REVIEW ARTICLE

STATUS OF SUGARCANE WILT: ONE HUNDRED YEARS AFTER ITS OCCURRENCE IN INDIA

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Abstract

Wilt of sugarcane was recorded almost 100 years ago in India and is one of the major fungal diseases affecting cane production and productivity. Many commercial varieties were withdrawn from cultivation due to theirsusceptibilityto the disease in the country. Even though the disease was recorded long back, information on pathogen(s) involved and their variability, host resistance, epidemiology, management was limited or scattered. Detailed studies taken up at Sugarcane Breeding Institute revealed that wilt incidence in different sugarcane varieties varied from trace to 75% in different agroclimatic regions of the country. Maharashtra, Tamil Nadu, Kerala and Karnataka showed trace incidence of wilt, whereas the states of Andhra Pradesh, Orissa, Gujarat, Bihar, Uttar Pradesh, Punjab and Haryana recorded moderate to severe wilt. East coast regions, South Gujarat and subtropical plains were identified as the disease endemic regions in the country. The disease occurred either alone or in association with red rot in different states and such combined infection was more commonly found in Bihar and Gujarat. Among the insect pests, root borer was found to aggravate wilt severity in different states. *Fusarium sacchari* was identified as the causal organism based on detailed morphological features and molecular characteristics and this has resolved conflicting claims regarding the true causal organism as species of *Fusarium*, *Cephalosporium* and *Acremonium*. ISSR and IGS-RFLP were found to be the efficient molecular markers to establish variation in *F. sacchari* and also to distinguish this species from other species of *Fusarium*. The present paper reviews sugarcane wilt scenario in the country, including variability in disease symptoms, impact on the crop, associated pathogens, pathogen variability and screening methods.

Key words: Sugarcane, wilt, *Fusarium sacchari*, disease impact, pathogen variability, epidemiology, screening for disease resistance.

Historical background

Wilt is one of the early known diseases of sugarcane in India. Although first reported by Butler (1906) from Bihar, detailed studies were made a few years later by Butler and Khan (1913). The disease continued to occur in Bihar for a long time and from there it spread to other states like Uttar Pradesh, Haryana and Punjab due to uninterrupted movement of diseased seed material, particularly through seed cane of Co 527. Wilt epidemics in India during the last century resulted in elimination of many commercial cultivars, such as Co 245, Co 321, Co 527, Co 951, Co 1107 and Co 1223, from cultivation (Kirtikar et al. 1972). In tropical India, the disease caused serious damage to the varieties Co 419, Co 449, Co 453, Co 527 and Co 1122 under cultivation in parts of Tamil Nadu during 1955-56. Many good

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South Africa, Thailand, Uganda, USA and Zimbabwe (Rao and Agnihotri 2000).

Impact of wilt in sugarcane

Wilt is a serious constraint to sugarcane production in India and is next to red rot caused by *Colletotrichum falcatum* in terms of 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 D.74 and purple varieties which had been inoculated. In one inoculation test with P.O.J. 213 in Louisiana, Abbott (1935) showed that the purple strain of *Fusarium* reduced the germination to 41% below that of 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 dried or dead canes found in the field after harvest and these may vary from 2 to 10 tonnes/ha (Parthasarathy 1972). Sarma (1976) reported that loss in the yield might go up as high as 65% and the incidence of the disease was more in ratoonthan in plant crop. Diseased plants produced less number of tillers than the healthy ones (Agnihotri and Singh 1989).

Wilt fungus in association with some insect pests of sugarcane, particularly stalk borer and scale insects, causes significant damage to the crop. In association with stalk borer, the disease has been reported to bring a loss of about 8.75 tonnes/ha (Kulshreshtha and Avasthy 1959). 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 the cv Co 1148 and the crop was almost unfit for milling. With conservative estimates of loss of 3-6 tonnes/ha, the disease may cause an annual loss of 12.7-25.4 million tonnes in different years. Hence, the loss to sugarcane production would be between Rs. 1250-2500 crores per annum (Viswanathan et al. 2006). The loss in production is borne by both the farmers and sugar industry. The impact of wilt infected canes on recoverable sugar in the mill is not assessed properly andsugar 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 - 25.8% reduction in juice extraction and 3 - 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 cvs Co 658 and Co 449.

Although wilt pathogen causes enormous damage to sugarcane in India, research work on wilt of sugarcane had received limited attention as compared to red rot and smut from sugarcane pathologists. Wilt either alone or in combination with red rot causes significant damage to cane yield and quality. Besides such well established synergistic activity of red rot and wilt pathogens in many varieties, wilt aggravates red rot susceptibility in some varieties. A combination of wilt with red rot, stalk borer or root borer further makes the crop unfit for downstream processing in mills.

Moreover, losses caused by wilt go unnoticed in the field since the pathogen infection takes place during maturity stage of the cropand the loss would be noticed only at the time of harvest. Thus under normal situations,even though the crop is harvested as healthy one, it still suffers from the disease. However, in epidemics, disease incidence is noticed during grand growth phase i.e. in 5 to 6 month canes.

Fig. 2. Wilt infected canes show leaf yellowing in the crown

Fig. 3. Wilt infection results in extensive foliage discolouration in sugarcane before drying

infected canes remain partially turgid with discoloured foliage and may not dry till harvest. In such plants, the crown remains green and other leaves show prominent yellowing. This may be due to sudden withdrawal of favourable environment for the disease to progress or weakening of influence of associated factors which aggravate the disease severity.

Recently the author has witnessed wilt development after *pokkah boeng* infection in some of the sugarcane varieties. In this situation, initially typical symptoms of *pokkah boeng* like shortening of leaves in the crown with deformation and chlorotic patches with irregular black streaks on the leaf lamina were observed. Later, the affected and other mature leaves show yellowing (Fig. 5) and this leads to systemic yellowing and drying of canes with typical wilt symptoms inside the canes. Since *Fusarium* sp. is associated with both the diseases, it is likely that the same pathogen may cause two

Fig. 4. Wilt infected canes show drying of canes with withering leaves

Fig. 5. Systemic yellowing of pokkah boeng affected sugarcane plant. The leaves in crown show typical *pokkah boeng* symptoms of deformation and shortening with lesions

cavities are evident in almost all the canes of an infected clump as a central hole surrounded by tissue discolouration as described before.

Fig. 8. Characteristic wilt symptoms in the stubbles after cane harvest in Western Uttar Pradesh

Wilt affected canes do not emit any odour, unlike canes infected with red rot or pineapple diseases. There are no white spots in internal tissues that are typical of red rot affected canes. Occasionally, scattered red streaks (vascular strands) in the internodes above the badly affected internodes are seen and such streaks pass from one internode to another. When the disease tissues are examined under a microscope, the walls of the xylem vessels look dark and fungal hyphae are seen in this lumen. Later, gum formation takes place concomitantly inside the affected vessels, intercellular spaces of parenchymatous cells and xylem parenchyma (Srinivasan 1964). Fungal hyphae ramify inside the cells and in the intercellular spaces, particularly at the point where tissues turn dark muddy red near the node. With the development of cavities, the fungi proliferate quickly, sporulate luxuriantly and produce abundant conidia (Ganguly 1964). Initially, the root system of the affected plant does not show any discolouration, lesions or damage attributable to the disease, but subsequently the affected roots die.Wilt endemic regions exhibited severe damage to the crop with brown discolouration and boat shaped cavity formation as noted in Karnal and areas with trace incidences of wilt showed pithiness and slight discolouration of internodes as recorded in Coimbatore.

Current scenario of the disease in the country

Survey for the disease in the country revealed that the disease occurred in almost all the sugarcane growing states . However, severity was the highest in Gujarat, east coastal regions of Andhra Pradesh and Orissa in tropical India. In subtropical regions, the states of Bihar, Uttar Pradesh, Haryana and Punjab showed severe incidences of wilt. The most affected cultivars were Co 7805 and Co 86032 in Andhra Pradesh and Orissa, Co 85036, Co 86032, CoSi 95071 and CoC 671 in Gujarat, Co 89003 in Punjab and Haryana, CoJ 64 and CoS 767 in Uttar Pradesh;in Bihar most of the cultivars exhibited wilt. The popular variety in the east coast region Co 7805 suffered due to wilt in Andhra Pradesh and Orissa. 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 areas of West Godavari district under upland conditions. In 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. Probably this may be a reason that the variety did not become popular in coastal Andhra Pradesh as compared to other regions in the peninsular region. Apart from popular varieties, some varieties which are under promotion also exhibited severe wilt in different states. For example, Co 0121 in Uttar Pradesh and Co 92012 in Tamil Nadu suffered severely due to wilt (Fig. 9). Currently moderate incidences of wilt occur in all the major sugarcane growing states in the country (Viswanathan and Rao 2011; Viswanathan 2013).

In general, the varietal susceptibility coupled with typical deltaic conditions like flooding during monsoon season and deep alluvial soils may aggravate the disease build up.

Sugar factories in coastal Andhra Pradesh have a system to remove dead canes/ immature canes in the factory yard before weighing and crushing. Critical analysis by the author very clearly showed that heaps of rejected lots contained wilt infected canes along with immature canes (Viswanathan 2012). Internal symptoms of the canes rejected in the yard showed typical cavity formation and pink to brown discolouration of the internodes. While harvesting, partially affected canes are bundled together with healthy canes for transfer to the factories and completely dried wilted canes, rejected by the farmers are left in the field. The author has witnessed several such fields where large numbers of dried canes were lying after harvest or trash burning (Viswanathan 2012). This scenario suggests yield loss caused by wilt is enormous in sugarcane. Wherever such sorting mechanism is followed, such losses become apparent but juice quality loss is averted. Probably in conventional milling systems, wilted canes are crushed together with healthy canes and juice quality loss is imminent. It was also not uncommon in villages where dried canes due to infection of red rot or wilt or both are gathered, stacked and used as fire wood (Viswanathan 2012). Such scenarios also indirectly project the huge losses caused by these diseases in sugarcane and to certain extent answer why the cane productivity remains static inspite of introducing high yielding varieties in the recent decades in the country.

Associated pathogens

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 and without sporodochia, while the latter with 1-3 septate conidia, macroconidia more commonly formed 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. Recent studies of Viswanathan et al. (2006) at SBI on pathogen recovery revealed that out of 95 nation-wide wilt infected samples subjected to isolation, only 53 samples yielded wilt pathogens and all are found to be species of *Fusarium*; *Acremonium* was not isolated in any of the samples. Further, Poongothai et al. (2013a) compared recovery of wilt fungus from nodal and internodal tissues which revealed that wilt fungi were recovered both from nodal and internodal tissues in 31 of the 125 cane samples subjected for pathogen isolation. Of the remaining 94 samples, 54 samples yielded wilt fungus only from nodal tissues and 40 samples yielded the fungus only from internodal tissues. Overall, higher recovery of the pathogen was made from nodal tissues than internodes. Detailed studies conducted on the identity of wilt associated *Fusarium* at SBI revealed *F. sacchari* as the causative agent (Viswanathan et al. 2011).

Pathogen variability

Detailed studies were carried out on phenotypic and genotypic variability in *F. sacchari* at SBI. Phenotypical characterization of the pathogen was done based on growth rate, pigmentation, texture, nature of phialides and conidia produced. Cultural pigment production had no influence on the dendrogram. Grouping of isolates in dendrogram was mainly based on morphology and pathogenicity. However, source of sample viz., internodal or nodal origin, soil and source variety did not influence isolate grouping. To some extent, geographical origin of the pathogen also correlated with the grouping. Correlation of the dendrogram generated by cultural and morphological characters with the molecular dendrograms revealed that the phenotypical data separated the different species to a limited extent. However, characterization based on RAPD, IGS-RFLP, ISSR and ITS sequencing clearly showed that the isolates that belonged to species other than *F. sacchari* formed a separate cluster. Findings on molecular characterization of *F. sacchari* made 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.

Association of other diseases and pests

During the surveys it was found that different cultivars showed combined infections of red rot caused by *Colletotrichum falcatum* and wilt. Many cultivars such as Co 0120, Co 89003, CoJ 64, CoS 767 and CoS 96268 showed combined infections of red rot and wilt in subtropical region (Table 1). Similar situation was observed in Co 94012, CoC 90063 and CoV 94101 in Tamil Nadu, Co 7805 and CoV 89101 in Andhra Pradesh, and Co 7805 in Orissa, in the tropical region. However, the phenomenon of combined infection was significant in Gujarat in the commercial cultivars viz. Co 85036, Co 88025, CoC 671, CoSi 95071 and Co 92020. Similarly, the promising sugarcane clones/ cultivars viz. Co 2001-10, 99N810 and 2001N193 in different trials also exhibited both diseases in the state. Although there were combined infections, only wilt was observed in Co 86032 in Orissa in close contact to Co 7805 which had both diseases together. In red rot-wilt combination, basal five to six internodes showed red rot infection followed by another five to six internodes with red rot lesions combined with characteristic wilt infection in each internode and top internodes showed mostly wilt symptoms. In certain cases, the top internodes with wilt infection have localized spots of red rot. Additionally, internodes show pith cavities covered with grey mycelium of *C. falcatum*, sparse growth of *Fusarium*, mixed mycelia of two fungi and

Fig. 10. Combined infections of red rot and wilt in sugarcane. Infected internodes become fibrous with pith cavities and also profuse growth of red rot pathogen

overlapping symptomsof both the diseases (Fig. 10). The extent of damage caused to the crop is more in wilt-red rot complex compared to the crop with wilt alone. Similar observation was recorded by many workers in the past (Agnihotri and Rao, 2002). Combined infection of red rot and wilt in sugarcane yielded cultures of both *C. falcatum* and *Fusarium* sp. on culture medium. In OMA plates kept for isolation, *C. falcatum* first emerged out of the infected stalk tissues followed by *Fusarium* which reflects the same order of emergence and infection of the pathogens on stalk tissues. The mycelium of *C. falcatum* spread to cover the entire culture plate; meanwhile wilt pathogen with pinkish pigmentation gradually established itself on the grey mycelium of *C. falcatum* and sequentially lysed *C. falcatum* mycelium (Viswanathan 2012) (Fig. 11). The observation points out that presence of red rot pathogen weakens the host thereby facilitating the entry of wilt fungus. Simasalit (1996) also found the stalk disease "red rot wilt" as a major sugarcane disease in Thailand. Metabolites from both the pathogens induced sugarcane red rot-wilt although metabolites from *C. falcatum* caused more disease induction. Severity of the disease was enhanced when metabolites from the two pathogens were combined.

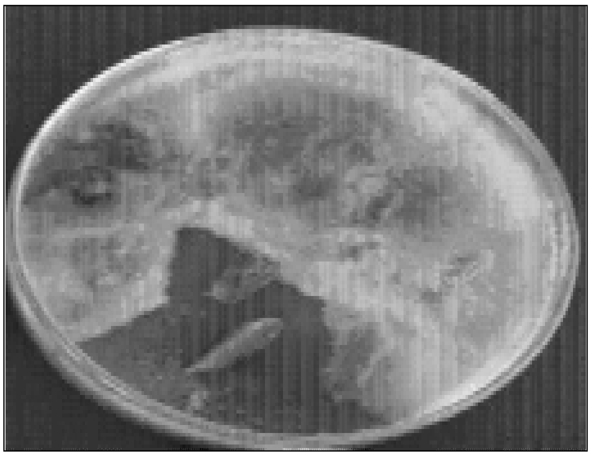

Fig. 11. Culture plate showing growth of *Colletotrichum falcatum* (greyish mycelium) and *Fusarium sacchari* (pinkish) from infected cane bits in oatmeal agar medium

Under Indian situations, wherever root borer, *Emmalocera depressella* infestation was severe, more wilt infection was noticed. Cultivars like Co 85036 and CoC 671 in Gujarat and Co 89003 in subtropical region showed such root borer-wilt complex. In Maharashtra also, root borer and wilt complex was noticed in Co 86032 and Co 94012 in Pune, Ahmednagar and Sangli districts. However, such borer-wilt complex was more pronounced in Gujarat State and the borer-wilt complex was confined to certain cultivars in other states. Two popular cultivars from the tropical region 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 in canes. This observation also revealed a new understanding on why the popular varieties from the tropical region failed in the subtropical region in the country. Probably the introduced varieties succumb to wilt due to their high susceptibility to root borer. Since the subtropical varieties are selected from the region they withstand against this borer or other pests which aggravate the crop to wilt infection. It was found that presence of root borer makes entry of pathogen easier in sugarcane stalks (Fig. 12a). Probably the injuries in underground portion of sugarcane stalks caused by root borer facilitated entry of the pathogen surviving in the soil.

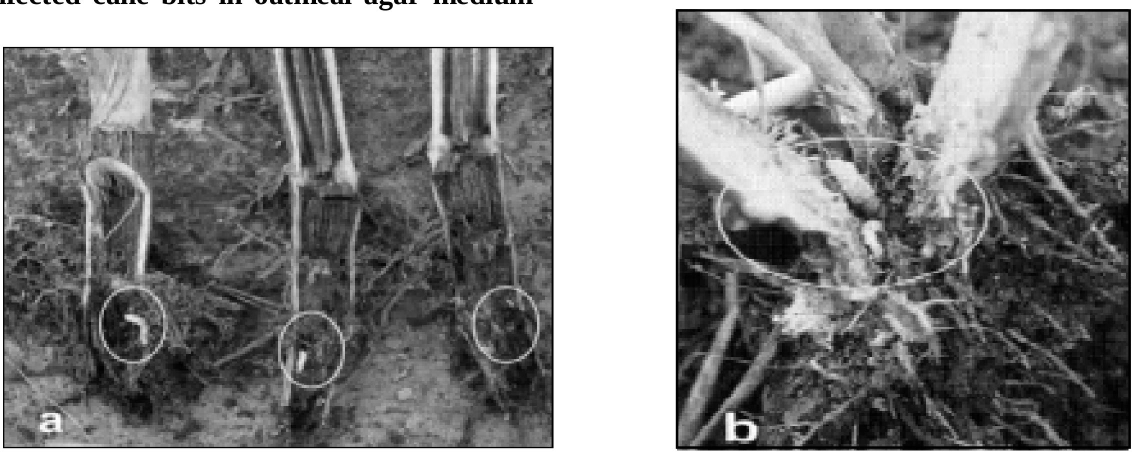

Fig. 12. Association of sugarcane wilt with insect pests infecting sugarcane. a: cv Co 0121 in Meerut district, Uttar Pradesh, exhibiting severe wilt with root borer infestation; b: Root grub infestation induced wilt in cv Co 86032 in Someshwarnagar, Maharashtra

Screening for disease resistance

Butler and Khan (1913) could not reproduce typical disease symptoms using *C. sacchari* whera slater workers were successful (Srinivasan 1964; Ganguly 1964; Kathirvelu and Mahadevan 1967; Bhatti and Chohan 1969). Four artificial inoculation methods viz., inoculation of setts, cut canes, standing canes and soil were reported during different periods with reasonable success in disease reproduction. In the first method, two tothree-buddedsettswere dipped in a spore suspension or inoculated by plug method (Ganguly and Jha 1964) with a composite culture of the pathogen and treated settswere planted in plots along with uninoculated canes. The effect of the pathogen was assessed based on germination and subsequently on wilt development. In the second method, 4ft long cane-pieces were inoculated in the middle by the plug method and the opening is sealed with wax or plasticin. These canes were incubated for one month in moist straw or saw dust and thereafter, the pathogenspread was recorded in the canes. In the third method, 7 to 8-month old standing canes were inoculated (Menon and Singh, 1960) and the linear spread of the disease was recorded after 3-4 months of inoculation (Singh et al. 1975). In the fourth method, fungal inoculum grown on sand-maize-meal was inoculated in soil and development of wilt symptoms was observed.

Using these methods, claims and counter claims have been made regarding the reproduction of the disease on artificial inoculation. Some workers reported that reproduction of disease using *F. verticilloides* has not always been possible (Luthra, 1936; Ganguly and Ramanujam 1959). On the other hand, Kathirvelu and Mahadevan (1967) and Singh *et al*. (1975) were successful in reproducing the disease. Singh and Singh (1974) reproduced wilt by inoculating healthy stalks using the plug method. Singh and Singh (1975a) demonstrated prevalence of two types of *F. verticilloides* in sugarcane stalks, namely virulent and non-virulent types. They opined that since non-virulent forms usually outnumber the virulent types, reproduction of disease on artificial inoculation is not always positive. Singh et al. (1980) established pathogenicity of *F. moniliforme* var. *subglutinans*, *A. furcatum* and *A. terricola* by inoculation in 6 to 7-month old standing stalks and by growing plants in the infected soil.

Wilt pathogens in association with other pathogens like *C. falcatum* (Singh *et al.* 1965; Singh and Singh, 1975b) or *Ceratocystis paradoxa* (Srinivasan 1964) cause much more damage than when present alone. Srinivasan and Vijayalakahmi (1961) 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. In continuation with *C. falcatum*, however, 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. Between the two pathogens, red rot caused greater deterioration in the quality of sugarcane than wilt (Singh and Waraitch 1981). On the other hand, Singh et al. (1975) reported that there was neither a consistently synergistic nor antagonistic effect when the wilt fungus is combined with red rot organism. The author has found that in both tropical and subtropical conditions, inoculation of *C. falcatum* and *F. sacchari* together caused more disease symptoms. Even red rot resistant genotypes showed more symptoms of red rot along with wilt (Viswanathan 2010). However, he found that the synergistic effect is variety specific.

In order to evaluate the response of different sugarcane genotypes to infection by wilt pathogens, most of the workers either considered the percent diseased stalks over the total number of the stalks observed (Sathyanarayana 1975; Srinivasan 1964) or percent length of the cane affected with the disease (Alexander and Lal 1967; Singh et al. 1975). Mohanraj and Alexander (1984) described

Available information on the associated pathogen is scanty, shallow and contradictory; hence a detailed investigation was carried out to characterize the pathogenic isolates by cultural, morphological and molecular methods after a comprehensive survey for the disease in major sugarcane growing states. The pathogen exhibited enormous variation in cultural characters and that could not be used to characterize the isolates. Using morphological features and molecular profiles in ISSR and IGS-RFLP, the pathogen was identified as *F. sacchari*.

During the recent years, sudden outbreak of *pokkah boeng* across the country was noticed in several varieties (Viswanathan 2012). It was found that *Fusarium* sp. associated with *pokkah boeng* also caused stalk infections and produced wilt in certain varieties. It is expected that further studies in this area would bring a new dimension on the Fusaria associated with *pokkah boeng* 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.

The disease could not be reproduced at ease under field conditions using a standard inoculation technique. Artificial inoculation experiments under disease endemic and non-endemic regions clearly revealed that a specific soil / environment is required for disease reproduction in sugarcane. Hence, there is a need to develop a region-specific disease-simulation technique for the disease. Involvement of parasitic nematodes in aggravating wilt incidence is suspected; however, further studies are required to establish their role in enhancing wilt infection in sugarcane.

It was clear from the field observation that the disease could be managed successfully in disease susceptible cultivars by reducing the associated biotic factor, root borer. Other control practices include use of clean seed from uninfected fields, use of resistant varieties and crop rotation. Biocontrol approach holds promise to manage the disease in endemic locations. Recent studies conducted at SBI and previous studies from Navsari, Gujarat, suggest that pressmud based *Trichoderma* formulations are effective in suppressing sugarcane wilt under field conditions.

Experiences in the past indicate that the disease occurrence is rapid and unpredictable due to changes in environmental conditions and this area needs special attention to identify specific edaphic and environmental factors influencing disease development. Sugarcane wilt fits to be an ideal candidate to study the impact of climate changes on disease buildup and development of epidemics in the future.

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