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Source: Florida Entomologist, 99(4) : 769-773 Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.099.0432

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Resistance of rice varieties to *Sitophilus oryzae* (Coleoptera: Curculionidae)

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Abstract

Rice, *Oryza sativa* L. (Poaceae), is one of the world's most important food crops. Among the insects that damage rice grains, the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), is the most important species. The objective of this study was to identify rice varieties with resistance to *S. oryzae* as measured by grain feeding damage and *S. oryzae* reproduction. The rice varieties evaluated were obtained from the germplasm bank of the National Center of Research Rice and Beans, Embrapa, Brazil. The experimental design was completely randomized with 3 replications and 22 treatments (varieties). The varieties Bonança, Esmeralda, and Rio Verde had the antibiosis and/or antixenosis type of resistance, providing high rates of mortality, low rates of adult emergence, and low rates of grain consumption by *S. oryzae*. The varieties Pepita and Progresso were susceptible, with a high rate of *S. oryzae* adult emergence and grain consumption. The presence or absence of a fissure in the grain was a major morphological characteristic determining susceptibility or resistance to *S. oryzae* in rice varieties.

Key Words: Ozyza sativa; rice weevil; plant resistance to insects; stored pest

Resumo

O arroz (*Oryza sativa* L.; Poaceae) é uma das culturas alimentares mais importantes do mundo. Entre os insetos que danificam os grãos de arroz, o gorgulho do arroz *Sitophylus oryzae* L. (Coleoptera: Curculionidae) é a espécia mais importante. O objetivo deste trabalho foi identificar variedades de arroz com resistência a *S. oryzae*. As variedades de arroz avaliadas foram obtidas do banco de germoplasma do Centro Nacional de Pesquisas em Arroz e Feijão, EMBRAPA, Brasil. O delineamento experimental foi inteiramente casualizado com 3 repetições e 22 tratamentos (variedades). As variedades Bonança, Esmeralda e Rio Verde apresentaram resistência do tipo antibiose e/ou antixenose, tendo altas taxas de mortalidade, baixas taxas de emergência de adultos e baixo consumo de grãos por *S. oryzae*. As variedades Pepita e Progresso são suscetíveis com alta emergência de adultos e consumo de grãos por *S. oryzae*. As presença ou ausência de fissuras no grão é uma importante característica morfológica para determinar a susceptibilidade ou resistência a *S. oryzae* em variedades de arroz.

Palavras Chave: Ozyza sativa; gorgulho do arroz; resistência de planta a inseto; praga de grãos armazenados

Rice (*Oryza sativa* L.; Poaceae) is one of the most widely cultivated cereals and globally important food crops (Copatti et al. 2013; Nascimento et al. 2015). Brazil ranks ninth in the world in rice production, with a harvest of 12 million tons in the 2013/2014 season. The major producing states are Rio Grande do Sul, Santa Catarina, and Mato Grosso (MAPA 2014).

Among the insects that cause damage to rice grains in storage, the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), is the most important species (Nwaubani et al. 2014). Damage occurs from reduction of the grain weight, loss of nutritive value and germination, contamination by mites and fungi, and loss of commercial value of the product (Souza et al. 2012).

The main method of pest control of stored rice grain has been chemical, using phosphine (Ribeiro et al. 2003; Hossain et al. 2014). However, the development of resistance of *S. oryzae* to these products hinders its control, and alternative measures are necessary for its management (Lee et al. 2001; Ribeiro et al. 2013).

Integrated pest management (IPM) is considered the most sustainable way of controlling stored product pests (Ribeiro et al. 2003). Plant resistance, a component of IPM, reduces the population density of pests below the economic injury level without additional costs to the farmer, and is compatible with other pest control methods (Lara 1991; Eigenbrode & Trumble 1994; Seifi et al. 2013).

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Resistance to insects in stored grains can be manifested as antibiosis, wherein some property of the grain affects the insect's biology, increasing mortality and reducing longevity, reproduction, and damage. Alternatively, the grain may display antixenosis, wherein the behaviors of the insects are affected, normally resulting in reduced feeding and oviposition (Lara 1991; Smith 2005).

Among the factors that confer resistance to *S. oryzae* are the bracts surrounding the grain. The bracts consist of a palea and a lemma, and they enclose the grain. The opening (fissure) in the bracts affects the occurrence of *S. oryzae* infestation in stored grains (Ribeiro et al. 2012). This opening permits the infestation of *S. oryzae* and *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) in rice varieties and is an undesirable characteristic in the selection of genotypes with resistance to stored grain pests. Cohen & Russell (1970) reported a positive correlation between the presence of fissures in grains and infestation by *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) in rice varieties.

Another cause that confers resistance to stored grain pests is the relationship among amylase inhibitors. Marsaro Júnior et al. (2005) observed that high content of lipids and the presence of amylase inhibitors extended the biological cycle of *S. zeamais* and affected resistance in rice grains.

Potential use of resistant varieties in the control of *S. oryzae* and other stored grain insects was previously reported (Sousa et al. 2010; Bottega et al. 2012). However, there are few detailed studies to determine the resistance in rice varieties to *S. oryzae*. Fontes et al. (2003) observed resistance to *S. oryzae* and *S. zeamais* in the Cica 09 variety and concluded that varieties with the most defects in the shell are most susceptible. Sousa et al. (2010) observed resistance in Agulha and Nenenzinho varieties to *S. oryzae*, whereas Branco Tardão was the most susceptible. Thus, the objective of this work was to evaluate resistance in rice varieties in relation to damage, feeding behavior, and reproduction by *S. oryzae*.

Materials and Methods

The experiments were conducted in the Laboratory of Agriculture Entomology of the Federal Goiano Institute – Campus Urutai, Goias, Brazil. The varieties used were obtained from the germplasm bank of the National Center of Research Rice and Beans, Embrapa, Brazil. The varieties evaluated were Aimoré, Araguaia, Bonança, Cabaçu, Carisma, Caripuna, Centro América, Confiança, Curinga, Esmeralda, IRGA 22, Monarca, Pepita, Progresso, Rio Paraguai, Rio Paranaíba, Rio Verde, Sertaneja, Soberana, Tangará, Vencedora, and Xingú. These varieties were selected because they are grown commercially by farmers.

INSECT REARING

Insects were reared in 5 L capacity glass containers with a 7 cm diameter mouth covered with organdy (voile) held on by a screw cap. The containers containing grains of a commercial maize variety (AG 9010) were kept in a controlled environment (25 ± 2 °C, photoperiod of 12:12 h L:D, and 70% RH). Periodically, the grain and insects were removed and placed on a sieve. Then fresh grain was added to the rearing containers and the insects removed from the sieve were returned to the containers with the fresh grain.

RESISTANCE OF RICE VARIETIES TO THE RICE WEEVIL

The experiments were conducted in 2 L capacity glass jars, the opening of which was covered with an organdy mesh cloth. Each jar initially contained 50 g of a given variety and was infested with 10 newly emerged adults. The jars were maintained in a controlled environment

(25 ± 2 °C, photoperiod of 12:12 h L:D, and 70% RH). The resistance of the rice varieties to *S. oryzae* was evaluated at 30, 60, and 90 d after the initial infestation (DAI). At 30 DAI, the contents of the jar were placed in a 0.59 mm mesh sieve. The numbers of dead and live adults were determined and the remaining grains weighed. Grain consumption was determined by the equation: 50 g (initial grain weight) – g of grain remaining = grain weight consumed in 30 d. At 60 and 90 DAI, the dead and live adults were counted and grain consumption was determined. The experimental design was completely randomized with 22 treatments (varieties) and 3 replications.

STATISTICAL ANALYSES

Data were subjected to analysis of variance and means compared by the Scott–Knott test at 5% probability using the statistical software Sisvar (Ferreira 2011). The preference index for feeding was calculated at 90 DAI by using the formula: C = 2A / (M + A), where C = preference index; A = consumption of the tested variety; M = consumption of the susceptible check variety (i.e., Araguaia) (Fontes et al. 2003). The values ranged between 0 and 2. The interpretation of the data was according to the C value. When C > 1, the tested variety was preferred in comparison with the check variety (stimulant); when C = 1, the tested variety was similar to the check variety (neutral); and when C < 1, the tested variety was less preferred in comparison with the check variety (deterrent).

Hierarchical cluster analysis was performed and Euclidean distance calculated as a measure of dissimilarity to identify the groups as to the degree of resistance in rice varieties to *S. oryzae*. To determine the relationship of the susceptible varieties to the percentage of damaged grains, a correlation analysis between the percentage of grains with fissures and the percentage of damaged grains was conducted, using Statistica software version 7.0 (Statsoft 2004).

Results

The effect of varieties was significant ($P \le 0.05$, df = 21) for the total number of live insects that emerged and amount of grain consumed in the 3 storage periods (30, 60 and 90 d), indicating the presence of genetic variability among varieties to *S. oryzae* (Table 1).

At 30 d, the number of dead insects was greatest in Bonança (10.0 dead), Esmeralda (10.0), Rio Verde (10.0), and Sertaneja (9.3) varieties, demonstrating high levels of resistance in these varieties. In contrast, there was good survival of insects in the Cabaçú (7.3 alive), Centro América (8.0), Pepita (6.3), Tanagá (7.3), and Araguaia (6.0) varieties, indicating high susceptibility to the rice weevil. In evaluating the grain consumption during this period, the Pepita variety had the highest level of grain mass consumed (1.9 g) by *S. oryzae*.

After 60 d of storage and the weevils reproduced, the varieties Carisma (21.3) and Curinga (24.7) showed the highest mortality (dead insects) of adults whereas the varieties Aimoré (3.3), Vencedora (3.0), Confiança (0.0), Bonança (0.0), and Esmeralda (0.0) showed the lowest. The highest emergence (number alive) of adults occurred in Pepita (113.3) followed by Carisma (33.3) and Centro América (32.0). Rio Verde, Aimoré, IRGA 22, Esmeralda, and Bonança varieties had no live adults. The mean mass of grain consumed was highest in Pepita (9.5 g) and Cabaçú (5.6 g) varieties, and statistically different from all others.

At 90 d of storage, the Curinga (19.0), Rio Paranaíba (15.0), Progresso (12.0), and Bonança (10.0) varieties produced the largest number of dead adults. The other varieties with the lower values did not differ statistically. The largest number of live insects was found in the Pepita variety (238.0). There were no live adults on Sertaneja and Rio Table 1. Mean number (± SE) of alive and dead adults, and grain consumed (Cons.) by Sitophilus oryzae, during 3 periods of storage (30, 60, and 90 d) when cultured on different rice varieties.

	30 d			60 d			90 d		
Variety ^{a, b}	No. dead	No. alive	Cons. (g)	No. dead	No. alive	Cons. (g)	No. dead	No. alive	Cons. (g)
Aimoré	8.0 ± 2.0 a	2.0 ± 1.7 b	$1.0 \pm 0.1 \text{ b}$	3.3 ± 3.3 d	0.0 ± 0.0 d	1.0 ± 0.1 c	1.3 ± 0.9 b	6.7 ± 2.3 c	0.9 ± 0.1 c
Araguaia	4.0 ± 0.6 b	6.0 ± 0.6 a	1.2 ± 0.1 b	8.3 ± 2.6 c	14.7 ± 5.6 c	1.8 ± 0.2 c	4.0 ± 2.1 b	26.7 ± 9.9 b	2.1 ± 0.3 b
Bonança	10.0 ± 0.3 a	0.0 ± 0.0 b	1.3 ± 0.0 b	0.0 ± 0.0 d	0.0 ± 0.0 d	1.3 ± 0.0 c	10.0 ± 2.5 a	2.7 ± 0.7 c	0.8 ± 0.1 c
Cabaçú	2.7 ± 0.7 b	7.3 ± 1.7 a	1.4 ± 0.2 b	9.0 ± 1.2 c	5.3 ± 2.2 d	5.6 ± 2.2 b	8.3 ± 4.3 b	5.7 ± 2.0 c	1.7 ± 0.3 b
Caripuna	6.7 ± 1.7 a	3.3 ± 1.5 b	1.1 ± 0.1 b	6.3 ± 3.2 c	2.7 ± 1.8 d	1.2 ± 0.2 c	5.7 ± 2.3 b	1.0 ± 1.0 c	0.7 ± 0.3 c
Carisma	5.0 ± 0.6 b	5.0 ± 0.6 a	1.2 ± 0.1 b	21.3 ± 3.2 a	33.3 ± 11.9 b	2.3 ± 0.2 c	6.7 ± 1.9 b	15.0 ± 4.7 b	2.6 ± 0.3 b
Centro América	2.0 ± 0.6 b	8.0 ± 0.6 a	1.4 ± 0.1 b	8.3 ± 0.9 c	32.0 ± 6.8 b	2.1 ± 0.1 c	1.7 ± 0.9 b	24.3 ± 11.2 b	2.9 ± 0.8 b
Confiança	8.3 ± 0.9 a	1.7 ± 0.9 b	0.7 ± 0.0 b	0.0 ± 0.0 d	2.3 ± 1.5 d	0.9 ± 0.4 c	5.0 ± 3.6 b	1.0 ± 1.0 c	0.3 ± 0.1 c
Curinga	7.0 ± 0.3 a	3.0 ± 0.0 b	1.6 ± 0.1 b	24.7 ± 2.6 a	27.7 ± 2.9 d	2.3 ± 0.1 c	19.0 ± 1.2 a	14.0 ± 4.5 b	2.5 ± 0.1 b
Esmeralda	10.0 ± 0.0 a	0.0 ± 3.0 b	1.3 ± 0.2 b	0.0 ± 0.0 d	0.0 ± 0.0 d	1.3 ± 0.2 c	5.7 ± 2.3 b	5.0 ± 2.5 c	0.5 ± 0.3 c
IRGA 22	8.0 ± 1.5 a	2.0 ± 1.2 b	1.4 ± 0.8 b	6.7 ± 3.3 c	0.0 ± 0.0 d	1.4 ± 0.9 c	2.3 ± 1.9 b	2.0 ± 0.6 c	0.3 ± 0.3 c
Monarca	7.0 ± 0.9 a	3.0 ± 0.6 b	1.1 ± 0.1 b	13.3 ± 1.2 b	20.3 ± 7.7 c	1.6 ± 0.2 c	4.7 ± 1.3 b	25.0 ± 7.9 b	1.5 ± 0.2 b
Pepita	3.7 ± 1.7 b	6.3 ± 2.6 a	1.9 ± 0.1 a	16.0 ± 2.6 b	113.3 ± 22.8 a	9.5 ± 0.1 a	5.3 ± 0.9 b	238.0 ± 3.2 a	5.8 ± 0.1 a
Progresso	2.7 ± 1.2 b	7.3 ± 1.2 a	1.3 ± 0.0 b	13.0 ± 2.5 b	17.7 ± 2.6 c	2.1 ± 0.2 c	12.0 ± 7.0 a	18.7 ± 7.8 b	5.3 ± 3.2 a
Rio Paraguai	5.7 ± 1.5 b	4.3 ± 1.3 a	1.1 ± 0.0 b	10.7 ± 0.7 c	4.3 ± 1.5 d	1.1 ± 0.0 c	8.0 ± 1.2 b	0.0 ± 0.0 c	1.0 ± 0.0 c
Rio Paranaíba	4.3 ± 2.4 b	5.7 ± 2.2 a	1.3 ± 0.1 b	8.7 ± 0.3 c	6.3 ± 2.0 d	1.5 ± 0.1 c	15.0 ± 4.4 a	2.3 ± 2.3 c	0.9 ± 0.2 c
Rio Verde	10.0 ± 0.0 a	0.0 ± 0.0 b	1.2 ± 0.1 b	10.0 ± 0.0 c	0.0 ± 0.0 d	1.2 ± 0.1 c	4.7 ± 2.4 b	2.0 ± 1.2 c	0.9 ± 0.1 c
Sertaneja	9.3 ± 0.7 a	0.7 ± 0.7 b	1.0 ± 0.0 b	10.0 ± 0.0 c	0.3 ± 0.3 d	1.0 ± 0.0 c	0.7 ± 0.3 b	0.0 ± 0.0 c	0.5 ± 0.0 c
Soberana	5.0 ± 0.6 b	5.0 ± 0.6 a	1.1 ± 0.1 b	10.7 ± 0.7 c	11.3 ± 4.3 d	3.5 ± 1.9 c	9.7 ± 2.3 a	18.7 ± 6.4 b	3.2 ± 1.7 b
Tangará	2.7 ± 1.7 b	7.3 ± 1.2 a	1.2 ± 0.0 b	8.7 ± 1.2 c	6.3 ± 2.3 d	1.5 ± 0.1 c	7.0 ± 4.7 b	3.0 ± 1.0 c	0.8 ± 0.1 c
Vencedora	8.7 ± 0.9 a	1.3 ± 1.0 b	1.1 ± 0.0 b	3.0 ± 3.0 d	0.3 ± 0.3 d	1.1 ± 0.0 c	5.0 ± 1.2 b	1.7 ± 1.7 c	0.3 ± 0.2 c
Xingu	8.0 ± 1.0 a	2.0 ± 1.0 b	1.6 ± 0.0 b	4.0 ± 0.6 c	1.0 ± 0.6 d	1.4 ± 0.1 c	2.3 ± 0.9 b	0.7 ± 0.7 c	0.8 ± 0.1 c
F (Treatment) ^d	5.82**	3.47**	1.48**	10.25**	39.69**	8.84**	2.61**	115.63**	3.68**
C.V. (%)	32.24	33.42	28.40	26.66	30.04	25.35	36.48	32.61	33.27

^aMeans in a column followed by the same letter did not differ by the Scott–Knott test at 5% probability. ^bThe initial infestation was 10 weevils per iar.

"Susceptible check variety.

Asterisks indicate significance level; *, significant at 5% probability; **, significant at 1% probability.

Paraguai varieties, but they did not differ statistically from Xingú (0.7), Caripuna (1.0), Confiança (1.0), Rio Verde (2.0), Rio Paranaíba (2.3), Bonança (2.7), Tangará (3.0), Vencedora (1.7), IRGA 22 (2.0), Cabaçú (5.7), Esmeralda (5.0), and Aimoré (6.7) varieties. The highest grain consumption (g) was seen in Pepita (5.8) and Progresso (5.3) varieties, lower consumption in Soberana (3.2), Centro América (2.9), Carisma (2.6), Curinga (2.5), Araguaia (2.1), Cabaçú (1.7), and Monarca (1.5), and the lowest in the remaining varieties.

The preference index for feeding showed differences between the susceptible check variety (Araguaia) and Carisma, Centro América, Curinga, Pepita, Progresso, and Soberana varieties, which were classified as stimulants (Fig. 1). The rest were classified as deterrent to *S. oryzae*. The correlation between the mass of damaged grains and the presence of fissures in the grains was the highest in the Pepita and Progresso varieties. The Curinga variety showed the lowest presence of fissures on the shell of the grain but experienced relatively high damage by adults of (Fig. 2).

The hierarchical cluster analysis, based on data of the total number of live insects, dead insects, and grain mass consumed during the 3 storage periods, revealed differences between each group of rice varieties according to the degree of similarity (Fig. 3). The varieties Rio Verde and Sertaneja were similar in the dendrogram by having the smallest Euclidean distance, followed by Aimoré, Vencedora, Xingú, IRGA 22, Esmeralda, Bonança, Caripuna, Rio Paraguai, Monarca, and Confiança varieties; these formed the group that showed a high degree of resistance to *S. oryzae* (Fig. 3). Centro América, Tangará, Araguaia, and Curinga varieties formed a second group of intermediate varieties, and the Pepita variety formed the last group, having the largest Euclidean distance and being distinct from other groups formed. Pepita along with Curinga had little similarity to the other varieties and showed a high susceptibility to *S. oryzae*.

Thus, considering the Euclidean distance of 3.0, different levels of resistance can be established for the rice varieties, based on the numbers of live insects and grain consumption by *S. oryzae*: the first group (Pepita and Curinga) was highly susceptible, the second group (Araguaia, Tangará, Centro América, Rio Paranaíba, Progresso, Soberana, Cabaçú,



Fig. 1. Preference index of feeding by *Sitophilus oryzae* (Coleoptera: Curculionidae) on 22 rice varieties.



Fig. 2. Correlation between the percentage of grains with fissures in the shell and mass of grain consumed by *Sitophilus oryzae* (Coleoptera: Curculionidae) in 22 rice varieties. 1-Aimoré; 2-Araguaia; 3-Bonança; 4-Cabaçu; 5-Caripuna; 6-Carisma; 7-Centro América; 8-Confiança; 9-Curinga; 10-Esmeralda; 11-IRGA 22; 12-Monarca; 13-Pepita; 14-Progresso; 15-Rio Paraguai; 16-Rio Paranaíba; 17-Rio Verde; 18-Sertaneja; 19-Soberana; 20-Tangará; 21-Vencedora; 22-Xingu.

and Carisma) was susceptible, and the third (Confiança, Monarca, Rio Paraguai, Caripuna, Bonança, Esmeralda, IRGA 22, Xingú, Vencedora, Aimoré, Sertaneja, and Rio Verde) was moderately resistant.

Discussion

The antibiosis type of resistance is characterized by alterations in the biology of insects such as high mortality in the immature stages and low adult emergence (number of live adults). Antixenosis is characterized by effects on the ability of the insect to select the host for food, shelter, or oviposition (Smith 2005). These resistance mechanisms normally include the presence of morphological features or allelochemicals in the host (Lara 1991; Smith 2005).

The results showed different behavior of *S. oryzae* with regard to extent of grain damage and number of live adults among the varieties (Table 1). In general, during the 3 evaluation periods, the Bonança, Esmeralda, and Rio Verde varieties were resistant as expressed by antibiosis and/ or antixenosis, and associated with high rates of mortality, low rates of adult emergence, and low grain consumption by *S. oryzae*. Pepita and Progresso varieties were susceptible, as shown by high adult emergence rates and grain consumption by *S. oryzae*. According to the preference index, Bonança, Esmeralda, and Rio Verde varieties were classified as having deterrent effects, and Pepita and Progresso possessed stimulant effects, as compared with the standard susceptible check variety Araguaia (Fig. 1).

In rice varieties, the main factors associated with resistance are related to morphological characteristics of the shell, which include the absence of a fissure or the hardness of the grains, and/or to chemical inhibitors such as the presence of an amylase inhibitor (McGaughey et al. 1990; Fontes et al. 2003; Marsaro Júnior et al. 2005). As reported by Ribeiro et al. (2012), the resistance in rice genotypes to *S. zeamais* and *S. oryzae* was positively correlated with the presence of a fissure in the shell of the grains. The authors concluded that varieties without a fissure and those that have a closed shell are resistant to pests of stored grain. Link et al. (1971) evaluated the resistance in rice varieties to *S. oryzae, S. zeamais*, and *S. cerealella* and observed that varieties showing a high frequency of grains with defects in the shell were the most damaged. The results reported herein confirm that this feature is



Fig. 3. Dendrogram based on the data of the total number of *Sitophilus oryzae* adults emerged (alive) and dead, and grain mass consumed on rice varieties during 3 storage periods. The hierarchical cluster analysis was performed using Ward's method with the Euclidean distance as dissimilarity measure. The arrow indicates the Euclidean distance used for separating the groups (Phenon line).

very important in relation to choice of *S. oryzae* for rice varieties. The resistant varieties Rio Verde and Bonança showed low percentages of grains with a fissure, confirming the importance of this morphological characteristic in the selection of resistant varieties.

The Esmeralda variety, although characterized as resistant, had a relatively high percentage (20%) of grains with fissures. Therefore, it is believed that other characteristics, in addition to the presence of fissures, are associated with the manifestation of resistance; these may include grain hardness or the presence of amylase inhibitors (McGaughey et al. 1990; Marsaro Júnior et al. 2005). Jha et al. (2012), when evaluating resistance in wheat varieties, found low losses of grain mass and low adult emergence of *S. oryzae* in varieties DDW 12 and C 306, which had hard grains.

Levels of resistance can be classified as immunity, high resistance, moderate resistance, susceptibility, and high susceptibility (Smith 2005; Campos et al. 2011). The results presented by hierarchical clustering analysis and adopting the Euclidean distance of 3.0 allowed the formation of 3 distinct groups in terms of resistance levels (Fig. 3). Pepita and Curinga were highly susceptible; Araguaia, Tangará, Centro América, Rio Paranaíba, Progresso, Soberana, Cabaçú, and Carisma were susceptible; and Confiança, Monarca, Rio Paraguai, Caripuna, Bonança, Esmeralda, IRGA 22, Xingú, Vencedora, Aimoré, Sertaneja, and Rio Verde were moderately resistant to *S. oryzae*.

Acknowledgments

We thank the Instituto Federal Goiano–Campus Urutaí for funding the present study, and the Brazilian Council for Scientific and Technological Development–CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico–CNPq) for the Productivity in Research grant awarded to the last author.

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