ASPECTS OF SUGARCANE RUST RESEARCH IN SOUTH AFRICA

By

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KEYWORDS: Puccinia melanocephala, Puccinia kuehnii, Strobilurin, Quantitative Resistance, Bru1.

Abstract

BROWN RUST (Puccinia melanocephala) has been present in South African sugarcane since 1941. Currently, a new unidentified rust pathogen is emerging which has been observed on a number of brown rust resistant cultivars. With orange rust (P. kuehnii) also present in Africa, it is a matter of time before the South African industry becomes challenged by three rust species. Since resistance to three rust pathogens in a single cultivar seems unlikely, effective fungicides have been identified. Additionally, to assist growers in the decision to use fungicides as well as the timing of application, simple linear models based on temperature and relative humidity are being developed. There has also been an increased focus on releasing brown rust resistant genotypes, which has resulted in most current cultivars containing major gene resistance. While the effects of major genes are large and detection is straightforward, they confer resistance to one race of a single pathogen and resistance may not be durable. We are now investigating quantitative resistance, which may provide partial resistance to multiple pathogen species when these have similar pathogenic strategies. Here we discuss the results of previous and current rust research in the context of developing strategies applicable to multiple rust pathogens.

Introduction

The two most widely occurring sugarcane rusts are brown rust, caused by Puccinia melanocephala, and orange rust, caused by Puccinia kuehnii. While brown rust was first reported in South Africa in 1941, orange rust has not yet appeared, although it is present elsewhere on the African continent. Because the arrival of orange rust seems imminent, the South African Sugarcane Research Institute has been active in creating awareness through various biosecurity initiatives in the southern African region.

African sugarcane rust

Partly as a result of increased awareness, an unusual rust was observed on cultivar N25 in Swaziland in 2008. Examination of urediospores indicated that the pathogen was neither P. melanocephala nor P. kuehnii which was confirmed by USDA-ARS researchers using species specific PCR. The disease appeared in South Africa in 2009 in Umfolozi. By 2011, it had spread to two other areas in South Africa, infecting a number of important cultivars.

Sequences spanning 1450 bp of the nuclear-encoded ribosomal RNA (rDNA), including a portion of the 5.8S subunit, the internal transcribed spacer region 2 (ITS-2) and the large subunit (28S) were generated using the primers Rust2Inv and LR6 (Aime, 2006). Appropriately referenced and accessioned sequences (Aime, 2006; Dixon et al., 2010) spanning the same rDNA region were obtained from Genbank representing 16 Puccinia species.
In a phylogenetic analysis using these data, African sugarcane rust separates from *P. melanocephala* and *P. kuehni* and clusters with *P. sparganioides* (Ash rust) and *P. physalidis* (host plants, *Physalis* species in the Solanaceae).

**Fungicides**

Whereas fungicidal rust control has not been routinely practised, a need has been identified with the appearance of African rust and the threat of orange rust. Initially product registration was sought for brown rust. Pre-screening of available fungicides resulted in the selection of two formulations for field trials.

In this trial, environmental conditions favoured brown rust development in the early stages of growth and drought progressively affected the later stages. Two applications of PYR+EPZ reduced brown rust infection by 85% and gave a 94% increase in yield (t/ha) (Figure 1).

Yield increases attributed to strobilurins have been observed in the absence of foliar diseases due to non-fungicidal physiological changes in wheat and barley.

Pyraclostrobin has been reported to increase chlorophyll content, photosynthetic rates, nitrogen use efficiency and stress tolerance, and to delay senescence (Venancio *et al.*, 2003).

Such changes may have contributed to the yield increases observed following treatment with strobilurins in sugarcane, particularly since a drought was experienced. Interestingly, in a second trial where rust incidence was very low and water stress was not evident, PYR+EPZ treatment increased yield by 10%.

The PYR+EPZ fungicide has received registration for use against brown rust and registration is being sought for African rust.
Rust risk models

Strobilurin fungicides are generally considered to be preventative products and are less effective in curing disease. To ensure that preventative fungicides are used timeously, forecasting models have been developed and used in management of many plant diseases (e.g. Moschini and Perez, 1999).

One of the most important factors influencing the outbreak and severity of foliar diseases is the duration of leaf wetness. A fully controlled dew chamber experiment was conducted to enhance our quantitative understanding of the effects of temperature and leaf wetness duration on rust caused by \textit{P. melanocephela}.

Results from this study (Ramouthar 2009; optimal temperature 20–25 °C, leaf wetness duration >9 hours, humidity >98%) were used to develop a tentative model (v1) to indicate periods of favourable conditions for rust infection. Validation field trials were conducted, in which rust incidence was monitored, leaf wetness conditions were recorded and weather conditions were recorded on nearby automatic weather stations (AWS).

Field trial results, together with model v1, were used to develop an amended model (v2), to predict periods of favourable conditions on the basis of leaf wetness and temperature conditions in the canopy, as well as a further amended model (v3), based on AWS data. These models are currently being refined and validated. Additionally, plots of an African rust susceptible cultivar have been planted adjacent to AWS sites.

Quantitative resistance

Anticipating the future presence of three rust pathogens, the possible use of quantitative resistance (QR), effective against multiple related pathogens, is being explored. The effects of individual QR genes can be small and phenotyping must be performed with precision if molecular markers are to be detected.

To this end whorl inoculation with brown rust spores was performed according to Sood et al. (2009) on a linkage disequilibrium (LD) mapping population. It was found that 88% of these genotypes were resistant, 8% were intermediate and only 4% were susceptible.

Furthermore, 76% of the resistant genotypes were found to contain the major rust resistance gene Brown rust 1 (Bru1) using methodology of Costet et al. (2012). Nevertheless, from an existing AFLP dataset, a number of putative markers associated with brown rust QR have been detected.

For future work a new LD population has been assembled having equal numbers of resistant, intermediate and susceptible genotypes, all of which are Bru1 negative. This population will also be screened with African rust.

Rapid phenotyping

Effective field screening depends on the presence of proper environmental conditions and is time-consuming and expensive. A method involving the direct inoculation of detached leaves has been developed to overcome these limitations.

Rather than relying on the assessment of visible symptoms, which can take up to 14 days, alternative methods for determining reaction phenotype are being explored. For example, chlorophyll fluorescence performance index (PI) has been found to be a sensitive indicator of leaf vitality in different crops. A major advantage of the PI lies in the fact that it can detect stress in plants even before visible symptoms appear (Christen et al., 2007).

By monitoring PI during the course of a detached leaf assay, resistant genotypes could be distinguished from intermediate and susceptible genotypes after only 4 days post inoculation (Figure 2). In a further development, preliminary results suggest that near infrared spectroscopy (NIRS) is capable of even greater discriminating ability, since all three categories could be separated. One reason for this might be that the NIRS calibration also takes constitutive resistance into account. This methodology will be extended to screening for African rust resistance.
Concluding remarks

A combination of marker assisted breeding for broadly effective QR, with rapid phenotyping of progeny is envisaged. However, the occasional release of high yielding cultivars of intermediate resistance remains likely.

Their protection using fungicides is a cost effective option, particularly if a plant physiological response also occurs. The ability to so respond is likely to be genotype dependent and this could be assessed across genotypes.

REFERENCES


INVESTIGATIONS ON THE OCCURRENCE OF YELLOW LEAF IN SUGARCANE PRODUCTION AREAS OF GUANGXI

By

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KEYWORDS: Sugarcane yellow leaf virus, Seed Cane, RT-PCR.

Abstract

THE PRESENT STUDY was conducted to determine the occurrence of Sugarcane yellow leaf virus (SCYLV) in sugarcane growing areas, and to provide a scientific basis for healthy seed cane production in Guangxi, China. Leaf samples with SCYLV symptoms at different stages were collected from different sugarcane fields of Guangxi. The virus was detected using the one-step RT-PCR method with SCYLV specific primers, and its amplified cDNA products were sequenced. The survey of SCYLV showed that 42 samples were positive by RT-PCR from a total of 84 samples. The infected cultivars accounted for 50% of the total number of cultivars. The crop was highly infected by SCYLV in northern Guangxi including Liuzhou, Liucheng, Yizhou and Luzhai as 19 sugarcane cultivars were found infected among the 25 tested (76%). The susceptible varieties followed the pattern: Liucheng 03-182 > GT21 > YT55 > YT93-159. The major commercial variety ROC22 was also found to be affected by SCYLV in 3 state farms, the most important sugarcane area in Guangxi. It is concluded that SCYLV has been building-up and spreading in sugarcane areas of Guangxi.

Introduction

Sugarcane yellow leaf virus (SCYLV) is regarded as the causal agent of yellow leaf and it belongs to the genus Polerovirus, family Luteoviridae (van Regenmortel et al., 2000). The major symptom of the disease is an intense yellowing of the midrib on mature leaves, and this discoloration subsequently spreads to the leaf blade, proceeding from the tip toward the base of the leaf, and tissue necrosis can be observed eventually.

The disease also leads to a reduction in sucrose content in stalks, accumulation of sucrose in leaf midribs, and causes significant yield loss (Grisham, 2001, 2002; Rassaby et al., 2003).

The aim of our study was to conduct a survey to find out the occurrence of yellow leaf in Guangxi, China by detecting SCYLV using reverse transcription–polymerase chain reaction (RT-PCR), and to provide a scientific basis for the need to produce healthy seed cane in Guangxi, China.

Materials and methods

Plant material and RT-PCR Detection of SCYLV

Symptomatic and asymptomatic leaves were collected from commercial fields of 15 sugarcane planting locations in Guangxi from August to December in 2011. Total RNA was extracted and purified from leaves with the RNeasy Plant Mini Kit(Qiagen) according to the manufacturer’s protocol.
The total RNA prepared from leaf tissue was prepared to first strand cDNA synthesis with Revert Aid™ First Strand cDNA Synthesis Kit following the manufacturer’s instructions (Fermentas Life Sciences). The virus-specific primers (Xu Dong-lin et al., 2005) P1: 5′-aatcagtgcacacatccgag-3′ and P2: 5′-ggagcgtcgcctacctatt-3′ were used to detect SCYLV in the PCR assay.

Results

Detection and survey of SCYLV

RT-PCR amplification of SCYLV with primers P1/P2 resulted in a major product of ≈630 bp, which contained an open reading frame of 591 bp. Taking nucleotide sequence of SCYLV 3 as an example to blast with other published SCYLV sequences submitted in NCBI, the nucleotide sequence of the RT-PCR product was found to be 99% identical to sequences of the isolates from China (including Guangxi, Guangdong, Yunnan, Fujian), United States, Brazil, Argentina, Germany, Guatemala, Peru, Colombia et al.

The results indicated that the CP gene of SCYLV is amplified successfully and samples displayed positive by RT-PCR are infected by SCYLV (Figure 1).

![RT-PCR detection of SCYLV in sugarcane leaves collected from fields.](image)

Fig. 1—RT-PCR detection of SCYLV in sugarcane leaves collected from fields. M: Marker DL 2000, 1: healthy leaf, 2: negative control, 3-8: sugarcane leaf samples collected from fields.

The survey of SCYLV results showed that 42 samples were positive by RT-PCR detection from a total of 84 samples. Infected cultivars made up 50% of the total number of cultivars.

The sugarcane crop was extensively infected by SCYLV in northern Guangxi including Liuzhou, Liucheng, Yizhou and Luzhai as 19 sugarcane cultivars were found infected among the 25 tested, which accounted for 76%.

The infection was the most serious in Liucheng, where 100% of cultivars were infected by SCYLV. It was found that 50% of sugarcane cultivars were infected by SCYLV in western Guangxi, 28.2% in southern Guangxi, and 16.6% in eastern Guangxi. The results also showed that SCYLV was variety-dependent as the variety of Liucheng 03-182 was highly susceptible. It is grown in eight locations but was found positive in five places. Cultivar Guitang21 was detected positive in three of five cane growing locations. Yuetang55 and Yuetang93-159 both were infected by SCYLV in two out of four areas where it is planted. The susceptible varieties followed the pattern: Liucheng 03-182 > GT21 > YT55 > YT93-159.

The major commercial sugarcane variety of ROC22 was also found to be affected by SCYLV in Liucheng, Yizhou and Jinguang state farm, the major sugarcane area in Guangxi.

In conclusion, SCYLV was of common occurrence in cane growing areas of Guangxi. It appeared to have built-up and has been spreading.
REFERENCES
GENETIC DIVERSITY IN THE POPULATION OF \textit{Fusarium proliferatum} CAUSING KNIFE – CUT DISEASE IN SUGARCANE IN KHUZESTAN USING VEGETATIVE COMPATIBILITY GROUPS AND PATHOGENICITY

By

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KEYWORDS: Pokkah Boeng, Nitrate Non-Utilising Mutants.

Abstract
DURING THE PERIOD of 2005–2006, 81 isolates of \textit{Fusarium proliferatum}, a causal agent of knife-cut disease of sugarcane in Khuzestan province, Iran, were examined for their pathogenicity and vegetative compatibility groups (VCGs). All isolates were found to be pathogenic on sugarcane. In population studies, nit mutants were generated from all isolates using PDC medium containing 3% and 5% KClO₃ and Czapeck medium containing 3% KClO₃. In total, 497 nit mutants were obtained. The phenotype of the isolates was determined based on their growth on basal medium containing one of the following salts: sodium nitrate, sodium nitrite, hypoxanthine and ammonium tartrate as a sole carbon source. It was found that 45%, 19% and 35% of \textit{Fusarium proliferatum} were respectively nit1, nit3 and NitM. Complementation of the nit mutants carried out on minimal medium revealed that sugarcane isolates belonged to 27 vegetative compatibility groups. No vegetative self-incompatibility was found. No specific relation was found between VCGs and pathogenicity level of the isolates. Moreover, no specific relation was found between VCGs and geographic origin of the isolates, although some of the VCGs were found only in some areas, but some were common in different sites.

Introduction
In the lower internodes, some scars similar to knife cuts were observed in one or both sides of the stem that caused it to bend. The causal agent of these symptoms was the same as pokkah boeng in shoots. The disease termed ‘knife-cut’ (pokkah boeng) is caused by the fungus \textit{F. proliferatum}. Pokkah boeng is common in most, if not all, sugarcane-producing areas of the world (Whittle and Irawan, 2000). Knife-cut rarely causes serious yield losses in commercial plantings. Reported outbreaks of the disease, while looking spectacular, have caused small economic losses (Whittle and Irawan, 2000). In Iran, sugarcane is mainly grown in Khuzestan province and the incidence of knife-cut has been reported in sugarcane fields all over Iran.

The genetic diversity of \textit{F. proliferatum} populations has been investigated in other crops such as asparagus by VCGs (Elmer, 1991). The formation of heterokaryons between different strains is an important and common component of the life cycle of many filamentous fungi.

Lineages that are capable of fusing (anastomosis) and forming stable and functional heterokaryons are known as sexually or vegetatively compatible, the former being frequently described as members of the same group of vegetative compatibility or vegetative compatibility group (VCG) (Leslie, 1993).
VCG analysis provides an identification tool and a way to assess genetic variability in the *Fusarium* spp. population. In addition, it increases our understanding of the population biology of this genus. Data on the morphological characteristics, pathogenicity test in sugarcane and information on VCGs can be integrated to correctly identify the *Fusarium* species causing the knife-cut disease.

Studies of the genetic diversity of *F. proliferatum* from sugarcane have recently been conducted and this paper provides the first research report on the diversity of *F. proliferatum* isolated from sugarcane fields using the vegetative compatibility group technique.

**Materials and Methods**

Plants showing knife-cut symptoms were randomly sampled from sugarcane fields in Iran from 2005–2006. Each sample was disinfected and cultured on Nash and Snyder selective medium (Jo *et al.*, 2008). *Fusarium* colonies were identified based on colony morphology and fungal characteristics according to Leslie and Summer Ell, 2006.

Pathogenicity tests were performed as follows: a wound was made on the stalk using a sterilised cork borer and a PDA agar plug from a 5-day old culture of *F. proliferatum* was placed in the wound. The inoculated portion was wrapped in parafilm. A blank PDA plug was used as control. The wrap was removed from the stalk two weeks after inoculation. Plants were monitored for the development of disease symptoms and isolations were carried out to confirm pathogenicity in inoculated stalks.

Vegetative compatibilities: (VCGs) were determined using the complementation of nitrate non-utilising (nit) mutants as a visual indicator of heterokaryon formation (Jo *et al.*, 2008). Nit mutants were generated from each of 81 *F. proliferatum* isolates on PDA, minimal medium (MM) and Czapeck agar amended with 1.5%, 3% and 5% potassium chlorate, respectively (Puhalla, 1985; Klittich and Leslie, 1988).

The fast-growing, chlorate-resistant sectors originating from the initially restricted colony, which grew thinly but expansively on Puhalla's minimal medium (Puhalla, 1985), were considered nit mutants. Nit mutants were phenotypically classified by their growth on basal medium (MM without NaNO₃) amended with one of several nitrogen sources (Pasquali *et al.*, 2005). Several nitl, nit3 and nitM mutants from all isolates were stored in sterile distilled water at 4 °C.

Before complementation tests among isolates, vegetative self-incompatibility of each isolate was examined (Pasquali *et al.*, 2005). Vegetatively compatible isolates were recognised by the robust growth at the interface of the two colonies after 10 days (Klittich and Leslie, 1988).

**Results and discussion**

Two hundred and seventy isolates in *Fusarium* section Liseola were recovered from sugarcane stalks infected by knife-cut disease including 81 isolates of *F. proliferatum*. The latter was identified based on the characteristics of macroconidia, phialides, microconidia, chlamydospores and colony growth traits.

The results showed that all *F. proliferatum* isolates were pathogenic to sugarcane. The fungus produced chlorate-resistant sectors on media complemented with chlorate. Large differences in sectoring frequency occurred between isolates. The majority of the chlorate-resistant isolates recovered were unable to utilise nitrate as the sole nitrogen source and consequently grew as thin expansive colonies without aerial mycelium on MM. However, a few chlorate-resistant sectors were able to utilise nitrate. The frequency of resistant sectoring was different when different concentrations of chlorate were supplemented. The phenotypes of 497 nit mutants were determined by their colony morphology on media containing nitrate, nitrite, hypoxanthine, uric acid, or ammonium tartrate as a sole nitrogen source. The nit mutants were divided into three classes; nit1, nit3 and nitM. Among the tested isolates, 225 (45%), 98 (19%) and 174 (35%), nit1, nit3 and nitM were generated, respectively. According to Puhalla (1985), the frequency of nit1 mutants is higher than the frequency of the other types of nit mutants.
The segregation of *F. proliferatum* isolates obtained from sugarcane into VCGs in Iran is reported for the first time. We identified 27 VCGs among 81 isolates. These results are in agreement with results from previous studies on *F. proliferatum*, demonstrating that this fungus is genotypically highly diverse (Elmer, 1991). In this study no relation was observed between pathogenicity and VCGs.

The differences in pathogenicity between the members of a VCG showed that these differences were because of mutations in locuses related to pathogenicity of *F. proliferatum*. In the case of telemorph, meiosis, recombination and crossing over are reasons for these mutations.

In some areas, VCGs were specific to the area and were not observed elsewhere. Thus the geographical region of an isolate could be predicted by these VCGs. On the other hand, some areas, in addition to specific VCGs, shared VCGs with other regions. A specific pattern between VCG distribution and geographic region of isolates was not observed.

Genetic similarity among isolates with respect to areas is possibly due to distribution of setts infected by *F. proliferatum* in the past years. Further studies on VCGs of this species in sugarcane in various countries would provide information about genetic diversity in the population of *F. proliferatum* that could be applied in its management.

**REFERENCES**


FIRST REPORT OF LEIFSONIA XYLI SUBSP. XYLI, CAUSAL AGENT OF RATOON STUNT OF SUGARCANE IN IRAN

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KEYWORDS: RSD, Isolation, Molecular Identification.

Abstract

RATOON STUNT (RSD) is often cited as the most important sugarcane disease in the world. For determining the incidence of RSD in Iran, ten mature stalks of 60 cultivars were collected randomly. Functional and non-functional vessels of each stalk were determined by staining following the Stalk Transpiration Method (STM). Among the 60 cultivars sampled, the percentage of non-functional vessels resulting from colonisation by the bacterium ranged between 11% in CP48-103 to 69% in NCo293. For isolating the bacterium, a modified sugarcane (M-SC) axenic medium was used. Colonies were observed after two weeks. The colonies were 0.1–0.3 mm in diameter, circular with entire margins, convex and non-pigmented. Taking into account the morphological and biochemical characteristics, the bacterium was identified as Leifsonia xyli subsp. xyli (Lxx). The isolate from cultivar CP50-28 was named Lxx-Iran. Further identification of the bacterium was confirmed using polymerase chain reaction (PCR) with samples from STM-positive stalks of cv. CP48-103, CP63-588, CP50-28 and CP45-3 and Lxx-Iran lysate and Lxx-Iran non-lysate samples. Primers used for PCR were Cxx1 and Cxx2 and the thermocycler parameters were as follows: denaturation at 94°C for 5 min, 30 cycles at 95°C for 1 min, 57°C for 1 min, 72°C for 30 s, and a final extension at 72°C for 10 min. The PCR product (437 bp) of each sample was sequenced and analysed. It showed 98–99% identity with the 16S-23S intergenic spacer region of Lxx, thus confirming the occurrence of ratoon stunt in Iran. This is the first report of the disease in Iran.

Yield losses from ratoon stunt vary, depending on host tolerance and environment. It may cause up to 50% yield losses in susceptible cultivars under drought and water logging conditions. The pathogen is extremely fastidious in its nutritional requirements and can only be grown in axenic culture on special media as the medium (Davis et al., 1980; Croft et al., 1993).

This study was conducted on commercial sugarcane cultivars in Khuzestan province in the southwest of Iran. In these areas, sugarcane is grown at latitude of 31°–32° N and longitude 48° E. Daily average maximum temperature in July is 45 °C with a maximum of 53 °C and average minimum in January is 5.1 °C with a minimum of –10 °C.

The annual average rainfall is about 280 mm and evaporation rate is 3000 mm. The relative humidity depends upon the field location and ranges between 30–38% in the north and 10–60% in the south of the province where sugarcane is cultivated.

Materials and methods

Introduction

Ratoon stunt (RSD) caused by the bacterium Leifsonia xyli subsp. xyli (Evtushenko et al., 2000) formerly named Clavibacter xyli subsp. xyli (Davis et al., 1984) infects the xylem vessels of sugarcane. Since the disease was first discovered in Queensland, Australia in 1944–45 in the cultivar Q28 (Steindl, 1961), it was then reported from other countries. Diseased plants do not show external symptoms.

Internal symptoms may include a salmon pink discoloration just below the growing point of young cane of susceptible cultivars and a yellow, orange, pink and red to reddish-brown discoloration of the vascular bundles in the shape of dots, commas and short lines near the lower part of the node of the mature stalks when split longitudinally.

The pathogen is readily transmitted mechanically to cuttings or to stubble cut by contaminated harvest equipment (Davis and Bailey, 2000). Planting infected seed cane also spreads RSD. The disease impedes or limits circulation of water and nutrients, toward the leaves.
Surveys were conducted in sugarcane fields of 10 Agro-Industry Companies (north and south of Khuzestan province) to investigate the presence of ratoon stunt in commercial cultivars and three germplasm museums.

For evaluating incidence of RSD, ten mature stalks of 60 cultivars at 9–12 months age were collected randomly. Functional and non-functional vessels of each of them were determined by staining following the Stalk Transpiration Method (STM) (Chagas and Tokeshi, 1994). For isolating the bacterium, a modified sugarcane (M-SC) axenic medium was used (Croft et al., 1993).

Further identification of the bacterium was confirmed using polymerase chain reaction (PCR) reported by Pan et al. (1998) with samples from STM-positive stalks of cv. CP48-103, CP63-588, CP50-28 and CP45-3 and Lxx-Iran lysate and Lxx-Iran non-lysate samples.

Primers used for PCR were Cxx1 (5’CCGAAGTGAGCAGATTGACC-3’) and Cxx2 (5’-ACCCTGTGTGTTTTTCAACG-3’).

The reactions contained 50 ng template DNA, 0.2 µM of each dNTP (dATP, dTTP, dGTP, dCTP), 0.5 µM each primer, 1 U of Ex-Taq DNA polymerase, Taq DNA Ext-10x buffer, 20 µM MgCl2 in 25 µL. Total volume was adjusted with sterile distilled water. The above components were obtained from Iran Co. Cinagene.

The thermocycler parameters were as follows: denaturation at 94 °C for 5 min, 30 cycles at 95°C for 1 min, 57 °C for 1 min, 72 °C for 30 s, and a final extension at 72 °C for 10 min. The PCR product (437 bp) of each sample was sequenced and analysed by BLAST (Altschul et al., 1990) with the available DNA sequences in the web site www.ncbi.nlm.nih.gov/NCBI/.

Results and discussion

Among the 60 cultivars sampled, the percentage of non-functional vessels due to plugging of xylem vessels resulting from colonisation by the bacteria ranged between 11% in CP48-103 to 69% in NCo293. For isolating the bacterium, a modified sugarcane (M-SC) axenic medium was used. Colonies were observed after two weeks following aerobic incubation at 28°C.

The colonies were 0.1–0.3 mm in diameter, circular with entire margins, convex and non-pigmented. The bacteria are aerobic, gram positive, non-motile, non-spore forming, non- acid-fast, oxidase-negative and catalase-positive.

Taking into account the morphological and biochemical characteristics, the bacterium was identified as Leifsonia xyli subsp. xyli (Davis et al., 1984). The isolate obtained from cultivar CP50-28 was named Lxx-Iran.

The PCR product (437 bp) of each sample showed 98–99% identity with the 16S-23S intergenic spacer region of L. xyli subsp. xyli, thus confirming occurrence of ratoon stunt in Iran.

Similarly, Gao et al., (2008) explained that the isolate Lxx-Fuzhou China (EU723209) showed 100% identity with the isolates AE016822 (Monteiro Vitorello et al., 2004), AF034641 (Fegan et al., 1998) and DQ232616 (Young et al., 2006) and 99% identity with the isolate AF056003 (Pan et al., 1998). Falloon et al. (2006) reported the isolate Lxx of Jamaica showed 99 to 100% identity with other isolates of Leifsonia xyli subsp. xyli existing in the International Gene Bank.

REFERENCES


PATHOGENICITY OF *Fusarium proliferatum*, A NEW CAUSAL AGENT OF POKKAH BOENG IN SUGARCANE

By

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KEYWORDS: *Fusarium verticillioides*, *F. moniliforme*, *F. subglutinans*, *F. semitectum*, *F. proliferatum*.

Abstract

POKKAH BOENG IS A JAVANESE term denoting a malformed or distorted top. A knife-cut symptom is also associated with pokkah boeng. During 1993–2001, samples were collected from infected stalks showing ladder-like lesions near the growing point. All samples were thoroughly washed in tap water and disinfected with 70% ethanol. After rinsing with distilled water, small pieces of infected tissues were plated on modified Nash & Snyder and potato dextrose agar (PDA) media and incubated at 25°C for 10 to 14 days. PDA, carnation leaf agar (CLA), sucrose nutrient agar (SNA) and KCl agar media were used for identification of isolates. They categorised four *Fusarium* species identified as *Fusarium verticillioides* (*F. moniliforme*), *F. proliferatum*, *F. subglutinans* and *F. semitectum* with 55%, 21.5%, 17.6% and 5.9% frequency, respectively. Pathogenicity studies with these fungi indicated that all isolates were pathogenic to sugarcane except isolates of *F. semitectum*. Among pathogenic isolates, *F. proliferatum* was less pathogenic than *F. verticillioides* and *F. subglutinans*. This is the first report of the pathogenicity of *F. proliferatum* on sugarcane in the world.

Introduction

Pokkah boeng is one of the most common sugarcane diseases. It is caused by the *Fusarium verticillioides* (Sacc.) Nirenberg, (1976) formerly named *F. moniliforme* Sheldon (teleom. Gibberella fujikuroi (Sawada) Wollenw) and *F. subglutinans* (Wollenw. & Reinking) Nelson, Toussoun & Marasas (teleom. Gibberella subglutinans (Edwards) Nelson, Toussoun and Marasas).

Pokkah boeng, a Javanese term denoting a malformed or distorted top, was originally described by Wakker and Went (1896) in Java, but no causal agent was established. Bolle (1927) was the first to demonstrate that the disease is caused by a fungus, identified as *Fusarium moniliforme* Sheldon (Martin et al., 1989).

The knife-cut symptom is also an abnormality sometimes associated with pokkah boeng (Figure 1).
Pokkah boeng is potentially damaging in highly susceptible cultivars. This research was conducted on commercial sugarcane cultivars in Khuzestan province in the south west of Iran. In this region, sugarcane is grown at latitude 31°–32° N and longitude 48° E.

Daily average maximum temperature in July is 45°C with a maximum of 53°C and average minimum in January is 5.1°C with a minimum of –10°C. The annual average rainfall is around 280 mm and evaporation rate is 3000 mm. The relative humidity depends on field location and could range between 30–38% in the north and 10–60% in the south of the province.

Materials and methods

Surveys were conducted in sugarcane fields of Karun, Haft-Tappeh and Imam Khomeini Agro-industry Companies (North of Khuzestan province) to determine the pathogen associated with pokkah boeng in commercial cultivars CP57-614, CP48-103, CP69-1062 and NCo310.

For isolating *Fusarium* species, samples were collected from infected stalks. Small pieces of ladder-like lesions near the growing point and knife-cut lesions from lower internodes were disinfected with 70% ethanol and washed with sterile distilled water. They were then plated on potato dextrose agar (PDA) and modified Nash & Snyder media and incubated at 25°C for 1 week. Single-spore method was used for each isolate (Nelson *et al*., 1984).

Morphology of single conidia on PDA medium was studied after 10–14 days incubation at alternating day and night temperatures of 25°C/20°C and a 12 h photoperiod. Diagnostic characters were followed on carnation leaf agar (CLA), sucrose nutrient agar (SNA) and KCl media after incubation for 1–2 weeks under the same temperatures and photoperiod as above. For production of sporodochium, cultures were incubated in light from fluorescent tubes (F4055/fluorescent) or in diffuse daylight from a North window (Nelson *et al*., 1984).

Two experiments were conducted to determine pathogenicity of *Fusarium* species. Detached stems (10 3-eye cuttings) in the laboratory and five mature stalks of 9-month cultivar CP57-614 growing in the pots were inoculated between two nodes with a 7-day old fungus mycelial disk (Koike, 1978). The surface of the cutting was washed thoroughly with tap water and sterilised with 70% ethanol. A cork borer method was used for inoculation.

The inoculated internodes were covered with cotton and parafilm. The cuttings were incubated at 25–27°C with RH > 90. At the end of the 10-day incubation period, the cuttings were split longitudinally and lesions inside the stem tissue were examined.

The mature stalks growing in pots were inoculated using the same method as above in midsummer. The stalks were split longitudinally and were evaluated after 40 days. Symptoms were noted. Koch’s postulates were conducted to determine the causal agent.
Results and discussion

Isolates of *Fusarium* were obtained from the ladder-like lesions and knife-cut symptoms. Based on colony morphology and diagnostic characteristics, the *Fusarium* isolates were categorised into four species (Nelson et al., 1984) and identified as:

**Fusarium verticillioides (F. moniliforme) (Sacc.) Nirenberg**

The white aerial mycelium grew rapidly and often became tinged with purple on PDA. Macroconidia were produced in pale orange sporodochia, which may be obscured by the mycelium and rarely from phialides on hyphae. Macroconidia were long, slender, and falcate to almost straight with the dorsal and ventral surfaces almost parallel, in delicate walls. The basal cell was foot-shaped (Figure 2a).

Microconidia were abundant and primarily single-celled, oval to club-shaped with a flattened base. They were formed in long chains (Figure 2b). Conidiophores were unbranched and branched monophialides (Figure 2c). Chlamydospores were absent.

**Fusarium proliferatum (Matsushima) Nirenberg**

The white aerial mycelium grew rapidly and was sometimes tinged with purple on PDA. Macroconidia were produced in tan to orange sporodochia and rarely from phialides on hyphae. Macroconidia were only slightly sickle-shaped to almost straight, with the dorsal and ventral surfaces parallel for most of the length of the macroconidium. The walls were thin and delicate and the basal cell was foot-shaped (Figure 3a). Microconidia were abundant, usually single-celled or club shaped with a flattened base. Pear-shaped microconidia may also occur but generally were rare. Microconidia were borne in chains of varying length (Figure 3b). Conidiophores were unbranched and branched polyphialides and monophialides (Figure 3c). Chlamydospores were absent.
**Fusarium subglutinans** (Wollenw. & Reinking) Nelson, Toussoun & Marasa

Colony morphology and macroconidia were similar to those produced by *F. moniliforme*. However, the microconidia were produced only in false heads, oval and usually single-celled, but may be 1–3 septate. Macroconidia were abundant, only slightly sickle-shaped to almost straight with the dorsal and ventral surfaces almost parallel, and with thin, delicate walls.

The basal cell was foot-shaped (Figure 4a). Conidiophores were unbranched and branched polyphialides and monophialides (Figure 4b).

The main differences between *F. subglutinans* and *F. moniliforme* were the absence of microconidal chains and the presence of polyphialides in *F. subglutinans*.

![Fig. 4 (a)—Macroconidia, (b) Monophialides and polyphialides of F. subglutinans (original).](image)

**Fusarium semitectum** (Fr.) Sacc. [& R,G,B,J] W

Dense aerial mycelium grew rapidly and often became tan to brown on PDA. Microconidia may be of two types. Those borne in the aerial mycelium were spindle-shaped straight to slightly curved, and the basal cell had a papilla but was not foot-shaped (Figure 5a).

The majority of the sickle-shaped macroconidia were borne from sporodochia, in those clones that produced them.

These macroconidia were slightly curved, with a foot-shaped basal cell. Conidiophores were unbranched and branched monophialides and polyphialides (Figure 5b). Chlamydospores were present (Figure 5c).

![Fig. 5 (a)—Mesoconidia, (b) mono and polyphialid and (c) chlamydospore of F. semitectum (original).](image)

Results of pathogenicity tests with cuttings under lab conditions indicated that, excluding *F. semitectum*, the rest were pathogenic to sugarcane, but *F. proliferatum* caused less extensive reddish-purple discoloration than *F. verticillioides* and *F. subglutinans* (Figure 6a).

Observation of pathogenicity tests with *F. proliferatum* on mature stalks confirmed the above results, such that inoculated stalks showed knife-cut symptom after 40 days (Figure 6b).

In both experiments, the pathogenic *Fusarium* spp. was re-isolated from the discoloured nodal or internodal tissues of the inoculated stalks or seed pieces. Neither pathogen was isolated from tissue of the controls.

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Pokkah boeng affected young cane growing vigorously under warm, humid conditions. Most cultivars recovered from the disease as the plants mature (Koike, 1984). Cultivar CP57-614 was susceptible to pokkah boeng and the disease may be observed from November to December in young plant cane.

Knife-cut symptoms were observed without malformed or damaged tops on sugarcane in the Khuzestan province. The disease is generally considered to be of minor economic importance in Iran.

REFERENCES


OUTBREAK OF RED SPOT OF THE LEAF SHEATH IN SUGARCANE IN SÃO PAULO STATE

By

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KEYWORDS: Saccharum spp., Passalora vaginae, Mycovellosiella vaginae, Cercospora vaginae.

Abstract

The disease known as red spot of the leaf sheath of sugarcane (Saccharum spp., interspecific hybrids), caused by the fungus Passalora vaginae, was previously reported in the states of São Paulo and Pernambuco, Brazil, in 1930, showing no significant losses for the crop at that time. The fruiting of the fungus is black, effuse and usually occurs in the centre of the lesions. Conidia are hyaline to diffuse olivaceous, cylindrical to obclavate-cylindrical, with zero to five septate, thickened hilum and dimensions ranging from 15–55 × 3–6.5 µm. Symptoms of premature dryness and breakage of leaves, purple-red spots associated to necrotic lesions in the sheath, were observed in sugarcane crops in the counties of Ribeirão Preto, Piracicaba, and Caçapava, state of São Paulo, Brazil, in September 2011 and March 2012. Samples of infected sugarcane from these areas were collected and assayed according to regular lab procedures. The direct analysis of tissues and BDA isolation pinpointed the fungus P. vaginae as the causal agent of the symptoms. The incidence of the disease in some currently planted sugarcane varieties has increased in the 2012 crop and expanded to other areas. Among the factors that may have favoured the high incidence and severity of the disease in the crop 2011–2012, were: rainfall above 500 mm 7 months after planting, high water deficit 10 months after planting, and harvesting of sugarcane without burning in the last 8 crops.

Introduction

Sugarcane is one of the major Brazilian agricultural crops since the early colonisation by the Portuguese. The crop enables Brazil to produce half the sugar trade worldwide, and is the raw material for ethanol production, a biofuel used in light vehicles that contributes decisively to make the Brazilian energy matrix one of the world's most renewable, by reducing carbon emissions, and promoting less generation of particulate pollutants (MAPA, 2012).

According to Tokeshi (s.d.) and Nass et al. (2001), among the phytosanitary problems that affect the production of sugarcane, red spot of the leaf sheath, caused by the fungus Passalora vaginae (W. Krüger) U. Brown & Crous 2003, had always been considered of secondary importance, showing no significant losses at that time. Previous occurrences in the states of São Paulo and Pernambuco did not cause significant economic damage to the affected crops.

However, between September 2011 and March 2012, samples were collected from sugarcane crops in the regions of Piracicaba, Ribeirão Preto, and Caçapava in the state of São Paulo, with severe symptoms that resembled the red spot of the sheath. In the field, the disease was initially characterised by the formation of reds spots with undefined rounded borders (Figure 1).
The lesions expand and surround the whole sheath, becoming dark red in the centre with reddish borders. The disease caused premature dryness and breakage of leaves, with significant yield losses. This study aimed to establish the etiology of the disease.

Materials and methods
Sugarcane samples from Piracicaba, Ribeirão Preto and Caçapava, in the state of São Paulo, were collected and assayed according to the regular laboratory procedures. Affected tissues with different levels of severity were directly observed under a stereo microscope.

Abundant fungal fruiting structures were transferred to slides and subsequently examined under optical microscopy in order to identify the pathogen.

The fungus was isolated from infected areas by transferring the spores to Petri dishes containing potato dextrose agar culture medium (PDA) and incubated at 24 ± 1 °C for 12 h.

Results and discussion
The direct examination of tissues with different levels of infection, the cultural characteristics on PDA medium and measurement of conidia confirmed the fungus, Passalora vaginae W. Krüger) U. Brown & Crous 2003, as the causal agent of the disease, with morphological characteristics similar to those described in Index Fungorum (Farr and Rossman, 2012).

Red spot of the leaf sheath was previously described in sugarcane producing areas in Brazil (São Paulo and Pernambuco) and various countries of the world, especially in the tropics and subtropics, without causing significant yield losses.

The fungus was previously known as Mycovellosiella vaginae and Cercospora vaginae which are synonyms found in the literature for this pathogen.

The fruiting bodies of the fungus are black, effuse and usually occur in the centre of the lesions. The conidia are hyaline to light olivaceous, cylindrical to cylindrical-obclavate, with one to five septate, thick hilum and dimensions ranging from 3 to 6.5 × 15–55 μm (Figure 2).

Conclusion
The results confirmed that the severe epidemics causing economic losses in the regions of Piracicaba, Ribeirão Preto and Caçapava, in the state of São Paulo, was caused by the fungus P. vaginae.
The unusual and main factors that may have favoured the high incidence and severity of the disease in the 2011–2012 crop season were attributed to rainfall above 500 mm, 7 months after planting, water stress at 10 months after planting, and harvesting of sugarcane without burning for the last eight crops.

REFERENCES


STUDIES ON THE ROOT KNOT NEMATODES IN SUGARCANE IN GUANGXI

By

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KEYWORDS: Meloidogyne spp., Perineal Pattern, Morphological Identification.

Abstract

GUANGXI IS THE most important sugarcane producing province in China and root-knot nematodes are potential damaging pests. However, little information is available concerning the species present and their incidence. We used morphological methods to identify the root-knot nematode species, and to compare their genetic diversities from different areas. Based on female characters of and on perineal patterns, we identified the sugarcane nematode as primarily southern root-knot nematode. A field survey showed that the root knot nematode mainly infected sugarcane roots that were 5–15 cm below ground, and frequently occurred from April through to June, and late October. The root-knot nematode was also able to damage newly-formed roots of sugarcane and formed large galls on the roots. Distributions of the root-knot nematode on Guangxi sugarcane were very asymmetrical (spotty pattern), and it more frequently occurred in Yulin than other areas. Identification and investigation of nematodes may have important implications for integrated pest management (IPM) and greatly improve sugarcane production in Guangxi.

Introduction

The fast growth of China’s economy in recent decades has led to increasing demand for sugar and bio-ethanol (Qiu et al., 2010, Liu, 2012). Sugarcane, has a good potential for bio-ethanol and sugar,(Li, 2010). It is one of the important economic plants of southern China (e.g. Guangxi and Yunnan provinces). Guangxi is the largest sugarcane production province, accounting for 60% of total cane output, and over 60% of sugar output in China (Li, 2006, Long et al., 2011).

However, sugarcane production in Guangxi is influenced by various biotic and abiotic stresses including extreme climatic conditions (low temperature in winter and drought in autumn), lacking irrigation water, weed competition, diseases, and stem borers (Zhou et al., 1998, Li, 2010). Apart from aboveground pest stress, sugarcane also suffers from various endoparasitic or ectoparasitic nematodes (Liu et al., 1993). Among them, the root knot nematode (Meloidogyne spp.) is one of the predominant species in sugarcane (Liu et al., 1993, Wei et al., 2012). It has been reported that root knot nematodes cause yield losses in Australia and Pakistan (BSES, 2010; Chaudhary et al., 2011). In China, Meloidogyne spp. (M. incognita, M. javanica and M. arenaria) is widely distributed in tropical and subtropical regions (Meng et al., 2004), and account for the majority of crop losses caused by root knot nematodes (Lei and Li, 1996, Feng, 2001).

These nematodes have a broad host range including mulberry, Fructus momordicae, tobacco and banana (Pu et al., 1983; Yu et al. 1993; Huang et al. 2011; Qiao et al., 2011).
It has been reported that *Meloidogyne* spp. infected sugarcane in the Guangdong and Fujian provinces (Zhang, 1992; Li, 1996). Information about distribution of root knot nematodes in Guangxi sugarcane is very limited. Only (Liu et al., 1993) and (Wei et al., 2012) reported that *Meloidogyne* spp. were distributed in some areas of Guangxi.

As aboveground symptoms caused by root knot nematodes are not always obvious, the frequency of as well as the damage caused by root knot nematodes in sugarcane has been poorly studied. The present study aimed to identify *Meloidogyne* species, and determine their distribution and incidence in sugarcane in Guangxi.

**Materials and methods**

Field surveys were conducted to determine the incidence of root knot nematodes on sugarcane in the major sugarcane producing areas of Guangxi (Baishe, Yulin and Beihai counties) in 2010. Root and soil samples were collected from the field. Nematodes were extracted by using Baermann funnel and sieving.

The roots were washed free of soil, and teased apart with forceps and a half spear to remove adult females and egg masses. The head and neck regions of nematodes were excised with a surgical blade, and the perineal patterns of females were trimmed. The perineal patterns were examined at 1000× magnification for species identification.

**Results and discussion**

The frequencies of root knot nematode varied among the surveyed areas. It was much higher in the Yulin area than the other areas, while none was detected from Tianyang, Baishe (Table 1, Figure 1).

**Table 1**—Frequency of occurrence of sugarcane root knot nematode in Guangxi subzone.

<table>
<thead>
<tr>
<th>Name of the area</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baise</td>
<td>6.67</td>
</tr>
<tr>
<td>Tianyang</td>
<td>0.00</td>
</tr>
<tr>
<td>Beihai</td>
<td>4.76</td>
</tr>
<tr>
<td>Chongzuo</td>
<td>0.00</td>
</tr>
<tr>
<td>Qingzhou</td>
<td>0.00</td>
</tr>
<tr>
<td>Yulin</td>
<td>8.00</td>
</tr>
</tbody>
</table>

*Frequency= Number of samples with RKN ÷ Total Samples × 100*

Fig. 1—Geographic distribution of sugarcane root knot nematode.
This result is partly similar to inferences made by Liu et al. (1993) and Wei et al. (2012) in that root knot nematode were likely distributed in the southern areas of Guangxi province (Liu et al., 1993; Wei et al., 2012).

The disappearance of root knot nematodes in Tianyang County may be due to expansion of the city, displacement of cane fields from irrigated land to hillside and insecticide application. The frequency of root knot nematodes varied among seasons and mainly occurred from April through to June, and late October (Figure 2).

The female is snow-white, pear-shaped, and is enclosed by galled tissue. Perineal pattern is oval to rounded, and has a high, squared, dorsal arch (Figure 3A). The second juvenile stage (J2) is slender with a rounded tail tip (Figure 3B). Based on the morphometric traits of worms and perineal patterns of females, the sugarcane nematode was primarily identified as southern root-knot nematode. As sugarcane is a monoculture, it may favour the persistence of root knot nematodes.

In addition, this study demonstrated that perineal pattern, which is the most frequently used characteristic for Meloidogyne species identification, is also a reliable trait to identify southern root-knot nematode in Guangxi sugarcane. However, morphological identification can only be carried out on a particular development stage and requires scientific expertise. Molecular technologies have shown great potential to distinguish among plant-parasitic nematodes.
Morphological examination combined with molecular tools could be a better way to identify root knot nematodes as suggested by Marcelo and Oliveira (Oliveira et al., 2011).

Acknowledgments

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REFERENCES


EFFECT OF THERMOTHERAPY FOR
THE CONTROL OF RATOON STUNT

By

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KEYWORDS: Ratoon Stunt,
Hydrothermotherapy, Sugar Cane.

Abstract
RATOON STUNT (RSD) caused by the bacterium Leifsonia xyli subsp. xyli causes losses ranging from 5 to 60% in countries where sugarcane is grown. Hot water treatment or hydrothermotherapy (HTT) of sugarcane cuttings has proven to be the most effective treatment in controlling this disease. In Tucuman, Argentina, its incidence was reported to be 54.8% in 2002. The aim of this study was to evaluate the effect of HTT on emergence and production of nine commercial varieties of sugarcane infected by RSD in Tucuman. Single and multi-nodal cuttings were obtained by manually chopping the stalks of nine varieties of sugarcane in three positions (basal, middle and apical). Single node cuttings consisted of portions of 4 to 5 cm with a healthy bud. After treatment with HTT, cuttings were planted in individual pots in blocks of 20 pots with three replications. Sprouting of single node cuttings was evaluated for two consecutive years and twice a year. Multi-node cuttings consisted of portions of 40 to 60 cm containing 3 to 5 healthy buds. Multi-node cuttings were treated with HTT, and planted in the field in randomised complete blocks with three replications. The production originating from multi-nodal cuttings was evaluated over two ratoons. HTT caused a decrease of 40% to 68% germination in single nodal cuttings compared to the control. HTT of multi-nodal cuttings resulting in a 21% increase in the number of stems, 15% increase in weight of stems and 32% increase in production (tonnes cane/ha). There was no difference in sucrose concentration. Ten years of application of this technology (2003–2012) has reduced the incidence of RSD from 54.8 to 34% in areas of direct monitoring; this represents a decrease of 20% for the province of Tucuman, Argentina.

Introduction
Ratoon stunt (RSD) caused by the bacterium Leifsonia xyli subsp. xyli, is one of the major diseases of sugarcane. It is present in every country in the world where this crop is grown, causing losses that can reach 60% of production, depending on the variety and growth conditions (Würschmidt et al., 1990; Rago et al., 2002a).

The bacterium colonises the vascular bundles of the stalks of sugarcane. Once infection occurs, the bacteria reproduce and spread rapidly. Exudates from the bacteria clog the xylem vessels, hindering the free movement of plant fluids (Davis et al., 1980, Rago et al., 2002b).

A few studies report genetic tolerance to infection in commercial varieties of sugarcane (Steindl, 1961). RSD is often undiagnosed due to the absence of visible external symptoms. The disease is most severe in years of drought, poor, waterlogged, compacted soil or if the cane is stressed from the simultaneous occurrence of other diseases (Gillaspie and Teakle 1989; Rago et al., 2002b).

Thermotherapy is a practice based on the use of heat to inactivate pathogens. The heat acts by destroying the pathogen with less effect on plant tissues (Würschmidt et al., 1989).
The aim of this research was to evaluate the effect of hot water treatment (HTT) on incidence of RSD and production in nine commercial varieties of sugarcane in Tucuman, Argentina.

**Materials and methods**

Single and multi-nodal cuttings were obtained manually by chopping the stems of nine varieties (Table 1) of sugarcane in three positions (basal, middle and apical).

**Table 1**—Yield of plots in nine varieties planted with multimodal cuttings.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Weight 10 stalks Control</th>
<th>% Diff.</th>
<th>No. stalks/m Control</th>
<th>% Diff.</th>
</tr>
</thead>
<tbody>
<tr>
<td>TUCCP 77-42</td>
<td>9.03</td>
<td>1.50</td>
<td>10.91</td>
<td>3.38</td>
</tr>
<tr>
<td>LCP 85-376</td>
<td>8.38</td>
<td>0.75</td>
<td>7.34</td>
<td>4.71</td>
</tr>
<tr>
<td>FAM 89-686</td>
<td>9.53</td>
<td>1.01</td>
<td>12.72</td>
<td>2.31</td>
</tr>
<tr>
<td>L91-281</td>
<td>8.93</td>
<td>0.44</td>
<td>4.64</td>
<td>7.10</td>
</tr>
<tr>
<td>LCP 85-384</td>
<td>7.06</td>
<td>1.88</td>
<td>17.73</td>
<td>0.72</td>
</tr>
<tr>
<td>CP 65-357</td>
<td>8.85</td>
<td>2.03</td>
<td>9.58</td>
<td>-0.95</td>
</tr>
<tr>
<td>RA 87-3</td>
<td>9.47</td>
<td>1.96</td>
<td>11.15</td>
<td>2.21</td>
</tr>
<tr>
<td>HoCP 89-888</td>
<td>5.80</td>
<td>2.18</td>
<td>4.18</td>
<td>5.34</td>
</tr>
<tr>
<td>NA 63-90</td>
<td>8.93</td>
<td>0.44</td>
<td>7.38</td>
<td>-1.57</td>
</tr>
<tr>
<td>Average</td>
<td>8.44</td>
<td>1.35</td>
<td>9.55</td>
<td>2.58</td>
</tr>
</tbody>
</table>

Single nodal cuttings consisted of portions of 4 to 5 cm with a healthy bud. After hot water treatment or hydrotherapy (HTT), the cuttings were planted in individual pots in blocks of 20 pots with 3 replications.

Sprouting of single nodes was recorded weekly until there was no change and was evaluated for two consecutive years, twice a year, and then averaged.

Multi-nodal cuttings consisted of portions of 40 to 60 cm containing 3 to 5 healthy buds. Multi-nodal cuttings were treated with HTT, and planted in the field in randomised blocks with 3 replications.

The hydrothermotherapy treatment was performed by immersing a basket containing the buds for two hours in water at 50.5 °C, then cooled to ambient temperature with tap water. Production was evaluated by measuring the number, height, and weight of 10 stalks and performance in two ratoons.

We determined the percentage of RSD infection in both the control and HTT treated plots, in both years by Tissue Blot Immunoassay (TBIA).

**Results and discussion**

**Single node cuttings**

In the nine varieties tested, the HTT treatment reduced germination by 41%. Germination in the HTT treatment did not exceed the control in any variety (Table 2). Findings in other countries have shown similar results (Rozeff, 2002; Rosler, 1974).

**Table 2**—Average percentage germination of single bud cuttings from nine varieties.

<table>
<thead>
<tr>
<th>Variety</th>
<th>% of shoots Control</th>
<th>HTT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>68</td>
<td>40</td>
</tr>
</tbody>
</table>

333
Multi-nodal cuttings

The number of stalks increased after HTT compared to the control by 21% while stalk weight increased by 15%. However, analysis at the factory did not show any significant difference in sugar content (Table 3).

Table 3—Yield of plots planted with multinodal cuttings. Average for nine varieties.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>HTT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight 10 stalks (kg cane)</td>
<td>8.28</td>
<td>9.74</td>
</tr>
<tr>
<td>Number of stalks/m</td>
<td>9.56</td>
<td>12.14</td>
</tr>
<tr>
<td>Sugar content (%)</td>
<td>10.54</td>
<td>10.39</td>
</tr>
</tbody>
</table>

The variety LCP 85-384 had the highest number of stalks in both the control and HTT treatments. The highest stalk weight was recorded in the varieties FAM 89-686 and RA 87-3, both in the control and HTT treatments.

The cuttings coming from varieties that were multinodal HTT had a 32% increase in the production of sugar.

The control plots had 57% of plants infected with RSD but, in the HTT treated plots, infection was reduced to 0%.

REFERENCES


GENETIC CHARACTERISATION OF ACIDOVORAX AVENAE, CAUSAL AGENT OF RED STRIPE OF SUGARCANE FROM NORTHWESTERN ARGENTINA

By

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KEYWORDS: Genetic Diversity, Isolation, Molecular Identification.

Abstract

THE RED STRIPE is a bacterial disease of sugarcane caused by Acidovorax avenae. This disease has become more important in recent years in all sugarcane areas of Argentina causing losses of up to 30% at milling and also affecting the juice quality. In this study, the isolation and molecular identification of the causative microbial agent was carried out. A total of 150 bacterial isolates were collected from sugarcane in different areas of Tucumán and Salta (northwestern region of Argentina) and were subsequently identified by molecular methods. Species-specific PCR using Oaf1/Oar1 primers allowed amplifying a 550 bp fragment from approximately 50% of the isolates. Molecular analysis of genetic diversity by means of RAPD-PCR revealed the presence of at least four different biotypes among the isolates. REP-PCR and ARDRA showed a lower level of discrimination. Pathogenicity tests were performed to confirm that A. avenae was the pathogenic agent causing red stripe symptoms. The results constitute the first report on the identification and characterisation of this pathogen from Argentina. The genetic diversity detected among the isolates constitutes an important finding to devise management strategies for accurate diagnosis and/or the selection of clones tolerant to the predominant strains.

Introduction

The sugarcane agroindustry in Argentina is mostly concentrated in the Northwestern (NW) provinces (Jujuy, Tucumán and Salta) representing one of the main economic resources. Even when productivity varies due to climatic and phytosanitary conditions, sugarcane output experienced a marked increase in recent years.

The major limiting factors affecting sugarcane productivity are diseases caused by different pathogens such as viruses, fungi and bacteria. Among these, red stripe, also known as ‘polvillo’, affects sugarcane practically worldwide. The agricultural practices implemented in Argentina such as green-cane harvesting and crop rotation including soybean, resulted in significant increases in disease occurrence.

The causative organism, Acidovorax avenae subsp. avenae has been recently reclassified to species level as A. avenae for sugarcane (Schaad et al., 2008). Red stripe causes the loss of cane for milling (up to 30%) as well as a decrease in juice purity (Pérez Gómez et al., 2010).

Despite significant production losses, red stripe has been poorly studied and no report on the identification and characterisation of A. avenae for a precise diagnosis is available. Since the identification of the red stripe causative agent is of utmost importance for efficient crop management, classical and molecular techniques were applied in this study for its isolation, identification and genetic characterisation of isolates from northwest of Argentina.
Materials and methods

Sampling and A. avenae isolation

Fourteen leaf samples from sugarcane with red stripe symptoms were collected in the 2008–09 growing season from six sugarcane cultivars in 12 different sugarcane producing areas of Tucumán and Salta, Argentina. Isolation of pathogenic microorganisms was performed according to Schaad et al. (2001).

A. avenae species-specific PCR

DNA extraction from cell cultures was according to Ausubel et al. (1992). PCR was performed using species-specific primers for A. avenae described by Song et al. (2003). PCR fragments were visualised by electrophoresis using 1.5% agarose and stained with Gel Red (Genbiotech, Argentina).

Genetic diversity analysis

All isolates characterised as Acidovorax avenae were subject to REP-PCR, ARDRA and RAPD-PCR analysis. REP-PCR reactions were performed according to Silva (2005) using the primers REP, ERIC and BOX. Enzymes TaqI, HincII, HaeIII and HindII were used for ARDRA protocol. Primers M13 and RAPD2 were used in RAPD reactions according to Fontana et al. (2005). The RAPD and REP profiles were normalised and submitted to Cluster Analysis with BioNumerics software.

Pathogenicity tests

Three sugarcane cultivars TUC 77-42, RA 87-3 and LCP 85-384, susceptible, intermediate and resistant to red stripe, respectively, were used. Bacterial suspensions adjusted to approx. 10⁸ CFU/mL were inoculated on leaves adaxial and abaxial surfaces by rubbing the leaf surfaces with sterile cotton wetted with the suspension.

Observations were carried out every 24 h to determine the evolution of symptoms, which were identified as ‘typical symptoms’ for the occurrence of red stripe on leaves or ‘severe symptoms’, for the apical bud rot.

Results and discussion

A. avenae isolation and identification

Cream coloured colonies consisting of Gram negative cells were selected and subjected to species-specific PCR amplification. Approximately 50% out of 150 isolates exhibited a positive signal for Acidovorax, amplifying a specific fragment of 550 bp (Figure 1).

PCR result indicated that other bacterial groups with the same A. avenae colony characteristics are associated with the infection as a sugarcane Acidovorax-red stripe pathosystem. Genera such as Erwinia and Pantoea, together with some Pseudomonas species, are described as epiphytic bacteria coexisting with A. avenae in/on rice seeds (Song et al., 2004).

One representative A. avenae strain, isolated from Tucumán and Salta, was used for pathogenicity tests. Both strains used were able to successfully reproduce red stripe symptoms on sugarcane leaves; the inoculated plants did not show any differences in pathogenicity or aggressiveness for each of the A. avenae biotypes. The lesions intensity developed on leaves was called ‘typical symptoms’.

Genetic diversity of A. avenae isolates

Primer M13 was more discriminative than RAPD2, enabling the differentiation of 4 biotypes among 39 isolates of A. avenae analysed.

Figure 2 shows the dendrogram obtained from RAPD profiles using BioNumerics software in which A. avenae strains were assembled in two major clusters at a similarity level of 85%. Cluster 1 contained 12 A. avenae strains with biotype ‘a’ which were isolated from TucCP 77-42, RA 87-3, Fam 91-209 and 5 strains with biotype ‘b’ which were isolated from Fam 89-686 Tucumán sugarcane commercial genotypes.
Fig. 1—PCR amplification products (550 bp) using species-specific primers for *A. avenae*.

Fig. 2—Dendrogram obtained from RAPD-PCR patterns using M13 primer from *A. avenae* strains isolated and analysed by *BioNumerics* software. S—Salta, and T—Tucumán biotypes are indicated by letters: a, b, c and d. Left column indicates commercial genotype of sugarcane.
On the other hand, cluster 2 grouped biotypes ‘c’ and ‘d’ isolated from NA 85-1602 and NA 02-2320 genotypes, respectively from the Salta region. From these results, it can be inferred that clustered strains were mostly associated with the different sugarcane genotypes as well as the geographical distribution of the phytopathogen.

The strategy used in this study (RAPDs and PCR amplification of ITS regions) for the rapid and consistent discrimination among \textit{A. avenae} strains from sugarcane had been also applied for variability studies of \textit{Ustilago scitaminea} causing smut in sugarcane as well as in different pathovars of the genus \textit{Xanthomonas} (Khoodoo and Jaufeerally-Fakim, 2006).

REP profiles obtained with primer REP1 revealed the occurrence of genetic diversity among isolates from Tucumán, whereas for isolates from Salta, the discrimination was not sufficiently clear as was evidenced by RAPD analysis. (Figure 3).

ARDRA results showed a low level of intra-species discrimination. However, using \textit{TaqI} and \textit{HaeIII} enzymes, it was possible to discriminate \textit{A. avenae} from other epiphytic bacteria such as \textit{Pantoea} and \textit{Erwinia} (Figure 4)

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{REP_fingerprints.jpg}
\caption{REP fingerprints of \textit{A. avenae} isolates obtained with REP1 primer. Two dominant profiles were found: a and b for Tucumán isolates.}
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\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{ARDRA_profiles.jpg}
\caption{ARDRA profiles obtained using \textit{HaeIII} enzyme for different strains of \textit{A. avenae} from Tucumán and Salta. The lanes 31, 51, 72 and LR are profiles obtained from non \textit{Acidovorax} genera.}
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REFERENCES


