RESISTANCE SCREENING OF PROMISING SUGARCANE CLONES TO TWO RACES OF GUMMING DISEASE BACTERIUM

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Abstract

TWO RACES of the gumming disease bacterium (*Xanthomonas axonopodis* pv. *vasculorum*), Races 1 and 2, commonly infect sugarcane varieties in Mauritius. Clones selected from the 3rd clonal stage (years 4–5) of the breeding program are screened for resistance to these two races. From 1998 to 2007, 878 clones were tested in disease resistance trials in localities where the races are prevalent. Inoculated spreader rows of a susceptible variety act as the source of inoculum for the test varieties. Evaluation to Race 1 was carried out at one site; whereas, testing against Race 2 was conducted at two to four sites. Screening showed that 99% of clones were immune to Race 1. Of the 9 clones that showed infection, seven were classified as resistant, one slightly susceptible and one susceptible. In contrast, the percentage of clones showing immunity to Race 2 was much lower (14%). Resistance, slight susceptibility, susceptible, and high susceptibility to Race 2 were 44%, 23%, 13% and 6%, respectively, among the remainder of the clones. Ratings were consistent across locations for 78% of clones. Evaluation at more than one site was consequently justified. The pathogen was isolated from commercial fields in regions prone to the disease and in germplasm collections during the period 1998–2007. Race characterisation was based on cultural characteristics and pathogenicity tests. Race 1 was readily recovered in noble cane collections. Apart from one instance in 1998, it was absent in commercial fields. These surveys confirmed that commercial varieties were highly resistant to Race 1 and, therefore, of insignificant importance in *Saccharum* hybrids. Gumming disease prevalent in plantations was therefore almost exclusively caused by Race 2. This race was predominant and resistance screening against it in different environments is necessary.

Introduction

Gumming disease of sugarcane caused by *Xanthomonas axonopodis* pv. *vasculorum* is present in some 25 countries (Rott and Girard, 2000). The population of the pathogen is heterogeneous and five groups have been characterised on the basis of fatty acid analysis and genetic variability (Dookun *et al*., 2000; Saumtally, 1996). It is considered as the most important bacterial disease of sugarcane in Mauritius and three races, Races 1, 2 and 3 have been reported in order of their appearance (Ricaud and Autrey, 1989). Race 1 dates back to 1848, infects noble cane (*Saccharum officinarum*) and was presumably introduced when sugarcane was brought for cultivation on the island. Noble canes are no longer cultivated but this race can be readily found in germplasm collections. Race 2 appeared in 1964 and caused an epidemic on cultivar M 147/44. It is frequently encountered in parts of the island on susceptible varieties. Race 3 appeared in epidemic proportion in 1980 and particularly affected varieties M 442/51, M 31/45 and M 377/56. The latter two varieties were released as resistant to the disease. With the eradication of M 377/56, Race 3 has

been absent in sugarcane fields for a number of years. Control of gumming disease relies on preventive measures such as sanitation, disease-free planting material and, more importantly, the cultivation of resistant varieties. The latter has been very effective in minimising the spread of the disease and has led to its eradication in Australia and West Indies as well as reducing its impact in Brazil, the Caribbean Islands and Fiji (Ricaud and Autrey, 1989). In Mauritius, screening of clones is during years 4–5 of the 9–12 year selection program, when the number of varieties is reduced to less than 150. Variety evaluation is conducted at different sites, in relationship to the distribution of the races of the pathogen. Because of the absence of Race 3 during recent years, screening to this race has been discontinued. This paper analyses the results of variety evaluation to Races 1 and 2 from 1998 to 2007, and discusses the necessity of screening varieties to the disease in the light of the distribution of the races around the island.

Materials and methods

Resistance screening and disease ratings

Varieties selected from the 3rd clonal stage of the breeding program (years 4–5) were planted in July each year in resistance trials for evaluation of resistance to *X a* pv. *vasculorum*. Testing for resistance to Race 1 was conducted at one site (Réduit), while trials for resistance to Race 2 were established at two to four sites (Case Noyale, Ferney, Médine and Yemen). In all trials, highly susceptible varieties, acting as spreader rows, were inoculated in January with a bacterial suspension of either Race 1 or Race 2 at a concentration varying from 1 x 10^8–10^9 cells/mL. Varieties under evaluation were planted on 3 m-row flanked by the artificially infected varieties. Test varieties along a row were also separated by a strip of inoculated varieties. Mixed *S officinarum* clones acted as spreader rows for Race 1, while varieties M 147/44 and M 377/56 were used as inoculum lines for Race 2. The pathogen was naturally transmitted by leaf contact to the test varieties planted without replication.

Standards included in Race 1 trials were varieties M 147/44, M 31/45, M 377/56, M 3035/66 (all resistant), B 34104 and M 55/1182 (both highly susceptible). With the exception of M 31/45, also a resistant standard for Race 2, all resistant controls for Race 1 were included in Race 2 trials as susceptible checks, while M 442/51 was the slightly susceptible control for this race. Assessment was carried out 6 and 8 months after inoculation on a qualitative scale based on the severity of infection as follows: absence (immune), short stripes on old leaves (resistant), medium length stripes on old leaves (slightly susceptible), long stripes on old leaves with or without short stripes on young leaves (susceptible). Varieties are considered highly susceptible when they exhibited one or more of the following symptoms: heavy striping with several long stripes, leaf chlorosis, gum exudation and death of stalks. A second 3-point scale was also used to quantify abundance of foliar and systemic infection and further assess the degree of infection: I (low), II (intermediate) and III (high). For any given year, the same clones were evaluated at the different sites.

Collection of isolates and race characterisation

Isolates of *X a* pv. *vasculorum* were collected from commercial sugarcane fields during island-wide surveys and from variety collections from April to October during 1998–2007. Inspections for gumming disease in susceptible varieties were made at locations known to be foci of infection. Sites surveyed were Belle Rive, Étoile, Ferney, Le Val, Quatre Soeurs (East), Case Noyale, Le Morne, Médine, Yemen (West), Quatre Bornes and Réduit (Centre). Varieties examined for symptoms were: *S officinarum* clones, M 147/44, M 377/56, M 351/57, M 555/60, M 3035/66, M 1557/70, M 1286/89, M 2454/95, R 570 and R 579. The pathogen was isolated from sugarcane leaves or stalks. Infected leaf strips were surfaced disinfected with 70% ethanol for 1 min, washed in sterile distilled water (SDW) and directly streaked on sucrose peptone medium (composition per litre: peptone, 5.0 g; KH_2PO_4, 0.5 g; $MgSO_4.7H_2O$, 0.25 g; sucrose, 20 g; agar 15.0 g; pH 7.0). Diseased stalks were washed and the node thinly sliced after removing the rind. The sliced pieces

were allowed to diffuse in 5 mL SDW for 30 min before streaking the suspension on sucrose peptone medium. The incubation period was 3–5 days at 30°C.

Races were distinguished by their growth habit on TZC agar slants (composition per litre: peptone, 10 g; glucose, 5 g, casein hydrolysate, 1 g; tetrazolium chloride, 0.05 g; agar 15 g; pH 7.2) after 7–10 days incubation at 30°C. Race 1 is distinguished from Race 2 by the formation of a compact growth at the top of the tube owing to its viscous exopolysaccharide (EPS). In contrast, the EPS of Race 2 is more fluid and the colony flows down to the bottom of the slant. Confirmation of race identity was obtained by pathogenicity tests using noble cane varieties Iscambine Stripe, Senneville (*S officinarum*) as well as hybrids M 147/44, M 31/45 and M 3035/66. Inoculated plants required monitoring for symptom development for up to two months. Race 1 infects the noble canes but none of the hybrids. Race 2 induces symptoms in all varieties, except M 31/45. Symptoms expression also differed between the two races. Race 1 only rarely causes slight leaf chlorosis. This symptom is common and intense in plants inoculated with Race 2.

Results

Resistance screening and disease ratings

Table 1 shows the annual and total number of clones tested from 1998 to 2007 across the various sites.

Table 1—Sites of gumming disease resistance trials and number of sugarcane clones tested from 1998 to 2007. The same clones were tested at all sites in the same year.

Years	Réduit (Race 1)	Case Noyale (Race 2)	Ferney (Race 2)	Médine (Race 2)	Yemen (Race 2)
1998	80	80	80	80	80
1999	135	135	135	135	–
2000	143	–	143	143	143
2001	69	69	69	69	69
2002	79	79	79	–	79
2003	94	94	94	–	94
2004	90	90	90	–	90
2005	61	61	61	–	61
2006	65	65	65	–	–
2007	62	–	62	–	62
TOTAL	878				

Inoculum pressure was adequate in the trials. For Race 1, the highly susceptible checks displayed the most severe symptoms of the disease. On the other hand, *Saccharum* hybrid controls were all immune to this race. For Race 2, the highly susceptible cultivars M 147/44, M 377/56 and M 3035/66 exhibited susceptibility to high susceptibility in all trials in conformance to their observed field reactions. The resistant variety M 31/45 displayed a resistant reaction to the two races, while M 442/51 was slightly susceptible to Race 2.

Screening to Race 1 revealed that most of the clones were immune. Of the 878 clones tested, 99% did not show any symptom to this race. Of the 9 clones that were infected, seven were resistant (M 1226/91, M 1954/91, M 1487/92, M 2773/94, M 2655/95, M 1641/97 and M 2181/00), one slightly susceptible (M 3096/94) and one susceptible (M 1866/90). When comparing the reactions of Race 1 resistant clones to that obtained with Race 2, all were more susceptible to the latter race, except clone M 1954/91 which was also resistant. Clone M 3096/94 was slightly susceptible to both races.. However, inversely to the other clones, M 1866/90 susceptible to Race 1 was resistant to Race 2.

Resistance trials to Race 2 from 1998–2007 showed that the ratings were consistent for most of the clones across two or more sites where the varieties were evaluated. However, in 196 cases (22%), the reactions were variable. Overall, the percentage of promising clones immune, resistant, slightly susceptible, susceptible and highly susceptible to Race 2 were 14%, 44%, 23%, 13% and 6% respectively (Figure 1). Combining immune, resistant and slightly susceptible reactions, more than 80% of the varieties had adequate resistance to Race 2.

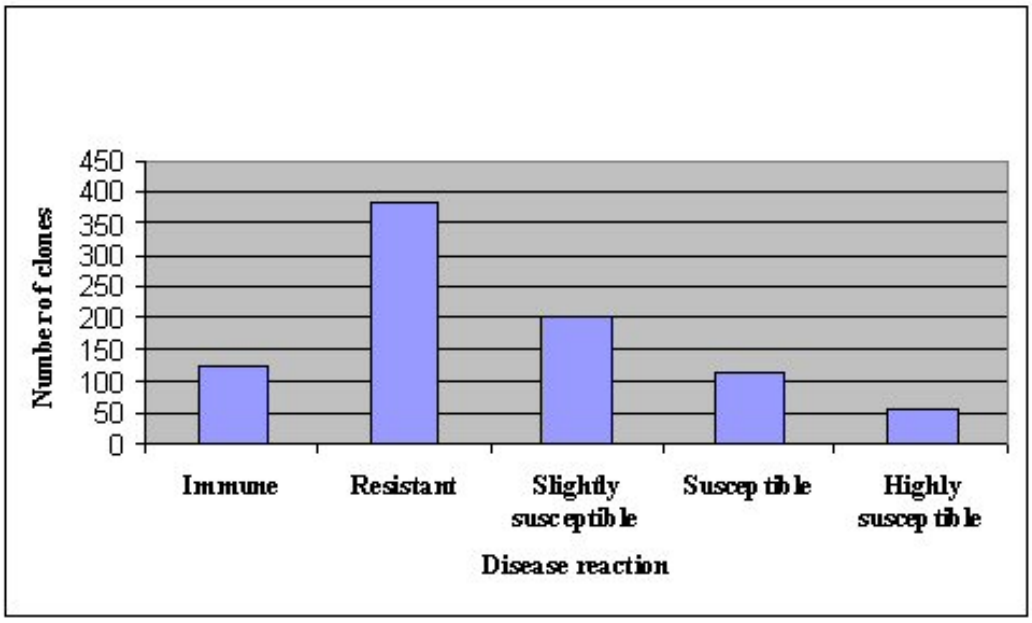

Fig. 1—Summary of reaction of clones to Race 2 of gumming disease evaluated from 1998–2007.

Collection of isolates and race characterisation

Two hundred and sixty isolates were collected between 1998 and 2007 from 10 sugarcane varieties and 13 *S officinarum* clones situated at 11 locations. Isolations on culture media were taken mostly from foliar stripes, as these were the most frequently encountered symptom, but also from systemically infected stalks. In the latter cases, the varieties concerned were *S officinarum* and the hybrid M 3035/66. Smooth, circular and very mucoid yellow colonies appeared after 3–4 days on sucrose peptone and recognisable as that of *X a* pv. *vasculorum.*

The race of the pathogen was determined by sub-culturing the colonies from sucrose peptone agar to tube slants of TZC medium, where the bacterium produced dark red colonies. Race 1 could be differentiated by its compact mucoid growth in contrast to the less viscous Race 2 that flowed down the tube after 4–5 days incubation. Pathogenicity tests were used to differentiate Races 1 and 2.

For Race 1 inoculated in *S officinarum* clones Iscambine Stripe and Senneville, foliar stripes were yellow at first, then turning necrotic along most of the length of the stripe. Leaf chlorosis in noble canes was slight and of rare occurrence, as the infection progressed and became systemic. Race 1 was not capable of causing lasting infection for more than two weeks in hybrid varieties and foliar symptoms eventually disappeared. In contrast, Race 2 produced long yellow stripes and intense leaf chlorosis in hybrids M 147/44, M 377/56 and M 3035/66 and *S. officinarum* clones. None of the two races could infect variety M 31/45.

Of the 260 isolates obtained, 97 of them belonged to Race 1. The race was isolated from *S officinarum* clones in germplasm collections present at Réduit. It was absent at all other sampling sites, except in one instance in 1998 when it was found in variety R 570 at Quatre Soeurs. It was not

found in subsequent surveys, except at Réduit. Race 2 was not isolated from *Saccharum* hybrids at Réduit but could be isolated from plantations at all other locations.

Discussion

In breeding programs across the world, resistance to main diseases is one of the major objectives. In Australia, clones are tested for resistance to diseases such as chlorotic streak, Fiji leaf gall, leaf scald, mosaic, orange rust, pachymetra root rot, ratoon stunt, red rot, smut and yellow spot in disease resistance trials and Final Assessment Trials before being approved for cultivation by the industry (Croft *et al*., 2000, Croft, *et al*., 2008, Magarey *et al*., 2009). In Louisiana and Florida, USA, varieties are evaluated for dry top rot, eye spot, leaf scald, mosaic, ratoon stunt, rust, smut and yellow leaf. In Mauritius, sugarcane varieties are tested against gumming, leaf scald, rust, smut and yellow spot. The general policy is that a variety susceptible to a particular disease is not recommended in an area where the disease is prevalent.

Variety improvement programs aim at crossing parents with adequate resistance to obtain a high proportion of resistant progeny. Nonetheless, owing to the heterozygous polyploid nature of sugarcane, resistance screening is necessary. Resistance is an important component in the management of sugarcane diseases and has proved powerful for controlling gumming disease. Screening needs to take into account the occurrence of variants of the pathogen that exist in the environment. Thus, evaluation of clones to gumming disease was started in Mauritius in 1932 and at that time resistance trials were established against Race 1.

With the appearance of new races, clones were simultaneously screened in 1964 and then in 1982 to Race 2 and Race 3 respectively. Gumming disease is considered to be a serious threat and, during the past 25 years, only two susceptible varieties, M 3035/66 and R 579, have been released in Mauritius for specific localities where the disease is not prevalent. This approach has been instrumental in managing the disease effectively.

In countries such as Brazil and Australia, the cultivation of resistant clones has been successful to the extent that the disease has been eradicated or reduced to low levels. Legislation for the replacement of susceptible varieties by resistant ones has been promulgated in Australia and Mauritius during gumming disease epidemics with positive effects (Ricaud and Autrey, 1989). In Mauritius, with the replacement of variety M 377/56, Race 3, the cause of an epidemic on this variety, can no longer be observed in sugarcane fields.

Data obtained from resistance trials from 1998 to 2007 showed that 99% of clones tested were immune to Race 1. Resistance to Race 2 was also high (58%), reaching over 80% if slightly susceptible varieties are included. However, compared to Race 1, the magnitude of resistant clones was much less. Race 1 occurs mostly at Réduit and, in that respect, this location had been chosen for variety testing against this race.

In contrast, Race 2 is more widely distributed, hence the choice of diverse sites and also as a measure of its importance in commercial fields. Screening against Race 2 at more than one location is justified owing to discrepancies recorded between sites. The final disease rating of a variety to this race is based on the highest score obtained. Surveys conducted during 10 years showed that Race 1 was recovered only once in commercial fields. In contrast, Race 2 was more predominant on *Saccharum* hybrids and is almost exclusively the race isolated from commercial sugarcane fields. The results of screening and race distribution indicate that the potential of Race 1 in causing disease in hybrids is low compared to Race 2.

The marked difference in variety reactions calls for a reassessment of the status of the races of the bacterium. The variability could well be more profound. Races 1 and 2 are distinguishable by several methods, including cultural characteristics, pathogenicity tests, serology, fatty acids profile, restriction fingerprinting and restriction fragment length polymorphisms (Dookun *et al*., 2000; Saumtally, 1996; Vauterin *et al*., 1995).

Despite the fact that the occurrence of Race 1 is insignificant in commercial fields and almost all the clones evaluated are resistant, it is proposed to continue screening with regards to the development of new types of varieties, with different genetic background compared to current ones, meant for biomass and fibre production. Furthermore, gumming disease is known to infect palms, broom bamboo, Guatemala grass and maize. A thorough search of *X a* pv. *vasculorum* on these hosts would provide a more comprehensive study on race distribution of the pathogen.

Conclusions

The establishment of systematic resistance trials to races of the gumming disease pathogen in Mauritius is a unique situation in sugarcane variety screening. The disease reactions of the various clones and island-wide collection of isolates have allowed an assessment of the threat posed by the disease.

The low ability of Race 1 to cause disease in current sugarcane genotypes, the absence of Race 3 in sugarcane fields and an overall decrease in gumming disease pressure during the last 25 years is comforting. Nevertheless, the identification of resistant varieties should continue so as to further decrease inoculum pressure.

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ÉVALUATION DES CLONES PROMETTEURS DE CANNE À SUCRE À DEUX RACES DE LA BACTÉRIE DE LA GOMMOSE

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Résumé

DEUX RACES de la bactérie de la gommose (*Xanthomonas axonopodis* pv. *vasculorum*), Races 1 et 2, causent régulièrement une infection de la canne à sucre à Maurice. Les clones issus du 3ème stade clonal (4–5 années) du programme d'amélioration génétique sont évalués pour leur résistance aux deux races. De 1998 à 2007, 878 clones ont été testés dans des essais d'évaluations dans divers sites où les races sont présentes. Des rangs de canne des variétés sensibles artificiellement inoculées agissaient comme source de disséminement de la maladie aux variétés en évaluation. Le criblage à la Race 1 fut effectué sur un site et sur deux à quatre sites pour la Race 2. Les résultats de ces essais ont montré que 99% des clones étaient immunes à la Race 1. Des 9 clones qui étaient infectés à divers degrés, sept étaient résistants, un légèrement sensible et un sensible. Le pourcentage de clones démontrant une immunité à la Race 2 était bien plus faible (14%). Les pourcentages de clones résistants, légèrement sensibles, sensibles et très sensibles étaient de 44%, 23%, 13% et 6% respectivement. L'évaluation était consistante d'un site à l'autre pour 78% des clones. L'établissement des essais sur plus d'un site est conséquemment nécessaire pour une évaluation fiable. Le pathogène a été isolé des champs industriels dans les régions où la maladie est présente ainsi que dans les collections du germoplasme pendant la période 1997–2007. La race de la bactérie fut déterminée sur la base des caractéristiques en milieu de culture et des tests de pathogénicité. La Race 1 était couramment isolée des cannes nobles conservées en collection. À part un cas en 1998, cette race était absente des champs commerciaux. Ces prospections ont confirmé que les variétés industrielles étaient très résistantes à la Race 1 et elle était de ce fait, d'importance mineure. La maladie de la gommose présente dans les champs industriels était donc presque exclusivement causée par la Race 2. Le criblage des variétés contre la Race 2 dans différents environnements est conséquemment nécessaire.

ESQUEMA DE RESISTENCIA A LA GOMÓSIS BACTERIANA EN CLONES AVANZADOS DE CAÑA DE AZÚCAR

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Gomósis de la Caña de Azúcar, *Xanthomonas axonopodis* pv. *vasculorum.*

Resumen

EXISTEN DOS RAZAS de la bacteria causal de la gomósis (*Xanthomonas axonopodis* pv. *vasculorum*), razas 1 y 2 que comúnmente infectan a las variedades de caña de azúcar en Mauricio. Los clones seleccionados en el tercer estado clonal (a los 4–5 años) del programa de mejoramiento son examinados para detectar la resistencia a estas dos razas. Desde 1998 hasta 2007, 878 clones fueron evaluados en ensayos de resistencia en las localidades donde son más frecuentes las dos razas. Surcos de una variedad susceptible se inocularon para que se constituyeran en fuente de inóculo para las variedades de la prueba. La evaluación contra la raza 1 se llevó a cabo en un sitio, mientras que contra la raza 2 se llevó a cabo en dos a cuatro sitios. Los resultados mostraron que el 99% de los clones fueron inmunes a la raza 1. De los 9 clones que mostraron infección, siete fueron clasificados como resistentes, uno como levemente susceptible y uno como susceptible. En contraste, el porcentaje de los clones que mostraron inmunidad a la raza 2 fue mucho menor (14%). La resistencia, levemente susceptible, susceptible, y alta susceptibilidad a la raza 2 en el resto de los clones fue de 44%, 23%, 13% y 6%, respectivamente. Las calificaciones fueron consistentes entre localidades para el 78% de los clones. La evaluación en más de un sitio por tanto se justificó. El patógeno siempre se aisló en los campos comerciales en las regiones propensas a la enfermedad y en las colecciones de germoplasma durante el período 1998–2007. La caracterización de las razas se basó en las características culturales y pruebas de patogenicidad. La raza 1 se ha aislado continuamente en colecciones de cultivares nobles de caña. Excepto por un caso en 1998, siempre ha estado ausente en los campos comerciales. Estos estudios confirmaron que las variedades comerciales son altamente resistentes a la raza 1 y, por tanto, de poca importancia para los híbridos de *Saccharum*. La presencia de la gomósis en las plantaciones comerciales es exclusiva de la raza 2, por tanto, su detección y evaluación por resistencia en contra de ella en diferentes ambientes se considera necesario.