MONITORING THE SEVERITY AND VARIABILITY OF BROWN RUST (*Puccinia melanocephala*) IN SUGARCANE VARIETIES IN THE CAUCA VALLEY, COLOMBIA

By

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KEYWORDS: Sugarcane, Brown Rust, Disease Incidence, Ribosomal DNA (Rdna), Resistance, Physiological Races.

Abstract

THE VARIETIES CC 85-92 and CC 84-75 are grown on more than 80% of the area planted with cane in the Colombian sugar industry; these varieties were initially resistant to brown rust disease. Brown rust has been present in Colombia since 1979. Genetic variability in *Puccinia melanocephala* is thought to have affected resistance in some varieties through the appearance of what are thought to be new races of the pathogen. This situation has been observed in some resistant varieties (e.g. CC 85-92, CC 84-75, CC 93-3895), where recently the disease has occurred at low severity. Therefore, an evaluation was made of the severity and possible variability of brown rust in the varieties selected by CENICAÑA in the Cauca River Valley. Samples were taken from plants from 1–14 months of age in the varieties CC 84-75, CC 85-92, CC 93-3895, CC 92-2804 and MZC 74-275 on 91 estates (10 sugar mills). On each plantation 20 stalks were selected at random, and the third leaf from the top visible dewlap leaf was taken from each stalk. Both disease reaction and severity were evaluated. Morphological and microscopic analyses of the structures found in the rust pustules were undertaken in leaf samples taken from each variety. Simultaneously, pathogen samples were collected and molecular techniques used (focusing on initiators of the ribosomal DNA (rDNA)) to detect possible genetic variation of *P. melanocephala*. The results showed that the disease reaction type in the varieties evaluated was 5 or less, with severities ranging from 0–12% leaf area affected. Variety MZC 74-275 showed susceptibility, with a reaction of 6 and a severity of 20% on the estates where it was evaluated. No differences were found among the morphological structures in the samples evaluated, all of which corresponded to *P. melanocephala*. The results obtained from the amplification of the ITS1 and ITS2 regions of the rDNA and from the PCR-RFLP did not show differences among the samples evaluated. These results could indicate that the variation of the pathogen is not reflected in its rDNA or that the molecular technique used was not sufficiently sensitive to detect small variations in the genome. Initial results about the presence or absence of gene Bru1 and susceptibility to brown rust are discussed.

Introduction

Brown rust of sugarcane caused by the fungus *Puccinia melanocephala* H. and P. Sydow was reported for the first time in the western hemisphere in 1978, spreading rapidly throughout the American continent.

In 1979, it was detected in the variety B 4362 in the provinces of Norte de Santander and Cesar in Colombia; two years later, it was found in the Cauca River Valley (Victoria *et al*., 1984; Victoria *et al*., 1988).

In Colombia, the disease occurs at greater severity in plants that are 4–5 months of age (Victoria *et al*., 1995); however, in older crops, the plant shows a certain degree of recovery, depending on varietal susceptibility (Victoria *et al*., 1995). In general, *P. melanocephala* delays plant development, which is manifested in a reduction in stalk length and the number of stalks per stool (Victoria *et al.*, 1984) resulting in a decrease of up to 40% in crop production in some susceptible varieties (Raid and Comstock, 2006).

The best strategy for controlling diseases is cropping resistant varieties; therefore, a prime objective of the CENICAÑA varietal improvement program is to select rust-resistant varieties. To do this, the disease is assessed at 4.5 months crop age, using the scale of Purdy and Dean (1981), which determines the genotype-pathogen interaction (type of pustule: 0–9) and percent severity in the third leaf from the top visible dewlap leaf.

Depending on pustule type, varieties with a score over 5 are considered susceptible and 5 or less, resistant. Variety selection considers disease severity but seeks high stability of the varieties in their reaction to the disease, thereby achieving vertical and horizontal resistance simultaneously (Victoria *et al*., 1989). Nevertheless, according to Raid and Comstock (2006), rust resistance has not been stable in some cane varieties due to the genetic variability of the pathogen. Various authors have proposed the existence of pathogen races, based on changes in the susceptibility of varieties previously reported to be resistant (Liu, 1980; Dean and Purdy, 1984).

The existence of rust races, based on differential varietal reactions, has been found in India and Florida (Ryan and Egan, 1989; Shine *et al*., 2005). Evaluation of *P. melanocephala* races requires an effective methodology for identifying genetic variability among the possible different pathogen populations. Molecular markers like AFLP and ITS provide a useful tool for monitoring genetic changes in populations. In addition, analysis of rDNA sequences have been used to establish phylogenetic tree and genetic variations in fungi (Hibbet, 1992

CENICAÑA's varietal improvement program developed varieties in the series 84, 85, 92 and 93 and these have stood out in recent years for their agronomic and factory traits. These varieties are being multiplied and tested on a commercial scale by the sugar mills. Varieties CC 85-92 and CC 84-75 currently hold first and second place in area under cultivation respectively; but at this time there are other outstanding varieties such as CC 92-2804, CC 93-3895, which are still in the process of being multiplied.

In the past, these varieties had low rust infection; but, in the last three years, there has been a gradual increase in the incidence of the disease. It is possible that this response is due to an interaction between the pathogen and varietal resistance.

The purpose of this study was to evaluate the presence and severity of rust in varieties under different environmental conditions; to evaluate isolates of the fungus collected in commercial crops throughout the Cauca Valley; to use ITS and PCR-RFLP molecular markers to determine the possible existence of pathogen races; to assess the possible presence of resistance genes or whether the increased incidence of rust is due to environmental factors.

Materials and methods

Evaluation of rust under different environmental conditions

Brown rust incidence was assessed in plantations on 91 estates, in the following sugar mills: Castilla (4), Cabaña (4), Incauca (3), María Luisa (2), Mayagüez (3), Manuelita (22), Providencia (8), Risaralda (16), Riopaila (8), Sancarlos (18) and Sicarare (3) between 2006 and 2008.

The evaluation was conducted in crops ranging from 1–14 months of age in areas destined for both seed and commercial production. Varieties CC 85-92, CC 84-75, CC 93-3895, and CC 92-2804 were evaluated; the susceptible variety MZC 74-275 was used as the check.

In each plantation, a sample was taken from 20 randomly-selected sites within a crop. At all sites and in each variety, the third leaf from the top visible dewlap leaf on stalks was used to

evaluate pustule type or disease reaction (scale of 0–9) and the percent severity of the disease (0–100), based on the guidelines defined by Purdy and Dean (1981).

Molecular evaluation

Samples

Ten leaves of varieties CC 92-2804, CC 93-3895, CC 85-92, CC 84-75, MEX 52-29, MZC 74-275, CP 57-603, CC 98-68 and CC 94-5827 were collected in different commercial crops. The samples were placed in plastic bags and conserved in a Styrofoam (foamed polystyrene) cooler with ice until they reached the laboratory where the molecular evaluation was undertaken.

Conservation of the pathogen and induction of sporulation

Leaf pieces (approx. 20 cm long) were taken from diseased leaves with a Type 4 reaction (Purdy and Dean, 1981); that is, with chlorotic or red spots and unopened pustules. The leaf pieces were washed with distilled water, then by ethyl alcohol at 70% and again washed with distilled water. The fragments were placed in glass jars containing 50 mL benzimidazole solution (12.5 mg/L) (Asnaghi *et al*., 2001), each of which was placed within PVC cylinders with the openings covered with netting (tulle). They were then incubated under glasshouse conditions at 25°C, 60–85% RH for 1–2 weeks until sporulation.

Extraction of the fungal DNA

The methodology described by Virtudazo *et al*. (2001a) was used to enable DNA extraction from the spores contained in one pustule, so preventing the mixing of possible races in different pustules found on the same leaf.

Amplification of DNA fragments

ITS markers were used to amplify the ribosomal DNA (rDNA), a technique used in different phylogenetic and population fungal genetics studies (because there are multiple copies in the genome these are easy to amplify). Different pairs of initiators from both the rDNA and other regions of the genome were evaluated. The PCR products obtained after amplification were visualised in agarose gels al 1.5% (for fragments <1000 pb), run in TBE at 0.5 X and tinctured with ethidium bromide.

***Amplification of the ITS 1 and ITS 2 regions for the harvested population of* P. melanocephala**

The regions with the best amplification were selected for evaluating the total population collected in the Colombian sugar zone. The ITS 1 region was selected for amplification (240 isolates in total), following the conditions described by Virtudazo *et al.* (2001a) with an annealing temperature of 56°C. The results were observed in agarose gels at 1.5%, tinctured with ethidium bromide.

Enzymatic restriction of the resulting PCR products

Different restriction enzymes (e.g. *Hinf I, Xba I, Hae III, Alu I, Taq I, Mse I* and *Ava II*) were used to find one that would digest the DNA and make it possible to visualise differences among the samples analysed. Fragments from the ITS 1 and ITS 2 regions (annealed PCR) were used. For the enzymatic restriction, 2.5 units of each tested enzyme were used with the associated buffer supplied by the commercial house, the latter at a final concentration of 1 X. The digestion temperature for the different enzymes was 37°C, except for *Taq* I, whose optimum temperature is 55°C. The digestion time for the different restriction reactions was 3 h 30 min., followed by a cycle of 80°C for 20 min. to denature the enzyme used. The results obtained with the different restriction enzymes were observed in agarose gels at 2.0%, run in TBE 0.5 X and tinctured with ethidium bromide.

Results and discussion

The measurement of pathogen paraphyses and urediniospores contained in rust pustules on leaves of the varieties CC 85-92 and CC 93-3895 confirmed that the infection was caused by *P.*

melanocephala, causal agent of brown rust (Virtudazo *et al*., 2001b). Abundant paraphyses were observed, the urediniospores were ovoid in shape, and brown-coloured teliospores were observed. In general, all the traits observed coincided with the descriptions of *P. melanocephala* and not *P. kuehnii* (Virtudazo *et al*., 2001b).

Evaluation of the rust under different environmental conditions

Variety CC 85-92, evaluated on 23 estates in 9 sugar mills, had one Type 5 disease reaction with a rust severity less than 8% in plants 4–6 months of age. At younger ages (4 months), the greatest severity was 8% with a Type 5 reaction in plants on the Palosecal 5 estates of Incauca. At older ages, the severity ranged from 0–4%, with types 0–5 reaction.

Variety CC 84-75 was evaluated on 9 estates of the Providencia, Riopaila and Castilla sugar mills. The highest disease severity was 10% with a Type 5 reaction at crop ages of 4.6 and 5.0 months on the Venecia 340 and La Luisa 80 estates of the Riopaila Sugar Mill. At crop age less than 4 months, disease severity ranged from 0–8% with reactions from 0–3. At older ages, disease severity and reaction were both zero (Table 1).

Table 1—Incidence of brown rust in variety CC 84-75 on different estates and sugar mills in the Cauca Valley.

Sugar mill/estate	Age	Rust*	
	(Mo)	R	S
PROVIDENCIA			
El Edén	4	3	1
Zabaletas Racines 7	3.5	4	8
RIOPAILA			
Valparaíso	3.5	0	0
Venecia	4.6	5	10
La Luisa 080	5	5	10
Riopaila 590	6	4	2
Riopaila 092	7.5	0	0
CASTILLA			
La Porfina 050	7	0	0
Potrerillo 050	4	4	1
* R: Reaction (Scale 0–9); S: Severity (0–100%)			

Variety CC 93-3895 was evaluated on 39 estates in 7 sugar mills. The greatest severity (15%) was found on the Esmeralda Estate of the Sancarlos Sugar Mill with a Type 5 pustule score at 4 months of age. At younger crop ages, disease severity ranged from 0–12% with reactions from 0–5. From 6–8 months, the score remained at 5, with severities ranging from 10–12%. At older ages (9 months), the severity was lower (Table 2). Variety CC 92-2804 was evaluated on three estates in the Sancarlos, Providencia and Risaralda sugar mills. Disease severity in these lots did not exceed 2%, with types 4 and 5 reactions. The check MZC 74-275 continued to show its susceptibility on all three estates with disease severities of 20% and a Type 6 reaction (Table 3).

It should be noted that every year CENICAÑA selects new varieties resistant to smut, brown rust and mosaic. This does not mean that these varieties are immune, given that immunity can easily cause the generation of pathogenic variations (new races) of the causal agents. In the case of brown rust, varieties with disease reactions from 0–5 are considered resistant, with severities less than or equal to 15% leaf area affected.

A reaction score of up to 5 is acceptable because climate (temperature, rainfall and RH) has little effect on disease severity with varieties of this rating; however, at higher scores, severity is highly affected by the climatic conditions (Victoria *et al*., 1989).

Table 2—Incidence of brown rust in variety CC 93-3895 on different estates and sugar mills in the Cauca Valley.

Sugar mill/estate	Age	Rust*	
	(Mo)	R	S
MANUELITA			
Palmera Guzmán 80	3.2	5	4
Santa Anita 27 B	3.3	5	12
Palmera Guzmán 60B	3.5	5	2
Palmera Guzmán 60A	3.5	5	2
Florencia 222A	3.5	5	2
Santa Anita 31 A	3.8	5	12
Santa Anita 27 B	4.5	5	12
Florencia 226	6.0	5	12
Santa Anita 32	6.7	5	12
Rita 4	6.7	5	12
Cascajal 42 A	7.0	5	10
Hacienda Real	7.0	5	12
Santa Anita 27 B	7.4	5	12
Rosario 149 B	7.4	5	12
Palmera Guzmán 60 A	8.8	5	12
Florencia 222 A	9.3	5	7
Olga 145 A	10.5	5	7
Florencia 226	11.4	5	7
Cascajal 42 A	12.4	5	7
Cascajal 46	14.5	4	5
RISARALDA			
San Luis 8	2.5	5	1
Riogrande 2	3.9	5	1
Estampilla 4	4.1	5	6
Santa Lucía 6	7.0	5	10
Bohios 24	7.8	5	7
Bohios 24	8.8	5	4
San Luis 7	11.0	0	0
SICARARE			
Pororo 9-12	4.0	0	0
Yerbera 5	4.0	0	0
Pororo 9-12	8.5	0	0
MARÍA LUISA			
Remolinos	8.0	5	5
MAYAGÜEZ			
Margarita 4 A	7.0	5	12
RIOPAILA			
Riopaila 56	6.0	5	8
Riopaila 092	8.5	0	0
SANCARLOS			
Esmeralda 87A	4.0	5	15
Esmeralda 86	7.0	5	10
Esmeralda 86	10.0	0	0
* R: Reaction (Scale 0–9); S: Severity (0–100%)			

Brown rust severity evaluation in varieties CC 85-92, CC 84-75, CC 93-3895 and CC 92-2804 showed that the reactions reached a maximum of 5 with a severity under 12%, which means that these varieties remain at rust-resistant levels (Victoria *et al.*, 1989).

Varietal selection and regional trial records suggest that disease severity has remained in the stated ranges.

Table 3—Incidence of brown rust on variety CC 92-2804 and MZC 74-275 on different estates and sugar mills in the Cauca Valley.

Variety/sugar mill/estate	Age	Rust*	
	(Mo)	R	S
CC 92-2804			
SANCARLOS			
Mojón 13	3.0	4	1
PROVIDENCIA			
Aurora 107B	6.0	4	2
RISARALDA			
Estampilla 2	4.1	5	1
MZC 74-275			
SANCARLOS			
Argelia 212A	5.0	6	20
RISARALDA			
Galia sopinga 32	2.2	5	7
* R: Reaction (Scale 0–9); S: Incidence (0–100%)			

Molecular evaluation

Genetic variability was assessed in the different rust samples collected on the affected plantations, using different ITS ribosomal markers and the PCR-RFLP methodology.

The results obtained did not show differences in the molecular size of the bands among the samples evaluated (Figure 1).

All the samples evaluated with the ITS initiators showed one intense band of approximately 670 pb, thereby making it impossible to obtain differences demonstrating pathogen variability. The PCR-RFLP technique increased the number of bands in each analysis, but did not show differences among the samples evaluated.

Figure 2 shows the results obtained after digestion of the ITS 1 region with restriction enzyme *Alu* I. The results obtained with the other enzymes were similar with no polymorphism among the samples.

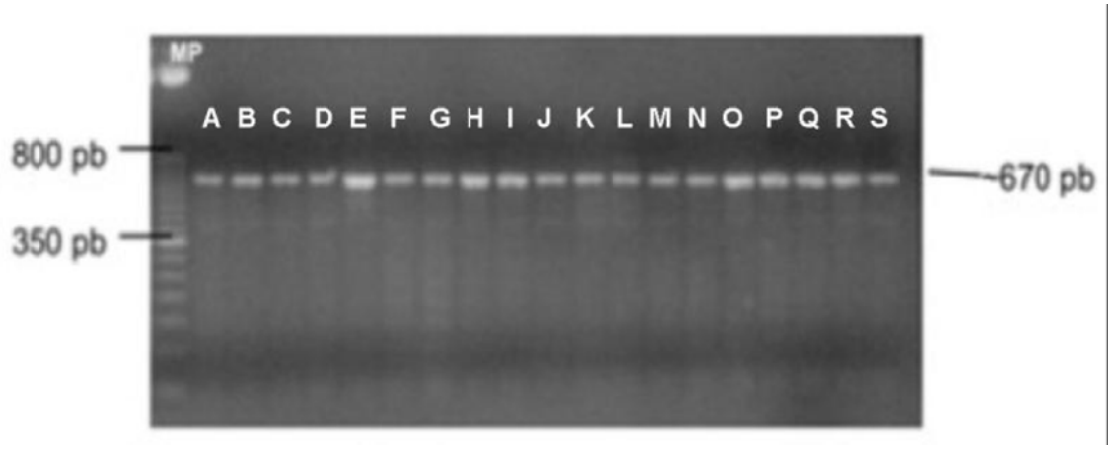

Fig. 1—PCR products (670 bp) of the amplification of the ITS 1 region of *P. melanocephala* in different isolates collected in the Colombian sugar zone. The result for 240 samples evaluated was similar to that recorded for these 19 samples. MP: Molecular weight marker, 50 pb.

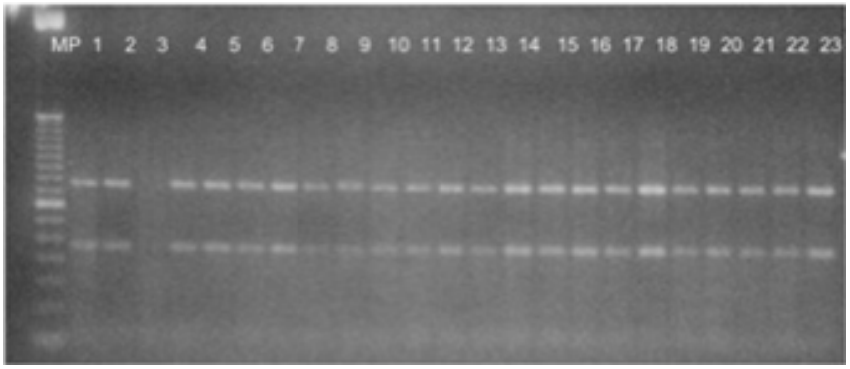

Fig. 2—Enzymatic digestion with enzyme *Alu* I of the amplified product from the ITS 1 region. It can be observed that the pattern of bands obtained with this restriction is similar for the different samples evaluated. Sample 3 does not have the pattern of bands due to one poor amplification that made it impossible to observe the result of the restriction.

In addition to the ITS 1 region of the rDNA, other DNA regions of the pathogen were evaluated; however, again there was no polymorphism with the different initiators, in agreement with results obtained with the ITS 1 region analysis. Similarly, the results obtained using the amplified ITS 2 region after digestion with restriction enzymes did not show differences among the samples evaluated, as can be seen in Figure 3.

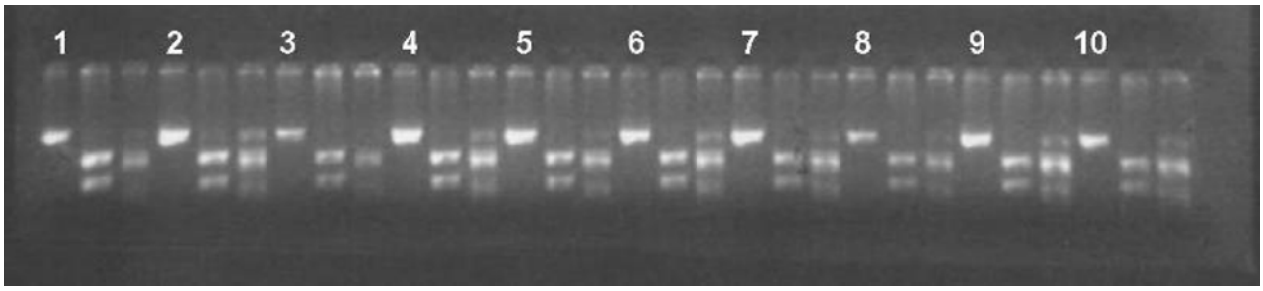

Fig. 3—Enzymatic restriction with enzymes *Mse* I and *Taq* I from the ITS 2 region. In Lane 1, one nondigested sample is observed, followed by the same sample digested with the *Taq* I enzyme and, in the next lane, digested with *Mse* I. In total there are 10 samples of rust under the same conditions.

Molecular tools failed to link pathogen genetic variability with variation in disease severity in varieties. However, this situation does not mean that there are no allelic differences among different pathogen samples.

This could be the result of the low resolution of the techniques used, which are only capable of detecting macro modifications at the pathogen DNA level. Limiting the study to the ribosomal region of the DNA limits the scope of the evaluation, given that many of the changes at the genomic level of the populations are not necessarily reflected at the rDNA level, especially if these changes imply an increase in the performance of a group of individuals due to specific changes in genes related to pathogen virulence.

The exclusive utilisation of rDNA in this study was primarily due to the impossibility of obtaining large quantities of DNA from the limited fungal tissue available and the impossibility of *in vitro* cultivation given that it is a biotrophic organism, incapable of growing in axenic media (Braithwaite *et al.,* 2009).

This situation limited the use of other molecular markers such as AFLP. This technique is capable of the rapid discrimination of samples due to the great polymorphism that it generates, but this also requires larger quantities of DNA (200–500 ng); this is not possible to obtain from just one rust pustule.

The potential limitation of the molecular techniques for detecting gene variation is reflected by the ability to separate genetically 1305 varieties of CENICAÑA's germplasm using five microsatellites, but it was not possible to separate somatic variations (highly susceptible to rust) found in normally resistant variety CC 01-86 (Macea *et al*., 2009). The microsatellite analysis showed that both genotypes were similar. Recently, Garsmeur *et al.* (2009) reported the presence of a major gene (*Bru1*) associated with brown rust resistance in the variety R 570 and common to most rust-resistant varieties. According to these authors, only 13% of the resistant varieties lack the *Bru1* gene, suggesting that their resistance is due to the presence of another resistance gene. The *Bru1* gene found in the original CC 01-86 was not found in the somatic variations. Nevertheless, in CENICAÑA, brown rust has been observed with equal severity in varieties that do have the *Bru1* gene (21.6% of the affected varieties have the gene) and those that lack it. In addition, the disease has also been absent in varieties that lack the *Bru1* gene (34% of the resistant varieties) (Gutiérrez, A.F., unpublished information).

Conclusions

Brown rust was found affecting some resistant varieties developed by CENICAÑA.

Rust resistance has been consistent in the varieties CC 85-92, CC 84-75, CC 93-3895 and CC 92-2804.

The results obtained in this study did not show genetic variability among different samples of brown rust collected from Colombian sugarcane crops.

Studies that use other more informative molecular markers, such as AFLP and cDNA-AFLP, or that include sequencing of the fragments similar to that obtained with PCR may be required to detect nucleotide changes among the evaluated samples. This will help confirm the existence or not of genetic diversity in the rust isolates.

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SURVEILLANCE CONTINUE DE LA SÉVÉRITÉ ET LA VARIABILITÉ DE LA ROUILLE BRUNE (*PUCCINIA MELANOCEPHALA*) DANS LES VARIÉTÉS DE CANNE À SUCRE DANS LA VALLÉE DE CAUCA EN COLOMBIE

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MOTS CLÉS: Canne à sucre, Rouille Brune, Incidence de Maladie, Résistance, ADN Ribosomique (rDNA), Races Physiologiques.

Résumé

LES VARIÉTÉS CC 85-92 et CC 84-75, initialement résistantes à la rouille brune, sont cultivées sur plus de 80% de la superficie sous canne à sucre en Colombie. La maladie est présente en Colombie depuis 1979. La variabilité génétique du *Puccinia melanocephala* est estimée avoir affecté la résistance de certaines variétés de par l'apparition de nouvelles races du pathogène. Cette situation a été observée dans quelques variétés résistantes (e.g. CC 85-92, CC 84-75, CC 93-3895) où la maladie était apparue à faible intensité. En conséquence, une évaluation de la sévérité et de la variabilité de la rouille brune a été effectuée dans les variétés sélectionnées par le CENICAÑA dans la vallée de la Rivière Cauca. Les échantillons ont été collectés des variétés CC 84-75, CC 85-92, CC 93-3895, CC 92-2804 et MZC 74-275, âgées de 1–14 mois, sur 91 établissements (10 sucreries). Pour chaque plantation, 20 tiges ont été choisies au hasard, et la troisième feuille de TVD collectée de chaque tige. La réaction et la sévérité face à la maladie étaient évaluées. Des analyses morphologiques et microscopiques de la structure des pustules de rouille étaient entreprises sur les échantillons de feuille pour chaque variété. Simultanément, des échantillons du pathogène ont été collectés et analysés par les techniques de biologie moléculaire (en se focalisant sur les initiateurs d'ADN ribosomique (rDNA)) pour détecter une éventuelle variabilité génétique du *P. melanocephala*. La réaction des variétés testées atteignait au maximum le niveau 5 sur l'échelle d'évaluation, avec des sévérités s'étendant de 0-12% de la surface de la feuille. La variété MZC 74-275 a montré une sensibilité de niveau 6 et une sévérité de 20% sur tous les établissements où elle a été évaluée. Aucune différence n'a été observée parmi les structures morphologiques dans les échantillons, toutes correspondaient au *P. melanocephala*. Les résultats obtenus de l'amplification des régions ITS1 et ITS2 de la rDNA et de la PCR-RFLP n'ont pas montré de différence parmi les échantillons examinés. Ces résultats démontreraient que la variabilité du pathogène ne se situait pas au niveau de son rDNA ou bien que les techniques moléculaires utilisées n'étaient pas suffisamment sensibles pour détecter des variabilités minimes dans le génome. Les résultats initiaux sur la présence ou l'absence du gène *Bru1* et la sensibilité à la rouille brune sont discutés.

SEGUIMIENTO Y VARIABILIDAD DE LA ROYA CAFE (*Puccinia melanocephala*) EN VARIEDADES DE CAÑA DE AZÚCAR EN EL VALLE DEL CAUCA

Por

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PALABRAS CLAVES: Roya Café, Incidencia, ADN ribosomal (ADNr), Resistencia, Variación.

Resumen

LAS VARIEDADES CC 85-92 y CC 84-75 ocupan más de los 80% del área sembrada con caña en el sector azucarero colombiano, variedades inicialmente resistentes a la roya café. Esta enfermedad se encuentra presente en Colombia desde 1979. La posible variación genética de *P. melanocephala* ha afectado la resistencia en algunas variedades probablemente debido a la aparición de nuevas razas del patógeno. Esta situación se ha observado en algunas variedades resistentes (CC 85-92, CC 84-75, CC 93-3895, etc.) y en donde recientemente se ha observado la enfermedad con baja severidad. Por lo cual se procedió a evaluar la severidad y variabilidad de la roya café en las variedades seleccionadas por Cenicaña en el valle del río Cauca. Se realizaron visitas a 91 haciendas de 10 ingenios sembrados con las variedades CC 84-75, CC 85-92, CC 93-3895, CC 92-2804 y MZC 74-275. En cada plantación se tomó al azar la tercera hoja a partir de la hoja TVD de 20 tallos y se evaluó la reacción y severidad de la enfermedad. Un análisis morfológico y microscópico de las estructuras encontradas en las pústulas de las diferentes variedades se realizó en las muestras recolectadas. Simultáneamente se realizó la recolección de diferentes muestras del hongo y mediante el uso de técnicas moleculares (ADN ribosomal (ADNr)), se determinaron las relaciones filogenéticas y variaciones genéticas en el hongo. Se evaluaron plantaciones desde los 1 a 14 meses de edad. Los resultados mostraron que en las variedades evaluadas el tipo de pústula fue de 5 o menos con severidades entre 0 y 12%. En las haciendas evaluadas con MZC 74-275, la variedad presentó susceptibilidad con reacción 6 y severidad del 20 %. No se encontraron diferencias entre las estructuras morfológicas en las muestras evaluadas y correspondieron a *P. melanocephala*. Los resultados de la amplificación de las regiones ITS1 e ITS2 del ADNr no mostraron diferencias entre las muestras evaluadas. Por esto se procedió a realizar digestión de los productos de PCR con enzimas de restricción, sin embargo tampoco se observaron diferencias entre las muestras. Estos resultados podrían indicar que la variación del patógeno no se refleja en su ADNr o que la técnica molecular utilizada no es lo suficientemente sensible para detectar pequeñas variaciones en el genoma. Resultados iniciales sobre la presencia o ausencia del gen Bru1 y la susceptibilidad a la roya son discutidos.