REVIEW

THE CONCEPT OF FIELD TOLERANCE AND ITS RELEVANCE IN SCREENING FOR RED ROT OF SUGARCANE

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Abstract

In sugarcane crop improvement programmes, screening for red rot resistance using the plug and nodal methods of inoculation results in the rejection of several high yielding and high quality genotypes for want of resistance, causing a severe constraint in the availability of suitable cultivars for improving productivity of the crop. However, some cultivars rated as susceptible by these methods are observed to show very low natural incidence of red rot even under endemic conditions suggesting their field tolerance to the disease. Studies have been carried out to develop suitable methodologies to screen for such field tolerance by inoculating field planted setts with the red rot pathogen multiplied on a suitable substrate simulating debris borne primary infection under field conditions. Results have indicated the potential to identify such field tolerant genotypes using certain standardized inoculation and evaluation procedures. Also, the possibility of identifying superior commercial clones or genetic stocks with field tolerance to red rot from the early seedling stage in the selection process has been indicated. Systematic use of the techniques, after some fine tuning, is expected to make available more superior cane cultivars for deployment in red rot endemic areas to substantially improve the productivity of the crop.

Key words: *Sugarcane, red rot, Colletotrichum falcatum, screening, field tolerance*

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Introduction

The importance of red rot disease in sugarcane needs no special emphasis since its role in severely reducing the yield and quality of the crop is well known. The disease is a major constraint in the productivity and production of the crop in the Indian sub-continent (Agnihotri, 1983) so much so that several very high yielding and high quality cane varieties had to be replaced in view of their susceptibility at some stage or the other during their cultivation under field conditions. The disease is caused by the fungal pathogen *Colletotrichum falcatum* (*Glomerella tucumanensis*) which is primarily transmitted through infected seed setts or pathogen propagules harboured in left over debris of a preceding red rot affected cane crop such as stubbles and trash, as well as by irrigation or flood water flowing from disease affected to healthy fields. Secondary cane to cane transmission may take place through air borne or rain splash borne conidia under monsoon conditions. Till recently, chemical control of the disease through the use of fungicides has not been successful for various reasons. Hence, the most practical measure to manage red rot is the use of resistant/tolerant cultivars.

Current red rot evaluation methods

The traditional methods of screening for resistance to red rot involve the plug and nodal methods of inoculation and evaluation. The plug method which is considered to be more severe is used in the early stages of the breeding programme such as the Preliminary Zonal Varietal Trial (PZVT). Clones which show resistant or moderately resistant reaction in this evaluation are promoted to further evaluation of yield and quality and the promising ones identified are considered for commercial cultivation. The moderately susceptible clones are evaluated by the nodal method of inoculation and those free of red

rot infection are considered for deployment in less endemic or disease free locations after further evaluation in the Zonal Varietal Trial (ZVT) and yield evaluation trials of the All India Co-ordinated Research Project (AICRP). In the AICRP, the promising varieties are also evaluated for their reaction to the diverse pathotypes of red rot prevailing in the different endemic locations of the country. Various methods of screening for red rot resistance in sugarcane have been reviewed in detail by Viswanathan (2010).

Loss of superior genotypes

Although the nodal method of red rot evaluation is considered more natural, it still results in the elimination of a large number of high yielding and high sugar genotypes for lack of resistance to the disease. This renders very few clones available for commercial deployment particularly in endemic locations. Since cane productivity largely depends on suitable location specific cultivars, availability of a large number of superior varieties with red rot resistance is desirable to select suitable ones and deploy them in specific locations. Thus, the loss of a large number of varieties during the process of selection for red rot resistance needs to be minimized while ensuring that the finally selected clones do not suffer yield and quality loss due to the disease under field conditions.

Observed field tolerance

Most cane varieties rated as susceptible or moderately susceptible by the plug and nodal methods of evaluation show high natural incidence of red rot under endemic conditions. However, in the last decade or so it has been observed that some varieties which showed susceptible reaction to red rot by the stalk inoculation and evaluation methods remain free of the disease and show very little natural incidence even under endemic locations. Typical among them are varieties such as Co 86032 and Co 8021 which are susceptible by the stalk inoculation procedures but show no high levels of red rot incidence even in endemic locations while varieties such as CoC 671, CoC 92061 and CoC 90063 grown in the same locations are severely affected by red rot. Such differences observed in fields planted with red rot free seed setts clearly show that the varieties Co 86032 and Co 8021 largely escape from the debris borne inoculum while the other varieties get easily infected.

The varieties Co 86032 and Co 8021 occasionally show a few infected clumps under extreme adverse conditions such as flooding but usually the incidence of the disease is very low, not high enough to cause significantl yield or quality loss. They also do not show significantly high levels of secondary spread of the disease. Thus, they appear to be relatively tolerant to the disease under field conditions.

The concept of field tolerance

The concept of field tolerance in crop protection, particularly with reference to insect pests, is well known. In pest management, it is observed that under certain situations cultivars with total resistance to particular pests are not available for cultivation. However, some cultivars consistently show only very low levels of pest incidence which does not significantly affect the yield and quality parameters of the crop. Crop protection scientists have arrived at different threshold levels of pest incidence with reference to different pests and crops up to which incidence of specific pests does not cause significant yield or quality loss. Such host cultivars which constantly show a level of pest incidence below the economic threshold level are considered field tolerant to specific pests. Using a similar analogy, an evaluation system was sought to be developed to screen and identify sugarcane clones with field tolerance to red rot even though they may be susceptible by the stalk inoculation procedures. Any variety with resistance/tolerance to primary debris borne red rot infection will show low levels of subsequent disease build up and secondary spread resulting in more productivity of the crop under field conditions.

Early studies on field tolerance to red rot

When the field tolerance phenomenon was first observed about a decade ago, attempts were made to understand it and simulate it under experimental conditions. The plug and nodal methods of inoculation and evaluation are stalk inoculation methods and represent secondary spread of the disease. Since prevention of initial primary onset of disease would involve resistance/tolerance to debris borne infection of planted setts, it was initially sought to screen for

field tolerance by using the traditional sick plot technique. Sugarcane research stations in the sub-tropics had earlier developed sick plots by applying red rot affected cane debris in selected fields over a period of time and planted the test clones in the infested soil in an attempt to screen them for field reaction. The results were not very encouraging since high or consistent levels of red rot could not be reproduced by this technique even in susceptible varieties.

Inoculum survival

A review of literature reveals that the red rot pathogen *C. falcatum* does not behave as a typical soil borne plant pathogen. Most plant disease causing *Colletotrichum* spp. are foliar pathogens and their ability to survive in a soil environment is much restricted. In sugarcane, the rapid inactivation of the inoculum in the soil by other soil microbes had been recognized for a long time (Abbot and Hughes, 1961). This explains well the failure of the sick plot system in screening for resistance since the accumulated soil inoculum quickly becomes inactive and high or consistent reproduction of the disease becomes impossible. This suggested the need to use fresh infected cane debris as inoculum.

Use of fresh inoculum

Mohanraj and Padmanaban (2006) reported the use of fresh red rot infected cane debris to screen for field tolerance. When this inoculum was applied in the soil followed by planting of setts of a susceptible cane variety immediately, high levels of disease incidence were produced in the crop. Although fresh infected cane debris resulted in good disease expression, inconsistencies were observed in the levels of disease incidence depending on the variations in the inoculum. Factors such as the progress of infection in the canes used as inoculum, sporulation of the pathogen and presence of saprophytic microbes in the debris caused heterogeneity of the inoculum resulting in varied levels of red rot incidence in the inoculated crop. This indicated the need to ensure uniformity of the pathogen inoculum used to screen for field tolerance.

Use of laboratory multiplied inoculum

To overcome the variability in red rot incidence caused by lack of homogeneity of the debris borne inoculum, the possibility of artificially multiplying the pathogen on a suitable substrate in the laboratory to obtain uniform cultures and use it as inoculum to screen for field tolerance was examined. This would also ensure continuous availability of the inoculum whenever required unlike in the case of naturally infected cane debris. Preliminary studies (Mohanraj and Padmanaban, 2006) indicated that the red rot pathogen multiplied on a mixture of sand and sorghum grains was suitable as inoculum. Profusely sporulating 10 day old cultures resulted in reasonably high and consistent levels of red rot in systematically planned experiments. The method could clearly bring out the differences in reaction to primary red rot incidence among different varieties. The studies also suggested the feasibility of screening sugarcane genotypes from the seedling stage in the breeding programme.

Some of the findings of a detailed study of field tolerance to red rot and screening for it in sugarcane undertook by Mohanraj (2010 & 2011) are discussed hereunder.

Suitable substrates to multiply the inoculum for testing field tolerance

Different media such as sand-maize medium, sand-oats medium, sand-sorghum medium, chopped sugarcane stalk tissue and chopped sugarcane stalk tissue mixed with sand were used to multiply the red rot pathogen for use as inoculum. Among these, sand-maize medium was found to be the most suitable for growth and sporulation, combining good pathogenicity in inducing primary red rot incidence. Although sugarcane stalk tissue was also relatively good, its continuous availability has to be ensured if it is considered a substrate for inoculum multiplication.

Effect of inoculum load on primary red rot infection and field tolerance

Since the quantum of inoculum applied can affect the expression of field tolerance, this aspect was studied by using different quantities of the pathogen multiplied on sand-maize medium to inoculate specific number of planted setts. The results showed that about 1-2 g of sand-maize multiplied inoculum per bud of planted cane was able to cause red rot levels in a susceptible variety, CoC 671 comparable to levels observed in naturally red rot infested fields

with a highly diseased preceding sugarcane crop. This corresponds to about 100 x 10^6 propagules of the pathogen per bud of planted cane derived at on the basis of earlier studies on the rate of sporulation of the red rot pathogen on different media. However, environmental conditions profoundly affected the relationship between inoculum load and red rot incidence levels in different cane varieties. Both during summer and monsoon conditions, the tolerant variety (Co 86032) showed only low levels of disease even with increase in inoculum load. However, the susceptible variety showed considerable increase in disease incidence with increase in inoculum level. Since cane setts with different numbers of buds are used for planting, standardization of the inoculum quantity as grams per bud of planted cane has been found to be appropriate.

Inoculum survival

For effective screening of field tolerance, it is important to determine the appropriate time of inoculation. Studies were carried out to study the duration for which the inoculum was effectively pathogenic and virulent either as infected cane debris or as laboratory multiplied pathogen culture to cause sufficient levels of disease. Both long-term and short-term experiments involving planting of setts of a susceptible cane variety at different intervals after inoculum application indicated that the highest level of red rot incidence occurred when the setts were planted immediately after inoculum application. Pathogenicity of the inoculum gradually declined and it was totally lost by about a month after application in the field. Inoculum as infected cane debris remained active for a slightly longer duration than the pathogen culture.

Inoculation of settlings

In addition to inoculation of planted setts, inoculation of young growing settlings was attempted to observe differences in red rot reaction among cane varieties. Inoculation of growing settlings by applying the inoculum on and around the setts during the early growth and tillering phase resulted in varietal differences in red rot incidence as indicated by the production of midrib lesions, total stalk symptoms and subsequent death of the plants. Symptom development was markedly enhanced by maintaining high soil moisture and humid conditions in the experimental fields. Observing the inoculated clumps for symptom development until maturity phase of the crop ensures effective identification of tolerant genotypes.

Identification of field tolerance

The most important symptom of red rot susceptible sugarcane genotypes in response to the sett inoculation procedure is total or high germination failure. Both pre- and post-emergence necrosis is common. In some cases, sprouted shoots subsequently dry up and die. The field tolerant clones are identified based on the proportion of infected clumps (%) compared to that in a highly susceptible variety such as CoC 671. When this index was used, Co 86032 was observed to show very low levels of red rot incidence while CoC 671 showed very high levels of disease incidence, even up to 100% mortality. Thus the criteria for identifying field tolerant clones using the grain method of inoculation are different from the scoring system followed in the plug and nodal methods of inoculation and screening for red rot resistance.

Nature of seed cane and differences in field tolerance

Seed setts of varying age from the same variety showed some variations in the expression of field tolerance when the inoculum was applied along with the setts at planting. Systematic field experiments showed that the top most setts of the cane were most susceptible while the setts from the bottom of the cane were least susceptible. Setts from the middle parts of the cane showed intermediate red rot reaction. It appears that the hardened bottom buds well protected by tough bud scales do not have easy access to the debris borne inoculum resulting in less disease expression. The findings emphasize the importance of using seed cane of uniform quality for reliable results in studies to screen for field tolerance.

Screening for field tolerance from the seedling stage

In the current cane improvement programmes, the first screening for red rot reaction is taken up at the Preliminary Zonal Varietal Trial (PZVT) stage after initial selection of the clones for good stand and quality by the plug method of inoculation. Those

which show susceptible or highly susceptible reaction are rejected and the resistant and moderately resistant clones are promoted to the next stage of evaluation. In the Zonal Varietal Trial (ZVT), clones showing moderate reaction are evaluated by the nodal method of red rot inoculation which is considered less severe and more natural. However, during the last decade or so it is observed that even with the relatively milder method of nodal inoculation, a large number of high sugar and high quality clones are not selected for field deployment in red rot endemic locations for want of resistance to the disease.

In this backdrop, the possibility of screening for field tolerance to the primary/debris borne infection from the early seedling stage in the crop improvement programme was examined. Germinating true seeds of sugarcane from different crosses involving commercial varieties as well as three month old seedlings obtained from crosses between genetic stocks were inoculated by applying red rot inoculum from sand-maize medium on the special pot mixture in pans in which the seedlings were grown. After about a fortnight, characteristic yellowing and drying symptoms appeared in the seedlings. Typical reddish purple or brown lesions developed near the base of the seedlings at soil level. Successful re-isolation of the pathogen from these lesions indicated positive colonization of the susceptible seedlings by the pathogen. With increase in duration after inoculation, more and more seedlings showed red rot symptoms until finally in each cross there was no more increase in the number of diseased plants indicating that all the surviving healthy seedlings were tolerant/ resistant to red rot by this method of inoculation. The disease free seedlings were clonally multiplied and retested for field tolerance by inoculating the setts with grain based inoculum at planting in the field. From the tolerant clones, those with good yield and quality could be selected under field conditions for possible future evaluation in the red rot endemic locations.

In another set of studies, seedlings of inter-specific hybrids and genetic stocks were evaluated by inoculating with grain based inoculum. Their re-evaluation in the clonal stage for field tolerance and yield and quality parameters resulted in the identification of many superior clones to be possibly used as genetic stocks. Thus, the seedling screening procedure indicated the potential to identify superior commercial varieties as well as genetic stocks with field tolerance which can serve as suitable parents in hybridization programmes. Based on these findings, a schematic schedule to screen for field tolerance to red rot from the early seedling stage to identify superior clones with field tolerance for commercial cultivation as well as genetic stocks has been proposed.

Field tolerance in combination with systemic fungicides

Although until recently, fungicidal control of red rot has not been considered practical, during the last decade some encouraging results have been obtained in reducing primary debris borne red rot infection by treating the seed setts with systemic fungicides before planting a susceptible variety such as CoC 671 (Malathi et al. 2004; Padmanaban and Mohanraj 2010). Effect of such sett treatment in a susceptible (CoC 671) and a field tolerant variety (Co 86032) was compared by field experiments. The results indicated that primary red rot incidence in response to inoculation with grain based inoculum was markedly reduced in the susceptible variety by sett treatment with systemic fungicide. Similar treatment resulted in very low levels of disease in the field tolerant variety (Co 86032) which by its very nature showed only low levels of disease even without the fungicidal treatment. Thus, systemic fungicidal sett treatment promises to very effectively manage primary red rot incidence when used in combination with a field tolerant cane variety.

Correspondence between evaluated field tolerance and natural red rot incidence

Five commercial sugarcane varieties were evaluated for red rot reaction by sett inoculation with grain based inoculum at planting in endemic locations. It was recorded that varieties which were susceptible to the grain method of inoculation showed high natural incidence of the disease while those identified as field tolerant were relatively free of red rot consistently.

A quantitative scoring system to rate field tolerance

Unlike with the plug and nodal methods where red rot reaction of genotypes is scored based on severity

of symptoms, field tolerance is best assessed based on the proportion of inoculated setts showing disease or mortality. When setts are planted in soil infested with the pathogen inoculum, individual nodal regions of the setts (point of entry) may or may not come into contact with the pathogen resulting in infection. However, infected buds will certainly be killed either during or after germination. This is similar to the field situation where the naturally available inoculum may or may not come into contact with the planted setts and it is different from the plug and nodal methods of inoculation where the inoculation procedures ensure good contact of the inoculum with the host. In systematically conducted studies to screen for field tolerance, the proportion of setts infected was found to be consistent and repeatable with regard to particular varieties.

The proportion of buds/setts infected in relation to the total number of buds/setts inoculated is considered to be a suitable index of red rot reaction in experiments involving the use of grain based inoculum. Since primary sett infection results in reduced clump population, it was realized that a limit up to which reduction in clump population will not result in significant yield and quality loss in the crop would be suitable to indicate field tolerance to red rot. It is well known that in sugarcane any initial reduction in clump population up to a certain level is usually compensated by enhancement of the yield and quality parameters of the surviving clumps so that the productivity of the crop is not negatively affected. Based on this fact and by superimposing the percentage disease incidence scale in response to grain based inoculation over the 0-9 scale of the plug method of the red rot evaluation, a scoring system has been suggested to identify superior cane genotypes or genetic stocks with field tolerance to red rot.

Screening for field tolerance under more ideal conditions

Just as any method of screening for disease resistance, the debris or grain method of red rot inoculation and evaluation is also profoundly influenced by environmental conditions. Field evaluation during high humidity monsoon conditions resulted in higher levels of disease than evaluation under hot dry conditions. This is expected since the disease is favoured by high soil moisture and atmospheric humidity. This problem was effectively overcome by carrying out the evaluations under controlled conditions such as a poly house/ glass house/ net house with provision for artificially increasing the humidity level of the micro-environment. Another important measure to reduce environment induced variability in disease reaction is to evaluate the test genotypes always along with standard susceptible and tolerant varieties and determining their reactions in comparison to those of the standard varieties.

Follow up with field testing

Screening a large number of recently developed sugarcane clones with grain based red rot inoculum both under field and controlled conditions has enabled the identification of several superior clones and genetic stocks which are consistently field tolerant to red rot. For the purpose of standardization the studies reported were carried out with a single pathotype (CoC 671) of the red rot pathogen. In order to obtain broad based and durable field tolerance, the genotypes identified have to be evaluated repeatedly in red rot endemic locations so that clones with field tolerance against multiple prevailing pathotypes can be identified and selected.

Conclusion

The investigations have demonstrated that by using standardized procedures and inoculating sugarcane setts at the time of planting with red rot inoculum, promising high yielding and high quality cane varieties with field tolerance to red rot can be identified. The various aspects of the methodology for such evaluation can be further fine tuned with more detailed and specific experimentations. With further improvements in biotechnological techniques and knowledge of molecular basis of field tolerance to red rot, further refinements to more effectively screen for field tolerance could be achieved. This would reduce the rejection of many high sugar and high yielding sugarcane genotypes because of their susceptibility to red rot evaluated by the plug and nodal methods of inoculation. Such increased availability of superior cultivars with field tolerance to red rot for deployment in red rot prone sugarcane areas would markedly enhance the production and productivity of the crop.

Acknowledgements

The authors gratefully thank Dr. N. Vijayan Nair, Director, Sugarcane Breeding Institute, for all facilities provided for the investigations.

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