

**ADVANCE IN GENETIC IMPROVEMENT TO RESISTANCE
OF SUGARCANE TO *XANTHOMONAS ALBILINEANS* (ASHBY)
DOWSON IN CUBA**

By

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Abstract

LEAF SCALD, caused by *X. albilineans*, is a quarantined disease in Cuba and not much is known on how it behaves under the environmental conditions of the country. Because of this, we did a molecular characterisation of the inoculum employed to artificially inoculate sugarcane plants at two varietal resistance trials and we developed a scale to characterise the severity of the infection in the progeny. The work has allowed progeny evaluation of the families resulting from the hybridisation program for their reaction to leaf scald. The months of September and October were the best times to plant genotypes for selection and evaluation of leaf scald resistance.

Introduction

Leaf scald, caused by *Xanthomonas albilineans* (Ashby) Dowson, was first reported in Cuba at the end of the 1970s.

During 1978–1982, leaf scald was detected in 14 foci scattered through 7 provinces of the country (Rivera *et al.*, 1979).

Years later, *X. albilineans* was detected in latently infected plants obtained through tissue culture and in commercial plantations. The danger of a resurgence of the disease was pointed out (Peralta *et al.*, 1997).

Leaf scald is found in more than 54 countries and, during recent years, there have been several outbreaks in different cane areas of the world, with significant losses being reported in highly susceptible varieties (Rott and Davis, 2000).

The most effective methods used by geneticists and plant pathologists to control the disease have been the use of resistant varieties and the use of healthy seed.

Because leaf scald has been reported to be influenced by the environment, there is a need to study the different phytopathological principles that determine the behaviour of leaf scald in Cuba and to obtain a greater efficiency in identifying new resistant varieties.

Materials and methods

The research was done at two research centres: one for the study of the diseases in Cuba, part of the Provincial Sugar Cane Experiment Station of Camaguey located in Tayabito, on a brown soil with carbonates, using two different planting times, March (Cycle A) and September (Cycle C), and the second at the Matanzas Sugar Cane Experiment Station in Jovellanos on a red ferrous soil, using the same planting cycles.

The methodology and experimental design were directed towards phytopathological concepts necessary to work with the disease caused by *X. albilineans* under Cuban conditions consisting of the following phases:

- Characterisation of isolates of *Xanthomonas albilineans* using AFLP, at two experiment stations in Cuba studying the disease.
- Determination of an evaluation scale to characterise the different disease reactions.
- Determination of the periods and times of greater occurrence of the disease under Cuban environmental conditions.

Characterisation of isolates of *Xanthomonas albilineans* through AFLP at the two test centres for the disease in the country

Bacterial cultures The bacterial cultures were centrifuged at 5000 rpm for 20 min and the sediment was used for the purification of the genomic DNA utilising the commercial method and reagents Wizard Genomic DNA (cat #A1120, Promega, USA). The integrity of the DNA was verified with agarose gels (0.8%) visualised with EtBr.

Genomic Analysis Genomic analysis was done by means of Amplified Fragment Length Polymorphism (AFLP) following the procedure described by Vos *et al.*, (1995) and according to European Patent 0534858 (Keygene, Belgium).

Determination of an evaluative scale for the characterisation of the different effects caused by the disease

To examine the role of bacterial concentration on genotypes resistance, as well as to define an evaluation scale, the study was divided into 4 fundamental aspects:

1. Define the symptoms and the severity of the infection.
2. Determine the dilutions required to make the counts of colony making units (ufc).
3. Compare the number of ufc in symptomatic and asymptomatic plants.
4. Determine the relationship between the ufc and the severity of the infection.

Afterwards, monthly evaluations were done, registering the number of plants with each degree of intensity in the scale that the proper methodology establishes. Having information on the severity of the disease and in order to differentiate intermediate responses from the varieties to this pathogen, a discriminant analysis was conducted.

Determining the seasonal periods of greater occurrence of leaf scald under Cuban environmental conditions.

The resistance against leaf scald of 377 new varieties of sugarcane and check varieties, (My5514, resistant; L 55-5, susceptible), was tested, utilising two different periods, Fall (September) and Spring (March), of planting and inoculation.

Results and discussion

Molecular characterisation of the samples of DNA of *Xanthomonas albilineans* analysed by means of AFLP with the combination of primers P (PstI) and M (MseI) is shown in Figure 1.

In the 4 combinations used, a great consistency and resolution of the amplified bands of DNA was observed.

The sections of gels with the greater resolution show they were amplified with different intensities, approximately 38 bands for the combination of primers A, 39 bands for B, 34 bands for C and 37 bands for the combination D.

At the same time, in all cases, a high similarity in the amplification bands is demonstrated and polymorphic bands of DNA were not identified.

The conclusion was that, under the conditions of this work, no significant variability was detected, demonstrating that both cultures are similar at this genomic level. Table 1 shows the primers used, and Figure 1 shows the amplification bands.

Table 1. Primers utilised in reactions of AFLP.

Code	Primers	Sequence the primers DNA	Kiss selective
P1	PstI-1	5'GACTGCGTACATGCA.3"	aa
P2	PstI-2	5'GACTGCGTACATGCA.3"	ac
M1	MseI-1	5'GATGAGTCCTGAGATAC.3"	acc
M2	MseI-2	5'GATGAGTCCTGAGATAC.3"	ggc

Copy of primers Isolations: (1. Jovellanos, 2. Camagüey)
 A. P1/M1
 B. P1/M2
 C. P2/M1
 D. P2/M2

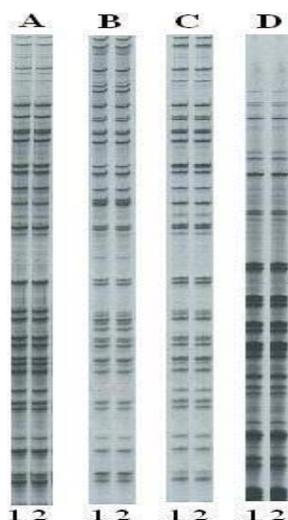


Fig. 1—Amplification bands (AFLP) using 4 combinations of primers from cultures of the pathogen *Xathomonas albilineans* from Matanzas and Camaguey provinces.

With no differences detected in the inocula used in the two Test Centres, a rating scale was developed for use under Cuban environmental conditions.

Based on a review of the results of the severity of the infection on plant cane and on ratoon crops and after applying the discriminatory analysis, 5 groups of varieties were characterised, based on their response to the infection (Table 2).

The definitive groups were decided on the maximum severity of infection shown in any of the evaluations on both ages of the crop.

Table 2—Degrees of severity of the infection to be used in the evaluation of leaf scald.

Grade	Denomination	Severity	
		Plant	Ratoon
1	Very resistant	0–2	0–2
2	Resistance fighters	2.1–10	2.1–10
3	Intermediate	010,1 2	10,1 20
4	Susceptible	20.1–30	20.1–30
5	Very susceptible	30	30

■ Resistants ■ Intermediates ■ Susceptibles

When the group of varieties showing the greatest severity of infection in the ratoon stage was examined, it was noted that when the severity did not go over 10%, neither has the level exceeded 10% in the plant cane stage, emphasising the criteria of several researchers that the 10% level of severity could be the limit of expression of resistance in leaf scald.

An interesting element of concern is determining those times when the disease presents itself with greatest incidence in Cuba, thus making it possible to evaluate varieties more efficiently.

A comparison of the effect of the disease on both ages of the cane, whether as plant cane or ratoon, in relation to their classification degree can be seen in Figure 2. As can be seen, material planted in September showed greater damage by the disease as plant cane, whereas those planted in March showed greater losses as ratoon crop, which indicates that genotypes in September plantings can be evaluated quicker, but it is evident that both planting dates should be evaluated.

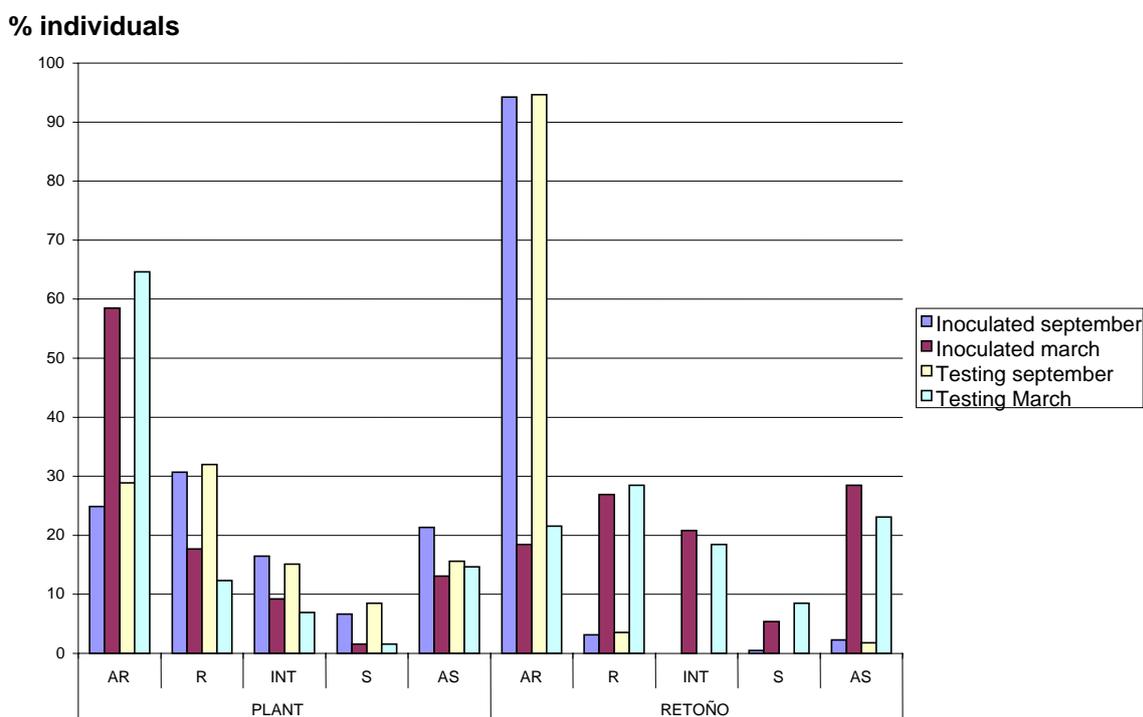


Fig. 2—Comparison of the effect of the disease in relation to both age of the crop (plant cane and ratoon) and different planting dates (fall and spring).

Conclusions

- Isolates of *Xanthomonas albilineans* used in the two Test Centres for varieties and parents in Cuba are similar at this genomic level; therefore, the information that is obtained can be extrapolated.
- A new scale showing different degrees of the severity of leaf scald infection is now available for Cuba.
- In fall planted cane (September), individuals can be separated as per their resistance without the need to get to the ratoon stage; to get more conclusive results, both plant cane and ratoons should be evaluated.

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LES AVANCÉES DE L'AMÉLIORATION GÉNÉTIQUE DE LA CANNE À SUCRE POUR LA RÉSISTANCE AU *XANTHOMONAS ALBILINEANS* (ASHBY) DOWSON À CUBA

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MOTS CLÉS: Echaudure, Canne à Sucre, Test de Résistance.

Résumé

L'ÉCHAUDURE, causée par *X. albilineans*, est une maladie de quarantaine à Cuba et il existe peu d'informations sur le comportement de la maladie sous les conditions environnementales prévalant dans ce pays. De ce fait, nous avons effectué une caractérisation moléculaire de l'inoculum utilisé pour l'inoculation artificielle de la canne à sucre de deux essais de résistance à la maladie et nous avons développé une échelle d'évaluation pour déterminer la sévérité de l'infection de la descendance. Cette étude a permis d'évaluer la progéniture des familles issues du programme d'hybridation génétique pour leurs réactions à l'échaudure. Les mois de septembre et d'octobre étaient les plus indiqués pour la plantation des génotypes, leurs sélections et l'évaluation de leur résistance à l'échaudure.

AVANCES EN EL MEJORAMIENTO GENÉTICO POR RESISTENCIA EN CAÑA DE AZÚCAR A *XANTHOMONAS ALBILINEANS* (ASHBY) DOWSON EN CUBA

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PALABRAS CLAVE: Escaldadura de la Hoja, Caña de Azúcar, Resistencia.

Resumen

LA ESCALDADURA de la hoja, causada por *X. albilineans*, es una enfermedad de cuarentena en Cuba y no se sabe mucho sobre cómo se comportamiento bajo las condiciones ambientales del país. Debido a esto, se hizo una caracterización molecular del inóculo empleado para infectar artificialmente las plantas de caña de azúcar en dos ensayos de resistencia varietal, usando una escala para caracterizar la gravedad de la infección causada. El trabajo permitió evaluar la progenie de las familias derivadas del programa de hibridación en su reacción a la escaldadura de la hoja. Los meses de septiembre y octubre fueron la mejor época para seleccionar y evaluar los genotipos en su resistencia a la escaldadura de la hoja.