DIAGNOSIS OF FIVE SUGARCANE DISEASES USING THE SAME LEAF SAMPLE

By

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Abstract

Both viral and bacterial diseases affect the sugarcane crop in Colombia. Disease control is mainly achieved through the use of resistant varieties and healthy seedcane. In order to select healthy seedcane, it is necessary to verify the absence of pathogens, for which reliable and sensitive methods of diagnosis are required. This paper presents the results of using tissue-blot (TBIA) and dot-blot (DBIA) enzyme immunoassays for diagnosing five sugarcane diseases of importance in Colombia: ratoon stunting disease, leaf scald, sugarcane mosaic virus, bacilliform virus and yellow leaf syndrome, caused by the sugarcane yellow leaf virus. Both methods showed good specificity and sensitivity; and a combination of both made it possible to determine the prevalence of the diseases in different areas. Diagnostic tests for the five diseases were done using the same leaf sample. This is a non-destructive sampling technique that facilitates fieldwork as well as the transport and storage of the samples. The methods are fast, simple, and economical; they do not require sophisticated equipment; and a large number of samples can be evaluated simultaneously.

Introduction

A number of diseases affect the sugarcane crop but do always not present external symptoms, which would facilitate their diagnosis. Control of these diseases is based on the use of resistant varieties, if available, and pathogen-free seedcane. This requires diagnostic techniques that are easy-to-use, economical and permit accurate diagnosis of pathogens. The method of dot-blot enzyme immunoassay (DBIA), based on extracting the pathogen from the leaf tissue, has been used to detect ratoon stunting disease (RSD) (Harrison *et al.,* 1990); but it presented problems with false positive results. A tissue-blot enzyme immunoassay (TBIA) was developed to detect the sugarcane yellow leaf virus (SCYLV) that is the cause of one form of yellow leaf syndrome (YLS) (Shenck *et al.,* 1997). In these enzyme immunoassays, specific antisera are used to detect pathogens that are bound to nitrocellulose membranes (NCM). This paper presents modifications of these techniques for the more accurate diagnosis of two bacterial pathogens, *Clavibacter (Leifsonia) xyli* subsp x*yli*, the causal organism of RSD, and *Xanthomonas albilineans,* the causal organism of leaf scald, and three viral pathogens, sugarcane yellow leaf virus (SCYLV), sugarcane bacilliform virus (SCBV) and sugarcane mosaic virus (SCMV).

Methods and results

Sample collection and storage

Different methods of storing the samples were tested. Packages of 20 leaves, collected from randomly selected plants in the field, were transported in completely sealed plastic bags. These were placed in a refrigerated cooler, or in a refrigerator at 4°C if processing was delayed for several days.

Antisera

The antisera for detecting SCBV and SCYLV were supplied by B.E.L. Lockhart (University of Minnesota, USA). Antisera for SCMV, leaf scald and RSD were prepared at CENICAÑA.

Optimisation of DIA and TBIA protocols

The pathogen was bound to the membranes for DBIA and TBIA and the same protocol was followed for both tests. The membranes were incubated for one hour on a horizontal shaker with 1% skimmed milk and 0.5% bovine serum albumin in tris-buffered saline (TBS). After two rapid washes with TBS, the corresponding conjugate, consisting of anti-rabbit immunoglobulin conjugated with alkaline phosphatase was added. For the conjugate it was concluded that a titre of 1:8000–1:10 000 worked well for all five pathogens.

To visualise the reaction, a mixture of bromo chloro-indolyl-phosphate (BCIP) and nitro blue tetrazolium (NBT) was added in substrate buffer with agitation and the membranes were washed with water and left to air dry. In the case of DBIA, the presence or absence of violet-blue-coloured spots in the samples was assessed. For TBIA, the presence or absence of violet-blue-coloured spots in the xylem vascular vessels in the leaf veins was determined using a stereomicroscope.

Dot-blot technique (DBIA)

Tissue was cut from the leaf laminae and midribs at different parts of the stalk: the basal part of the top visible dewlap leaf (TVD), leaf #2 and leaf #5 of affected plants. For all five diseases and healthy checks, samples were weighed.

KEYWORDS: Sugarcane, Viral Diseases, Bacterial Diseases, Diagnosis, TBIA, DBIA.

Detection of RSD and leaf scald

Weighed material was suspended in 1 mL of sterile water and left at ambient temperature to facilitate the diffusion of the bacteria from the diseased tissue. Samples were filtered through a Bio-Dot microfiltration apparatus (Bio-Rad®), which contained a NCM of 0.45 µm, previously moistened in TBS (pH 7.5). The membrane was then dried at ambient temperature and either used immediately or stored at 4°C for several days. It was found that 100 mg/mL of midribs was sufficient for detecting a positive reaction. The optimum sample volume was 10 µL for leaf scald and 30 µL for RSD.

Diagnosis of SCYLV, SCBV and SCMV

One sample of 100 mg of tissue/ml was sufficient to obtain a good reaction in the test. The optimum tissue/DIECA ratio for both SCYLV and SCBV was 1:10, the ideal sample volume was 10 µL and the optimum dilution was 1:500. SCYLV produced a stronger reaction when tissue from the leaf lamina was used than with tissue from the midrib. For SCBV and SCMV, no reaction was observed when using tissue from the midrib.

Tissue-blot immunoassay (TBIA)

Impressions were made of the central vein of the TVD leaves and leaves 2 and 5 on a NCM, evaluating the effect of distance from the base of the leaf. The blots were processed immediately or stored at 4°C before processing.

For the bacterial diseases, the proportion of positive vascular bundles was highest in the lowest leaves. For SCYLV, a stronger reaction was observed from the TVD leaf than from leaves 2 and 5. In all the leaves, the proportion of positive vascular bundles was highest in the basal part of the midrib, up to 30–40 cm from the base. At greater distances from the leaf base the reaction became weaker, and after 60–70 cm, no positive vessels were detected. For SCBV and SCMV, it was not possible to visualise the staining of the vessels by this method.

Sensitivity

Different mixtures of diseased and healthy leaves were made for all five diseases, mixing one diseased leaf with 19 healthy ones, two with 18 and so on until there were absolute checks of 20 diseased leaves and 20 healthy ones. DBIA was capable of detecting one diseased leaf among 19 healthy ones. The level of detection of RSD and leaf scald was also determined by counting bacteria by immuno-fluorescent direct count on filter (FADCF). Samples with concentrations greater than 4.8×10^4 cells/mL were detected.

Conclusions

The main advantages of DBIA and TBIA are their high sensitivity and specificity. They are also simple, rapid and economical. They do not require sophisticated equipment such as an epifluorescent microscope, which is necessary for FADCF.

Combining DBIA and TBIA resulted in highly efficient evaluation of a large number of samples for RSD, leaf scald and SCYLV. The initial evaluation used a mixture of 20 leaves. Diagnosis was first done using DBIA. Samples that tested positive by DBIA were then evaluated individually by TBIA or DBIA to determine the percentage incidence of each pathogen. It was not possible to combine the two methods for the diagnosis of SCBV and SCMV because TBIA did not detect these two pathogens. For these pathogens, the leaves of samples that initially tested positive were re-evaluated individually by DBIA to determine percentage incidence.

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REFERENCES

Hammond, J. and Jordan, R.L. (1990). Dot blots (viruses) and colony screening. In: Hampton, R., Ball, S. and de Boer, S. ed. Serological methods for detection and identification of viral and bacterial plant pathogens: A Laboratory Manual. APS Press, Minnesota, 237–248.

Harrison, N.A. and Davis, M.J. (1990). Comparison of serological techniques for diagnosis of ratoon stunting disease. Sugar Cane 1990, Spring Supplement: 5–9.

Schenck, S., Hu, J.S. and Lockhart, B. (1997). Use of a tissue blot immunoassay to determine the distribution of sugarcane yellow leaf virus in Hawaii. Sugarcane 1997, No. 4: 5–14.

DIAGNOSTIC DE CINQ MALADIES DE LA CANNE A SUCRE A PARTIR D'UN MEME ECHANTILLON FOLIAIRE

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Résumé

La culture cannière de Colombie est affectée à la fois par les maladies virales et bactériennes. La lutte contre ces maladies repose sur la plantation de variétés résistantes et de boutures saines. Pour sélectionner des boutures saines, il est nécessaire de vérifier l'absence de pathogènes, et ceci nécessite des méthodes de diagnostic précises et sûres. Ce papier présente les résultats de l'utilisation des tests immuno-enzymatiques par empreintes de tissus (TBIA) et par empreintes des sections (DBIA) pour le diagnostic de cinq maladies très importantes: le rabougrissement des repousses (RSD), l'échaudure des feuilles (LSD), le virus de la mosaïque, le virus bacilliforme et le virus du syndrome de la feuille jaune. Les deux méthodes ont montré à la fois une forte sensibilité et une bonne spécificité; et la combinaison des deux a rendu possible la détermination de la présence et l'incidence d'une maladie dans la zone observée. Le diagnostic des cinq maladies a été effectué à partir d'un même échantillon foliaire. Ceci représente une technique d'échantillonnage non destructive qui facilite le travail au champ aussi bien que le transport et la conservation des échantillons. Ces méthodes sont rapides, simples, et économiques; elles ne nécessitent pas d'équipement élaboré; et permettent d'évaluer un grand nombre d'échantillons à la fois.

Mots clés: Maladies virales, maladies bactériennes, méthodes de diagnostic, TBIA, DBIA, techniques d'échantillonnage.

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Resumen

El cultivo de la caña de azúcar en Colombia es afectado por enfermedades virales y bacterianas. El control de las enfermedades se realiza utilizando de semilla sana para lo cual es necesario verificar la ausencia de patógenos. Para seleccionar ésta semilla es necesario contar con métodos de diagnóstico confiables y sensibles. Este trabajo presenta los resultados de la implementación de los métodos inmunoenzimáticos del Tissue-blot (TBIA) y dot-blot (DBIA) para el diagnóstico de cinco enfermedades de gran importancia en el cultivo como son: raquitismo de la soca, escaldadura de la hoja, virus baciliforme, virus del mosaico y el virus causal del síndrome de hoja amarilla. Para el TBIA se hicieron impresiones de tejido foliar y para DBIA extracciones virales, y suspensiones bacterianas, en ambos casos se colocaron las muestras sobre membranas de nitrocelulosa, se emplearon antisueros específicos para cada enfermedad, un conjugado con fosfatasa alcalina y un sustrato para visualizar la reacción. Los dos métodos mostraron buena especificidad y sensibilidad; la combinación de los dos permite determinar la presencia e incidencia de la enfermedad en el área evaluada.. El diagnóstico se realiza con la misma muestra foliar para las cinco enfermedades. El muestreo no destructivo facilita la labor en el campo, el transporte y conservación de las muestras. Estos métodos son rápidos, sencillos, económicos y no requieren equipo sofisticado y se pueden evaluar simultáneamente un gran número de muestras.

Palabras claves: enfermedades virales, enfermedades bacterianas, diagnóstico, TBIA, DBIA.