Resistance of Sugarcane Cultivar R 570 to *Puccinia melanocephala* Isolates from Different Geographic Locations

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ABSTRACT

Asnaghi, C., D'Hont, A., Glaszmann, J. C., and Rott, P. 2001. Resistance of sugarcane cultivar R 570 to *Puccinia melanocephala* isolates from different geographic locations. Plant Dis. 85:282-286.

Two different inoculation techniques were investigated before studying the reaction of the major rust resistance gene of sugarcane cultivar R 570 against isolates of *Puccinia melanocephala* from different geographic locations. Cultivar R 570 exhibited severe rust symptoms when in vitro plantlets were inoculated with a rust isolate from Réunion Island, but a good correlation with field resistance was observed when detached leaves were inoculated with the pathogen. This latter technique was then used to inoculate R 570 and a sample of its self progeny with rust isolates from Brazil, Colombia, Florida (three isolates), Guadeloupe, Réunion Island, and Zimbabwe. R 570 was resistant to all isolates of *P. melanocephala*, and the segregation of resistance in the progeny did not change with the isolates, suggesting that a single gene, or a single chromosomal region, was involved in the resistance against all tested isolates. This major resistance gene has, therefore, potential value to improve resistance to rust in various geographic regions.

Sugarcane (*Saccharum* spp.) common rust is caused by *Puccinia melanocephala* H. Syd. & P. Syd. The pathogen causes yellowish spots on both leaf surfaces. Subsequent flecks become reddish brown, and sporulating pustules develop on the lower leaf surface of susceptible cultivars within 10 to 14 days. The disease has a wide distribution and occurs in almost all sugarcane-growing areas (23). It was responsible for epidemics causing important economic losses; for example, yield losses of 50% were estimated in Mexico in 1981 to 1983 for the susceptible cultivar B 4362 (Rai et al., 1991, cited by Comstock [7]).

Resistant cultivars provide the most efficient control of sugarcane common rust. Although sugarcane breeding is complicated by complex genome structure of cultivars (high level of ploidy, aneuploidy, and interspecific hybrid origin), progress in breeding for rust resistance has been rapid, as this trait has high narrow and broad-sense heritability (8); moreover, evaluation of plant reaction is relatively easy. Rust resistance is generally considered to be a quantitatively inherited trait (3,28,31), and different rating scales, based on symptom severity, have been developed (see 30 for review). Two methods are commonly used to evaluate rust reaction. The first method

is field assessment following natural infection. The second method is a controlled condition test by artificial inoculation of whole plants (7,17,26) or detached leaves (15,29) with rust spores. Evaluation of inoculated in vitro plantlets (whole plantlets generated by axial bud culture and grown under aseptic conditions in glass tubes) has also been attempted (16,18).

The development of resistant cultivars has resulted in the control of sugarcane common rust and, therefore, in the decrease of economic losses caused by this disease (23). Nevertheless, existing resistant cultivars are threatened by the establishment of new races of *P. melanocephala*. The existence of different races is supported by the changes of rust reaction by different sugarcane cultivars at different stages of selection grown in different countries (10,14,15,21,27). However, as expression of the disease is influenced by the interaction of genetic, environmental (primarily air temperature and leaf wetness), and physiological (age of infected plants) factors (5), pathogenic race descriptions based on evidence from these earlier reports should be considered with care because evaluation methodologies were not standardized and test environments differed (30). For example, cultivar CP 78-1247 was considered to be resistant or moderately resistant until 1988, when it then exhibited extremely high rust susceptibility throughout south Florida (21). Occurrence of different common rust races has been postulated using controlled conditions in India (26), Hawaii (4), and Florida (6). Taylor (29) tested different rust isolates from Australia (Queensland) and

concluded that only one race was present in that location, and that changes observed in rust reaction of cultivars were probably caused by differences in environmental conditions. Rust isolates from different countries have never been compared, and little is known about the existence of *P. melanocephala* races throughout the world.

Inheritance studies of resistance to common rust in a progeny derived from selfing a modern cultivar, R 570, resulted in the identification of a major rust resistance gene (9). This gene is the first major gene involved in a trait of agricultural interest to be determined in sugarcane. R 570 is one of the most widely cultivated varieties in Réunion Island and Mauritius, and it is included in many breeding programs worldwide. The objective of this work was to determine if the gene that confers resistance to rust in Réunion Island also confers resistance to isolates of the pathogen from other parts of the world. Two different inoculation techniques were investigated before studying the reaction of R 570 and a sample of its self progeny against rust isolates from Brazil, Colombia, Florida, Guadeloupe, Réunion Island, and Zimbabwe.

MATERIALS AND METHODS

Plant material. In vitro plantlets of the cultivars R 570 (resistant) and two controls, H 49-5 (susceptible, 26) and B 4362 (susceptible, 14), were derived from axial buds and grown in glass test tubes (2.5 × 20.0 cm) in 20 ml of Murashige and Skoog medium (MS) modified by Feldmann et al. (11). Plantlets were grown and propagated at 28°C in a controlled environment chamber with a 12-h photoperiod. Twenty- to 30-day-old plantlets (approximately 2.0 × 15.0 cm) were used for inoculation.

Leaf material used in the detached-leaf inoculations was obtained from plants (1 to 6 months old) grown from cuttings in a quarantine greenhouse at Montpellier, France (22), with only the youngest leaves (i.e., 0 to +2, with +1 being the youngest visible leaf defined by Artschawager [1]) being used. Each leaf was cut into three to five pieces approximately 6 to 8 cm long. Leaves of the above-mentioned cultivars and 18 progeny derived from selfing R 570 were evaluated. The 18 progeny issued from seedlings of R 570 selfing were asexually propagated by cuttings and are subsequently referred to as 18 clones (9,12).

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Accepted for publication 12 November 2000.

Publication no. D-2001-0102-01R
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Inoculum. Rust isolates from various sugarcane-growing areas (Argentina, Australia, Brazil, Colombia, Florida, Guadeloupe, Hawaii, Louisiana, Mauritius, Réunion Island, South Africa, and Zimbabwe) were cooperatively gathered within the framework of the International Society of Sugarcane Technologists (ISSCT). Spores were collected from the lower surface of infected leaves and sent to Montpellier, France. Each rust isolate sample was represented by a mixture of spores collected from several pustules from the same cultivar, and this inoculum was neither monosporic derived nor purified. Spore germination percentage of each sample was determined by depositing spores on 4.5% water agar glass slides placed on top of water-soaked paper in petri plates. After 24 h at 25°C, the slides were observed under a microscope and the percentage of spore germination was assessed. Eight spore samples with a germination percentage greater than 5% (ranging from 5 to 70%) were used for subsequent inoculation tests. These isolates originated from Brazil (BRA), Colombia (COL), Florida, Guadeloupe (GUA), Réunion Island (REU), and Zimbabwe (ZIM). Three isolates from Florida, namely Florida 43 (FL43), Florida 78 (FL78), and Florida 97 (FL97), were collected from cultivars B 4362, CP 78-1247, and CP 97-1283, respectively. Spores of each isolate were placed in 0.5-ml Eppendorf tubes and stored at -20°C until used for inoculation. The percentage of spore germination was verified at the time of inoculation by the water agar slide method described above.

Inoculation tests. *In vitro* plantlets inoculation. The inoculation technique described by Péros (16) was utilized with some modifications. Spores were removed from the storage tube with a lancet needle, placed in 10 µl of sterile distilled water, and streaked on the lower leaf surface of plantlets removed from the glass tubes. The plantlets were then replaced into the tubes with either MS medium or MS supplemented with geneticin (0.25%) and benomyl (0.05%) or thiram (0.4%). Inoculated plantlets were stored in a controlled environment chamber at 25°C with a 12-h photoperiod. The experiments with MS growth medium and MS supplemented with geneticin and benomyl were repeated once, whereas the experiment with MS supplemented with geneticin and thiram was not repeated.

Detached-leaf inoculation. Two inoculation methods were used. In the first method, rust spores were spread onto 4.5% water agar from which 10 small agar squares (0.2 × 0.2 cm), containing about 20 viable spores each, were excised and applied to the lower surface of each sugarcane leaf piece. In the second method, each leaf piece was sprayed with 70 µl of a spore suspension containing 4×10^3 to 1.4×10^5 (average = 7.5×10^4) viable spores

per ml of sterile distilled water with a glass test tube atomizer. This latter method was used to inoculate the three cultivars R 570, H 49-5, and B 4362 and the 18 clones.

Inoculated leaf pieces were placed in test tubes (2.5 × 20.0 cm) containing 5 ml of a 12.5 mg/liter benzimidazole solution. According to Person et al. (1957, cited by Browder [2]), benzimidazole prevents chlorophyll degradation in detached leaves. The closed tubes were randomly arranged on racks, maintained for 24 h in the dark at 25°C, and then placed at 25°C with a 12-h photoperiod.

The number of independent inoculations with each isolate varied from two to six for the three cultivars R 570, H 49-5, and B 4362, and from one to four for the 18 clones. For each repetition, the three cultivars or the 18 clones were treated simultaneously. Two and three spray inoculations of 26 to 30 R 570 leaf pieces were performed with the Brazil and the Réunion Island rust isolates, respectively.

Rust resistance evaluation. The field reaction to rust of R 570 and the 18 clones was determined on Réunion Island using natural infections (9; J. H. Daugrois, *personal communication*). Rust resistance was evaluated using a scale from 1 (the most resistant) to 9 (the most susceptible) as described by Tai et al. (28). This scale is based on visual assessment of the disease symptoms. Scores from 1 to 2 indicate resistant plants with no sporulation of the fungus, but some necrotic or chlorotic spots may appear on the leaves. Scores from 3 to 9 indicate susceptible plants, with fungal sporulation increasing with each higher number. Eight clones with a score of 1 and one clone with a score varying between 1 and 2 were considered resistant. The other nine clones formed the susceptible group: two clones (II10 and II9) were slightly to moderately susceptible, with scores varying between 2 and 5, five clones (I32, II25, II28, III21, and III33) were moderately susceptible (scores of 4 to 5), and two clones (I04 and I25) were susceptible (scores of 6 to 7).

The reaction of these plants to rust isolates from various origins was determined under controlled conditions in Montpellier, France. Resistance was determined by the proportion of plantlets or leaf pieces ex-

hibiting sporulating pustules. A reaction was rated positive when at least one sporulating pustule was observed.

Statistical analysis. Chi-square analyses were performed using the SAS software (SAS Institute, Cary, NC) with FREQ and CATMOD procedures.

RESULTS AND DISCUSSION

Comparison of inoculation methods.

In a first experiment, *in vitro* plantlets of the three cultivars R 570, H 49-5, and B 4362 were inoculated with the isolate from Réunion Island. Sporulating pustules were observed on the leaves of all three cultivars after 10 to 14 days (Table 1). The number of plantlets showing sporulating pustules was greater when the plantlets were grown on MS medium with benomyl and geneticin (Table 2). Similar results were obtained when benomyl was replaced by thiram (data not shown). There were no differences in number and size of sporulating lesions or the number of diseased plants between the resistant cultivar and the two susceptible ones (Table 2).

The high level of infection in plantlets observed for both resistant and susceptible cultivars indicated that resistance observed under field conditions was not expressed in tissue culture plantlets: cultivar R 570 showed *in vitro* numerous sporulating pustules and a high incidence of rust, whereas in the field, pathogen sporulation has never been reported in this cultivar. Reports (16,18) about *in vitro* expression of varietal rust resistance are inconsistent, and our results confirm observations of Péros and Bonnel (18), who described occurrence of *in vitro* sporulation on three different resistant cultivars (Q 75, H 37-1933, and B 37172). More rust symptoms occurred when a fungicide and an antibiotic were added to the growth medium. These chemicals prevented growth of saprophytic microorganisms on the medium and consequently favored growth of the sugarcane plantlets and development of the disease (Table 1). Therefore, inoculation of *in vitro* cultured sugarcane was, in our hands, not suitable for determining the rust reactions of sugarcane clones.

In a second experiment, detached leaves of cultivars R 570, H 49-5, and B 4362 were inoculated with five rust isolates (one

Table 1. Inoculation of *in vitro* plantlets of three sugarcane cultivars with *Puccinia melanocephala* from Réunion Island

Cultivar	Growth medium		Total
	Medium 1	Medium 2	
R 570 (resistant)	4/18 ^a	14/21	18/39
H 49-5 (susceptible)	4/12	12/20	16/32
B 4362 (susceptible)	6/19	15/20	21/39
Total	14/49	41/61	

^a Number of plantlets showing sporulating pustules per total number of inoculated plantlets (data correspond to the total data of two independent trials of 6 to 11 plantlets each). Medium 1 is a modified Murashige and Skoog medium (11), and medium 2 is medium 1 supplemented with benomyl (0.05%) and geneticin (0.25%).

each from Brazil, Réunion Island, and Zimbabwe and two from Florida) by placing water agar pieces containing spores and by spraying spore suspension (15,29). These two inoculation techniques were tested because they are less spore-consuming than the inoculation method with compressed air in a tower that was used by Taylor (29). No sporulation was observed with R 570 leaves, whatever the inoculation method (Table 3). Both susceptible cultivars had sporulating lesions for all five rust isolates, with the exception of cultivar B 4362 inoculated with the isolate from Zimbabwe. The number of leaf pieces that showed sporulating lesions did not differ between the two inoculation methods or between the two susceptible cultivars (Table 2). Additionally, the resistance level of the three cultivars was in agreement with field observations with natural rust infection. The detached-leaf technique appeared, therefore, to discern

resistant from susceptible cultivars effectively.

From a practical point of view, utilization of water agar pieces to inoculate the leaf pieces was more time-consuming. Moreover, agar is favorable to the development of many other microbial organisms that could interfere with the rust fungus. The spraying of rust urediniospores on sugarcane leaves thus appeared more effective.

Comparisons of rust isolates have to be performed under controlled conditions, or in the field with a range of standard cultivars of known reactions, because rust disease incidence is largely influenced by environmental conditions. All our experiments were performed in an area where sugarcane is not grown commercially, so the amount of background rust spores was limited. It was therefore imperative to use an inoculation method utilizing a minimal amount of spores. The detached-leaf meth-

od matches all these conditions and was thus chosen for further experiments.

Reaction of R 570, H 49-5, and B 4362 against eight rust isolates. Cultivar R 570 and two susceptible cultivars, H 49-5 and B 4362, were tested against eight rust isolates collected from diverse geographic locations (Table 4). All inoculations were performed with the original collected spores in order to maintain spore source integrity. The difference between the resistant and susceptible cultivars was clearly demonstrated: 2.2% of R 570 inoculated leaves showed sporulating pustules, as opposed to 60 and 68% for B 4362 and H 49-5, respectively (Table 4). The rust incidence of the two susceptible cultivars varied according to the isolate of the pathogen (Table 2). For example, sporulation occurred on each leaf piece inoculated with the isolate from Colombia. In contrast, only 8 out of 20 leaf pieces of cultivar H 49-5 inoculated with an isolate from Florida (FL97) and 5 out of 17 leaf pieces of cultivar B 4362 inoculated with the isolate from Zimbabwe showed sporulating pustules (Table 4). However, the reaction pattern of the two susceptible cultivars appeared similar against the different rust isolates (Table 2).

Cultivar R 570 was resistant against all eight rust isolates, but this also might indicate the involvement of several rust resistance genes different from the major one identified so far. Several sources of resistance to rust have been described in other plant species such as flax or maize (13,20), and these sources can be held concurrently in one cultivar. This issue was consequently addressed with the evaluation of 18 clones derived from selfing R 570.

Reaction of an R 570 selfing progeny sample against seven rust isolates. Leaf pieces of 18 clones were inoculated with seven rust isolates from different geographic locations (Table 5). The resistance of these clones in the field in Réunion Island varied from 1 (resistant) to 7 (susceptible with sporulating pustules of medium size). Nine clones were rated resistant (score 1 or 2), and the other nine clones were rated susceptible (score 2 to 7). The resistant clones did not develop any sporulating lesions, with the exception

Table 2. Probability values from chi-square (χ^2) analysis of the number of sporulating leaf pieces or plantlets

Experiment, effect	df	χ^2	Probability
In vitro plantlets inoculation (Table 1)			
Medium	1	15.53	<0.001
Cultivar	2	0.85	0.655
Medium $\times$ cultivar	2	0.77	0.681
Inoculation technique comparison (Table 3)			
Technique	1	2.89	0.089
Cultivar ^a	1	0.63	0.425
Technique $\times$ cultivar	1	0.90	0.089
R 570, H 49-5, and B 4362 inoculation (Table 4)			
Rust isolate ^b	6	21.80	<0.001
Cultivar ^a	1	2.47	0.1160
Rust isolate $\times$ cultivar	6	10.84	0.0936
Inoculation of 18 clones (Table 5)			
Rust isolate ^c	4	96.16	<0.001
Clone ^d	8	10.57	0.227
Rust isolate $\times$ clone	32	36.19	0.279
Position of the leaf piece	3	3.86	0.277
Level of the leaf ^e	1	3.73	0.053

^a Data of resistant cultivars were not taken into consideration because no or very little sporulation occurred on leaf pieces of these cultivars (including these data in the analysis would hide a possible significant effect between susceptible cultivars).

^b Data of rust isolate from Colombia were not taken into consideration because data reached maximum values, which would cause null data that could not be used to perform Pearson chi-square test (25) or logit model used by the SAS CATMOD procedure.

^c Data of rust isolates from Guadeloupe and Colombia were not taken into consideration because of reduced number of data and maximum values, respectively.

^d Data of +2 leaf level were not taken into consideration because of reduced number of data.

^e Only data of susceptible clones were taken into consideration.

Table 3. Comparison of two detached-sugarcane-leaf inoculation techniques with rust: spraying leaves with a rust spore suspension (P) and application on leaves of water agar pieces containing rust spores (A)

Rust isolate		Cultivar and inoculation technique					
		R 570 (resistant)		H 49-5 (susceptible)		B 4362 (susceptible)	
		P	A	P	A	P	A
BRA	Brazil	0/5 ^a	0/3	4/5	2/3	5/5	2/2
FL78	Florida	0/5	0/3	1/4	1/3	2/5	2/3
FL97	Florida	0/5	0/3	4/5	1/3	4/5	1/3
REU	Réunion Island	0/15	0/11	11/14	3/10	11/15	5/9
ZIM	Zimbabwe	0/5	0/3	2/5	2/3	0/3	2/3
	Total	0/35	0/23	22/33	9/22	22/33	12/20

^a Number of leaf pieces showing sporulating pustules per total number of inoculated leaf pieces (data correspond to the total data of one to three independent trials two to five leaf pieces each).

of clone I12, which showed a few cases of a single pustule with isolates from Brazil and Réunion Island. In contrast, the percentage of leaf pieces showing sporulating pustules in susceptible clones ranged from 25% (isolate from Zimbabwe) to 92% (isolate from Colombia). The same resistance pattern was observed for the seven isolates of the pathogen: the nine sugarcane clones that were resistant to the isolate from Réunion Island were also resistant to the other six rust isolates, and similarly the clones susceptible to *P. melanocephala* from Réunion Island were susceptible to the isolates from Brazil, Colombia, Florida, Guadeloupe, and Zimbabwe. The same progeny pattern for the different rust isolates indicated that the

gene or the chromosomal region identified in R 570 as providing resistance against the *P. melanocephala* isolate from Réunion Island also conferred resistance against other rust isolates and appeared to be the only major gene involved in the genetic control of resistance in R 570. No races of rust were detected by the three cultivars and the 18 clones used in our experiments. This may indicate that if there are rust races among the tested isolates, they are differentiated by other genes.

The reaction of susceptible clones was not significantly different when comparing the percentage of leaf pieces showing sporulating pustules for all isolates (this percentage varied from 54% for the clone III33 to 67% for the clone II25), or for any

single rust isolate (except for the isolate from Réunion Island, whose associated chi-square probability level was $P = 0.047$) (Table 2). Comparing the different rust isolates, the percentages of leaf pieces showing sporulating pustules were significantly different (Table 2) and varied, in the susceptible clones, from 25% (isolate from Zimbabwe) to 92% (isolate from Colombia) (Table 5). Variation in rust aggressiveness could explain this result, but it could also be due to large differences in the quality or purity of each spore sample. Rust samples could contain other microorganisms, such as hyperparasites (24), affecting rust spore germination and progress of disease symptoms in sugarcane; however, inoculum used in this study was not microscopically or culturally checked for contaminating microorganisms. To evaluate the importance of these factors, the rust samples should be first cloned and aseptically propagated. This, however, could also lead to selection of particular isolates of the pathogen.

The influence on symptom appearance of the leaf level on the plant or the position of the leaf piece used for inoculation was tested with the 18 clones. The numbers of sporulating leaf pieces were not significantly different when the leaf 0 or +1 was used or when pieces were taken from the lower, middle, or upper part of the leaf (Table 2).

Attempts to select spores with increased virulence on R 570. Considering all experiments by leaf-spray inoculation,

Table 4. Detached-leaf inoculation of a rust-resistant sugarcane cultivar (R 570) and two susceptible cultivars (H 49-5 and B 4362) with eight isolates of *Puccinia melanocephala*

Rust isolate	Origin	Sugarcane cultivar		
		R 570 (resistant)	H 49-5 (susceptible)	B 4362 (susceptible)
BRA	Brazil	1/20 ^a	18/18	15/19
COL	Colombia	0/20	20/20	20/20
FL43	Florida	1/10	7/10	6/10
FL78	Florida	0/15	6/14	5/15
FL97	Florida	0/20	8/20	8/15
GUA	Guadeloupe	0/15	7/15	10/15
REU	Réunion Island	1/29	21/29	15/28
ZIM	Zimbabwe	0/20	11/19	5/17
	Total	3/149 (2.2%)	98/145 (68%)	84/139 (60%)

^a Number of leaf pieces showing sporulating pustules per total number of inoculated leaf pieces (data correspond to the total data of two to six independent trials of three to five leaf pieces each).

Table 5. Inoculation of 18 clones derived from selfing R 570 with seven isolates of *Puccinia melanocephala*^a

Sugarcane clone	Field resistance (score)	Isolate of <i>Puccinia melanocephala</i> (origin)							Total (%) ^b
		BRA (Brazil)	COL (Colombia)	FL78 (Florida)	FL97 (Florida)	GUA (Guadeloupe)	REU (Réunion Island)	ZIM (Zimbabwe)	
I 04	S (7)	15/20 ^c	16/20	4/10	11/20	6/7	15/20	3/20	70/117 (58.0%)
I 25	S (6)	16/20	20/20	7/10	10/20	4/7	11/20	4/19	72/116 (62.0%)
I 32	S (5)	16/20	20/20	4/10	13/20	7/7	6/20	7/20	73/117 (62.0%)
II 25	S (5)	14/20	18/20	6/10	15/20	3/7	10/14	9/20	75/111 (67.0%)
III 33	S (5)	11/20	18/20	3/10	12/20	7/7	9/20	3/20	63/117 (54.0%)
II 28	S (5)	15/20	15/20	4/10	15/20	3/7	10/20	5/20	67/117 (57.0%)
III 21	S (4 to 5)	17/20	20/20	8/10	14/20	2/7	9/20	3/20	73/117 (62.0%)
I 10	S (2 to 4)	16/20	18/20	5/10	13/20	7/7	9/20	6/20	74/117 (63.0%)
II 09	S (2 to 5)	16/20	20/20	3/9	15/20	7/7	5/19	5/19	71/114 (62.0%)
Total (%) ^d		136/180 (75%)	165/180 (92%)	44/89 (49%)	118/180 (65%)	46/63 (73%)	84/173 (48%)	45/178 (25%)	
I 06	R (1 to 2)	0/20	0/20	0/10	0/20	0/7	0/20	0/20	0/117
I 11	R (1)	0/20	0/20	0/10	0/20	0/7	0/20	0/20	0/117
I 12	R (1)	1/20	0/20	0/10	0/20	0/7	3/20	0/20	4/117 (3.0%)
II 08	R (1)	0/20	0/20	0/10	0/20	0/7	0/20	0/20	0/117
II 32	R (1)	0/20	0/20	0/10	0/20	0/7	0/20	0/20	0/117
III 01	R (1)	0/20	0/20	0/10	0/20	0/7	0/20	0/20	0/117
III 03	R (1)	0/20	0/20	0/10	0/20	0/7	0/20	0/20	0/117
III 30	R (1)	0/20	0/20	0/10	0/20	0/7	0/20	0/20	0/117
III 32	R (1)	0/20	0/20	0/10	0/20	0/7	0/20	0/20	0/117
Total (%) ^d		1/180 (0.5%)	0/180	0/90	0/180	0/63	3/180 (1.7%)	0/180	

^a Field resistance to rust was evaluated through several replications in Réunion Island (9). S: susceptible, R: resistant. Reaction to rust was defined on a 1 (most resistant) to 9 (most susceptible) scale.

^b Percentage of leaf pieces showing sporulating pustules for each progeny clone.

^c Number of leaf pieces showing sporulating pustules per total number of inoculated leaf pieces (data correspond to the total data of one to four independent trials of four to seven leaf pieces each).

^d Percentage of leaf pieces showing sporulating pustules for each rust isolate.

rust sporulation was observed on three out of 184 (1.6%) leaf pieces of cultivar R 570 and on four out of 1,053 (0.4%) leaf pieces of the nine resistant progeny derived from selfing R 570. In the study involving R 570, H 49-5, and B 4362 with eight isolates of *P. melanocephala*, single and separate cases of rust sporulation on R 570 leaves were observed with the isolates from Brazil, Florida (FL43), and Réunion Island (Table 4). In the study involving the clones derived from selfing R 570, the resistant clone I12 showed sporulating pustules on four different leaf pieces; however, only one sporulating lesion was observed in each case, and the size of this pustule was markedly smaller than the size of the pustules on susceptible cultivars, which are usually 2 to 10 mm long by 1 to 3 mm wide (23). Spores from the pustule of the R 570 leaf infected with the rust isolate from Réunion Island (Table 4) were collected and propagated on detached leaves of susceptible cultivar H 49-5. The resulting spores were abundant and used to reinoculate R 570 and H 49-5 leaf pieces. Cultivar H 49-5 again developed numerous sporulating pustules, but R 570 showed only some brown spots that never formed sporulating lesions. In order to evaluate the importance of sporulation on resistant clones, two additional sets of 89 and 56 R 570 leaf pieces were inoculated with isolates from Réunion Island and Brazil, respectively. One pustule with very few spores was obtained for the isolate from Réunion Island, but no sporulation for the isolate from Brazil. The spores obtained on R 570 were collected and used to inoculate leaves of H 49-5 and R 570. Three sporulating pustules appeared on H 49-5, whereas about 10 chlorotic spots were observed on R 570. The spores from H 49-5 were collected and used to again inoculate H 49-5 and R 570 leaves. The susceptible cultivar H 49-5 showed about 100 sporulating pustules, whereas R 570 showed only nonsporulating spots. These results confirmed that the rare spores occurring on R 570 were not a new race of the pathogen. Péros (17) observed the development of numerous nonsporulating lesions on R 570 leaves inoculated under controlled conditions. R 570 resistance against rust thus appears not to be complete. In vitro plantlets of R 570 did not show rust resistance in our study (Table 1), and Péros et al. (16,19) suggested that high level of nitrogen content in plantlet leaves or physiological effects due to tissue culture may favor in vitro expression of rust disease in field resistant cultivars.

Sugarcane has a complex genetic nature, and the rust resistance gene identified in cultivar R 570 represents the first major gene of disease resistance identified in sugarcane. Its extent of reaction is an important characteristic to determine its possible utilization for varietal improvement

of sugarcane. In our study, none of the isolates of *P. melanocephala* overcame this resistance gene. Our data confirm the wide range of field resistance to rust of cultivar R 570, which is grown in numerous locations where rust occurs (Brazil, Chad, Congo, Côte d'Ivoire, Guadeloupe, Martinique, Mauritius, Réunion Island, Trinidad, Vietnam, and Zimbabwe), and provide evidence, but no direct proof, that this resistance is controlled by a single gene or a single chromosomal region. This major gene of resistance of R 570 can therefore be used to improve resistance to rust in different geographic areas.

ACKNOWLEDGMENTS

We thank M. J. Darroussat, M. Muller, J. F. Bousquet, and J. Milazzo for technical support; J. C. Girard, J. Notaise, M. V. Casagrande, P. Zvoutete, J. C. Comstock, J. Victoria, J. H. Daugrois, B. J. Croft, R. Bailey, K. McFarlane, J. Hoy, R. Ullivarri, S. Schenck, and S. Saumtally, who provided rust spore samples; P. Letourmy for helpful advice with statistical analyses; and M. Chatenet, J. C. Girard, M. V. Casagrande, and J. P. Péros, who shared their valuable experience with rust. Our thanks also go to J. C. Comstock, M. J. Davis, J. C. Girard, and J. P. Péros for critical reviewing of the manuscript.

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