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BIOLOGY

Screening of Brazilian sugarcane genotypes for smut reaction

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ABSTRACT: The main method of sugarcane smut (*Sporisorium scitamineum*) control is the genetic resistance. This study assessed the reaction of Brazilian sugarcane genotypes to the smut. The design used was randomized blocks, with 41 genotypes and four replications. The experimental unit consisted of five seedlings per genotype. The genotypes were inoculated by immersing the buds in a smut spores' suspension. For each genotype, it was obtained the disease incidence in percentage of stalk with whip and with the disease incidence data over time the area under the disease progress curve (AUDPC) were calculated for each genotype. Based in the AUDPC, three genotypes were classified as highly resistant (7.3%), eighteen as resistant (43.9%), twelve as moderately resistant (29.3%), three as moderately susceptible (7.3%) and five as susceptible (12.2%). Genotypes with higher levels of resistance to smut sugarcane can be used for integrated disease management and can be used as parents in new crosses in order to obtain progenies with higher proportion of genotypes resistant to smut.
Key words: *Saccharum* spp., *Sporisorium scitamineum*, AUDPC.

Triagem de genótipos brasileiros de cana-de-açúcar à reação ao carvão

RESUMO: O principal método de controle do carvão da cana-de-açúcar (*Sporisorium scitamineum*) é o controle genético. O objetivo deste estudo foi avaliar a reação de genótipos brasileiros de cana-de-açúcar ao carvão. O experimento foi conduzido em blocos casualizados, com 41 genótipos e quatro repetições. A unidade experimental foi composta por cinco mudas por genótipo. Os genótipos foram inoculados através da imersão das gemas em uma suspensão de esporos do fungo. Para cada genótipo, obteve-se a incidência da doença em percentagem de colmos com chicote de carvão e com os dados de incidência da doença ao longo do tempo foi calculada a área abaixo da curva de progresso da doença (AACPD) para cada genótipo. Baseado na AACPD, três genótipos foram classificados como altamente resistentes (7,3%), dezoito como resistentes (43,9%), doze como moderadamente resistentes (29,3%), três como moderadamente suscetíveis (7,3%) e cinco como suscetíveis (12,2%). Genótipos com os maiores níveis de resistência ao carvão da cana-de-açúcar que podem ser utilizados para o manejo integrado da doença e como genitores em novos cruzamentos para obter progênies com maior proporção de genótipos resistentes ao carvão.
Palavras-chave: *Saccharum* spp., *Sporisorium scitamineum*, AACPD.

Sugarcane smut is caused by the fungus *Sporisorium scitamineum* (Syd.) and the losses caused by the disease occurs due to death of stalks and need to early renew the cane field, or yet making the planting of more productive cultivars unfeasible and reduction of the quantity and quality of the juice cane (SANTOS et al., 2004; MANSOOR et al., 2016).

The most effective way of smut control is the use of resistant cultivars (TOKESHI & RAGO, 2005), in this way, during the development process of a cultivar, the sugarcane genetic breeding programs perform genotype evaluations to select those with the highest level of disease resistance. Resistance to smut in sugarcane seems to be a moderately heritable trait, and proportion of resistant progeny can be increased minimizing the use of susceptible parents (CHAO et al., 1990). Besides that, after the cultivar release, it is important that the producers know the level of resistance of the cultivars to sugarcane smut. Thus, the present study assessed the resistance of 41 Brazilian sugarcane genotypes to obtain information to be used for integrated disease management.

The experiment was carried out in field conditions at the Experimental Station of Paranavaí of the Federal University of Paraná (UFPR), in the municipality of Paranavaí, PR (23º05"S, 52º26" W, 470 m asl), in the period from February, 2019 to January, 2020. The design used was randomized blocks with 41 genotypes and four replications. The experimental unit consisted of five seedlings per

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genotype, maintaining 0.5 m between seedlings and 1.5 m between rows (3.75 m^2). The 41 genotypes used were chosen based on their economic and genetic importance within the breeding program (Table 1). The resistant standards used were RB867515 (DAROS et al., 2015), while as intermediate standards were used RB92579 and RB966928 (ARCOVERDE et al., 2018; CURSI et al., 2021) and as susceptible standards, the genotype RB935621 was chosen based in field observations during the selection stages of the breeding program. Likewise, in this group are several clones in different phases of the Sugarcane Genetic Breeding Program (PMGCA), which is linked to RIDESA (Inter-University Network for the Development of the Sugarcane Industry).

The inoculum was collected approximately 60 days before inoculation. Young whips (still covered by a natural silver film, which is the external part of the whip) were collected, avoiding the oldest uncovered whips, which could compromise their viability. Whips were collected from several cities in the northern region of Paraná (Paranavaí, Terra Rica, Nova Esperança, Paranacity, Guairaçá, Alto Paraná and Santa Fé), from 6-8-month-old plants of different sugarcane cultivars, with the aim of increasing the genetic variability of the pathogen. The whips were spread out on a canvas placed over a plastic tarp in a well-ventilated room to dry for 4 to 5 days. After complete drying of the collected whips, the leaf cover and the silver film of the whips were manually removed, and a gentle scraping was performed using a switchblade to remove the teliospores, which were then sieved with a sieve (100 mesh) attached to a vacuum cleaner to remove impurities. The teliospores obtained were packed in paper bags containing 10g of spore/bag and identified, being stored in bottles containing 2/3 silica gel and kept in refrigerator (5 ± 2 °C) until the moment of inoculation. The viability of the teliospores was evaluated according to MINCHIO et al. (2011), using inoculum with a minimum germination of 80%.

The seedlings used came from individualized buds. Before planting, thermotherapy was carried out by immersing the buds in water at 52 °C for 30 minutes, acclimated to room temperature per 5 days, and then followed by fungus inoculation through immersion of the shoots in a suspension containing 1.0 x 10^6 viable teliospores mL^{-1} inside a plastic container with a volume equal to 500 L, where all the treatments were inoculated together at the same time. The immersion time was 30 minutes, with agitation being performed approximately every five minutes. After this period, the inoculated shoots were stored in closed black plastic bags and maintained at an approximate temperature of 30 ºC for 24 hours, being afterwards planted (February 27, 2019) in tubes containing commercial substrate:filter cake (solid residue from sugar or etanol production) (1:1). These were kept in a greenhouse with sprinkler irrigation until 60 days after planting, when the seedlings were transplanted to the field (May 1, 2019).

The evaluations started at 48 days after transplanting (DAT) (June 18, 2019, when the first whips appeared, being performed eight evaluations at approximately every 30 days until 268 DAT (January 24, 2020). The number of tillers with young whips (presence of full or partial silver film) emitted in the period between evaluations and the number of healthy plants per plot were evaluated at the field. The total number of stalks per plot was calculated adding the number of whips (infected stalks) and the number of healthy stalks. From the collected data, of each genotype, was obtained the disease incidence (I) according to the following equation:

$$I = \left(\frac{number\ of\ infected\ stalks}{total\ number\ of\ stalks}\right) * 100$$

With the disease incidence data over time, the area under the disease progress curve (AUDPC) was calculated as proposed by SHANER & FINNEY (1977). The data were submitted to the Shapiro-Wilk normality and Bartlett's homogeneity of variance tests. The results were obtained through analysis of variance (ANOVA), with test of comparison of means (Scott-Knott), at the level of significance of 5%. Statistical analyzes were performed using the R software (R CORE TEAM, 2022). After the genotypes were classified according to each resistance level based in the AUDPC and Scott-knott test and then the progress curves over time with average incidence values for each resistance level were obtained.

The genotypes classification and their respective AUDPC values are presented in Table 1. Based on Scott-knott test, the genotypes were grouped in 4 levels of resistance denominated resistant (R), moderately resistant (MR), moderately susceptible (MS) and susceptible (S). The group of resistant genotypes was divided in two: highly resistant (HR), composed by genotypes that showed absence of symptoms; and resistant (R) formed by the ones, with the presence of the disease (whips). Of the 41 tested genotypes, three were classified as HR (7.4%), eighteen as R (43.9%), twelve as MR (29.3%), three as MS (7.3%) and five as S (12.2%).

The sugarcane smut epidemic started in 48 days after transplanting (108 days after buds inoculations), when the observation of the first whips

Table 1 - Resistance level of sugarcane genotypes to resistance to smut (*Sporisorium scitamineum*) based their AUDPC (area under the disease progress curve) and highest incidence (%) of smut observed in each genotype.

Genotype	-------Female--------	---------Male---------	-------AUDPC-------		--Resistance level--	Highest incidence (%)
RB985476	H53-3989	RB855206	0.0*	a	HR**	0.00***
CV6945	-	-	0.0	a	HR	0.00
RB956911	RB855206	RB855035	0.0	a	HR	0.00
RB041443	RB805203	?	23.0	a	R	0.50
RB056363	RB855063	?	35.1	a	R	0.70
RB011941	BJ7504	RB72454	45.3	a	R	0.80
RB006970	RB855536	SP80-1816	73.4	a	R	1.50
RB106822	NA5679	?	101.3	a	R	1.50
RB056351	RB855206	?	102.0	a	R	3.20
RB126202	RB036066	?	158.2	a	R	2.20
RB127825	CTC14	RB867515	183.5	a	R	2.60
RB046258	RB855002	?	195.8	a	R	2.20
RB946903	RB765418	RB72454	209.4	a	R	2.20
RB046215	SP-931322	RB946903	210.0	a	R	1.60
RB066498	RB966229	RB825548	211.0	a	R	3.10
RB066484	RB966229	RB825548	219.4	a	R	3.80
RB867515	RB72454	?	232.4	a	R	3.40
RB056396	SP-801816	?	235.4	a	R	3.40
RB046221	RB935903	?	236.0	a	R	3.50
RB046210	RB855546	RB962012	248.5	a	R	4.20
RB056320	BJ7015	?	259.9	a	R	4.20
RB006995	SP80-1816	RB855536	335.7	b	MR	7.10
RB106814	RB867515	RB867515	369.4	b	MR	3.80
RB046209	RB855063	MP	377.6	b	MR	4.60
RB106832	RB931536	?	382.3	b	MR	4.10
RB006629	SP80-1816	RB855536	419.8	b	MR	6.00
RB966928	RB855156	RB815690	450.7	b	MR	5.10
RB056349	RB855206	MP	484.5	b	MR	4.90
RB056300	RB912525	?	531.2	b	MR	5.80
RB056301	RB956911	?	534.2	b	MR	5.00
RB036066	SP70-1143	SP77-5181	563.5	b	MR	9.00
RB92579	RB75126	RB72199	578.5	b	MR	7.90
RB036145	SP83-2847	TUC71-7	685.0	b	MR	8.70
RB136301	RB956911	?	1098.2	c	MS	17.00
RB835486	L60-14	?	1346.3	c	MS	11.00
RB006984	SP80-1816	RB855536	1354.4	c	MS	14.20
RB046299	SP83-2847	RB855127	1682.3	d	S	16.10
RB126276	?	?	1713.8	d	S	17.60
RB016916	SP80-3280	RB855589	1836.4	d	S	17.40
RB126201	RB036066	?	1892.7	d	S	20.10
RB935621	RB835089	SP70-1143	1983.5	d	S	20.90

? = Unknown pedigree and - = Pedigree not available.

*Averages followed by the same letter in the column do not differ statistically from each other by Scott Knott's 5% probability.

**HR - highly resistant, R - resistant, MR - moderately resistant, MS - moderately susceptible and S- susceptible.

***The highest incidence was not obtained at the same time for all genotypes.

was possible. The peaks of incidence curves were observed in the fourth evaluation (142 days after transplanting) for all classification groups. Except for genotypes classified as HR, in which whips were not observed during the evaluations, the other curves showed similar pattern (Figure 1). The highest observed incidence value was 20.9% in the RB935621 genotype (susceptibility standard genotype) and 3.4% in the RB867515 genotype (resistant standards genotype) (Figure 1 and table 1).

In the present study it was possible to observe a variability in smut resistance levels in the different genotypes, being this knowledge of disease resistance important to the culture of sugarcane for recommending the use of cultivars with the highest levels of resistance in areas where smut is an issue, ensuring high productivity.

The variations that occur in the culture with the emission of new whips over time demonstrates the importance of periodic evaluations of the disease, as if only one or two evaluations are done during the culture cycle, these values can be under or overestimated, depending on the epidemic phase in which they were observed. Thus, the usage of a variable that encompass the whole period of the epidemic can be more efficient for genotype classification, as is the case of area under the disease progress curve (AUDPC).

Most of the tested genotypes in this study (~81%) were classified as HR, R or MR to the disease. This shows that most of the evaluated genotypes has some level of resistance to smut. This is justified due to the evaluation of cultivars which have already undergone a breeding process. For instance, genotypes that have already been released as cultivars and with their resistance well known fit in the levels of resistance in this study, as is the case of cultivars RB92579 and RB966928 that have an intermediate resistance to smut (ARCOVERDE et al., 2018; CURSI et al., 2021), as well as the cultivar RB867515, which is considered resistant to the disease (DAROS et al., 2015). Sugarcane genotypes with higher levels of resistance to smut sugarcane must be used for integrated disease management. The screening of smut resistance is also an important information for selecting genotypes that will be used as parents in sugarcane crossings. As smut resistance in sugarcane seems to be a moderately heritable trait, the use of susceptible genotypes such as RB046299, RB126276, RB016916, RB126201 and RB935621

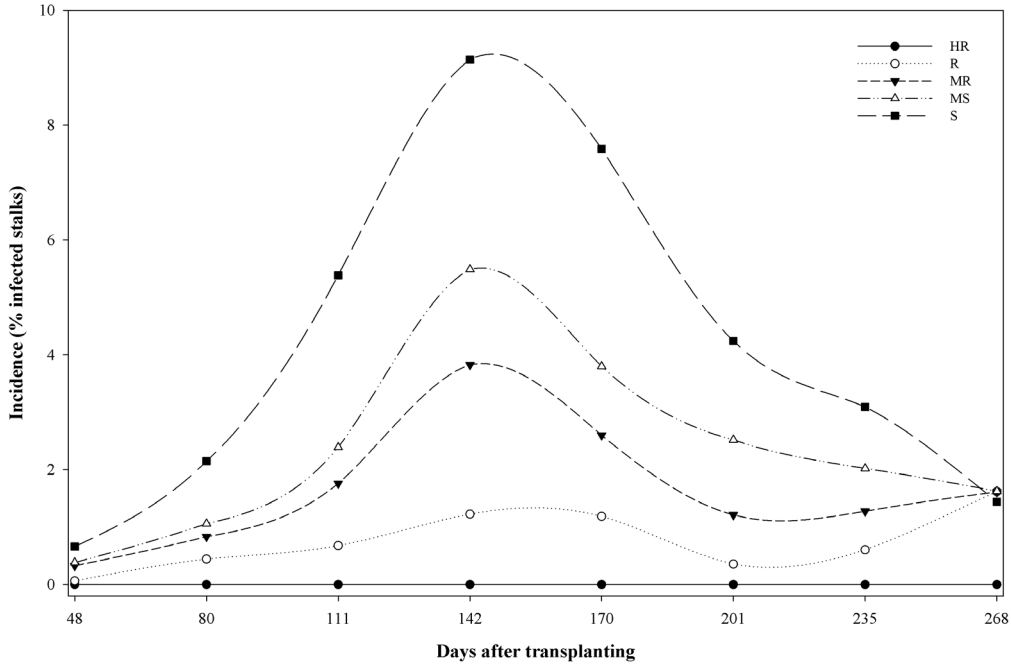

Figure 1 - Incidence of smut (%) over time in sugarcane according to genotypes to the resistance levels: HR – highly resistance, R – resistant, MR – moderately resistant, MS – moderately susceptible and S – susceptible.

(Table 1) as parents should be avoided to increase the proportion of resistant progeny (CHAO et al., 1990).

In conclusion, of the 41 tested genotypes, 21 were classified as highly resistant or resistant, and can be useful for integrated disease management and also can be used as parents in new crosses in order to obtain progenies with higher proportion of genotypes resistant to smut.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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