GMR

The Original by FUNPEC-RP

Resistance to orange rust associated with the G1 molecular marker in parents of Brazilian RB sugarcane varieties

J. Borella, B.P Brasileiro, A.A.C. de Azeredo, L. Ruaro, R.A. de Oliveira and J.C. Bespalhok Filho

Departamento de Fitotecnia e Fitossanidade, Universidade Federal do Paraná, Curitiba, PR, Brasil

Corresponding author: J. Borella
E-mail: borella.juli@gmail.com

Genet. Mol. Res. 21 (1): gmr18980
Received October 07, 2021
Accepted January 13, 2022
Published February 27, 2022
DOI http://dx.doi.org/10.4238/gmr18980

ABSTRACT. Orange rust, caused by the fungus *Puccinia kuehnii*, results in high productivity losses in sugarcane. Selection of resistant genotypes is one of the aims of sugarcane breeding programs. Phenotypic and molecular characterization of the parents of such crosses is essential to obtain superior varieties. We evaluated the reaction to orange rust in the field, the pattern of disease evolution, the molecular marker G1, and the usefulness of this information for the prediction of resistant phenotypes in the main sugarcane parents of the Brazilian Interuniversity Network for Development of the Sugarcane Energy Sector (RIDESA). For this evaluation, 63 sugarcane parents conserved in the RIDESA germplasm bank and that participated most in crosses from 1970 to 2000 were included. The experiment was carried out in a complete block design with three replications, using the clone RB036145 (susceptible to orange rust) as an infective line. Eleven severity assessments were performed using a diagrammatic scale. From the disease severity data, the relative area under the disease progress curve (rAUDPC) was calculated, and the genotypes were classified as resistant, intermediate, and susceptible, compared with the rAUDPC value of the SP79-2233 variety (susceptible to orange rust) and of the variety

RB867515 (resistant to orange rust). Of the 63 sugarcane parents, 43 were classified as resistant, of which 27 were positive for the G1 marker; 10 as intermediate, including seven positives for G1; 10 were susceptible, among which four were positive for this marker. The molecular marker G1 showed an accuracy of 71% in predicting the resistant phenotype and could be used as a tool for the characterization of resistant germplasm.

Key words: Marker-assisted selection; Plant breeding; *Puccinia kuehnii*; *Saccharum* sp

INTRODUCTION

Orange rust, caused by the fungus *Puccinia kuehnii* (Pucciniaceae), is a disease of economic importance in the main sugarcane producing countries, as it causes a reduction in productivity in susceptible cultivars due to the formation of pustules on the leaves and consequent reduction of photosynthetic rate (Zhao et al., 2011). The first outbreak of orange rust was in Australia in 2000, where the susceptible cultivar Q124 accounted for 45% of the crop area at that time, causing devastating consequences for sugarcane production (Magarey et al., 2001). In Brazil, orange rust was first reported in late 2009 (Barbasso et al., 2010). The most widely planted sugarcane variety RB72454 in Brazil between 1995 and 2010 (Cursi et al., 2021) had to be replaced due to its susceptibility to orange rust (Barbosa et al., 2012).

The use of resistant varieties is one of the main methods of controlling the diseases that affect the sugarcane crop. The use of resistant varieties facilitates crop management and reduces production costs by not requiring agrochemicals, in addition to not harming the environment. Disease resistance is among the most important characteristics of the sugarcane improvement program of the Inter-University Network for the Development of the Sugarcane Industry (RIDESA- Rede Interuniversitária para o Desenvolvimento do Setor Sucroenergético) (de Morais et al., 2015). RIDESA's sugarcane breeding program usually evaluates the reaction of sugarcane cultivars to rust (brown and orange) from natural field infection. In disease assessments, diagrammatic scales are used, where scores are assigned visually (Araújo et al., 2013; Klosowski et al., 2013a).

Molecular markers associated with resistance alleles can be extremely useful in identifying resistant genotypes in the early stages of breeding programs and can be used in the process of marker-assisted selection (MAS) (Alzate-Marin et al., 2005). In addition, it can be used to indicate potential parents for future crosses and establish resistance alleles (Fier et al., 2020). Genotype classification by molecular techniques in sugarcane has been used for resistance to brown rust (*Puccinia melanocephala*) (Barreto et al., 2017).

The G1 molecular marker was the first marker to be published associated with the resistance of sugarcane to orange rust and showed an efficiency of 65.8% in the prediction of resistant phenotypes in mapping studies of a F1 segregating population obtained from the cross between clones CP95-1039 (resistant) and CP88-1762 (susceptible) (Yang et al., 2018). Fier et al. (2020) reported an evaluation efficiency in predicting resistance to orange rust of 71.43% using the molecular marker G1 when evaluating Brazilian commercial

cultivars, whereas Hoepers et al. (2020) observed an efficiency in predicting the resistant phenotype of 22.86% using clones in the selection phase and commercial cultivars.

Characterization of germplasm regarding the reaction to the main diseases is a measure that helps in the selection of parents, since it would focus on specific crosses, allowing to increase the number of seeds per cross and, consequently, selection of a greater number of promising clones. Considering that the RIDESA sugarcane germplasm bank lacks information about the reaction to orange rust, the aims of this work were: evaluate the reaction to orange rust in the field and the pattern of disease evolution, the presence of the molecular marker G1 and the accuracy in the prediction of the resistant phenotype in the main sugarcane parents of the Interuniversity Network for Development of the Sugarcane Energy Sector (RIDESA).

MATERIAL AND METHODS

The field experiment was conducted at the Experimental Station of Paranavaí (latitude 22º58'41.21"S; 52º28'4.84"W longitude; altitude of 470 m), belonging to the Federal University of Paraná, from March 2019 to May 2020. The region's climate is classified as Cfa according to Köppen, with annual precipitation from 1,200 to 1,400 mm and average annual temperature between 22 and 26ºC (Aparecido et al., 2016) dystrophic Red Latosol soil, with smooth and undulated relief (Santos et al., 2013). The temperature (ºC), precipitation (mm) and relative humidity (%) data (Figure 1) were obtained from an automatic meteorological station, model Davis Vantage PRO2, located at the experimental site.

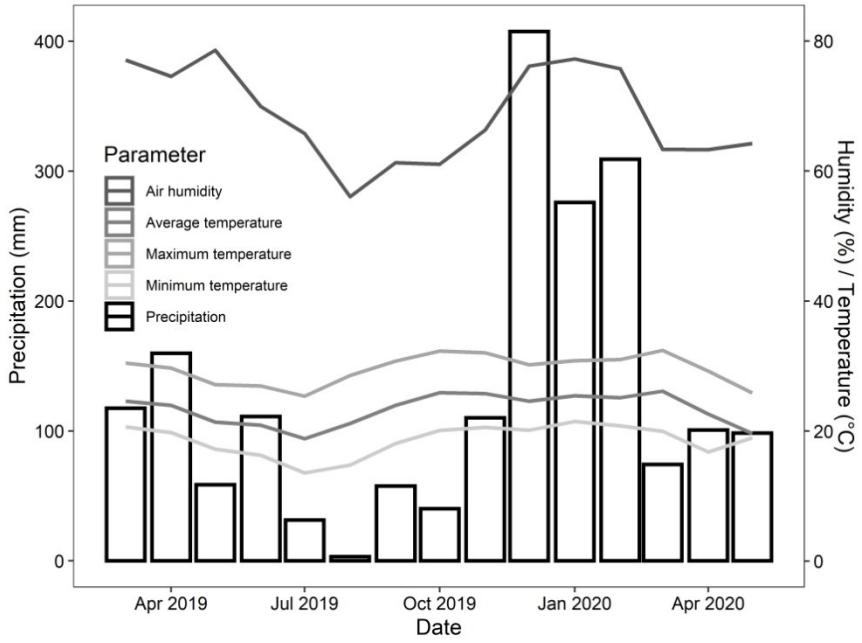

Figure 1. Air humidity, average temperature, maximum temperature, minimum temperature and precipitation in the period from March 2019 to May 2020, in the sugarcane cycle, in the municipality of Paranavaí.

For the experiment, 63 genotypes were used, belonging to the RIDESA germplasm bank, located in the municipality of Murici in the state of Alagoas (latitude 09º 13 'S, longitude 35º 50' W and height 515 m). The genotypes selected for study represent the parents with the highest participation in hybridizations by RIDESA in the 1970s, 1980s, 1990s and 2000s.

The experimental design used was a randomized block design with 63 parents (treatments) and three replications. Each plot consisted of two three-meter furrows spaced at 1.4 m and planting 15 buds per meter. To increase the orange rust inoculum concentration in the area, the clone RB036145 (highly susceptible to orange rust) was used as a donor line, being present in every two plots.

Monthly evaluations of orange rust severity were carried out using a diagrammatic scale developed by Klosowski et al. (2013a) with scores ranging from 1 to 9, 1 (0.06%), 2 (0.14%), 3 (0.36%), 4 (0.89%), 5 (2.17%), 6 (5.18%), 7 (11.87%), 8 (24.9%) and 9 (45%) of leaf area affected by symptoms. The severity assessments started in July 2019, totaling 11 assessments. The disease severity was determined on the +3 leaf, in ten plants per replicate, and scores were assigned to the position with the highest incidence of the disease.

From the severity data, the area under the disease progress curve (AUDPC) was calculated for each parent (Shaner and Finney, 1977). In addition to the AUDPC, the relative area under the disease progress curve (rAUDPC) was obtained, dividing the AUDPC by the time of epidemic as proposed by Fry (1978). Parents were classified as resistant (rAUDPC $\leq$ 0.02), intermediate (0.02 < rAUDPC > 1.87) and susceptible (rAUDPC $\geq$ 1.87). For classification, the rAUDPC value obtained in the present work was used for the SP79-2233 and RB867515 varieties, considered susceptible to orange rust and resistant to orange rust, respectively (Garcés et al. 2014; Klosowski et al., 2015; Chapola et al., 2016; Fier et al., 2020).

Total DNA was extracted according to the CTAB protocol (Ferreira and Grattapaglia, 1995), where 0.3 g of young leaves, without the midrib of each parent, were macerated with liquid nitrogen. The extracted DNAs were resuspended in 50 µL of ultrapure water and the concentration of the samples was performed with the NanoDrop equipment (ND-1000 UV-VIS). After quantification, 5 µL of DNA from each sample was diluted in ultrapure water to a concentration of 120 ng/µL, and then used to perform PCR.

DNA samples were submitted to PCR and the reactions were performed in a final volume of 25 µL, following the GoTaq Master Mix (Promega) protocol containing: 1 x GoTaq Master Mix, 1 µl of each of the primers at a concentration of 10 mM, 3 µl of DNA template at a concentration of 120ng/µl and ultrapure water to make up the volume. The molecular marker G1 was used to amplify a 970 bp fragment, according to the program described by Yang et al. (2018), with the reaction mixture incubated at 95°C for 5 min, followed by five cycles of 60 seconds of denaturation at 96°C and 5 minutes of annealing at 68 °C with a decrease of 2°C each cycle, and 1 minute extension at 7 °C. For another five cycles, the annealing temperature started at 58°C for 2 minutes with a 2°C decrease for each cycle; PCR was continued for an additional 25 cycles of 60 seconds at 96°C, 1 minute at 50°C and 1 minute at 72°C with a final extension at 72°C for 5 minutes. The following pairs of primers were used: Forward: 5'ACCATGGAAATCCATACGTC3' (Forward) and 5'GGCCAACACTTAGGCCAATA3' (Reverse).

The PCR products were visualized on a 1% agarose gel stained with GelRed (Biotium®), using the 100 bp ladder as a molecular weight marker. Cultivars RB855156

and RB72454 were used as a positive controls and cultivar RB845210 as a negative control (Fier et al., 2020).

The accuracy of G1 molecular marker selection in predicting the resistant phenotype was obtained from parental reaction data. To this end, the sum of the number of parents classified as resistant and positive for G1 was divided by the number of parents that presented the molecular marker and multiplying the result by 100 (Yang et al., 2018).

RESULTS

The orange rust progress curve in intermediate and susceptible parents is shown in Figure 2. The first symptoms of orange rust were observed in July 2019, four months after planting, with an increase in severity at the end of November in susceptible parents (Figure 2 A and B). The highest severity values were observed from January onwards for most parents. In the intermediate parents (Figure 2 C and D) a lower intensity of the disease and slow evolution of the disease were observed.

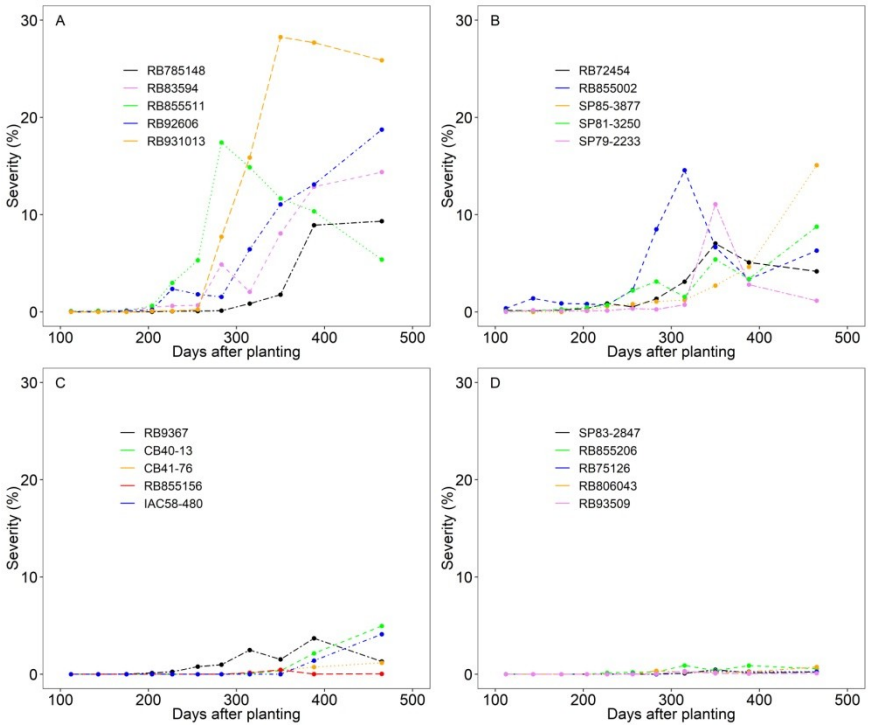

Figure 2. Disease progress curve for orange rust (percentage of leaf area affected by symptoms) in sugarcane parents from the RIDESA germplasm bank, for parents classified as susceptible (A and B) and intermediate (C) and D).

Parents that presented the fragment referring to the G1 molecular marker, amplified via PCR with an expected size of 970 bp, were classified as positive (+). Parents that did not amplify the fragment corresponding to the marker were classified as negative (-) for G1 (Figure 3).

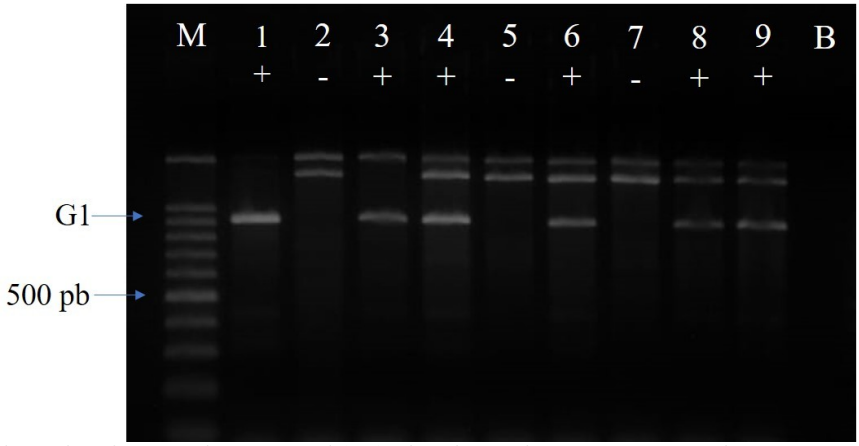

Figure 3. Electrophoretic pattern in agarose gel (1%) of marker G1 in sugarcane parents from RIDESA. Ladder 100pb (M), RB91539 positive (1), RB83102 negative (2), RB835019 positive (3), RB855536 positive (4), CTC 4 negative (5), RB855511 positive (6), RB845210 negative control (7), RB855156 positive control (8), RB72454 positive control (9), white (B).

The results regarding classification of the reaction to orange rust, maximum severity and presence of the molecular marker G1 (+) in the main sugarcane parents are shown in table 1. Of the 63 parents evaluated in the field, 43 (68%) were classified as resistant to orange rust, 10 (16%) as intermediate and 10 (16%) as susceptible. Of the 43 parents classified as resistant to orange rust, 27 (63%) presented the fragment related to the molecular marker G1, 7 (70%) of the 10 classified as intermediate and 4 (40%) of the 10 classified as susceptible, were positive to the marker. The frequency of resistant parents in the 1970s, 1980s, 1990s and 2000s was 75, 70, 67, and 68% respectively.

Table1: Reaction of RIDESA sugarcane genitors to orange rust and presence or absence of the G1 molecular marker in the 1970s (70), 1980s (80), 1990s (90) and 2000s (00).

Genitor	Genealogy		Decades				G1	rAUDPC	Maximun severity	Classification
	♀	♂	70	80	90	00				
M253-48	B34104	M21340	x				+	0	0	R
CO331	CO213	CO214	x				+	0	0	R
CB41-14	?	?	x				-	0	0	R
CB41-76	POJ2878	?	x				+	0.31	1.18	I
CO740	P3247	P4775		x			+	0	0	R
L60-14	CP521	CP48103		x			+	0	0	R
RB735220	CB4495	?		x			+	0	0	R
NA56-79	CO419	CO419		x			+	0	0	R
SP70-1143	IAC4865	?		x			-	0	0	R
SP71-1406	NA56-79	?		x			+	0	0	R
TUC71-7	CP5268	CP62258		x	x		+	0	0	R
IAC58-480	POJ2878	CP44101		x			+	0.55	4.12	I
CB40-13	?	?		x			+	0.93	4.95	I
RB72454	CP5376	?		x	x	x	+	2.74	7.04	S
RB735200	CO331	?			x		-	0	0	R
RB765418	M25348	?			x		+	0	0	R
RB835019	RB72454	NA56-79			x		+	0	0	R

RB835054	RB72454	NA56-79	x	+	0	0	R
RB835089	RB72454	NA56-79	x	+	0	0	R
RB835486	L6014	?	x	-	0	0	R
RB855453	TUC71-7	?	x	+	0	0	R
RB855563	TUC71-7	SP70-1143	x	+	0	0	R
SP79-1011	NA56-79	CO775	x	+	0	0	R
RB751194	CB4049	?	x	+	0	0	R
RB75126	C278	?	x	+	0.11	0.29	I
RB806043	NA56-79	?	x	-	0.19	0.75	I
RB855206	RB72454	TUC71-7	x	+	0.38	0.89	I
RB785148	IAC47-31	?	x	-	2.76	9.33	S
RB855002	SP70-1143	RB72454	x	+	4.53	14.57	S
BJ7504	?	?	x	-	0	0	R
CO62175	CO951	CO419	x	-	0	0	R
CTC4	SP83-5073	?	x	-	0	0	R
F150	CO310	PT4352	x	+	0	0	R
IAC862210	CP5258	CO798	x	-	0	0	R
RB72910	?	?	x	-	0	0	R
RB745464	CP5376	?	x	+	0	0	R
RB83102	NA56-79	SP70-1143	x	-	0	0	R
RB83160	NA56-79	SP70-1143	x	+	0	0	R
RB845210	RB72454	SP70-1143	x	-	0	0	R
RB855035	L6014	SP70-1284	x	+	0	0	R
RB855113	SP70-1143	RB72454	x	-	0	0	R
RB855536	SP70-1143	RB72454	x	+	0	0	R
RB867515*	RB72454	?	x	+	0.02	0.20	R
RB91539	H59-9018	?	x	+	0	0	R
RB928064	SP70-1143	?	x	-	0	0	R
RB931011	RB83160	RB72454	x	+	0	0	R
RB931530	Q107	?	x	+	0.01	0.06	R
RB931566	RB721012	RB835089	x	+	0	0	R
RB931602	Q113	RB72454	x	-	0	0	R
SP80-1816	SP71-1088	H57-5028	x	-	0	0	R
SP80-1842	SP71-1088	H57-5028	x	-	0	0	R
SP80-3280	SP71-1088	H57-5028	x	+	0	0	R
RB855156	RB72454	TUC71-7	x	+	0.07	0.45	I
RB93509	RB72454	?	x	-	0.06	0,33	I
SP83-2847	HJ5741	SP70-1143	x	-	0.11	0,46	I
RB9367	RB72454	RB83102	x	+	1.31	3,70	I
SP79-2233*	H562954	?	x	-	1.87	11.07	S
SP81-3250	CP701547	SP71-1279	x	-	2.77	8.76	S
SP85-3877	H65606	?	x	-	3.00	15.09	S
RB83594	RB72454	B3337	x	+	5.27	14.38	S
RB855511	SP71-1406	?	x	+	7.06	17.42	S
RB92606	Q107	RB72454	x	-	6.38	18.74	S
RB931013	RB72454	RB83100	x	-	12.47	28.25	S

* SP79-2233 Susceptible Standard, RB867515 Resistance Standard; rAUDPC – area under the relative disease progress curve.

The percentage of positive parents for the molecular marker G1 is 60%. These results suggest that the presence of the marker is frequent among the main sugarcane parents used in crosses by RIDESA.

Selection accuracy (efficiency in predicting the resistant phenotype) of the G1 marker was 71% with 27 genotypes being resistant to orange rust (phenotypic and genotypic) among the 38 genotypes that were positive for presence of the G1 marker (Table 1). Considering the severity of the parents belonging to the intermediate and susceptible groups, there was a 31.4% reduction in the mean value of severity when the molecular marker G1 was present, decreasing from 3% to 2%, according to descriptive analysis (Figure 4).

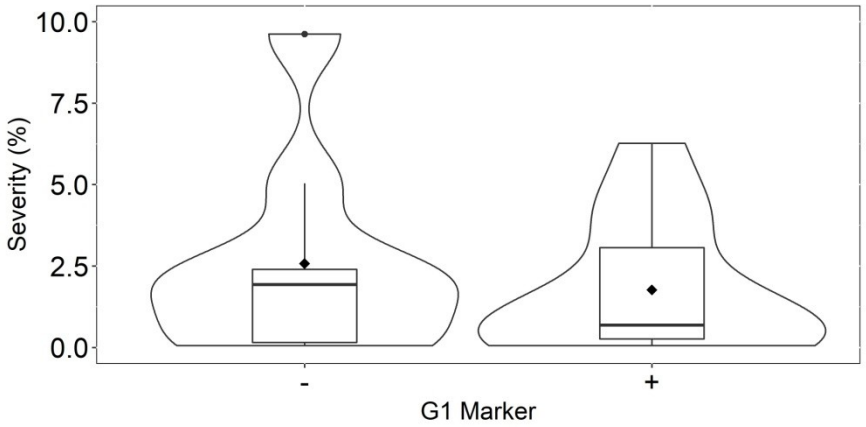

Figure 4. Boxplots with medium severity (percentage of leaf area of sugarcane with symptoms of orange rust) from the intermediate and susceptible parent groups, for the presence of the G1 marker (+) and absence of the marker (-). The black dot indicates the overall mean severity of each group and the line within the boxplots indicates the median.

DISCUSSION

The characterization of germplasm reaction to major diseases is extremely useful for breeding programs. The use of molecular markers as a tool in this characterization process is also important, as it increases the germplasm information, facilitating the choice of parents (Guimarães et al., 2016).

The occurrence of orange rust depends on environmental conditions, with greater severity being observed in conditions of high humidity and warm temperatures (Minchio et al., 2017) in adult plants (Garcia et al., 2007; Braithwaite et al., 2009). In susceptible parents, an increase in the disease progress curve was observed at the end of November and greater disease severity from January onwards, corroborating the research by Araújo et al. (2013), Chapola et al. (2016) and Valeriano et al. (2021).

Sugarcane parents with an intermediate reaction (Figure 2 C and D) presented lower severity for orange rust and the evolution of the disease tended to occur more slowly over time in relation to the parents with a susceptible reaction (Figure 2 A and B), indicative of horizontal/quantitative resistance. Quantitative resistance is defined as a type of resistance that affects both disease onset and development speed, phenotypically incomplete resistance and characterized by reduced disease intensity, but not absence when compared to more susceptible genotypes (Amorim et al., 2018).

The percentage of resistant parents to orange rust under field conditions observed in this work was 68%, indicating that although the disease appeared in Brazil only in December 2009, a large percentage of resistant parents were already being used in the crossbreeding combinations in RIDESA sugarcane breeding program. This contributed to the disease not causing major losses in productivity with its emergence in Brazil because a larger number of varieties were already resistant and susceptible cultivars were quickly replaced (Fier et al., 2020). The arrival of orange rust in Colombia in July 2010 also caused susceptible clones to be quickly eliminated from the experiments, with the most planted commercial cultivars being resistant to the disease (Cadavid et al., 2012).

Although orange rust appeared in Brazil in 2009 (Barbasso et al., 2010), we can observe that most of the parents used in crosses in the 1970s and 1980s were resistant to the disease, and there was an increase in the number of intermediate and susceptible parents being used in crosses in subsequent decades. This increase in the number of parents with intermediate and susceptible responses over the decades may have occurred due to the participation of RB72454 (susceptible to orange rust) on the genealogy of others parents (as female or male parent). In a work carried out by Borella et al. (2021) that evaluated the resistance to brown rust in the same group of genotypes, the frequency of resistant parents had an opposite tendency of what occurs with orange rust.

RB72454 (susceptible to orange rust and positive to G1 mark) is the most important parent of RIDESA, having participated in crosses during the 1980s, 1990s and 2000s, playing an important role in the development of new cultivars. In this study, RB72454 participated in 17 combinations of crosses as a genitor (female or male) and gave rise to 9 resistant genotypes. A successful example in which the parent RB72454 participated as a mother was the cultivar RB867515 (resistant to orange rust and also positive to G1 mark). This reinforces the possibility of considering the use of susceptible parents to orange rust in mating combinations when they present other favorable characteristics, as was the case for RB72454 (Klosowski et al., 2013b, Yang et al., 2019).

For 12 (twelve) parents it was possible to track the presence of the G1 marker in their genitors. Six of these parents came from crosses were both genitors were positive to G1 marker and all of them were also positive. From this six, 4 presented a resistant reaction (RB835019, RB835054, RB835089 and RB931011) while two presented an intermediate reaction to orange rust (RB855206 and RB855156).

For the others six parents, one genitor was positive and the other negative to G1 marker. Half of these parents (3) were positive to G1 and half (3) were negative. Regarding the reaction to orange rust, five parents were resistant (RB83102, RB83160, RB845210, RB855113 and RB855536) and one was susceptible (RB855002). Thus, it looks like that the resistance gene(s) associated with the G1 marker is involved in quantitative resistance and that more than one copy (allele) of the resistance gene linked to the G1 marker may increase the levels of resistance to orange rust, as suggested by Fier et al. (2020).

Yang et al. (2018) have reported the resistance provided by G1 marker is quantitative and stronger resistance were achieved if combined with other loci (additive effect). This could be the cause why in crosses between the parent RB72454 (susceptible to orange rust and positive for G1) and the parent NA56-79 (resistant and positive for G1) only resistant progenies were observed, while intermediate genotypes were observed between RB72454 and TUC71-7 (resistant to orange rust and positive to G1).

The molecular marker G1 was developed by researchers at the Sugarcane Field Station in Canal Point based on studies from a segregating population obtained by crossing sugarcane clones (CP95-1039 and CP88-1762) and showed an efficiency of 65.8% (Yang et al., 2018). In the work by Fier et al. (2020), using the molecular marker G1 in Brazilian sugarcane cultivars, observed an efficiency in the prediction of resistance of 71.43%, indicating that this marker could be used for assisted selection in sugarcane breeding programs. In a study carried out by Hoepers et al. (2020), the authors did not validate the molecular marker G1 because it showed an accuracy of 22.86% in the prediction of the phenotype resistant to orange rust, using clones selected in a Brazilian breeding program and commercial sugarcane cultivars. For the group of parents used in this study, the

molecular marker G1 showed an accuracy of 71% in the prediction of resistant parents, a result similar to that obtained by Yang et al. (2018) and Fier et al. (2020) and higher than those obtained by Hoepers et al. (2020).

The absence of the G1 molecular marker in parents resistant to the disease (such as SP70-1143, RB735200, RB835486, RB845210, CTC4, RB83102, SP80-1842), and the presence of the G1 marker in intermediate and susceptible parents, suggests that other genes may be related to resistance to orange rust and that the single presence of G1 marker is not enough to bring resistance to the disease. Klosowski et al. (2013b) and Yang et al. (2018) have already reported the possibility of quantitative inheritance for orange rust, in which the resistance response is based on the interaction of the effect of several genes, each one contributing quantitatively to the plant's resistance level, which may be influenced by the environment.

In a work carried out by Klosowski et al. (2013b) with orange rust, the authors observe that the reaction of progenies from crosses involving resistant parents do not supported the hypothesis of 3:1 segregation that conditions the presence of a major gene for resistance, differently from what Daugrois et al. (1996), with the Bru1 gene for brown rust. In a study by Yang et al. (2019), the authors suggested that resistance to orange rust was quantitative with the contribution of multiple genes. In addition, others QTLs associated with resistance to orange rust have been identified, which agrees with the theory that multiple genes are involved in the sugarcane response to orange rust (Yang et al., 2018; Yang et al., 2019). Other molecular markers associated with sugarcane resistance to orange rust were reported by McCord et al. (2019), in an experiment with genotypes from the Sugarcane Research Station germplasm collection in Canal Point (FL, United States).

In the present study, the G1 marker has shown a high efficiency for prediction of resistant genotypes for orange rust (71%) and to be effective in reducing the severity of the disease, proving to be a helpful tool to be used in the characterization of sugarcane germplasm, complementing the information from field evaluations, and contributing to the selection of parents for crosses. More studies aimed at understanding the inheritance of resistance to orange rust and the mechanisms linked to the resistance response should be developed, as well as new studies in order to investigate new sources of resistance to the disease.

CONCLUSIONS

Of the main parents used by RIDESA's sugarcane genetic improvement program, 68% were classified as resistant to orange rust. The molecular marker G1 showed 71% accuracy in predicting the resistant phenotype, being a valuable tool for germplasm characterization.

ACKNOWLEDGMENTS

This study was financed in part by the Coordination of Superior Level Staff Improvement (CAPES/Brazil) Finance Code 001. We are grateful to the Sugarcane Genetic Improvement Program of the Federal University of Paraná belonging to the 'Inter-University Network for the Development of the Sugarcane Industry—PMGCA/ RIDESA/UFPR for financial support.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Alzate-Marin AL, Cervigni GDL, Moreira MA and Barros EG (2005). Seleção assistida por marcadores moleculares visando ao desenvolvimento de plantas resistentes a doenças com ênfase em feijoeiro e soja. *Fitopatol. Bras*. 30: 333–342.

Amorim L, Rezende JAM and Bergamin Filho A (2018). Manual de Fitopatologia Volume I Princípios e conceitos. 5ª Ed, Editora Agronômica Ceres Ltda.

Aparecido LEO, Rolim GS, Richetti J, Souza PS, et al. (2016). Climate classifications for climatic zoning in the State of Paraná, Brazil. *Ciênc. Agrotec*. 40(4): 405-417.

Araújo KL, Canteri MG, Gilio TAS, Neubauer RA, et al. (2013). Resistência genotípica e monitoramento da favorabilidade para ocorrência da ferrugem alaranjada da cana-de-açúcar. *Summa.* Phytopathol. 39: 271-275. https://doi.org/10.1590/S0100-54052013000400007.

Barbasso D, Jordão H, Maccheroni W, Boldini J, et al. (2010). First report of *Puccinia kuehnii*, causal agent of orange rust of sugarcane, in Brazil. *Plant. Dis*. 94: 1170. https://doi.org/10.1094/PDIS-94-9-1170C.

Barbosa MHP, Resende MDV, dos Santos Dias LA, Barbosa GVS, et al. (2012). Genetic improvement of sugar cane for bioenergy: The Brazilian experience in network research with RIDESA. *Crop Breed. Appl. Biotech*. 12: 87-98. https://doi.org/10.1590/S1984-70332012000500010.

Barreto FZ, Balsalobre TWA, Chapola RG, Hoffmann HP, et al. (2017). Validação de marcadores moleculares associados à resistência à ferrugem marrom em cana-de-açúcar. *Summa*. *Phytopathol*. 43: 36-40. https://doi.org/10.1590/0100-5405/168917.

Braithwaite KS, Croft BJ, Magarey RC and Scharaschkin T (2009). Phylogenetic placement of the sugarcane orange rust pathogen *Puccinia kuehnii* in a historical and regional context. *Australasian Plant. Pathol.* 38(4): 380-388.

Borella J, Brasileiro BP, Azeredo AAC, Ruaro L, et al. (2021). Reaction to Brown Rust and Presence of the Bru1 Gene in Brazil/RIDESA Sugarcane Parents. *Sugar Tech*. 23: 1037-1044.

Cadavid M, Ángel JC and Victoria JI (2012). First Report of Orange Rust of Sugarcane Caused by *Puccinia kuehnii* in Colombia. *Plant Dis*. 96(1): 143. https://doi.org/10.1094/PDIS-05-11-0406. PMID: 30731887.

Chapola RG, Hoffmann HP and Massola NS (2016). Reaction of sugarcane cultivars to orange rust (*Puccinia kuehnii*) and methods for rapid identification of resistant genotypes. *Tropical Plant. Pathol*. 41: 139-146.

Cursi DE, Hoffmann HP, Barbosa GVS, Bressiani JA, et al. (2021). History and Current Status of Sugarcane Breeding, Germplasm Development and Molecular Genetics in Brazil. *Sugar Tech*. 24: 112-133.

Daugrois JH, Grivet L, Roques D, Hoarau JY, et al. (1996). A putative major gene for rust resistance linked with an RFLP marker in sugarcane cultivar R570. *Theor. Appl. Genet*. 92: 1059-1064.

de Morais LL, de Aguiar MS, Silva PA, Câmara TMM, et al. (2015). Breeding of sugarcane. In Industrial crops: Breeding for bioenergy and bioproducts, eds. Von Mark V. Cruz, and David A. Dierig, p.29-42.

Ferreira ME and Grattapaglia D (1995). Introdução ao uso de marcadores RAPD e RFLP em Análise Genética. Brasília: Embrapa, Cenargem.

Fier I, Balsalobre TWA, Chapola RG, Hoffmann HP, et al. (2020). Field resistance and molecular detection of the orange rust resistance gene linked to G1 marker in Brazilian cultivars of sugarcane. *Summa. Phytopathol.* 46: 92-97. https://doi.org/10.1590/0100-5405/221803.

Fry WE (1978). Quantification of general resistance of potato cultivars and fungicide affects for integrated control of potato late blight**.** *Phytopathology.* 68: 1650-1655.

Garcés FF, Fiallos FF, Martinez F, Aime MC, et al. (2014). First Report of Orange Rust of Sugarcane Caused by *Puccinia kuehnii* in Ecuador. *Plant. Dis*. 98(6): 848. https://doi.org/10.1094/PDIS-05-13-0574-PDN.

Garcia EO, Casagrande MV, Rago AM and Junior NSM (2007). Preservação de urediniósporos de *Puccinia melanocephala*, agente causal de ferrugem em cana-de-açúcar. *Summa. Pphytopathol*. 33(2): 152-156. https://doi.org/10.1590/S0100-54052007000200009.

Guimarães CT, Schuster I, Magalhães JV and Souza Júnior CL (2016). Marcadores moleculares no melhoramento de plantas. In: Borém A and Caixeta E. *Marcadores Moleculares*. p129-177.

Hoepers LML, Koch G, Silva MCC, Ruaro L, et al. (2020). Validation of the G1 Molecular Marker Associated with Resistance to Orange Rust in Brazilian Sugarcane Genotypes. *Sugar Tech*. 23: 108-117.

Klosowski AC, Bespalhok Filho JC, Ruaro L and May de Mio LL (2013a). Proposta e validação de escala para a ferrugem alaranjada da cana-de-açúcar. *Tropical Plant. Pathol.* 38: 166-171.

Klosowski AC, Bespalhok Filho JC, Ruaro L and May de Mio LL (2013b). Inheritance of Resistance to Orange Rust (*Puccinia kuehnii)* in Sugarcane Families from Crosses Between Parents with Different Orange Rust Reactions. *Sugar Tech*. 15: 379-383.

Klosowski AC, Bespalhok Filho JC, Ruaro L, Fragoso RB, et al. (2015). Reação de cultivares e época de avaliação da ferrugem alaranjada da cana-de-açúcar. *Biosci. J.* 31: 489-498.

Magarey RC, Croft BJ and Willcox TG (2001). An epidemic of orange rust on sugarcane in Australia. *Proc. Int. Soc. Sugarcane Technol.* 24: 410-416.

McCord P, Glynn N and Comstock J (2019). Identifying markers for resistance to sugarcane orange rust (*Puccinia kuehnii*) via selective genotyping and capture sequencing. *Euphytica.* 215: 150.

Minchio CA, Fantin LH, Oliveira KB, Rocha JA, et al. (2017) Morphological changes of the Urediniospore of *Puccinia kuehnii* germ tube in function of temperature. *Agron. Sci Biotechnol*. 3: 19-24.

Santos HG dos, Jacomine PKT, Anjos LHC, Oliveira VA, et al. (2013). *Sistema Brasileiro de Classificação de solos*. 3.ed. Brasília, EMBRAPA, 353p.

Shaner G and Finney RE (1977). The effect of nitrogen fertilization on the expression of slow-mildewing in knox wheat. *Phytopathology*. 67: 1051-1056.

Valeriano TTB, Rolim GS, Manici LM, Giustarini L, et al. (2021). Process-based model to simulate sugarcane orange rust severity from weather data in Southern Brazil. *Int. J. Biometeorol.* 65: 2037-2051.

Yang X, Islam MS, Sood S, Maya S, et al. (2018). Identifying quantitative trait loci (QTLs) and developing diagnostic markers linked to orange rust resistance in sugarcane (Saccharum spp.). *Front. Plant Sci.* 9: 350. https://doi.org/10.3389/fpls.2018.00350.

Yang X, Sood S, Luo Z, Todd J, et al. (2019). Genome-Wide Association Studies Identified Resistance Loci to Orange Rust and Yellow Leaf Virus Diseases in Sugarcane (Saccharum spp.). *Phytopathology*. 109(4): 623-631. https://doi.org/10.1094/PHYTO-08-18-0282-R.

Zhao D, Glynn NC, Glaz B, Comstock JC, et al. (2011). Orange Rust Effects on Leaf Photosynthesis and Related Characters of Sugarcane. *Plant Dis*. 95(6): 640-647. https://doi.org/10.1094/PDIS-10-10-0762.

