

Energy-cane: resistant to major sugarcane diseases?

Caña para energía: ¿Resistente a las principales enfermedades de la caña de azúcar?

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ABSTRACT

This study's objectives were to compare the occurrence of brown rust and smut in conventional and energy cane families and clones in field conditions and to understand how the new hybridizations could contribute to increasing the allele frequency of resistance against the main crop diseases in breeding populations. Disease occurrence was evaluated in two selection stages. Of the 2.400 clones constituting the material for selection in the first stage, 88 clones ($\approx 4\%$ of the total) were selected for the second stage. Considering the overall performance of the clones without symptoms of either disease in the evaluated families that would be possibly selected in the second stage, the percentage was at least 28% lower in the families selected for high biomass (37.33%) than of the selected conventional (57.14%) and intermediate biomass (66.67%) families. In the intermediate biomass and high biomass sugarcane families, brown rust and smut rates were higher in the first stage. In the second stage, brown rust incidence was higher in high biomass families. However, at the end of both selection phases, intermediate biomass and high biomass sugarcane clones without simultaneous brown rust and smut symptoms could be selected.

Keywords: *Saccharum* spp, *Puccinia melanocephala*, *Sporisorium scitamineum*, biomass, plant breeding.

RESUMEN

El objetivo de este estudio fue comparar la incidencia de roya marrón y carbón en familias y clones de caña de azúcar convencional y caña de azúcar para energía, a fin de evaluar el efecto de las hibridaciones en la frecuencia alélica de resistencia a estas enfermedades en el mejoramiento de los cultivos. La ocurrencia de las enfermedades fue observada en dos etapas de selección. En la primera etapa se evaluaron 30 familias, clasificadas según tipo de familia: cinco familias de caña de azúcar convencional (CO); trece familias de caña de azúcar para producción de energía con alta biomasa (HB) y, doce familias de caña de azúcar para producción de energía con biomasa intermedia (IB). Durante la segunda etapa, se evaluaron 88 genotipos libres de enfermedad: siete genotipos CO; 60 genotipos HB y 21 genotipos IB. En la primera fase de selección se observaron tasas más altas de carbón y roya en familias de caña de azúcar de biomasa intermedia (IB) y alta (HB). En la segunda fase de selección, la incidencia de la roya de las familias de alta biomasa (HB) fue mayor. En las familias con biomasa media y alta las tasas de roya marrón y carbón resultaron mayores en el primer estadio. En el segundo estadio, la incidencia de roya marrón fue mayor en las familias con alta biomasa. Al final de dos fases, se seleccionaron clones de caña de azúcar de biomasa intermedia y alta, sin síntomas de ninguna de las enfermedades evaluadas.

Palabras clave: *Saccharum* spp, *Puccinia melanocephala*, *Sporisorium scitamineum*, biomasa, mejoramiento genético.

Introduction

The possibility of profitable use of biomass for power cogeneration and cellulosic ethanol production of has inspired new sugarcane research lines, mainly to increasing fiber content and stalk yield of future varieties (Ramos *et al.*, 2017). To raise the fiber content in future varieties, researchers proposed the exploitation of *Saccharum spontaneum* and *S. robustum* accessions crossed with high-performing commercial hybrids (*S. spp.*), intending

to breed energy cane varieties (Ramos *et al.* 2017). These species' accessions should also be used to increase the frequency of resistance genes to the major crop diseases (Matsuoka *et al.* 2014).

Brown rust (*Puccinia melanocephala*) is one of the main sugarcane diseases. This fungal parasite attacks leaf tissues, where it produces the typical rust symptoms, prominent pustules, which are easily diagnosed in the field (Tokeshi and Rago 2005). For Brazil's field conditions, a productivity reduction of up to 47% was reported,

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and for the most susceptible varieties, the damage level can exceed 60% (Matsuoka 1993). Resistant plant breeding is the only economic measure of brown rust control (Tokeshi and Rago 2005). The heritability of disease resistance is high, indicating that the elimination of susceptible parents is a good strategy for developing resistant varieties (Ramdoyal *et al.* 2000).

Another important sugarcane disease is smut (*Sporisorium scitamineum*). Caused by a fungal parasite that attacks plant's meristematic tissues, it produces a structure known as "whip," a typical symptom of infected stalks. This structure is an alteration of the stem morphology and consists of plant tissue tapered and covered with a black mass of the fungus teliospores. The "whip" is easily identified in the field and allows a safe diagnosis of the disease (Tokeshi and Rago 2005).

Smut is considered a major concern in all breeding programs, causing breeders to choose resistant parents (Rago *et al.*, 2009). Damage levels of 50% to 73% lower in ratoon crop yields caused by the disease were reported (Bergamin Filho *et al.* 1987, Ido *et al.*, 2006). In view of the need to develop new sugarcane biotypes, using hybridizations of *S. spontaneum* and *S. robustum* accessions, the objective of this study was to compare the occurrence of brown rust and smut

in conventional and energy cane families and clones under natural field conditions, to understand how these new hybridizations can contribute to increasing the allele frequency of resistance to the major sugarcane diseases in breeding populations.

Material and methods

First selection phase (T1)

The crosses were made in 2012, at the Station of Serra do Ouro for flowering and crosses, of the Federal University of Alagoas (UFAL), in Murici, Alagoas, Brazil (latitude 9°13 'S, longitude 35°50 'W; 450 m asl) and at the Station of Devaneio for flowering and crosses, of the Federal Rural University of Pernambuco (UFRPE), in Amaraji, Pernambuco, Brazil (latitude 8°19,8' S, longitude 35°24,8 'W; 514 m asl). The caryopses derived from the crosses were prepared and sent to the Experimental Station Paranaíba, of the Federal University of Paraná (UFPR), in Paranaíba, Paraná (latitude 23°05'S, longitude 52°27'W; 503 m asl).

Seeds were sown, and seedlings were produced to plant the first selection phase of the experiment (T1) in February 2013. In this phase, 30 families were evaluated (Table 1) in a randomized complete block design. Each plot consisted of 20 seedlings,

Table 1. Parents of 30 evaluated families and classification of the crosses according sugarcane ideotype.

Families*			Families*		
Mother	Father	Ideotype	Mother	Father	Ideotype
RB855156 ⁽¹⁾	RB987935 ⁽¹⁾	CO	Co62175 ⁽¹⁾	28NG289 ⁽³⁾	HB
RB867515 ⁽¹⁾	RB965518 ⁽¹⁾	CO	RB93509 ⁽¹⁾	Co285 ⁽⁴⁾	HB
RB867515 ⁽¹⁾	RB996961 ⁽¹⁾	CO	SP81-3250 ⁽¹⁾	US85-1008 ⁽⁴⁾	HB
RB966928 ⁽¹⁾	TUC77-42 ⁽¹⁾	CO	Co421 ⁽⁵⁾	POJ2878 ⁽⁶⁾	IB
SP80-3280 ⁽¹⁾	RB867515 ⁽¹⁾	CO	Co421 ⁽⁵⁾	RB93509 ⁽¹⁾	IB
F150 ⁽⁶⁾	IN84-88 ⁽²⁾	HB	CoS245 ⁽⁶⁾	RB813804 ⁽¹⁾	IB
IAC87-3396 ⁽¹⁾	US85-1008 ⁽⁴⁾	HB	Co617 ⁽⁵⁾	POJ2878 ⁽⁶⁾	IB
IM76-228 ⁽³⁾	RB867515 ⁽¹⁾	HB	F160 ⁽⁶⁾	MEX68-200 ⁽⁶⁾	IB
RB01640 ⁽¹⁾	IN84-58 ⁽²⁾	HB	MEX68-200 ⁽⁶⁾	F150 ⁽⁶⁾	IB
RB036066 ⁽¹⁾	? ⁽²⁾	HB	MEX68-200 ⁽⁶⁾	RB04823 ⁽¹⁾	IB
RB04813 ⁽¹⁾	US85-1008 ⁽⁴⁾	HB	MEX68-200 ⁽⁶⁾	RB93509 ⁽¹⁾	IB
RB83102 ⁽¹⁾	IM76-229 ⁽³⁾	HB	RB01623 ⁽¹⁾	MEX68-200 ⁽⁶⁾	IB
RB867515 ⁽¹⁾	B70710 ⁽⁴⁾	HB	RB93509 ⁽¹⁾	Co421 ⁽⁵⁾	IB
RB92579 ⁽¹⁾	IM76-228 ⁽³⁾	HB	RB93509 ⁽¹⁾	MEX68-200 ⁽⁶⁾	IB
RB92579 ⁽¹⁾	IM76-229 ⁽³⁾	HB	US74-103 ⁽⁵⁾	RB92579 ⁽¹⁾	IB

*Family ideotypes based on genealogical information; CO: conventional sugarcane family; HB: high biomass energy cane family; IB: intermediate biomass energy cane family - (2nd and 3rd generation of *S. spontaneum*). ⁽¹⁾ Sugarcane hybrid (*Saccharum* spp.), ⁽²⁾ *S. spontaneum*, ⁽³⁾ *S. robustum*, ⁽⁴⁾ 1st generation of *S. spontaneum*, ⁽⁵⁾ 2nd generation of *S. spontaneum*, ⁽⁶⁾ 3rd generation of *S. spontaneum*; ?Unknown parent (multiparent cross).

spaced 0.50 m apart, distributed in two 5-m long rows, spaced 1.40 m apart, with a total of 3.000 seedlings. In the T1 phase, the 30 families were classified according to the genealogy of their parents (family type), as follows: five conventional sugarcane (CO) families, derived from crosses between conventional sugarcane clones and varieties; 13 energy cane families with high biomass (HB) from crosses between clones of the species *S. spontaneum* and *S. robustum* with sugarcane hybrids; and 12 energy cane families with intermediate biomass (IB), from crosses of second and third generation *S. spontaneum* clones with sugarcane hybrids (Table 1).

Cane plant cutting occurred in December 2013. Disease incidence was evaluated in the ratoon crop in November 2014, 11 months after the first cut, in a favorable period for the occurrence of diseases of major importance for the crop.

Second selection phase (T2)

The second selection phase (T2) was installed in December 2014, with 27 families and 88 genotypes disease-free in T1, in which 7 were CO, 60 HB, and 21 IB, according to Table 1. Planting of T2 occurred in December 2014, at the Experimental Station Paranaíba, of the Federal University of Paraná, in Paranaíba, Paraná. This phase was arranged in an incomplete block design, with five blocks, i.e., four with 20 and one with only eight plots. Each experimental plot consisted of two 5-m rows, spaced 1,40 m apart, in which 18 buds per meter were planted. Disease occurrence

was assessed in December 2015, in the cane plant cycle, 12 months after planting.

Disease assessment

Brown rust

For the evaluation of brown rust, the +3 leaves of all tillers of a plot were observed in both experimental stages, although in the first selection cycle (T1), the scores were assigned according to the mean plot performance (composed of a full sib family). The 1 - 9 diagrammatic scale score system was modified to a 4-grade scale, to assess the percentage of disease-affected leaf area (Purdy & Dean, 1981; Amorim *et al.* 1987) (Table 2).

Smut (*Ustilago scitaminea*)

The clones' smut incidence was only evaluated in the T1 phase, whereas smut incidence on the ratoon tillers was evaluated in all families in both phases (T1 and T2). In T1, smut incidence was determined as the ratio between the number of clones (clumps) with at least one whip and the total number of clones per family. The ratio of the total number of ratoon tillers by the number of ratoon tillers with whips per plot was determined to determine smut incidence on the ratoon tillers. The total frequencies of smut incidence on clones and smut incidence on the ratoon tillers in each family type were determined. In T2, only the clones smut incidence of each family with or without symptoms were considered.

Table 2. Brown rust severity scales proposed by Amorim *et al.* (1987) and modified scaled.

Amorim <i>et al.</i> (1987)			Modified Scale	
Score	LAS (%)*	LBRR	Score	LBRR
1	0	Highly Resistant	1	Highly Resistant (HR)
2	0.5	Resistant	2	Resistant (R)
3	1	Resistant		
4	5	Moderately Resistant		
5	10	Moderately Susceptible	3	Moderately Susceptible (MS)
6	25	Susceptible		
7	35	Susceptible		
8	50	Susceptible	4	Susceptible (S)
9	>50	Highly Susceptible		

*percentage of leaf area with symptoms (LAS (%)) proposed by Purdy & Dean (1981); levels of brown rust reaction (LBRR) associated with different score scales.

Results and discussion

Brown rust and smut are diseases that cause losses in sugarcane yield worldwide. Genetic resistance is the most commonly used control method in disease management. Therefore, studies that evaluate new families and clones of high biomass disease reaction should be further explored within genetic breeding programs. The higher resistance to diseases in sugarcane crosses is due to the knowledge and mastery of breeding for the development of varieties, especially regarding the parents' response to the diseases. We evaluated progenies from 30 crosses in 2 selection phases, involving elite cultivars (*Saccharum* spp.), *S. spontaneum*, and *S. robustum* from different generations and named three family type according to the species involved in the crosses: conventional (CO), intermediate biomass (IB) and high biomass (HB).

Family performance in the first selection phase (T1)

According to the scores of brown rust severity in the family plots in the first selection phase (T1), the rust reactions levels of the HB families RB93509 × Co285, RB867515 × B70710, RB83102 × IM76-229, IM76-228 × RB867515, and F150 × IN84-88, which represented 38% of the evaluated families, were moderately susceptible (MS) and susceptible (S) (Figure 1A). Approximately 15% of the HB families (SP81-3250 × US85-1008 and RB01640 × IN84-58) were classified as highly resistant (HR) in all plots (Figure 1A).

Of the intermediate biomass families (IB), 16% (MEX68-200 × F150 and Co617 × POJ2878) reacted MS and S to brown rust (Figure 1A). The only IB family with resistance score HR in all plots was MEX68-200 × RB04823. Conversely, the plots of all five CO families, with the exception of RB867515 × RB965518, were classified as HR (Figure 1A).

Considering the overall performance of the family types in the evaluations of T1, the HB and IB families were evaluated with the highest brown rust susceptibility scores (3 and 4) on more than half the plots, i.e., classified as MS and S (Figure 1B). Of the plots with CO families, 88% were classified as highly resistant (Figure 1B).

The parents should be characterized for disease susceptibility to promote crosses between

resistant parents, and consequently breed progenies with a higher frequency of resistant plants (Croft *et al.* 2008), to avoid obtaining progenies with susceptibility, mainly to brown rust. In Brazil, high biomass sugarcane clones have been developed in the Northeastern region, where rust and smut problems are smaller, despite recent reports of the occurrence of these diseases.

In the first selection phase, the rust frequency was higher (MS and S) in the families' plots denominated as HB and IB, and smut incidence was higher in the ratoon tillers and clones. In T1, 88 clones were selected without smut and brown rust symptoms and with greater vigor.

The unmeasured characteristics coupled with the fact that field breeders are familiar with the selection of conventional clones resulted in a higher rigor in the selection of this type of family and, consequently, in a lower number of conventional clones (CO) selected for T2, in spite of the low incidence of smut and rust in the families evaluated in T1.

The high biomass (HB) families based on crosses between hybrid sugarcane (*Saccharum* spp.), *S. robustum*, and *S. spontaneum* (until the third generation) had similar smut incidence percentages to those of the other family types. However, the incidence of clones with brown rust symptoms was at least 19% higher than that of the other family types, which resulted in a lower number of clones without symptoms of either disease in T2.

S. spontaneum and *S. robustum* genes' introgression are the main source of resistance to the major sugarcane diseases (Matsuoka *et al.* 2014). In contrast, we observed a higher brown rust and smut incidence in crosses of sugarcane hybrids with *S. spontaneum* and *S. robustum* in T1 than of the progenies derived from crosses among commercial hybrids.

The divergence from the results reported in the literature allows the conclusion that this type of cross will not always favor progenies with a high frequency of smut and brown rust resistant clones. Some symptom-free clones for the diseases studied in T1 presented symptoms in all three types of families in T2. The appearance of symptoms on clones previously selected as resistant is common in breeding programs.

Over a period of five years, 15 genotypes apparently resistant to brown rust were discarded from the breeding program conducted at Canal

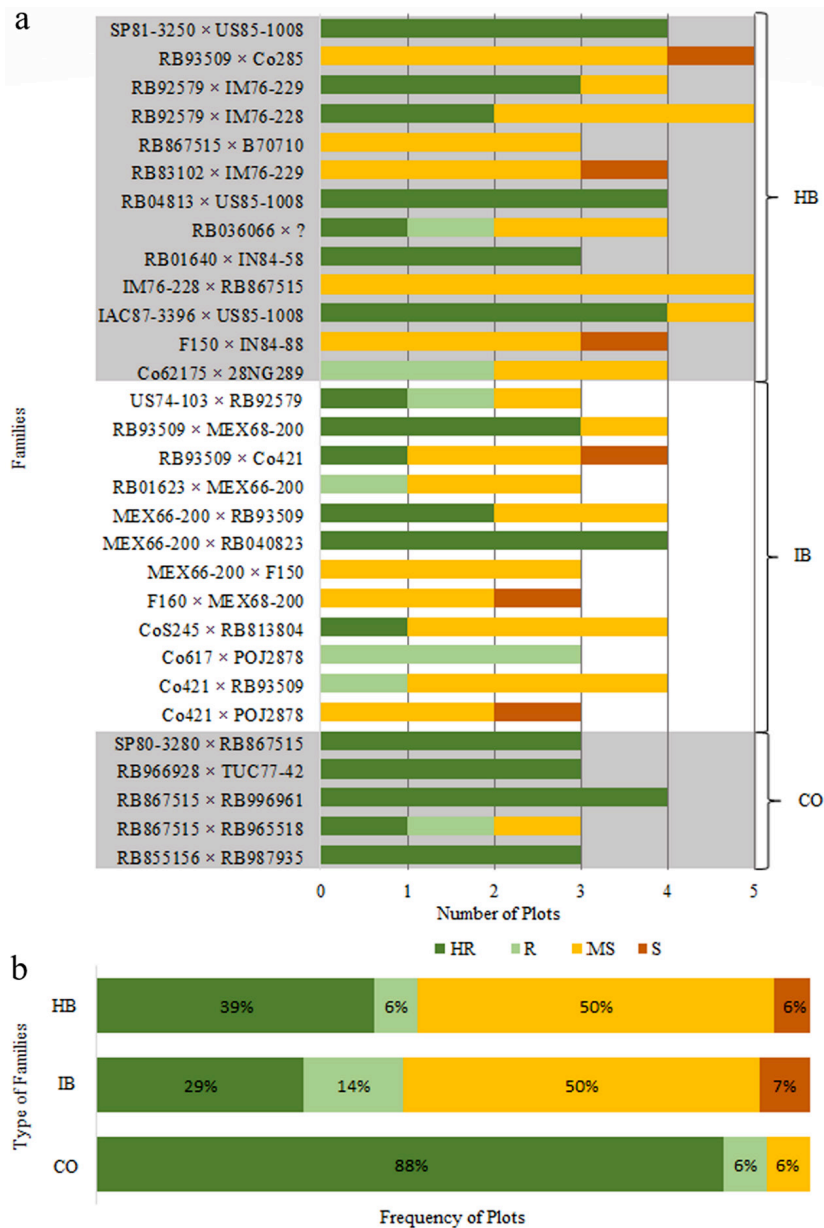


Figure 1. Reaction levels to brown rust (*Puccinia melanocephala*) on plots of conventional (CO), intermediate (IB) and high biomass (HB) sugarcane in the first selection phase (A) and the respective percentages of each family type (B). HR = Highly resistant, R = Resistant, MS = Moderately susceptible and S = Susceptible.

Point - Florida, about one year prior to release after the appearance of disease symptoms (Glynn *et al.*, 2013). In 2007, L79-1002, a variety with high biomass yield, was released in the United States, with CP52-68 as female and Tainan, an *S. spontaneum* clone, as the male parent. After the expansion of the cultivation area of L79-1002 to the

states of Louisiana and Florida, smut susceptibility was stated (Bischoff *et al.* 2008).

The non-appearance of symptoms on susceptible clones may be related to differences between experimental field conditions. In other words, the clones are exposed to unequal inoculum levels. Artificial inoculation would be an interesting tool

to be used for providing a more uniform exposure of the clones (Sood *et al.*, 2009). The clone selection efficiency could also be improved using molecular tools to study genes related to brown rust and smut resistance in sugarcane (Li *et al.*, 2018).

Smut incidence on all HB families varied from 1 to 32% of the clones evaluated in the crosses (Figure 2A), with a mean of 16.69% of smut-infected clones (Figure 2B), i.e., smut incidence was highest on this family ideotype. In this family's ideotype, the smut incidence on clones was < 10% only for the crosses SP81-3250 × US85-1008, RB867515 × B70710, and IAC87-3396 × US85-1008 (Figure 2A).

The smut incidence on clones on the crosses RB92579 × IM76-229, RB92579 × IM76-228, and RB01640 × IN84-58 exceeded 25% and was associated with the highest incidence of ratoon tillers (>15%) (Figure 2A). In the HB families,

smut incidence on the ratoon tillers ranged from 1 to 34% (Figure 2A), and smut symptoms were observed on an average 10% of the ratoons of this family type (Figure 2B).

Only two IB families (MEX68-200 × F150 and Co421 × POJ2878) were smut-free at the time of the evaluation, and 25% (US74-103 × RB92579, RB01623 × MEX68-200, CoS245 × RB813804, and Co421 × RB93509) had a smut incidence on clones of 10%. However, none of the IB families' incidence levels exceeded the mean percentage of 17%, as observed in HB families (Figure 2A and 2B). The families with smut-free progenies were RB867515 × RB965518 (CO), RB966928 × TUC77-42 (CO), Co421 × POJ2878 (IB), and MEX68-200 × F150 (IB) (Figure 2A, of which the latter two were graded with scores of > 3 for brown rust in all plots (Figure 1A).

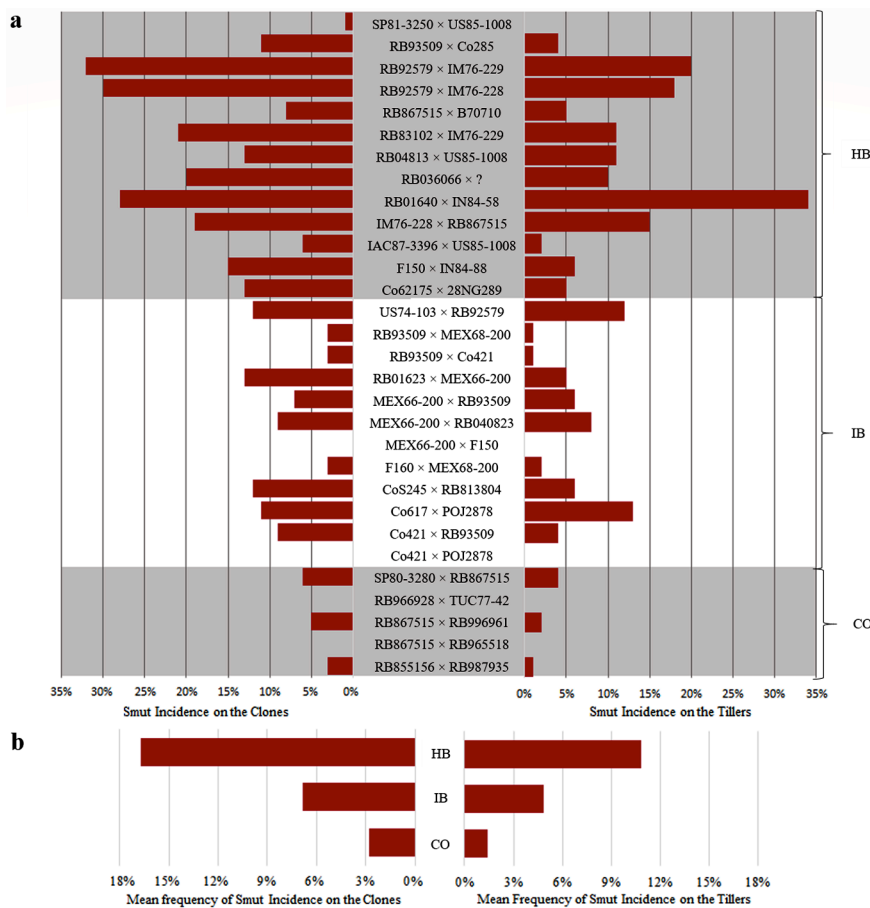


Figure 2. Incidence of clones and tillers with smut (*Sporisorium scitamineum*) in families (A) and family types (B) conventional (CO), intermediate biomass (IB) and high biomass (HB), in the first selection phase (T1).

Of the three CO families with smut symptoms, the highest smut incidence on clones of 6% was observed on cross SP80-3280 × RB867515. This percentage is lower than the overall mean of the IB families (Figure 2A). Concerning the CO families, the progenies of two families (RB966928 × TUC77-42 and RB867515 × RB965518) were smut symptom-free, and in terms of overall performance, had the lowest percentage (<3%) of mean smut incidence on clones and ratoon tillers, considering all three evaluated family ideotypes (Figure 2A and 2B). Of the clones evaluated in T1, 88 (≈ 4% of the total) were selected for T2, free of symptoms of the studied diseases, and good performance for relevant agronomic traits such as high vigor and tillering capacity.

Recurrent selection is an efficient method to increase the fiber content of *S. spontaneum* and *S. robustum* populations' fiber contents since high-fiber parents can generate clones superior to those of the currently cultivated varieties. This strategy can also contribute to the search for disease resistance sources, and that must be implemented in breeding programs for the development of high biomass clones and varieties (Matsuoka *et al.* 2014).

Family performance in the second selection phase (T2)

The results of the clone performance of the different family types evaluated in T2 are listed in Table 3. High resistance to brown rust and the

Table 3. Number and ideotype of clones per family at each Level of Brown Rust Reaction (LBRR) and Smut symptoms during the second selection phase (T2).

TYPE ¹	Family	LBRR				SMUT		HR∩A ²
		HR	R	MS	S	A	P	
CO	RB855156 × RB987935	1	0	0	0	0	1	0
CO	RB867515 × RB965518	0	0	1	0	1	0	0
CO	RB867515 × RB996961	2	0	0	0	1	1	1
CO	RB966928 × TUC77-42	1	0	0	0	1	0	1
CO	SP80-3280 × RB867515	2	0	0	0	2	0	2
IB	Co421 × RB93509	1	0	0	0	1	0	1
IB	Co617 × POJ2878	4	1	1	1	5	2	4
IB	CoS245 × RB813804	1	0	0	0	0	1	0
IB	F160 × MEX68-200	2	0	0	0	1	1	1
IB	MEX68-200 × RB04823	0	1	0	0	1	0	0
IB	MEX68-200 × RB93509	2	0	0	0	2	0	2
IB	RB01623 × MEX68-200	1	0	0	0	1	0	1
IB	RB93509 × Co421	4	0	0	0	3	1	3
IB	RB93509 × MEX68-200	1	0	0	0	1	0	1
IB	US74-103 × RB92579	1	0	0	0	1	0	1
HB	Co62175 × 28NG289	1	0	0	0	0	1	0
HB	F150 × IN84-88	2	0	0	0	1	1	1
HB	IAC87-3396 × US85-1008	4	0	0	0	2	2	2
HB	IM76-228 × RB867515	3	1	0	0	4	0	3
HB	RB01640 × IN84-58	2	0	0	0	2	0	2
HB	RB036066 × ?	4	3	2	2	7	4	1
HB	RB83102 × IM76-229	2	2	4	3	9	2	1
HB	RB867515 × B70710	3	0	0	1	3	1	3
HB	RB92579 × IM76-228	11	1	1	0	7	6	6
HB	RB92579 × IM76-229	2	0	0	1	3	0	2
HB	RB93509 × Co285	2	0	0	2	4	0	2
HB	SP81-3250 × US85-1008	1	0	0	0	0	1	0
TOTAL CO (%)		86	0	14	0	71	29	57.14
TOTAL IB (%)		80.95	9.52	4.76	4.76	76.19	23.81	66.67
TOTAL HB (%)		61.67	11.67	11.67	15	70	30	38.33

¹CO: conventional sugarcane family; IB: intermediate biomass family; HB: high biomass family. LBRR: HR: highly resistant, R: resistant, MS: moderately susceptible and S: susceptible and absence (A) or presence (P) of symptoms during the second selection phase (T2). ²HR∩A: number of clones per family with high rust resistance and absence of smut symptoms

absence of smut symptoms are indispensable characteristics (HR∩A) for selecting clones that constitute the next selection phase since susceptibility to any disease would impair the release of a new variety (Table 3). In T2, 41 clones had no brown rust symptoms (i.e., 4 conventional sugarcane, 14 intermediate biomass, and 23 high biomass energy cane clones).

Of all five CO families evaluated in T1, at least one clone was selected for the second selection phase, with a total of seven clones. Five CO clones (57.14%) were symptom-free for both studied diseases (Table 3). Six CO clones (86%) were classified as highly resistant (HR), and only one clone of the family RB867515 × RB996961 was classified as HR and free of smut symptoms (A) (Table 3), when evaluated in T1, this CO family, had no smut symptoms but was the only one of those selected for smut-symptom absence with plots that were not classified as HR for brown rust (Figures 1A and 2A).

Among the 12 IB families evaluated in T1, two families (MEX68-200 × F150 and Co421 × POJ2878) had no clones selected for the T2 phase (Table 3). The MS and S notes for brown rust influenced these 2 families' exclusion during breeding phases, although not smut incidence was observed in the T1 selection phase (Figure 2A and 3A). In the IB families, 14 clones were identified as HR∩A in stage T2 (Table 3). From the cross for IB, Co421 × RB93509 and the reciprocal, RB93509 × Co421, five HR clones were derived, of which only one had brown rust symptoms.

The HB families with the highest number of clones selected for T2 were: RB036066 × ?, RB83102 × IM76-229, and RB92579 × IM76-228, with at least 11 clones selected in T1, although only the family RB92579 × IM76-228 generated more than one clone without symptoms of either disease (Table 3). The overall frequencies of HR against brown rust in T2 in the selected CO (86%) and IB families (80.95%) were higher than the frequencies for the absence of smut symptoms (A) of 71 and 76%, respectively (Table 3). The families selected for HB had a higher overall frequency of clones with no smut symptoms (70%) than the clones evaluated as highly brown rust-resistant (61.67%) (Table 3).

Considering the overall performance of clones without symptoms of either disease (HR∩A) in the evaluated family types, which may be selected in T2, in the families selected for HB (37.33%), the

percentage obtained was at least 28% lower than that of the selected CO (57.14%) and IB families (66.67%) (Table 3). Of the 88 clones evaluated in T2, 41 clones (≈ 46%) were free of brown rust and smut symptoms (Table 3).

For the achievement of energy cane varieties, more studies on the parents that can contribute to increasing the fiber content are needed, particularly *S. spontaneum* and *S. robustum* accessions, to better understand the general and specific combining abilities for the traits biomass production as well as disease susceptibility.

Therefore, the introduction of new accessions and recurrent selection within *S. spontaneum* and *S. robustum* populations is now necessary to broaden the genetic base of the germplasm banks (BAG) of RIDESA and, thus, to be able to exploit the variability that can be generated from there, to raise the fiber content and resistance to the major crop diseases of the new populations.

These strategies should be adopted because, while conventional breeding has generated new combinations, testing and selecting the new clones in the field under the selection pressure caused by newly arisen pathogen strains, the *S. spontaneum* and *S. robustum* accessions maintained by the RIDESA BAG were not subjected to the same selection pressure. This may explain the fact that the HB crosses evaluated in this study were not efficient in the generation of brown rust and smut resistant clones.

Considering the evaluation in other stages of the breeding program, the clones used in this study that are disease-free and high-performing for the yield variables will become candidates to be released as new varieties and/or will be maintained in a germplasm bank as parents for the development of conventional and intermediate and high biomass sugarcane varieties.

Conclusion

Higher brown smut and rust rates were observed in intermediate and high biomass sugarcane families in the first selection phase. In the second selection phase, the brown rust incidence of high biomass families was higher. However, at the end of two selection phases, intermediate and high biomass sugarcane clones without disease symptoms were selected.

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