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Screening for Red Rot Resistance in Sugarcane

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ABSTRACT

Red rot of sugarcane caused by *Colletotrichum falcatum* Went is a serious fungal disease affecting sugarcane stalks, the economical part of sugarcane in different countries. In India, the disease has been a menace for sugarcane cultivation and many elite varieties were removed from cultivation due to their susceptibility to the disease. The commercial varieties are recommended for cultivation in India if they possess red rot resistance. Hence screening for red rot resistance became an integral part of varietal development in the country. Plug method of pathogen inoculation is the most common method used for red rot screening in sugarcane and here, severity score is being assigned based on the red rot symptoms inside the canes. The key parameters of the lesion such as lesion width, nodal transgression and nature and severity of white spots determine the disease severity. Since a bore-hole injury is caused on the stalk to inoculate the pathogen, there is an apprehension that natural barrier is breached to introduce the pathogen in this method. So many other methods were developed to facilitate the pathogen to enter the cane tissues in a natural way. However the disease reaction in these methods is influenced by environmental factors which lead to disease escapes under field conditions. Recently a controlled condition testing was developed to screen large number of progenies using cane tops in a short time. Overall, the plug method is used to assess resistance potential of a genotype in a foolproof way in sugarcane varieties proposed for commercial cultivation and the controlled condition testing is followed initially to screen large population.

Keywords: controlled condition testing, disease screening, field tolerance, nodal method, plug method, red rot, sugarcane
Abbreviations: CCT, controlled condition testing; **ELISA**, enzyme linked immunosorbent assay; **IRS**, internodal rot severity; **ITS**, internally transcribed region; **PCR**, polymerase chain reaction; **RAPD**, random amplified polymorphic DNA; **rDNA**, ribosomal DNA

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INTRODUCTION

Among the diseases of sugarcane in the Indian subcontinent red rot caused by the fungal pathogen *Colletotrichum falcatum* Went (Melanconiales, Deuteromycetes, Perfect stage: *Glomerella tucumanensis* (Speg.) Arx and Muller) is recognized as the most important one and is a very serious constraint in the production and productivity of the crop (Viswanathan *et al.* 2002).

In fact, effective management of red rot has become an imperative in the successful cultivation of sugarcane in India and neighbouring countries in Asia. Although several components are involved in the integrated management of sugarcane red rot, by far the most effective and practical strategy is the deployment of resistant cultivars. Cultural practices such as planting healthy disease free cane, roguing, avoiding ratoons of affected crops, crop rotations particularly with rice etc., are known to be helpful in reducing the occurrence and spread of red rot. But in practice they all play a supplementary role in the management of disease. Further, as of now no chemical control measures are found to be effective to manage red rot. While non systemic fungicides may be inhibitory to the pathogen inoculum available in the field they have no effect on the pathogen present inside the seed setts or stalks. Even most of the systemic fungicides available in the market till recently have not been found to be very effective against the internally sett borne infection or internally infected stalks in the field due to their limited absorption, uptake, translocation and persistence in the cane tissues. Of late, systemic fungicides such as Thiophanate methyl (Malathi *et al.* 2004) show promise in the management of red rot and their wide spread use under field scale is yet to be adopted. Similarly though the potential to manage red rot with systemic acquired or induced resistance by synthetic signal molecules has been indicated from some research findings many of them are not yet commercially available and require much studies and standardization before they can become practical under field conditions. In view of all these limitations it is generally acknowledged that growing resistant/tolerant varieties is the

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most important strategy to manage sugarcane red rot.

The above facts make it clear that screening and identification of sugarcane genotypes with resistance/tolerance is a vital requirement in the successful management of red rot. Screening for red rot resistance usually has the following objectives:

- Identification of commercially superior (high sugar and high yielding) varieties with resistance/tolerance to red rot;
- Identification of genetic stocks as resistance donors in the breeding programmes;
- Evaluation of differential reaction to study pathogenic variability in *C. falcatum*;
- Molecular and biotechnological studies such as development and evaluation of transgenics, understanding mechanisms of resistance, identifying molecular markers for resistance, etc.

Disease symptoms in relation to disease severity and reaction

Most techniques presently used to screen for resistance to red rot are primarily based on the various symptoms associated with the disease and the resulting differences in disease severity. Hence it would be appropriate at first to examine the different symptoms of red rot. The different symptoms of red rot can be broadly classified as external and internal symptoms.

1. External symptoms

External symptoms are yellowing and drying of the leaves, death of the entire top (**Fig. 1**), shrinking and drying of the stalks, discolouration of the rind, discoloured internodal rind lesions, presence of pink spore masses on the lower nodes during humid and waterlogged conditions, etc.

2. Internal symptoms

Internal symptoms include continuous or discontinuous reddish lesions extending along the length of the stalks, areas of white spots dispersed amidst the reddish lesion (**Fig. 2**), formation of pith cavities in the advanced stages of the disease (**Fig. 3**), grayish fungal growth of the pathogen in the cavities, etc. The reddish colour of the lesions is due to the production of anthocyanidin pigments by the host tissues in response to colonization by the red rot pathogen. Resistant varieties show dark reddish well defined restricted lesions while in susceptible varieties the lesions are light red colored and diffused spreading over large areas of the host tissues.

In India, next to yield and quality, red rot is the most important constraint in the production and productivity of sugarcane and hence screening for red rot resistance relevant to Indian conditions is discussed first.

3. Disease situation

In the early decades of the last century when red of sugarcane was first reported from the Indo Gangetic plains of Northern India the sugar industry was mostly dependent on the species clones of *Saccharum officinarum* and *S. barberi* which are known for their high sugar content. However they were also highly susceptible to red rot and many high sugar and very popular hybrid varieties such as 'BO 3', 'BO 10', 'BO 11', 'BO 17', 'BO 54'; 'Co 213', 'Co 312', 'Co 453', 'Co 1148', 'Co 1158', 'CoS 443', 'CoS 510, etc. had to be abandoned (Viswanathan 2010). This situation prompted researchers to explore the possibility of hybridizing *S. officinarum* clones with other cane species such as *S. spontaneum* which seemed to possess inherent resistance to the red rot. The process necessitated procedures to identify the resistant genotypes from a large number of progeny genotypes generated in the hybridization programme. Probably in the earlier times identification of resistant genotypes was done by growing the promising genotypes in red rot prone endemic locations and recognizing the resistant ones by the natural elimination of the susceptible genotypes. It was likely that the results of this approach were variable and quite inconsistent rendering it necessary to develop suitable screening techniques for red rot resistance under artificial inoculation conditions.

Red rot being a disease that primarily affects the stalks, most of the techniques used to screen for red rot resistance were based on inoculation of the stalks and scoring disease severity taking into account the different symptoms (Viswanathan 2010).

Disease screening based on natural infection process

In these methods attempts were made to identify resistant varieties by planting test varieties in pathogen-infested fields and selecting those showing low or no disease incidence.

1. Sick plot techniques

Here red rot inoculum in the form of infected cane debris was added to the experimental field over a period of time and the fields planted with the test sugarcane varieties to select those with low or no disease incidence. However, these methods were not very successful as they did not result in uniform or consistent disease expression due to rapid inactivation of the inoculum, its loss of virulence, heterogeneous nature of the inoculum, etc., and these techniques were discontinued.

2. Seedling blasting technique

Attempts were made to screen true sugarcane seedlings raised from fluff from the germination stage itself for red rot resistance. Srinivasan (1962) tried to screen seedlings by spraying them with a heavy spore suspension of the red rot pathogen and maintained under high humidity conditions in pans or beds. Susceptible genotypes were identified by the development of foliar lesions while resistant ones were symptom free or produced only necrotic spots after 3 days of incubation. The seedlings were grouped based on the symptoms they produced as follows:

1. Seedlings with long spots coalescing into irregular patches with profuse sporulation of the pathogen.
2. Seedlings with spindle shaped spots 1-2 mm long with dark brown margins.
3. Seedlings with no symptoms or only chlorotic spots.

Seedlings showing different symptoms were marked, the diseased leaves were clipped off and the seedlings re-inoculated by spraying the spore suspension. Seedlings showing resistant reaction were replanted in the field after removing the diseased leaves for evaluation of adult plant reaction in the clonal stage.

The technique was utilized as part of the programme on breeding for horizontal resistance to red rot by screening seedlings of a large number of progenies obtained from several crosses and eliminating the susceptible ones at a very early stage.

Disease screening based on artificial testing methods

These techniques involve application of the red rot inoculum on the canes and rating the reaction of the varieties to the disease based on the development of different symptoms.

1. Plug method of inoculation and screening based on average lesion length

Chona (1950) reported his observations on the extent of tissue invasion by red rot and the resultant damage caused to the cane as nodal restriction was an important factor in the development of the disease in many varieties. After exten-

Table 1 Red rot categories in relation to different lesion lengths (Chona 1954; Srinivasan and Bhat 1961).

Average lesion length (inches)	Red rot reaction
<5.0	Highly resistant
5.0-15	Resistant
15-30	Moderately resistant
>30	Susceptible

Table 3 Categorization of red rot reactions (Srinivasan and Bhat 1961).

Score on the 0 – 9 Scale	Reaction category
0.0 – 2.0	Resistant (R)
2.1 – 4.0	Moderately resistant (MR)
4.1 – 6.0	Moderately susceptible (MS)
6.1 – 8.0	Susceptible (S)
8.1 – 9.0	Highly susceptible (HS)

sive studies, Chona (1954) attempted to establish the relationship between lesion extension and red rot severity and utilize it for screening for disease reaction. The red rot screening scale based on average lesion length suggested by him is presented in **Table 1**.

Chona had fully recognized the effect of environmental factors on red rot development and had critically observed the differences in the reaction of varieties rated based on lesion length from season to season caused by weather factors. To overcome this problem he emphasized that reaction of test varieties based on lesion length should be recorded in comparison to the lesion length of a susceptible variety/ varieties to ensure reliability of the scoring system.

Disadvantages of considering lesion length only

Screening for red rot resistance based on lesion extension (nodal transgression) only was a common practice for quite some time during the early years of sugarcane improvement. However, many sugarcane pathologists soon recognized the limitations of this procedure. It was often observed that the extent of lesion extension in many varieties did not show much direct relationship to the damage caused to the cane or the degree of yield and quality loss. In many varieties despite considerable lesion extension there was little damage to the plants, most of the lesion free tissues appearing healthy with the absence of other symptoms of red rot. On the contrary, many varieties with restricted lesion extension but with other prominent symptoms such as white spots, greater lesion width and dried tops (leaves) showed severe stalk damage resulting in much yield and quality loss. These observations indicated the need to consider all the important symptoms of the disease for the screening procedure so that the system would be comprehensive and the reaction ratings would be realistic.

2. Plug method of inoculation and the 0 to 9 red rot scale

Srinivasan and Bhat (1961) standardized a comprehensive red rot screening method taking into account all the different symptoms of the disease. This method is commonly adopted by many sugarcane pathologists. A standing cane crop of about 7 months is considered suitable for evaluation. Cultures of *C. falcatum* usually grown on media such as oatmeal agar are used for inoculation. About 10 day cultures with profuse sporulation are found most suitable (**Fig. 4**). A fresh spore suspension of the pathogen containing about one million conidia/ml is prepared. A bore hole is punched in the middle of the 3rd internode from bottom (**Fig. 5**) with a cork borer or metal inoculators of about 0.5 cm diameter and the plug of cane tissue with the rind (**Fig. 6**) removed. A small quantity (about 0.5 ml) of the spore suspension is dropped into the bore hole (**Fig. 7**), the hole replugged with the removed cane tissue (**Fig. 8**) and sealed tight with plastic clay (**Fig. 9**). For best results and good disease expression, the inoculation is carried out at the beginning of the monsoon season so that the incubation period coincides with high humidity conditions suitable for disease development. To ensure reliability in each test clone, a large number of canes are inoculated. Also suitable susceptible and resistant check varieties are inoculated simultaneously for comparison.

After an incubation period of about 60 days the inoculated canes are split open longitudinally and the severity of red rot symptoms expressed is scored on a 0 – 9 scale based on condition of tops (leaves), progress of the red rot lesion along the length of the cane, width of the lesion and the amount of white spots present. The plug method is suitably adapted and currently used commonly to screen for red rot resistance as described in **Table 2**. The values for the three symptoms are added and the red rot score on the 0 – 9 scales is arrived at (**Table 3**).

Implications of different symptoms on red rot reaction

As mentioned the evaluation method indicated above takes into account all the major symptoms associated with red rot which contribute to significant loss in yield and quality of the crop.

1. Condition of tops

Yellowing and drying of the leaves and death of the entire spindle indicate extreme susceptibility of the genotype. It is caused by the total collapse, disintegration and breakup of the vascular system in the plant resulting in the loss of conduction of water and nutrients upwards. The red rot pathogen is known to produce cell wall degrading enzymes which bring about this disorganization of conducting tissues resulting in leaf drying. It is often observed that in many varieties even with extensive lesion development there is no leaf drying indicating the unsuitability of considering lesion length only to screen for red rot reaction. Infected varieties with dry tops usually show most of the other important symptoms of the disease also. Studies have shown that the red rot pathogen produces phytotoxic metabolites which are

Table 2 Red rot rating based on the 0 – 9 scale (Srinivasan and Bhat 1961).

Symptoms	Numerical scale	Severity of symptoms
Condition of tops	0	Green.
	1	Yellow or drying.
Nodal transgression	0	No lesion spread. Lesion restricted to the inoculated node.
	1	Lesion transgression of one node above the inoculated node.
	2	Lesion transgression of 2 nodes above the inoculated node.
	3	Lesion transgression of more than 2 nodes above the inoculated node.
Lesion width	0	No lesion spread.
	1	Lesion spread to about 25% of the width of the stalk.
	2	Lesion spread to about half the cane width.
	3	Lesion spread to more than half the width of the cane.
White spots	0	No white spots.
	1	Sparse presence of white spots.
	2	Moderate to profuse presence of white spots.

Fig. 1 Red rot affected cane stalks showing total drying of leaves. **Fig. 2** White spots dispersed in the reddish tissue lesions. **Fig. 3** Reddish tissue lesions extending along the cane in red rot affected stalks with pith cavities. **Fig. 4** Actively sporulating cultures of *Colletotrichum falcatum* grown on different media to be used for inoculation. **Fig. 5** A hole being punched in the internode in preparation for red rot inoculation. **Fig. 6** Bore hole after removal of tissue plug in preparation for inoculation. **Fig. 7** *C. falcatum* conidia suspension being dropped into the bore hole. **Fig. 8** Bore hole re-plugged with the removed tissue bit. **Fig. 9** Bore hole and tissue plug sealed with plastic clay. **Fig. 10** *C. falcatum* inoculum being dropped inside the leaf sheath to reach the node. **Fig. 11** Controlled condition testing to screen for red rot resistance. **Fig. 12** Cotton swab with *C. falcatum* inoculum wound around exposed node to screen for resistance under controlled condition testing. **Fig. 13** Resistant variety evaluated under controlled condition testing with no red rot symptoms. **Fig. 14** Susceptible reaction. **Fig. 15** Highly susceptible reaction. **Fig. 16** Rotting cane with profuse sporulation of *C. falcatum* on nodal and intermodal tissues. **Fig. 17** Red rot infected cane debris used to screen for field tolerance. **Fig. 18** *C. falcatum* multiplied on sand-sorghum medium to screen for field tolerance. **Fig. 19** Red rot inoculums applied on and around setts before covering with soil. **Fig. 20** Response of two susceptible and one tolerant variety (right) to grain based red rot inoculation. **Fig. 21** Susceptible (dried) and field tolerant (healthy) reaction to *C. falcatum* inoculation with grain-based inoculum. **Fig. 22** Midrib lesion in a variety lacking field tolerance in response to inoculation with grain-based inocula. **Fig. 23** Detection of *C. falcatum* colonization of sugarcane tissues by histological staining by 72 h. All photos unpublished.

capable of causing leaf yellowing and drying. It appears that in susceptible varieties the pathogen which heavily colonizes the stalk produces large quantities of these toxins which rapidly get translocated to the leaves where they cause yellowing and drying symptoms indicating the susceptibility of the variety.

2. Nodal transgression of lesions

It has been recognized that the nodal tissues in the cane stalk offer resistance to the spread of the pathogen along the length of the cane in many host varieties. The hardy fibrous nature of many cane varieties restricts the rapid passage of the pathogen to adjacent nodes. Thus the extent of lesion development can indeed be a criterion to indicate the red rot reaction of varieties. However as stated earlier this relationship does not appear to be consistent. Further, quantification of this lesion extension poses a problem. Though Chona's method (Chona 1954) of actually measuring the lesion length was useful, considerable variability was observed in this. This was further confounded by differences in the age, length of cane, internodal length, etc., of the stalks rendering it rather difficult to precisely relate lesion length to red rot reaction. Also it was often observed that the advancing end of the lesion ended in a narrow strip and extended as a series of small red lesions making it difficult to decide the exact length of the lesion. These factors prompted Srinivasan and Bhat (1961) to modify the method of quantifying the lesion extension by measuring it in terms of the number of internodes transgressed by the lesion. Experience over the years has proven that such measurement could be one of the important criteria in red rot screening when considered along with other symptoms of the disease also.

***Lesion width*:** It is usually observed that when the red rot pathogen is inoculated in cane stalks the resulting lesions are of varying widths. The lesion width may range from a narrow strip to the entire width of the cane stalk. It is observed that clones which show very wide stalk lesions show more damage to the stalk with greater effect on yield and quality, even though lesion extension may be restricted. This positive relationship between lesion width and disease severity has been recognized as one of the valid criteria in the procedure to screen for red rot resistance. Lesion width is usually measured leaving the inoculated node since it often shows a slightly greater lesion width possibly due to high pathogen concentration, injury caused by the inoculators, attempted colonization by other non-pathogenic organisms, etc. It is logical to conclude that greater lesion width indicates the pathogen has invaded and degraded a large volume of host tissue resulting in greater derangement in water and nutrient flow increasing the severity of the disease.

***Presence of white spots in the lesions*:** In many susceptible varieties the reddish lesions are interspersed with white or less pigmented areas randomly or extending across the width of the stalk. Extensive presence of white spots and their wide spread distribution along the lesions are a characteristic association with susceptibility to red rot and hence scoring for white spot intensity is a very important criterion in the methodology to screen for resistance. Even when other disease severity criteria may be somewhat ambiguous due to several factors, presence of white spots invariably indicates susceptibility. This effect is explained by the histopathological basis responsible for this phenomenon. As stated earlier the red color of red rot lesions is due to the production of anthocyanidin pigments and resistant varieties produce more of them. Even within the cane stalks there appear to be areas of varying tissue resistance to red rot. As the colonization by the pathogen proceeds those tissue areas which show resistance to the pathogen produce the pigments in large quantities and turn red in colour. Those tissue areas which lack resistance remain pigment free and allow free colonization by the pathogen. In varieties with narrow lesions white spots are very rare and if present are very small surrounded by dark necrotic tissue. In varieties with broad lesions white spots are very common, extensive and are surrounded by light red colored ground tissue (Atkinson 1939). Studies of Srinivasan and Bhat (1961) had revealed that while the pathogen could be readily isolated from the white spots it could be only rarely isolated from the dark red colored areas of the lesions clearly indicating that in the dark red areas the tissues actively resist the spread of the pathogen while white pigment-free areas lack resistance and in these white spots there is an equilibrium between the host and the pathogen which colonizes these tissues without causing much tissue necrosis. Thus the white spots constitute tissues which facilitate rapid spread of the pathogen along the cane and their presence is a certain indicator of susceptibility.

***Presence of serial spots and streaks*:** Some times in certain varieties it is observed that beyond the leading end of the red rot lesion there is a series of somewhat circular spots distributed randomly along the length of the stalk progressing towards the top nodes. In resistant varieties these spots are dark red in colour, small with well defined sharp margins. In susceptible varieties the spots are larger light red with a white centre. These spots are produced by colonization of the pathogen in regions where conidia of *C. falcatum* happen to get lodged as they move along the vascular stream of the cane stalks away from the point of initial infection. In some varieties with continuous vessels there is a severe reaction of parenchyma cells close to the vessels resulting in long dark vascular streaks.

***Criteria to decide disease reaction*:** As discussed above the three quantifiable attributes viz. nodal transgression, lesion width and white spots are correlated to red rot reaction. Usually varieties showing yellowing and drying of tops (leaves) in response to inoculation also show high values of the other parameters also. Thus such yellowing and drying by itself is able to conclusively indicate susceptibility. In clones which show green tops, it is more appropriate to record the severity of other symptoms as criteria to determine the different grades of red rot reaction.

***Environment induced variability*:** It is well recognized that a warm humid climate is ideally suitable for the development of red rot and to achieve this objective red rot screening is usually taken up during the monsoon periods. However, due to fluctuations in the seasonal conditions the environmental parameters prevailing during screening at different places/times may differ. These results in variations in the red rot reactions of the same varieties tested during different seasons. However, observations have indicated when the clones are screened for red rot reaction using the scale of Srinivasan and Bhat (1961), the variation in disease reaction is usually limited to just one category above or below. Thus the system has been established to be quite reliable and relatively well reproducible. Only in the case of moderately susceptible varieties some caution is needed to confirm the reaction since due to such environmental effects they may become susceptible.

A comparative evaluation of the 0 – 9 scale and the ave-

Table 4 Number of clones showing different red rot reactions as rated by the 0-9 scale and the average lesion length method.

Reaction category	0 – 9 scale	Average lesion length method
Highly resistant	2	–
Resistant	2	9
Moderately resistant	3	77
Moderately susceptible	30	–
Susceptible	57	30
Highly susceptible	33	11
Total	127	127

Source: Srinivasan and Bhat (1961)

rage lesion length method shows that when the same number of clones is screened, they both result in different proportions of clones showing different reactions (**Table 4**). Overall, the experience of sugarcane pathologists is that the plug method of red rot evaluation is considered very reliable since the results are well defined and there is not much disease escape (Viswanathan 2010).

***Types of inoculators*:** Different methods have been used to introduce the red rot pathogen into the cane stalks for the inoculation process. The different methods cause different levels of injury in the cane stalks during inoculation. Wang and Lee (1982) suggested a method where a small hole is drilled into the internode and a toothpick soaked with inoculum broth introduced into the hole. Instead of pre-colonized toothpicks Agnihotri (1983) used sterile toothpicks dipped in a conidial suspension of the pathogen. Virk and Satyavir (1989) inoculated by making a slanting hole in the first or second internode from the bottom and introducing a pre-colonized toothpick into the hole. All the methods generally aim at causing less injury to the stalk while ensuring insertion of the pathogen into the stalk tissues.

***Inoculum requirements*:** For the plug and nodal inoculation methods, *C. falcatum* culture multiplied on oatmeal agar medium has been found to be satisfactory to result in adequate disease expression for screening purposes. Originally Chona (1954) used small bits of mycelial plugs as inoculum. Instead of this, Prakasam *et al*. (1971) used a conidial suspension of the pathogen. A minimum conidial concentration of at least one million conidia/ml is considered essential to ensure sufficient red rot development for screening purposes. Satyanarayana *et al*. (1984) observed that a spore concentration of less than 60,000 conidia/ml resulted in very low disease development. Young cultures about 10 days old with good sporulation are ideal.

***Incubation period*:** Different researchers have suggested different durations of incubation from the time of inoculation for red rot evaluation. Agnihotri (1983) stated that an incubation period of 3-5 months is needed for red rot evaluation. In general, experience has shown that a minimum incubation period of 60 day is required. Based on this observation, the All India Coordinated Research Project on sugarcane also follows a 60 days incubation period for the red rot screening studies. Environmental conditions also play a significant role in the rate of development of the disease. Warm and humid conditions result in fast development of symptoms while under dry climatic conditions disease development is rather slow.

***Disadvantage of the plug method – the injury factor*:** Although being commonly used, the plug method of inoculation is also considered as too severe by many researchers since it introduces the pathogen directly into the cane tissues causing injury and breaking the natural barriers of resistance operating on the host surface. It evaluates the tissue resistance and the ability of the pathogen to spread within the cane stalk and ignores the resistance mechanisms which may prevent the pathogen from entering the host. Under natural field conditions injuries do not play a significant role in red rot incidence. However, experience has shown that information on tissue resistance is quite necessary since it indicates the ultimate potential vulnerability or otherwise of a host genotype being considered for deployment in a red rot endemic cane growing location. To overcome the disadvantages of the plug method, a different inoculation and evaluation method *viz*. the nodal method has been suggested.

3. Nodal method of red rot evaluation

The method originally developed by Singh and Budhraja (1964) with certain modifications is presently followed to evaluate promising sugarcane clones for red rot reaction. A fresh spore suspension of the red rot pathogen containing about 1 million conidia/ml is prepared. About 7 month old cane stalks are selected for inoculation. The top few leaf sheaths of the cane are slightly pulled out and about 2 ml of the spore suspension is dropped between the pulled out leaf sheaths and the stalk so that the spore suspension comes into contact with the selected nodes (**Fig. 10**). The inoculation is carried out during the monsoon season so that suitable high humidity conditions prevail for good disease development. Also sprinklers can be operated in the experimental field to ensure high humidity. After 60 days of incubation, the top leaves and leaf sheaths are stripped off and the inoculated and adjacent nodes are examined for red rot symptoms. The nodal regions and the adjoining rind of the internodal regions are also examined. Based on the presence or absence of typical red rot symptoms the clones are rated either as resistant or susceptible to red rot. Rana and Gupta (1968) have indicated a key to rate the red rot reactions of the varieties to the nodal method of inoculation based on different symptoms.

The nodal method of red rot evaluation is considered more natural since it involves only applying the inoculum on the canes without causing any injury simulating nature as it would occur under field conditions.

4. Inoculation of exposed nodes

Rana and Gupta (1968) and Kirtikar *et al*. (1974) described a method of evaluating first clonally multiplied seedlings for red rot reaction. Setts from seedling canes were planted in the field and in the month of September when the canes are about 6 months old, two or three lower most green leaves with sheaths are gently stripped off without causing much injury exposing the fresh leaf scar region and *C. falcatum* conidial suspension of is sprayed on them. In the month of January the inoculated canes are split open and the red rot severity scored based on symptoms such as the nature of lesions and also early symptoms on the surface of the nodal regions. Rana and Gupta (1968) suggested a scoring scale to screen sugarcane clones for red rot in this method (**Table 5**).

Clones with good yield and quality which are either resistant or moderately resistant are straightaway multiplied for deployment for commercial cultivation. Superior clones showing susceptible reaction are selected for re-evaluation to consider their suitability for cultivation in less disease prone areas. The highly susceptible clones are totally rejected. The authors observed that in their study nearly 53% of the clones are highly susceptible by this method of red rot screening. Further it was reported that the pathogen infected the host primarily through the fresh leaf scar rather than the root primordia or the buds.

5. Spray inoculation of grown up seedlings

A method to spray inoculate standing seedlings of about 5-6 months old was described by Singh *et al*. (1978). The pathogen inoculum is prepared by mixing and blending cul-

Table 5 Red rot screening by inoculation of exposed nodes (Rana and Gupta 1968).

Red rot reaction	Symptoms
Resistant	No nodal infection.
Moderately resistant	Small light to dark reddish spots restricted to the nodal tissues only.
Susceptible	Lesions extend beyond the inoculated node and cross the inoculated nodes. Tops remain green.
Highly susceptible	Lesion spread to one or more nodes above the inoculated node.

Table 6 Red rot rating of seedlings in response to spray inoculation (Singh *et al*. 1978).

Red rot reaction	Symptoms
Resistant	No disease symptoms
Moderately resistant	Dark red spots about 2-7 mm long on the leaf and leaf sheath with or without sporulation.
Susceptible	Elongated midrib lesions on young leaves bearing plenty of acervuli with conidia
Highly susceptible	Large number of red or reddish brown elongated or irregular spots about 1-5 cm in size on the leaves and leaf sheaths with necrotic spots bearing plenty of acervuli.

Table 7 Controlled condition testing for red rot screening (Mohanraj *et al*. 1997).

Reaction category	Symptoms
Resistant (R)	No symptoms or minute dark brown specks only.
Moderately resistant (MR)	Dark brown small restricted necrotic spots on the nodal region. No internal symptoms. No sporulation of pathogen.
Moderately susceptible (MS)	Light brown necrotic lesions spreading towards the internodal regions, reddish discolouration, mild disintegration, slight necrosis of growth ring and leaf scar, no rotting or oozing of sap.
Susceptible (S)	Light brown diffused lesions spreading from the inoculated node towards the internodal region, light yellowish brown discoloration and moderate shredding of internal tissues, bud necrosis, mild to moderate sporulation of the pathogen on the nodes, sparse white spot formation, necrosis of growth ring, root eyes and leaf scar.
Highly susceptible (HS)	Yellowish brown discolored lesions spreading fast from the inoculated node to one or more internodes, severe shredding and disintegration of internal tissues with rotting, profuse sporulation of the pathogen and white spot formation, total bud necrosis, severe necrosis of root eyes, growth ring and leaf scar.

tures of different pathotypes of *C. falcatum* as a viscous sticky suspension and sprayed on the plants after sun set during the monsoon season. Such a preparation of inoculum enhanced conidial viability and at the same time ensured moist conditions necessary for successful infection. Typical red rot symptoms are reported to develop in about a fortnight and the varieties are graded for disease reaction based on the symptoms (**Table 6**).

The above red rot screening techniques were suggested by different researchers under different circumstances but they are not uniformly followed as commonly accepted procedures by all the sugarcane pathologists, and hence often the results are not consistent showing considerable variation under different experimental and environmental conditions. Also compared to the plug method these techniques resulted in much disease escape since in the absence of injury and positive insertion of the pathogen into the cane, any lack of ideal environmental conditions may result in failure of infection rendering susceptible clones to be identified as resistant.

6. Red rot rating based on mathematical relationship

With a view to precisely quantify the different red rot grades Prasada Rao *et al*. (1978) attempted to develop a method based on discriminant function to score different grades of red rot severity by assigning relative weightage values to the three important red rot severity parameters viz. lesion width (X_1), presence and nature of white spots (X_2) and nodal transgression of the lesion (X_3). To arrive at the red rot score (Z), the values for X_1 and X_2 are multiplied by constants 3.5 and 36.7, respectively and the value of X_3 added. A value for Z above 89 is considered susceptible, a value between 42 and 89 is considered as moderate reaction and a value less than 42 is considered as resistant. For red rot scoring by this method a minimum of 35 cane stalks have to be inoculated. In this method of red rot evaluation among the three attributes, presence of white spots is given major importance to discriminate reaction of a variety. Though this system was reported as an equally effective method to screen for resistance to red rot as the 0 – 9 scale, Viswanathan (2010) compared the discriminant function method and the 0 – 9 scale of Srinivasan and Bhat (1961) and observed that the discriminant function mainly indicates only the susceptible nature of a clone in view of its greater emphasis on white spots and fails to identify resistance as indicated by other parameters. When selected varieties were evaluated for red rot by the 0-9 scale of Srinivasan and Bhat (1961) and the discriminant function there were differences in the reaction of the individual varieties between the two methods. By the discriminant function method the reaction within the same variety was found to range from moderately resistant to susceptible which is not considered feasible. Further, it showed values representing susceptible reaction only even in varieties with yellow and dry tops in response to inoculation based on the internal symptoms. Such varieties, in view of the total crop loss they suffer are classified as highly susceptible by the 0-9 scale which is quite appropriate.

In spite of claims that discriminant function rates red rot reaction with much precision, in view of the above mentioned discrepancies in its value and the complications involved in recording the data and computing the function values with the formula, the method has not become popular and is not accepted to screen for red rot resistance in sugarcane.

7. Controlled condition testing (CCT) for red rot reaction

Evaluation of red rot resistance under field conditions usually involves one full crop season and during this period it is virtually impossible to have much control over the environmental conditions in the field to ensure adequate disease development.

With a view to rapidly evaluate red rot reaction of sugarcane clones under ideal environmental conditions minimizing disease escape, a controlled condition testing method has been developed (Mohanraj *et al*. 1997; Viswanathan *et al*. 1998). Top one third to one half of 7-8 month old non-flowering canes with top leaves are vertically arranged on sand beds in a humidity chamber maintained at about 30°C (**Fig. 11**). It is ensured that the bottom nodes of the stalks are well in contact with the sand bed leaving the spindle intact with tips of the spindle leaves just trimmed. Freely removable leaves with leaf sheaths are stripped off without causing any injury exposing the top nodes and internodes. A conidial suspension of *C. falcatum* containing about 1 million conidia/ml is prepared. Absorbent cotton strips of about 10 cm long and 3 cm wide are soaked in the spore suspension and these cotton swabs are wound around the two top exposed nodes of the prepared stalks (**Fig. 12**). Humidity inside the chamber is maintained above 90% by operating a timer controlled mist blower inside. Also the interior of the chamber is illuminated for 8 hrs a day with fluorescent lamps to provide about 320 watts of light energy. The inoculated canes are maintained in the humidity chamber for 7-10 days, removed from the chamber and examined for symptoms. The nodal and internodal regions between the inoculated nodes are scraped with a sharp knife and the symptoms caused by the inoculation are examined. The major criteria which determine red rot reaction are nature of nodal and internodal lesions, colour and spread of the lesions, tissue disintegration, rotting, sporulation of the pathogen, bud necrosis, leaf drying, etc. (**Fig. 13-16**). Based

on the symptoms, the test clones are identified as resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible (**Table 7**).

***Advantages of the new method*:** The conventional disease screening techniques like plug and nodal methods take one year to assess clones for disease reaction. Further these methods have limitations of land, labour, rapidity, repeatability, restriction in using various pathotypes for screening and also environment induced disease escapes especially in the nodal method. This method circumvents these constraints in field testing and the new method has been very effectively used to evaluate several thousands of clones including those in the pre-release stage in the advanced stage of selection in the breeding programs, germplasm clones, inter-specific and intergeneric hybrids, somaclones, etc. Parental clones with resistance to multiple pathotypes of the red rot pathogen have been identified for use in the hybridization programs. During the course of the past 15 years or so, more than 25,000 clones from various sources have been evaluated for red rot reaction by this method at this method. At our institute normally in the breeding programs, superior clones in the final stages of selection (Initial Varietal Trial, Advanced Varietal Trial) are screened for red rot reaction by the plug or nodal methods. Presently in many programs even in the earlier stages of selection, the progeny clones are screened for red rot reaction by this method before they are forwarded to the next stages of selection.

8. Screening for field tolerance to red rot

It has been observed that sometimes a few sugarcane varieties such as Co 8021, Co 86032 etc., which have been rated as susceptible by the above stalk inoculation methods remain free of red rot incidence under endemic conditions also suggesting that these varieties could posses field tolerance to the primary infection process of the disease. Under natural endemic conditions, primary infection of healthy cane setts planted in the field takes place from the pathogen inoculum available in the left over debris of a preceding diseased crop in the form of trash, stubbles, etc. This fact suggested the possibility that red rot screening could be carried out by planting setts in a field either naturally or artificially infested with the pathogen. Earlier some researchers attempted to use the sick plot technique to screen for red rot resistance. Red rot infected cane material was applied in the field over a period of time and the test sugarcane clones planted and the red rot reaction rated based on the intensity of disease development starting from the germination phase. The sick plot technique was not found to be effective since *C. falcatum* not being a typical soil-borne pathogen was unable to survive for long durations saprophytically in the absence of the host resulting in inadequate or inconsistent disease expression rendering it unsuitable for screening purposes.

***Screening with fresh red rot debris*:** To overcome the shortcomings of the sick plot technique, attempts were made to screen for tolerance by planting setts in fields after applying large quantities of fresh red rot affected cane debris (**Fig. 17**). Though this method showed better results than the sick plot technique it resulted in some inconsistencies in disease expressions due to lack of uniformity of inoculum in the cane debris and inability to apply specified quantities of inoculum in relation to the canes planted to ensure reliable results. Also non availability of quality inoculum continuously throughout the year was a serious limitation.

***Screening with laboratory cultured inoculum*:** In order to ensure uniformity and virulence as well as continuous availability of inoculum, efforts were made to multiply the red rot pathogen on a suitable substrate for use to screen for field tolerance by planting the test clones in the field after application of such inoculum (Padmanaban *et al*. 2010). Inoculum multiplied on media such as sand sorghum medium was used (**Fig. 18**). Encouraging results have been obtained in identifying field tolerant clones if the evaluations were carried out under ideal high humidity conditions either in the field or greenhouse (**Fig. 19**). Reaction of the clones to red rot is recorded based on the proportion of plants showing early red rot symptoms such as germination failure (**Fig. 20**), pre and post emergence necrosis of shoots, leaf yellowing and drying (**Fig. 21**), development of midrib lesions (**Fig. 22**), etc. Studies are underway to further standardize the procedure with reference to factors such as age of inoculum, suitable substrate for inoculum multiplication, quantum of inoculum to be used, seed cane to be used, stages of inoculation, red rot scoring scale, etc.

This evaluation system is expected to reduce the rejection of many high sugar and high yielding sugarcane clones for want of resistance to the stalk inoculation procedures presently being followed. Such clones identified could be deployed on a limited scale in red rot endemic locations and monitored critically for red rot development and spread. After confirming their field tolerance they can be adopted for large scale commercial cultivation. Further, the low level of disease incidence in these varieties can be effectively reduced with agronomic management measures and use of systemic fungicides. This could significantly improve the productivity of the crop which is presently seriously limited by the rejection of several high sugar and high yielding varieties in the breeding programs for want of resistance to red rot.

Indices of red rot resistance

In addition to symptoms, certain serological, biochemical and physiological indices can serve as supplementary factors to indicate the resistant or susceptible nature of sugarcane varieties.

1. Serology

Usefulness of an ELISA technique to detect and quantify colonization of sugarcane tissues by *C. falcatum* has been demonstrated earlier at this institute (Viswanathan *et al*. 1999, 2000a). The intensity of such colonization was related to the resistance or susceptibility of the varieties to red rot. Thus the technique could be used to determine the reaction of the varieties even before symptom expression. Our studies indicated that the pathogen colonization in the nodal regions was higher than in the internodal regions. Bud tissues and root eyes recorded higher pathogen titer similar to the levels in the white spots of the lesions. While the technique is rapid and can be used to screen a large number of varieties within a short time it needs to be further refined to eliminate non-specific reactions observed sometimes with certain samples. Further sampling procedures have to be standardized.

2. Phytoalexins

Enhanced accumulation of 3-deoxyanthocyanidin phytoalexins such as apigeninidin, leutolinidin and related compounds in certain varieties in response to pathogen inoculation is known to be associated with red rot resistance (Viswanathan *et al*. 1994, 1996a, 1996b, 2000b; Viswanathan 2001). While in the susceptible varieties, phytoalexin accumulation is low and at a slower pace in resistant varieties they accumulate rapidly and in large quantities.

3. Response to pathogen toxin

Susceptible varieties express some symptoms of red rot when treated with partially purified toxin of the red rot pathogen while resistant varieties show less severe reaction. Also susceptible varieties show increased electrolyte leakage while resistant varieties show increased accumulation of phytoalexins in response to treatment with partially purified toxin of *C. falcatum* (Mohanraj *et al*. 2003). Further

studies in this regard with fully purified red rot toxin may enable its more effective use in screening for red rot resistance.

Standardization of the above indices can be useful to develop pathogen free and rapid screening procedures to identify red rot resistant host varieties.

4. Histological criteria

Rapid colonization of inoculated canes and sporulation of the pathogen within 48 hrs after inoculation under CCT conditions as determined by histological examination indicates their high susceptibility (**Fig. 23**). Comparison of serological, histological and biochemical methods by Nallathambi *et al*. (1999) indicated that they can serve as effective indices to identify red rot resistant cane varieties.

5. Molecular markers

Efforts are underway to identify molecular markers in sugarcane either constitutive or expressed in response to pathogen inoculation to select genotypes with resistance to red rot. PCR (Polymerase chain reaction) based diagnostic techniques have been developed to detect colonization of sugarcane genotypes by the red rot pathogen and establishment of a relationship between host colonization and disease reaction can help in the rapid and effective identification of resistant genotypes. Much progress has been achieved in understanding the mechanisms of red rot resistance at the molecular level which may ultimately lead to precise screening for resistance (Viswanathan *et al*. 2008, 2009; Viswanathan 2010).

6. Red rot reaction based on damage index (DI)

In Brazil, Giglioti and Canteri (1999) attempted to develop a red rot screening system taking into account, the loss caused by the disease in relation to the volume of stalk attacked and the damage caused. A damage index was worked out based on the correlation between the percentage of the area along the cane stalk colonized by the pathogen and the loss caused. They validated the method using a scale with four severity levels and linear regression between the estimated and actual disease severity.

***Screening for horizontal resistance*:** The realization of the occurrence of pathotypes of *C. falcatum* and differential host reaction led to the need to identify cultivars with resistance to multiple pathoypes. Since pathotypes appeared to be distributed in the different regions of the country a project was implemented in the 1970's and 1980's to breed, screen and identify superior sugarcane clones with resistance to pathotypes of the different regions by screening them with the pathotypes of the respective regions (Alexander *et al*. 1987). This has resulted in the development of several promising clones with multiple pathotype resistance to red rot.

Other related issues in red rot screening

1. Virulence maintenance

Continuous sub-culturing of *C. falcatum* on artificial media is known to result in gradual loss of virulence resulting in insufficient disease development for screening purposes. The problem is avoided by regularly inoculating and re-isolating the pathogen from a susceptible cane variety which helps to maintain virulence. Also regular isolations of the pathogen from naturally infected canes in the field and their multiplication ensure adequate virulence and pathogenicity.

2. Problem of pathogen variability

During the earlier years (before 1970), two types of *C. falcatum* cultures were recognized viz., the dark and light colored isolates. The dark colored isolates were less sporulating and less virulent and were found unsuitable for screening purposes. The light colored isolates were profusely sporulating and very virulent and hence routinely used in screening for resistance. However, from the 1970's differences were observed in the pathogenicity of *C. falcatum* isolates on different host genotypes (Jothi 1989). Clones rated as resistant to certain isolates behaved as susceptible to certain other isolates suggesting the prevalence of different pathotypes/strains of the pathogen and the operation of differential host reaction (Padmanaban *et al*. 1996). Attempts have been made to characterize the red rot pathotypes of tropical and sub-tropical India on molecular basis using RAPD markers and internally transcribed sequences (ITS) sequences of rDNA (Mohanraj *et al*. 2002; Malathi *et al*. 2010). This problem is presently being sought to be solved by using a mixture of virulent pathotypes of the particular region for the screening process so that clones with broad based and durable resistance can be identified.

3. Environmental requirements for red rot screening

Irrespective of the method of inoculation and evaluation, the environmental conditions prevailing during and after inoculation profoundly influence red rot development and the reaction of varieties. Of these factors, humidity and temperature are of particular importance. From early times it has been recognized that severe red rot occurrence is usually associated with high rain fall, high atmospheric humidity, high soil moisture, flooded conditions and warm temperatures. In the subtropics under Shahjahanpur conditions second and third week of September were found to be suitable for red rot development under artificial inoculation conditions (Rana and Gupta 1968) when average relative humidity was about 90% and the maximum and minimum temperatures were 33 and 24°C, respectively. Effect of environmental factors on red rot incidence by the nodal method is more marked. Even with the plug method of inoculation which is considered to be rigorous there is reduction in red rot severity with decrease in mean temperature from 31.0 to 21.1°C (Beniwal and Satyavir 1991). They have observed that sugarcane varieties differ in their red rot reaction in relation to their response to temperature variations. These include effect on specific symptoms such as top drying. Under Haryana conditions best results with nodal method of inoculation were obtained when inoculations were made during the early part of the monsoon particularly in the month of July (Satyavir *et al*. 2002). Inclusion of standard susceptible and resistant varieties in the evaluations for comparison will enable to ascertain whether suitable environmental conditions had prevailed during the screening process.

Screening for red rot resistance under the All India Coordinated Research Project (AICRP) on sugarcane

The All India Coordinated Research Project on sugarcane is a national programme to develop sugarcane varieties in India and which follows a system of screening for red rot resistance developed after much experience. Initially the promising clones are screened for red rot resistance by the plug method of inoculation. From these those which are susceptible but have high yield and quality are screened again by the nodal method of inoculation. Screening is carried out at locations representing different red rot endemic regions of the country using a mixture of *C. falcatum* pathotypes of that particular region during the monsoon period to ensure high humidity.

The protocols for red rot screening using different methods under the AICRP are presented next (Viswanathan 2010).

1. Plug method

Plug inoculation is carried out in the middle of the 3rd internode by dropping two drops of the spore suspension into the bore hole with a syringe. Red rot rating is recorded after 60 days based on the 0 – 9 scale. At least two canes in each of 20 clumps are inoculated and evaluated.

2. Nodal method

Inoculation is carried out by dropping about 1ml of the spore suspension (1 million spores/ml) in the axils of 4th and 5th node from the top between leaf sheath and stalk at two opposite buds after slightly pulling out the leaf sheaths. After 60 days the red rot reaction is evaluated based on symptoms such as spindle lesions, mid rib lesions, acervuli and spores on leaf scar, root eyes, root primordia and growth ring. The nodes are scraped and examined for development of lesions. At least 15 stalks are evaluated for each variety.

3. Cotton swab method

Here the lower most leaves with sheaths are removed. Strips of cotton swabs dipped in conidial suspension of *C. falcatum* are wound around these exposed nodes and covered by wrapping with Parafilm®. At the time of evaluation, the cotton swabs are removed, the nodal area scraped with a sharp knife and examined for presence or absence of lesions. Two canes each in at least 20 clumps each are inoculated and evaluated. For inoculation, a mixture of about 7 day old cultures of *C. falcatum* isolates representing the North West, North Central, East coast and Peninsular zone isolates are used in the respective centres.

Screening for red rot resistance in different countries

In addition to India, red rot is also a commonly occurring disease in many other countries such as the United States of America, South American countries such as Brazil, Argentina and Asian countries such as Pakistan, Bangladesh, Thailand, etc. Depending on their individual circumstances they follow different red rot screening methods and some of them are discussed below.

Pakistan and Bangladesh which are geographically contiguous with India use the normal plug method for red rot evaluation.

Red rot is known to occur in the southern parts of the United States of America where it primarily affects seed cuttings severely inhibiting germination resulting in poor crop stand and much crop loss. In the United States, the United States Department of Agriculture evaluates red rot reaction by inoculating mature cut canes under laboratory conditions by a modified plug method of inoculation. Sugarcane stalks with about 10 nodes with no borer damage are collected and the leaves and the leaf sheaths are removed. The surface of the stalks is disinfected with a dilute solution of sodium hypochlorite. A hole of about 3 mm diameter is punched in the middle internode of each stalk. A conidial suspension of about 2.5 million spores of the pathogen/ml is prepared and about 0.1ml of the suspension is introduced into the hole punched in the internode. The inoculated canes are maintained at a temperature of about 25°C for 20 – 25 days, split open and the red rot reactions are graded based on symptoms in comparison to symptoms produced on standard varieties of known red rot reaction (Abbott 1938; Abbott *et al*. 1967). The reactions of the varieties are graded as Class 1 to 5, Class 5 being the most susceptible.

Varieties which show Class 3 or 4 reactions under laboratory conditions are re-evaluated again under field conditions to confirm the reactions. Seed cuttings are inoculated with the pathogen and planted in the field during the month of October. Inoculation is carried out either by introducing the inoculum into a hole punched in the sett or by smearing it on the nodes in the sett. In the following spring, red rot reaction of the genotypes is recorded based on the effect of the disease on sett germination. Multiple replications are maintained with suitable checks.

Table 8 Red rot scoring (Yin *et al*. 1996).

Red rot reaction	Value range for NP	Value range for IRS
Resistant	<2	<18%
Susceptible	2-3	18-38%
Highly susceptible	>3	>38%

A method of red rot evaluation involving inoculation of cane stalks and incubating them in wet paper towels covered over by perforated polythene sheets was described by Yin *et al*. (1996). The inoculated canes were maintained at about 25°C for about 5 to 7 weeks, split open longitudinally and examined for internal symptoms. The criteria considered for disease rating are nodal transgression of lesions in both directions from the point of inoculation, number of nodes showing rotting symptoms and the internode rotting symptoms upto 4 internodes on either side of the inoculated internode. Numerical values were assigned to represent nodes passed by the lesion (NP) and internodal rot severity (IRS) and the red rot index (RI) was arrived at by using the formula RI = NP × IRS. The range of different values of NP and IRS representing different red rot grades is given in **Table 8**.

In Thailand (Manjaroenchot 1986), when different inoculation techniques were compared, it was found that injecting 1 ml of a suspension containing more than a million conidia/ml into the cane stalks with a hypodermic syringe resulted in an infection rate of more than 91.7%. On the other hand, when the spore suspension was drenched in the soil with or without wounding of the roots, it resulted in only 33.3 and 25.0% infection, respectively.

In South America, Argentina is one of the countries where red rot is an important disease of sugarcane. Here inoculation of standing cane stalks was found to be a suitable method to screen for red rot resistance. It was reported that when the Abbott scale of red rot scoring was adopted the disease severity index ranged from 0.23 to 1.40 (de Ramallo and Ploper 1976).

Indo – US collaborative work on screening for red rot resistance

Since the problem of red rot is common to India and the USA, collaborative research programs were undertaken to screen for red rot resistance. The technique involved the inoculation of cut canes in the laboratory or the greenhouse. For this purpose the red rot pathogen was grown on a suitable medium such as oatmeal agar. Cane stalks of the test clones were collected from the field when they were about 7 months of age, bore holes were punched in the top and bottom internodes, the pathogen culture was introduced into the bore holes and the bore holes sealed. After a fixed incubation period the cane stalks were longitudinally split and examined for progression of internal symptoms. Clones in which the lesions extending from both ends meet were considered highly susceptible. Greater the distance between the two extending lesions higher was the resistance of the clone. Thus the primary criterion deciding red rot reaction was only the progress of the lesion.

Limitations

The above screening method was found to have serious limitations. It was often observed by later researchers that in many clones even when there was much lesion extension they showed no drying of the leaves or other symptoms without much reduction in yield or quality attributes of the clone under filed conditions. The method was not found to be suitable under Indian conditions since many factors

relating to disease development here are quite different. This necessitated the development of a screening technique which was more comprehensive taking into account the nature of all the symptoms and their effects on the cane.

Future scenario of red rot screening

Although many aspects of screening for resistance to red rot in sugarcane have been standardized there is much scope for further improvements and fine tuning in the screening techniques to render the results more reliable and consistent while ensuring that high sugar and high yielding clones which can survive in endemic locations are not unnecessarily rejected. Better understanding of the epidemiology of disease development and spread particularly with reference to environmental factors will enable evaluation of the clones under ideal conditions with suitable techniques so that the screening process would simulate natural field conditions at the same time reducing disease escapes. Further knowledge of the mechanisms of resistance would help to identify more precise physiological and biochemical indices to select resistant clones. Detailed investigations on characterization of *C. falcatum* pathotypes is expected to enable the use of the most suitable isolates to select cultivars for deployment in relation to the prevalence of these pathotypes. Advances in molecular biological techniques could render marker assisted selection possible to identify resistant genotypes. More and more application of biotechnological techniques is expected to markedly improve the precision of the screening process.

The promising results observed with systemic fungicides, biocontrol agents and resistance inducing signal molecules in the management of red rot suggest that their combined use with field tolerant varieties identified by suitable techniques will go a long way by making more high sugar and high yielding varieties available for deployment. Perhaps combination of different methods which supplement and complement each other at different stages in the varietal improvement programme may prove to be much advantageous to identify more superior varieties suitable for red rot endemic locations, thereby significantly improving the production and productivity of the crop.

REFERENCES

Abbott EV (1938) *Red Rot of Sugarcane*, USDA Technical Bulletin, 641, 96 pp

Abbott EV, Zummo N, Tippert RL (1967) Methods of testing sugarcane varieties for disease resistance at the US Sugarcane Field Station, Houma, Louisiana. *Proceedings of International Society of Sugar Cane Technologists* **12**, 1138-1141

Agnihotri VP (1983) *Diseases of Sugarcane*, Oxford & IBH Publishing Co., New Delhi, 363 pp

Alexander KC, Rao MM, Mohanraj D, Seshadri K, Padmanaban P (1987) A new approach to improvement of red rot resistance of sugarcane. *Indian Journal of Mycology and Plant Pathology* **16**, 108-112

Anonymous (2011) Report of Principal Investigator. All India coordinated Research Project on Sugarcane, Plant Pathology, Indian Institute of Sugarcane Research, Lucknow, India, 140 pp

Atkinson RS (1939) On the nature of resistance of sugarcane to red rot. *Proceedings of International Society of Sugarcane Technologists* **6**, 684-692

Beniwal MS, Satyavir (1991) Effect of atmospheric temperature on the development of red rot of sugarcane. *Indian Phytopathology* **44**, 333-338

Chona BL (1950) Studies on the diseases of sugarcane in India. III Sources and mode of red rot infection. *Indian Journal of Agricultural Sciences* **20**, 363-385

Chona BL (1954) Relative resistance of sugarcane varieties to red rot. *Indian Journal of Agricultural Sciences* **24**, 301-315

De Ramallo VNE, Ploper LD (1976) Red rot in sugarcane: technique of testing for resistance. *Revista Industrial y Agricola de Tucuman* (Argentina) **53 (2)**, 15-20

Giglioti EA, Canteri MG (1999) Phytopathometry applied to the integrated management of borer-rot complex in sugarcane: (I) A standard diagram to assess disease severity. In: Rao GP, Bergamin Filho A, Autrey JC, Magarey RL (Eds) *Sugarcane Pathology*, Science Publishers, Enfield (NH), USA, pp 183-194

Jothi R (1989) Studies on variation in red rot pathogen *Colletotrichum falcatum* Went on sugarcane, PhD thesis, Bharathiar University, Coimbatore, 245 pp

Kirtikar, Gupta SC, Kureel DG (1974) Screening of sugarcane varieties for red rot resistance. *Proceedings of the International Society of Sugarcane Technologists* **15**, 189-193

Malathi P, Padmanaban P, Viswanathan R, Mohanraj D, Ramesh Sundar A (2004) Efficacy of Thiophanate methyl against red rot of sugarcane. *Acta Phytopathologica Entomologica et Hungarica* **39**, 39-47

Malathi P, Viswanathan R, Ramesh Sundar A, Prakasam N, Padmanaban P, Jothi R, Renuka Devi SR, Poongothai M (2010) Variability among *Colletotrichum falcatum* pathotypes used for screening red rot resistance in sugarcane. *Sugar Cane international* **28 (2)**, 47-52

Manjaroenchot A (1986) A stem rot and root rot of sugarcane. Office of the Permanent Secretary, Bangkok (Thailand). Office of the Cane and Sugar Board; Kasetsart University, Bangkok (Thailand), 99 pp

Mohanraj D, Padmanaban P, Viswanathan R, Alexander KC (1997) Sugarcane screening for red rot resistance. *Sugar Cane* **3**, 18-23

Mohanraj D, Kumaresan S, Sreenivasan TV (2002) Molecular characterization of isolates of the sugarcane red rot pathogen *Colletotrichum falcatum* Went. *Indian Phytopathology* **55**, 147-151

Mohanraj D, Padmanaban P, Karunakaran M (2003) Pathogen toxin induced electrolyte leakage and phytoalexin accumulation as indices of red rot (*Colletotrichum falcatum* Went) resistance in sugarcane. *Phytopathologia Mediterranea* **42**, 129-134

Nallathambi P, Padmanaban P, Mohanraj D, Viswanathan R, Jothi R (1999) Histological, biochemical and immunological markers for red rot (*Colletotrichum falcatum* Went) resistance in sugarcane genotypes. *Sugar Cane*, **4**, 13-16

Padmanaban P, Mohanraj D, Viswanathan R, Rao MM, Prakasam N, Jothi R, Alexander KC (1996) Differential interaction of sugarcane clones to pathotypes of *Colletotrichum falcatum* Went. *Sugar Cane* **4**, 16-20

Padmanaban P, Mohanraj D, Viswanathan R (2010) Field tolerance to red rot disease. *SBI News* **30 (2)**, 1-3

Prakasam P, Appalanarasiah TC, Satyanarayana Y (1971) A note on an improved method of screening sugarcane varieties against red rot disease. *Indian Journal of Agricultural Sciences* **41**, 1131-1133

Prasada Rao KK, Sarma MN, Satyanarayana V, Rao MA (1978) Discriminant function as a reliable guide for assessing varietal reaction to red rot of sugarcane. *Proceedings of the International Society of Sugar Cane Technologists* **16**, 395-400

Rana OS, Gupta SC (1968) An easy method of screening out red rot susceptible varieties at initial stages of multiplication. *Indian Sugar* **18**, 447-452

Rott P, Bailey RA, Comstock JC, Croft BJ, Saumtally AS (2000) *A Guide to Sugarcane Diseases*, CIRAD-ISSCT, CIRAD Publications Services, Montpellier, France, 339 pp

Satyanarayana Y, Achutharamarao M, Ramapandu S (1984) Influence of conidial concentration of *Physalospora tucumanensis* Speg. in the inoculum on the reaction of sugarcane varieties. *Sugar Cane* **2**, 4

Satyavir, Kumar A, Raj K, Virk KS (2002) Red rot of sugarcane: The research scene in Haryana. In: Singh SB, Rao GP, Eswaramoorthy S (Eds) *Sugarcane Crop Management*, SCI TECH Publishing LLC, Houston, Texas, USA, pp 109-126

Singh K, Budhraja TR (1964) Method for inoculating sugarcane for red rot. *Plant Disease Reporter* **48**, 991-993

Singh HN, Gupta SC, Singh SB, Singh MP (1978) Improvement of the technique for screening against red rot at seedling stage. *Sugarcane Pathologists' Newsletter* **21**, 29-31

Srinivasan KV (1962) A technique for evaluation and elimination of red rot susceptible sugarcane seedlings at an early stage. *Current Science* **31**, 112-113

Srinivasan KV, Bhat NR (1961) Red rot of sugarcane – criteria for grading resistance. *Journal of Indian Biological Society* **40**, 566-577

Virk KS, Satyavir (1989) Evaluation of screening methods for red rot resistance. *Indian Sugar* **39**, 621-622

Viswanathan R (2001) Accumulation of anthocyanin compounds in sugarcane tissues as a factor responsible for red rot resistance. *Madras Agricultural Journal* **88**, 571-576

Viswanathan R (2010) *Plant Disease: Red Rot of Sugarcane*, Anmol Publishers, New Delhi, India, 306 pp

Viswanathan R, Mohanraj D, Padmanaban P (1998) Comparison of three testing methods for evaluation of resistance to red rot caused by *Colletotrichum falcatum* in sugarcane (*Saccharum officinarum* L.). *Indian Journal of Agricultural Sciences* **68**, 226-230

Viswanathan R, Mohanraj D, Padmanaban P (2000b) Possible involvement of anthocyanin compounds in resistance of sugarcane red rot. *Indian Phytopathology* **53**, 311-313

Viswanathan R, Mohanraj D, Padmanaban P, Alexander KC (1994) Possible role of red rot pigments in host pathogen interaction in sugarcane with red rot pathogen. *Indian Phytopathology* **47**, 281

Viswanathan R, Mohanraj D, Padmanaban P, Alexander KC (1996a) Accumulation of 3-deoxyanthocyanidin phytoalexins Leuteolinidin and Apigenidin in sugarcane in relation to red rot disease. *Indian Phytopathology* **49**, 174-175

Viswanathan R, Mohanraj D, Padmanaban P, Alexander KC (1996b) Synthesis of phytoalexins in sugarcane in response to infection by *Colletotrichum falcatum* Went. *Acta Phytopathologica et Entomologica Hungarica* **31**,

229-237

Viswanathan R, Padmanaban P Mohanraj D, Jothi R (1999) Rapid detection of red rot (*Colletotrichum falcatum*) in sugarcane and evaluation of host reaction by ELISA. In: Rao GP, Bergamin Filho A, Autrey JC Magarey RL (Eds) *Sugarcane Pathology*, Science Publishers, Enfield (NH), USA, pp 36-45

Viswanathan R, Padmanaban P Mohanraj D, Jothi R (2000a) Indirect ELISA technique for the detection of the red rot pathogen in sugarcane (*Saccharum* spp. hybrid) and resistance screening. *Indian Journal of Agricultural Sciences* **70**, 308-311

Viswanathan R, Ramesh Sundar A, Padmanaban P, Mohanraj D (2002) Red rot disease of sugarcane and its management. In: Upadhyay RK, Mukherji KG, Dubey OP (Eds) *IPM Systems in Agriculture (Vol 8) Key Pathogens and Diseases*, Aditya Books, New Delhi, pp 277-301

Viswanathan R, Ramesh Sundar A, Malathi P (2008) Identification and characterization of differentially expressed genes/proteins involved in sugarcane – red rot pathogen interaction. *Annual Report*, Sugarcane Breeding Institute for 2006-07, Coimbatore, pp 51-52

Viswanathan R, Ramesh Sundar A, Malathi P, Rahul PR, Ganesh Kumar V, Banumathy R, Prathima PT, Raveendran M, Kumar KK, Balasubramanian P (2009) Interaction between sugarcane and *Colletotrichum falcatum* causing red rot: Understanding disease resistance at transcription level. *Sugar Tech* **11**, 45-50

Viswanathan R, Samiyappan R (1999) Red rot disease in sugarcane: A major constraint for the Indian Sugar Industry. *Sugar Cane* **5**, 9-15

Wang ZN, Lee CS (1982) Improvements on the methods of testing sugarcane varietal resistance to leaf blight and ret rot. *Report of Taiwan Sugar Experiment Station* **95**, 1-7

Yin Z, Hoy JW, Milligan SB (1996) Evaluation and heritability of resistance to sugarcane red rot. *Phytopathology* **86**, 662-667