

Red rot of sugarcane (*Colletotrichum falcatum* Went)

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Abstract

Red rot of sugarcane was recorded more than 100 years before in Java, India, Argentina, USA and other countries, and it is one of the most devastating diseases of sugarcane. Since the cultivated sugarcane (*Saccharum officinarum*) has failed across the countries, systematic inter-specific hybridization between *S. officinarum* and the wild species *S. spontaneum* referred as 'nobilization' was done to develop resistant varieties and the disease was managed in most of the countries. However, in the countries especially in Asia, varietal breakdown to red rot caused severe epiphytotic, by which the resistant varieties failed in the field at regular intervals. New pathogenic strains of *Colletotrichum falcatum* with higher virulence were found responsible for varietal breakdown in sugarcane. Extensive cultivation of a single variety over large areas led to extensive crop damages due to 'vertifolia' effect in different decades in India. The current epiphytotic on the ruling variety Co 0238 has caused loss of more than one billion US dollars in the current season in the country. Detailed studies were done on pathogenic variation, epidemiology, screening methods, disease resistance mechanism, identifying effectors, pathogenicity determinants, antifungal genes and transgenics. Recently, complete genome and transcriptomes of *C. falcatum* were sequenced and pathogenicity hot spots and candidate secreted effector proteins were identified and this will further help to identify the candidate genes for further genetic manipulation. In spite of poor understanding on inheritance of resistance to *C. falcatum* in sugarcane, new varieties with red rot resistance were developed and deployed after each of the epiphytotic to save the crop. Further, other management practices including bioagents, chemicals and inducers were attempted and improved efficacy by mechanized sett treatment showed promising results to manage the disease under field conditions.

Keywords: sugarcane, red rot, *Colletotrichum falcatum*, life cycle, epiphytotic, varietal breakdown

Review Methodology: The review has been an update of classical and recent research works done on the important plant pathogen, affecting sugarcane production worldwide. The following web sources <https://www.isosugar.org/sugarsector/sugar> and <https://indiansugar.com/Statics.aspx> were referred for global scenario of sugarcane and its importance. Research works of Dr C.A. Barber and Dr E.J. Butler, who laid foundation for red rot work during the early part of the last century, were referred for historical perspectives on the area of research. CABI abstracts of the last 40 years were referred to collect recent research works on the area of review. The site of <https://www.cabi.org/isc/datasheet/25361#toDistributionMaps> was referred for disease distribution in the world. The following books, Diseases of Sugarcane: Major Diseases (Elsevier), Plant Disease: Red Rot of Sugarcane (Anmol Publications), Red rot of sugarcane (Technical Bulletin, USDA), Sugarcane Crop Management (SCI TECH Publishing) and Sugarcane Improvement through Breeding (Elsevier) were referred for disease symptoms, pathogenicity, disease cycle and disease management. Online papers from different sources were referred for detailed information on recent developments on pathogenic variation, host-pathogen interaction, sugarcane breeding, disease management, transgenic approaches etc.

Sugarcane and its importance

Sugarcane (*Saccharum* spp.) is a monocotyledon and member of the family *Poaceae*, tribe *Andropogoneae*. The genus *Saccharum* consists of 6 to 37 species depending on taxonomic interpretation and the members are of tall grasses, native to warm temperate to tropical regions of South and South East Asia. Sugarcane has thick, jointed and fibrous stalks of 2 to 6 metres tall that store sugar. Six *Saccharum* spp. viz. *S. officinarum*, *S. sinense*, *S. barberi*, *S. edule* (cultivated species), *S. robustum* and *S. spontaneum* (wild species) are well characterized and the cultivated sugarcane is an interspecific hybrid involving two or more species of *Saccharum* [1].

Sugar is extracted by evaporating the water from cane juice. Crystallized sugar production was reported 2500 years ago in India [1]. Arabs introduced sugar to the Mediterranean around the eighth century AD and Spain has started sugarcane cultivation by that time [1]. Sugarcane was among the early crops brought to the Americas by Spaniards. Although sugarcane was grown principally for sugar in the previous decades, now it is also grown for fibre and energy, primarily ethanol (biofuel), electricity from bagasse and bio-manure from filter cake. Since sugarcane is a C4 crop, it is viewed as one of the most capable biomass producer. The crop faces a wide range of issues from sugarcane production to sugar processing. Research institutions across the globe and industrial groups are pursuing ways and means to tackle the constraints associated to sugar manufacture, bioethanol production and sustaining cane farming (<https://www.isosugar.org/sugarsector/sugar>). Traditionally, sugarcane is propagated from stem cuttings (referred as setts) of one to three buds. The crop duration of sugarcane is 12–14 months and it can be harvested two to ten times; after each harvest, new stalks come from the stools, called ratoons; hence, the crop is cultivated like a plantation crop in many countries [2].

Sugarcane contributes approximately 80% of the global sugar requirement; sugar beet meets the remainder. Brazil, India, Thailand, China, the USA, Mexico, Pakistan, Australia and Guatemala are the major sugarcane-producing countries (<http://www.fao.org/faostat/en>). In India, sugarcane is grown in 5.2 M Ha area, which is approximately 3.0% of the total cultivable area in the country, and it contributes 7.5% gross value of agricultural production (<https://indiansugar.com/Statics.aspx>).

Disease incidence

Red rot of sugarcane was first reported as a disease in sugarcane in Java in 1893 [3]. Within a decade after Went's description of the disease and its economic damages to sugarcane milling in Java, its occurrence was reported in several other parts of the world. All the reports indicated that the disease was widely spread and recognized as a new disease of sugarcane in the countries like Australia;

India; the USA, including Hawaii and mainland; West Indies; Brazil; Mauritius; Philippines etc. [4–6] (Fig. 1).

Red rot is one of the most serious diseases of sugarcane in many countries including India, Pakistan, Bangladesh, Thailand, Myanmar, Nepal, Vietnam and other countries. In Louisiana, red rot was described as one of the factors causing stubble deterioration of sugarcane [7, 8]. The disease severely affected sugarcane production in Thailand during 1991–1992 and 2004–2005 due to cultivation of susceptible varieties. During 2011–2012 also, a severe red rot epidemic occurred only in Lopburi province where the variety K93-236 was grown [9, 10]. In Myanmar, red rot is a constraint to cane cultivation in all the sugarcane-growing areas. The disease is managed in the country by releasing resistant varieties. They regularly screen new sugarcane clones for red rot resistance [11, 12].

Red rot has been reported in different countries from Asian, African and American continents like Malaysia [13], Nigeria with 5%–12% incidences [14], Sudan [15], Indonesia, [16], Philippines [17], China [18], Colombia [19], Peru [20], Taiwan [21], Australia [22] and South Africa [23]. In South Africa, red rot was first identified in 1941 and the disease caused considerable destructions in the varieties POJ 2725 and Co 290 [24]. Red rot was found as one of the diseases that is economically affecting cane production in Guatemala and Nicaragua [25]. In Thailand, red rot occurs along with wilt; hence, it is referred as 'red rot wilt' or sugarcane 'root and stem rot' or 'red rot Fusarium stem rot' [26]. Currently, the disease occurs in all the sugarcane-growing continents and it is reported in 77 countries [5].

Red rot in India

In India, Dr C.A. Barber did foundation work on the disease when it struck for the first time in the then Madras Presidency [27–29]. Butler [30], the Imperial mycologist, at the Imperial Agricultural Research Institute, Pusa, Bihar (India), studied extensively on the causal organism and its portals of entry into the cane stalk. Based on the most distinctive feature of rotting of the internal stalk tissues with reddish discolouration, he named the disease as 'red rot'. Both Barber and Butler recognized the importance of the disease and devised management strategies of healthy seed and avoidance of waterlogging to reduce the crop damage in India. Severe red rot epiphytotic in the Godavari delta and North Indian plains caused extensive damages to sugarcane; however, this scenario resulted in the establishment of Sugarcane Breeding Institute (SBI) at Coimbatore, India, in 1912 by Dr Barber to develop red rot-resistant varieties through interspecific hybridization. Development of interspecific hybrids involving *S. officinarum* and *S. spontaneum* from Coimbatore started from Co 205 in 1918 and later hundreds of 'Co' varieties were released for commercial cultivation and adopted in India and in many other countries. Dr Barber was instrumental in developing many such interspecific hybrids; his contributions to



Figure 1. Distribution of sugarcane red rot in the world (<https://www.cabi.org/isc/datasheet/25361#toDistributionMaps>).

sugarcane have been recognized by naming one of the species of *Saccharum* native to India as *S. barberi* [31]. Similarly, sugarcane breeding centres in Java and Barbados also developed new varieties and these efforts have promoted growth of sugar industry in many countries [6].

Economic Impact

During the 1938–1939 season, a red rot epiphytotic of exceptional severity occurred in the subtropical region predominantly in Uttar Pradesh (UP) and Bihar, the major sugarcane region in India. This devastation resulted in failure of the major commercial variety Co 213, in which thousands of hectares were devastated. Due to the poor supply of canes, the sugar mills in the eastern UP crushed only one-third of their normal canes during 1938–1939 and half during 1939–1940 [32]. Severe infections of red rot can cause loss of nearly two-third of cane stalks produced in subtropical India [33, 34]. In Pakistan, 28.5% losses in cane weight was reported at initial infection by the red rot pathogen and it reached 82.7% when the disease intensity increased to 75% in the cvs L 116 and B 4360. Sucrose content, the main economic produce, was reduced in the range of 31%–75% at different infection levels [35,36]. Severe red rot epiphytotic in peninsular India during 1990s caused losses of 30%–50% in cane yield in the varieties like Co 6304, CoC 671, CoC 85061, CoC 86062, CoC 92061, CoSi 86071 etc. Yield losses of up to 100% were found in ratoon crops in different factory areas [37–40].

Red rot-affected canes show a decline of 29 to 83% in cane weight and 24 to 90% in juice extraction [6]. In the history of red rot epiphytotic in India, the country

currently faces one of the worst crop losses due to sudden failure of the most popular variety Co 0238 in the states of Uttar Pradesh (UP) and Bihar (Fig. 2). In UP state alone, nearly 0.5 M ha out of 2.6 M ha area has severe red rot during the 2020–2021 season (S.P.Singh, Unpublished). The crop losses due to red rot in the state have increased from few thousand ha during 2016–2017 season to this mammoth figure [41]. The total loss caused due to this disease outbreak works out to be 1.0 to 1.414 billion US\$ during the current season alone. The previous year's loss could be at least 40%–50% of the current loss. Overall, the disease has affected nearly 10% of the cane area in the country and it may further increase in the coming seasons. This is the economic repercussions caused by the disease in India; hence, it is called as 'cancer of sugarcane' [42]. Due to the disease, losses are encountered in different sectors such as farmers, small industries that manufacture *gur/khandsari* sugar, sugar mills etc. in the country (Fig. 3).

In South Africa, in the 1970s, widespread occurrence of the disease was found in the cooler, southern and inland parts, causing damage in many varieties including NCo 376 [43]. In Thailand, red rot caused cane yield losses from 34.6% to 73.7% in plant crop and up to 100% losses in the ratoons. The crop losses were estimated to approximately US\$20 million during severe epiphytotic of red rot wilt in 1990s in the country [26].

C. falcatum infects nodal tissues and kills the buds; hence, it causes losses in bud germination and crop establishment in the field. This leaves gaps in the field and a poor crop stand leading to losses in cane production. When setts of latent infections were planted in spring crop season, a maximum reduction in germination of up to 73% was recorded, whereas in autumn season, only 19%–56% reduction was recorded [44]. In a set of 13 varieties, impact



Figure 2. Complete destruction of sugarcane crops due to red rot in Uttar Pradesh, India.

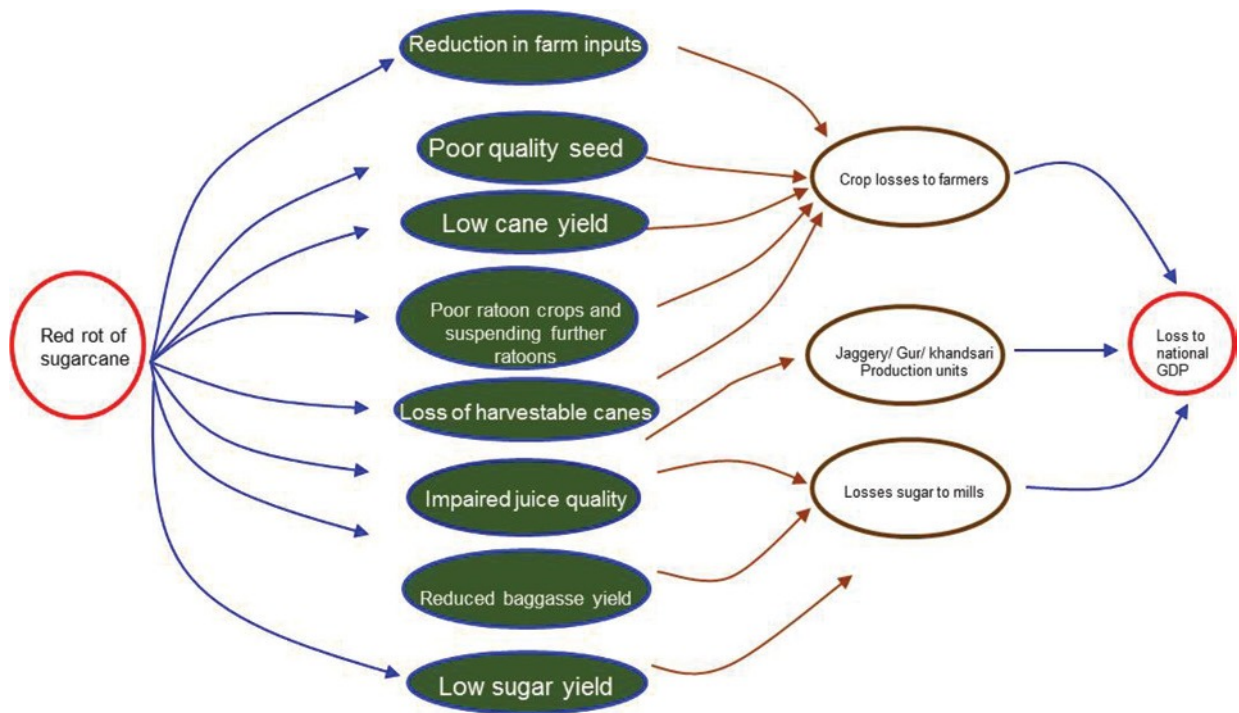


Figure 3. Impact of red rot on sugar(cane) economy.

of 9 isolates of *C. falcatum* inoculum in the soil at the time of planting of setts on bud germination and crop stand was studied for two seasons at Coimbatore under tropical conditions in India. In the first-year trial, the healthy controls recorded an overall germination of 74.0%, while the pathogen-inoculated plots recorded a mean germination of 43.6% only, showing a drastic reduction of 41.4% in bud germination. In the subsequent year, the same set of host varieties recorded an overall mean germination of 46.0% in the presence of the pathogen as compared to 75.3% in uninoculated control plots, showing a reduction

of 39.0% in sett germination [45]. Subsequently, it was found that the soil inoculum slowly builds up and causes disease in susceptible (S) and moderately susceptible (MS) varieties to varying extent. By 360 days, the nine *C. falcatum* isolates caused a mean reduction of 80.1% and 86.1% in cane population in the susceptible cvs CoC 671 and Co 94012, respectively, in a favourable season. Likewise, the MS cvs Co 06030, Co 06022, CoV 09356 and Co 06027 recorded a loss in cane population of 63.1%, 52.6%, 48.0% and 42.5%, respectively [45]. This study indicated that poor crop establishment due to red rot can cause a huge loss to

cane production in the plant crop in the disease-endemic regions. From such plant crops, ratoons cannot be taken up, which causes further losses in cane farming.

Red rot symptoms

The disease is recognized based on its characteristic rotting and reddening symptoms of stalk tissues. However, the pathogen infects the crop at all the stages of its growth. Earlier, the author has described detailed symptoms of the disease [6, 46]. Here, the disease symptoms are grouped into young crop, stalk and foliage symptoms.

Symptoms in young crop

During the germination phase, symptoms of pre-germination death of buds and drying of germinated sprouts/shoots are observed (Fig. 4). Infected canes or seed cane infections immediately after planting show progress of disease from the seed canes to new sprouts. In such cases, typical red rot symptoms can be seen inside the canes along with foliage discoloration in the sprouts (Fig 5). In case of infections of post-germination phase, the seedlings show yellow to orange discoloration and this leads to drying of entire shoots (Fig. 5). During late germination phase of the crop, drying of whorl alone within healthy leaves is seen due to passage of infection from shoot base to the apex. Later, the affected plant may gradually die or the plant may recover from the disease with new side tillers.

During tillering phase or before cane formation, the symptoms of the disease are witnessed in the form of mostly orange-yellow discoloration of spindle leaves and drying of the tillers (Fig. 6). Sometimes, the leaves in the whorl may show typical midrib lesions of varying intensities. Similar to the symptoms in plant crop, ratoons also show

death of new sprouts from the stools and this leads to poor crop establishment. Death of the stools in the ratoons shows gaps of varying sizes, depending on the severity of stools' infection.

Stalk symptoms—external

The disease-affected canes show drying of canes either singly or in clumps. The top two to three leaves in the spindle of the affected stalks show orange to yellow discoloration, which can be easily recognized from the background of green foliage (Fig. 7). The discoloured leaves dry soon in a few days. In severe cases, the entire field shows drying and complete crop failure occurs (Fig. 8). Similar to red rot, wilt or infestation of insect pests also manifests such as drying of canes in a clump. By critical examination of the affected stalks, red rot can be recognized based on rind discoloration of varying intensities and internal symptoms. Infection of internal core tissues leads to expression of characteristic rind discoloration, usually at varying intensities of brown or paleness of rind (Fig. 9). Other characteristic symptoms of red rot on the canes are typical nodal necrosis around the region of bud, growth ring and leaf scar; pinkish spore masses of the pathogen on nodal region, especially at root eyes, leaf scar or rind surface (Fig. 10); break-off of canes at nodes and de-topped canes in the field and appearance of fruiting bodies of the fungus (acervuli) on rind surface (Fig. 11). Rampant colonization of the fungus inside the canes severely impairs sap movement and translocation of photosynthates to the roots. Hence, the affected cane dries out in course of time.

Internal symptoms

Splitting of the affected canes longitudinally shows characteristic red rot symptoms inside the stalk as



Figure 4. Death of germinated sprouts/shoots due to red rot.



Figure 5. Primary infections of *C. falcatum* cause red rot inside the planted canes and progressive foliage discoloration in the sprouts.



Figure 6. Drying of the entire sugarcane with prominent midrib lesions (blackish) on the top leaves.



Figure 7. Red rot–affected sugarcane shows typical orange yellow discolouration of spindle leaves.

reddening of internal tissues with white spots that are usually elongated laterally covering the entire width of the cane (Fig. 12). Further, immediately after opening, a slightly acidic starchy odour emits from the affected cane tissue. The white spots may vary in size and number and occasionally they are so abundant as to give the tissue a mottled appearance (Fig. 13). Later, the affected canes show longitudinal pith cavities filled with greyish mycelia of *C. falcatum* (Fig. 14) and drying of the cane tissues. The red rot–affected canes also show other kinds of symptoms like dull brown discolouration of the ground tissue, characteristic serial spots in the internode tissues, reddish discolouration of the ground tissue without white spots, pits of varying sizes and numbers on pith region etc. (Fig. 15). Nature of varieties, stage of the crop, soil moisture, prevailing weather conditions etc. influence expression of these symptoms in the affected canes.

Foliar (midrib) symptoms

Usually, *C. falcatum* infects stalk tissues and foliar symptoms are observed as typical midrib lesions, occasionally, reddish areas on the sheaths and small reddish brown spots on the leaf lamina. On the midrib, the infection first appears as small brown to red spots on the upper and lower surface. These small spots expand rapidly in both directions and coalesce to form long lesions of few inches to entire length of the leaf or they may remain as a series of unconnected lesions. When favourable condition prevails, all the newly emerging leaves show midrib lesions (Fig. 16). During



Figure 8. Red rot–affected canes exhibit drying in large numbers in the entire field.



Figure 9. Red rot-affected canes show discolouration of greenish rind.



Figure 10. Diseased cane shows *C. falcatum* conidial mass on the nodal tissues.



Figure 11. *C. falcatum* fruiting bodies (acervuli) on nodal tissues and rind.

monsoon/ post-monsoon season, rarely top rot-like symptoms are noticed with rotting of entire undifferentiated leaves within the whorl with lesions (Fig. 17). Usually, a higher intensity of midrib lesions is observed after summer rains and during monsoon months in the field.

Combined infections of red rot and wilt

Since *C. falcatum* and *Fusarium sacchari*, the wilt pathogens, infect stalk tissue in sugarcane, combined infections of the pathogens are common in India and many other countries.



Figure 12. Typical red rot symptoms of reddish discoloration of ground tissue with white spots.

Usually, *F. sacchari* follows *C. falcatum*, and in such canes, progress of the latter inside the cane is ceased and the rotten tissues show drying with varying tinges of purple to pink or pinkish red in the affected tissues. After its entry, *F. sacchari* moves rapidly through the infected internodes from the base, reaches upper uninfected internodes and causes pithy cavities with pinkish discoloured patches that are characteristic to wilt (Fig. 18). Generally, combined infections of these two fungi cause quick drying of cane stalks than to their separate infections. Occasionally, red rot-affected canes show *F. sacchari* and *Ceratocystis paradoxa*, the pathogen associated with pineapple disease in standing canes (Fig. 19). Under field conditions, the stalk diseases of sugarcane like wilt, stalk rot and pineapple disease show certain overlapping symptoms with red rot [46, 47]. However, red rot-affected canes can be easily

identified with the characteristic reddening of internal tissues with white spots.

Cut ends of the affected canes show characteristic reddish patches interspersed with whitish or pale ground tissue or in some occasions reddish lesion accompanied with greyish mycelium in pith cavities (Fig. 20). This symptom is unique to red rot-affected canes, whereas wilt-affected canes exhibit typical hollowness in the pith region with brownish discolouration of the rim of the pith cavities (Fig. 21).

Pathogen

The Ascomycete fungus, *Glomerella tucumanensis* (Spegazzini) von Arx & Muller, is the associated pathogen. Under field conditions, only anamorphic stage of the pathogen *Colletotrichum falcatum* F.A.Went is recorded. The fungus comes under Phylum: Ascomycota, Subphylum: Pezizomycotina, Class: Sordariomycetes and Family: Glomerellaceae. After describing the disease in detail with the symptoms, Went [3] demonstrated the parasitism of the fungus he isolated from the diseased tissues, calling it *Colletotrichum falcatum* Went and further carried out life history studies.

Cultural characteristics

The fungal colony on oatmeal agar consists of abundant aerial mycelium, broadly spreading, sometimes zonate, densely woven into compact, velvety turf in some isolates, or a cottony, floccose one in others, referred as dark and light races, respectively [7]. Colour ranges from almost white through light ashy grey to dark grey, growing darker with age (pale olive grey to pearl grey) with no colour or pigmentation in reverse or in the medium. Hyphae are densely interwoven anastomosing in definite ropes. Conidia develop in a pink- or salmon-coloured, water soluble, mucilaginous mass, and when produced rapidly on the upper portion of the acervulus, it is covered with a shining droplet. The isolates show enormous phenotypic variation on the plates and broadly they are grouped as light and dark isolates; however, there are intermediate types with variation for sporulation and colony characters [6]. Several of the isolates studied at Coimbatore, India, were able to produce acervuli in culture with diameter ranging from 0.639 to 1.54 mm and setae are found in all the isolates, and in few cases, it was rare. The number of setae ranged from 3 to 20 per acervulus and length ranged from 90 to 220 μm . Conidiophore length ranged from 120 to 330 μm . In the past, several authors have reported emergence of new isolates with variation in morphological and cultural characters in *C. falcatum*. However, the dimensions of all the isolates may fall within the broad range for conidial, acervuli, conidiophore and setae.



Figure 13. Red rot-affected stalks show a mottled appearance of white spots.

Abbott [7] has observed that in artificial culture the dark-type isolates produce chlamydospores in much greater abundance than the light ones. Certain isolates of *C. falcatum* are reported to produce black hard structures, that is, stromatic bodies on culture media that consist of fertile hyphae, some of which perform true function as conidiophores and bear typical falcate conidia of *C. falcatum* at their tips. Earlier, a stroma-forming strain of *C. falcatum* was reported from infected sugarcane varieties from Karnal, India [48]. It is presumed that they may play an important role in perpetuation of *C. falcatum*; however, the conditions that favour development of stroma in the field are unknown.

Cultural instability of *C. falcatum* with the formation of sectors has been reported. Usually, the cultures grow less luxuriantly or become mycelial with low sporulation after a prolonged period of artificial cultivation. The successive

batches of cultures of an isolate obtained at different times varied in sporulation. It was reported that incubation in a dry atmosphere tends to reduce fructification, whereas incubation in a humid atmosphere favours it [7]. However, it was also reported that the isolates are morphologically stable without significant changes in virulence as a result of long continued cultivation on oatmeal medium [7]. The author has recorded instability of the fungal cultures in the long run and they have lost their virulence after repeated sub-cultures on oatmeal agar. Hence, many sugarcane pathologists pass the cultures through their respective host cultivars regularly, re-isolate and maintain in culture collections. Sometimes, the poorly defined 'sectors' or 'patch' variants appear in the plates, but transfer of these sectors to fresh plates produces colonies apparently identical with the original. Numerous reports on physiology of *C. falcatum* including media, nutrients, pH,



Figure 14. Pith cavities filled with greyish mycelia of *C. falcatum* during later stages of disease development.

temperature, conidial germination etc. were reported earlier in detail [6, 49]; hence, more emphasis is given on pathogenicity, pathogenic and molecular variation in the sub-chapter.

Although asexual phase of the pathogen is commonly recovered from the infected tissues, Spegazzini [50] and Carvajal and Edgerton [51] described and characterized the perfect state of the pathogen. In India also, the occurrence of perfect state of the fungus was recorded both under field conditions and in culture [52–54]. Duttamajumder [49] made a detailed account of perfect state of the fungus in India. He felt that the red rot pathogen is basically a leaf parasite similar to *Colletotrichum* infecting sorghum and it completes its life cycle on sugarcane leaf. He also opined that so long as *C. falcatum* restricts itself on the midrib, it does not cause any significant harm to the cane crop. It was emphasised that most of the midrib



Figure 15. Red rot-affected canes show serial spots (left) and pits (right) in the internodes tissue.

population survives on the midrib without causing much harm to sugarcane and only a few of them adopt to invade the stalk. However, studies conducted at Coimbatore revealed that most of the midrib isolates of *C. falcatum* from susceptible varieties are found to be more virulent than stalk isolates, which is in contrast to the previous observations [55].

Pathogenicity

The historic red rot epiphytotic on the popular cv Co 213 in the subtropical India led to the emergence of light-coloured cultures of *C. falcatum* with highly sporulating phenotype, which caused severe devastation on the variety during 1936–1939 [56]. For the first time in the country, the researchers were prompted to further study on the



Figure 16. Entire midrib in the spindle leaves show continuous lesions.

pathogen side since the research till that time was focussed only on deploying high-quality and high-yielding varieties. Successive failures of the varieties such as Co 301, Co 312, Co 313, Co 385, Co 421, Co 453, Co 513, BO 11, CoS 443 etc. were attributed to the highly virulent strains of the pathogen [57, 58]. Due to these developments, 1940 onwards, the researchers have initiated varietal screening for red rot resistance including germplasm [33].

The pathogenic isolates were maintained in different names in different centres. Historic failures of the popular variety Co 1148 in 1970s and later prompted the scientists to characterize the new virulent pathogenic strains on a set of differentials. In 1980s, a clear report on occurrence of three distinct pathotypes of *C. falcatum* from the subtropical region was reported based on the pathogenicity on a set of host varieties [59]. Subsequently, to characterize the pathogenic variation in *C. falcatum*, detailed studies were conducted in tropical and subtropical locations to identify suitable host differentials from 48 *Saccharum* spp. and hybrid cultivars [60]. A set of 13 host differentials were identified and established occurrence six pathotypes in *C. falcatum* in the country [61]. Later, prevalence of a new pathotype from the cv 85A261 along with three identified pathotypes from the cvs Co 419, CoC 671 and Co 997

was reported in coastal Andhra Pradesh and Odisha [62]. The new isolates were phenotyped on the host differentials; about 11 of them were characterized as designated pathotypes from 4 agro-climatic zones in the country till 2010 and used for screening of sugarcane progenies in the respective zones [6]. Another pathotype CF12 was recently designated from the tropical region [63].

Molecular variation

Earlier RAPD was used to group *C. falcatum* isolates and it was reported that the isolates had a general agreement with their pathogenicity on different sugarcane varieties [64, 65]. ITS nucleotide sequence analysis of 15 *C. falcatum* isolates in Thailand showed 95.32–100% identity with each other and 96.30–97.74% related to *C. falcatum* [12]. Nine isolates from red rot-affected locations possessed a high degree of pathogenicity on the susceptible cvs E-Heaw and K93-236, whereas those from non-epidemic were nonpathogenic. Furthermore, in the same study, the isolates from endemic areas had a light type of colony character and it was concluded that in Thailand *C. falcatum* is differentiated into two distinct races (pathogenic and



Figure 17. Red rot-affected plant shows death of young leaf with prominent midrib lesions on the leaves in the whorl.

nonpathogenic) based on their pathological character on the stalks of sugarcane.

Nine major *C. falcatum* pathotypes used for disease screening were characterized based on sequencing of 5.8-internal spacer (ITS) region of rDNA into two phylogenetic groups. Further, by using nitrate non-utilizing (nit) mutants, heterokaryon formation was demonstrated and vegetative compatibility grouping (VCG) in *C. falcatum* was standardized. The VCG grouped the mutants into five categories and through this different isolates of the same pathotype were recognized [66]. Grouping of the pathotypes based on VCG, pathogenicity and ITS was similar with certain deviation; however, these tools distinguished the two contrasting pathotypes CfI 148 and Cf7717 as reported earlier by RAPD [61, 66]. Additionally, serological reactions between antisera of the pathotypes and the antigen in diffusion agar plates gave a clear relationship between the pathotypes [61, 67]. Subsequent molecular grouping of the *C. falcatum* isolates revealed that the Indian isolates fell into three separate assemblages as Group I, II and III. Among them, the latter had a distinct phenotype of dark coloured, least virulent and non-sporulating had a similarity to other country isolates, whereas the first two subgroups had overlapping phenotypic and pathogenic features [68]. Apart from ITS

spacer region sequencing, other conserved genes calmodulin, actin and GPDH were used to further refine molecular grouping, which revealed the presence of one major group of virulent isolates and a minor group of least virulent isolates, which was established by definite nucleotide variation and further confirmed by RAPD and ISSR. Some of the primers were able to differentiate the virulent isolates with specific markers with the least virulent isolates. In addition, *C. falcatum* proteomes were established between virulent and least virulent isolates and identified distinctive and differentially expressed proteins related to virulence. Pathogenicity-related genes identified from *Colletotrichum* spp. and other fungi like glutathione S-transferase, DJ-1/Pfpl family protein and serine protease were identified from *C. falcatum* [69]. Recently, utilizing the NGS data, virulent strain-specific simple sequence repeat (SSR) marker from the genome of *C. falcatum* was identified [70].

Virulence diversity in *C. falcatum*

C. falcatum occurs in nature with enormous diversity for pathogenicity. It is dictated either by the varietal diversity or large spread of a ruling variety over large areas. The new



Figure 18. Sugarcane stalks exhibiting symptoms of combined infections of *C. falcatum* and *F. sacchari*.

pathogenic variants may be evolving from the prevailing pathotype(s) in the field with a significant gain in their pathogenicity and virulence due to adaptation to the new host varieties or to a new environment for the pathogen carried through seed canes. Diversity in the new *C. falcatum* isolates was carried out in the respective locations in India and no study was made to assess pathogenic diversity in huge collection of the isolates simultaneously in the country. The author has made an elaborate study to document pathogenic behaviour for nearly 117 isolates originated from tropical and subtropical states in two locations, Coimbatore (tropical) and Karnal (subtropical) on a highly susceptible cv CoC 671 for five seasons by following plug method of testing under field conditions [71]. The isolates displayed variation in their behaviour from season to season for red rot reactions at both the

locations. In the subtropical conditions, more number of less and least virulence reactions was recorded; however, more highly virulent categories also recorded there and it may probably be due to weather conditions prevalent after pathogen inoculation. Though both the locations showed similar behaviour for moderate virulence, at the subtropical location, it ranged from 5.22% to 21.15% during the five seasons with a mean of 11.94%, whereas in the tropical location, it ranged from 10.08% to 16.36% with a mean of 12.73%, indicating a stable behaviour in the latter than the former. Some of the isolates from the tropical region maintained a higher virulence in both the locations, whereas many of the subtropical isolates and pathotypes exhibited less virulence in both the locations. Virulence of the isolates was not static in the locations over the years for many of the isolates due to their stable and unstable



Figure 19. Combined infections of *F. sacchari* and *C. paradoxa* in red rot-affected cane (right) against healthy canes (left).

behaviour and place of phenotyping, source of host variety and prevailing weather conditions. Assessing more than 100 *C. falcatum* isolates simultaneously brought out existence of vast diversity in *C. falcatum* isolates for their pathogenicity in India. Probably such a high pathogenic variation is responsible for frequent varietal breakdown in the country for the 100 years.

Pathogenicity factors

It is well established that *C. falcatum* pathotypes vary in their virulence and this specific trait decides the pathogenicity of a particular pathotype. The pathotypes produce secondary metabolites and hydrolytic enzymes,

considered as bioweapons that probably contribute aggressiveness/virulence during pathogen infection and colonization. Several workers studied production of hydrolytic enzymes, viz. pectolytic and cellulolytic by *C. falcatum*. It was reported that virulent isolates of the pathogen produce more hydrolytic enzymes than weakly pathogenic ones [72–74].

C. falcatum produces a toxic metabolite initially reported as anthraquinone compound, which may facilitate pathogen infection and spread in the stalk. The phytotoxin is soluble in water and in many organic solvents, capable of producing symptoms of red rot in the stalks barring white spot [75, 76]. Later studies established a correlation between disease expression and production of phytotoxins and hydrolytic enzymes by the pathogen. *C. falcatum* is a typical hemibiotroph, initiates infections as a biotroph and turns to necrotrophic phase for colonization and ramification inside the host tissue to complete life cycle. The phytotoxins and hydrolytic enzymes produced during necrotrophic phase of the pathogen determine the pathogen colonization and tissue damage. When pathogenicity is interfered by co-inoculating *Trichoderma harzianum*, production of these metabolites was reduced with no symptoms production on the host [77]. Further, less virulent pathotype produces low levels of phytotoxins and hydrolytic enzymes compared to a virulent pathotype CF06 [78].

When major *C. falcatum* pathotypes were studied for the relation between toxin production and symptom production on leaves, it was found that the virulent pathotypes caused more severe symptoms along with more loss of electrolyte leakage. Further, the virulent isolates produced higher levels of pectinolytic and cellulolytic enzymes especially exo-polygalacturonase and melanin [79]. All these biochemical studies clearly revealed a positive association on the production of secondary metabolites like toxins and melanin and hydrolytic enzymes in *C. falcatum* virulence and disease expression. Recently, molecular basis of the pathogenicity factors were studied using both genomic and proteomic tools. A study of 28 pathogenicity gene homologues in two distinct pathotypes that vary in their virulence revealed a specific role of some of these pathogenicity genes in *C. falcatum* pathogenesis with a clear differential expression [80]. Tricyclazole interferes in germination of the conidia and appressoria production and its melanisation in many fungal pathogens. Using tricyclazole, the melanin inhibitor, the role of melanin in *C. falcatum* pathogenesis has been proved. The role of melanin was further established by studying the expression of melanin biosynthesis genes such as PKS1, SCD1 and THR1 [81]. Subsequently, knockdown mutants of *C. falcatum* were developed through RNA silencing strategy and *Agrobacterium*-mediated transformation to functionally analyse polyketide synthase I (PKS1), the major gene that regulates dihydroxy naphthalene melanin production. The loss-of-function mutants for PKS1 showed reduced pathogenicity in leaf and stalk tissues and established a clear role for melanin in pathogenic virulence in *C. falcatum* [82].



Figure 20. Cut ends of red rot–affected canes show reddish discolouration with white patches and fungal mycelium filled in the pith cavities.



Figure 21. Wilt-affected canes exhibit typical hollowness in the pith region with brownish discolouration in the rim of the pith cavities.

C. falcatum effectors

Proteomics-based investigations lead to characterization of cellular and extracellular virulence and pathogenicity factors produced by pathogens as well as to identify changes in protein levels in host plant upon infection by pathogenic organisms and symbiotic counterparts [83]. *In vitro* secretome of *C. falcatum* cultured under light and dark conditions using 2DE coupled with MALDI TOF/TOF MS was analysed. The study has identified nine differentially expressed proteins and revealed a major portion of alterations occurred in low molecular weight (LMW) proteins of less than 30 kDa. In dark cultures, the LMW proteins were either less abundant or absent, except a ceratoplatenin protein called eliciting plant response like protein I (CfEPLI), very high abundant LMW protein. While in light cultures, a novel protein named as 'plant defence inducing protein I' (CfPDIP1) was highly abundant [84]. Further studies functionally characterized distinct domains of CfEPLI and CfPDIP1 by *in vitro* expression and purification, which indicated that CfEPLI Δ NI-92 and CfPDIP1 Δ NI-21 induce hypersensitive reaction in tobacco and systemic resistance in sugarcane against *C. falcatum*. These studies have identified proteins that putatively contribute to *C. falcatum* virulence and demonstrated the potential role of PAMPs/ effectors of *C. falcatum* inducing PAMP-triggered immunity (PTI)/effector-triggered immunity (ETI) in sugarcane [85].

Complete genome of C. falcatum

For the first time, the *C. falcatum* genome was sequenced to be of 48.16 MB in size with 12,270 genes [86]. Subsequently, the transcriptome of *C. falcatum* (*in vitro*) was reported to be 31 MB with 23,136 predicted CDS [87]. Mining of *C. falcatum* genome and transcriptome data yielded putative 768 and 884 small secreted proteins (SSPs), respectively. The predicted secretory proteins were further divided into classical and non-classical proteins and discovered that signal peptides have an apparent role during pathogenesis by stabilizing fungal secretory proteins in the host environment. The SSPs contained a large number of esterase, proteinase, CAZy families, cytochrome P450, peptidases, secondary metabolites, transporters and transcription factors. In *in planta* transcriptomic studies, these SSPs were recognised as major pathogenic determinants [88].

Recently, the *C. falcatum* pathotype Cf671 (MTCC accession number-12142) and CfROC (isolated from the sugarcane variety ROC), R-1 (virulent isolate of CfROC recovered from the cv CoC 671) maintained at the red rot culture collection facility of ICAR-SBI, Coimbatore, were used for whole genome and consecutive transcriptome sequencing and also for the phenotypic and genotypic studies. Phenotypic studies were carried out to identify the infection process, mating type and population structure using high mobility group (HMG) proteins. Further

characterization had been done using SEM analysis, which revealed the nature of *C. falcatum* isolates. This finding shows that *C. falcatum* is a definite stalk-intriguing pathogen that establishes itself as a precursor in attributing gene families for its virulence. During interaction with sugarcane, *C. falcatum* expresses 2/3 of CAZy genes during biotrophic and necrotrophic phases.

Diagnosis

Serology-based diagnostics especially ELISA and dot-blot assays were developed earlier with antisera developed against mycelial proteins or specific polypeptide of *C. falcatum*. These assays were sensitive to detect the pathogen colonization in the nodal tissues like buds, root eyes, leaf scars and pith and rind tissue of an infected cane [89–91]. However, they have not been put into use later. Chandra *et al.* [92] amplified *C. falcatum* DNA with high specificity, efficiency and rapidity under isothermal conditions in LAMP assay. In this assay, they demonstrated that visual judgement of colour change in 1 h without further post-amplification processing makes the LAMP method convenient, economical and useful in *C. falcatum* diagnosis. However, here also the assay could not be adopted under field conditions. Dot-blot-based assay with non-radioactive probes was standardized at the institute and was found efficient to detect the pathogen in the soil [93]. It has been applied to assess *C. falcatum* propagule load in the soil before taking up new crop in the endemic locations in Tamil Nadu, India.

Infection process and life cycle

C. falcatum pathogenesis and infection process in sugarcane have been well documented. Recently, using modern tools, the process has been explained in detail with more clarity. On contact, the conidia germinate with germ tubes, produce appressoria, attach firmly to the sugarcane tissue and endure stably for varying periods. This may support the fungal pathogen to perpetuate when the infective mycelium is unable to proliferate further. The main portals of entry for the pathogen are leaf scar, root primordia and buds in the nodal region (Fig. 22). After penetration, the fungus makes inter- and intracellular colonization. Later, acervuli produce profusely around the infected tissues. Using *C. falcatum* isolate expressing green fluorescent protein (GFP) markers and other sensitive histological assays, infection, colonization and fructification of the pathogen on the leaf and stalk tissues were clearly established. On sugarcane leaf tissues, by 12 h post-inoculation (hpi), *C. falcatum* conidia germinate and form appressorial structures; by 24 hpi, after formation of primary hyphae, the fungus enters into the host cell; by 48 hpi, it spreads to nearby cells through secondary hyphae and this phase marks the end of biotrophic phase, in which



Figure 22. *C. falcatum* making progressive infection after entry through nodal tissues in sugarcane.

the fungus does not kill the cells. Later, necrotrophic phase of pathogenesis begins, in which the secondary hyphae continue to damage the cell structure with widespread colonization and kill the colonized cells to make rapid proliferation. The pathogen makes both intra- and intercellular colonization and emerges outside through the stomatal pores with sporulating acervuli structures with setae by 72 hpi (Fig. 23). On stalk tissue also, the pathogen shows similar infection cycle, unlike in the leaf tissue, and in stalks, the pathogen continues to make colonization due to availability of host tissue and make both upward and downward progress. In due course, it occupies the entire core tissues in the internodes and nodes. By 30–45 days, the pathogen macerates entire tissues and causes complete destruction of the stalk tissues. The pathogen also grows

inside and fills the pith cavities with greyish-black mycelia. The presence of acervuli throughout rind tissues and typical sporulation can also be seen on the root eyes, leaf scar and rind [6, 94–96]

In young sugarcane, pathogen infection occurs at the base of the shoot at the point of contact with old bud scales and scale-like leaves. The dormant mycelium/appressoria present in the buds and bud scales in the node and soil-borne inocula are the sources of infection for young shoots. This inoculum grows with the emerging bud and enters into the shoot, infecting the new leaves continuously and perhaps the growing point in a systemic way. Later in the season, the infection takes place through the nodal regions of the cane near ground level from conidia of the pathogen, carried through irrigation water or rainwater. The conidia of the fungus produced in great abundance on leaf midrib get washed down to soil by rain or dew into the cavity between the cane stalk and the leaf sheath and thus reach the nodes and cause infection mainly through the leaf scar region, root primordia and growth ring (Fig. 22).

The pathogen infection progresses considerably in the susceptible varieties in the same season or it may remain as dormant incipient infection. In highly susceptible varieties, the conidia landed in the whorl directly infect and penetrate the growing point to reach the stalk. Although *C. falcatum* may infect almost any part of the sugarcane plant, its importance is limited largely to its occurrence on the leaf midribs, the internal stalk tissues and the stubbles of the ratoons. Infection of the roots may occur; however, red rot is not important as a root disease.

Seed cane infection

Although in laboratory tests, infection of setts is possible through cut ends of the setts, and this mode of infection is of little practical importance. Agnihotri *et al.* [97] reported that diseased setts are the main sources of *C. falcatum* infection, and the disease spreads from the mother setts to the new shoots. Setts having both internal and external infections cause much more damage than the setts having either internal or external (nodal) infections. Nodal infections are mostly responsible for the spread of the disease in nature because of their very small size. They also reiterated that healthy setts can become infected if planted in soil that harbours red rot-affected cane debris. The inoculum in the debris readily infects nodal region, gradually proceeds to bud sprouts and causes death of young settlings (Figs. 4 and 5).

Leaf infection

In general, the pathogen rarely affects leaf lamina. Lesions produced on the leaf midribs are the characteristic symptoms on leaf tissues. In Louisiana, the first leaf infections are usually noted during May or June and

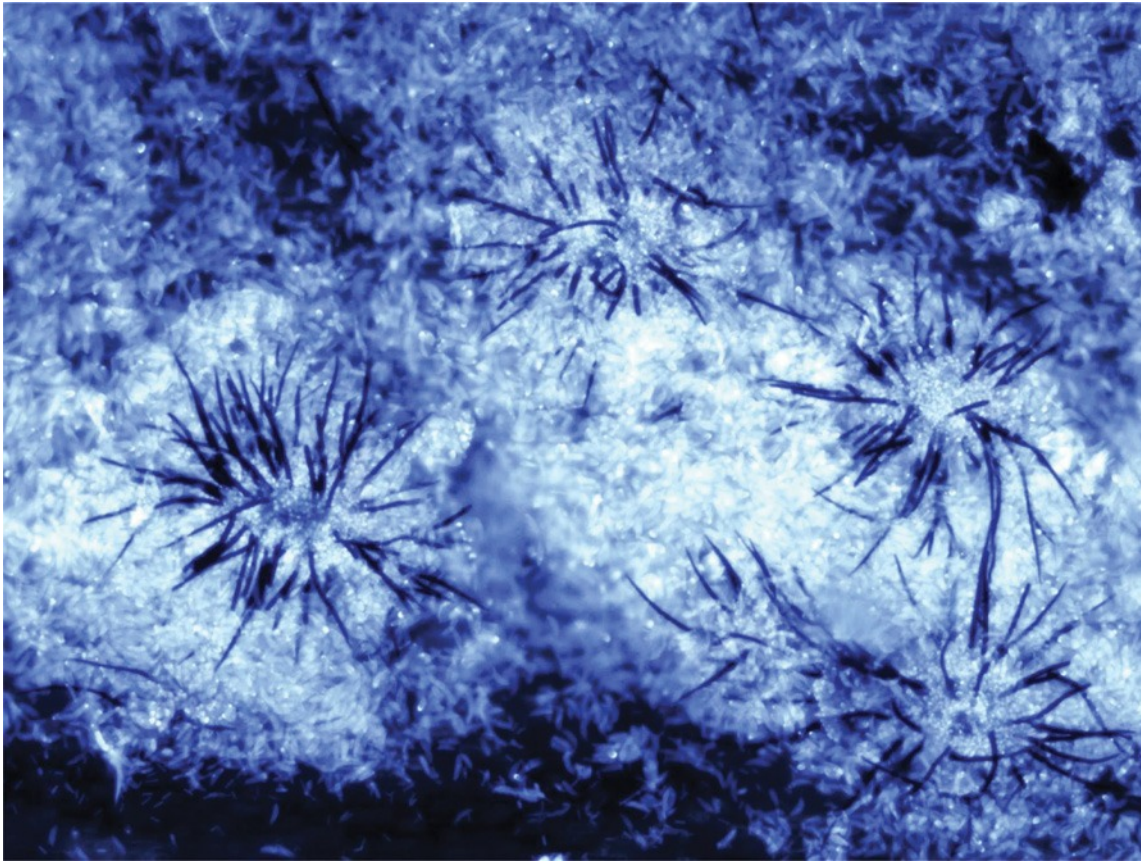


Figure 23. *C. falcatum* produces acervuli (with black paraphyses) with extensive conidial discharge on the surface of the cane tissue (Staining with fluorescence brightener shows conidia in white in the background).

infections on the midrib arise from small punctures made by leafhoppers when ovipositing. However, in India, researchers confirmed that infection takes place through the apparently uninjured epidermis [98, 99]. Fructification begins in 10 to 14 days following inoculation, the lesions extend longitudinally along the midrib and the fruiting area likewise increases so that old lesions are usually covered with black masses of acervuli.

Chona and Bajaj [52] observed only the perithecial stage of red rot fungus on the blade. Edgerton [100] reported stray occurrence of small spots on the lamina. Prakasam and Appalarasiah [101] reported dark brown spots with well-defined margins and mostly on matured leaves. In the advanced state, the area between the spots dried up presenting a straw-coloured appearance. In some of the top leaves, they found lesions on leaf lamina and sheath. The canes from such infected plants upon replanting exhibited disease and thereby confirmed the lesions caused by *C. falcatum*.

There is confusion regarding virulence associated with profuse sporulation in midrib isolates. Not all the highly sporulating isolates are found to cause stalk infections on the susceptible varieties [102, 103]. There are reports that most midrib isolates of *C. falcatum* are weak pathogens or less virulent and do not present any serious threat to cane cultivation. There is an opinion that the pathotypes that

cause midrib lesions are markedly different from those causing infection in stalk [104–106]. However, the author has found that midrib isolates are highly virulent on the stalks [55].

The author has studied development of laminar lesions under field as well as controlled conditions. On the leaf lamina, the pathogen causes reddish-brown lesions of varying sizes. The lesions are always linear in shape parallel to veins with irregular margin. Very often, the centre of the lesions will be broader with narrow ends. Yellow halos of 2–3 mm breadth surrounding the lesions are observed under high humidity conditions and lesion margin is not clear in such lesions. Usually, the centre of the lesions is lighter in colour than the margins where the colour intensity is more. Sometimes progressive lesions with yellow discoloration alone are noticed on the infected leaves. Such lesions may extend up to 60 cm in length. Later, such lesions turn pale and that particular region dry off. Adjoining lesions coalesce to cause extensive linear lesions with yellow discoloration covering most of the laminar region under favourable conditions. Sometimes the yellow halo at the ends extend on both directions as yellowish runners as observed in eye spot caused by *Bipolaris sacchari* on sugarcane leaves. Gradually, the runners extend on the sides and cause extensive lesions. In the large patch of discoloured lesions, laminar tissue shows

shot holes on the primary lesions and subsequently leaf shredding (Fig. 24).

Stalk infection

The fungus may enter the cane stalk through various channels. Went [3] concluded that natural infection occurs chiefly through the holes made by boring insects. Various other workers have reported influence of borer holes in aggravating red rot incidence [6]. In Louisiana, 50% of the *Diatraea saccharalis*-infested plants were badly infected with red rot [107]. Abbott [7] established that red rot infection in the growing stalks occurs principally through

the tunnels of the moth borer (*D. saccharalis*) in Louisiana and Southern Florida and, in some varieties, through the root primordia. However, studies of Singh *et al.* [108] revealed that stalk borer infestation does not play a conspicuous role in accelerating *C. falcatum* infection but showed a possible role in secondary transmission of the pathogen. Under Indian conditions, both in the tropical and subtropical regions, no association was found between infestations of stalk borer (*Chilo auricilius*), internode borer (*Chilo sacchariphagus indicus*) or top borer (*Scirpophaga excerptalis*) and red rot severity in sugarcane varieties. The author has found no changes in the disease progress from the bottom internodes to the upper nodes due to borer injury or there is a mild change in the lesion spread (Fig. 25).



Figure 24. Laminar infection of *C. falcatum* in sugarcane. Left: sugarcane cv CoS 8436 exhibits lesions on lamina, leaf sheath and midrib under natural conditions in Haryana. Right: laminar lesions of varying sizes with yellow halo extending to several cm under artificial inoculation (cv CoC 671).

Hence, borer pests of canes do not favour stalk infection under Indian conditions.

Chona [109] reported that red rot infection in varieties like Co 233, Co 396, Co 548 and BO 4 took place rapidly through growth cracks. Edgerton [110] also reported similar findings with Co 290. Later, it was clarified that pathogen infection takes place only when the propagules land on the split before the sealing process starts [99]. Some of the varieties have tendency of producing growth cracks in the internodes, especially during high rainfall or waterlogging. The longitudinal splits in the cane receives fresh conidia washed from midrib lesions in the canopy,

rain splash or wind. When the split injury is fresh, infection occurs and the disease progress is witnessed on both directions (Fig. 26). The fungus continues to develop in the tissues of both midrib and the stalk until the cane is harvested for seed or for milling.

Nodal infection

The conidia lodge at the nodes of mature stalks and cause infection in various tissues of the node such as the bud scales, root primordia, growth ring and leaf scar. Among



Figure 25. *C. falcatum*-inoculated canes show limited or no impact to lesion development with internode borer infestation. Yellow arrows indicate the pathogen-inoculated bore holes by plug method of inoculation. White arrows indicate borer tunnels.

them, leaf scar is the main portal of entry for red rot pathogen into the stalk [110–112]. Immediately after abscission, the ends of the vessels of the leaf scar are open and conidia may be sucked in or infection hyphae may affect entry. Srinivasan and Alexander [112] showed that dormant infections might occur in leaf scar tissue and on the bud scales, which could remain in that condition for long periods. Similar observations have been reported from Louisiana [113, 114]. It is probable that dormant infections under certain favourable conditions are activated and leads to the development of characteristic symptoms



Figure 26. Progress of red rot lesions through internodal splits. Arrows indicate the cracks or lesions below the split region.

of red rot. Hence, such infections are epidemiologically important.

Great differences were also observed in different sugarcane varieties for their susceptibility to nodal infection. The varieties that possess a comparatively greater resistance to nodal infections are likely to remain free from disease even if growing with plenty of *C. falcatum* inoculum in the field. The popular sugarcane cv Co 86032 of tropical India though susceptible to *C. falcatum* by plug method showed greater levels of nodal resistance to most of the prevailing pathotypes. Similar observations were made in many of the varieties tested under advanced varietal trials at Coimbatore, where attempted infections of the pathogen are noticed after nodal swab method of inoculation as reddish discolouration of leaf scar region to varying intensities in terms of spread and pigmentation (Fig. 27). The dark pigmentation due to accumulation of 3-deoxyanthocyanidin compounds restricts the pathogen progress through the leaf scar injury, whereas in the susceptible varieties, disease development occurs within few days after inoculation.

Further, certain *C. falcatum* isolates have tendency to cause infections on the nodal tissue while making progress inside the stalks, whereas other isolates skip the nodal region that is of fibrous nature to the next internode (Fig. 28). In case of the former, after completing infections on the nodal tissue, the fungus comes out through nodal tissues, mostly, root eyes and leaf scar, exposing the fungal propagules (Fig. 10). Through such events, the fungus spreads quickly in the field; however, in



Figure 27. Attempted penetration of *C. falcatum* in a resistant sugarcane variety shows dark reddish discolouration on nodal tissues.

case of the latter, the infective propagules release to the environment occurs after the death of the canes, probably in the next season as cane debris inoculum.

During 1990s, the author has observed no red rot in the cv Co 86032 in many fields that had 60%–80% red rot with the susceptible varieties like CoC 671, CoC 92061, CoSi 96071 etc. in the previous season. Subsequent observations for the last 25 years in the tropical region revealed that the cv Co 86032 remained free from the disease due to its nodal resistance and existence of field tolerance may have been due to resistance to nodal infection [31, 115]. It was also reported that the power

of nodal infection is not possessed by all the isolates of *C. falcatum* [109]. Evidently, the predominance of such isolates that possess this trait could cause any appreciable secondary infection of the crop and bring about an epiphytotic. Probably, the variety like Co 86032 may survive in the field till emergence of such matching pathotype(s) with capability to penetrate through node. The author has recorded occurrence of red rot in this variety in different occasions; however, it was confined to few clumps in Tamil Nadu state and probably new isolates have not emerged with complete pathogenic weapons to cause knockdown effect in this variety.



Figure 28. Nodal infection in sugarcane stalks as a trait of virulence in *C. falcatum* pathotypes. Left: The virulent pathotype causes susceptible reaction with extensive nodal necrosis and abundant fungal growth; Right: Less virulent pathotype causes moderately susceptible reaction with poor nodal necrosis.

Pathogen spread

The rate of *C. falcatum* spread within the seed canes after planting depends on the degree of red rot susceptibility of sugarcane varieties. Soil temperature and moisture significantly influence disease spread; however, both have a significant bearing on the growth of the sugarcane itself. Under favourable conditions, the pathogen spreads rapidly through both the vascular bundles and the parenchyma, and the entire tissues of the stalk may be invaded within 2 or 3 months after infection.

Pathogen spread within crop

Midrib lesions serve as the principal source of inoculum for stalk infections. Midrib lesions appear from two months after planting in the field and the frequent winds and dashing summer rains provide an excellent medium for spreading millions of propagules by splashing and blowing them to the stalks or to other leaves or by trickling over the leaf lesions and down to the stalk, where drops of water containing conidia may be held for several hours by the cup-like ligule. Detailed studies were carried out in the past on *C. falcatum* pathogenesis and disease spread under field conditions. Most of the *Colletotrichum* spp. infect foliar tissues or fruits in different plants, whereas *C. falcatum* infects stalk tissues in sugarcane. Foliar infection of the pathogen in sugarcane is rare and it never infects other plants under natural conditions. It has developed specific adaptation to infect sugarcane tissues at different stages. Most significant adaptation of *C. falcatum* is its capability to infect hard nodal tissues in the cane and cause midrib lesions. These two characters of *C. falcatum* make it a successful pathogen on sugarcane. After infection of nodal tissues, either it spreads both vertically and horizontally to cause damage to the cane stalks in the same season or the restricted infection as dormant infections on the nodal tissues help it to initiate fresh infection in the next crop

(Fig. 22). Midrib lesions directly help the pathogen to spread in the current season. Here also cane infection initiated from the midrib lesions in the whorl may cause dormant infections in the later stages of the crop. Detailed studies are required on the pathogenicity mechanisms developed by *C. falcatum* to understand more about its pathogenesis.

Pathogen spread from plant crop to ratoon

During harvesting, all the infected canes are cut and removed along with the healthy canes for sugar extraction. Although above-ground portion of the infected canes are removed, there is residual inoculum in the stubbles (Fig. 29). The pathogen remaining in the stubbles spreads to new sprouts depending upon the prevailing conditions at the time of ratooning. Some sugarcane varieties may show more of upward movement of the pathogen in the stalks. However, in some, the pathogen moves rapidly towards bottom and colonize effectively in the stool. Such varieties favour disease spread substantially from plant to ratoon crops. If we consider the leftover inoculum, it will be less in the former when compared to the latter. Overall, the pathogen completes life cycle within few months of planting of the crop or takes one season depending upon the variety, source of inoculum, prevailing weather conditions and agronomic practices followed in the field (Fig. 30).

Adaptation of *C. falcatum* to sugarcane varieties

Continuous emergence of new variants of *C. falcatum* is the major concern in the field as they pose challenge to host resistance in the new varieties. However, only limited information is known on the adoption of the pathotypes to the newly released varieties under field conditions. Earlier, Srinivasan [116] studied in detail on the role of host varieties on emergence of new isolates. He has shown that some sugarcane varieties induce rapid development



Figure 29. Red rot in the harvested canes, which serves as primary source for new ratoon crop.

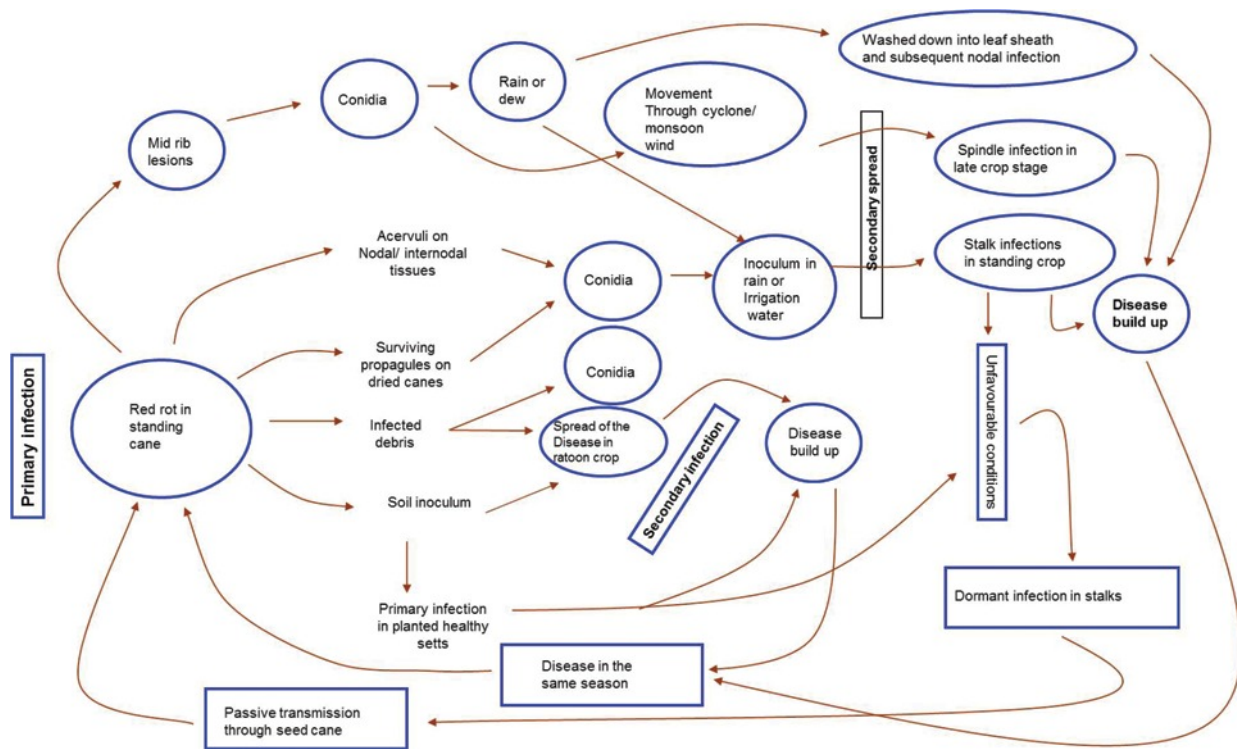


Figure 30. Disease cycle of red rot in sugarcane.

and dominance in infected tissues of the dark, avirulent type of variant, while others appear to favour the dominance of the virulent parental clone. Sometimes a more virulent isolate than its parental clone has also appeared. Srinivasan [117] also opined adaptive changes in cultivated *C. falcatum* in relation to the host varieties, with subsequent alterations in the virulence patterns of the fungus. He also observed that the pathogenic isolates are often unstable in their pathogenicity and have a tendency to pass irrevocably into an avirulent phase. Earlier studies of the author explained how a less virulent isolate gains virulence after several rounds of repeated inoculation and isolation on an incompatible host variety [118]. After repeated inoculations, the dark isolates at initial phases become light with increased sporulation on their adapted hosts. Development of light isolates and reduced latent period for symptom expression by repeated inoculations on incompatible host varieties indicated gain of virulence or pathogenicity of that pathotype for adaptation on a particular cultivar [119]. The adapted cultures were able to tolerate to the new cytoplasm as suggested by Srinivasan [116].

A detailed study was conducted by the author with 12 *C. falcatum* isolates and 20 varieties varying in disease resistance for 10 years to identify how the isolates change their behaviour on the host varieties under tropical conditions [118]. The isolates always exhibited their virulence on the susceptible varieties but not on the varieties with moderately susceptible or intermediate reactions when they were inoculated by plug method under field conditions. Also the isolates originated from susceptible or MS varieties had expressed their virulence

similarly, indicating that all the new pathotypes completely evolved with potential virulence to cause knockdown effect on the host. The study revealed that although the resistant varieties remained free from infections from the isolates, on few occasions, the isolates have broken barriers of incompatibility and exhibited disease severity. On susceptible host varieties, the expression of the virulence was in the range of 62.9%–97.9%, whereas on MS hosts, it was 21.3%–40%, clearly revealing that the pathogen has to evolve further to completely adapt on the latter group of varieties. Such evolution happens under field conditions after varietal introduction and the existing pathotype adapts to the host variety and slowly gain its virulence to cause knockdown effect. This study also demonstrated on the gain of virulence by the new pathotypes over the old pathotypes that were isolated 30 years ago, designated as CF04 (*Cf*419), CF05 (*Cf*997) and CF06 (*Cf*671) and used for the resistance screening in the tropical states. Meanwhile, in the last two to three decades, new varieties were deployed and correspondingly new pathotypes have evolved from the new varieties suggesting the gain of virulence from the new hosts.

In another interesting study, the author [63] has again demonstrated on the continuous evolution of *C. falcatum* in tune with the new varieties deployed in the field. He used the case study with *C. falcatum* pathotype CF06, the predominant pathotype of the tropical region in India used for varietal screening, and assessed how it has given way for new pathotype CF12 with 32 host varieties under field conditions for seven years. The then-popular cv CoC 671, which was cultivated extensively during 1980s and 1990s

in the tropical region, had a devastating red rot epiphytotic and led to the emergence of highly virulent pathotype CF06 during that time [6, 31, 39, 61]. However, many varieties like Co 86002, Co 87012, Co 92012, Co 92020, Co 94003, Co 99006, Co 2001-13, Co 06022, Co 06027, CoC 24, CoSi 6, 81V93, 89V44, CoV 92102, CoV 09356, PI 96-843, PI 1110, Si 7, Si 8 etc. deployed for cultivation from 1990s onwards succumbed to the pathogen though they were rated as resistant at the time of their introduction to the field. It was suspected that the pathotype CF06 either lost its virulence or new pathotypes with matching virulence have emerged in the field. Hence, the pathotype CF06 and a highly virulent pathotype Cf94012 were compared for their comparative behaviour on a set of 32 varieties varying in red rot resistance. On three R varieties, both the pathotypes behaved similarly, whereas on another six R varieties, the latter caused MS or S reactions. Further, on the S and MS varieties, the pathotype Cf94012 exhibited a very high virulence during the seven seasons indicating acquired virulence of the new pathotype, reflecting that the virulent isolates arose in the field on the above varieties. The new pathotype was designated as CF12 and has been used for varietal screening during the last five years. Even though the study has clearly demonstrated higher virulence of the pathotype CF06, it could not exhibit virulence like the new pathotype CF12 on the new varieties developed in the recent years. The reason could be that the pathotype CF06 is no longer in contact with the host in the field to gain virulence and the new pathotype CF12 emerged after the year 2000, an adapted one from CF06 to attack the new varieties by gaining virulence. This specific adaptation in the new pathotype matched to the new varieties; hence, they failed in the field. This is how new pathotypes adopt to the new varieties in the field. A role of high sugar in sugarcane varieties in acquisition of high virulence by *C. falcatum* was demonstrated with a set of species clones from *Saccharum* and *Erianthus* [120].

Gain of virulence by *C. falcatum*

Over the decades, it was found that the fungal pathogen gained virulence and the gain of virulence is directed by the host varieties deployed for cultivation in the field. Report of light-coloured *C. falcatum* cultures with highly sporulating phenotype from the cv Co 213 during 1936–1939 was first reported on the virulence in *C. falcatum* [56]. Later, another disease outbreak in the cv Co 313 led to the emergence of 'D' strain (isolate No 244) of the fungal pathogen [57]. Although *C. falcatum* that caused destruction in the field in the tropical region was known, major gain of virulence in the pathogen was found after failure of the popular high sugar variety CoC 671 in the states of Tamil Nadu, Kerala, Andhra Pradesh, Puducherry and Gujarat. For the first time, the origin of highly virulent pathotype Cf671, later designated as CF06, was recorded in the tropical region. It was also found to be the highly

virulent pathotype of all the major isolates available in the country that time. It had surpassed other isolates and pathotypes like CF04, CF05, Cf658, Cf6304, Cf8001 etc. in virulence [39]. It has maintained the virulence for more than 20 years in the tropical states. Another pathotype CF12 was recently designated from the tropical region with high virulence than CF06 [63].

It is reported earlier that in Indian scenario especially in the Indo-Gangetic plains in UP and Bihar, red rot epiphytotic followed a 'boom' and 'bust' cycle in the last 100 years [31]. Every time when the popular varieties were grown over large areas, the pathogen gained virulence substantially with something like super-virulent strains after failures of the varieties like Co 213, Co 312, Co 453, Co 1148 and CoJ 64 in the previous century. Recently, the popular variety Co 0238 was spread unscrupulously to the entire command area that resulted in evolution of another super-virulent strain in the region. In the state of UP, the variety was grown in 2.2 M ha (82.21% of total cane area) and in Bihar 0.16 M ha (64.12% of total cane area) in 2019–2020 cropping season [121]. This monoculture of single variety over a large area has favoured swift evolution of highly aggressive strain of the pathogen (vertifolia effect). In this situation, the pathogen has evolved very quickly and caused varietal breakdown within a few years. First incidence of the disease was recorded during 2016–2017 season in few districts and this historical epiphytotic engulfed nearly 0.5 M ha area in the current season, indicating very rapid changes in the pathogen virulence. In the past, such gain of virulence was witnessed after severe epiphytotic in the cv CoC 671 in the tropical region [39]. Although emergence of highly aggressive strains of the pathogen has caused havoc in the country, the breeders have always undermined the pathogen onslaught by promoting a single variety over several thousands of hectares. Greedy sugar millers also spread the variety in an unscientific manner for a short-term gain and finally end up losing to the pathogen. The loss does not stop here; due to the high virulence and aggression, the new pathotype causes varietal breakdown quickly and this poses difficulty to identify replacement varieties. Hence, the origin of new aggressive strains of the pathogen solely depends on the host side as suggested by Srinivasan [116].

Epidemiology

Primary infection

Infected seed canes serve as the principal source of primary inoculum for the annual recurrence of red rot in many countries. The setts may carry internal, external or both types of *C. falcatum* infection. High percentage of the nodes developed red rot and the infections developed from leaf scars and bud scales in Louisiana. When these apparently healthy stalks are planted under favourable conditions, they display development of the disease [114]. In India, infected setts and soil are reported as the primary

sources of *C. falcatum* inoculum for the disease recurrence in endemic regions [97]. *C. falcatum* forms abundant acervuli in nodal tissues and rind (Fig. 11). In nature, such nodal infections are mainly responsible for spread of the disease since latent infections are difficult to be detected by naked eyes [4].

Along with sett-borne infections, soil-borne inocula play a major role in red rot perpetuation and initiate disease cycle in endemic locations. Detailed studies were conducted in different decades on the role of soil-borne inocula in initiating disease cycle, their survival and influence of weather conditions. It was reported that various factors influence survival of *C. falcatum* in soil under field conditions [109, 122]. When one-cm long pieces of red rot-affected stalks and midribs were used, the fungus survives in the soil for two months [123]. Later, it was reported that *C. falcatum*-infected nodal and internodal tissue bits on soil surface survive for nine months. After burial of the stalk pieces, the presence of different types of fungal structures viz. conidia, thick-walled hyphae, setae, appressoria and chlamydo-spores were found for two months [124].

Survival of *C. falcatum* in the soil is influenced by the factors such as soil depth, soil moisture, sterilized or natural soil, nature of affected canes like split or un-split etc. Further saprophytic survival of *C. falcatum* in soil is low [6]. Furthermore, the occurrence of midrib lesions in the field especially in the young crop is an indication of disease spread from the infected setts/soil or ratoons from affected plant crop (Fig. 5).

The application of certain organic and inorganic amendments increased red rot incidence especially in the susceptible cv Co 312 [125]. Studies conducted in Coimbatore revealed disease development from *C. falcatum*-infected cane debris or multiplied on sorghum grains. The susceptible varieties readily picked up infections from such inoculum, whereas MS varieties had shown pathogen infection less frequently. However, MR varieties had shown infections rarely and both MS and MR varieties showed a differential interaction when different isolates are used [45]. Under field conditions, *C. falcatum* survives in the soil or stubbles for more than one year, especially when the stubbles are left undisturbed after one or two ploughings with or without green manure crop [6, 126]. However, Singh *et al.* [123] reported that the fungal pathogen survives in affected cane pieces in the soil up to 34 and 63 days in autumn and winter, respectively, under subtropical India. They found approximately 50 propagules per g of soil are sufficient to initiate infection and root primordia and leaf scars are the main portals of entry for the pathogen from soil.

The survival of red rot pathogen in the soil as thick-walled mycelium, appressoria, setae, chlamydo-spores and conidia that are liberated to soil during the process of decomposition of red rot-affected tissues are reported by many workers [124]. After two months of burial of infected tissues, all types of fungal structures, namely conidia, thick-

walled hyphae, hyaline hyphae setae, chlamydo-spores and appressoria, were present in about 30 to 50% of them. Five months later, they found setae, appressoria, chlamydo-spores and thick-walled mycelium and eight months later only setae and thick-walled mycelium were found [124].

Further, disease development of the soil-borne inocula of 11 *C. falcatum* isolates on 10 sugarcane varieties varying in disease resistance showed that the disease susceptible and MS varieties picked up infections and exhibited disease from germination phase onwards. Apart from a differential interaction of the *C. falcatum* isolates against the host varieties, the varieties showed a year-to-year variation in disease development from the isolates. For example, the cvs Co 403 and Co 06027 that remained disease-free against all the isolates during 2017–2018 season had red rot from four and eight isolates, respectively, during 2018–2019 season. Overall, the *C. falcatum* isolates from soil caused very severe red rot incidences of 71.4%–100%, in the two susceptible cvs CoC 671 and Co 94012. The differential interaction of the host varieties and the pathogenic isolates were clearly visible in the MS varieties. Among the MS varieties, the cv Co 06030 exhibited disease against 10 of 11 isolates with 83.3% mean disease incidence, whereas the cvs Co 06022, Co 06027 and CoV 09356 recorded red rot against 5–7 isolates with mean disease incidences of 23.4%, 47.5% and 27.1%, respectively [45]. Earlier, Singh [125] reported that the *C. falcatum* can survive in the rhizosphere of an MR variety without causing symptoms and the residual inoculum is able to infect a succeeding crop of a susceptible variety.

The general hypothesis is that high soil moisture favours red rot severity in sugarcane. However, other workers have reported that dry or drought period during growing season favours severe attack of the disease [127, 128]. Singh *et al.* [129] observed maximum red rot incidence in plant and ratoon crops in the fields, where irrigation after two months of cane germination was avoided as compared to other fields with normal irrigation or with waterlogging during monsoon in subtropical conditions. They explained that moisture stress in the summer predisposes the plant to attack of the pathogen and promotes formation of more dormant structures. Further, the presence of more amounts of dead parenchyma cells may enable the spread of the pathogen in stalk tissues. Due to water stress, more dead tissues occur; hence, the resistance offered by the host tissues against the pathogen spread appears to be reduced, which hastens the conquest of the pathogen [130]. The fungus surviving in dormant stages on young plants or infected sugarcane debris gets favourable environment upon commencement of rain at the end of June in subtropical regions to sporulate and produce abundant acervuli laden with conidia, which may initiate fresh infections. However, the formation of dormant infections in ratoon does not differ significantly with moisture levels [131]. Desiccation has been found to increase disease severity in the stalks of certain cultivars in Louisiana. It was found that red rot and desiccation have a

synergistic effect on spring shoot population reductions in all the tested cultivars, except one that was very sensitive to desiccation, suggesting that drought condition during the initial growth processes in sugarcane increases red rot severity [132].

In compost, the fungus was not found to survive for more than a month. This shows that the mycelium is unable to hold its own against microorganisms, which offer a keen competition [132]. Higher rhizosphere population of *Trichoderma* and other microbes was recorded in healthy sugarcane as compared to red rot-infected sugarcane in the fields [133]. There were also reports that rhizosphere soil of red rot-resistant cultivars was more lytic to *C. falcatum* than the rhizosphere soil of susceptible cultivars [125, 134].

Soil-borne inoculum and varietal breakdown

The experience of various workers indicates that sugarcane varieties easily succumb to *C. falcatum* under waterlogged conditions and even the MR varieties cannot withstand against the attack of the pathogen [6]. Various hypotheses have been put forth to explain varietal breakdown under waterlogged conditions. Several authors have opined that predisposition of the host to infection under such condition is the major factor [27, 30, 56, 135]. In Louisiana, the red rot is more severe in the heavy or black soils, which are usually poorly drained [7]. Such conditions favour the disease through the negative influence they have on the normal growth activities of the sugarcane plant, as well as by the direct favourable effect of high moisture on the fungus.

On stagnant water, aggregation of floating mycelia occurs with the production of conidia in acervuli. Further, the conidia germinate and fuse with each other, leading to aggregation of fused conidia. After fusion of conidia, some of the fused conidia germinate to produce conidia directly on them [136]. Waterlogged condition may favour exchange of genetic material between variable isolates through heterokaryosis and possibly new variant in the pathogen population may originate under field conditions. Further, it is possible that waterlogged conditions commonly prevail during the southwest monsoon periods in sub-tropical region and during northeast monsoon periods in east coastal region in India and may favour origin of new pathogenic variants capable of knocking down existing variety in the field.

Although such phenomenon occurred regularly, how the varieties that were hitherto resistant to the pathogen succumb to *C. falcatum* has not been comprehensively explained. Also longevity of resistance to red rot in sugarcane varieties has been unpredictable after their introduction to commercial cultivation. Recently, Viswanathan and Selvakumar [115] made a detailed field study on how varietal breakdown occurs in the sugarcane varieties to red rot using soil-borne inoculum sources and artificial inoculation by plug method. MR/MS varieties do

not harbour sett-borne infections, and debris-borne inoculum is the only available inoculum available under field conditions. Hence, about 11 *C. falcatum* isolates with different virulence potential were tested to assess behaviour on 10 varieties varying in red rot resistance from MR to HS under tropical conditions in the field. The varieties maintained their disease reactions in the plug method of inoculation, whereas they exhibited a differential behaviour against soil-borne inoculum of the respective isolates in the field trials. When the pathogenic behaviours in the two inoculation methods were compared, their phenotypic behaviour was similar in MR and HS varieties. However, four of the five MS varieties behaved differently to soil-borne inoculum of *C. falcatum* isolates, that is, they tend to show very high levels of disease incidences even up to 100% like susceptible varieties. Under field conditions, *C. falcatum* exhibits enormous variation, when a new variety is introduced although the interaction is incompatible initially, it makes continuous effort to adopt to the new host variety and make the incompatible to compatible in due course of time. Such a scenario was observed during the three years that the isolates with varied virulence were somehow able to cause disease in the MS varieties akin to their behaviour under endemic locations. Although the MS varieties succumbed to the pathogen under endemic locations, they were not totally knocked down as in the case of susceptible varieties. This observation also revealed that the study mimicked the natural adaptation of the pathogen to the new varieties and caused varietal breakdown under simulated conditions. This study also clearly established certainty of field tolerance possessed by the ruling variety of the tropical region in India Co 86032, which survived in the field for more than two decades in spite of its continuous exposure to abundant inoculum in endemic locations [31]. And, the same variety remained immune to soil-borne inoculum of all the isolates in the field trials, whereas it showed MS or S reactions by the plug method to different isolates. Additionally, it was found that due to its adaptation to withstand attack from the soil-borne inoculum, probably the variety remained in the endemic regions for considerably extended period in the field without perishing like other varieties as witnessed in the trials. This study also illustrated how a variety with field tolerance potential survived in the field and others even though they are MR or MS to red rot failed in the field conditions. This is the first study to explain and experimentally demonstrate varietal breakdown to red rot in sugarcane varieties.

By contrast, another popular variety Co 0238 cultivated in more than 2.5 million ha in the subtropical states in India succumbed to the pathogen after its adoption to large areas in Uttar Pradesh and Bihar states. The variety could not withstand the pathogen onslaught within two to three seasons especially in Central and Eastern Uttar Pradesh and Bihar. Currently, more than 0.4 M ha area is affected in the Central and Eastern Uttar Pradesh (Fig. 2), whereas in Western Uttar Pradesh, Haryana and Punjab regions, the

variety remained free from the disease till now. The major cause of varietal breakdown in the cv Co 0238 is attributed to severe waterlogging during monsoon season in Central and Eastern Uttar Pradesh and Bihar regions combined with sudden emergence of highly virulent pathotypes that were able to cause knockdown effect on most of the designated differentials used to characterize *C. falcatum* pathotypes [137]. Here, the major cause of the disease epiphytotic is attributed to soil-borne inoculum carried through monsoon rain over a large tract in the region. This is not the first time that a prominent variety is knocked down in the subtropical India and the same region witnessed such dreadful red rot epiphytotics after varietal breakdown for the past 100 years [31].

Secondary infection

Other than the primary infections caused by soil- and sett-borne inocula, secondary infections initiated by the inocula carried through rain and irrigation water play an important role in disease build up in the field. These infections carry the disease from plant to plant and from field to field. During active monsoon periods in India, inocula are carried for several kilometres and if a susceptible variety is grown over large areas, with a single monsoon flooding, the disease spread is expected in the entire command area. This is what happened in the last few seasons in Uttar Pradesh and Bihar states in India, where the predominant variety Co 0238 collapsed due to both primary infections facilitated by infected seed canes and secondary infections after the monsoon over several districts. Further, midrib lesions serve as a source for secondary infections and favour disease spread within the field. The conidia liberated through cool wind carried through air to a limited distance and rain-washed conidia drip down, reach the soil and become an infection source to be carried by irrigation or rain water. In addition, rain splashes from midrib lesions distribute the inoculum to a limited distance in the field during the rainy season. Virk *et al.* [138] found that spindle infection of *C. falcatum* has a positive indication of varietal susceptibility. They also reported that spindle infections are seen only in the susceptible varieties after inoculation, whereas the varieties with MS, MR or R remained free from infections. Midrib lesions that are usually rare in Florida suddenly appeared after the appearance of rust in the affected leaves [139].

Water plays a major role in dissemination of *C. falcatum* inoculum and probably predisposes the host to pathogen infection and invasion. The fungus in tissue bits survived for 7–8 months on the surface of damp soil [137]. The spread of *C. falcatum* to underground stems is an important factor that decides perpetuation of the disease in the stubbles and initiation of fresh disease build up in the ratoons. Build-up of the disease was found in ratoons in some of the susceptible varieties after plug method of inoculation; hence, there were suggestions to grade genotypes for

disease resistance based on development and spread of the disease in subsequent ratoons [140]. The spread of the pathogen occurs from the primary focus up to 6 m with dormant infections; however, the plants within 3.6–5 m showed drying in a susceptible variety [131]. This observation indicates inoculum threshold required for initiating disease development in the field. Usually, the *C. falcatum* inoculum is disseminated by irrigation or rain water to nearby plants in the field, which will serve as new infection foci in the ratoon fields.

Synergism with other pathogens

Synergistic activity of *C. falcatum* and *Fusarium sacchari* causing wilt in the stalks was reported by several researchers. Srinivasan and Vijayalakshmi [141] found natural infection of *C. falcatum* with *F. sacchari* in sugarcane. In artificial inoculation, they found synergistic effect of combined inoculation in disease-susceptible clones. *F. sacchari* traversed more number of internodes than *C. falcatum* when both were inoculated simultaneously and *F. sacchari* had no effect on pathogenicity of *C. falcatum* spread inside the canes, whereas the disease reaction of the former increased. Further, *F. sacchari* was isolated from internodal tissues without any visible symptoms; however, *C. falcatum* could be isolated only from the discoloured tissues [142]. Interestingly, it was reported that failure of the popular cv CoC 671 in Gujarat has been largely due to the combined infections of both the pathogens. Also, in adjacent fields of the cv CoC 671 having combined infections of red rot and wilt, the cv Co 86032 exhibiting wilt alone and the cv Co 92020 with red rot alone were commonly observed in Gujarat [143]. This kind of scenario exists in different states and such situation indicates the existence of variation in varietal resistance to both the pathogens. There are also possibilities of complementing factors between the two stalk pathogens. It is possible that *C. falcatum* may make available some growth-promoting factors needed by *F. sacchari*. It is also found that *F. sacchari* cannot cause severe damages in one season and it needs abiotic stress factors to cause more damages to the infected canes, whereas when both the pathogens infect the crop, the damage is quick and within few weeks, the entire stool dries. In Thailand, *C. falcatum* and *F. moniliforme* are reported to be associated with the red rot–*Fusarium* stem rot, the former found to play a more significant role in disease severity than the latter. A single infection by *C. falcatum* showed the same level of severity as infection from both *C. falcatum* and *F. moniliforme* [144]. Combined inoculations of *C. falcatum* and *Pythium arrhenomanes* causing root rot result in increased severity of red rot in the stalks under Louisiana conditions [145]. In addition to *F. sacchari*, *Ceratocystis paradoxa*, causing pineapple disease, was also found after *C. falcatum* attack. Here also, *C. paradoxa* follows *C. falcatum* in the stalks. Further, *C. falcatum* never infects the canes infected with these pathogens.

Sugarcane borer interaction

The incidence of red rot in South Africa was greater in the higher altitude areas, with long growing cycles, and also where canes are damaged by *Sesamia* or *Eldana* borers [23]. The disease severity in the country is aggravated when the incidence of stalk borers is high. Trenor and Bailey [146] found a direct relationship between the frequency of borer damage in the stalks and red rot incidence. *Diatraea saccharalis* is reported to transmit *C. falcatum* in Cuba [147]. Infestation of the cane stalks by sugarcane borers provides a main opportunity for *C. falcatum* infection in the stalks in any climatic conditions [148]. In the borer–red rot complex, nitrogen fertilisation was found to aggravate the incidences of both the insect and red rot. The number of larval bore holes had a correlation with the incidence of red rot. Increase in the number of red rot-affected internodes proportionately reduced sugar percent in the canes. Though nitrogen doses did not affect the attractiveness of sugarcane, higher N doses increased internodal tissue damages by *D. saccharalis* larvae [149]. Although N fertilisation through irrigation gave higher returns in stalk and sugar yields as compared to rainfed crops in Brazil, it had favourable effects on borer-rot complex. However, the gains of stalk and sugar yields due to N application were found to compensate for the increase in borer-rot complex infestation [150].

Red rot pathogen is associated with injury of the sugarcane borer *D. saccharalis* in Brazil. The effect of *C. falcatum* infection in sugarcane and borer weight gain and the attractiveness of herbivore-induced plant volatiles to the parasitoid *Cotesia flavipes* were examined. The wasps preferred *C. falcatum*-infected canes when a choice was given between mock-inoculated and herbivore-damaged infected plants. When fed on healthy plants, *D. saccharalis* larvae were larger as compared to those fed on *C. falcatum*-infected sugarcane plants and this indicated that the parasitoids are likely to benefit from being attracted to volatiles of healthy than the infected/damaged sugarcane. Further, *C. falcatum* infection was not likely to disrupt the parasitoid host location; however, the disease attenuates the attractiveness of herbivore-induced sugarcane volatiles to *Cotesia flavipes* [151].

Weather factors

After planting of seed canes, soil temperatures have an important bearing on the development of red rot within the stalk. Contrary to what might be expected from the fact that the optimum temperature for *C. falcatum* is relatively high (30 to 32°C), the disease is more destructive to seed cane when soil temperatures following planting are low rather than when they are high in Louisiana [7]. In the field trials conducted in Coimbatore, a higher red rot severity was recorded from soil-borne inocula during 2018–2019 season than the previous season. It was found that the latter

was warmer as compared to the former with more variations between maximum and minimum temperatures due to scanty summer rains during April–June. However, the 2018–2019 season that was conducive for disease development had limited variation for temperature and continuous rain during March to September [45]. Due to the favourable weather, the soil inoculum survived in the soil and caused disease during different stages of crop growth in the trial, whereas higher day temperature with a climate of drought during germination and grand growth phase do not favour disease development. A low day temperature due to frequent rains during grand growth phase is ideal for red rot incidences from the soil-borne inoculum or expression of the disease from the dormant infections in the underground tissues. This applies to the ratoon crops also, where the residual inocula in the stools cause fresh infections in the newly emerging shoots, if cool environment prevails. In the young affected shoots, midrib lesions appear very frequently and readily serve as a source for secondary spread (Fig. 16 and 17). In warm conditions of 16.5°C–35°C prevailing in September under Lucknow conditions favoured pathogen infection sugarcane varieties and the pathogen reaction was seen within 15 days of inoculation, whereas at low temperature, the susceptible cultivars tested behaved as moderately susceptible and the MS ones became resistant. Further, the age of the plant and sugar content did not have an effect on disease development in the internodal tissues [152].

Cold weather may check the spread of the rot but does wipe out the progress it has already made. Steib [153] reported that when the fungus was exposed to subfreezing temperature (–19°C) for 2–5 months it was not destroyed. This indicated that the red rot fungus in the mycelial state in the soil or in sugarcane residues survived winter conditions in Louisiana, USA.

A multiple linear model was developed for predicting secondary spread of red rot by Kumar *et al.* [154] under Haryana conditions in India. Their model accounted for 94% of variation in red rot incidence at different periods within the cropping season. The results also revealed that rainfall and number of rainy days along with minimum temperature and morning relative humidity are the major factors that affect the spread of the disease.

Host-pathogen interaction

In the absence of clear-cut evidences on inheritance of red rot resistance, the exact mechanism governing red rot resistance in sugarcane has not been understood clearly. Earlier, different biochemical features that impart resistance in a particular genotype have been studied. In response to *C. falcatum* infection, disease-resistant varieties develop gum deposits that block the spread of the advancing hyphae by filling up the intercellular spaces. This process takes place in advance of infection and seals off further spread of the pathogen in adjoining tissues [100, 155].

Although the role of phenolic compounds in red rot resistance was reported, subsequently no correlation was found between total phenolic content and degree of resistance to red rot in sugarcane [156]. The role of enzymes involved in oxidative and phenyl-propanoid pathways was reported to be associated with resistance in many sugarcane varieties [156–158]. Also at cellular level, these enzyme activities varied in sugarcane varieties; calli from a resistant variety recorded very high enzyme activities than the susceptible variety. Similarly, the pathogen toxin also elicited these defence enzymes differentially in the calli. Further, calli from the susceptible varieties recorded a more pronounced loss of electrolytes upon toxin treatment than in the resistant variety [159, 160]. The pathogen toxin caused more damages in the susceptible variety and it is reflected in electrolyte release to the medium.

Phytoalexins

The red substance released in the tissues including intercellular spaces near the invading hyphae in the stalks is referred as red rot pigment (RRP). Detailed studies were conducted on the RRP and their inhibition on conidial germination *in vitro* and mycelial growth was reported [161, 162]. Subsequently, the RRP showed presence of several compounds after fractionation and a differential induction of the compounds was found among the resistant (R) and susceptible (S) varieties after pathogen inoculation [161, 162]. Fractionation of the pigments by high-performance liquid chromatography (HPLC) revealed the presence of 3-deoxyanthocyanidin compounds, which were identified as luteolinidin, apigeninidin and caffeic acid ester of 5-O-apigeninidin. In incompatible interaction, these compounds present at much higher concentration, whereas in the compatible reaction, they were completely absent or present in very low concentration [163, 164]. Another interesting finding is that the S variety accumulated reasonable amount of these phytoalexins when the fungal toxin was used for inoculation, but in response to pathogen inoculation, the same variety failed to synthesize the antifungal compounds due to their degradation by the pathogen [164]. These findings clearly demonstrated that the phytoalexins are specifically accumulated only in incompatible host-pathogen interactions and compatible interactions had no such phytoalexins or with trace quantities. These three compounds were identified as phytoalexins in sorghum and *C. sublineolum* interaction, a similar host-pathogen interaction [165].

Further studies with a set of host differentials proved that there is accumulation of anthocyanin compounds in incompatible interactions and such induction/accumulation in compatible interactions is limited [166, 167]. While screening sugarcane clones for red rot reactions, phenotypic expression of incompatible (resistant)

interactions was always characterized by restricted dark pigmentation at the site of infection in the nodal or internodal tissues as compared to diffused pigmentation with spreading lesions in the compatible (susceptible) interactions (Fig. 31). Such rapid induction of the phytoalexin compounds in large quantities confines the pathogen at the site of penetration in the resistant varieties.

Later studies of Malathi *et al.* [168] conclusively proved that 3-deoxyanthocyanidin compounds luteolinidin and apigeninidin act as sugarcane phytoalexins and govern resistance in sugarcane to red rot. Recent HPLC assays revealed that the differential induction of nine different 3-deoxyanthocyanidin compounds upon the pathogen infection in cane varieties varies in disease resistance. Four of them were identified as luteolinidin, 5-methoxy luteolinidin, apigeninidin and arabinosyl-5-O-apigeninidin. The resistant cvs Co 93009, BO 91 and Baragua recorded multi-fold induction of luteolinidin, apigeninidin and arabinosyl-5-O-apigeninidin, respectively, in the stalk tissues after *C. falcatum* inoculation as compared to the susceptible varieties [169]. This study established that the phytoalexins are not induced in similar pattern in disease-resistant varieties and probably the compounds' induction varies based on genomic constitution of host varieties. Recently, it was established that phytoalexin fractions were found to exhibit a clear inhibition of *C. falcatum* on seeded agar [170].

Pathogenesis-related (PR) proteins

Detailed studies on pathogenesis-related (PR) proteins in red rot resistance showed higher activities of chitinase and β -1,3-glucanase in R varieties as compared to S varieties, constitutively and upon pathogen inoculation [171]. Further, Western blot assays revealed specific induction of chitinases and TLPs after pathogen inoculation in stalk and leaf tissue inoculation. The R variety showed more number of PR proteins with high intensity during early intervals than the S variety [172]. These studies gave a clear indication that the PR proteins may play an important defence mechanism operating in sugarcane against *C. falcatum*. This differential induction of chitinases was subsequently validated through transcript expression and real-time PCR assays [173, 174]. Sugarcane chitinase ScChiBI was characterized further based on its full-length sequence, and it had structural resemblances with the classes I/IV chitinase beginning with a signal peptide and ending with a signature domain. The results concluded that the predicted structure of ScChiBI belongs to class IV of family 19 glycosyl hydrolases. Wound-inducible protein in sugarcane SUGARWIN2, a homologue of a barley wound-inducible protein (BARWIN), was found to induce in response to stalk borer *Diatraea saccharalis* infestation but after pathogen infections. Recombinant SUGARWIN2 was found to exhibit anti-fungal activities on *C. falcatum* including alterations in morphological features like



Figure 31. Nodal tissues exhibit a diffused pigmentation with spreading lesions in the internodes of compatible interactions (left); incompatible reactions show a restricted dark pigmentation at the site of pathogen entry in the nodes (right). Yellow circles indicate inoculated nodes by cotton swabbing method.

increasing vacuolization, damages to fungal integrity and leak of intracellular material that led to conidial apoptosis [175]. Quantitative transcript analyses of mucoprotective genes *SUGARWIN1* and *SUGARWIN2* revealed maximum expression in the resistant genotype as compared to lowest in the susceptible genotype [176], and the study has suggested a probable antifungal activity of *SUGARWIN* proteins in sugarcane against *C. falcatum*.

Molecular basis of red rot resistance

To elucidate the mechanism underlying host-pathogen interaction, it is important to determine the host targets in sugarcane during its interaction with *C. falcatum*. To investigate the mechanism of red rot resistance, both genomic and proteomic tools were adopted and standardized for sugarcane [156, 177]. Since the pathogen

infects the stalk tissues, standardized protein extraction from the fibrous stalk tissues and MS analysis and developed a stalk specific proteome for the first time [178]. The 2D-based core proteome analysis classification was performed during the interaction in sugarcane with *C. falcatum* [179]. The study has identified proteins involved in several defence and cellular functions such as cyclic nucleotide-gated ion channel protein, R2R3-MYB transcription factor MYB6, WRKY transcription factor, flagellar-associated protein, class III HD-Zip protein HDZ34, calcium-dependent protein kinase, DNAJ heat shock protein, callose synthase, PISTILLATA-like protein, putative p-coumarate 3-hydroxylase, NB-ARC domain-containing protein, cytokinin oxidase 3, UDP glucose:flavonoid 3-O-glucosyltransferase, NADH dehydrogenase, ACC synthase, putative disease resistance protein etc.

Expression pattern of transcription factors (TFs) in sugarcane in incompatible interaction and systemic acquired resistance (SAR) inducer priming revealed that eight TFs are highly induced in both the cases. The TFs that are significantly induced early are expected to involve actively in triggering or co-ordinating defence against pathogen invasion [180]. Subsequently, temporal expression of 22 putative defence-related genes by reverse transcription (RT)-PCR in red rot-susceptible cv CoC 671 with response to priming using various SAR inducers, viz. benzothiadiazole (BTH), salicylic acid (SA) and *C. falcatum* elicitor, was studied. Upon SAR induction in response to pathogen challenge, differentially regulated phenylpropanoid pathway genes such as cinnamic acid 4-hydroxylase, 4-coumarate:coenzyme A ligase, chalcone synthase, and chalcone reductase and R genes such as NBS-LRR genes and basal layer antifungal peptide (BAF) genes were found upregulated. These upregulated genes are likely to play a potential role in SAR induction and might contribute to defence against *C. falcatum* [181].

Later, throughput genomic approaches like differential display, subtractive libraries and next-generation sequencing platforms were employed to unravel red rot resistance in sugarcane. In differential display, reverse transcription polymerase chain reaction (DD-RT-PCR) identified altered expression of genes in response to *C. falcatum* infection in red rot resistant cv Co 93009. Differentially expressed genes represented five categories, of which the defence/stress/signalling group was the largest, with clones homologous to genes known to be actively involved in various pathogenesis-related functions in plant species. The study reported overexpression of several transcripts related to ethylene-mediated and jasmonic acid pathway of plant defence mechanisms [182]. Later, an *in vitro* system of using sugarcane suspension cells and crude elicitor of *C. falcatum* was standardized for transcriptome analysis and identifying defence-related genes in sugarcane. The results revealed upregulation of many potential defence-related transcripts like putative chitinase, glycine-rich protein, 14-3-3-like protein, xylanase

inhibitor protein, calmodulin-related protein, Myb-related transcription factor CBM2-like, basal layer antifungal peptide etc. Further, by adopting RACE-PCR approach, complete gene sequences of 14-3-3-like protein and xylanase inhibitor were identified and the genes were characterized to domain level. It was reported that the transcript profile in *in vitro* system of sugarcane suspension cells and Cf-elicitor has been close to the cane tissue challenged with the pathogen and the approach was useful to identify defence-related traits in sugarcane against *C. falcatum* [183].

Suppression subtractive hybridization (SSH), a more efficient molecular tool, was employed to a series of SSH libraries, developed from sugarcane stalk samples to understand sugarcane defence responses during the initial phase of *C. falcatum* pathogenesis. A total of 139 ESTs were obtained, which were functionally categorized as belonging to recognition and signal transduction, oxidative stress, redox maintenance, membrane trafficking and transport, defence and programmed cell death, energy and photosynthesis, metabolism, secondary metabolite biosynthesis, cell/nuclear structure and unknown categories. For the first time, this study identified a network of early defence responses and related signals in *C. falcatum*-resistant sugarcane in response to the fungal pathogen infection. Also for the first time, a set of differentially expressed EST clusters in red rot-resistant sugarcane variety was identified in response to *C. falcatum* infection [184, 185].

Subsequently, the next-generation sequencing (NGS) approach was combined with SSH and large transcriptomic data specific to red rot resistant and susceptible libraries were obtained. After sequencing, 10,038 and 4,022 high-quality transcripts were obtained from resistant and susceptible libraries, respectively. Based on mapping of the transcripts to KEGG-KASS database, the presence of a CEBiP receptor and the signals ROS, Ca²⁺, BR, jasmonic acid (JA) and ABA was identified in both the responses. However, MAPK, ethylene (ET), phosphoinositide (PI) signals and JA amino conjugation were found only in the resistant interaction and validated expression of the transcripts involved in these pathways through qRT-PCR assays. This study concluded that perception of PAMPs occurs in both compatible and incompatible systems; however, downstream signalling through MAPK, ET, PI and JA amino conjugation and activation of R genes happens only in the resistant interaction. Further, this is the first detailed transcriptomic analyses of compatible and incompatible interactions in a sugarcane variety exhibiting differential interaction with two *C. falcatum* pathotypes that vary in their virulence through SSH and the NGS platform [186]. When two varieties were used to generate SSH libraries, interference of their varied genetic background cannot be ruled out, but the use of a variety that behaves differentially to two pathotypes circumvented these cross reactions. This study has clearly brought out a library of expressed genes during resistant and susceptible

interactions with *C. falcatum* in sugarcane. Further studies are envisaged on applying genome-editing approaches to specifically manipulate susceptibility-associated genes to obtain genome-edited plants with tolerance to the dreaded pathogen. This strategy may bring more dividends to safeguard elite sugarcane varieties that failed in the field due to varietal breakdown.

Induced resistance

In line with induced systemic resistance (ISR) against *C. falcatum* in sugarcane, detailed studies were conducted on systemic acquired resistance (SAR) by synthetic signal molecules in sugarcane. Initially, acibenzolar S-methyl (ASM) was studied along with salicylic acid (SA) and ASM and was found to persist up to 30 days in the pre-treated cut canes. Increased phenolic content and accumulation of pathogenesis-related (PR) proteins, viz., chitinase, β -1,3-glucanase and thaumatin-like protein (TLP) (PR-5), were observed in sugarcane cvs CoC 671 and BO 91 [187]. Subsequently, the synthetic elicitors like benzothiadiazole (BTH), SA and isonicotinic acid were found to reduce *C. falcatum* colonization in cane stalks by triggering a range of defence-related compounds [188]. Later it was found that Cf-elicitor, a glycoprotein extracted and purified from the cell wall of red rot pathogen, exhibited induction of many defence-related compounds and PR proteins similar to BTH in susceptible sugarcane cvs CoC 671 and CoC 92061 [189].

Comprehensively, studies on induced resistance involving synthetic/biotic inducers have demonstrated the disease-suppressive effects of BTH and Cf-elicitor against red rot of sugarcane under field conditions. However, many factors like cost-effectiveness and lack of protein sequence information have deterred scaled-up applications of BTH and glycoproteinaceous Cf-elicitor, respectively, for field situations. Efficacy of the inducers BTH (125 μ M) and Cf-elicitor (60 μ g glucose equivalents ml^{-1}) was studied for induced resistance in reducing red rot incidence during the early phases of crop growth against soil-borne inoculum and in that they suppressed disease intensity significantly. During grand growth phase also, the inducers exhibited induced resistance in the stalks upon pathogen challenge in the susceptible variety [190]. Further studies were continued to identify pathogen-associated molecular patterns (PAMPs) and *C. falcatum* effectors to improve induced resistance through pathogen-derived resistance approaches. In this regard, recently, PAMP/effector molecules, viz. CfEPLI and CfPDIP1, are found to suppress *C. falcatum* colonization in the stalks by activating systemic resistance. Molecular basis of induced resistance revealed an upregulation of the master regulator NPR1 and PR genes, a similar phenomenon demonstrated earlier for BTH-mediated induced systemic resistance. The study further revealed involvement of SA-mediated defence pathway during priming of sugarcane with BTH, CfEPLI

and CfPDIP1 [95]. The recent studies on host-pathogen have opened opportunities to explore further to identify interacting partners of the characterized PAMPs/effectors to reveal the series of molecular signals and downstream activation of defence molecules that could lead to delineation of PTI/ETI mechanism in sugarcane.

Management

Developing resistant varieties to red rot has sustained sugarcane cultivation over a century. Hence, breeding in sugarcane revolves around red rot resistance in many Asian countries [31]. However, other management strategies were developed time to time to manage the disease in different countries to reduce the damages caused by the disease in the field. Moreover, in many occasions, the ruling varieties fail due to varietal breakdown to *C. falcatum*, and alternate strategies are required to moderate the disease severity and to save the crop.

Breeding for red rot resistance

Managing red rot through host resistance is the most sustainable way; hence, research efforts are being made to develop and deploy resistant varieties for the past 100 years in different countries. In India, cv Co 205, the first successful interspecific hybrid involving *Saccharum officinarum* and *S. spontaneum*, was introduced for cultivation [31]. Though the variety became popular in the subtropical India, it picked up red rot within few years after its introduction. Subsequently, many varieties were developed and deployed for cultivation; however, many of them succumbed to the disease. The adoption of improved cvs Co 210 and Co 213 in northern India immensely contributed in quantum jump in the cane yield and replaced all the *S. barberi* varieties under cultivation. However, during expansion of cane cultivation to larger areas with the new elite varieties, precautions of good quality and healthy seed were totally ignored. Hence, during 1938–1940, cv Co 213 perished in several thousands of hectares in that region in the severe red rot epiphytotic. Later, the disease gradually destroyed other varieties like Co 290, Co 312, Co 313, Co 357, Co 370, Co 393, Co 421, Co 453, CoS 5 etc. in the region. This made a policy change in sugarcane researchers in India to focussed breeding with red rot resistance as one of the major component [6,31]. In tropical India, replacement of *S. officinarum* cultivated in the early decades of the last century with interspecific hybrids reduced the disease severity. Similarly, *S. officinarum* clones were replaced with the hybrids in West Indies in the past and red rot as a major disease became a minor disease [6].

Experience of different researchers over the decades revealed only limited information is available on inheritance of resistance to major diseases. In case of smut and rust diseases, additive genetic variance is important, which

clearly indicates that substantial progress can be made by selecting resistant parents [191, 192], whereas little is known about the genetics of resistance to red rot [193]. Earlier, it was suggested that the dominant genes from *S. officinarum* might be involved in masking the effect of *S. spontaneum* resistance genes [194]. However, there were conflicting reports that either there is no association between the degree of resistance of the progenies and parents [195] or crosses involving resistant parents tend to have more resistant progenies than from crosses involving susceptible parents [196, 197].

Information available from a large number of crosses made in Coimbatore and elsewhere indicates that in crosses that involve resistant parents, a high percentage of the progenies tend to be resistant or moderately resistant. This was also true with respect to a directed breeding programme for red rot resistance initiated by Alexander *et al.* [198] at ICAR-SBI, Coimbatore. This was a directed breeding programme to evolve varieties with broad spectrum of resistance against two or more major pathotypes of red rot. Under this programme, by inter-crossing selected resistant parents and subsequently by inter-crossing and selecting resistant progenies, a number of clones that are resistant to 2 to 4 pathotypes were obtained. This study indicated that as in the case of other diseases, for red rot also, additive genetic component is important and the incorporation of parents with resistance in crosses will help in building up a source population. Alexander *et al.* [199] identified horizontal resistance in sugarcane progenies by testing them at various locations in India and screening them with the respective zonal pathotypes in Karnal in Haryana and Chakia in Bihar in subtropical region, and Tanuku in Andhra Pradesh and Nellikuppam in Tamil Nadu in the tropical region. Natarajan *et al.* [200] investigated the relative importance of vertical (race specific) and horizontal (essentially race non-specific) resistance in sugarcane to red rot biometrically. They pointed out a progressive shift against HR brings out the importance of gene complexes from *S. spontaneum* in imparting stable horizontal resistance against red rot in sugarcane.

Ram *et al.* [201] identified the parental clones Co 8347, ISH 021 and Co 86011 as good general combiners for imparting red rot resistance. Of the 30 parental combinations studied for their contribution to impart red rot resistance, Bora *et al.* [202] found only 12 combinations involving elite parents viz. Co 1148, Co 88039, Co 6806, C 79180, Co 356, CoS 70 and Co 6304 in developing agronomically superior progenies with resistance to red rot. Slow red rot development in sugarcane was described in sugarcane varieties based on drying of tops, nodal sporulation and spindle infection after pathogen inoculation by nodal, nodal injury and plug methods. The cvs CoH 110, CoH 119 and CoPant 84211 show slow red rot as they did not show any of the three symptoms and nodal sporulation was found to be positively correlated with spindle drying [203]. Babu [204] analysed association of red rot resistance

in sugarcane with major yield and quality traits for effective family selection. He reported a positive correlation between red rot resistance and tiller counts at 90 and 120 days. The author claimed the presence of *S. spontaneum* genomic complement in the progenies, which is characterized by high tillering and resistance to red rot. In the individual families, he found a positive correlation with sucrose per cent in two families Co 98006 × 987001 and CoJ 72 × Co 62198. Among the two families, the former produced the highest proportion of resistant progenies (58%) with moderate level of sucrose content (14.98%). This cross combination offers much scope for future exploitation in breeding programme targeting to combine red rot resistance and appreciable juice quality. However, the conclusion made by the author is that the association of red rot resistance with tiller count has no meaning. Scientists should not be carried away by the statistical analyses and they have to introspect genetic control of disease resistance with parental genome.

There were many attempts to find a relation between sucrose content and red rot resistance in the past, though it is clearly known that red rot resistance is a dynamic one and any such established relation will be subjective. Ram *et al.* [205] also made such an attempt in that they showed a significant positive correlation between disease reaction and juice quality during October and January with the clones with higher HR Brix during August. They suggested that the clones with high HR Brix during August (>10.6) are likely to be more susceptible to disease. However, the claim is not supported by robust data and analyses. Further, such relation is less likely to exist with a dynamic pathogen like *C. falcatum*. Shanthi and Alarmelu [206] reported that three crosses Co 7201 × Co 62198, Co 85002 × CoT 8201 and CP 49-50 × CoT 8201 gave more clones combining high sucrose and red rot resistance. To negate the earlier findings, Singh and Govindaraj [207] found 23.9% progenies of six crosses resistant at low to medium HR Brix; however, at high to very high HR Brix level, they also found the progenies are resistant. They concluded that HR Brix value in the progenies, an indicator for high quality, is not associated with the resistance.

Role of parental clones in red rot resistance

The degree of resistance of the parents is reported to have little difference in the percentage of resistant seedlings [208]. In Louisiana, heritability studies indicated that red rot resistance can be augmented through careful cross-based selection [145]. Singh *et al.* [209] reported that R × R parental combination gave higher frequency of resistant progenies than in R × S parents. Their findings contradicted the earlier assumption that the degree of resistance of the parent made little difference in the frequency of resistant seedlings in the progenies [208, 210, 211]. They expressed that genes for red rot resistance and ratooning ability are incorporated from *S. spontaneum*. In juice analysis, they found most of the low-sugar clones are resistant to the

disease and vice versa. However, experience of the author suggests that not all the low-sugar genotypes are resistant to red rot. In a set of crosses involving parents that vary in red rot resistance, 57.1% of the clones were found to be resistant when either the female or the male parent was resistant and 20.5% clones were resistant when both the parents are susceptible [212]. However, the progeny behaviour is not uniform in $R \times R$, $R \times S$ or $S \times S$ combinations, since in five $S \times S$ crosses, resistant population ranged from 66.7% to 2.5% indicating that there is no definite pattern of segregation. Similarly, in case either one of the parents is resistant, it varied from 100% to 33.3% among five crosses. However, Chaudhary *et al.* [213] observed different ratios (1:3, 3:1 and 1:5) for resistant to susceptible clones. They further suggested that a few genes with additive effect may govern the disease resistance. The number of loci without having dominant recessive alleles in a particular genotype and interaction thereof probably decides the proportion of R to HS categories in the progenies. Further, meiotic irregularities are also responsible for such a ratio in sugarcane. Their further studies revealed that specific cross combinations like Co 1148 \times Co 775, CoH 7803 \times CoS 510, CoH 7803 \times Co 775, CoJ 83 \times Co 62198 etc. give red rot-resistant progenies with desirable agronomic traits [214]. Sugarcane hybrids are highly complex polyploids with chromosome number ranging from 100 to 120 (2n), where basic chromosome number (x) is only 8. It indicates the representation of genes in 12 or more dosages. Due to this inherent complexity, loss of some genetic material is not always reflected in the phenotype. Mishra [215] reported that switching over from Co to BO varieties reduced red rot in Bihar where epiphytotic are common. Without much scientific proof, he concluded that crosses made in tropics and selections made in the subtropics in India will probably enhance the red rot problem and cultivation of BO varieties and raising of seedlings from the crosses made in subtropics will minimize the red rot menace.

The highly polyploid genome of the parental clones in these studies poses problems for objective conclusions on the significance of the segregative ratios observed on the progeny. The allelic interactions and the extreme variability expected in the gametes by the meiotic process involving clones with different chromosome numbers do affect the reliability of conclusions on the number of genes involved. The heritability of the resistant trait is evidenced from the different studies. The self-pollinated progeny of the susceptible cv CoC 671 were almost all susceptible and the hybrids between CoC 671 and the resistant cv BO 91 were segregated with 25%–30% resistant progeny.

Sources for red rot resistance

S. spontaneum clones appear to have built up resistant genes through adaptation in red rot-endemic conditions and so they are valuable sources of resistance. However, while many hybrids carrying 'spontaneum' genes possess a high degree of resistance, they also appear, for this reason,

to be potent source of origin of new virulent strains through natural selection of variants. Since new pathogenic strains are believed to arise through mutation and parasexual recombination *in vivo* in the host [116, 216], the appearance of strains with increased virulence and concomitant periodical losses through epiphytotic appears to be a price that has to be paid for increased productivity through the development of interspecific hybrid varieties [217]. Hence, the emphasis should be shifted towards safeguarding the level of resistance already available in hybrid sugarcane and then building it up rather than trying to infuse resistance against specific isolates of the fungus.

Srinivasan and Alexander [217] screened about 660 *Saccharum* spp. clones for red rot resistance and found poor resistance in the clones of *S. officinarum*, *S. robustum* and *S. sinense*, and a third of the entries in *S. barberi* and more than half in *S. spontaneum* were resistant. They opined that the origin of *S. officinarum* and *S. robustum* in a milieu where they were not subjected to the disease hazard probably contributed to their lack of resistance. In the germplasm utilization programme initiated during 1980s at ICAR-SBI, Coimbatore, many useful hybrids involving hitherto unutilized clones of *S. officinarum*, *S. barberi*, *S. sinense*, *S. robustum*, *S. spontaneum* and a few commercial varieties have been identified. Resistance to red rot in these clones was evaluated at Karnal and Motipur in subtropical India. Although there are many clones with higher yield levels and with other desirable traits, the level of resistance to red rot is low [218]. Recent screening of 419 sugarcane germplasm clones comprising 206 *S. officinarum*, 141 *S. robustum*, 30 *S. sinense* and 42 *S. barberi* for red rot resistance revealed 7 are R and 34 are MR against red rot and rest are MS to HS, indicating very low resistance in the germplasm clones. The *S. officinarum* clones Baragua, Koelz 11131 and Koelz 11132, *S. robustum* clones 28 NG 251 and 57 NG 238, *S. barberi* clones Chin, DhaurKalig, Kansar, Maneria IMP-1552, Mungo 254, Nargori, Kewali-14G, Manga (SIC) and *S. sinense* clones Reha, Ikhri and Kalkya consistently proved to be resistant in the plug method and controlled condition testing [219].

Natarajan *et al.* [200] suggested introduction of new variants of *S. spontaneum* in breeding programmes to enhance the probability of obtaining varieties with stable resistance expeditiously as a long-term measure. They reported creation of genetic variability by introducing gene sources from 10 clones of *S. spontaneum* besides 12 *S. officinarum*, which were hitherto unutilized in varietal development, and hence they suggested that the new gene pool offers a rich repository of variability for red rot resistance as well as productivity. This has become all the more important since it was observed that the cultivars cultivated earlier world over had a narrow genetic base with 19 clones of *S. officinarum* and 2 clones of *S. spontaneum* [220].

Overall, the frequency of red rot resistance is low among the breeding population and progenies in the USA [145]. Hence, there were suggestions to introgress genes from wild relatives of *Saccharum* spp. to enhance red rot

resistance in the parent population. Ram *et al.* [221] have introgressed sugarcane with *Erianthus* for cold tolerance and red rot under subtropical conditions in India. Singh *et al.* [222] reported that the cv CoS 8436 resistant to major pathotypes of *C. falcatum* contributed more resistance in the progenies in the range of 41.5%–58.3% when used as female or male parent in crossing with R/MS or S parents than the other resistant parents viz. Co 7201, Co 87267 and BO 91. However, the latter produced more resistant progenies when it was used as female parent and crossed with susceptible male parents. Hale *et al.* [223] detected significant differences among the species and accessions within species clones of sugarcane germplasm for all traits contributing to rot index. Among the *Saccharum* spp., *S. spontaneum*, *S. barberi* and *S. robustum* displayed the lowest red rot symptom severity means, whereas *S. officinarum* and *S. sinense* had the highest rot severity means. Clones of *S. spontaneum* showed a high level of variation with RI means among themselves in the range of 2–29 and *S. barberi* also exhibited a high level of resistance with the values in the range of 1–20 RI. *Erianthus* accessions were found to possess high resistance with all of them showing very less values of RI. They suggested the use of *Erianthus* and selected accessions of *S. spontaneum* and *S. barberi* to impart red rot resistance in sugarcane. Nair *et al.* [224] diversified the genetic base of the sugarcane varieties with *Erianthus procerus* and suggested this approach to obtain red rot resistance with cane yield and drought tolerance. Recently, interspecific hybrids (ISH) and intergeneric hybrids (IGH) involving *Erianthus* spp. with *Saccharum* spp. or other hybrids were screened and resistant genotypes were identified in Coimbatore. It was found that some of the cytoplasmic derivative clones and IGH clones in specific cross combinations displayed greater levels of red rot resistance. This study provided the status of red rot resistance in wild relatives of *Saccharum* spp. and their derivatives for further utilization to develop red rot-resistant progenies [225].

Molecular markers

There have been few attempts to study host resistance in sugarcane against *C. falcatum* through molecular markers especially using resistance gene analogues (RGAs). In different host-pathogen interactions, many resistant (R) genes were discovered and they are found to have conserved domain of NBS-LRR. The NBS-LRR genes were completely characterized and have ATP/GTP binding activity and kinase 2a, kinase 3, P-loop and GLPL-conserved motifs [226]. These R genes confer resistance against different pathogens, but they have similar motifs with conserved DNA sequences. These conserved DNA sequences are being exploited for the isolation of new RGAs. In this regard, conserved regions of R genes were targeted using degenerate oligonucleotide primers for RGAs through PCR [227]. Many attempts were made to study RGAs from sugarcane and their role in imparting red rot resistance. RGAs specific for R and S reactions in

sugarcane to red rot were identified and sequencing of resistance fragments showed 80%–99% similarity with other resistant gene sequences especially reported from rice, sugarcane and maize [228, 229]. From a resistant variety, Hameed *et al.* [230] identified 15 RGAs from conserved motifs of nucleotide-binding site (NBS) class and kinase class of RGAs and these RGAs were found to have a close similarity with RGAs from other plants. In addition, Singh *et al.* [231] reported close association of two microsatellite markers UGSM316₈₅₀ and UGSM316₆₀ with MR varieties and tight linkage of UGSM316₄₀₀ marker with HS varieties. Parida *et al.* [232] characterized and genotyped single nucleotide polymorphisms (SNPs) and insertion or deletion (InDels) in disease resistance genes along with sugar pathway of *Saccharum* complex and sugarcane varieties using amplicon sequencing and CAPS assays. Five SNPs were found to have a strong genetic association with the pathways and evidenced an InDel marker in the promoter sequence of sucrose synthase-2 gene, with sugar content and red rot resistance. The functionally related SNPs and InDels, in disease resistance genes, and designed genic CAPS markers are expected to be of immense use in marker-assisted genetic improvement of sugarcane for red rot resistance. Earlier, ESTs were generated from tissue and red rot-specific cDNA libraries and identified EST clusters from Indian sugarcane varieties [233].

To identify marker-trait associations (MTAs) for resistance in sugarcane to red rot, Singh *et al.* [234] fingerprinted a set of 119 Indian sugarcane genotypes for 944 SSR alleles. Four MTAs were detected after mixed linear model containing population structure and kinship as co-factor, and among them, three were found to have homology with sorghum genome. They found many genes encoding plant defence-related proteins like cytochrome P450, MAP Kinase-4, serine/threonine-protein kinase, ring finger domain protein, glycerol-3-phosphate transporter-1 and other genes that present close to these MTAs. This is an important finding to identify reliable molecular marker towards marker-assisted breeding in sugarcane. In this direction, in the ongoing Indo-Australian research project between ICAR-SBI and Sugar Research Australia/CSIRO, Brisbane, genomic markers associated with red rot resistance trait were identified in sugarcane through genome-wide association study using an SNP marker array. These markers suggested the presence of a major effect QTL in sugarcane for red rot resistance. It is expected that these SNP markers can predict red rot resistance with good accuracy, and identification of these novel markers may aid in developing red rot-resistant varieties and provide further insight into the molecular mechanism of red rot resistance (Prakash Lakshmanan, personal communication).

Somaclonal variation

After standardization of tissue culture technique in 1980s in sugarcane, there were many attempts to exploit

somaclonal variation and select red rot-resistant types. Jalaja *et al.* [235] reported the release of the first somaclone Co 94012 for commercial cultivation with red rot resistance. However, it was never found to be resistant to red rot as reported by the author on several occasions [46, 63, 71, 156]. Kumar *et al.* [236] reported that somaclones from donor cv CoS 88230 were better in red rot resistance. Kumar *et al.* [237] also reported the development of red rot-resistant somaclone. In Bangladesh, Mahmud *et al.* [238] found varied red rot reactions in the somaclones from that of source varieties on MS tissue culture media containing 2, 4-D. Using RAPD and SSR markers, they verified the presence of variability in the red rot-resistant somaclones in comparison to the parent. In Pakistan, Ahmed *et al.* [239] reported that approximately 10% of the somaclones from S and MS varieties are found resistant to *C. falcatum*. In the resistant somaclones, four candidate genes such as catalase, sucrose phosphate synthase, gibberellin 2-oxidase 4 and tillering branched 1 showed no SNP changes in their exon regions with the parental clones. They claimed that somaclonal variation is a good source of genetic improvement in sugarcane for disease resistance with no SNP changes in candidate genes.

Screening for red rot resistance

Chona [211] had done detailed studies to standardize screening sugarcane clones for red rot resistance by plug method. He has taken average linear spread of infection in the inoculated canes as the sole criterion to judge the comparative resistance or susceptibility of the clones. However, the relative importance of drying of tops, lesion width, occurrence and nature of white spots and nodal transgression was ignored in this plug method; subsequently, Srinivasan and Bhat [155] have found only these four characters mentioned above are critical and dependable to rate resistance in sugarcane to *C. falcatum*. The 0–9 scale developed by them is used across the countries to rate the clones into different categories viz. resistant (R: 0.0–2.0), moderately resistant (MR: 2.1–4.0), moderately susceptible (MS: 4.1–6.0), susceptible (S: 6.1–8.0) and highly susceptible (HS: 8.1–9.0). There were attempts to modify the 0–9 scale and on delivery of inoculum, discriminant function equation by giving relative weightage to the three important characters viz. lesion width, occurrence of nature of white spots and nodal transgression by the pathogen [240, 241], insertion of a toothpick soaked with inoculum broth on a small hole drilled onto the internode [99, 240, 241], a modified hypodermic syringe for making puncture on the internodes and placing the inoculum [242] and a revised criteria to rate sugarcane clones after plug method by combining the lesion width and nodal transgression [243]. However, these methods did not add any advantages over the 0–9 rating system developed by Srinivasan and Bhat [155]; hence, they were rejected by the scientists due to complications in inoculation and evaluation.

The plug method of inoculation with 0–9 rating scale has been adopted in most of the research centres around the world to screen sugarcane varieties for red rot reaction. Since rind tissue is injured to create bore hole, there were apprehensions that it as rigorous and abnormal, breaking barriers of resistance. Hence, new inoculation methods were attempted to deliver the inoculum in a natural way by targeting it to reach nodal tissues that contain the portals of *C. falcatum* entry. Since the approach is to target nodal tissues for the pathogen entry, they are called as 'nodal method'. Initially, *C. falcatum* conidial suspension dipped in cotton was wrapped around three clean nodes, 10 to 20 cm above the ground level and covered with a polythene tubing [111]. Subsequently, few drops of conidial suspension were placed between the leaf sheath and stalk [244]. After about three months, the inoculated nodes are scrapped to record the extent of infection and then the stalks are sliced longitudinally to measure the spread of pathogen. All these methods were not followed due to practical difficulties and failure to induce disease even in disease-susceptible varieties. However, the nodal swabbing method developed at Coimbatore has been found an ideal method to identify the clones with nodal resistance to *C. falcatum*. Here, cotton strips dipped in conidial suspension are swabbed around two nodes of 6–8th nodes from the top, immediately after removing the leaves. The cotton pads are tightly held by a wrapping with parafilm® and also to prevent loss of moisture. This method ensures ideal disease development from the inoculated nodes in the susceptible varieties (Fig. 32) and the clones are categorized into resistant and susceptible (Fig. 31) [245, 246].

In general, red rot development by nodal method is highly influenced by environmental factors. Temperature also plays a crucial role in disease development after artificial inoculation by plug method. The climatic conditions prevailed at Shahjahanpur in the subtropical India during 2nd to 3rd weeks of September with mean relative humidity (RH) of approximately 90% and the maximum and minimum temperatures of about 33°C and 24°C, respectively, are found most favourable for *C. falcatum* infection in artificial inoculations [244]. Rapid red rot development in the temperature range of 25°C–30°C under Gorakhpur location in Uttar Pradesh by nodal method was reported. An optimum temperature of 28°C–30°C was found ideal for disease development by spray inoculation method [247]. The environmental conditions with mean temperatures ranging from 29.4°C to 31.0°C prevail during second week of August to first week of September and are found to be optimum for red rot at Hisar in subtropical India [248]. The disease development is maximum, when canes are inoculated nodally during the month of July and later inoculations resulted in poor red rot development [249].

Apart from stalk inoculation of *C. falcatum* approaches, there were attempts to apply the inoculum in the soil to favour disease development without injury. One such method was applying pathogen-infected crop debris to



Figure 32. Cotton swab inoculation of *C. falcatum* on the top nodes of a sugarcane variety in a field trial at Coimbatore (left); the pathogen causes spreading lesions from the inoculated nodes. The lesions are seen as irregular dark patches of discoloration on the rind (right).

induce disease development [250]. Subsequently, the inoculum was multiplied on sorghum grains and optimized disease development in sugarcane varieties [251]. Recently, disease development from soil-borne inoculum sources was demonstrated in sugarcane varieties varying in disease resistance [45]. This method of inoculum application and selection of clones is expected to identify the clones with field tolerance, where susceptible clones pick up infections at regular intervals, whereas the tolerant clones remain free from the disease infections [45].

In Cuba, Yakutkin and Rodrigues [147] successfully assessed red rot resistance on a 1–5 scale after injecting conidial suspension of the pathogen into 8–9 months old stalks. Later, Alfonso *et al.* [252] reported screening of sugarcane varieties and progenies for red rot resistance in Cuba. In Louisiana, harvested stalks are inoculated with *C. falcatum* and incubated for 6 weeks. After split open, parameters of number of nodes crossed, number of nodes rotted and internode rot severity are recorded and based on the extent of node damage and internode rot, a rot index (RI) is developed [244]. Artificial inoculation by plug

method is followed to screen sugarcane varieties for red rot resistance in many countries, including Thailand, Argentina, Myanmar, Pakistan, Bangladesh and in other countries [6, 9, 253, 254].

Rapid/early screening for red rot resistance

In sugarcane varietal improvement programmes, a rigorous selection is required in early stages because of practical difficulties in handling a large variable population. Further, the scientists are disappointed when a large number of agronomically superior clones are rejected after a number of years of evaluation due to their susceptibility to red rot. Hence, seedling blast technique was developed in which six weeks old seedlings are inoculated by spraying *C. falcatum* conidial suspension and later kept under a polythene shed to favour high humidity for infection. In this, more than 80% of red rot-susceptible seedlings are eliminated at an early stage of growth [255]. The method is even now found useful to screen out the susceptible population in a cross and forward the resistant types for further evaluation in varietal selection process (Fig. 33).



Figure 33. View of testing for red rot resistance in sugarcane seedlings inside the chamber at Coimbatore. The susceptible lines show death of seedlings.

Whereas in mature seedling method, conidial suspension of the pathogen is sprayed on the five- to six-month-old seedlings on the nodes after removing two to three green leaves under field conditions [244, 256, 257]. In 1990s, a new controlled condition testing was developed, in which cane tops of 6–8 months old canes are inoculated on two nodes with a cotton pad dipped in *C. falcatum* conidial suspension. The inoculated canes are kept inside a temperature and humidity controlled chamber to favour disease development and disease severities are categorized from R to HS based on disease symptoms on leaf scar, bud, growth ring, root eyes and rind and the evaluation is completed in 10 days (Fig. 34a(a)–(c)) [93]. The clones behave similarly in their disease reactions in this method and plug method [258, 259]. By this method, thousands of clones comprising progenies of different crosses, somaclones, germplasm, inter-specific/inter-generic clones etc. are screened every year at this institute, which is the major sugarcane breeding centre in India. Unlike field screening methods that require one year to complete screening cycle, in this method, it is completed in 10 days. This rapid method enabled identification of red rot reactions from very early stages of varietal selection. In addition, it reduced resources significantly in terms of land and labour to screen such a large number of clones every year [31]. In Brazil, phytopathometry was used to assess the damage caused by *Fusarium verticillioides* and/or *C. falcatum* in sugarcane [260].

Biocontrol approaches

Detailed studies conducted earlier revealed effectiveness of plant growth-promoting rhizobacteria (PGPR) belonging to *Pseudomonas fluorescens* and *Trichoderma* isolates against red rot pathogen under *in vitro* and *in vivo* conditions [261–264] in sugarcane. PGPR-mediated induced systemic resistance (ISR) was demonstrated against *C. falcatum* in sugarcane. Additionally, the bacterial strains enhanced sett germination, tillering and growth of the cane under field conditions. The induced resistance has persisted up to 90 days in plants and significantly reduced disease incidence in red rot-susceptible varieties by effectively reducing the pathogen colonization [261, 265–267]. Mechanism of induced resistance by the fluorescent pseudomonads was investigated and found different PR proteins such as β -1, 3-glucanases, chitinases and thaumatin-like proteins (TLPs) and enzymes involved in phenyl-propanoid and oxidative pathways [268, 269]. Further, *P. fluorescens* efficacy was improved by integrating the PGPR strains with fungicides under field conditions governing PGPR - mediated ISR in sugarcane [270]. Additionally, the PGPR strains were found to suppress the soil-borne pathogen inoculum surviving in the rhizosphere [271]. The PGPR strains produced various antibiotics, plant growth-promoting hormones, siderophores, chitinases etc. to cause beneficial effects on sugarcane and deleterious effect on *C. falcatum* [272–274]. Senthil *et al.*

[275] reported effective control of *C. falcatum* in sugarcane using liquid formulation of *P. fluorescens* Pfl by applying the same @ 4 litres ha⁻¹. The application of the bacterial culture through drip system showed establishment of

bacterial colonies in the rhizosphere region and enhanced plant growth and yield of sugarcane.

In Pakistan, six PGPR strains are identified with prolonged survival in the sugarcane rhizosphere with a



Figure 34. Continued

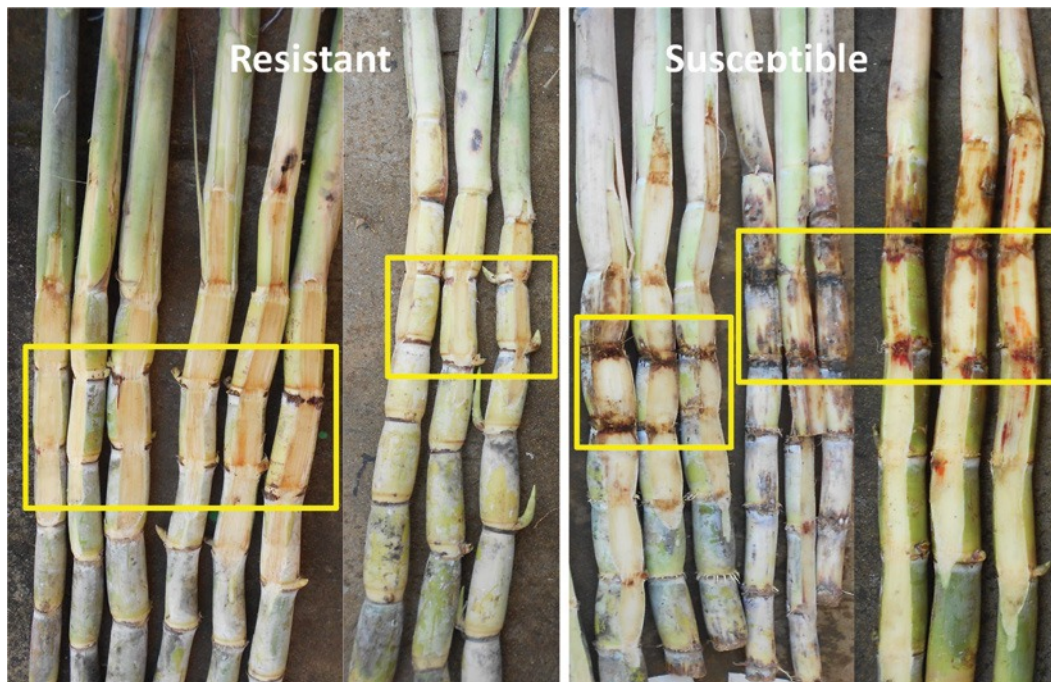


Figure 34. (a). Two nodes with cotton pads in the cane tops were inoculated with *C. falcatum* and incubated inside the humidity-controlled red rot testing chamber at ICAR-SBI, Coimbatore. (b). Closer view of the inoculated canes show disease development in five days in the susceptible clones amidst resistant types. (c). Resistant clones show no disease development or restricted lesions on the nodes (left); the susceptible clones exhibit extensive lesions on the nodal and internodal tissues (right); the inoculated nodes are marked in yellow rectangles.

potential to suppress red rot as *Bacillus subtilis*, *Pseudomonas putida*, *Ochrobactrum intermedium*, *B. subtilis*, *Bacillus* sp. and *Stenotrophomonas maltophilia* by 16S rDNA. Three antagonistic *Bacillus* strains reduced red rot incidence by 45%–49% in susceptible sugarcane upon challenge inoculation of the pathogen in the stalks and by 48%–56% when inoculated in the soil near the roots. It was speculated that high performance of the antagonists was due to induced systemic resistance as well as direct suppression of the pathogen [276, 277]. Recently, characterization of antagonists from sugarcane rhizosphere grouped 20 bacterial strains into four groups, viz. *Paenibacillus alvei*, *Alcaligenes faecalis*, *Pseudomonas fluorescens* and uncultured bacterium [278]. Efficient endophytic PGPR strains were isolated from sugarcane and their antagonistic activities and biocontrol potential were established against *C. falcatum* [279, 280]. On the basis of the 16S rRNA gene sequencing, PGPR strains native to sugarcane that are antagonistic to *C. falcatum* were characterized. Among them, 13 of 26 were found to be proteobacteria, 10 Firmicutes and 3 Bacteroides. Some of the selected biocontrol strains protected the crop from red rot disease in a susceptible variety [281]. Inoculation of *Bacillus xiamenensis* strain PM 14 claimed to be multi-stress tolerant to sugarcane plants, suppressed red rot symptoms and enhanced plant growth under greenhouse experiments. Augmented production of antioxidative enzymes and proline content was reported to the ISR against *C. falcatum* in sugarcane [282]. Pressmud

(filter cake), a sugar industry by-product, is used as an organic substrate and is very often used as a carrier for bioinoculants in sugarcane. Hassan *et al.* [283] assessed survival of two antagonistic strains of *Bacillus* sp. and found that the formulation maintained $9.0 \log \text{CFU g}^{-1}$ population till nine months. Further, introduced antagonists were found to be compatible to the indigenous bacteria in the organic substrate, which maintained a population at $7.8\text{--}8.0 \log \text{CFU g}^{-1}$. Under field conditions, the bacterial bioformulation induced defence-related enzymes, reduced red rot and improved cane yield. The study revealed that pressmud is a viable carrier to formulate antagonists, and such formulation could be used as a potential biopesticide to manage red rot of sugarcane. In Thailand, the application of *Bacillus subtilis* strains on red rot wilt-affected setts at the time of planting reduced the disease incidence and improved sett germination. By the third month after planting approximately 36.17% disease reduction was observed in the treated plots [284].

Among the antagonistic fungi, the *Trichoderma* spp. were effective against red rot pathogen under *in vitro* and greenhouse conditions [263]. Efficacy of fungal antagonists belonging to *Trichoderma* has been reported in sugarcane by several authors from India and other countries. *T. harzianum* has been found to have the potential to suppress red rot in sugarcane [285, 286]. Healthy sugarcane setts were treated by applying *T. harzianum* strains grown in sterilized maize bran (2 kg) and later mixed with sterilized

press mud or farm yard manure @ 20 kg ha⁻¹. The treatments of *T. harzianum* spore suspension containing 10⁶ conidia ml⁻¹ and metabolites as 2.5% culture filtrate were also compared along with combinations to improve biocontrol efficacy. The results revealed protection of 45%–55% against *C. falcatum* infection in sugarcane. The protected plants had no pathogen infection as against 97%–100% infection in unprotected plants. In 20%–25% plants, the pathogen infection was considerably suppressed where severity of infection was reduced to 2–4 in 0–9 scale [285]. Similarly, 27 *Trichoderma* strains were grouped into four strains such as *T. asperellum*, *T. harzianum*, *T. aureoviride* and *T. atroviride*. Among them, the most effective strains against *C. falcatum* were found to be *P. alvei* BA2 and BA19 and *T. harzianum* T17 and T20 [278]. Studies of Joshi *et al.* [287] revealed that among the 29 sugarcane rhizospheric *Trichoderma* spp., 21 as *T. harzianum* and the remaining 8 as *T. longibrachiatum*, indicating that the former is the predominant species in sugarcane rhizosphere. Further, delivery of a combination of sett and soil treatment of *T. longibrachiatum* isolates exhibited maximum disease suppression accompanied by higher cane yield as compared to untreated control. However, their earlier studies on direct application of secondary metabolites of antagonistic *Trichoderma* isolates as sett treatment did not appreciably reduce red rot [288].

Antifungal proteins and metabolites produced by the antagonists

The effectiveness of the antagonists is mainly attributed to mycoparasitism action, by the action of cell wall-degrading enzymes (CWDEs) such as chitinase, β -1,3-glucanase, β -1,4-glucanase, β -1,6-glucanase, protease and xylanases. Earlier CWDEs produced by *T. harzianum* inhibited the red rot pathogen [289] and *P. fluorescens* strains inactivated the pathogen toxin, which failed to elicit characteristic symptoms on the host [290]. Recently, PGPR strain BA2 has been found to produce higher cellulase, chitinase, β -1,3-glucanase and protease enzymes to suppress *C. falcatum*. Similarly, the fungal isolates, *T. harzianum* T20 was found more effective in producing CWDE cellulase, chitinase, β -1,3-glucanase and protease enzymes and it was confirmed through expression assays during its interaction with *C. falcatum*. Production of polygalacturonase (PG) by the pathogen was also significantly reduced during interaction [291]. Antagonistic microbes produce different types of biosurfactants based on their physicochemical properties as neutral lipids, phospholipids, glycolipids, lipopeptides, polymeric compounds and fatty acids. Several biosurfactants from microbes exhibit antibacterial, antifungal and antiviral activities and hence they become relevant molecules to combat many diseases and infections [292]. The rhamnolipid biosurfactant produced by the bacterial strain *Pseudomonas aeruginosa* DS9 had a strong antifungal activity against *C. falcatum* [293].

Secondary metabolites of organic compounds were not directly involved in the normal growth, development and reproduction of microorganisms. But the mechanism of secondary metabolites plays an important role to defend against diseases and interspecies competition. In this context, GC-MS analysis of secondary metabolites from *C. falcatum*, *P. alvei* and *T. harzianum* and their respective interaction samples revealed that 11 new volatile compounds during *C. falcatum* and *P. alvei* interaction and 20 new compounds during interaction of *C. falcatum* and *T. harzianum* were produced, in addition to constitutive compounds of antagonistic strains and correspondingly there were massive inhibition of *C. falcatum* metabolites. Volatile compounds of *P. alvei* (Phenol; Eicosane; Dibutyl phthalate; Pyrrolo etc.) and *T. harzianum* (Styrene; 1-Undecanol, Decane, Eicosane etc.) identified during interaction with *C. falcatum* were reported to be antifungal in nature. The crude metabolites obtained during interaction of *C. falcatum* with *P. alvei*/*T. harzianum* inhibited the *C. falcatum* growth in plates. Further, there was no symptom production by these metabolites on detached leaves as compared to well-developed symptom by *C. falcatum* metabolites. These results also confirm the suppression on production of *C. falcatum* metabolites [294].

Interactive proteomics with red rot pathogen and antagonists

The antagonists *P. alvei* BA2 and *T. harzianum* T20 specifically expressed approximately 10 and 25 kDa and 22, 45 and 65 kDa proteins, respectively, on culture filtrate of *C. falcatum* and these purified proteins exhibited inhibition of conidial germination, germ tube elongation and appressorium formation in *C. falcatum*. The identified proteins from two-way interaction of *C. falcatum* with *P. alvei* or *T. harzianum* inhibited *C. falcatum* *in vitro* and they were characterized and identified through 2-D gel electrophoresis and MS [295]. Further, three-way interaction studies were conducted in sugarcane leaf bits, cut canes and standing canes with *T. harzianum* and *C. falcatum* to identify the antagonists' antifungal proteins and their expression. Expression analysis identified upregulated proteins related to defence and stress-related transcripts induced by *T. harzianum* in sugarcane and the pathogenicity/virulence-related proteins of *C. falcatum* cytochrome p450 and Hsp20-like proteins were downregulated during interaction [294, 295]. These studies clearly demonstrated ISR-mediated suppression of *C. falcatum* by *T. harzianum* in sugarcane and weakening of pathogenicity mechanism of *C. falcatum*.

Heat treatment

Gupta *et al.* [296] reported failure of hot water treatment at 50°C for 2 h or 52°C for 1.5 h to eradicate *C. falcatum* from infected setts. Subsequently, sett treatment in hot water (50°C for 2 h) combined with fungicide treatment

was found to eliminate *C. falcatum* infections in the planting materials [297]. Singh *et al.* [298] reported MHAT for 2 h eliminated *C. falcatum* infection from seed cane; however, success rate varied from 68.75% to 100%, depending on the variety. Singh [299] reported killing of red rot pathogen deep-seated in the sett tissues or latent in the nodal region by applying hot air at 54°C for 8 h. However, hot air treatment is found to harm germinating buds. Jha *et al.* [300] also reported its ineffectiveness to control *C. falcatum* from the setts; in addition, it adversely affected sett germination. In the USA, aerated steam therapy (AST) at 51°C for 4 and 5 h killed *C. falcatum* in 61 and 75% of naturally infected buds, respectively, and similarly at 52°C for 4 and 5 h killed the fungus in 88 and 91% of buds, respectively. AST at 52°C for 4 and 5 h killed the pathogen in naturally infected leaf sheaths and leaf midribs, stalk borer–tunnelled internodes and inoculated internodes. AST of stalks at 52°C for 4 h did not affect bud germination in the cv CP 65-357; however, it significantly reduced in the cv Co 290 [301]. In Tamil Nadu, India, AST improved germination from 24% to 34% in the infected canes; however, complete control of the disease was not found [302, 303].

Chemical control

Several fungicides, both systemic and non-systemic in mode of action, were found effective in suppressing the pathogen under *in vitro* conditions, but their efficacy under field conditions was comparatively less. Various reports on the efficacy of fungicides against *C. falcatum* were reported earlier [6]. Poor efficacy of fungicides under field conditions is mainly due to impervious nature of rind and nodal tissues that prevent the entry of fungicides or its metabolites. Hence chemicals move slowly reach the site of pathogen colonization inside the stalks or concentration of the translocated toxic compound is insufficient to kill the fungus. Therefore, for effective control of red rot under field conditions, chemicals alone may not be effective, but they should be used in combination with other means of control like seed selection, sanitation, crop rotation and rouging of infected stools [6].

Before planting, two- or three-budded setts are immersed in fungicide suspension for 30 min under field conditions and this is a prophylactic measure to reduce disease caused from sett and soil-borne inocula. In this way, the primary infection from *C. falcatum* surviving in the soil/debris may be arrested. Sett treatment with fungicides carbendazim or thiophanate methyl was found to reduce pathogen entry from soil through cut ends [297]. There were also reports on incomplete control of the disease by fungicides by treatment of disease-affected setts with fungicides for varying time intervals and partial reduction in disease development with single-budded setts [304]. The fungicide efficacy was improved by combining fungicide treatment with hot water treatment. Hot water (50°C; 2h)

in combination with aretan treatment was found highly effective in eliminating the infection of *C. falcatum* in two-budded setts [297]. In Thailand, stool spray application at least twice during the one- to five-month stage with different fungicides is recommended to control red rot. Further, fungicides are applied on the whole stalks placed in the furrows before planting instead of dipping setts in the fungicides [305]. Recent studies revealed efficacy of azoxystrobin 18.2% + difenoconazole 11.4% w/w SC (Amistar Top 325 SC) at the rate of 1.00 ml per litre to manage red rot and smut and rust diseases in sugarcane and to increase cane yield in sugarcane [306].

Studies conducted at ICAR-SBI proved effectiveness of the systemic fungicide thiophanate methyl against *C. falcatum* [307]. Further, sett treatment with thiophanate methyl at 0.25% was found to be effective against debris-borne inoculum of the pathogen in the soil [308]. These studies showed reduction in the disease incidence by prophylactic application of the fungicide, improved sett germination and better plant survival in the field. Further, compatibility of thiophanate methyl with *Pseudomonas fluorescens* strains was found more effective against primary inoculum of *C. falcatum* surviving in the soil [270]. Subsequently, eight systemic fungicides of azoles and strobilurins were evaluated in comparison with thiophanate methyl and carbendazim for their efficacy against *C. falcatum*. Screening of fungicides against the pathogen under *in vitro* conditions at 1, 5 and 10 µg mL⁻¹ concentrations indicated that the fungicides significantly differed in their efficacy in inhibiting the pathogen growth. Of all the fungicides, carbendazim required only 1 µg mL⁻¹ for the complete inhibition of the pathogen followed by thiophanate methyl, which required 10 µg mL⁻¹ [309]. The new-generation fungicides were less inhibitory, azole-strobin, strobin-metiram, baycor, flusilazole and propiconazole gave more than 80% inhibition. Although carbendazim and thiophanate methyl were more effective under *in vitro* conditions, combinations of azole, strobilurin and thiophanate methyl were more protective than their separate applications under field conditions. Protection of setts with effective fungicides individually or in combination resulted in improvement in cane yield and cane juice quality in sugarcane under field conditions [309].

Based on the studies conducted at ICAR-SBI revealed that thiophanate methyl is highly compatible with other fungicides and biocontrol agents; however, sett treatment for 30 min is ineffective and needs more than 12 h or overnight soaking is required for effective fungicide diffusion in the setts and significant reduction in red rot. Since handling seed canes of high volume for prolonged fungicide treatment has practical difficulties, further attempts were made to develop mechanized sett treatment system for easy, effective and rapid fungicide treatment. In this regard, sett treatment device employing high-pressure fungicide diffusion technique was developed. Improved fungicide delivery through the mechanized sett

treatment was validated under red rot–endemic locations in Cauvery delta in Tamil Nadu, India, with disease-susceptible varieties. The field trials clearly indicated that the mechanized sett treatment with the fungicide thiophanate methyl (TM) (1000ppm) protected the planted setts from soil-borne inoculum of red rot and significantly improved the plant survival in the field. Further, cane yield was improved by 1.4- and 1.2-fold increase in sett treatment + soil drenching with TM and sett treatment with TM + *Pseudomonas fluorescens* soil drenching, respectively, whereas cane yield doubled in case of sett treatment alone with TM as compared to pathogen-inoculated control treatment, which recorded only 35% of cane yield of uninoculated control plots. The efficacy of fungicide delivery was again confirmed in another season in disease-endemic area, revealing the potential of mechanized sett treatment to protect sugarcane from red rot and also against smut [310–312]. Based on its efficacy against red rot and smut, new sett treatment devices were installed in many states viz. Uttar Pradesh, Haryana, Tamil Nadu, Gujarat, Andhra Pradesh and Maharashtra in India and benefiting sugar mills to manage red rot from primary sources of the inoculum (Fig. 35). By this, young crops are protected from the disease and chances for disease build-up through secondary spread are reduced. In the history of disease management in sugarcane through fungicides, this is a success story benefiting the industry and saving the crops from red rot. Further, treating the single-budded setts with fungicide, insecticide and nutrients has largely benefitted commercial nurseries to raise vigorous and healthy settlings intended for direct planting in the field (Fig. 36). Earlier in the USA, an added air pressure of 10 lb/in² for 15 min was devised for treating three-budded setts in fungicide suspension in water. The treated setts under air pressure recorded normal growth and the fungicides

exhibited more effective control of red rot, when applied under an added pressure of 10 psi than under normal atmospheric pressure. In bioassays, the zone of inhibition of the pathogen around fungicide-treated stalk-core sections from setts under pressure was much larger than that from setts treated at atmospheric pressure [313]. However, no further reports are available on the application of this approach to manage red rot in sugarcane.

There were claims from some sugar mills that distillery effluents are effective to contain red rot under field conditions. However, studies of Nallathambi *et al.* [314] clearly established that such efficacy is false under field conditions. Further, under *in vitro* conditions, the effluents promoted *C. falcatum* growth and no inhibition was found.

Transgenic approach

Nayyar *et al.* [315] reported the expression of β -1,3-glucanase gene from *Trichoderma* spp. in red rot–susceptible sugarcane cv CoJ 83 with CaMV 35S promoter through *Agrobacterium* mediated in planta genetic transformation. The transgenic lines have expressed resistant reaction to the *C. falcatum* pathotypes CF08 and CF09 under *in vivo* conditions. Inside the parenchymatous tissues, the expressed gene caused inhibition of fungal growth by lysis. They further reported that upregulation of the transgene after *C. falcatum* inoculation up to 2.0-fold in leaves and 5.0-fold in roots and the expressed protein cleaved β -1,3-glycosidic bonds, which leads to lysis of the invading fungus. In Pakistan, transgenic lines in sugarcane expressing barley chitinase class-II gene and *HarChit* and *HarCho* encoding chitinase and chitosanase proteins, respectively, were developed and the *C. falcatum* challenged canes were found to show strong resistance against the pathogen [316, 317].



Figure 35. Mechanized sett treatment device to treat sugarcane setts with fungicides and other agro inputs. Left: View of the sett treatment device installed at Vellode, Erode District, Tamil Nadu, India; Right: Inside view of the device with setts dipped in the treatment solution.



Figure 36. Production of disease-free sugarcane settlings from single-node cuttings in a nursery at Vellode, Erode District, India.

Conclusion

The disease caused enormous damage to sugarcane cultivation across the countries amounting to billions of dollars for more than 100 years. Though many countries such as the USA, Australia, Brazil, South Africa, Argentina, Indonesia, Cuba etc. suffered heavily during the first few decades of the last century, they are either free from the disease or the disease severity is at the minimum. It is no longer a threat to sugarcane cultivation in these countries, whereas many Asian countries especially those in the South and South East Asia encounter serious impact every year due to red rot. In many of the countries in the New World and Africa, the impact of *C. falcatum* is associated with stalk borer as 'borer-rot complex' or causing germination failure in the planted stalks as in Louisiana. These countries managed the severe epiphytotic of red rot through systematic varietal and seed replacements. Further, sugarcane being an introduced and industrial crop, replacement of the varieties is not challenging, whereas in the Asian countries, the crop is grown traditionally for sugar industry, cottage industries to manufacture different sweeteners and chewing; hence, complete replacements of the affected varieties have not been successful. In addition, growing of sugarcane as plantation in large holdings of thousands of hectares enabled them to effectively replace the seed and variety, as compared to Asian countries where cane is cultivated by thousands of growers in small holdings. Over the

decades, the islands of red rot-affected gardens were common in Asian countries that served as reservoir of inoculum for continuous attack in the new varieties. Apart from the nature of farm holdings, lack of domestic quarantine and ineffective government policies failed to remove disease-affected varieties and prevent red rot spread in the Asian countries.

In a country like India where sugarcane is grown in more than 5 M ha, the disease severely affects the productivity in many of the states. Varietal breakdown to red rot in sugarcane is witnessed over the decades and practically no variety withstood the attack of red rot for the past 100 years [31, 115, 318]. Though the disease was contained after each of the varietal breakdown and epiphytotic, the disease severity has not been reduced and pathogen gained virulence from one epiphytotic to another [31]. Though the impact of 'boom and bust cycle' enacted by the red rot pathogen is well known, the policy makers have not learnt from the past epiphytotic. For example, the current popular variety Co 0238 has occupied more than 80% area in the state of Uttar Pradesh in subtropical India, which is grown more than 50% of sugarcane in the country [121]. Due to 'vertifolia' effect, the pathogen became more aggressive on the variety and it resulted in the destruction of thousands of hectares in the ongoing season. The total loss caused is estimated to be 1.0 to 1.414 billion US\$ due to this disease outbreak during the season. The unscrupulous spread of a single variety over large areas happened before the eyes of

scientific community and policy makers and it was partly due to sugar mills who wanted to make fast cash without anticipating such a catastrophe.

Sugarcane pathologists have developed effective screening techniques to identify resistance in the breeding progenies, parents and germplasm sources and identified several hundreds of resistant clones every year. Only red rot-resistant varieties are recommended for commercial cultivation; however, unscientific promotion of some varieties for the benefit of sugar millers and others has failed the scientific advances made for 14 years to develop and release a new variety.

C. falcatum genome sequencing and transcriptome profiling is a proven approach to bring insights into pathogenicity mechanisms to invade host tissues, strategies of acquiring nutrients, avoid plant defence and to provoke disease symptoms. These developments have identified gene families that attribute to virulence and other active enzymes similar to other hemi-biotrophic pathogens. It is envisaged that characterizing the genes involved in *C. falcatum* pathogenicity with the help of whole genome analyses as background information has identified molecular biomarkers and a breakthrough by identifying unique genes in sugarcane × *C. falcatum* interaction. Identifying effector molecules in the pathogen, interactive proteomics with biocontrol agent, identifying resistant and susceptible genes through subtractive libraries, characterizing red rot pathotypes and host adaptation of *C. falcatum* pathotypes have paved way for better understanding of host-pathogen interaction, pathogenic virulence, emergence of new isolates and varietal breakdown. The new knowledge on genomic and proteomic studies will further strengthen to manipulate host through genetic engineering and genome editing.

The new disease management through mechanized fungicide delivery in the setts has for the first time showed ways and means to efficiently manage the disease using fungicides; further, the system also enables delivery of biocontrol agents through setts. Additionally, micro-irrigation system needs to be optimized to deliver fungicides or bioagents during grand growth phase of the crop to arrest disease development from secondary infections. Also, the inefficient heat treatment systems should be discouraged for disease management.

An important area of work by the extension workers at sugar mills is to extend field life of elite and agronomically superior varieties by preventing varietal breakdown. This can be achieved by avoiding varietal mixtures, clean seed nursery programme, proper disease surveillance in the field, maintaining field hygiene by removing and destroying infected canes and, if needed, withdrawing the variety for a short time. Further, scientific varietal deployment in the disease-prone areas based on *C. falcatum* pathotype mapping will protect the new varieties from the contact of the pathotype with potential resistance breaking. It is also valuable to take advantages of recent developments on

GWAS using SNPs to identify red rot resistance in the parental clones and progeny population precisely, to develop disease-resistant varieties through marker-assisted selection.

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