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Quantification of brown rust infection in sugar cane progeny

Cuantificación de la infección por roya parda en progenies de caña de azúcar

 Yaquelin Puchades-Izaguirre*,  Wilfre Aiche-Maceo,
 Reynaldo Rodríguez-Gross,  Mónica Tamayo-Isaac

Instituto de Investigaciones de la Caña de Azúcar (INICA), carretera CUJAE Km 1½, Rancho Boyeros, CP 19390, La Habana, Cuba

ABSTRACT: Brown rust is one of the most important diseases for sugar cane worldwide. In Cuba, resistance to the disease is a criterion for the selection of new cultivars and it is evaluated in the genetic improvement program from early stages. This work was developed with the objective of quantifying, in Clonal Lot Stage 1, the incidence and severity of brown rust by crosses. For this purpose, data were collected from individuals managed by the genetic improvement program in the southeastern region of Cuba. The variables degree, incidence and severity of the disease among families and parents were determined. Results showed that there is high variability in the development of brown rust in Clonal Lot 1. The degree and severity variables are the most useful for identifying families with progenies resistant or tolerant to the disease. The C90-501 x Mex66-1235 and C90-501 x B6368 families provide high yielding individuals with resistance to brown rust. The analysis allows breeders to develop crosses that provide high-yielding sugarcane and sugar individuals with resistance to the disease.

Key words: cross, incidence, *Puccinia melanocephala*, parents.

RESUMEN: La roya parda es una de las enfermedades más importantes para la caña de azúcar en todo el mundo. En Cuba, la resistencia a la enfermedad es criterio de selección de nuevos cultivares y se evalúa en el programa de mejoramiento genético desde las etapas tempranas. Este trabajo se desarrolló con el objetivo de cuantificar, en la Etapa Lote Clonal 1, la incidencia y la severidad de la roya parda por cruces. Para ello, se recolectaron los datos de los individuos manejados por el programa de mejoramiento genético en la región suroriental de Cuba. Se determinaron las variables grado, incidencia y severidad de la enfermedad entre familias y progenitores. Los resultados mostraron que existe alta variabilidad en el desarrollo de la roya parda en el Lote Clonal 1. Las variables grado y severidad resultan las más útiles para identificar familias con progenies resistentes o tolerantes a la enfermedad. Las familias C90-501 x Mex66-1235 y C90-501 x B6368, aportan individuos de alto rendimiento y con resistencia a la roya parda. El análisis permite a los mejoradores el desarrollo de cruzamientos que aporten individuos de altos rendimientos en caña y azúcar, con resistencia a la enfermedad.

Palabras clave: cruce, incidencia, *Puccinia melanocephala*, padres.

INTRODUCTION

Sugarcane brown rust is a disease widely distributed in most sugarcane growing areas of the world (1). It is caused by the phytopathogenic fungus *Puccinia melanocephala* Sydow & P. Sydow, and is considered among the most important diseases affecting the crop (2,3).

Although first reported in Java in 1890, epiphytophthora increased in frequency since 1949. In the 1970s, severe outbreaks occurred in major sugar cane producing countries such as Cuba, Jamaica, Australia, United States, Mexico, India, Thailand and Mauritius, leading to the abandonment of high yielding sugarcane and sugar cultivars, e.g., B4362, Co475, T34362 and CP78-1247.

*Author for correspondence: yaquelin.puchades@inicasa.azcuba.cu

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In the absence of control measures, brown rust causes yield losses of 10 to 50% in susceptible sugar cane cultivars. In regions where the pathogen is well established, the disease is generally managed by using resistant cultivars. However, the durability of resistance has been erratic, due to the constant variability of the causal agent (5).

In Cuba, the behavior against the disease is a criterion for the selection of new sugarcane cultivars. From the first stages of the breeding scheme, infection methods are applied with high inoculum pressure that allow discriminating the greatest possible amount of susceptible population (6). In this way, it is guaranteed that there are no high percentages of clones rejected for this reason in the final stage of the process.

The breeding and selection of cultivars resistant to *P. melanocephala* is a challenge, due to the complex genome of sugar cane and the long breeding process that normally takes ten or more years (7,8). These reasons also make the replacement of susceptible cultivars very slow (9).

The selection of parents to be used in the breeding program is a critical decision for plant breeders. Knowledge and a better understanding of the variability in the incidence and severity of brown rust in progenies from early stages provide useful information to increase the efficiency of breeding programs. The present work aims to quantify the incidence and severity of brown rust among crosses (families), with data collected from individuals in Clonal Batch Lot Stage 1 of the genetic improvement program in the southeastern region of Cuba.

MATERIALS AND METHODS

Experiment planting: Clonal Lot 1 of the 2017 series, LC1-2017, was established in October 2018 with individuals (one seedling) planted, with two bud cuttings, at 1.0 x 1.6 m spacing. With the scheme used, infection methods with high inoculum pressure are applied that allow discriminating as much of the susceptible population as possible (6). In LC1-2017, 18 combinations of the crossing program were evaluated, including 671 individuals, 13 females and 14 male parents.

Data collection: The brown rust evaluation was carried out between three and five months of crop age. For this, based on the observation of symptoms, the disease was categorized using a scale of four grades (10), where: 0= without infection (highly resistant); 1= isolated pustules (resistant); 2= union between pustules (intermediate) and 3= massive presence of pustules with necrotic areas by the union between pustules (susceptible).

All families (crosses) included in the crossing program, which at the time of planting had 40 or more individuals, were analyzed. For the quantification of infection, the following variables were determined: mean degree of infection per family, percentage of infection (incidence) and severity. The formulas used were:

Mean degree of infection = $\sum \text{degree of infection} / \text{total clones}$ (It includes individuals with grade 0).

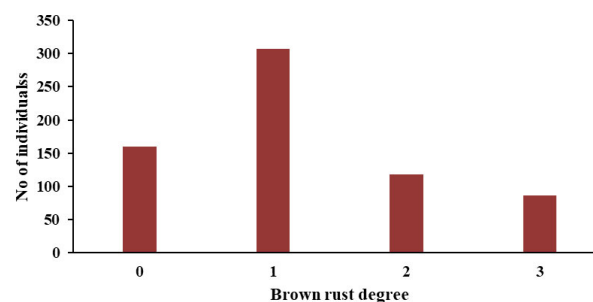


Figure 1. Distribution by degree of brown rust in Clonal Lot 1-2017 of the genetic improvement program in the southeastern region of Cuba

Incidence = $(\# \text{ of infected clones} / \text{total clones}) * 100$

Severity = $\sum \text{degree of infection} / \# \text{ of infected clones} * 100$ (excludes individuals with grade 0).

Data analysis: Data were analyzed by crosses and parents. Coefficients of variation (CV) between families and parents for each parameter were estimated with the mean of each family and parent. While, the CVs within families and parents were determined with the overall mean. The relationship between incidence and severity with disease grades was tested by linear regression. The data from the crosses were compared with the estimated genetic value (EGV) and its classification, according to the mathematical model of Rodriguez *et al.* (11,12), using SAS software (13). The LC1-multivariate model was chosen, which includes seven variables related to sugar content of the progenies, components of agricultural yield (diameter and length of stems) and resistance to charcoal and brown rust.

RESULTS AND DISCUSSION

The general parameters evaluated in LC1-2017 are shown in Table 1.

Table 1. Parameters related to brown rust evaluated in Clonal Batch 1-2017

Total number of clones evaluated	671
No. of uninfected clones	160
No of infected clones	511
% Infection	76.2
Severity (mean value of infected clones)	1.57
Overall mean grade (includes non-infected clones)	1.2

The distribution of the disease, according to the grades of the scale used, it is shown in Figure 1. The highest percentage of individuals (45.7 %) were categorized as grade 1 (resistant), while 12.8 % were susceptible (grade 3) and, therefore, will be eliminated from the selection scheme.

The number of clones per family was variable (Table 2), which is associated with differences in the number of postings per cross and in percentages of selection in the previous stage (postings). High variability was observed in

Table 2. Maximum, minimum, mean and coefficient of variation values of clone number and brown rust (BR) degrees of the families evaluated in Clonal Batch 1-2017

Parameter	No. clones	BR degree
Maximum	42	3
Minimum	28	0
Mean	37	1.2
SD	4.55	0.94
CV % (between families)	12.3	78.3
CV % (within families)		70.6

SD-Standard deviation, CV- coefficient of variation

the degrees of brown rust, both within and between families, the latter being higher, suggesting the usefulness of the evaluations carried out in Clonal Lot 1, for the identification of crosses and parents that provide resistance or tolerance to brown rust.

The incidence and severity variables showed a positive and significant linear correlation with the mean degree of disease infection between families (Figure 2). This means that both parameters can be used to determine differences between crosses in response to brown rust at the Clonal Batch Lot 1 stage; however, the coefficient of determination (R^2) for severity is higher, indicating that it is more suitable for evaluations.

In general, variables mean brown rust degree between families, incidence and severity, ranged from 0.6-2.56, 52.5-100 % and 1.13-2.56, respectively (Table 3).

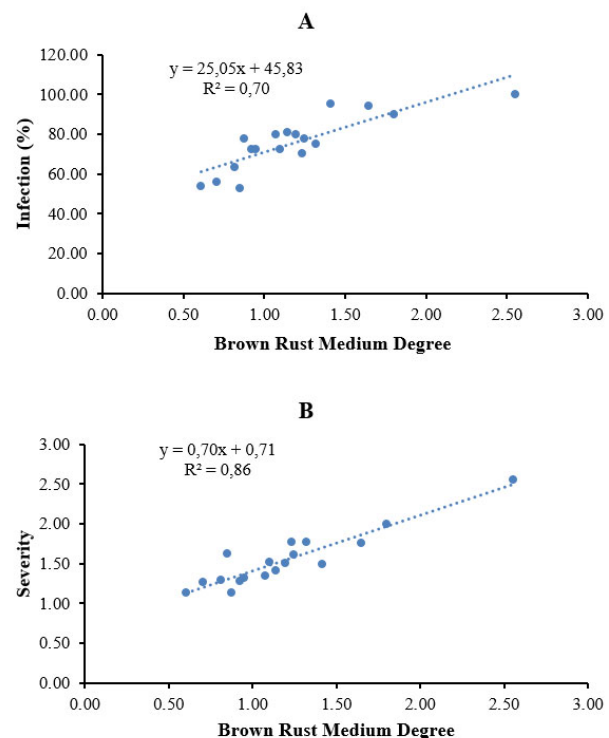
The crosses were ordered according to the average degree of the disease and greater variability was detected in the degree and severity of the disease, which confirms the usefulness of both variables to determine crosses with progenies resistant or tolerant to brown rust.

The 18 families analyzed belong to the national crossing program, but only nine have been previously evaluated with 40 or more individuals in LC-1 and have a classification based on the EGV (Table 4). Particularly, the cross C90-501 x Mex66-1235, is categorized as proven by its EGV, which denotes its importance in providing high yielding individuals with resistance to brown rust. Meanwhile, C187-68 x CP70-1133 is a family that provides selection, although with susceptibility to the disease.

The crosses C90-501 x B6368, C323-68 x CP70-1133 and C92-325 x C86-407, classified as exploratory, have provided selection, but in the last two, the incidence and severity of brown rust show high proportions. This implies future evaluations to decide whether to keep them in the crossing program.

From crosses classified as moderately discarded, C323-68 x CP72-2086 provides individuals with resistance or tolerance to brown rust. While C1616-75 x CP70-1133 shows low EGV and considerable disease affection. These same characteristics are evident in C323-68 x C227-59 and B6368 x Co997, which confirms them as possible to discard in the Cuban crossing program.

In the feminine parents, the variables mean brown rust degree, incidence and severity were between 0.9-2.6, 59.4-100 % and 1.3-2.6, respectively (Table 5). Greater



A) Incidence and B) severity of 18 families at the Clonal Lot 1 stage of the of genetic breeding program in the southeastern region of Cuba

Figure 2. Relationship between the average degree of brown rust and the variables incidence and severity

variability was observed in the variables brown rust degree and severity. The importance of C90-501 in the crossbreeding program is noteworthy, as it provides individuals resistant to the disease.

Values obtained for the brown rust degree, incidence and severity variables of the male parents are shown in Table 6. In this sense, Mex66-1235 is of lower value in the three variables evaluated, which confirms its importance in the crossing program. Further studies of families including B45181, CP72-2086 and CP70-1133 will provide a better estimate of their contribution in obtaining disease resistant or tolerant cultivars.

The high variability observed in the Clonal Lot 1 stage, in the southeastern region of Cuba, denotes the importance of family selection for the evaluation of progenies resistant or tolerant to the disease. Brown rust is one of the most

Table 3. Variables brown rust degree (BR), incidence (%) and severity of the families evaluated in Clonal Lot 1-2017

Family Code	Progenitors		BR degree	Incidence (%)	Severity
	Femenino	Masculino			
465	C90-501	Mex66-1235	0.6	53.6	1.1
687	CP72-2086	B45181	0.7	55.9	1.3
1640	C323-68	CP72-2086	0.8	63.2	1.3
476	C90-501	B6368	0.9	52.5	1.6
278	C92-325	C86-407	0.9	77.5	1.1
320	C86-56	C187-68	0.9	72.5	1.3
650	C323-68	C227-59	1	72.5	1.3
1636	C323-68	CP70-1133	1.1	80	1.3
1975	C266-70	B45211	1.1	72.5	1.5
1552	C120-78	C1051-73	1.1	81	1.4
1676	C86-407	B6368	1.2	80	1.5
450	C92-325	CP70-1133	1.2	70	1.8
107	CR63-124	M165/38	1.3	77.5	1.6
463	C90-501	C90-101	1.3	75	1.8
151	C187-68	CP70-1133	1.4	95.1	1.5
1499	B6368	Co997	1.6	94.1	1.8
206	C1616-75	CP70-1133	1.8	90	2
1856	C86-503	C88-553	2.6	100	2.6
Mean			1.2	75.7	1.5
CV %			38.6	18.2	22.6

CV- coefficient of variation

Table 4. Estimated genetic value and category of families evaluated in Clonal Lot 1 -2017

Family Code	Progenitors		EGV	Category (according EGV)
	Femenino	Masculino		
465	C90-501	Mex66-1235	87.9	Verified
151	C187-68	CP70-1133	83.5	Moderately verified
476	C90-501	B6368	80.4	Exploratory
278	C92-325	C86-407	79.4	Exploratory
1636	C323-68	CP70-1133	78.2	Exploratory
1640	C323-68	CP72-2086	76.9	Moderately discarded
650	C323-68	C227-59	75.8	Very discarded
206	C1616-75	CP70-1133	74.6	Discarded
1499	B6368	Co997	73.1	Discarded

EGV- Estimated Genetic Value

Table 5. Variables brown rust degree (BR), incidence (%) and severity of femenine parents evaluated in Clonal Lot 1-2017

Femenine	BR degreee	Incidence (%)	Severity
C90-501	0.9	59.4	1.5
C86-56	0.9	72.5	1.3
C323-68	0.9	72.0	1.3
C92-325	1.0	74.3	1.4
C266-70	1.1	72.5	1.5
C120-78	1.1	81.0	1.4
C86-407	1.2	80.0	1.5
CR63-124	1.3	77.5	1.6
C187-68	1.4	95.1	1.5
B6368	1.6	94.1	1.8
C1616-75	1.8	90.0	2.0
C86-503	2.6	100.0	2.6
Mean	1.3	78.8	1.6
CV %	38.2	16.9	22.3

CV- coefficient of variation

Table 6. Variables brown rust degree (BR), incidence (%) and severity of male parents evaluated in Clonal Lot 1-2017

Masculine	BR degree	Incidence (%)	Severity
Mex66-1235	0.6	53.6	1.1
B45181	0.7	55.9	1.3
CP72-2086	0.8	63.2	1.3
C86-407	0.9	77.5	1.1
C187-68	0.9	72.5	1.3
C227-59	1.0	72.5	1.3
B6368	1.0	66.3	1.5
B45211	1.1	72.5	1.5
C1051-73	1.1	81.0	1.4
M165/38	1.3	77.5	1.6
C90-101	1.3	75.0	1.8
CP70-1133	1.4	84.8	1.6
Co997	1.6	94.1	1.8
C88-553	2.6	100.0	2.6
Mean	1.2	74.7	1.5
CV %	41.9	17.4	24.2

CV- coefficient of variation

devastating diseases for sugarcane production in the world (14). The use of disease-resistant parents is the fundamental basis of breeding programs.

Families and parents with higher values in the variables degree, incidence and severity increase the risk of obtaining susceptible cultivars. These should be limited in the crossing program; even more if it is considered that, the disease is present in all sugarcane areas of Cuba (15) and constitutes a limitation for sugarcane and sugar production.

Studies similar to this work allow discriminating the parents with the best chances of producing elite clones (10). Worldwide, multiple trials have been developed to eliminate the negative effects of brown rust on sugarcane yields, quality and profits. These include conventional breeding, molecular marker-assisted selection, disease monitoring, fungicide application, and selection of resistant cultivars by intensive methods (16,17).

Evaluation of parents based on progeny response to brown rust and other agronomic traits early in the breeding scheme can help improve the choice of crosses and parents in the breeding program. There is evidence on the effectiveness of family selection to identify the best individuals in families with a higher proportion of clones with suitable characteristics (18). The availability of brown rust evaluation data in families will allow breeders to develop crosses with disease resistance and high sugar cane and sugar yields.

CONCLUSIONS

- Sugar cane brown rust has high variability in degree, incidence and severity among families at the Clonal Lot 1 stage.
- The variables brown rust degree and severity are the most useful for identifying families with progenies resistant or tolerant to the disease.

- Families C90-501 x Mex66-1235 and C90-501 x B6368 provide high yielding individuals with resistance to brown rust.

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