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Ratoon stunting disease (*Leifsonia xyli*) of sugarcane

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Abstract

Sugarcane, being one of the most important cash crop of India, is being widely cultivated in several parts of the country. Like other crops of economic importance, sugarcane is also threatened by several diseases. Diseases caused by fungus, bacteria, viruses and phytoplasmas pose a serious threat to sugarcane cultivation which adversely affect the cane yield and sugar recovery in different sugarcane growing regions. Among the Bacterial diseases Ratoon Stunting Disease (RSD) results in severe losses in cane yield and quality attributes. RSD has been identified from different regions of the world using serological and molecular tools. In this article, information on occurrence, symtomatology, molecular characterization, transmission, and management approaches regarding RSD has been reviewed.

Keywords: Sugarcane, Ratoon Stunting Disease, *L. xyli*, Diagnosis, Management.

Introduction

Sugarcane is cultivated in about 4.2 million ha in tropical and subtropical parts of India. Being an important agricultural crop of the country, it is affected by several pathogens such as fungai, bacteria, viruses and phytoplasmas which are responsible for reducing the yield and quality of the crop (Tiwari et al., 2011). In the beginning of the 19th century, only few diseases were recognized as important ones. After rapid extension of sugarcane cultivation to different agroclimatic zones, monoculture of sugarcane, practices of ratooning over large areas and due to unrestricted seed movements many disease epiphytotics have occurred in different sugarcane regions (Alexander and Vishwanathan, 2002). About 100 diseases of sugarcane caused by fungi, bacteria, viruses and phytoplasmas have been reported from India and other countries (Rott et al., 2000). There are several known sugarcane viruses in India, *Sugarcane mosaic virus*, *Sorghum mosaic virus*, *Maize dwarf mosaic virus*, *Johnson Grass mosaic virus* (Shukla et al., 1989; Mckern et al., 1991), *Zea mosaic virus* (Seifers et al., 2000), *Cocksfoot streak virus* (Gotz and Maiss, 2002) and *Pennisetum mosaic virus* (Fan et al., 2003). Sugarcane Phytoplasma has reached at alarming in India. Grassy shoot disease (GSD), White leaf disease (WLD), Green grassy shoot disease (GGS) and Sugarcane yellow leaf phytoplasma (SCYLP) is affecting sugarcane (Tiwari et al., 2010 a; Tiwari et al., 2011). Among the fungal diseases red rot (*Colletotrichum falcatum* Went), Wilt (*Fusarium molniforme*), root rot (*Pythium graminicolum* Subr.), pineapple disease (*Thielaviopsis parodoxa* (de Seynes) C. Moreau), and smut (*Ustilago scitaminea* Syd.) causes severe losses to the sugarcane. Among bacterial diseases, gumming disease caused by *Xanthomonas vasculorum* Dows., leaf scald, caused by *Xanthomonas albilineans* Dows, and Ratoon stunting disease (RSD) caused by *Leifsonia xyli* have been the major constraints to sugarcane (Tiwari et al., 2010a). Among these, RSD is gaining international importance because of it's unspecific symptoms and losses.

Ratoon stunting disease

Ratoon stunting disease (RSD) was first detected in 1944 in the ratoon crop of Q 28, in Queensland (Australia) by Steindl. He coined the terminology ratoon stunting for the disease. Later on, it was noticed that the disease also affects the plant crop and, therefore, the terminology ratoon stunting is a misnomer in present day context, However, this terminology is still being used in phyto-pathological literature. The crop did not exhibit any discernible symptoms, but in general, showed loss of vigour and stunted growth. To date, the disease has been reported from Australia, USA, India, Brazil, Florida, China, Fiji, Philippines and South Africa (Antoine, 1958; Spaull and Bailey, 1993; Mayeux et al., 1998; Rao and Singh, 2000; Vishwnathan, 2001; Dela et al., 2002; Xu et al., 2008; Johnson and Tyagi, 2010). The disease flares up under drought conditions and the impact of the disease is more in the ratoon crop as compare to the plant crop (Agnihotri, 1990). The RSD pathogen seems to impair normal metabolic growth processes in the roots of infected canes which are, to some extent, checked by sulphur application. After having established the importance of sulphur in arresting the RSD pathogen, studies on physio-biochemical changes in RSD-affected sugarcane supplied with sulphur were under taken by Saxena et al. (1983). Their studies showed that tillering in RSD-affected sugarcane was delayed however, the adverse

effects were marked by the application of sulphur, as ammonium sulphate. The number of tillers per plant also increased significantly in the RSD-affected crop which showed beneficial effect of the element on tillering. The stunting effect of RSD pathogen was reduced by sulphur application and there was about 50% increase in the yield of the sugarcane in the RSD-affected crop, while in a healthy crop the increase was about 20%. This increase in the yield of RSD-affected crop by sulphur application was attributed to a favorable N:S ratio for the crop. The amount of chlorophyll also increased in the foliage of sulphur treated crop.

Status in India

In India the disease was first observed in CoS 510 at Golagokarannath (U.P.) by S.J.P. Chilton in 1956. RSD is now widely spread in the country and it caused appreciable losses in Bihar, few regions of Uttar Pradesh, Haryana, Punjab and Madhya Pradesh (Agnihotri, 1990; Agnihotri and Duttamajumder, 1992). The symptoms of RSD have been described in detail by Gillaspie and Devis (1992).
Occurrence of RSD in all the varieties grown in Punjab and Haryana was revealed by Sandhu et al. (1970). Poor germination of RSD infected buds of BO was attributed to the absence of acid invertase (Madan et al., 1986). Ratoon stunting disease was noticed in 87 genotypes of *S. officinarum*, 64 genotype of other clones at SBI, India (Rao, 1981). Rao (1982) reported clear symptoms on Co 281, Co 312, Co 356, Co 421, Co 449, Co 617, Co 740, Co849, Co 975, Co 997, Co 1148, Co 1158, Co 1287, Co1305, Q 28 and CP 52-68. Rao (1983) reported that RSD was more prominent in partially wilt affected canes. Significant differences in yield and quality upon RSD infection were observed in Co 213, Co 281, Co 997, CP 44-101 and CP 52-68 (Rao, 1984). Madan et al. (1982) reported that acid invertase activity was completely absent in RSD infected buds. The inhibition of acid invertase by RSD pathogen was probably due to a factor which either inhibited acid invertase directly or interfered with the synthesis of DNA dependent m- RNA synthesis, The factor is probably thermolabile and the inhibition is reversible, since MHAT of the infected sets circumvents the inhibitory effects of the causal agent on enzyme activity. The absence of acid invertase in the RSD affected buds was perhaps responsible for poor sprouting of buds (Madan et al., 1986). The region for low incidence of disease in tropical India as compared to north India needs critical investigation. Recent work on existence of this important pathogens has been conducted in several area of Uttar Pradesh and was confirmed through molecular analysis. The incidence of the disease was low, but significant in the drought condition

Symptoms

Symptomology of the RSD has not been clearly understood. Only identification of the infected host through indicator plants is possible. Ratoon stunting disease causes no characteristic external symptom. Because of the lack of readily recognized external symptoms, low to moderate reductions in plant growth caused by RSD may not be discernible during visual inspection of sugarcane fields. Even when growth is less than expected, loss may be attributed to other factors including soil type, fertility, weather, cultural or environmental stresses or other pathogens. Yield loss caused by RSD is the result of reduced stalk height and diameter and, sometimes, reduced numbers. Yield loss caused by RSD is enhanced by stress, particularly moisture stress (Gao et al., 2008; Comstock and Gilbert, 2009). The characteristics of *L. xyli* subsp. *xyli* that made it difficult to determine the etiology of RSD contribute to the problems of diagnosis today. Internal symptoms of diseased stalks may include a salmon pink discoloration just below the growing point of the young cane. An orange red discoloration of the vascular bundles may also be observed at the nodes of mature cane when they are split longitudinally (Gao et al., 2008). The discoloration does not extend into the internodal regions of the stalk. The presence of color, and intensity of the discoloration of the growing point and vascular bundles may vary with the stage of growth of the stalk and will differ among cultivars.

Transmission

Transmission of the pathogen from field to field or from one geographical area to another is by propagating cuttings from infected plants. Sugarcane is the only known natural host of *L. xyli* subsp. *xyli*. The xylem-limited bacterium is mechanically transmitted from infected plants to healthy plants in sap on tools and equipment during planting, harvesting, and cultivation of the crop. The RSD bacterium is transmitted through seed cane taken from diseased plants. Because symptoms of the disease are not readily visible, the bacterium may be spread unwillingly from one area to another. Stalks in potential seed fields can be randomly sampled and serologically assayed to determine RSD incidence. RSD can be readily transmitted by knives and mechanical harvesting machines that become contaminated with pathogen that is contained in juice of diseased stalks. Transmission by harvesting machinery is very significant. Cane chewing animals may be capable of transmitting the disease when they graze on a diseased stalk and then a healthy one. Not much is known about this means of transmission or its significance. There are new reports that the pathogen survives in the soil after harvest to re-infect healthy plants. The extent of infection by the pathogen surviving in the soil is not known (Comstock and Gilbert, 2009).

Diagnosis

Because of the lack of distinct or reliable visual symptoms, diagnosis is primarily by laboratory techniques. Diagnostic techniques include light and electron microscopy, culture of the bacterium, serological tests, host induced responses, and DNA-based methods.

a) Serological

L. xyli subsp. *xyli* is a small xylem-limited, coryneform bacterium that can be observed *in situ* by transmission electron micrography. Early TEM studies (Worley and Gillaspie, 1975; Kamiunten and Wakimoto, 1976; Weaver et al., 1977) were valuable in verifying the etiology of RSD and observing the infection process and the tissues of the host infected by the pathogen. Kao and Damann (1978) using scanning electron micrography observed the bacterium in the vessels, tracheids, parenchyma, and lacunea of the xylem. Xylem sap extracts can be examined by either phase-contrast (PCM) or dark-field microscopy (1000-1600X) for the presence of the pathogen (Gillaspie et al., 1973; Stendl, 1976; Davis and Dean, 1984; Amiet, 1985). A positive diagnosis depends on the diagnostician's ability to recognize the characteristic morphology of the bacterium and to detect the pathogen even when the titer of the organism is low. Culture

of *L. xyli* subsp. *xyli* is extremely difficult because of the fastidious nature of the bacterium. Davis et al. (1980) formulated a special medium for the axenic culture of the bacterium, but growth was slow. The development of the semi-selective medium has been important for the study of *L. xyli* subsp. *xyli*, but diagnosis by isolation of the pathogen is rarely used. Antisera was developed to the RSD bacterium by Gillaspie (1978). Other serological tests for the RSD bacterium have been developed including fluorescent-anitbody-direct-count-microscope (FADCS), fluorescent-antibody-direct-count-on-filters (FADCF) (Davis and Dean, 1984; Davis, 1985), tissue-blot enzyme immunoassay (TB-EIA) (Harrison and Davis, 1988), dot-blot immunoassay (DB-EIA) (Harrison and Davis, 1990), liquid transfer enzyme immunoassay (LT-EIA) and evaporative-binding immunoassay (EB-EIA) (Croft et al., 1994). For each of these protocols, purified immunoglobulin G (IgG) to *L. xyli* subsp. *xyli* was prepared from rabbit antiserum using whole cell suspensions of the bacterium. A host response is induced in stalks infected by *L. xyli* subsp. *xyli* when a cross section of mature basal internodes of sugarcane are treated with 0.1 M Tris (tris[hydroxymethyl]aminomethane) at pH 10 (Damann, 1988). The alkaline-induced metaxylem autofluorescence (AIMA) can be seen in the secondary walls of metaxylem cells as a red autofluorescence in infected plants but not in similarly treated healthy plants. Chung et al. (1994), used dot-blot hybridization with seven DNA probes specific for *L. xyli* subsp. *xyli*. A tissue blot DNA hybridization assay (TB-DHA) was developed by Pan et al. (1998a).

b) Molecular

PCR-based assays have been developed for the detection and identification of *L. xyli* subsp. *xyli* (Damann, 1992; Astua-Monge, 1995; Davis et al., 1998; Fegan et al., 1998; Pan et al., 1998b; Pan et al., 2001). Multiplex PCR for the simultaneous detection of *Lxx* and *Xa* (Fegan et al., 1998) were also developed. Davis et al. (1998) described a nested, multiplex PCR assay in which two reactions are performed sequentially with the amplification product of the first reaction serving as the target for the next reaction. Both the sensitivity and specificity of diagnosis can be enhanced by nested PCR. However, both multiplex PCR and nested PCR are more complex procedures and, therefore, are subject to more error if not carefully designed. In some situations such as sugarcane quarantine programs and international shipments of sugarcane germplasm, a single procedure to simultaneously test for PCR procedures developed for detection of both *Xa* and *Lxx* performed well for detecting these pathogens in sugarcane samples at or above population sizes (10 to 1,000 cfu/reaction) that are generally considered the lower limits of resolution of other diagnostic techniques, except for isolation of the pathogens (Davis et al., 1998). However, PCR provides an added level of specificity compared with isolation. Young et al. (2006) performed the phylogenetic analysis between *Lxx* and related genera. Monteiro et al. (2004) sequenced the whole genome of a Brazilian *Lxx* strain, CTCB07, which had a size of 2.6 Mb with 68% GC and containing 2,351 open reading frames, of which 307 were pseudo-genes. This work will provide a valuable database for the further characterization of genes involved in the interaction with sugarcane. On a broader level, molecular characterization of *Lxx* permits comparisons with other bacteria, especially related forms that may pose a future threat to the sugarcane industry.

Management

Control of RSD aims at reducing the incidence of the disease by ensuring only disease free plants are used for propagation, and reducing transmission by implementing hygienic cultivation and harvesting practices. This involves the three related components of heat therapy, farmer education and diagnostic services. Sugarcane can be effectively cured of RSD by heat therapy. The two main methods used are hot water at 50ºC for 2-3 hours (Johnson and Tyagi, 2010), or hot moist-air at 58ºC for 8 hours. However, there are some drawbacks associated with heat treatment. Depending on the variety, heat treatment can have either positive or negative effects on germination, often leading to inconsistent planting strikes. Use of thermotherapy to control the disease in India has been reported (Menon et al., 1971: Singh et al., 1973). Rao (1981) found that treatment with tetracycline-hydrochloride, polycide and tridemoform were not found effective against the pathogen in eliminating the disease from the cane and hot water treatment at 50^0C for 2 hrs was better in controlling the disease. Successful elimination of RSD through aerated steam therapy was achieved by Mayeux et al. (1998). As it is a bacterial disease, effective and economical control measure should be developed using antibiotics. There is also a need to study the losses caused by RSD in different agroclimatic regions of India. Suitable techniques should also be developed for quick screening of sugarcane genotypes. RSD is exceedingly prevalent in industries that have not implemented control strategies. Bailey and McFarlane (1999) reported RSD incidences approaching 100% in many central African industries where control measures were not practiced. Although heat-treatment and clean-seed schemes are acknowledged as having great value for the management of RSD, they are costly and labour-intensive for farmers, who will generally tend to use plants available on their properties. The role, therefore, of diagnostics is pivotal to management of RSD. The axenic isolation of *Lxx* (Davis et al., 1980) facilitated the development of immunological and molecular based diagnostics. The detection of *Lxx* in leaves is useful because it is nondestructive and may increase the rapidity of sample collection and diagnosis. For example, the application of either PCR or immunological assays on pooled leaf samples would facilitate greater coverage of the field and lower the incidence threshold for detection of the disease. Unfortunately, however, low relative sample sizes are an inescapable facet of RSD control. Under-diagnosis of RSD is undoubtedly widespread, and must surely lead to the persistence of a low level of incidence throughout sugarcane growing regions. Another approach for the elimination of this important pathogen is use of tissue culture techniques. A number of pathogens viz, Sugarcane viruses, Phytoplasmas have been eliminated through meristem culture and successfully healthy seed material has been produced (Parmessur et al., 2002; Mishra et al., 2010; Tiwari et al., 2008, 2010b, 2011). This approach could also be applied on RSD affected sugarcane for producing healthy seeds. Cryotherapy, thermotherapy and chemotherapy with the combination of tissue culture would also be helpful, as these techniques have been used in case of viruses in the other crops (Wang and Valkonen, 2007). Due to the possibility of reinfection, both heat therapy and disease-free plants produced from tissue culture must continually be produced to ensure disease-free seed cane for planting. So, there is a need to conduct awareness programme by the authorities at the farmer level for the disease identification and to use healthy and pure seed materials, avoiding the infected ones. There

should be strict management strategies for checking the further spread of the infected canes.

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