**Xanthomonas albilineans** and **Sugarcane yellow leaf virus** (SCYLV) in Guatemala: assessment of methodologies for sanitation of infected sugarcane material

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Abstract An important problem in sugarcane crop is the clonal propagation from plant material that is infected by pathogens but does not show disease symptoms. In this investigation we compared DBIA (dot blot immunoassay) and PCR techniques for detecting *Xanthomonas albilineans*. We also used RT-PCR for detecting *Sugarcane yellow leaf virus* (SCYLV). We performed a random location survey to evaluate the incidence of both pathogens in order to confirm their presence in asymptomatic plants. By sequencing the DNA amplified fragments of *X. albilineans* isolates, we found an A/G SNP. Such single-base change does not necessarily imply a change in phenotypic expression. We compared thermotherapy in combination or without meristem-culture for plant sanitation. Meristem culture was suitable for rescuing *X. albilineans*-free plants, while meristem culture or thermotherapy, alone or combined, could partially or completely eliminate the virus.

**Key words**  Leaf scal, sugarcane yellow leaf, DBIA, PCR, disease-free

**INTRODUCTION**

The vascular bacterial disease leaf scald, caused by the bacterium *Xanthomonas albilineans* (Ashby) Dowson, is one of the major diseases of sugarcane (Rott and Davis 2000). Firstly, this bacterium colonizes the leaf surface, spreading later within the tissues through the xylem (Rott and Davis 2000; Birch 2001). Latent infection or the absence of symptoms is a characteristic feature of the disease, which occurs in tolerant varieties and under favorable conditions for plant growth. This latency comes to an end for unknown reasons, and symptoms of the chronic or acute forms of leaf scald disease can then appear. Sugarcane plants can be infected by *X. albilineans* for weeks or months without exhibiting symptoms or showing inconspicuous symptoms, hindering control disease (Martin and Robinson 1961; Ricaud and Ryan 1989). Prevention and control of leaf scald include the cultivation of resistant varieties supplemented by disinfection of cutting instruments regularly, the use of disease-free cuttings from nurseries, and the early detection of infection in fields. The treatment most used to obtain *X. albilineans*-free cuttings is immersion in running water for 24 h and then incubation in hot-water at 50°C for 3 hours (Ovalle 2012).

The yellow leaf syndrome of sugarcane (YLS) was first reported in Hawaii in 1989. Since then, it has been seen in more than 30 countries. Initially it was shown that the *Sugarcane yellow leaf virus* (SCYLV) was responsible for YLS, but subsequent studies revealed a phytoplasma associated with the disease. Recently YLS has been differentiated as ‘yellow leaf’ caused by SCYLV and ‘leaf yellows’ caused by sugarcane yellow phytoplasma (SCYP) (Joomun et al. 2007). SCYLV symptoms are more evident in mature and stressed plants. They start with the yellowing of the lower side of the midrib of the leaves 3-6 counting from the first fully expanded leaf at the apex downwards. Yellowing then expands to the lamina from the tip of the blade towards the sheath (Comstock and Miller 2003). Because SCYLV infected plants are often asymptomatic and efficient diagnostic methods were unavailable until the end of 1990, the disease spread worldwide through infected germplasm (Girard et al. 2010). To control the disease, the use of resistant varieties and the use of healthy plants are recommended. For the latter, tissue culture has proven to be an efficient tool. Snyman et al. (2005) and Parmessur et al. (2002) reported the elimination of SCYLV and have regenerated healthy plants using tissue culture. Moreover, Chatenet et al. (2001) found that hydrothermal treatments to control other diseases in sugarcane, were not effective in eliminating the virus in infected material.

Seeking better disease control in Guatemalan sugarcane production, we evaluated different treatments for eliminating *Xanthomonas albilineans* and SCYLV from infected plants.
MATERIALS AND METHODS

To make sure the bacteria infected the plants, we used and compared our DBIA routine diagnosis method with PCR. The diagnosis method for the virus was RT-PCR. The DNA amplified fragment of *X. albilineans* from each plant was sequenced and the sequences compared to determine its diversity. Assessment of genetic variation of the SCYLV is in progress. We have conducted a survey of symptomless plants in sugarcane-growing areas as a preliminary assessment of infection and the identification of hot spots.

RESULTS AND DISCUSSION

Diagnosis and characterization *Xanthomonas albilineans*

Analyzing symptomatic plants, we found that, although PCR showed a little higher efficiency for detecting *X. albilineans* due to its higher sensitivity, DBIA is still an adequate method that can be used routinely.

![Fig. 1. Comparison of DBIA and PCR diagnostic methods for X. albilineans on 54 symptomatic stalks from two locations](image)

The *X. albilineans* DNA sequence that was amplified in the diagnosis of symptomatic plants showed a SNP (Fig. 2) but its effect on the phenotype is unknown.

![Fig. 2. A/G SNP found in the sequence of 23 PCR amplified fragments of X. albilineans.](image)
Heat treatments alone and combined with meristem culture for pathogen elimination from infected plants

We found that meristematic culture (MC) alone or combined with heat treatments was the best for eliminating \textit{X. albilineans}, while meristem culture or thermotherapy, alone or combined, could partially or completely eliminate SCYLV from infected plants (Fig. 3).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{Proportion of disease-free plants obtained after treatments of stalks infected with (left) \textit{X. albilineans} and (right) sugarcane yellow leaf virus.}
\end{figure}

Survey of \textit{Xanthomonas albilineans} and SCYLV in some sugarcane growing areas

Our random sampling of symptomless plants showed that \textit{X. albilineans} is less widespread than SCYLV and that, the latter is more frequent on higher ground. We also found that some varieties classified as resistant can be infected, although they did not show symptoms.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4.png}
\caption{Sugarcane growing regions of Guatemala showing 56 plots surveyed for (left) \textit{X. albilineans} and (right) SCYLV in symptomless plants.}
\end{figure}

CONCLUSION

Our study showed that both leaf scald and yellow leaf are present in some sugarcane-growing regions of Guatemala. We also found that meristematic tissue culture and heat treatment are essential for disease control.

ACKNOWLEDGEMENT

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Xanthomonas albilineans et Sugarcane yellow leaf virus (SCYLV) au Guatémala: évaluation de méthodes d’assainissement de cannes à sucre infectées

Resumen. La multiplication clonale à partir de plantes infectées par des pathogènes mais qui ne présentent pas de symptômes est un problème important dans la culture de la canne à sucre. Dans cette étude nous avons comparé le DBIA (dot blot immunoassay) et les techniques PCR pour détecter Xanthomonas albilineans. Nous avons également utilisé la RT-PCR pour détecter le Sugarcane yellow leaf virus (SCYLV) dans des plantes asymptomatiques. Nous avons réalisé une enquête sur des sites aléatoires pour évaluer l’incidence des deux pathogènes afin de confirmer leur présence dans des plantes sans symptômes. En séquençant les fragments d’ADN amplifiés à partir des isolats de X. albilineans nous avons trouvé un polymorphisme nucléotidique A/G. Ce changement d’une seule base n’implique pas forcément un changement dans l’expression phénotypique. Nous avons comparé la thermothérapie en combinaiosn avec ou sans la culture de meristème pour l’assainissement des plantes. La culture de meristème était adaptée à la récupération de plantes assainies de X. albilineans, tandis que la culture de meristème ou la thermothérapie, seules ou combinées, pouvaient éliminer le virus de façon partielle ou complète.

Palabras clave: Echadure de la feuille, feuille jaune de la canne à sucre, DBIA, PCR, assainissement

Xanthomonas albilineans y Sugarcane yellow leaf virus (SCYLV) en Guatemala: evaluación de métodos de saneamiento de caña de azúcar infectada

Resumen. La propagación clonal de caña de azúcar a partir de material infectado por patógenos que no muestran síntomas de enfermedad es un problema importante en este cultivo. En esta investigación comparamos las técnicas DBIA (dot blot immunoassay) y PCR para la detección de Xanthomonas albilineans. También utilizamos RT-PCR para la detección del virus de la hoja amarilla de la caña de azúcar (SCYLV). Realizamos una prospección aleatoria para evaluar la incidencia de ambos patógenos y de esta forma poder confirmar su presencia en plantas asintomáticas. Por medio de la secuenciación de fragmentos de ADN provenientes de aislados de X. albilineans, encontramos un SNP A/G. Este cambio de base no necesariamente implica un cambio en la expresión fenotípica. Comparramos termosterapia con y sin cultivo de meristemas para el saneamiento de plantas. El cultivo de meristemas resultó apropiado para el rescate de plantas libres de X. albilineans, mientras que el cultivo de meristemos o la termosterapia, solos o combinados, pudieron eliminar el virus parcialmente o completamente.

Palabras clave: Escaldadura foliar, virus de la hoja amarilla de la caña de azúcar, DBIA, PCR, saneamiento