari* were found to be pathogenic on sugarcane.

The study on pathogenicity clearly points out that once the pathogen gets access into the host, it causes severe

damage to the crop. But pathogen entry into the host remains a mystery. Under favourable environmental conditions, the pathogen enters the plant system and causes severe losses. Sugarcane wilt has caused severe losses to the crop over years in different countries and reports were made on the isolations of different fungi including *viz*., *Fusarium*, *Acremonium* and *Cephalosporium. Cephalosporium* is now merged into *Acremonium.* Although the disease could not be reproduced earlier by several workers we have found plug method of inoculation as the best to induce the disease. Our finding resolves the problem in reproducing the disease in the field by imposing abiotic stresses on the crop. Ma *et al.*'s (2010) work on transposable elements points out that mobile chromosomes convert non-pathogenic strain into a pathogenic one. Probably this may a reason that several workers who were unable to reproduce the disease in the past. The present study clearly concludes 100-year-old confusions that existed on the causative agent of sugarcane wilt. Macroconidia production is a key factor that separates the genus *Fusarium* and *Acremonium*. Mere observation of growth character or conidia lead to wrong diagnosis of the pathogen as some mutated strains of *Fusarium* are also slow in growth as *Acremonium* and macroconidia production is sparse that is easily overlooked.

MANAGEMENT

Available management strategies to control wilt can be catalogued in to 5 categories viz., agronomical, physical, chemical, resistant varieties and biological. Agronomical practice includes use of disease free seed, avoiding ratoons and burning of infected canes. Physical method employs heat therapy but it was found ineffective in eliminating the wilt pathogen in infected canes. Breeding and selection of resistant varieties is cumbersome as the pathogen keeps changing in nature through adaptation, mutation, heterokaryosis, parasexual mechanism, etc. Chemical methods eradicate only the surface borne pathogens in sugarcane. It has been found that the amount of systemic fungicide absorbed by the setts steeped for 24 h was only fungitoxic and not fungicidal to the wilt pathogens. The low solubility of fungicides in water and poor intake of the fungicide inside the setts were some of the factors leading to poor efficacy of fungicides against sett-borne infections. Hence the disease could be easily managed with resistant varieties. In case if resistant varieties are not available, the following measures are to be taken in an integrated way to reduce the disease.

Cultural practices

As vegetative propagation in sugarcane favours harbouring of the pathogen in the planting setts, adequate care should be taken while selecting seed canes. If there is any infected clump in the field the seed cane plot is to be rejected. It is advised to select always a disease free area to raise seed crop. Seed cane plots are to be monitored periodically for the disease at regular intervals. Always seed canes should be selected from such carefully monitored fields.

It is well known that wilt pathogen survives in the soil for many years. In this situation going for monoculture of sugarcane over large areas, provide conditions for the rapid build up of inoculum. Crop rotation with paddy is recommended to reduce debris-borne inoculum of wilt pathogens under puddled conditions. Companion cropping of sugarcane with coriander has been observed to reduce wilt incidence (Singh *et al*. 1983). Three tier crop rotations have also been recommended in wilt sick soils (Viswanathan and Padmanaban 2008).

In the plant crop, the disease either is introduced through infected setts or through surviving inoculum in soil/crop debris and it takes time to build up disease from the source to a noticeable level. Hence, there are chances that the plant crop may escape from the disease completely or with partial damage. In ratoons, the pathogen residing in the stubbles readily causes more disease. Since build up of disease increases in successive ratoon crops when disease is noticed in plant crop it should be harvested on priority basis and ratooning in such fields should be strictly avoided.

From our experience we found that wilt severity is predisposed by water logging and drought and borer pests infestation favours wilt outbreaks in certain regions. Hence, adequate care should be taken to minimize many of these biotic and abiotic factors which predispose the crop to wilt. Providing optimum irrigation and drainage wherever required would be of paramount importance to manage the disease in endemic locations.

Disease surveillance

Major disease outbreak results in the field due to not reporting of the disease immediately after its first occurrence. In the field conditions, either the field staff failed to identify the disease correctly or ignoring the likely build up of disease in later stages of the crop or in the ensuing ratoon. After planting the setts and after proper regulations of irrigation water the field will have to be kept under periodical disease surveillance. A group of 5 to 10 persons should be trained in the art of detection of all major diseases so that the nursery and commercial plots can be kept free from all major diseases.

Chemical control

Although the organomercurials were found to be effective as sett treatment, it could not be recommended as they are not available in the market considering their demerits. Ganguli (1964) reported that wilt incidence under field conditions can appreciably reduced by applying 40ppm boron or manganese to wilt sick soils or by sett treatment. Soil amendment with boric acid (15 kg/ha) and sett treatment with aretan (0.1%) followed by 0.1% Carbendazim considerably reduced wilt incidence and pathogen population (Singh *et al.* 1985). These chemicals were found to increase the population of beneficial microbes which indirectly caused fungitoxic effects. Dipping setts in 0.1% Carbendazim helps to prevent entry of the pathogen through cut ends. By applying Chloropyriphos 20EC or Confidor at 0.5L a.i. /ha or Furadon 3G or Feproril at 1.5 kg a.i./ha during the last week of August, root borer can be managed. Managing root borer by these insecticides helps to control wilt in different parts of subtropical India (Viswanathan and Padmanaban 2008).

Use of resistant varieties

Whenever severe wilt epidemics devastated cane cultivation during the past, suitable replacement varieties with disease resistance were identified and sugar industry was saved from threats in India. For example, when wilt epidemics devastated cane cultivation in Gujarat state in Peninsular India in 1980s adoption of resistant variety Co 8338 saved the sugar industry in the state. Hence growing resistant variety is the cost effective and it will assure sustained cane cultivation in a region. So far there is no systematic screening programme for screening wilt resistance as it is difficult to reproduce the symptoms by artificial inoculation unlike red rot and smut which have different methods for screening. However the genotypes in zonal varieties are being screened for wilt resistance in the centres like Navsari (Gujarat), Ludhiana (Punjab), Lucknow (Uttar Pradesh) and Pusa (Bihar) under sick plot conditions. Here the disease reaction is assessed as resistant, moderately resistant, moderately susceptible and susceptible using their 0-4 scale. The newly developed disease simulation method needs to be validated under these locations to take up screening sugarcane germplasm for wilt resistance in different centres.

Biological control

As biological control is considered as economically and environmentally viable method for the management of soil-

borne pathogens, considerable progress has been made for the management of sugarcane wilt with biocontrol agents. Ganguli and Khanna (1955) employed proven antagonists of *Cephalosporium*, but did not obtain adequate control of sugarcane wilt under field conditions. However, Bhatti and Chohan (1970) indicated possibility of wilt suppression using *Bacillus* and *Streptomyces* antagonists.

The pressmud produced in a sugar mill is about 4 percent of the cane crushed. Pressmud is a good substrate and its composition suits well for the establishment of *Trichoderma* on soil. The pressmud contains considerable amount of major and minor nutrients. Application of pressmud improves, iron availability and also improves availability of iron, zinc, calcium, magnesium and manganese. In calcareous soils, it reduces soil pH, electrical conductivity (EC) and exchangeable sodium percent (ESP) and increases available status of the soil. Immediate and direct application of pressmud is not desirable as it has a wide C: N ratio and its decomposition generates lot of heat. Composting of pressmud using microorganisms helps in enriching it and makes it easier and safer to apply to the field. Hence use of pressmud as a tool for large-scale multiplication served in 2 ways: utilizing a sugar industry waste product and also multiplying a biocontrol organism for the control of a destructive disease. This reduces the cost of large-scale multiplication of antagonist and the hitherto unwanted byproduct of sugar industry is being efficiently disposed through value addition. Singh and Joshi (2007) used different temperatures (28-36°C) and a range of moisture levels (10-60%) for the multiplication of *Trichoderma harzianum* on pressmud. *T. harzianum* is reported to multiply well on pressmud under laboratory and compost pits at farmers' fields at 32°C at 30% moisture with a cfu of 7.66×10^6/g by 21 days.

During 2005-2008, Viswanathan *et al*. (2007, 2008) explored the possibility of fungal and bacterial bioagents for effective management of sugarcane wilt under field conditions. They isolated 15 strains of *Pseudomonas* and 26 strains of *Trichoderma* spp. from rhizosphere soils of wilt-affected clumps from different locations and screened against 10 *Fusarium* isolates. In bioassay, they found that *Pseudomonas* strains were not effective in controlling the pathogen and *Trichoderma* isolates were found to be effective (**Fig. 10**). The effective isolates belonged to *T. harzianum*, *T. viride* and *T. pseudokoningii*. They were multiplied on *Trichoderma* special medium and for large-scale field application we have standardized a pressmud based formulation. Detailed field trials on wilt management have been conducted at Bardoli in Gujarat and Chelluru in Andhra Pradesh. Results of the trials at Gujarat revealed that soil application of *Trichoderma* multiplied on pressmud is effective in managing wilt in endemic locations (Viswanathan, unpublished). However, to confirm the results more number of disease management trials are to be conducted in endemic locations.

CONCLUSIONS

Wilt was recorded 100 years back in India, which has witnessed many epidemics resulting in colossal loss to the farmers and industry. Researchers also find it difficult to find a suitable replacement to the elite varieties like 'Co 7805' or 'Co 89003' which failed due to wilt. Many times the loss caused by the disease goes unnoticed probably due to their late occurrence in the season and due to its camouflaging symptoms with red rot, it is wrongly diagnosed as red rot. Also during combined infections with red rot, importance of wilt pathogen's role in enhancing disease severity is being ignored. Due to the repeated epidemics of red rot in tropics and subtropics, importance of wilt could have been ignored. Also confusion prevailing on the associated pathogens and cumbersome process in reproducing the disease are the real bottlenecks for the sugarcane pathologists to start their career in sugarcane wilt. Another important disease, smut has also attracted researchers due to its easy reproduction in the field and easy to screen the progenies for disease resistance. Nevertheless past and recent studies conducted at SBI have given new understanding on disease spread, associated pathogens, pathogen variability, field technique to reproduce wilt and biological control of the disease and answered many unanswered questions on the disease. Further studies are required to validate the new screening technique and biocontrol of the disease for the further utilization of these findings. Adoption of a field level screening technique would ease the identification of wilt resistance in new sugarcane varieties and similarly biocontrol approach would sustain sugarcane productivity in the wilt endemic locations.

REFERENCES

Abbott EV (1932) Seed rots of sugarcane in Louisiana. *Proceedings of International Society of Sugar Cane Technologists*, 4th Congress Bulletin **48**, 1-2

Abbott EV (1935) Economic importance of red rot and comparative susceptibility of some sugarcane varieties in the southern United States. *USDA Circular* **350**, 1-26

Agnihotri VP (1983) *Diseases of Sugarcane*, Oxford and IBH publishing Co, New Delhi, 363 pp

Agnihotri VP, Rao GP (2002) A century status of sugarcane wilt in India. In: Singh SB, Rao GP, Eswaramoorthy S (Eds) *Sugarcane Crop Management*, Sci Tech Publishing LLC, Houston, USA, pp 145-160

Agnihotri VP, Singh RP (1989) Sugarcane wilt current status. In: Agnihotri VP, Singh N, Chaube HS, Singh US, Dwivedi TS (Eds) *Prospective in Plant Pathology*, Today and Tomorrow's printers and publishers, New Delhi, India, pp 227-237

Alexander KC, Lal MM (1967) Studies on wilt (*Cephalosporium sacchari* Butler) of sugarcane. Annual Report, Indian Institute of Sugarcane Research, Lucknow, p 63

Avasthy PN (1978) Sugarcane scale insect and its control. *Co-operative Sugar* **10**, 53-54

Ayson AA (1938) The role of *Fusarium* in the deterioration of sugarcane stalks and stubble pieces. Thesis (unpublished), Louisiana State University, pp 1-30

Bhatti DS, Chohan JS (1970) Antagonism of certain microorganisms to *Cephalosporium sacchari. Journal of Research (Punjab Agricultural University)* **7**, 631-635

Bourne BA (1922) Researches on the root diseases of sugarcane. Department of Agriculture, Barbados, pp 1-17

Bourne BA (1933) Sugarcane breeding experiments. *Annual Report, Florida Agriculture Experiment Station*, pp 180-181

Bourne BA (1953) Studies on sugarcane red rot in the Florida Everglades. *Proceedings of International Society Sugar Cane Technologists* **8**, 915-924

Butler EJ (1906) Fungus diseases of sugarcane in Bengal. *Memoirs of the Department of Agriculture (India), Botany Series* **1**, 2-24

Butler EJ, Khan AH (1913) Some new sugarcane diseases. Part I, Wilt. *Memoirs of the Department of Agriculture, India, Botany Series* **6**, 180-190

Edgerton CW, Moreland CC (1920) Effect of fungi on the germination of sugarcane. *Louisiana Agricultural Experiment Station Bulletin* **169**, 1-40

Fawcett GL (1922) Enfermedades de la cana de azucar en Tucuman. *Revista Industrial de Agricultura de Tucuman* **13 (1 & 2)**, 46 pp, illus (Translation of 2nd Edn, Library USDA, Sugarcane and Sweet Sorghum Section)

Gams W (1971) *Cephalosporium – Artige Schimmelpize (Hyphomycetes)*, G. Fischer, Stuttgart, 262 pp

Ganguli A (1964) Wilt. In: Hughes CG, Abbott EV, Wismer CA (Eds) *Sugarcane Diseases of the World* (Vol II), Elsevier Publishing Co., Amsterdam, pp 131-135

Ganguli A, Khanna KL (1955) Annual report of the scheme for investigation and control of wilt disease of sugarcane for the year ending 31st May 1955. Annual report Sugarcane Research Station, Pusa, Bihar (India), 53 pp

Gupta MR, Gupta SC (1976) Assessment of losses caused by wilt disease of sugarcane in western UP. *Indian Sugar* **26**, 151-152

Khanna KL, Chacravarti AS (1949) Studies on storage of *gur*. *Current Science* **18**, 127-128

Kirtikar, Singh GP, Shukla R (1972) Role of seed material in carry over of wilt disease of sugarcane. *Indian Sugar* **22**, 89-90

Kulshreshtha JPA, Avasthy PN (1959) An estimate of damage caused to sugarcane crop by the stalk borer *Chilotraea auricilia* Dudg. *Proceedings of All India Conference of Sugarcane Research and Development Workers* **3**, 27-28

Ma L, van der Does HC, Borkovich KA, Coleman JJ, Dabouss M-J, Di Pietro A, Dufresne M, Freitag M, Grabherr M, Henrissat B, Houterman PM, Kang S, Shim W-B, Woloshuk C, Xie X, Xu X-R, Antoniw J, Baker SE, Bluhm BH, Breakspear A, Brown DW, Butchko RAE, Chapman S, Coulson R, Coutinho PM, Danchin EGJ, Diener A, Liane R. Gale, Gardiner DM, Goff S, Hammond-Kosack KE, Hilburn K, Hua-Van A, Jonkers W, Kazan K, Kodira CD, Koehrsen M, Kumar L, Lee Y-H, Li L, Manners JM, Miranda-Saavedra D, Mukherjee M, Park G, Park J, Park S-Y, Proctor RH, Regev A, Ruiz-Roldan MC, Sain D, Sakthikumar S, Sykes S, Schwartz DC, Turgeon BG, Wapinski I, Yoder O, Young S, Zeng Q, Zhou S, Galagan J, Cuomo CA, Kistler HC, Rep M (2010) Compara-

tive genomics reveals mobile pathogenicity chromosomes in *Fusarium*. *Nature* **464**, 367-373

Mitter N, Srivastava AC, Renu, Ahamad S, Sarbhoy AK, Agarwal DK (2002) Characterization of gibberellin producing strains of *Fusarium moniliforme* based on DNA polymorphism. *Mycopathologia* **153**, 187-193

Mohanraj D, Alexander KC (1984) A qualitative numerical index for rating severity in sugarcane wilt. *Indian Sugar Crops Journal* **10**, 19-20

Nelson PE, Dignani MC, Anaissie EJ (1994a) Taxonomy, biology, and clinical aspects of *Fusarium* species. *Clinical Microbiology Reviews* **7**, 479-504

Nelson PE, Juba JH, Ross PF, Rice LG (1994b) Fumonisin production by *Fusarium* species on solid substrates. *Journal of AOAC International* 77, 522-525

Nirenberg H (1976) Untersuchungen Uber die morphologische und biologische Differenzierung in der Fusararium-Sektion Liseola (Investigations on the morphological and biological differentiation in the Fusarium section Liseola). *Mitteilungan aus der Biologischen Bundesanstalt fur Land und Forstwirtschaft Berling-Dahlem* **169**, 1-117

Nowell W (1920) *Diseases of Crop Plants in the Lesser Antilles*, The West India Committee, London, 383 pp

Ouvanich W, Long TB (1994) Relationship between *Colletotrichum falcatum* and *Fusarium subglutinans* or *F. moniliforme* sugarcane red rot-fusarium stem rot pathogens. *Thai Agricultural Research Journal (Thailand), Warasan Wichakan Kaset, (May-Aug)* **12 (2)**, 117-123

Ouvanich W, Kusalwong A, Handee C (1995) Red rot-Fusarium stem rot of sugarcane in Thailand. *Proceedings of International Society of Sugar Cane Technologists* **21**, 342-343

Parthasarathy SV (1972) *Sugarcane in India*, The KCP Ltd., Madras, India, 685 pp

Poongothai M (2010) Cultural, morphological, molecular and pathogenic characterization of pathogen(s) causing sugarcane wilt in India. PhD thesis, Bharathiyar University, 263 pp

Rands RD, Abbott EV (1938) Sugarcane diseases in the United States. *Proceedings of International Society of Sugar Cane Technologists* **6**, 202-212

Rashid A, Singh N (2002) Role of plant parasitic nematodes on the incidence and severity of sugarcane wilt. *Sugar Tech* **4 (l&2)**, 61-62

Sardhana HR, Singh N, Tripathi BK (2000) Investigation on the relationship between root borer and wilt disease of sugarcane. *Indian Journal of Entomology* **62**, 11-17

Sarma MN (1971) Some observations on sugarcane disease complex in Nizamabad distrcit in Andhra Pradesh. *Indian Sugar* **21**, 619-627

Sarma MN (1976) Wilt disease of sugarcane. *Sugarcane Pathologists Newsletter* **15/16**, 30-33

Satyanarayana Y (1975) Determination of causal organism involved in wilt syndrome. Sugarcane Research Station, Anakapalle, India. Report for 1972-73, pp 87-90

Singh V, Joshi BB (2007) Mass multiplication of *Trichoderma harzianum* on sugarcane press mud. *Indian Phytopathology* **60**, 530-531

Singh K, Singh RP (1974) Involvement and pathogenicity of *Acremonium* in wilt syndrome of sugarcane. *Sugarcane Pathologist's Newsletter* **11/12**, 24-25

Singh K, Singh RP, Agnihotri VP (1975) Taxonomy and pathogenicity of fungi causing sugarcane wilt syndrome. *Indian Phytopathology* **28**, 86-91

Singh N, Singh RP, Lal S (1985) Effect of sett borne inoculum of wilt pathogens on germination and subsequent growth of sugarcane. *Acta Botànica Indica* **13**, 212-217

Singh O, Waraitch KS (1981) Effect of wilt and red rot induced stress on quality deterioration of sugarcane. *Sugarcane Pathologists Newsletter* **27**, 25-30

Singh OP (1973) Wilt assumes a serious problem in the sugarcane riverine belt of Saraswati Sugar Mills Area, Yamunanagar Ambala. *Indian Sugar* **23**, 129-130

Srinivasan KV (1956) Report of the Mycologist, Annual Report for the year 1955-56, Sugarcane Breeding Institute, Coimbatore, India, pp 82-84

Srinivasan KV, Vijayalakshmi U (1961) Note on a synergistic asscoation between *Glomerella tucumanensis* Speg. and strains of *Fusarium moniliforme*. *Proceedings of All India Conference of Sugarcane Research and Development Workers, Waltair* **4**, 619-622

Subba Raja KT, Natarajan S (1972) Sugarcane wilt caused by *Cephalosporium sacchari* and *Fusarium moniliforme* in India, *Sugarcane Pathologists Newsletter* **8**, 21-23

Viswanathan R (2010) *Plant Disease: Red Rot of Sugarcane*, Anmol Publishers, New Delhi, 301 pp

Viswanathan R, Malathi P, Ramesh Sundar A Poongothai M, Singh N (2006) Current status of sugarcane wilt in India. *Sugar Cane International* **24 (4)**, 1-7

Viswanathan R, Malathi P, Ramesh Sundar A (2007) Wilt of crops with special reference to cultural, morphological, molecular characterization and pathogenic variability of isolates in India. Annual Report 2006-07, Sugarcane breeding Institute, Coimbatore, pp 56-57

Viswanathan R, Malathi P, Ramesh Sundar A (2008) Wilt of crops with special reference to cultural, morphological, molecular characterization and pathogenic variability of isolates in India, Annual Report 2007-08, Sugarcane breeding Institute, Coimbatore, pp 49-50

Viswanathan R, Malathi P, Ramesh Sundar A (2009) Final Report of the Network Project on Wilt of crops with special reference to cultural, morphological, molecular characterization and pathogenic variability of isolates in India, Sugarcane breeding Institute, Coimbatore, 46 pp

Viswanathan, Padmanaban P (2008) *Handbook on Sugarcane Diseases and their Management*, Sugarcane Breeding Institute, Coimbatore, India, 80 pp

Viswanathan R, Poongothai M, Malathi P (2011) Pathogenic and molecular confirmation of *Fusarium sacchari* causing wilt in sugarcane. *Sugar Tech* **13**, 68-76

Viswanathan R, Rao GP (2011) Disease scenario and management of major sugarcane diseases in India. *Sugar Tech* **13**, 336-353

Waraitch KS (1981) Wilt disease in Co 1148 in Punjab and assessment of losses caused by it. *Indian Sugar* **31**, 37-40